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Articles

Effects of zinc oxide nanoparticles and auxin on adventitious roots morphology and phytochemistry of Coleus forskohlii Briq. (Lamiaceae)

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Coleus forskohlii Briq., a medicinal plant from the Lamiaceae family, has been utilized worldwide to treat various disorders. The primary active compound in this plant is forskolin, a diterpene that accumulates in its rhizome. This study aims to assess the in vitro culture of *C. forskohlii* by investigating the effects of different concentrations of auxin hormone (1 and 2 mg L^{-1}) and zinc oxide (ZnO) nanoparticles (10 and 20 ppm) as elicitors on biometrical traits related to rooting as well as forskolin content. Each treatment was replicated three times, and the results were analyzed using SPSS version 20. We observed that most traits examined decreased in samples treated with 1 mg L^{-1} of IAA hormone. In contrast, samples treated with 2 mg L^{-1} of IAA exhibited the highest values compared to the control samples. However, the differences in the examined characteristics among the samples were not statistically significant. Notably, the number of adventitious roots and the forskolin content increased with each increment in IAA hormone concentration. In plants treated with 2 mg L^{-1} IAA hormone, all measured biometrical traits surpassed those of the control group as well as those of samples co-treated with IAA (2 mg L^{-1}) and ZnO nanoparticles (10 and 20 ppm). The forskolin content followed a similar trend. However, the lowest values for these traits were observed in the plants treated with 2 mg L^{-1} IAA and 20 ppm ZnO nanoparticles. These findings indicate that 2 mg L^{-1} of IAA hormone significantly impacts both root morphology and phytochemical characteristics compared to 1 mg L^{-1}. Furthermore, ZnO nanoparticles at a concentration of 20 ppm exhibited an inhibitory effect on root morphology and forskolin content, which could not be mitigated by treatment with 2 mg L^{-1} IAA.

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Keywords

Coleus forskohlii, Forskolin, nanoparticle, phytohormones, tissue culture

Introduction

Coleus forskohlii Briq., commonly known as Coleus, is a medicinal herb belonging to the Lamiaceae family. This plant is extensively used in traditional medicine to treat a variety of conditions, including inflammatory diseases, hypertension, respiratory disorders, aging, and weight management. Rich in various secondary metabolites such as terpenoids, flavonoids, and alkaloids, Coleus primarily contains forskolin, a labdane diterpene that accumulates in the plant's roots (Kulkarni et al. 2023). Forskolin enhances the accumulation of cyclic adenosine monophosphate (cAMP) without the hormonal stimulation of adenylate cyclase, thereby activating other enzymes through direct or indirect pathways (Sapio et al. 2017).

Research has shown that both environmental and genetic factors significantly influence the amount of forskolin in Coleus. Additionally, phytogeographical diversity and distinct chemotypes have been observed among natural populations of *C. forskohlii* (Srivastava et al. 2017).

To extract secondary metabolites from plants, various cells or organs can be cultivated in laboratory settings based on the site of biosynthesis for these compounds (Biswas et al. 2023). Since forskolin accumulates in the roots of Coleus, root culturing offers a promising method to obtain this compound in greater quantities. The exogenous application of elicitors is a well-known strategy to enhance the biosynthesis of secondary metabolites in root cultures (Biswas et al. 2023). In this context, Sun et al. (2023) demonstrated that various auxin phytohormones significantly promote the formation of adventitious roots by stimulating cell division and expansion in plants. The three primary types of auxin hormones in plants are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NNA) (Butova et al. 2023).

The application of nanostructures in agriculture aims to enhance crop production by improving plant resilience under various environmental conditions, such as water scarcity, salinity, and elevated CO2 levels (Usman et al. 2020; Kumari et al. 2023). Zinc (Zn) is an essential element for all living organisms, crucial for the activation of several enzymes in plant cells, including alcohol dehydrogenase, carbonic anhydrase, and RNA polymerase. Zinc stabilizes biomembranes by interacting with membranous molecules such as phospholipids and proteins and is involved in various metabolic pathways, including photosynthesis, glycolysis, and the synthesis of lipids and nucleic acids. Moreover, this cation plays a vital role in the production and detoxification of reactive oxygen species (Balafrej et al. 2020).

