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STRATEGIES TO ENHANCED PRODUCTION OF BIOMASS AND NATURAL ANTIOXIDANTS OF *CICHORIUM INTYBUS* L. HAIRY ROOTS BY USING NANOPARTICLE ELICITORS

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ABSTRACT

This study describes the influence of silver nanoparticles (AgNPs) and selenium nanoparticles (SeNPs) on the biomass and phytochemical production in the hairy root (HR) cultures of *Cichorium intybus* L. The HRs were grown in ½ Murashige and Skoog (MS) medium supplemented with sucrose (30 g/L) and AgNPs or SeNPs at two selected concentrations for 25 days on the shaker at 110 rpm in the dark. The results showed that SeNPs stimulated HR growth whereas AgNPs inhibited it at both concentrations used. Peroxidase activity was higher than catalase activity. In most cases, the antioxidant enzyme activities were significantly higher in the HRs elicited by elicitors than in control HRs. The activities of catalase and peroxidase reached their highest levels at approximately 16.7 and 486.4 u/mg protein, respectively. DPPH radical scavenging activity was highest in the HRs elicited by AgNPs. AgNPs (3.25 and 1.5625 mg/L) elicited HR extracts had significantly enhanced the production of total phenolic, and total flavonoid contents compared to the control HR extracts. The maximum total phenolic content was 87.04 ± 6.23 mg/g gallic equivalent, and it was 139.03 ± 3.56 mg/g ascorbic acid equivalent in total flavonoid content in the HRs elicited by AgNPs. The HRs elicited by SeNPs produce an average amount of total phenolic, flavonoid, and DPPH radical scavenging activity which were also significantly higher than those produced by control HRs. The results from our study suggested the effectiveness of the elicitation process in enhancing the root biomass, total phenolic, and flavonoid content. NPs-elicited Chicory HRs offered an effective and favorable *in vitro* method to improve the production of bioactive compounds for potential uses in pharmaceutical industries.

Keywords: antioxidant activity, *Cichorium intybus* L., flavonoids, growth curve, hairy root, phenolic.

INTRODUCTION

Cichorium intybus L., commonly known as chicory, has a broad distribution worldwide and is a member of the Asteraceae family. Over the past ten years, *Cichorium intybus* L. has become a significant vegetable and technological advancement crop in many

temperate countries, particularly Europe, Asia, and North America (Street *et al.*, 2013). Chicory has been reported to have a wide range of biological properties in many studies, including antibacterial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, antioxidant, anti-bacteria, tumor-inhibitory, and antiallergic properties (Janda *et al.*, 2021). These features are made by a variety of chemical compounds synthesized by chicory such as sesquiterpene lactones, flavonoids, coumarins, caffeine acid, and hydroxycinnamic acids (Matvieieva *et al.*, 2023). The phytochemical compound was found to be mainly synthesized in the root parts of chicory.

Hairy roots (HRs), which can be formed by the infection of *Rhizobium rhizogenes* bacteria into plants, act as an important biotechnological tool that helps to investigate the molecular basis of a variety of fundamental phenomena in biochemistry, plant manners, and physiology (Tepfer, 1990). Biotransformation, generation of high-value plant metabolites, and phytoremediation are the primary goals of hairy root culture-related applications (Sevon and Oskman, 2002). The HR culture can be utilized efficiently for research purposes thanks to its fast growth in biomass, stable genetics, and ability to synthesize different metabolites (Mai *et al.*, 2016).

Climate change has many negative impacts on plants. Elicitors, small chemical molecules, can be a solution to help the crop get through all of the challenging conditions of the environment. They can act as a potential factor to make the plant synthesize some target chemical compounds efficiently (Lone *et al.*, 2023). Nanoparticles (NPs) with a diameter smaller than 100 nm have recently exhibited a lot of interest because of their high surface area and nanoscale size (Khan *et al.*, 2019). NPs are utilized as fertilizers, herbicides, pesticides, or fungicides (Mittal *et al.*, 2020). If the nanoparticle concentration is present in low concentrations, NPs can also be used to trigger abiotic stress in plants, where plants will increase in biomass or produce reactive oxygen, *de novo* secondary metabolites (phenolic or flavonoid compounds) in response to stress (Mittal *et al.*, 2020). However, when present in greater concentrations, they induce phytotoxicity. In research, many scientists have utilized AgNPs in *Caralluma tuberculata* (Ali *et al.*, 2019), bitter gourd (Chung *et al*., 2018), or SeNPs in *C. tuberculata* (Ali *et al*., 2023), bittermelon (*Momordica charantia*) (Behbahani *et al.*, 2020) to elicit biologically active second metabolite production in plants.

