PLANT PRIMING WITH SODIUM CHOLATE INCREASES OXIDATIVE STRESS RESPONSE IN MAIZE

CRNKOVIĆ, M.[1*] – ŠUĆUR ELEZ, J.[1] – PETROVIĆ, K.[2,3*] – KLJAK, K.[4] – ANĐELKOVIĆ, V.[2] – TOLIMIR, M.[2] – MALENČIĆ, Đ.[1]

[1]*University of Novi Sad Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*

[2]*Maize Research Institute "Zemun Polje," Slobodana Bajića 1, 11185 Belgrade, Serbia*

[3]*Agriculture Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia*

[4]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

**Corresponding authors*
e-mail: marina.crnkovic@polj.uns.ac.rs; kristina.petrovic@mrizp.rs

(Received 23rd Oct 2025; accepted 11th Feb 2026)

Abstract. The elicitor activity of sodium cholate (NaC) was examined in maize (*Zea mays*, L.) leaves and roots. The effect of this bile salt on the oxidative status in maize metabolism was determined by spectrophotometric methods, measuring the production of superoxide anions ($O_2^{\bullet-}$ radicals), activity of three antioxidant enzymes (superoxide dismutase – SOD, EC 1.15.1.1; catalase – CAT, EC 1.11.1.6 and pyrogallol peroxidase – PPX, EC 1.11.1.7), content of malondialdehyde (MDA), an end product of lipid peroxidation, the contents of total phenols, tannins and flavonoids, as well as measuring scavenging capacity of plant extracts by the DPPH and ABTS assays. In addition, concentration of NaC remaining in the growth media was measured. The results showed that all tested NaC concentrations caused mild oxidative stress due to increased $O_2^{\bullet-}$ radical production, activity of enzymes, and contents of all measured phenolic compounds after seven days of treatment. The highest percentage of NaC remaining in the maize growth medium after seven days was measured at the lowest initial concentration, 20 mg L^{-1}, indicating that this concentration was the least absorbed by the maize. Results from this study provide important insights for sustainable agriculture.
Keywords: *bile salts, elicitor, oxidative status, spectrophotometry, sustainable agriculture*

Abbreviations: NaC, Sodium cholate; SOD, Superoxide dismutase; CAT, Catalase; PPX, Pyrogallol peroxidase; MDA, Malonildialdehyde-; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ROS, Reactive oxygen species; HS, Hoagland's nutrient solution; FW, Fresh weight; DW, Dry weight; GAE, Gallic acid equivalents; QE, Quercetine equivalents; TE, Trolox equivalents

Introduction

When a pathogen comes into contact with a plant, their molecular-level communications is initiated by the pathogen sending molecules called elicitors (Ponzio et al., 2016). However, not all elicitors originate from a pathogen; some of them are from the host plant or another source. A molecule with elicitor activity triggers a plant defense response. Several elicitors have been described in the literature, such as jasmonic, arachidonic and salicylic acids, some glycoproteins, peptides and phospholipids (Thakur and Sohal, 2013), and more recently, bile acids. They are not synthesized in plants, but can come into contact with them through stable manure. To our knowledge, among bile acids, only the elicitor activity of cholic (Koga et al., 2006) and deoxycholic acid (Zarattini et al., 2017) has been described so far.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

It has been proven that cholic acid in rice leaves caused the accumulation of phytoalexins, the induction of hypersensitive cell death, the synthesis of pathogenesis-related (PR) proteins and increased resistance to the next infection by a virulent pathogen (Koga et al., 2006); in the suspension of rice cells it induced the expression of enzyme genes that participate in the synthesis of only some types of phytoalexins (Shimizu et al., 2008), and in the leaves and roots of soybean plants it caused an increase level of oxidative stress (Kevrešan et al., 2009; Malenčić et al., 2012).

Oxidative stress is a process of imbalance between reactive oxygen species (ROS) production and the antioxidant defense system (Betteridge, 2000). Plants under the biotic factor e.g. pathogen attack, or abiotic factor e.g. heat, chilling, drought, salinity, chemical compounds, mechanical damage etc., may increase the level of superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), perhydroxyl radical ($^{\bullet}O_2H$) and hydrogen peroxide (H_2O_2). Furthermore, the antioxidant defense system could be negatively affected during abiotic and biotic stress (Ceron-Garcia et al., 2011). Excessive amounts of ROS and inadequate functioning of the antioxidant system lead to cell and tissue injury. Therefore, the efforts of farmers to bring up healthy crops could be reinforced with novel techniques focused on plant physiology and crop protection by means of the elicitation of plant defense responses against any kind of stress (Ceron-Garcia et al., 2011). In addition to many other plant defense responses, elicitors have also been shown to induce ROS production, as mentioned above in the treatment of soybeans with cholic acid as an elicitor. Increased ROS production, accompanied by activation of plant antioxidant systems, could prepare the plant defense system for abiotic and biotic stress.