The current study aims to investigate: (1) the effects of different concentrations of auxin (IAA) and zinc oxide nanoparticles on the induction of adventitious roots and their morphological traits in Coleus, (2) the impact of various percentages of auxin and zinc oxide nanoparticles on forskolin biosynthesis in the plant, and (3) the morphological traits of adventitious roots and forskolin biosynthesis in plants co-treated with different concentrations of auxin and ZnO nanoparticles.

Materials and methods

Establishment of adventitious roots and treatment

C. forskohlii (Wizard Mosaic cultivar) plants were sourced from the Iranian market (Markazi Province, Mahallat – code 452644). Healthy and intact parts were collected from the parent plant and disinfected in a 5% sodium hypochlorite solution for 10 minutes. Following this, the explants were thoroughly rinsed with sterilized water for 5 minutes to remove any residual disinfectant.

The explants were then immersed in a rooting hormone solution (IAA) for 5 to 10 minutes at three different concentrations: 0, 1, and 2 mg L^{-1}. After treatment, the explants were transferred to sterilized flasks containing 200 mL of MS liquid medium.

Three replicates were conducted for each treatment, resulting in a total of 9 flasks and 27 explants. All culture vessels were placed in a growth chamber maintained at 26±2 °C and illuminated with 5000–6000 lux light intensity for 4 weeks to facilitate the production of adventitious roots. At the end of this period, the adventitious root cultures were treated with zinc oxide nanoparticles at concentrations of 10 and 20 ppm for 10 days.

For each treatment, several morphometric parameters of the roots were measured, including the number of roots per explant, root length (in cm), as well as the fresh and dry weights of the roots (in mg). Root length and weight were measured using a digital caliper and an A&D digital scale, respectively.

Extraction and quantification of forskolin

Forskolin content was quantified according to the method outlined by Schaneberg and Khan (2003). To begin, 100 mg of freeze-dried root powder was weighed and transferred to a centrifuge tube, to which 6 mL of acetonitrile was added. The samples were sonicated for 15 minutes, after which they were centrifuged. The supernatant was carefully pipetted into either a 10 mL or 25 mL volumetric flask, and the volume was adjusted with acetonitrile to the final mark.

Prior to HPLC injection, each sample was filtered through a 0.45 µm nylon membrane filter. The relative forskolin content was then measured using an Agilent 1100 HPLC system (USA), employing a mobile phase of acetonitrile and water in a 1:1 ratio with a flow rate of 1 mL min^{-1}.

Statistical analysis

The collected data were analyzed using SPSS version 20 software, applying ANOVA and Duncan’s multiple range tests for statistical assessment. Diagrams were created using Excel version 2010 software.

Results

IAA hormone treatment on root biometry and phytochemistry

Number of adventitious roots in the control plant was 80, while it was increased to 120 and 180 in the treated samples with IAA hormone at the concentrations of 1 and 2 mg L^{-1}, respectively (Fig. 1A). Average length of adventitious roots was 2.75 cm in the control plant, but it decreased to 1.25 and 2.5 cm in the treated samples by IAA hormone at the concentrations of 1 and 2 mg L^{-1} (Fig. 1 B). Average weight of fresh roots was 0.81 g in the control plants. It decreased to 0.6 g in the coleus root samples were treated with IAA hormone at the concentration of 1mg L^{-1}. Meanwhile, roots weight increased to 1.38 g in 2 mg L^{-1} IAA hormone treated samples (Fig. 1 C). The weightiest dried roots (0.15 g) were detected in *C. forskohlii* treated samples with 2 mg L^{-1} IAA hormone. Meanwhile, the dried roots weight in the control plants was bigger than those were treated with 2 mg L^{-1} IAA hormone (Fig. 1 D). However, no significant variation was detected for the entire examined trait. The highest and lowest relative amounts of forskolin (without any significant variance) were belonged to 2 mg L^{-1} IAA hormone-treated plants and the controls, respectively (Fig. 2).

