

The Use of Silver Nitrate as an Elicitor to Increase Bioactive Compounds in Artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] Callus Culture

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Abstract

The globe artichoke belongs to the Asteraceae family and has become more and more popular among other vegetables due to its beneficial health-promoting features that are related to bioactive compounds present in leaves. The plant materials have inadequate proportions of valuable bioactive compounds in nature, so researchers are emphasizing on how to enhance their amounts. *In vitro* techniques with integrated novel practices can be employed to enhance phytochemicals from any plant. The current study aimed to determine and assess valuable bioactive components in 3 artichoke cultivars via callus cultures which were subjected to a treatment of 4 different concentrations (2.5, 5.0, 10.0, and 15.0 mg L⁻¹) of silver nitrate. Results indicated that well-balanced levels of plant growth regulators were necessary for stimulating the callus formation of globe artichoke. The findings of the current study also revealed the importance of cultivar differences regarding callus formation. Experimental results revealed that variation in silver nitrate concentrations had a significant effect on biomass, total phenolic content and total antioxidant activities. The results of the current, study may offer a good strategy by promoting bioactive compounds of globe artichoke leaves for utilizing in large-scale industries, pharmacology, and food supplements.

1. Introduction

Several researchers believe that in the upcoming years, the potential adverse consequences of global warming on agricultural ecosystems will become considerably more pronounced than before. If agricultural production becomes less productive, it will be difficult for people to get healthy food. This has led to greater interest in research aimed at improving the existing nutritional value of our goods, increased consumer awareness of the significance of consuming high-quality meals, and a shift toward functional foods. Plants/vegetables have the potential to be viewed as functional foods due to the phytochemicals they have, and among vegetables, in this context,

artichoke is known to be rich in valuable bioactive compounds.

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] plant has been used to meet a variety of human requirements, such as culinary and medicinal reasons since ancient times. Nowadays, it is appreciated as a functional food. The value of the artichoke plant in terms of being a rich source of vitamins, minerals, and bioactive compounds has once more come to light recently due to the growing interest in functional foods coupled with healthy and conscientious nutrition.

To meet the growing demand for functional foods, it is critical to expedite research into whether these valuable bioactive components, which can vary depending on the stage of plant development,

can be produced using *in vitro* techniques and whether there is a chance of increasing the amounts of the produced components. The artichoke plant may be used to make extracts, like many other plants. High-level contents of the desired extracts are also significant in this regard. Yet, as long as they are not impacted by harsh conditions, the contents of extracts obtained from conventionally grown plants are at a certain steady level. The beneficial bioactive chemicals already present in plants may be amplified utilizing *in vitro* methods and that approach has been tried on different crops. Based on *in vitro* approach, callus culture technique has been also used in previous studies by treating callus with different elicitors to see their effect on the contents of biomass and bioactive components.

Instead of helping individuals grow and develop, bioactive components offer "improvement in quality of life" by lowering the risk of developing certain medical problems (Pandino et al., 2011). They also assist in the process of plant growth and development while protecting them against biotic or abiotic stresses (Abu-Reidah et al., 2013). The quantity of medicinally important herbal bioactive components in plants is low in concentration, and they are synthesized at different growth and developmental phases of the plant, thus production rates often remain low. Nowadays, certain biotechnological techniques are applied to boost these important compounds' production rates. They may be acquired in this situation utilizing the *in vitro* callus cultivation approach in high quantities and under controlled circumstances and regardless of time. It is a known fact that callus culture is used on a large scale in different industries including pharmacy, cosmetics, and agriculture (Ozsan and Onus, 2020).

