Journal of Vasyl Stefanyk Precarpathian National University. Biology
https://journals.pnu.edu.ua/index.php/jpnubio/
Vol. 11 (2024), P57-P65

UDC **581.1**
doi: 10.15330/jpnubio.11.57-65

THE EFFECT OF SILVER NITRATE ON THE BIOCHEMICAL PARAMETERS OF *ROSA CANINA* L.

Viktor Husak[1], Uliana Kilchytska[1], Yulia Savchuk[1], Uliana Stambulska[2,3]

Abstract: Dietary supplements play a crucial role in providing the human body with phytochemicals that may be insufficiently represented in the daily diet. The dog rose (*Rosa canina* L.) exhibits anti-inflammatory, antioxidant, and antidiabetic properties due to its wide range of biologically active compounds, including anti-inflammatory galactolipid, vitamin C, phenols, lycopene, lutein, zeaxanthin, and other carotenoids. The high interest in the potential health benefits of *Rosa canina* has driven research and the development of technologies aimed at enhancing the content of biologically active compounds in this plant. It is well known that the activation of secondary metabolic pathways by elicitors and various stress factors can stimulate the production of biologically active substances in plants. This study investigates the effect of silver nitrate on several biochemical parameters of *Rosa canina* L. under conditions of microclonal propagation. The results demonstrated that the concentrations of chlorophylls a and b in the leaves of *Rosa canina* decreased under the influence of silver nitrate at concentrations of 1, 10, and 50 mg L^{-1}. The carotenoid content in the plant leaves remained unchanged under silver nitrate treatment, while the anthocyanin content increased. However, the levels of polyphenolic compounds and flavonoids decreased in plants exposed to all tested concentrations of silver nitrate.

Keywords: micropropagation, *Rosa canina* L., silver nitrate, plant pigments, polyphenols.

1. INTRODUCTION

The release of silver ions (Ag^+) from $AgNO_3$ induces significant toxicity in various organisms, including bacteria, algae, plants, and animals, primarily due to their inhibitory potential (Tripathi et al., 2017). However, the scientific literature addressing the phytotoxicity of silver ions presents contradictory findings. For instance, silver ions have been shown to improve certain morphological indices in *Cucumis sativus* L. (Sharma et al., 2012). Studies on kenaf (*Hibiscus sp.*) and cotton (*Gossypium hirsutum* L.) demonstrated that the presence of $AgNO_3$ in the initiation medium enhanced the formation of shoot buds on hypocotyl explants (Ouma et al., 2004). Conversely, Ag^+ has been reported to negatively affect the growth of *Oryza sativa* L. and *Triticum aestivum* L. (Vannini et al., 2014).

Silver ions can impact plant embryogenesis and the development of buds, roots, and shoots, which are crucial processes for normal organogenesis (Bais et al., 2000; Bais et al., 2001). Ag^+ influences plant organisms by inhibiting the action of ethylene, a plant hormone involved in processes such as root hair development, flower wilting, fruit drop, ripening, and senescence. Silver ions block ethylene binding sites, rendering plants insensitive to this gaseous hormone. The ethylene receptor, ETR1, has a single ethylene-binding site on the homodimer, with binding mediated by a single copper ion (Cu^+). Replacement of the copper cofactor with silver locks the receptor in a conformation that inhibits ethylene responses (Rodriguez et al., 1999).

An important property of silver compounds is their antiseptic effect (Ahmad et al., 2020). One mechanism underlying this effect is the ability of $AgNO_3$ to stimulate the production of reactive

oxygen species (ROS) in bacterial cells. ROS cause damage to critical cellular components, including DNA, proteins, and lipids, ultimately leading to bacterial cell death (Yin et al., 2020).

Rosa canina L. is capable of growing in both temperate and tropical climates; however, it thrives optimally under temperate conditions characterized by moist, warm summers and mild winters. The species exhibits notable frost resistance and favors well-drained, moderately fertile soils with a neutral to slightly acidic pH (Javanmard et al., 2017). Additionally, Rosa canina demonstrates tolerance to drought conditions. These adaptive characteristics, combined with its beneficial properties and diverse applications, were the basis for selecting this plant for our research.