To our knowledge, the influence of NPs on chicory and their possible stimulation on the production of phytochemicals in chicory hairy roots has not been widely investigated. Therefore, this study was conducted to determine the potential effects of AgNPs and SeNPs on chicory hairy root accumulation and bioactive compound production. The success of the study can produce more insights into the applications of NPs in chicory hairy root culture.

MATERIALS AND METHODS

Materials

Hairy roots of Cichocium intybus L.

Hairy roots of *Cichorium intybus* L. were indly provided by the Laboratory Biology of Plants and Innovation in Amiens, France.

Chemical reagents

Murashige and Skoog medium from Duchefa (Netherlands), AgNPs - synthesized by the Physic Institute, SeNPs synthesized by the University of Hue. Guanicol and H_2O_2 were sourced from China and Vietnam, respectively. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), $NaNO_2$, $AlCl_3$, Na_2CO_3, and Follin-Ciocalteu were from Sigma-Aldrich (Germany).

Methods

Growth kinetic

HRs were cultured in Erlenmeyer flasks containing 100 mL of half strength Murashige and Skoog (Murashige and Skoog, 1962) medium (½ MS) supplemented with 30 g of sucrose (Matvieieva et al., 2023). This medium had a pH of around 5.7-5.8. Each flask contained 0.33 g of hairy roots and was shaken continuously at 110 rpm and 26 ℃ in the dark. To elicit the growth of hairy roots and secondary metabolite production, AgNPs (1.5625 and 3.25 mg/L) and SeNPs (5 and 10 mg/L) were filtered using a 0.45 µM membrane and added directly to the autoclaved ½ liquid MS medium. The control was hairy roots grown in ½ MS medium. Every 5 days, the roots were taken out to measure their fresh weight. This step was repeated until 25 days of culture to build the growth curve of *C. intybus* L. hairy roots.

Peroxidase (POD) and Catalase (CAT) activity assay

Before conducting the POD and CAT assays, 0.2 g of HRs were taken for protein extraction. After the HRs turned into powder, homogenized them with 3 mL of 100 mM Phosphate-buffered saline (PBS) buffer at pH 7.8. All the samples were centrifuged at 4 ℃ and 10000 rpm for 20 minutes. The supernatant was transferred to a new falcon tube. The NanoDrop® ND-1000 UV-Vis Spectrophotometer was used to determine the amount of crude protein (mg/mL) in the supernatant.

The POD and CAT activity tests were adapted from Zhang's study with some modifications (Chen and Zhang, 2016). Briefly, the components of 10 reactions for testing POD activity contained 5,6 µL of 0.2% guaiacol, 10 mL of 100 mM PBS (pH 7.0), and 5.8 µL of 30% H_2O_2. The components of 10 reactions for testing CAT activity included 15,5 µL of 30% H_2O_2 and 10 mL of 100 mM PBS (pH 7.0). To measure the POD and CAT activity, mix 200 µL of the root extract with 1 mL PBS (pH = 7.8), and immediately record the absorbance every 15 seconds within 1 minute at the wavelengths of 470 nm (for POD) and 240 nm (for CAT) by using the UV-Vis spectrometer. The result will be applied to the below formula to calculate the POD and CAT activities:

$$\text{POD activity } \frac{\Delta A470 \text{ x } \left(\frac{V}{Vt}\right)}{\frac{0.1 \text{ x } t}{Cp}}$$

$$\text{CAT activity} = \frac{\Delta A240 \text{ x } (V/Vt)}{(0.1xt)/Cp}$$

where ΔA470 and ΔA240 are the absorbance alteration measuring at 470 nm and 240 nm for every 15 seconds in 1 minute; V: crude enzyme solution total volume; Vt is the amount of crude enzyme used in the testing tube; t stands for reaction time; Cp is the concentration of crude protein (mg/mL); and 0.01 is one unit of POD or CAT that can be defined as the quantity of enzyme that raises absorbance at 470 nm or 240 nm by 0.01 units per minute.