Elicitors are agents that cause stress and activate secondary pathways, which trigger the creation of bioactive substances. To rapidly produce a large number of phytochemicals, elicitors (biotic and abiotic) can be used in *in vitro* techniques. Regarding the subject, plant callus cultures have great potential to get beneficial phytochemicals rich in antioxidant activity. Besides, the integration of elicitors to callus cultures may enhance the accumulation of valuable phytochemicals in plants (Ali et al., 2018; Al-Khayri and Naik, 2020). It is known that silver nitrate has effects on enzyme activity, gene expression, and the synthesis of secondary metabolites in plants (Alirezaei et al., 2017), and that is why silver nitrate is a preferred abiotic elicitor to increase the synthesis of bioactive compounds in plant tissue culture experiments (Winson et al., 2020). Based on above stated information present study was conducted to provide solutions to issues like whether the culture process and elicitation might be improved the total phenolic contents and total antioxidant activities in artichoke. In this content, the total phenol and total antioxidant activities of the extracts produced as a consequence of the silver

nitrate elicitation treatments on callus culture of three different artichoke cultivars (Sakız OP, Bayrampaşa OP, and Olympus F₁ hybrid).

2. Material and Methods

2.1. Plant material

As plant materials two open-pollinated (OP) Sakız, Bayrampaşa, and one F₁ hybrid Olympus cultivar, that were grown in open fields of Faculty of Agriculture at Akdeniz University were employed. Plant collecting and surface sterilization of plant materials was conducted according to Ozsan and Onus (2020). Leaves were separately harvested for each cultivar from the inner part of the plants and subjected to the surface sterilization process. After washing by running the tap water leaves were kept for 15 min in an antibacterial soap solution with 5% then washed again. The following step for surface sterilization step was performed at laminar flow cabinets by utilizing a hypochlorite solution with 20% (active substance 5%) for ten minutes, then three rinsing with autoclaved distilled water.

2.2. The media preparation, callus cultures and physical culture conditions

In the current study, optimizing studies on callus formation were conducted with 22 different media combinations (Table 1). As the basic media Gamborg B5 (Gamborg et al., 1968) was employed with various concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 mg L⁻¹) 6-Benzylaminopurine (BAP) and naphthalene acetic acid (NAA). Approximately 0.5-1.0 cm size leaf explants were inoculated to prepared media and maintained the growths at certain physical culture room conditions, 24 ± 2°C, 16h/8h - light/dark, 3000 μ E.m⁻².s⁻¹ light intensity.

2.3. Evaluation of calli formation

The success of callus formation of media combinations was expressed as 0-40% of cultured leaf explants as insufficient growth (+), 41-75% as moderate growth (++), and 76-100% as fine growth (+++). Healthy growing calli in different media combinations were weighed as 1.0 g per treatment and cultivar under aseptic conditions for further elicitor treatments (Table 2).

2.4. Elicitor treatments

All obtained calli were sub-cultured 5 times. At the end of the 5th sub-culture, silver nitrate at different concentrations (2.5, 5.0, 10.0, and 15.0 mg L⁻¹), which were separately filter-sterilized with a 0.22 μm filter, was added to 10-day-old callus cultures for 7 days.

2.5. Growth kinetics and biomass production

Table 1. Media compositions for callus formation.

No	Media-Gamborg B5 (g L ⁻¹)	Media compositions			
		BAP (mg L ⁻¹)	NAA (mg L ⁻¹)	Sucrose (g L ⁻¹)	Plant agar (g L ⁻¹)
1	3.2	-	-	30.0	6.0
2	3.2	0.5	0.5	30.0	6.0
3	3.2	0.5	1.0	30.0	6.0
4	3.2	0.5	2.0	30.0	6.0
5	3.2	0.5	3.0	30.0	6.0
6	3.2	0.5	4.0	30.0	6.0
7	3.2	0.5	5.0	30.0	6.0
8	3.2	1.0	1.0	30.0	6.0
9	3.2	1.0	2.0	30.0	6.0
10	3.2	1.0	3.0	30.0	6.0
11	3.2	1.0	4.0	30.0	6.0
12	3.2	1.0	5.0	30.0	6.0
13	3.2	2.0	2.0	30.0	6.0
14	3.2	2.0	3.0	30.0	6.0
15	3.2	2.0	4.0	30.0	6.0
16	3.2	2.0	5.0	30.0	6.0
17	3.2	3.0	3.0	30.0	6.0
18	3.2	3.0	4.0	30.0	6.0
19	3.2	3.0	5.0	30.0	6.0
20	3.2	4.0	4.0	30.0	6.0
21	3.2	4.0	4.0	30.0	6.0
22	3.2	5.0	5.0	30.0	6.0