2. RESEARCH METHODS

Reagents

All other reagents were received from local suppliers (Ukraine) and they were of analytical grade.

Cultural conditions and plant material

The influence of different concentrations of silver nitrate on selected biochemical parameters was investigated in *Rosa canina* L. plantlets. Dog rose plantlets previously grown *in vitro* in our laboratory were used. The explants were cultivated in Murashige-Skoog medium with following concentrations of silver nitrate: 0 (control), 1, 10, or 50 mg L^{-1}. (Murashige and Skoog, 1962). Before the agar adding, the medium was adjusted to pH 5.8 and autoclaved for 30 min at 121°C. The plants cultivate at a temperature of 25-28°C, 2000 lux illumination and 16 hours of daylight. After three to four weeks regenerative shoots developed from the axillary buds. These shoots were used for the determination of the biochemical parameters.

Determination of pigments concentration

Preparation of extracts for the determination of pigment content in fresh dog rose leaves was conducted in accordance with the previously described protocol (Husak et al., 2024). The concentrations of chlorophyll *a* and *b*, carotenoids and anthocyanins in the total extract was determined by spectrophotometric method using the specific absorption coefficients (Lichtenthaler, 1987). To assayed the anthocyanins content hydrochloric acid was added to the total extracts to a final concentration of 1%. The anthocyanin concentrations were assayed spectrophotometrically at 530 nm using an absorbtion coefficient of 30 $mM^{-1} \cdot cm^{-1}$ (Semchuk et al., 2009, Stambulska and Lushchak 2013, Husak et al., 2024). The concentrations of pigments were calculated according to the following formulas (Gitelson et al., 2001).

Determination of polyphenolic compounds

The total content of phenolic compounds were measured by the Folin-Cicalteu reagent (Geremu et al. 2016). The contents of polyphenolic compounds were assayed spectrophotometrically at 765 nm (Geremu et al. 2016) and calculated using the formula described previously (Matic et al. 2017). The content of flavonoids were determined colorimetrically at 510 nm as described Shraim and colleagues (Shraim et al., 2021).

Statistical analysis

The data are presented as meas ± S.E.M. Romanowski's, Dixon's and Shovene's tests were used for identification and rejection of outliers. Statistical analyses were carried out using the StatOptima software (version 2.5), with ANOVA followed by Dunnett's test. Statistical significance was set at $P<0.05$.

3. RESULTS AND DISCUSSION

Effect of $AgNO_3$ on the pigments concentrations

Our experiment found that chlorophylls *a* and *b* content under the influence of different concentrations of silver nitrate was significantly lower compared to the control group. The chlorophyll *a* content decreased by 52%, 51% and 30% under the influence 1, 10, and 50 mg L^{-1} silver nitrate (Fig. 1a), while the chlorophyll *b* content decreased by 60%, 67% and 30%, respectively, at these concentrations compared to the control group (Fig. 1b).