Preparation of hairy root extract for chemical tests

The 25-day-harvested HR samples, which weighed from 5 to 10 g, were cleaned and dried in an incubator at 45 ℃ until they reached their constant weight mass. After the roots were dried, they were ground to powder with the mortar and transferred into falcon tubes. The falcon was added with 10 mL of methanol (MeOH) 80% and sonicated in an ultrasonic sonication bath for 30 minutes. Then, the obtained sample was centrifuged at 13000 rpm for 2 minutes. The upper phase was transferred to newly weighed falcons and put into an oven that was set at 60 ℃ overnight to eliminate all methanol. Sample stock was prepared by calculating the suitable volume of MeOH to get a stock of 40 mg/mL.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) activity

transferred to newly weighed falcons and put into an oven that was set at 60 ℃ overnight to eliminate all methanol. Sample stock was prepared by calculating the suitable volume of MeOH to get a stock of 40 mg/mL.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) activity

A DPPH free radical scavenging assay was used to estimate the antioxidant activity of HR extract. To prepare the DPPH assay, 39.4 g of DPPH were dissolved in 10 mL of absolute MeOH to obtain a 10 mM stock solution of DPPH (Khan *et al*., 2019). Then, in the mixture, the required ratio of the prepared sample to the DPPH solution was 1:19. After this step, the mixture was incubated in the dark for 20 minutes. Finally, 180 µL of the sample was added in triplicate to 96-well-plates. The absorbance was recorded using SpectraMax iD5 Multi-Mode Microplate Readers at a wavelength of 517 nm. Ascorbic acid was used as a reference. The radical-scavenging percentage was calculated by the formula below:

$$\text{Inhibition percentage} = \left(1 - \frac{\text{As}}{\text{Ab}}\right) X100$$

where Ab was the absorbance of the blank, and Ab was the absorbance of the sample.

Estimation of total phenolic content (TPC)

Based on the protocol of Singleton, the phenolic content was determined with some modifications (Singleton *et al.*, 1999). The sample stock solution was diluted in the range of 0.4 to 4 mg/mL by using MeOH 80%. After the dilution step, 40 µL of the diluted solution was added to the Eppendorf 1.5 mL, which was already filled with 480 µL of Folin-Ciocalteu reagent (diluted with MilliQ water at a ratio of 1:10) and incubated for 1 minute. After that, 480 µL of Na_2CO_3 6% was added to the mixture. The mixture was mixed well and incubated at 40 °C for 15 minutes. The gallic acid standard curve was built in the concentration range of 25 to 400 µg/mL. The absorbance of the standard solution and samples were measured at a wavelength of 765 nm using SpectraMax iD5 Multi-Mode Microplate Readers. Total phenolic content was calculated as mg of gallic acid equivalent by using an equation acquired from the gallic acid calibration curve.

Estimation of total flavonoid content (TFC)

To determine the amount of flavonoid in HR samples elicited by elicitors, the procedure followed the paper of Khan et al. with some modifications (Khan 2019). Forty µL of

$NaNO_2$ 10% and 40 µL of $AlCl_3$ 10% were added to 240 µL of HR extract and vortexed for 6 minutes. Next, 400 µL of NaOH and 280 µL of ethanol (30%) were added to the mixture and incubated for 20 minutes at room temperature. The quercetin standard curve was built in the concentration range of 0.025 to 0.4 mg/mL. SpectraMax iD5 Multi-Mode Microplate Readers were used to measure the absorbance of these reactions at a wavelength of 510 nm.

Statistical analysis

All the experiments were triplicated. The data was presented as mean ± standard deviation. Significant differences between parameters were analyzed using a one-way analysis of variance and a sTurkey post-hoc test at $p < 0.05$ (Nguyen *et al*., 2023).