This study was conducted to test the hypothesis that sodium cholate (NaC) is an elicitor that triggers mild short-term oxidative stress in maize (*Zea mays*, L.), which would subsequently contribute to a faster maize defense response to subsequent stress (e.g. pathogen attack). This is based on the concept of plant priming, which is defined as a unique physiological state induced by treating plants with some natural or synthetic compound, with the aim of eliciting a faster and stronger cellular defense response following pathogen attack or some abiotic stress (Conrath et al., 2006). Given that this is only a preliminary study, further research is needed that would include inducing biotic/abiotic stress in the maize plant after NaC treatment. If this hypothesis proves to be true, the plant priming method could contribute to sustainable agriculture by improving crop protection in a safe and environmentally friendly way.

Materials and methods

Plant growing procedure

Maize seeds were sterilized in a 1.8% NaClO solution and sown in the sterilized sand for seven days to grow. After that period, the seedlings were transferred to the pots with Hoagland's nutrient solution (HS). This solution contained several components dissolved in water: $Ca(NO_3)_2 \times 4H_2O$, KNO_3, $MgSO_4 \times 7H_2O$, KH_2PO_4, Fe-EDTA, as well as microelements: H_3BO_3, $MnSO_4 \times 4H_2O$, $ZnSO_4 \times 7H_2O$, $CuSO_4 \times 5H_2O$ and $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (Malenčić et al., 2012). The maize seedlings were growing hydroponically during another seven days at the average temperature of 25°C. Each pot consisted of eight maize seedlings and every treatment and control were performed in triplicate. After two weeks in total, the treatment with the salt of cholic acid, NaC, was performed, and control seedlings were grown without added NaC. Aqueous solution of NaC was used in measuring the remaining concentration of NaC in the growth media and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

NaC dissolved in HS was used in measuring of the effect of NaC on the induction of oxidative stress. Cholic acid was used in the form of salt because in that form it was soluble in water and applicable for treatment. Solutions of NaC were used as a growth media for maize seedlings, so absorption occurred through the roots. Leaves and roots samples were taken one, three, five and seven days after the treatment, and each time morphological characteristics (leaf and root fresh weight and root length) were measured.

Measurement of the remaining concentration of NaC in the growth media

Maize seedlings were grown as described in the previous section and then placed in an aqueous NaC solution, with a concentration of 20, 40, 60 and 80 mg L^{-1}. Immediately after dissolving NaC in water, medium samples were taken as a control (0 days), and the samplings were continued every other day until the seventh day.

The remaining concentrations of NaC in growth media were measured by the HPLC method (Zhu and Brown, 1990). After filtration through the syringe filters (CHROMAFIL Xtra RC-45/25, pores size 0.45 µm, Ø25; Macherey-Nagel GMbH Co KG, Düren, Germany), the samples were analyzed by the SpectraSystem HPLC instrument (Thermo Separation Products, Inc., Waltham, MA, USA). The apparatus included a Symmetry C18 column (150 × 4.6 mm, 3.5 µm; Waters Corporation, Wexford, Ireland) heated to 35°C, an SCM1000 degasser, a P4000 quaternary gradient pump, an AS3000 autosampler, and an RI-150 refractive index detector. ChromQuest 5.0 Software was used to acquire and process the results. The mobile phase used in this experiment was the mixture of methanol and 0.1 M acetic acid (70/30, v/v) (linear elution) at a flow rate of 1 mL min^{-1}. Injection volume was 20 µL, flush volume 400 µl and run time 20 min. The NaC concentrations in samples of growth media were quantified by external standardization with calibration curves using a stock solution of NaC (2400 mg L^{-1}) in water. Seven different concentrations: 5, 10, 20, 40, 80, 100 and 150 mg L^{-1} were used for the stock solution and the results are expressed as a percentage of the remaining concentration of NaC in the medium compared to the initial concentration.

Measurement of the effect of NaC on the induction of oxidative stress in maize

Collected leaves and root samples were prepared for biochemical analysis. Methods for determination of NaC effect on the induction of oxidative stress used in this study were: measuring of $O_2^{\bullet-}$ radical production, activity of antioxidant enzymes (SOD, CAT and PPX), intensity of lipid peroxidation (content of MDA), contents of total phenols, tannins and flavonoids, as well as two antioxidant assays, DPPH and ABTS assay.

It was weighted 1 g of fresh maize leaves and 2 g of fresh maize roots. The remaining fresh leaves were allowed to dry, then ground. Fresh leaves and roots samples were homogenized with 10 mL of phosphate buffer (0.1 M KH_2PO_4), at pH 7, and then centrifuged at 5000 rpm at 4°C, for 15 min, and the supernatant was separated. Dry leaf samples (0.2 g) were prepared with 70% acetone and the extraction lasted for 24 h, followed by filtration through filter paper (Whatman No. 4) and the resulting extracts were stored in the refrigerator until use. Obtained supernatants and extracts were used for the abovementioned assays, based on UV/VIS spectrophotometry.