Differences in callus biomass in response to varied elicitor treatments were measured as fresh (FW) and dry (DW) weights after 7 days of elicitor treatments. Calli were taken out from petri dishes, pressed softly on filter paper to the excess water, weighed, and expressed as fresh weight. The calli were harvested, weighed, and oven dried at 60°C for 48 h to determine the dry weight (DW).

2.6. Determination of total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu method as defined by Škerget et al. (2005). The sample (1.0 g) is extracted in 100 mL of extraction solution. The sample at the volume of 0.5 mL was added to 2.5 mL Folin-Ciocalteu reagent which was 10 times diluted with water. After adding 2.0 mL sodium carbonate (75.0 g L⁻¹) the mixture was maintained for five minutes at 50°C and then cooled. The absorbance values of samples were measured at 760 nm. The total phenolic content was expressed as mg gallic acid equivalent per 100 g dry weight of callus.

2.7. Determination of total antioxidant activity

The free radical-scavenging activity (2,2-diphenyl-1-picrylhydrazyl - DPPH) was analyzed according to Fernández-León et al. (2013). A 1.0 g dry sample was dissolved in a 20 mL extraction solution (80% methanol) for 1 minute with the help of ultraturrax. For analysis, 50 µL of the diluted extract was transferred to an Eppendorf tube and 950 µL of diluted DPPH was added into it and mixed with vortex for 30 minutes and kept in the dark. The absorbance of the DPPH solution prepared for analysis was determined and

spectrophotometrically readings were recorded at a wavelength of 515-517 nm. The total antioxidant activity was expressed as mg trolox equivalent per 100 g dry weight of callus.

2.8. Statistical analysis

The experiments of the current study were performed with a completely randomized factorial design in triplicates. The data obtained were subjected to variance analysis in the JMP package program and the differences between the averages were determined by the least significant difference (LSD) test also the differences were determined statistically significant at $P < 0.05$.

3. Results and Discussion

3.1. Evaluation of media combinations, callus formation and biomass accumulation

In vitro calli formation and maintenance as well as bioactive compound accumulation in cultivated explants are known to be influenced by a variety of variables, e.g. genotype, plant explant type, media, plant growth regulators, and physical circumstances of culture (Siatka, 2019). One of the most crucial variables to consider when measuring growth is the cell's biomass. Types, concentrations, and combinations of plant growth regulators that added medium had an important effect on callus formation and medium No.15 and medium No.19 were determined as the most responsive media combinations.

The experimental findings demonstrated that there were variations in cultivars' callus formation in

Table 2. Effects of different media combinations on cultivars.