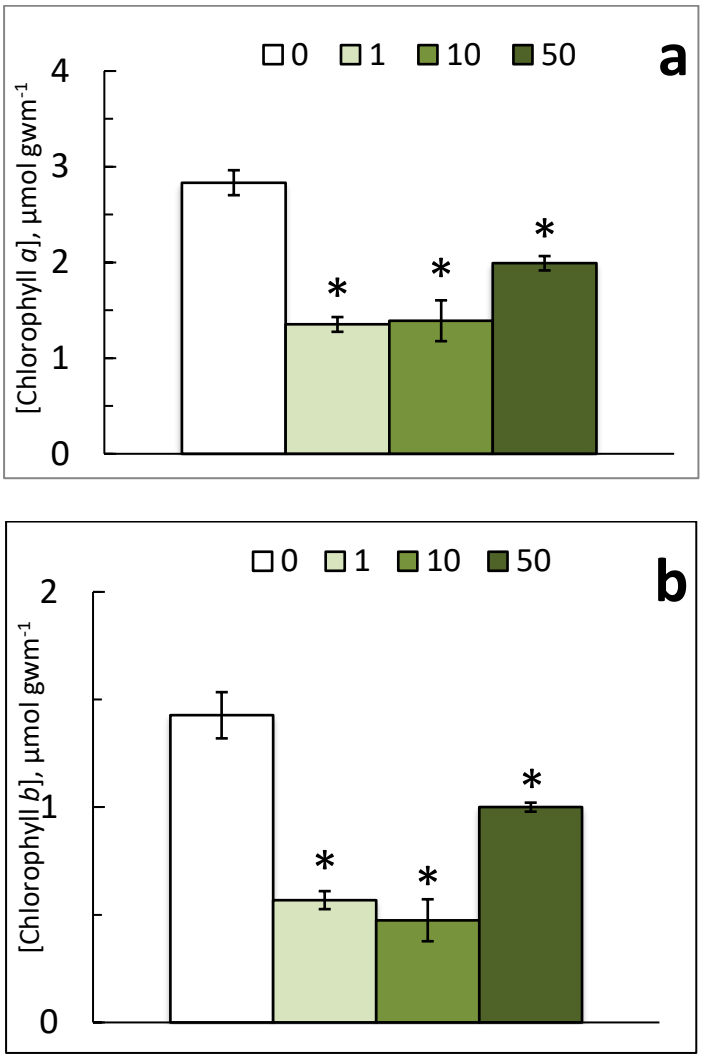

Figure 1. The content of chlorophyll *a* (a), chlorophyll *b* (b) in dog rose leaves, exposed to different concentrations of $AgNO_3$. Data are presented as means ± S.E.M, n = 6-8. *Significantly different from the control (without $AgNO_3$) group of plants ($P < 0.05$) according to ANOVA followed by Dunnett's test.

Prior studies have demonstrated the inhibitory effect of silver nanoparticles on two green algae *Chlorella vulgaris* B. and *Dunaliella tertiolecta* B. (Oukarroum et al., 2012). The chlorophylls content of chlorella at 1 and 10 mg L^{-1} AgNPs decreased by 34 and 51% compared to the control, respectively. Karimi and colleagues (2017) obtained similar results in a study of the physiological effects of silver nitrate at 10 and 100 mg L^{-1} on common wheat *Triticum aestivum* L. (Karimi et al., 2017). The decrease in chlorophyll content in plant tissues exposed to silver nitrate may be depended on various factors, including the disruption of pigment synthesis, pigment degradation, direct

inhibition of enzymatic steps associated with chlorophyll biosynthesis, and changes in the protein composition of photosynthetic membranes (Prasad et al., 1999). Reduction of photosynthetic pigments in treated $AgNO_3$ plants may be suggestive of oxidative stress. Silver ions have demonstrated the capacity to affect photosynthesis through competitive substitution of Cu^+ in plastocyanin (Pc). This interaction is significant bcause it affects the electron carrier function from cytochrome *b6/f* to photosystem 1 (PS1) in the photosynthetic electron transport chain (Gross, 1993). The result of these interactions is the replacement of Cu^+ by Ag^+ and its subsequent binding to Pc, which disrupts or inactivates the photosynthetic electron transport. Ag(I)-substituted Pc competitively inhibits electron transfer between normal Cu-containing Pc and PS1 (Jansson et al., 2008).

Carotenoid content in leaves of *Roza canina* L. was not statistically different between the control and experimental groups under the influence of different concentrations of silver nitrate (Fig. 2a). Previous research has shown that *Solanum tuberosum* plants exposed to 2 mg L^{-1} AgNPs exhibited a significant increasing in the carotenoids content, and no difference was observed when treated with $AgNO_3$. A significant decrease in carotenoid content was observed at higher concentrations (10 mg L^{-1} and 20 mg L^{-1}) in both the $AgNO_3$ and AgNPs treatments (Bagherzadeh et al., 2015). The investigation of the molecular effects of silver nanoparticles on rice seedlings showed that the carotenoid content was not significantly changed after exposure to 0.2 mg L^{-1} AgNPs but significantly decreased after exposure to 0.5 and 1 mg L^{-1} AgNPs (Nair et al., 2014).