Effect of co-treatment on root morphology and phytochemistry

The mean number of the adventitious roots in the samples treated with 2 mg L^{-1} IAA hormone was bigger than the control samples and those were co-treated with 2 mg L^{-1} IAA hormone and 10 and 20 ppm ZnO nanoparticles, respectively. In addition, no significant difference was detected in adventitious roots number among the treated samples (Fig. 3 A). The largest root (2.75 cm) belonged to the control samples compared with those were treated with 2 mg L^{-1} IAA hormone (2.6 cm), and in co-treated samples with 2 mg L^{-1} IAA hormone and 10 (2.25 cm) and 20 ppm (1.9 cm) ZnO nanoparticles. There was no significant variation among the treated plants (Fig. 3 B). The roots fresh and dried weights in the plants treated with 2 mg L^{-1} IAA hormone were bigger than the control sample, and those co-treated samples with 2 mg L^{-1} IAA hormone and both concentrations of ZnO nanoparticles. However, we did not find any significant difference among the treated plants (Fig. 3 C, D).

The highest forskolin amount was detected in 2 mg L^{-1} IAA hormone-treated plants, rather than the control samples, and those which were co-treated with 2 mg L^{-1} IAA hormone and 10 and 20 ppm ZnO nanoparticles. In this regard, the smallest amount belonged to the co-treated plants by 2 mg L^{-1} IAA hormone and 20 ppm ZnO nanoparticles. No significant difference was determined among the samples (Fig. 4).