No	Media composition	Cultivars		
		Sakız OP	Bayrampaşa OP	Olympus F ₁
1	Gamborg B5 (G.B5 - control)	-	-	-
2	G.B5 + 0.5 mg L ⁻¹ BAP + 0.5 mg L ⁻¹ NAA	+		
3	G.B5 + 0.5 mg L ⁻¹ BAP + 1.0 mg L ⁻¹ NAA	+	+	+
4	G.B5 + 0.5 mg L ⁻¹ BAP + 2.0 mg L ⁻¹ NAA	+	+	+
5	G.B5 + 0.5 mg L ⁻¹ BAP + 3.0 mg L ⁻¹ NAA	+	+	+
6	G.B5 + 0.5 mg L ⁻¹ BAP + 4.0 mg L ⁻¹ NAA	+	+	+
7	G.B5 + 0.5 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	+	+	+
8	G.B5 + 1.0 mg L ⁻¹ BAP + 1.0 mg L ⁻¹ NAA	+	+	+
9	G.B5 + 1.0 mg L ⁻¹ BAP + 2.0 mg L ⁻¹ NAA	+	+	+
10	G.B5 + 1.0 mg L ⁻¹ BAP + 3.0 mg L ⁻¹ NAA	+	+	+
11	G.B5 + 1.0 mg L ⁻¹ BAP + 4.0 mg L ⁻¹ NAA	+	+	+
12	G.B5 + 1.0 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	+	+	+
13	G.B5 + 2.0 mg L ⁻¹ BAP + 2.0 mg L ⁻¹ NAA	+	+	+
14	G.B5 + 2.0 mg L ⁻¹ BAP + 3.0 mg L ⁻¹ NAA	+	++	+
15	G.B5 + 2.0 mg L ⁻¹ BAP + 4.0 mg L ⁻¹ NAA	+++	+++	+
16	G.B5 + 2.0 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	+	+	+
17	G.B5 + 3.0 mg L ⁻¹ BAP + 3.0 mg L ⁻¹ NAA	+	+	+
18	G.B5 + 3.0 mg L ⁻¹ BAP + 4.0 mg L ⁻¹ NAA	++	+	+
19	G.B5 + 3.0 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	+++	+++	+
20	G.B5 + 4.0 mg L ⁻¹ BAP + 4.0 mg L ⁻¹ NAA	+	+	+
21	G.B5 + 4.0 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	+	+	+
22	G.B5 + 5.0 mg L ⁻¹ BAP + 5.0 mg L ⁻¹ NAA	++	++	+

Table 3. Calli fresh and dry weights (biomass).

AgNO ₃ treatments (T)	Fresh weight (g)		Dry weight (g)		Mean differences values – AgNO ₃ treatments	
	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Sakız OP (SOP)	Bayrampaşa OP (BOP)		
1. Medium No.15 (control) (X)	6.22 ef	0.00 h	0.55 df	0.00 h	3.11 E	0.27 EF
2. X + 2.5 mg L ⁻¹ AgNO ₃	6.25 ef	5.43 f	0.57 cf	0.45 fg	5.84 C	0.51 C
3. X + 5.0 mg L ⁻¹ AgNO ₃	7.19 be	7.51 bd	0.69 bd	0.71 bc	7.35 B	0.70 B
4. X + 10.0 mg L ⁻¹ AgNO ₃	6.81 ce	6.31 df	0.62 be	0.54 ef	6.56 BC	0.58 C
5. X + 15.0 mg L ⁻¹ AgNO ₃	10.41 a	8.25 b	0.89 a	0.74 b	9.33 A	0.81 A
6. Medium No.19 (control) (Y)	3.62 g	0.00 h	0.37 g	0.00 h	1.81 F	0.18 F
7. Y + 2.5 mg L ⁻¹ AgNO ₃	7.64 bc	0.00 h	0.71 bc	0.00 h	3.82 DE	0.35 DE
8. Y + 5.0 mg L ⁻¹ AgNO ₃	8.10 b	0.00 h	0.77 ab	0.00 h	4.05 D	0.38 D
9. Y + 10.0 mg L ⁻¹ AgNO ₃	7.09 be	0.00 h	0.65 be	0.00 h	3.54 DE	0.32 DE
10. Y + 15.0 mg L ⁻¹ AgNO ₃	6.05 ef	0.00 h	0.55 df	0.00 h	3.02 E	0.27 EF
Mean differences values-cultivars (C)	Fresh weight (SOP:6.94 A, BOP:2.75 B), Dry weight (SOP:0.64 A, BOP:0.24 B)					
LSD values-fresh weight	LSD (C) = 0.386, LSD (T) = 0.864, LSD (C × T)= 1.223					
LSD values-dry weight	LSD (C) = 0.043, LSD (T) = 0.097, LSD (C × T)= 0.137					

Different letters between cultivars and AgNO₃ treatments denote significant differences (LSD test, $P < 0.05$).

all investigated media compositions. The cultivar with the best response regarding callus formation was "Sakız" OP, which was followed by "Bayrampaşa" OP. "Olympus" F₁ did not form enough amount of callus and that is why excluded from further silver nitrate elicitor treatments.