RESULTS

Growth kinetics of chicory hairy roots

After pre-screening in terms of HR growth, two concentrations of AgNPs and SeNPs were chosen for this study. All the HRs were cultured with elicitors at different concentrations for 25 days, and the fresh HRs were collected every 5 days to measure their biomass. Figure 1 presents the growth of HR biomass under all the treatment and control conditions. The growth of chicory HRs experienced a lag phase from 0 to 10 days, a log phase from 10 to 25 days, and started to reach a stationary phase after 25 days. Overall, SeNPs increased the highest HR biomass among the treatments, and SeNPs at a concentration of 5 mg/L showed higher biomass than the biomass obtained from the medium containing SeNPs at a concentration of 10 mg/L. After 25 days of culture, HR that were treated with 5 mg/L of SeNPs reached a weight of 6.71 ± 0.2 g. In contrast, the HR cultured in medium containing AgNPs inhibited HR biomass in comparision to the control. Both concentrations of AgNPs show the same trend of inhibition. HR biomass cultured in medium containing AgNPs reached only 1.35 g while it was 3.71 g in the control condition after 25 days of culture.

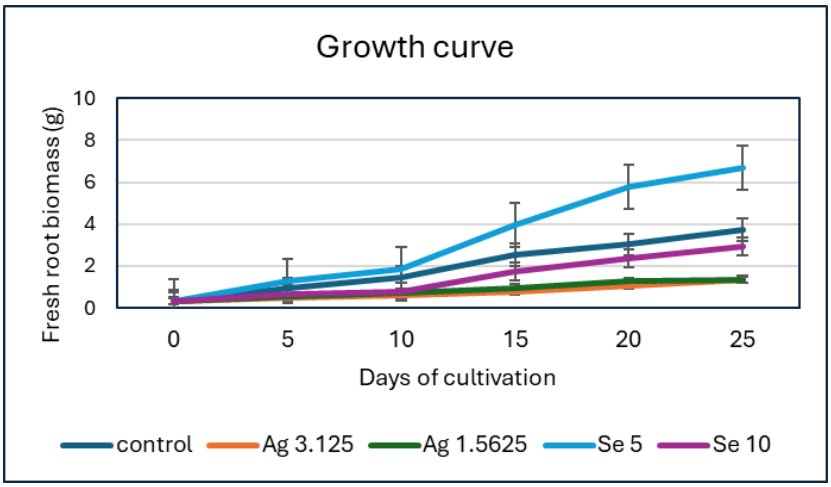

Figure 1. The *Cichorium intybus* L. hairy roots (top image) and the growth curve of *Cichorium intybus* L. hairy roots cultured in medium containing NPs (treatment conditions) and half-strength MS (control condition) for 25 days (bottom graph). The concentration of NPs is presented in mg/L.

Estimation of POD and CAT activities

The change in the CAT activity of *C. intybus* L. under different treatments is shown in Figure 2. Higher concentrations of NPs caused higher CAT activities. CAT activities (14.70 ± 1.18 u/mg protein) and (16.72 ± 1.38 u/mg protein) were respectively recorded in the HRs cultured in medium containing SeNPs at 5 and 10 mg/L, which were significantly higher than the ones obtained in the control roots ($p < 0.05$). There was also no significant difference in the CAT activity obtained in HRs cultured in medium containing 3.15 mg/L AgNPs compared to both concentrations of SeNPs (Figure 2).

In comparison with the control HRs, CAT activity in the HRs cultured in medium containing AgNPs was significantly higher than that in the control medium ($p < 0.05$). CAT in HRs cultured in medium containing 3.25 mg/L of AgNPs was also recorded with significantly higher CAT activity in HRs cultured in medium containing 1.5625 mg/L of AgNPs ($p < 0.05$). CAT activity in HRs cultured in medium containing 1.5625 mg/L AgNPs was 10.82 ± 1.76 u/mg protein, while it was 15.94 ± 2.67 u/mg protein in HRs cultured in medium containing 3.25 mg/L AgNPs.

Similar to CAT, higher concentrations of NPs also caused higher POD activities. POD activity was significantly higher in HRs cultured in a medium containing SeNPs at 10 mg/L and AgNPs at 3.25 mg/L than in the control medium ($p < 0.05$). Lower concentrations of SeNPs and AgNPs did not show a difference in the POD activity in HRs compared with control. Regarding HRs cultured in medium containing AgNPs, the samples in the 3.25 mg/L AgNPs treatment, which was 486.44 ± 35.6 u/mg protein, showed a significantly higher POD activity than the one cultured in medium containing 1.5625 mg/L AgNPs which was 42.43 ± 2.1 u/mg protein ($p < 0.05$). POD activity in HRs cultured in medium containing 10 mg/L SeNPs, which was 543.43 ± 51.15 u/mg protein, was also significantly higher than POD activity in HRs cultured in medium

containing 5 mg/L SeNPs, which was 143.5 ± 22.23 u/mg protein ($p < 0.05$).