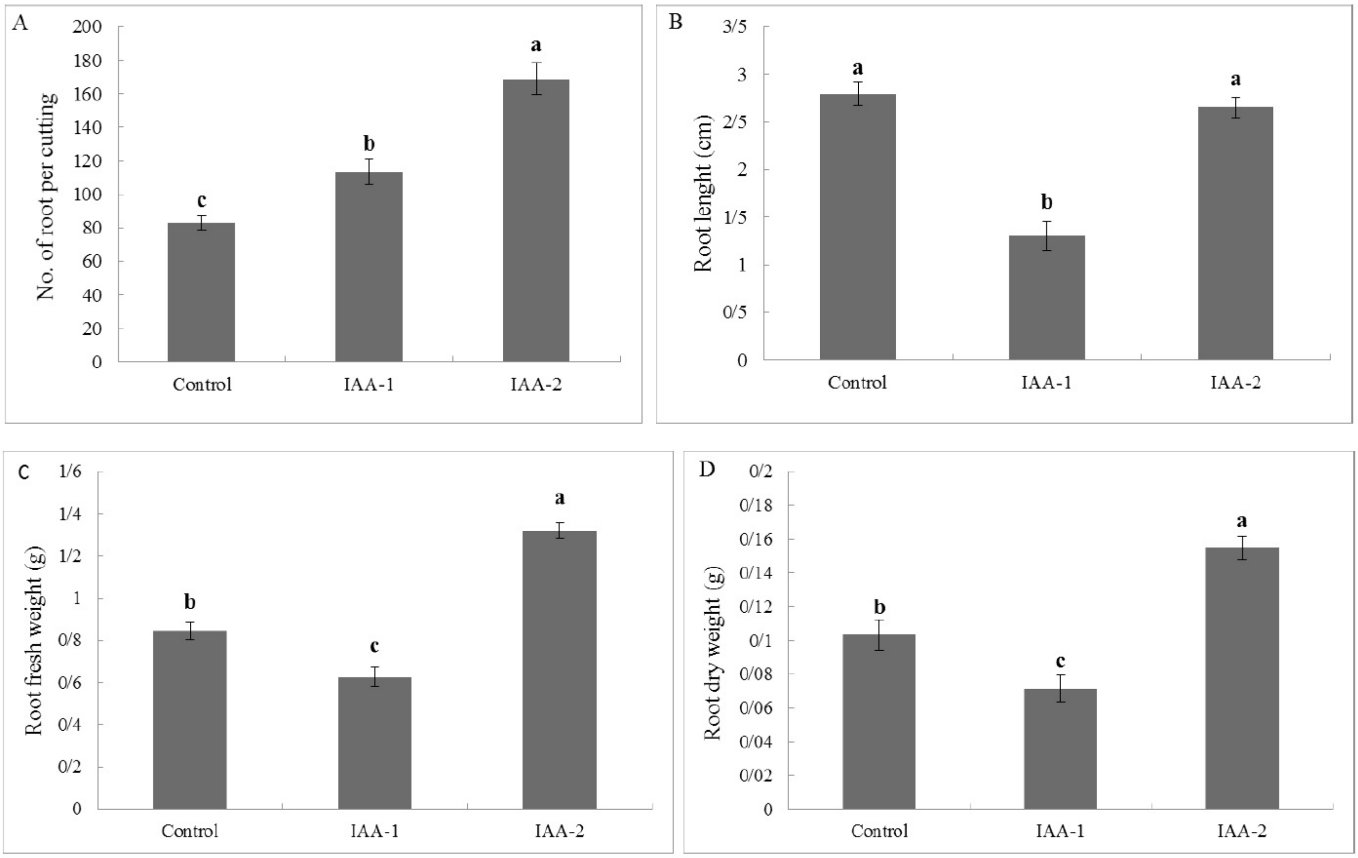

Figure 1. *Comparison of the effect of various concentrations of IAA hormone (0, 1 and 2 mg L^{-1}) on a) the number of adventitious roots, b) root length, c) fresh weight, and d) dry weight of C. forskohlii. Similar characters indicated a lack of significant difference between the mean values of the Duncan test.*