Determination of the production of $O_2^{\bullet-}$ radical is based on the reaction of inhibiting the autoxidation of adrenaline (Misra and Fridovich, 1972; Volkov et al., 2023). Absorbance of formed adrenochrome, the product of adrenaline autoxidation, was measured at 480 nm and the $O_2^{\bullet-}$ content was expressed as µmol g^{-1} FW. The activity of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

SOD was measured based on the its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT) (Mandal et al., 2008). The absorbance of formed formazan blue was measured at 560 nm and the results were expressed as U g^{-1} FW. One unit of the SOD activity is defined as the amount of the enzyme, required to inhibit reduction of NBT by 50%. The activity of CAT was determined by measuring the decomposition of H_2O_2, with a decrease in absorbance at 240 nm (Elevarthi and Martin, 2010), and the pyrogallol peroxidase by measuring the content of purpurogallin, a product of pyrogallol oxidation, at 430 nm (Morkunas and Gmerek, 2007). As with SOD, the results for CAT and PPX were also expressed as U g^{-1} FW. According to the same reference as for determining SOD activity (Mandal et al., 2008), with minor changes, the level of lipid peroxidation was determined. MDA production was evaluated as a measure of the level of lipid peroxidation. This method is based on the reaction between the formed MDA and thiobarbituric acid (TBA). Concentration of the resulting product was measured spectrophotometrically at 532 nm, and the results were expressed as nmol MDA g^{-1} FW. The content of total polyphenols and tannins was determined by the Folin-Ciocalteu procedure (Hagerman et al., 2000). Tannin content was determined as follows: tannins were removed from the extracts by adsorption on insoluble matrix polyvinylpolypyrrolidone – PVPP, and the content of non-tannin phenols was determined, in the same way as total polyphenols, by adding the Folin-Ciocalteu reagent and 20% Na_2CO_3, followed by the absorbance reading at 720 nm. Measured values of non-tannin phenols content were subtracted from the measured values of total polyphenols content, whereby the tannins content was calculated and both total polyphenols and tannins content were expressed as mg GAE g^{-1} DW. Assessment of flavonoids content was conducted by adding $AlCl_3$ reagent and measuring the absorbance of the formed complex at 430 nm (Harnafi et al., 2007). Results were expressed as mg QE g^{-1} DW. DPPH and ABTS assays (Chang et al., 2007) were performed in order to evaluate the antioxidant potential of the plant extracts. The DPPH assay is based on the radical scavenging ability of the extract's components, where transformation of the purple $DPPH^{\cdot}$ radical into a yellow reduced form is measured (maximum absorption at 517 nm). ABTS assay, similar to the DPPH, measures the ability of the extracts to neutralize $ABTS^{\cdot+}$ radicals (absorbance was measured at 734 nm). The results of DPPH and ABTS assays were expressed as mg TE g^{-1} DW.

Statistical analysis

All measurements were performed in triplicate and all results were expressed as mean ± standard error (SE), n = 9. Statistical significance was tested with a one-way ANOVA followed by comparisons of means by Fisher LSD test (Statistica - 14.0.0.15, TIBCO Software Inc.), and *P* values less than 0.05 were considered significant. In all figures vertical lines represent standard errors.

Results

The amount of NaC remaining in the aqueous solution in which maize seedlings were grown for seven days was measured. Other than that, the effect of NaC on oxidative stress in this crop was determined by measuring various parameters.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

Concentration of NaC in the growth medium of maize seedlings

Four concentrations of NaC were used in this test: 20, 40, 60 and 80 mg L^{-1}, and after one day, medium sampling was started to determine the remaining NaC concentration compared to the initial concentration in order to estimate the approximate amount of NaC absorbed by the maize. Sampling continued after 3, 5 and 7 days, and the results are shown in *Figure 1*.

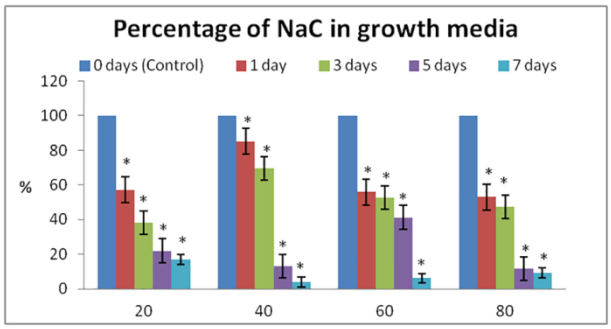

Figure 1. *Percentage of remaining NaC in the growth media of maize seedlings over seven days, compared with initial concentrations (20, 40, 60 or 80 mg L^{-1}) of NaC. The values are expressed as mean ± standard error (SE), n = 9, and those marked with an asterisk are significantly different from 0 days, the control ($P < 0.05$)*

Already after the first day, the remaining NaC concentrations in the medium were about 50% or more (40 mg L^{-1} NaC 85%) of the initial concentration. After that, the decrease in concentrations continued until the seventh day, when 4, 6 and 9% from the initial concentrations of 40, 60 and 80 mg L^{-1} NaC respectively remained in the medium and 17% from initial 20 mg L^{-1} NaC. This indicates that the lowest NaC concentration (20 mg L^{-1}) was the least absorbed by maize during this seven day-period. All decreases in NaC concentrations in this experiment are statistically significant compared to the initial concentrations.