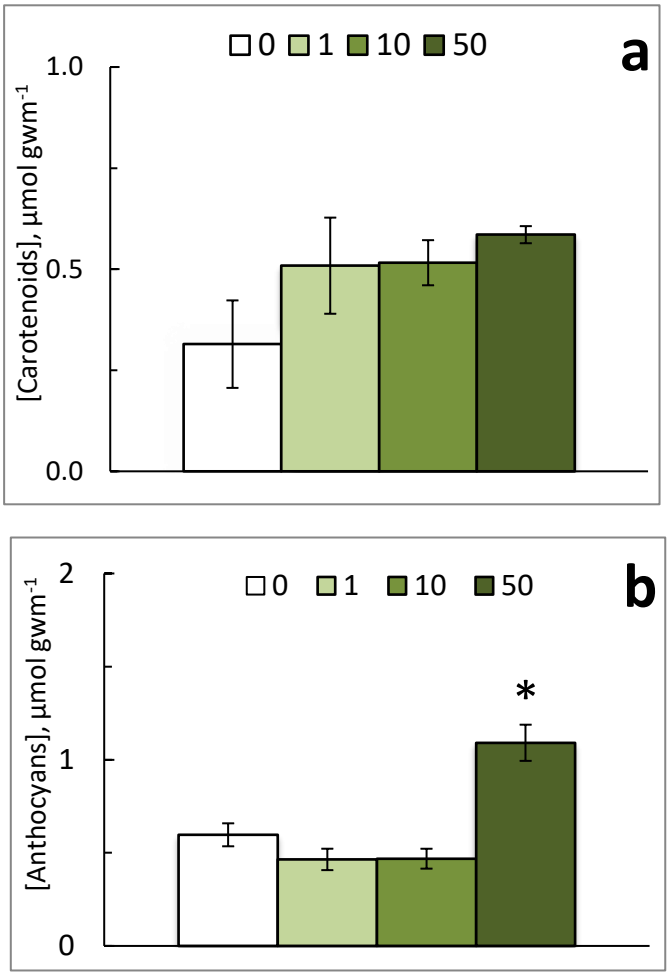

Figure 2. The content of carotenoids (a) and anthocyanins (b) in dog rose leaves exposed to different concentrations of $AgNO_3$. Data are presented as means ± S.E.M, n = 6-8. *Significantly different from the control (without $AgNO_3$) group of plants ($P < 0.05$) according to ANOVA followed by Dunnett's test.

As depicted in Fig. 2b, the anthocyanin content in explants of dog rose treated 50 mg L^{-1} $AgNO_3$ was 45% higher compared to the control value (Fig. 2b). Previous research has indicated a significantly increased anthocyanins accumulation in *Arabidopsis seedlings* exposed to silver nanoparticles (Syu et al., 2014). In the study of the biochemical properties of basil *Ocimum basilicum* L., the concentration of anthocyanins increased under the influence of silver nitrate (40 ppm and 80 ppm) (Nejatzadeh-Barandozi, 2014). Research suggests that anthocyanins mitigate the stress response by scavenging free radicals and limiting the movement of H_2O_2 in plant cells.

Effect of $AgNO_3$ on the content of Polyphenols and Flavonoids

Our results demonstrated that the polyphenols and flavonoids content in the leaves of *Rosa canina* L. was reduced under different concentrations of silver nitrate compared to the control (Fig. 3a). `The polyphenol content demonstrated a marked decrease, with a 76% reduction observed in the presence of 1 mg L^{-1} silver nitrate, an 81% reduction seen in the presence of 10 mg L^{-1} silver nitrate, and a 54% reduction evident in the presence of 50 mg L^{-1} silver nitrate.

The results showed that the content of flavonoids in the experimental groups under the influence of different concentrations of silver nitrate decreased compared to the control group (Fig. 3b). The results of this study demonstrated that the application of 1 mg L^{-1}, 10 mg L^{-1} and 50 mg L^{-1} silver nitrate reduced the flavonoid content by 61%, 49% and 44% respectively compared to the control group.