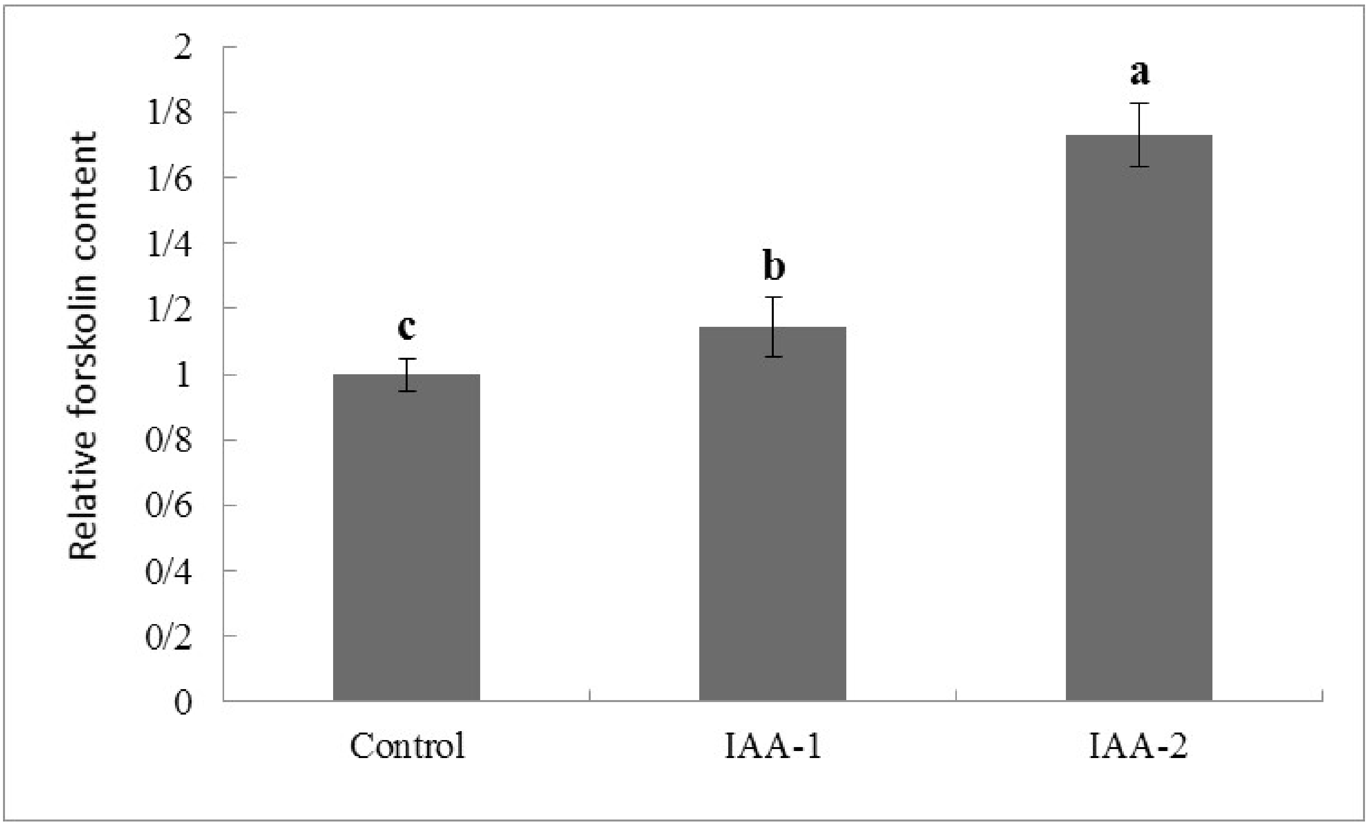

Figure 2. *Comparison of the effect of various concentrations of IAA hormone (0, 1 and 2 mg L^{-1}) on the forskolin content of the adventitious roots in C. forskohlii. Similar characters indicated a lack of significant difference between the mean values of the Duncan test.*