The effect of NaC on the oxidative stress parameters in maize

Parameters of oxidative stress determined in this experiment were: superoxide anion ($O_2^{\bullet-}$ radical) production, activity of antioxidant enzymes (superoxide dismutase – SOD, EC 1.15.1.1; catalase – CAT, EC 1.11.1.6 and pyrogallol peroxidase – PPX, EC 1.11.1.7), lipid peroxidation intensity – measured by the content of the end product, malondialdehyde (MDA), contents of total phenols, tannins and flavonoids, as well as scavenging capacities by DPPH and ABTS assays. The obtained results are given in *Figures 2* and *3*.

Treatment of maize with 80 mg L^{-1} NaC, after one day, led to a statistically significant reduction in MDA content in leaves, compared to the control. Also, at the same concentration, the activity of all tested antioxidant enzymes, SOD, CAT and PPX, was significantly reduced in roots. Other than that, at concentrations of 40 and 60 mg L^{-1}, after one day of treatment, there was a significantly reduced scavenging capacity in the ABTS assay in plant extracts, and at 20 and 40 mg L^{-1} there was a significant increase in tannin content compared to the control.

At all concentrations of NaC, three days after treatment, the activity of at least one enzyme was significantly reduced. By the influence of the lowest concentration, 20 mg L^{-1}

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

NaC, in the leaves was only reduced the activity of SOD, and in the roots the activity of all enzymes, by the influence of 40 mg L^{-1} NaC in the leaves were reduced activity of SOD and PPX, and in the roots only PPX, then with concentration of 60 mg L^{-1} in the leaves only the activity of PPX was reduced, and in the roots only CAT, while with the highest concentration of NaC, 80 mg L^{-1}, were significantly reduced the activity of CAT and PPX in the leaves and roots. Also, by all concentrations of NaC, the tannin content significantly decreased compared to the control. Furthermore, the results of the flavonoid content and ABTS assay were harmonized after three days of treatment. NaC concentrations of 20 and 40 mg L^{-1} significantly reduced flavonoid content and scavenging capacity in the ABTS assay, and the concentration of 60 mg L^{-1} significantly increased them.