3.2. Effect of silver nitrate on fresh and dry weights of calli

The supplementation of different concentrations of silver nitrate had a positive impact on calli fresh and dry weights (biomass) (Table 3). Experimental results revealed that calli fresh and dry weights were affected positively by 15.0 mg L⁻¹ silver nitrate treatment for successful media combinations of No. 15, No. 19 which was followed by silver nitrate treatment concentration of 5.0 mg L⁻¹.

When the cultivars are evaluated among themselves, it is seen that the Sakız cultivar gives a

more positive result than Bayrampaşa to silver nitrate concentrations regarding biomass. In previous studies some researchers indicated that the use of silver nitrate as an elicitor in the right quantities might have beneficial effects (Yan et al., 2006; Deepthi and Satheeshkumar, 2016). According to Yan et al. (2006), the addition of 15 µM Ag⁺ to *Salvia miltiorrhiza*'s hairy root culture significantly promotes growth and increases root dry weight. In addition, Zaker et al. (2015) reported that 25 and 50 µM Ag⁺ treatment decreased root growth whereas 5 µM of Ag⁺ application boosted root growth in adventitious root cultures of *Perovskia abrotanoides*.

3.3. Effect of silver nitrate on total phenolic content and total antioxidant capacity of calli

Table 4, display the total phenolic content and antioxidant capacity of calli samples that had been

Table 4. Total phenolic contents and total antioxidant activity of calli.

AgNO ₃ treatments (T)	Total phenolic content		Total antioxidant activity		Mean differences values – AgNO ₃ treatments	
	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Sakız OP (SOP)	Bayrampaşa OP (BOP)	Total phenolic content	Total antioxidant activity
1. Medium No.15 (control) (X)	417.42 b	0.00 i	331.35 a	0.00 o	208.71 D	165.67 E
2. X + 2.5 mg L ⁻¹ AgNO ₃	375.34 c	383.48 c	283.59 c	261.11 d	379.41 A	272.35 A
3. X + 5.0 mg L ⁻¹ AgNO ₃	302.96 d	440.99 a	197.89 i	304.66 b	371.97 A	251.27 B
4. X + 10.0 mg L ⁻¹ AgNO ₃	229.45 f	410.69 b	199.30 h	231.61 f	320.07 B	215.45 D
5. X + 15.0 mg L ⁻¹ AgNO ₃	228.89 f	269.85 e	223.18 g	233.01 e	249.37 C	228.09 C
6. Medium No.19 (control) (Y)	179.82 h	0.00 i	192.18 k	0.00 o	89.91 F	96.09 G
7. Y + 2.5 mg L ⁻¹ AgNO ₃	197.47 g	0.00 i	185.25 l	0.00 o	98.73 E	92.62 H
8. Y + 5.0 mg L ⁻¹ AgNO ₃	193.25 g	0.00 i	172.60 m	0.00 o	96.62 EF	86.30 I
9. Y + 10.0 mg L ⁻¹ AgNO ₃	189.05 gh	0.00 i	138.89 n	0.00 o	94.52 EF	69.44 J
10. Y + 15.0 mg L ⁻¹ AgNO ₃	196.62 g	0.00 i	196.49 j	0.00 o	98.31 EF	98.24 F
Mean differences values-cultivars (C)	Total phenolic content (SOP= 251.03 A, BOP= 150.50 B) Total antioxidant activity (SOP= 212.07 A, BOP= 103.03 B)					
LSD values-Total phenolic content	LSD (C) = 3.885, LSD (T) = 8.688, LSD (C × T)= 12.287					
LSD values-Total antioxidant activity	LSD (C) = 7.467 × 10 ⁻⁷ , LSD (T) = 1.669 × 10 ⁻⁶ , LSD (C × T)= 2.361 × 10 ⁻⁶					

Different letters between cultivars and AgNO₃ treatments denote significant differences (LSD test, $P < 0.05$).