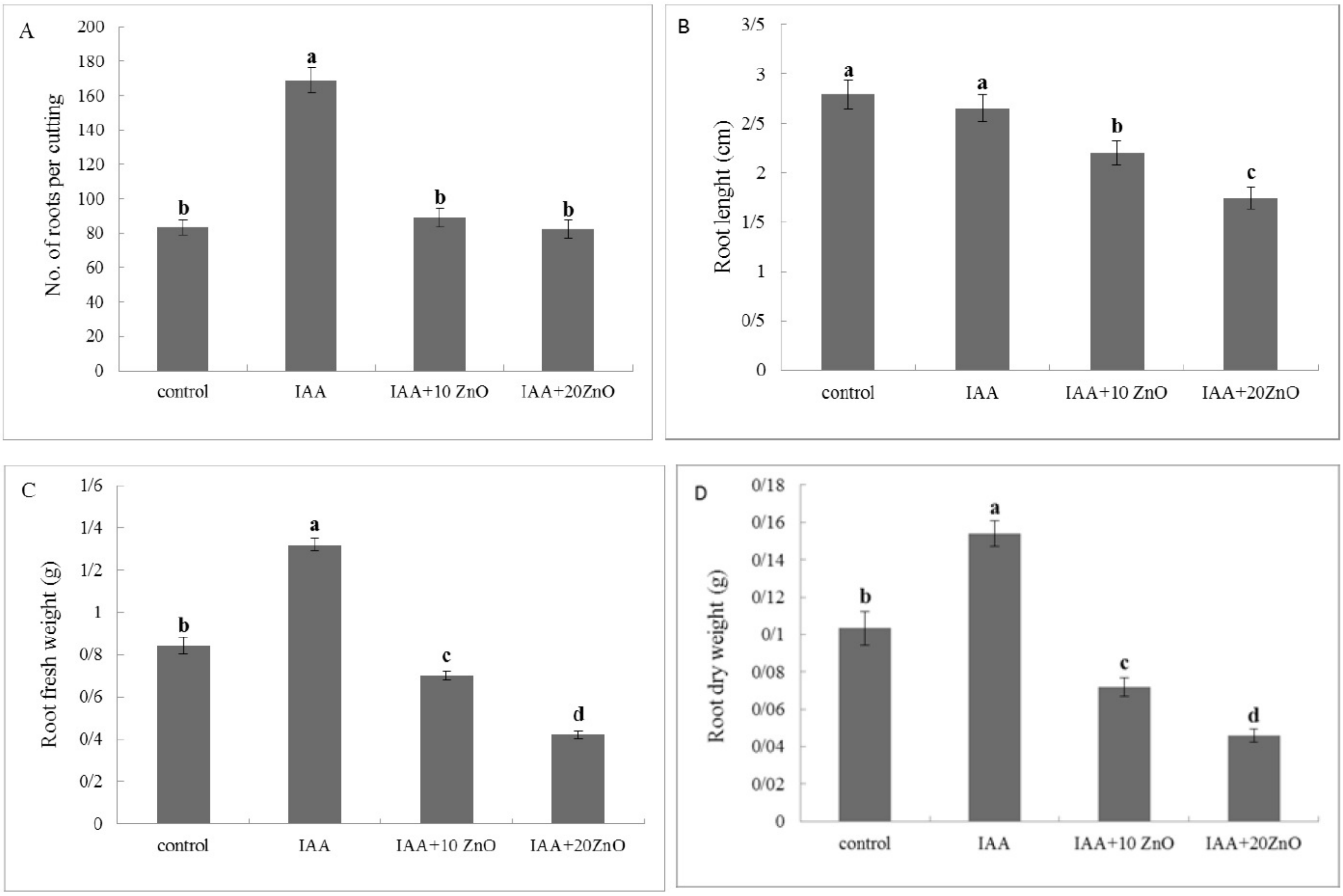

Figure 3. *Comparison of the effect of various concentrations of IAA hormone (0, 1, and 2 mg L^{-1}) and nano ZnO (10 and 20 ppm) on a) the number of the adventitious roots, b) root length, c) fresh weight and d) dry weight of C. forskohlii. Similar characters indicated the lack of significant difference between the mean values of the Duncan test.*