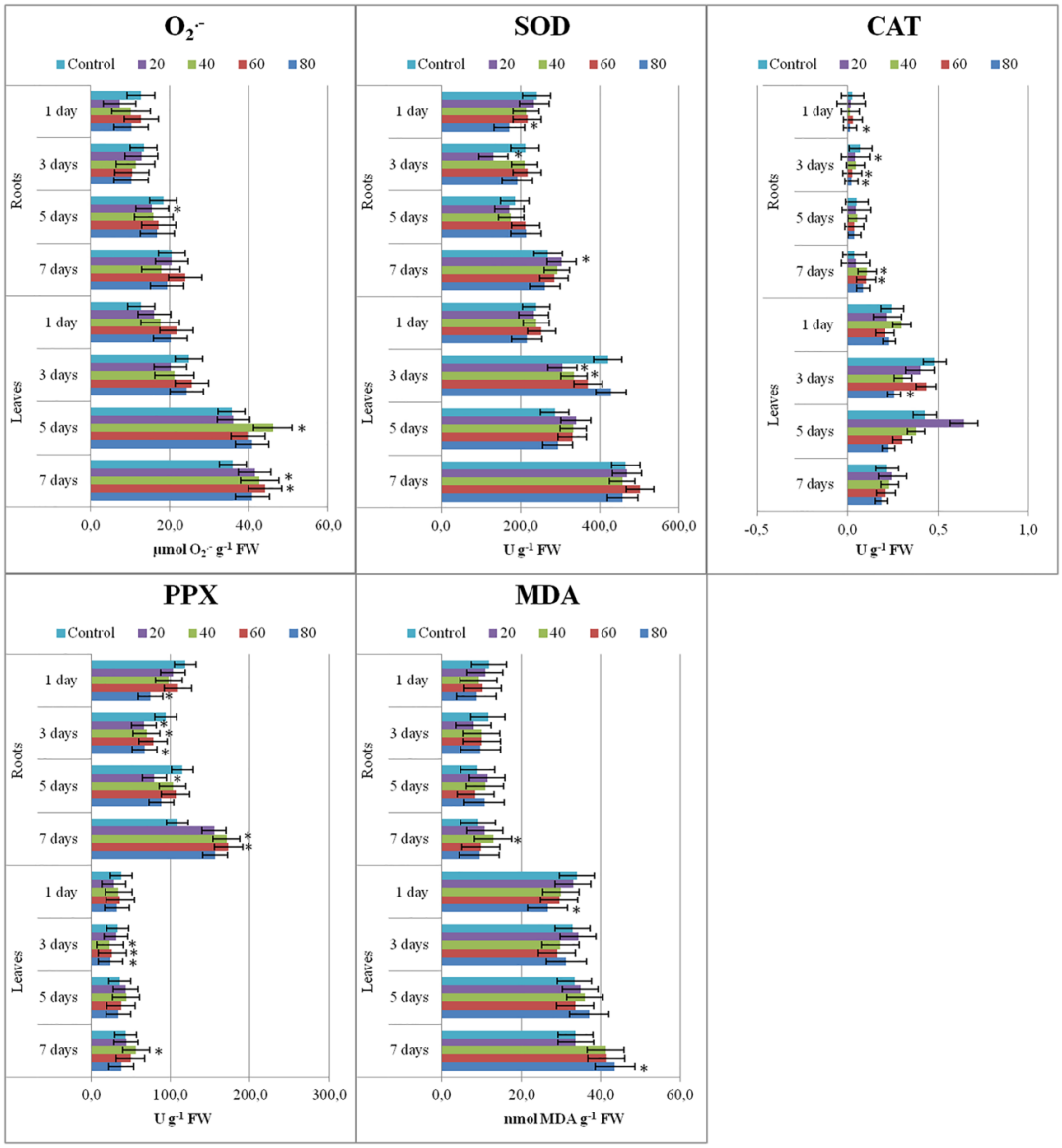

Figure 2. *$O_2^{\bullet-}$ radical production, SOD, CAT, PPX activities and MDA content in maize seedlings treated with NaC (20, 40, 60 and 80 mg L^{-1}) during seven days. The values are presented as mean value ± standard error, n = 9, and those marked with an asterisk are significantly different from the control ($P < 0.05$)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

Five days after the treatment of maize with NaC, the production of $O_2^{\bullet-}$ radicals significantly increased in the leaves compared to the control by the impact of 40 mg L^{-1} NaC, and in the roots it significantly decreased by the impact of 20 mg L^{-1} NaC, where it also affected the reduction of PPX activity. This lowest concentration of NaC significantly increased the content of tannins, flavonoids as well as the scavenging capacity in the DPPH assay. The increased content of phenolic compounds after five days was also observed with moderate concentrations of NaC: 40 mg L^{-1} increased the content of flavonoids and 60 mg L^{-1} of total phenols, tannins and flavonoids. In this period, after five days, higher concentrations of NaC, 60 and 80 mg L^{-1} significantly reduced the scavenging capacity in the ABTS assay.

On the seventh, last day of sampling, it was found that only moderate concentrations of NaC (40 and 60 mg L^{-1}) statistically significantly increased the production of $O_2^{\bullet-}$ radicals compared to the control, and only in the leaves. Also, in this period, all but the highest concentrations of NaC caused a significant increase in enzyme activity, especially in roots: 20 mg L^{-1} NaC caused an increase only in SOD activity in roots, 40 mg L^{-1} in CAT activity in roots and PPX activity in leaves and roots, and 60 mg L^{-1} CAT and PPX activities in roots. Furthermore, 40 mg L^{-1} of NaC caused a significant increase in MDA content in the roots, and 80 mg L^{-1} in the leaves which indicates an increased intensity of lipid peroxidation in these samples. After the seventh day of treatment, significantly higher amount of total phenols and tannins was recorded in the treatment with 80 mg L^{-1} NaC. In the treatment with 40 mg L^{-1} NaC significantly lower amount of total phenols and scavenging capacity in the DPPH and ABTS assays was recorded, as well as a significantly lower scavenging capacity in the ABTS assay in the treatment with 20 mg L^{-1} NaC.