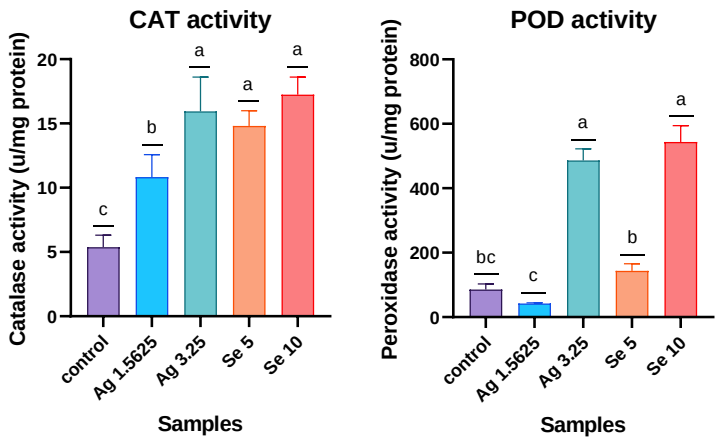

Figure 2: Catalase and peroxidase activity of *Cichorium intybus* L. hairy roots cultured in medium containing NPs (treatment conditions) and half-strength MS (control condition). The data was expressed as means ± the standard deviations of three replicates. Samples with the same letters are not significantly different at a significance level of $p<0.05$. The concentration of NPs is presented in mg/L.

DPPH assays

Table 1 shows the percentage of DPPH radical scavenging activity in *Cichorium intybus* L. HRs at five different culture conditions. The inhibition percentage varies from 48.41 ± 1.23 % to 120.11 ± 3.04 %. Both DPPHs of HRs cultured in medium containing SeNPs 5 and 10 mg/L or AgNPs 1.5625 and 3.25 mg/L were significantly higher compared with the control ($p < 0.05$). Although there were no significant differences between the two concentrations of SeNPs or AgNPs, they presented the ability to scavenge DPPH radicals at a significant level. The HR samples cultured in medium containing AgNPs at both concentrations had a scavenging percentage significantly larger than approximately 3-fold compared to the control extract, while it was approximately 2-fold in the HRs cultured in medium containing SeNPs.

Table 1. Estimation of the percentage of DPPH scavenging activity of *Cichorium intybus* L. hairy roots grown in medium containing NPs (treatment conditions) and half-strength MS (control condition).

Culture conditions	% of scavenging
Control	48.41 ± 1.23^{c*}
AgNPs 1.5625 mg/L	111.34 ± 1.15^{a}
AgNPs 3.25 mg/L	120.11 ± 3.04^{a}
SeNPs 5 mg/L	82.66 ± 3.15^{b}
SeNPs 10 mg/L	98.61 ± 10.09^{b}

***** Data was expressed in means ± the standard deviations of three replicates followed by the same letters are not significantly different (p<0.05)

Total phenolic and flavonoid content

The total phenolic and flavonoid contents of the samples were presented in Figure 3. Most of the HR samples cultured in medium containing NPs, TPC, and TFC were significantly higher than in the control ($p < 0.05$). The range of TPC, equivalent to gallic acid, in the samples was recorded from 31.9 ± 4.76 to 87.04 ± 6.23 mg/g. The observed TPC value was significantly higher in the HRs cultured in medium containing AgNPs than SeNPs ($p < 0.05$). There were no significant differences in TPC production obtained between two concentrations of two NPs ($p > 0.05$). Among the five culture conditions, the HRs cultured in medium containing 1.5625 mg/L AgNPs produced the highest TPC, which was 87.04 ± 6.23 mg/g gallic equivalent.

Similar to TPC, TFC was highest in HRs cultured in medium containing AgNPs. TFC varied from 5.44 ± 1.71 mg/g to 139.03 ± 3.56 mg/g ascorbic acid equivalent. The higher the NP concentrations, the significantly higher TFC was obtained ($p < 0.05$). The highest value of TFC was observed in the HRs cultured in medium containing AgNPs at 3.25 mg/L, followed by AgNP at 1.5625 mg/L, SeNP at 10 mg/L, and SeNP at 5 mg/L. The lowest TFC was obtained in HRs grown in a control ½ MS medium.