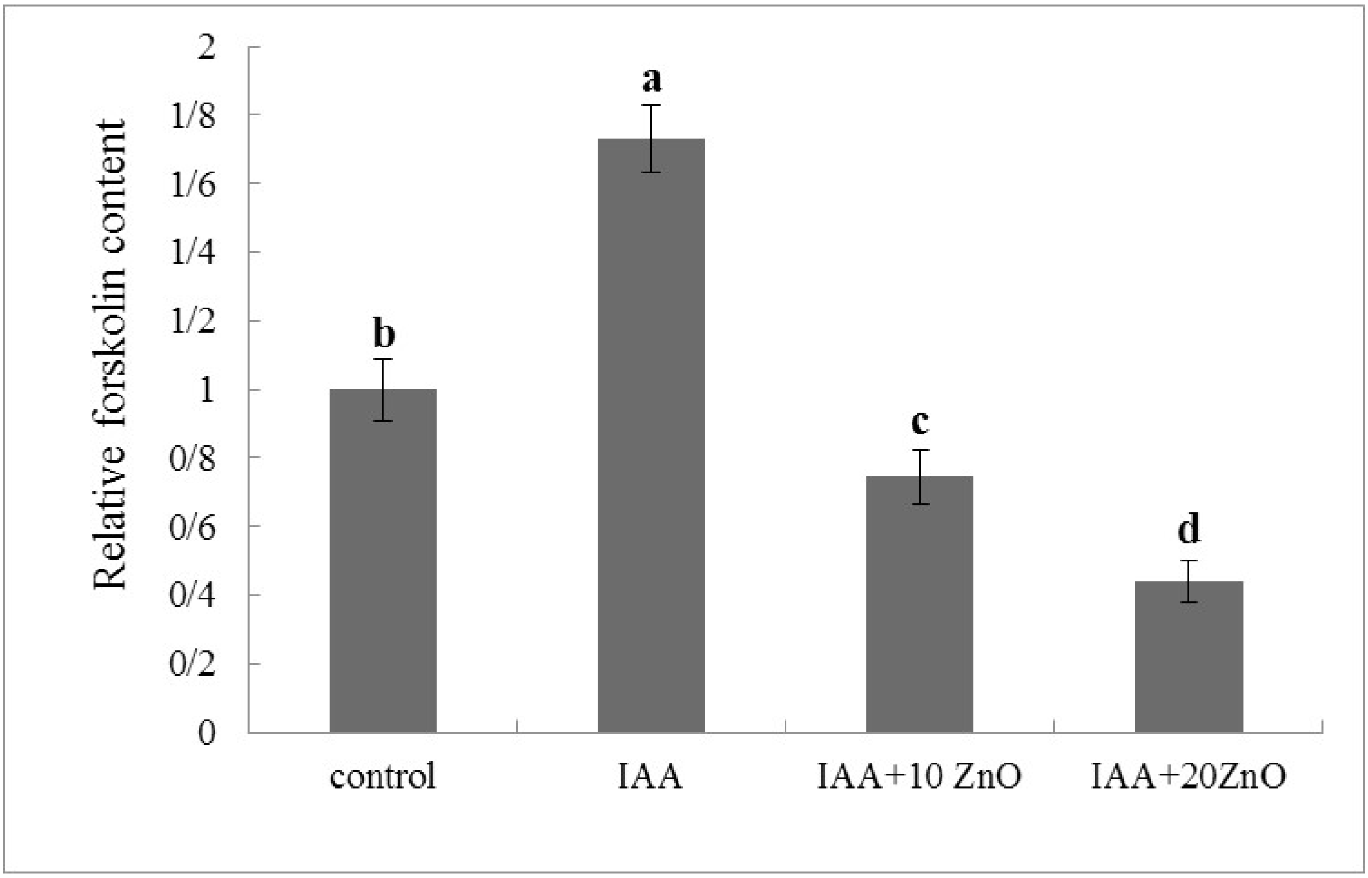

Figure 4. *Comparison of the effect of various concentrations of IAA hormone (0 and 2 mg L^{-1}) and nano ZnO (10 and 20 ppm) on forskolin content of the adventitious roots of C. forskohlii. Similar characters indicated the lack of significant difference between the mean values of the Duncan test.*

Discussion

In this study, we evaluated forskolin biosynthesis and root morphometric traits – including the number, length, and fresh and dry weights of adventitious roots—in one-month-old C. forskohlii plants treated with indole-3-acetic acid (IAA) at concentrations of 1 and 2 mg L^{-1}, both in the presence and absence of zinc oxide (ZnO) nanoparticles at concentrations of 0, 10, and 20 ppm.

Our results demonstrated a significant relationship between IAA concentration and root morphological variables, including the number and length of adventitious roots, as well as their fresh and dry weights. This finding aligns with the work of Sivakumar et al. (2021), who reported that IAA was more effective than other plant phytohormones in promoting root growth in *C. forskohlii* nodal segments. The formation of adventitious roots and their morphological characteristics are influenced by various internal and external factors (Ghimire et al. 2022). Among these, phytohormones – particularly auxins – play a crucial role (Zheng et al. 2020). Auxins regulate plant growth by promoting adventitious root development, which involves stimulating cell division in the cortex and phloem, fostering the proliferation of root tissue, and disrupting sclerenchyma structures. Furthermore, auxins synchronize cell division and formation patterns, facilitating the development of lateral roots by activating the cell cycle (Wei et al. 2019).

Successful rooting hinges on the transfer of natural auxins synthesized in young shoots and leaves to the ends of cuttings to prevent tissue death (Kasim et al. 2009). Numerous studies have demonstrated that the application of exogenous auxins can enhance adventitious rooting, with root development closely tied to auxin concentration and distribution. For instance, optimal auxin levels have been shown to stimulate rooting in *Malus hupehensis* (Zhang et al. 2017), *Salvia* *fruticosa* Mill. (Sağlam et al. 2014), and *Dalbergia sissoo* (Omar and Ahmed 2017). Palm et al. (2019) suggested that auxin enhances DNA transcription and RNA synthesis, particularly ribosomal RNA, thereby increasing protein biosynthesis.

Among the evaluated characteristics, IAA at a concentration of 2 mg L^{-1} exhibited positive effects, whereas a reverse pattern was observed at 1 mg L^{-1}. Notably, the number of adventitious roots increased with higher IAA concentrations. Paque and Weijers (2016) noted that auxins function as morphogens, altering cell specification in a concentration-dependent manner, whereby different concentrations yield diverse outcomes.