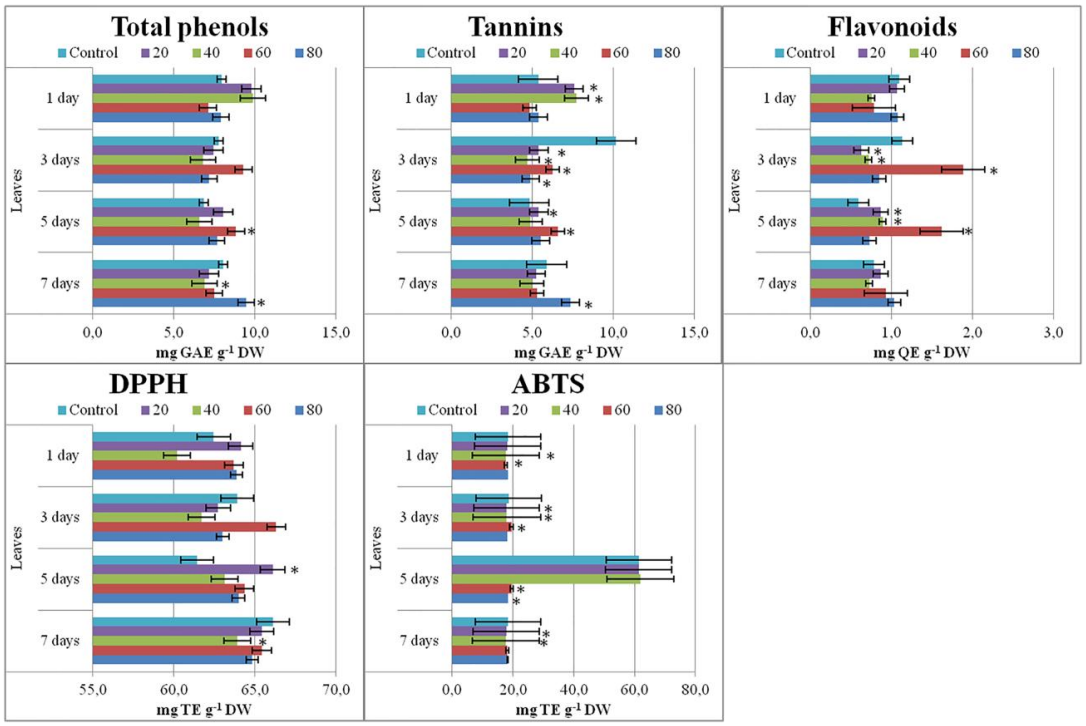

Figure 3. *Total phenols, tannins and flavonoids contents, as well as scavenging capacities by DPPH and ABTS assays in maize seedlings treated with NaC (20, 40, 60 and 80 mg L^{-1}) during seven days. The values are presented as mean value ± standard error, n = 9, and those marked with an asterisk are significantly different from the control ($P < 0.05$)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

At each sampling, the leaf and root fresh weight, as well as the root length, were measured. The results of morphological characteristics are shown in *Figure 4,* and the appearance of the plants in *Figure 5*. According to the results, NaC had the greatest effect on the leaf fresh weight, while in the roots it almost did not cause a significant change in either fresh weight or length. In addition, 40 mg L^{-1} NaC caused the most statistically significant changes in the morphological characteristics compared to the control, because it caused a significant increase in the leaf fresh weight and the root length of maize after three days of treatment and after seven days a significant decrease in the leaf and root fresh weight. Except for 40 mg L^{-1}, the highest concentration of NaC, 80 mg L^{-1}, caused a significant increase in leaf fresh weight compared to the control after the third day of treatment.

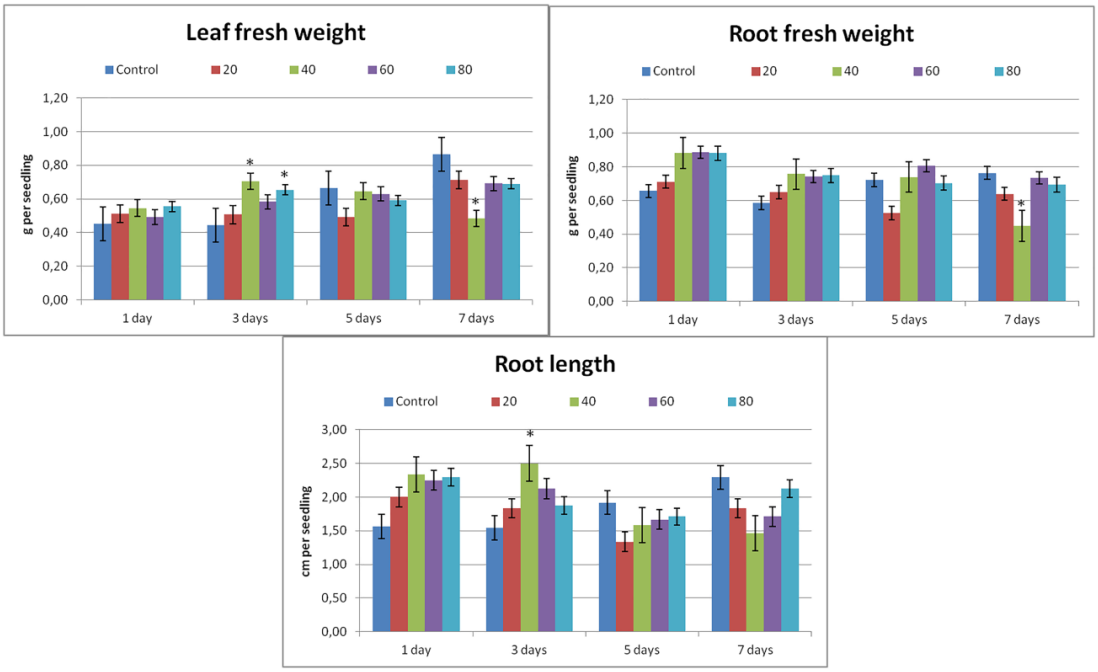

Figure 4. *Morphological characteristics of maize after seven days of treatment with 20, 40, 60 and 80 mg L^{-1} NaC. The values are presented as mean value ± standard error, n = 9, and those marked with an asterisk are significantly different from the control ($P < 0.05$)*

Figure 5. *Appearance of maize seedlings after seven days of treatment with 20, 40, 60 and 80 mg L^{-1} NaC. The first pot on the left was the control and next to it to the right were the treatments from lowest to highest concentration*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