**The total phenolic content (TPC) was expressed as mg gallic acid equivalent per 100 g dry weight of callus (mg GAE/g DW).

*** The total antioxidant activity (TAA) was expressed as mg trolox equivalent per 100 g dry weight of callus (mg trolox/g DW).

stimulated with various concentrations of silver nitrate. Accordingly, the highest total phenolic content among various silver nitrate concentrations was obtained from 2.5 mg L⁻¹, while the media with non-added silver nitrate (control) created undesirable results. It is seen that as the silver nitrate concentration increases, the total phenolic content decreases except for media with the non-application of silver nitrate regardless of cultivars and media. Besides, with regards to the total phenolic content between cultivars, Bayrampaşa was by far the best while Sakız has the highest total antioxidant activity. It was also determined that the calli of the Sakız cultivar obtained from the medium without silver nitrate application had the highest antioxidant capacity.

In some previous studies, exogenous treatment with elicitors has been shown to increase the synthesis of bioactive compounds in plant cells (Gheisary et al., 2018). Several researchers have shown that elicitor treatments are particularly successful in plant cell proliferation and the augmentation of phytochemicals (Namdeo, 2007). The efficiency of elicitor treatments can be increased by taking into account factors including the culture age, the length of exposure to elicitors, and the kind and concentration of the elicitor (Nazir et al., 2019). Açıkgöz (2020) reported that medium with the non-elicitor application (control) and medium treated with silver nitrate at 100 µM concentration has the lowest total phenolic content. In his research, it was determined that the treatment with silver nitrate as an abiotic elicitor increased the total contents of phenolic and antioxidants. According to previous research, employing low quantities of silver nitrate may enhance the overall quantity of phenolics and antioxidants, similar to the present report's findings (Cai et al., 2013; Nadeem et al., 2018; Açıkgöz, 2020). It is known that cells can respond to certain elicitor treatments at low

concentrations and in a short time. On the other hand, some of the elicitor treatments can respond at high concentrations and over a prolonged time. Gonçalves et al. (2019) reported that 50 µM silver nitrate treatments enhanced the total phenolic content by 12.5% at the end of six weeks, while Yan et al. (2006) demonstrated that for increasing the total phenolic content, the treatment with 15 µM Ag⁺, which was at low concentration, was more efficient at the 4th day after application.

Previous researches reported the differences between callus tissues of total phenolic content and total antioxidant capacities to be associated with the phenolics of cell and flavonoid types and concentrations (Sabir et al., 2012; Xu et al., 2016). As a result, discrepancies in antioxidant capabilities can be attributed to variations in phenolic acid and flavonoid concentration that emerge throughout elicitor treatment (Krishnan et al., 2015; Sarkate et al., 2017).

4. Conclusion

The present experiment results reveal the different elicitor concentrations' effects on biomass, total phenolic content, and total antioxidant activity from the callus culture of two globe artichoke cultivars. Using silver nitrate as an elicitor increased the biomass of Sakız and Bayrampaşa artichoke cultivars when applied at the concentration 15.0 mg L⁻¹. For further studies, higher concentrations of silver nitrate should be tested to determine the optimum concentration of silver nitrate for biomass increase. Regarding total phenolic and antioxidant contents, the low concentration of silver nitrate treatment as 2.5 mg L⁻¹ had a positive effect on the phenolic content of Sakız and Bayrampaşa cultivars, while silver nitrate treatment had no positive effect on antioxidant

contents. The current findings are expected to help those working on increasing globe artichoke's values as a functional food.

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