Our findings indicated that variations in IAA concentrations (1 and 2 mg L^{-1}) positively impacted forskolin biosynthesis in the adventitious roots of *C. forskohlii*. According to Sivakumar et al. (2021), forskolin is a principal bioactive compound found in the roots of the coleus plant. Thus, increasing IAA concentration, which correlates with higher root numbers, significantly enhances forskolin levels. The concentration of growth regulators is critical for the production, accumulation, and quality of secondary metabolites (Ghassemi-Golezani et al. 2022). For instance, treatments with 2,4-D, NAA, or IAA have been shown to enhance flavonoid production in *Cichorium intybus* L. (Fathi et al. 2018). Similarly, Linh et al. (2019) reported that hormonal elicitors like NAA and methyl jasmonate boosted biomass production and medicinal compound yield in capillary roots of *Panax vietnamensis* cultured in liquid media.

Additionally, various auxins have been applied to maximize gentiopicroside production in the capillary roots of *Gentiana scabra*, with the highest yields achieved using 1 mg L^{-1} of NAA in the liquid medium (Huang et al. 2014). Furthermore, Gangopadhyay et al. (2011) indicated that naphthalene acetic acid (NAA) significantly influenced growth and secondary metabolite production in *Plumbago indica* roots. However, it is essential to note that the optimal concentration of IAA varies by species, and in our study, IAA at 2 mg L^{-1} outperformed 1 mg L^{-1}. Plant growth regulators like auxins can modify gene expression, activate specific genes, and trigger enzymes across various biosynthetic pathways, ultimately mediating the production of secondary metabolites such as forskolin (Raei et al. 2017).

Our study also revealed a decrease in all root characteristics at both ZnO nanoparticle concentrations (10 and 20 ppm) compared to the control groups. The impact of nanoparticles on plants depends on factors such as nanoparticle properties, concentrations, application methods, and plant species (Talebi et al. 2022). Since zinc is classified as a heavy metal, high concentrations can act as a stressor. For instance, elevated levels of ZnO nanoparticles have been shown to negatively affect seed germination, root morphology, elongation, and leaf number in *Arabidopsis* (Yang et al. 2023), as well as similar adverse effects in wheat (Amirjani et al. 2016) and other crops such as radish, canola, ryegrass, lettuce, and cucumber (Lin and Xing 2007).

We observed that root length decreased with increasing ZnO nanoparticle concentrations, consistent with Balafrej et al. (2020), who reported that zinc stress reduces primary root length due to inhibited proliferation and subsequent elongation of root cells. Li et al. (2013) linked decreased root growth to cell death in meristematic tissue and heightened lignification levels in *Triticum aestivum* seedlings exposed to high zinc concentrations. Additionally, in some plants treated with high zinc levels, crystal accumulation in the parenchyma cells of xylem can obstruct nutrient transport and hinder root growth.

Furthermore, the forskolin content in all plants treated with auxin hormones and ZnO nanoparticles decreased compared to the control groups, indicating that ZnO nanoparticles had an inhibitory effect on forskolin biosynthesis in the coleus plant at both concentrations tested. Moreover, the IAA treatment failed to mitigate the toxic effect of the nanoparticles on forskolin production. Similar findings were reported by Riahi-Madvar and Shahbazi (2015), who noted that high concentrations of heavy metals like copper reduced forskolin biosynthesis.

Conclusions

In the current study, we investigated the effects of varying concentrations of IAA on the biometrical and phytochemical characteristics of adventitious roots. Our results showed that root length, as well as dried and fresh weights, increased in samples treated with 2 mg L^{-1} IAA compared to the controls. However, treatment with 1 mg L^{-1} IAA exhibited an inhibitory effect on these traits. Notably, both the number of adventitious roots and the forskolin content in *C. forskohlii* increased with each increase in IAA concentration. In contrast, the morphometric characteristics and forskolin levels in plants co-treated with 2 mg L^{-1} IAA and ZnO nanoparticles decreased as the concentration of nanoparticles increased. These findings suggest that IAA at 2 mg L^{-1} promotes root development and forskolin production, it does not mitigate the toxic effects of ZnO nanoparticles.

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