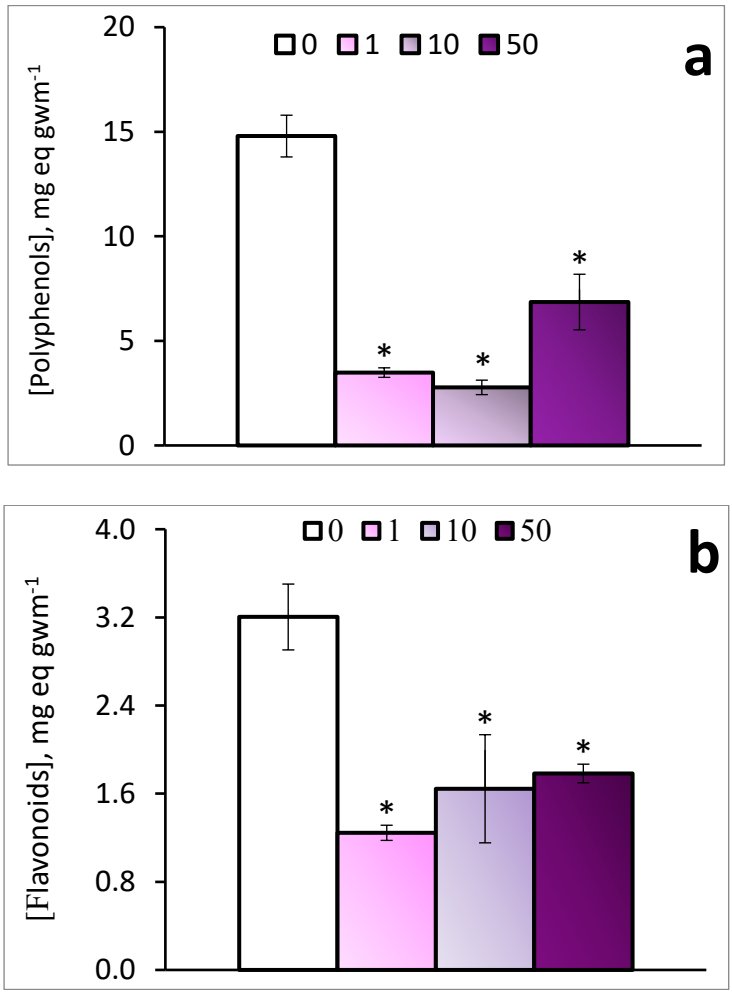

Figure 3. The content of polyphenols (a) and flavonoids (b) in dog rose leaves exposed to different concentrations of $AgNO_3$. Data are presented as means ± S.E.M, n = 6-8. *Significantly different from the control (without $AgNO_3$) group of plants ($P < 0.05$) according to ANOVA followed by Dunnett's test.

In contrast, in *Ocimum basilicum* L., an increase in polyphenol content was observed with rising levels of silver nitrate (Nejatzadeh-Barandozi et al., 2014). This phenomenon can be attributed to the polymerization of phenols in plant tissues, catalyzed by enzymes such as peroxidase, which chelates silver (Heredia et al., 2009; Elzaawely et al., 2007). Consequently, the presence of high concentrations of silver nitrate leads to an accumulation of phenols due to the toxic effects of silver on plant cells.

Furthermore, research by Tahoori et al. (2019) revealed that the application of silver nitrate at concentrations of 0, 2, 4, 8, and 10 mg L^{-1} resulted in increased flavonoid content in *Glycyrrhiza glabra* L., with the most pronounced effects observed at concentrations of 8 and 10 mg L^{-1}. It is well-established that exposure of plant materials to elevated concentrations of heavy metals induces the production of secondary metabolites and antioxidant compounds, including flavonoids (Díaz et al., 2001).

Flavonoids, in particular, have been shown to provide non-enzymatic protection through their antioxidant properties. These compounds contribute to the stability of the cell wall and act as a physical barrier, mitigating the detrimental effects of heavy metals on plant cells (Díaz et al., 2001).