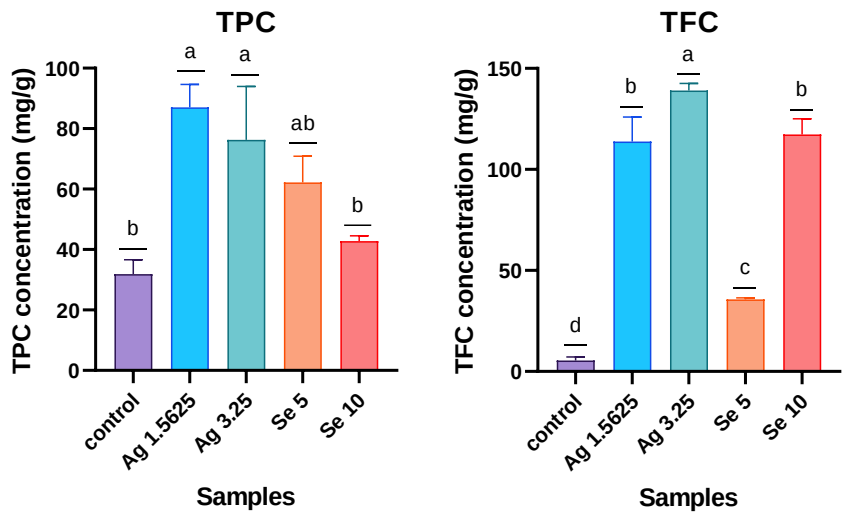

Figure 3. Total phenolics (TPC) and total flavonoid (TFC) contents of *Cichorium intybus* L. HRs cultured in medium containing NPs (treatment conditions) and half-strength MS (control condition). Data was presented as mean ± the standard deviations of three replicates. Samples with the same letters are not significantly different at a significance level of $p < 0.05$. The concentration of NPs is presented in mg/L. TPC: expressed as (mg gallic acid/g), TFC: expressed as (mg Quercetin/g).

DISCUSSION

The ingredients of the medium have a great influence on the growth process of hairy roots. According to the report by Matvieieva

et al. (2023), the HRs cultured in ½ MS medium supplemented with 3% sucrose had a significant growth tendency and that was observed during the 25 days of our study. The lag phase, known as the early growth phase, starts from day 0 to day 10. This is the phase of slow growth. From day 10 to day 25, the phase shows rapid growth and is maximal throughout the entire lifespan. This is also the log phase in plant growth. The stationary phase of HRs will fall on day 25, when the growth rate starts to decline and stops. It is also an essential phase when compounds and substances will be synthesized at the highest level during plant growth. Therefore, collecting the samples and chemical compounds at the end of log phases results in a high biomass yield and thus provides the ideal amount of chemical compounds. In our study, all the HR samples were collected on day 25. The growth kinetics of HRs can also be different in different culture mediums or plant species (Mai *et al.*, 2016).

In our study, two nanoparticles, including SeNPs and AgNPs, which were considered abiotic stress, were used. SeNPs showed the ability to stimulate HR biomass, while AgNPs inhibited HR growth. SeNP at a concentration of 5 mg/L is more dominant when the HR biomass index is more than 2-fold higher than that of SeNP at 10 mg/L, which has the same result as Garza's report. Garza showed that a lower concentration of SeNPs gave better growth stimulation than a higher one in vinca plants (Garza-García *et al.*, 2023). It can be explained by the fact that higher concentrations create more stress on the plant, which causes reduced growth of HRs. On the other hand, AgNP roots showed relatively slow and moderate growth, with no significant changes between the two concentrations. There have been a number of previous reports showing the potential of SeNPs on plant growth at low concentrations, but at higher doses, it can be harmful (Khan *et al.*, 2023). However, up to date, there have also been no reports of using only SeNPs as elicitor for hairy roots, especially *C. intybus* L. In the study of Il-min Chung and his colleagues, AgNPs were used to elicit the *Cucumis anguria* L. HRs. The growth of the HRs was significantly decreased at concentrations of 1 and 2 mg/L AgNPs, which is similar to our obtained data (Chung *et al.*, 2018). The effect of suppressing or stimulating the biomass growth of HRs depends strongly on the AgNP concentrations for different types of HRs. Higher concentrations of AgNPs, ranging from 0, 25, 50, 100, and 200 mg/L, also inhibited HR growth of *Hyoscyamus muticus* L. (Abdelkawy *et al.*, 2023). In general, from our study, it can be concluded that HR biomass growth was inhibited by AgNPs and promoted by SeNPs at the studied concentrations.