Discussion

The treatment of maize seedlings with NaC induced mild oxidative stress, which was expected as this bile acid salt has been previously shown to have elicitor activity in soybean plants (Kevrešan et al., 2009). In our preliminary studies (Crnković et al., 2022) of the effect of NaC, dissolved in water or HS, on oxidative stress in sunflower and maize based on measuring MDA content, it was also shown that NaC had good elicitor activity because it caused mild oxidative stress by increasing MDA content and thus the intensity of lipid peroxidation. Furthermore, in the same reference was reported that concentrations of 60 and 80 mg L^{-1} NaC in maize roots caused increased MDA content compared to the control even after five days, in contrast to sunflower roots in which MDA content was reduced compared to the control by treatment with 20, 40, 60 and 80 mg L^{-1} NaC already after the third day, which means that NaC maintained oxidative stress in maize for a longer period than in sunflower. This information could be useful in planning future experiments with a pathogen in terms of how long before the pathogen NaC should be applied to have the effect of accelerating the maize defense response.

The results from this study showed that first day after maize treatment with NaC, at low concentrations (20 and 40 mg L^{-1}) the first marker of high level of oxidative stress was increased tannin content compared to the control. Tannins, like all other phenolic compounds, are antioxidants that plants generate to protect against oxidative damage (Fedeli et al., 2004). The increased content continued for all other measured phenolic compounds with treatment with all NaC concentrations after five and seven days. Considering this and all other parameters, oxidative stress in maize was stronger in the second half of the NaC treatment period, because during that period, under the influence of all NaC concentrations, there was either increased production of $O_2^{\bullet-}$ radicals, higher activity of antioxidant enzymes or increased content of MDA and phenolic compounds compared to control (or a combination of several of them), all of which indicates that oxidative stress was still present after seven days.

In a study of soybean treatment with NaC in combination with inoculation with *Aspergillus niger* (Malenčić et al., 2012), it was concluded that NaC alone did not contribute to oxidative stress, except at the highest concentration of 80 mg L^{-1}, and when it acted together with a source of biotic stress (*A. niger*), it seemed to be too aggressive for the soybean defense system. Therefore, it is necessary to find the appropriate concentration of NaC and find the most optimal time when the treatment should be done before infection. It is also necessary to find a suitable maize pathogen for further research into how NaC will affect maize metabolism when applied prior to infection.

The ABTS assay, in this study, generally showed statistically significant changes in scavenging capacity (mostly decreases compared to control) in a larger number of maize samples than the DPPH assay. According to the number of samples that showed statistically significant changes compared to the control, enzyme activities (especially CAT and PPX in the roots), tannin content and ABTS assay stand out the most. Therefore, the influence of NaC on oxidative stress in maize was most evident in those parameters. Also, it is observed that during the first three days of treatment, the parameters of oxidative stress generally decrease compared to the control, i.e. enzyme's activity, phenolic compound contents, and scavenging capacities in antioxidant assays decreased (with a few exceptions), while after the fifth and seventh day, an increase in parameters is generally observed compared to the control: production of $O_2^{\bullet-}$ radicals, enzyme's activity and phenolic compound contents. According to the obtained results for MDA content, NaC significantly affected lipid peroxidation in only a few samples, more

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

precisely only the concentrations of 40 mg L^{-1} (in the roots) and 80 mg L^{-1} (in the leaves) caused a significant increase in MDA content, and this occurred only after the seventh day of treatment, which means that the effect of NaC on maize cells was not too aggressive.

Although there are not many recent studies on the elicitor activity of cholic acid, studies on other elicitors and the different plant defense responses they trigger can be found in the literature. It has been proven so far that some of the following responses can also be caused by cholic acid. The 12-oxophytodienoic acid (OPDA), precursor of the jasmonic acid (JA), has been found to triggers production of phytoalexins in rice cells (Shinya et al., 2022). Also, the elicitors salicylic acid (SA), gibberellic acid (GA), abscisic acid (ABA), 2,6-dicholorisonicotinic acid (INA) and γ-aminobutyric acid (GABA) are shown to induce the biochemical defense in maize against the maydis leaf blight disease. All of the measured parameters, for example total phenol content and SOD activity, were increased by all elicitors (Kumar et al., 2024). Other than that, Elshahawy and Abd El-Wahed (2025) reported that SA can be used as an environmentally safe fungicide against the late wilt disease of maize, as it induced increased total phenol and flavonoid content, peroxidase activity, and other parameters.

Conclusion

All concentrations of NaC induced oxidative stress in maize, with greater intensity after the fifth and seventh day, when most oxidative stress parameters were increased compared to control. The lowest applied concentration of NaC, 20 mg L^{-1}, appeared to induce the mildest level of oxidative stress, as after the seventh day of treatment it induced increasing of only one parameter compared to control, an increase in the SOD activity in maize roots. Also, this lowest concentration of NaC did not cause statistically significant changes in morphological characteristics. Since we found that 17% of the initial 20 mg L^{-1} NaC was still detected in the growth medium after the seventh day, which was the highest percentage of the remaining concentration in the medium of all other initial NaC concentrations, it can be concluded that the 20 mg L^{-1} NaC was the least absorbed by the maize. Based on all these findings, 20 mg L^{-1} NaC did not prove to be toxic to maize metabolism during the seven-day experiment. It has also been shown to be the most harmless concentration of NaC for use in plant priming of maize, as it has been shown to induce oxidative stress that is alleviated after seven days.