4. CONCLUSIONS

The results demonstrated that exposure to 1–50 mg L^{-1} $AgNO_3$ reduced the levels of chlorophylls a and b in the leaves of Rosa canina L. cultivated *in vitro*. In contrast, the carotenoid content in the leaves remained unaffected by silver nitrate treatment. Notably, cultivation on a medium containing 50 mg L^{-1} $AgNO_3$ resulted in an increased anthocyanin content compared to the control, indicating that silver nitrate may induce mild oxidative stress. This finding suggests that $AgNO_3$ potentially exerts phytotoxic effects, as reflected by a decrease in polyphenol and flavonoid levels.

Conflict of interest

The authors declare that they have no conflict of interest.

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Viktor Husak, PhD, [1]Associate Professor Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenka Str., Ivano-Frankivsk, 76018, Ukraine.
E-mail: viktor.husak@pnu.edu.ua
ORCID ID: https://orcid.org/0000-0001-9415-9837

Uliana Kilchytska, [1]student Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenka Str., Ivano-Frankivsk, 76018, Ukraine.
E-mail: uliana.kilchytska.21@pnu.edu.ua

Yulia Savchuk, [1]student Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, 57 Shevchenka Str., Ivano-Frankivsk, 76018, Ukraine.
E-mail: yuliia.savchuk.21@pnu.edu.ua

Uliana Stambulska, PhD, [2]Lead Engineer of Testing Center, Ivano-Frankivsk Scientific and Production Center for Standardization, Metrology and Certification State Enterprise, 127 Vovchynetska Str., Ivano-Frankivsk, 76006, Ukraine
[3]Senior Research Officer of Reforestation and Selection Laboretory, Ukrainian Research Institute of Mountain Forestry named after P.S. Pasternak, 31 Hrushevsky Str., Ivano-Frankivsk, 76000, Ukraine
E-mail: ustambulska@ukr.net
ORCID ID: https://orcid.org/0000-0003-1020-7112

Received: 15 November 2024; **Revised:** 14 December 2024; **Accepted:** 19 December 2024;
Published: 29 December 2024

ВПЛИВ НІТРАТУ СРІБЛА НА БІОХІМІЧНІ ПОКАЗНИКИ *ROSA CANINA* L.

Віктор Гусак, Уляна Кільчицька, Юлія Савчук, Уляна Стамбульська

Журнал Прикарпатського університету імені Василя Стефаника. Біологія, **11** (2024), С.57–С.65.

Анотація:
Дієтичні добавки відіграють важливу роль у забезпеченні організму людини фітохімічними речовинами, які можуть бути недостатньо представлені в повсякденному раціоні харчування. Шипшина звичайна *Rosa canina* L. проявляє протизапальні, антиоксидантні та антибіабетичні властивості завдяки широкому спектру біологічно активних сполук, таких як протизапальний галактоліпід, вітамін С, феноли, лікопін, лютеїн, зеаксантин та інші каротиноїди. Високий інтерес до

потенційно корисних властивостей шипшини стимулює проведення досліджень та розробку технологій, спрямованих на підвищення вмісту біологічно активних сполук у цій рослині. Як відомо, активація вторинних метаболічних шляхів еліситорами та різними стресовими чинниками можуть ініціювати в рослині утворення біологічно активних речовин. У цій роботі досліджено вплив нітрату срібла на деякі біохімічні показники шипшини звичайної (*Rosa canina* L.) за умов мікроклонального розмноження. Показано, що концентрація хлорофілів *a* і *b* у листках шипшини знижувалася за дії нітрату срібла в концентраціях 1, 10 і 50 мг/л. За дії нітрату срібла вміст каротиноїдів у листках рослини не змінювався, а вміст антоціанів зростав. Вміст поліфенольних сполук і флавоноїдів знижувався в рослинах, що зазнали впливу нітрату срібла за всіх обраних концентрацій.

Ключові слова: мікроклональне розмноження, *Rosa canina* L., нітрат срібла, рослинні пігменти, поліфеноли.