POD is considered an essential antioxidant, participating in the process of eliminating ROS, while CAT plays an important role in plant defense mechanisms because it can detoxify hydrogen peroxide and protect cells (Rao *et al.*, 2022). In our study, the results indicated that a high concentration of SeNPs had significantly higher POD and CAT activities compared to a low concentration. That result is completely consistent with the enhanced POD enzyme activity when increasing the concentration of SeNPs recorded in the study of Ahmad (Ahmad *et al.*, 2022). To explain that, SeNPs have been demonstrated to significantly reduce ROS in tomato plants (Ishtiaq *et al.*, 2023). Similarly, POD activity in HRs cultured in medium containing AgNPs also tends to increase with the increase of AgNP concentrations.

This relationship has been proven proportional in Khan's study (Khan *et al.*, 2019). Otherwise, low concentrations of AgNPs exhibited significantly low POD activity, which also demonstrated a rather low ROS scavenging capacity. Regarding CAT activities, the increase in SeNP concentration was not correlated with CAT activity, which was observed in the study of Rao and colleagues (Rao *et al.*, 2022). However, it was in agreement with the study of Ishtiaq in tomatoes (*Solanum lycopersicum* L.) (Ishtiaq *et al.*, 2023). In the study with *Spirodela polyrhiza,* the activity of CAT was not affected by exposure to AgNPs (Jiang *et al.*, 2014). Cameron explained that the change in enzyme activity might be due to either regulation of genes or direct surface interaction of the enzymes with AgNPs (Cameron *et al.*, 2018).

The DPPH assay is also important in determining antioxidant activity. In our study, the percentage of DPPH radical that were scavenged by both SeNPs and AgNPs was significantly higher than in control HRs ($p < 0.05$). However, there was no significant difference in the DPPH values among the two studied concentrations in each NP. The results are different from the studies of Hassan (Hassan *et al.*, 2022) or Khan (Khan *et al.*, 2019). According to their reports, the percentage of DPPH radical scavenging of SeNPs increased proportionally with the increase in concentration and was significantly different from each concentration of 25, 50, 100, 200, and 400 ppm (Hassan *et al.*, 2022) or 20 to 80 ppm (Khan *et al.*, 2019). The reason given may be due to the concentration range;in their report, the concentration ranges differ quite a lot compared with our range. We obtained a higher DPPH value in HRs elicited by AgNPs than by SeNPs. Our results suggested that the HRs suffered more stress when cultured with AgNPs compared to SeNPs.

Finally, the TPC and TFC in the HRs cultured in medium containing NPs were investigated. Our results showed significantly higher TPC in HRs cultured in medium containing AgNPs but not SeNPs. TFC was higher in HRs cultured in medium containing both NPs than in normal HRs ($p < 0.05$). Until now, there have not been many studies investigating the TPC and TFC produced by HR cultured in medium containing SeNPs. The results of El-Saadony *et al.*, (2021) demonstrated that SeNPs increased TFC and TPC production by wheat plants. TFC and TPC were also increased in cell suspension cultures of bitter gourd treated with 10 mg/L AgNPs (Chung, Rekha, *et al.*, 2018). In terms of concentration, TFC is proportional to the increase in AgNP concentrations in our study. This trend can also be seen in Il-min Chung's study of *C. anguria* hairy roots (Chung *et al.*, 2018), and *H. muticus* L (Abdelkawy *et al.*, 2023). The increased TFC and TPC can be explained as a mechanism for plants to improve their growth by decreasing the toxic effects of NPs.

CONCLUSION

In conclusion, our study has made significant advancements in increasing secondary metabolites produced by Chicory HRs. The results showed the stimulation of HR biomass by SeNPs, whereas AgNPs at two studied concentrations inhibited HR growth. In most cases, higher antioxidant enzyme activity as well as TFC, TFC, and DPPH were obtained in HRs cultured in medium containing NPs than in control HRs. This investigation provides valuable insights into *in vitro* strategies to increase the interested metabolite production, which

could be applied for pharmaceutical applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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