Acknowledgements. This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant numbers 451-03-33/2026-03/200117, 451-03-34/2026-03/200117 and 451-03-33/2026-03/200040. The authors would like to thank the Institute of Field and Vegetable Crops for their valuable collaboration.

REFERENCES

[1] Betteridge, D. J. (2000): What is oxidative stress? – Metabolism. 49(2): 3-8. DOI: 10.1016/S0026-0495(00)80077-3

[2] Ceron-Garcia, A., Vargas-Arispuro, I., Aispuro-Hernandez, E., Martinez-Tellez, M. A. (2011): Oligoglucan Elicitor Effects During Plant Oxidative Stress. – In: Bubulya, P. (ed.) Cell Metabolism—Cell Homeostasis and Stress Response. InTech, Rijeka. DOI: 10.5772/26057

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

[3] Chang, H. Y., Ho, Y. L., Sheu, M. J., Lin, Y. H., Tseng, M. C., Wu, S. H., et al. (2007): Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts. – Botanical Studies 48: 407-417. https://www.researchgate.net/profile/Sheng-Hua-Wu/publication/285533793_Antioxidant_and_free_radical_scavenging_activities_of_Phellinus_merrillii_extracts/links/63a12c87e42faa7e75d8fd0b/Antioxidant-and-free-radical-scavenging-activities-of-Phellinus-merrillii-extracts.pdf

[4] Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., et al. (2006): Priming: getting ready for battle. – Molecular Plant-Microbe Interactions 19(10): 1062-1071. DOI: 10.1094/MPMI -19-1062

[5] Crnković, M., Šućur, J., Malenčić, Đ., Jovanović-Šanta, S. (2022): Assessment of oxidative stress of maize and sunflower treated with cholic acid in different media—screening test. – In: Proceedings of the 27th Symposium on Biotechnology with International Participation. University of Kragujevac Faculty of Agronomy, Čačak. DOI: 10.46793/SBT27.099C

[6] Elevarthi, S., Martin, B. (2010): Spectrophotometric Assays for Antioxidant Enzymes in Plants. – In: Sunkar, R. (ed.) Plant Stress Tolerance, Methods and Protocols. Springer Science + Business Media, Stillwater, OK. DOI: 10.1007/978-1-60761-702-0_16

[7] Elshahawy, I. E., Abd El-Wahed, M. S. (2025): Efficacy of plant resistance elicitor salicylic acid against *Magnaporthiopsis maydis* and its exogenous application for controlling late wilt disease of maize. – Cereal Research Communications 53: 1545-1558. DOI: https://doi.org/10.1007/s42976-025-00631-w

[8] Fedeli, D., Berrettini, M., Gabryelak, T., Falcioni, G. (2004): The effect of some tannins on trout erythrocytes exposed to oxidative stress. – Mutation Research 563: 89-96. DOI: 10.1016/j.mrgentox.2004.06.008

[9] Hagerman, A., Harvey-Mueller, I., Makkar, H. P. S. (2000): Quantification of tannins in Tree Foliage—A Laboratory Manual. – FAO/IAEA, Vienna. https://www.iaea.org/sites/default/files/21/06/nafa-aph-manual-pubd31022manual-tannin.pdf

[10] Harnafi, H., Bouanani, N. H., Aziz, M., Caid, H. S., Ghalim, N., Amrani, S. (2007): The hypolipidaemic activity of aqueous *Erica multiflora* flowers extract in Triton WR-1339 induced hyperlipidaemic rats: A comparison with fenofibrate. – Journal of Ethnopharmacology 109: 156-160. DOI: 10.1016/j.jep.2006.09.017

[11] Kevrešan, S., Malenčić, Đ., Popović, M., Kuhajda, K., Kandrač, J. (2009): The effect of cholic acid treatment on the oxidative status of soybean plants. – Journal of Serbian Chemical Society 74(8-9): 857-865. DOI: 10.2298/JSC0909857K

[12] Koga, J., Kubota, H., Gomi, S., Umemura, K., Ohnishi, M., Kono, T. (2006): Cholic acid, a bile acid elicitor of hypersensitive cell death, pathogenesis-related protein synthesis, and phytoalexin accumulation in rice. – Plant Physiology 140: 1475-1483. DOI: 10.1104/pp.105.070334

[13] Kumar, C., Chand, P., Choudhary, C. S., Maurya, S., Kumar, A., Priya, S. (2024): Activity of different defense related antioxidative biochemical compound upon exogenous application of different elicitors on maize against *Helminthosporium maydis* Y. Nisik & C. Miyake. – Cereal Research Communications 52: 721-731. DOI: https://doi.org/10.1007/s42976-023-00408-z

[14] Malenčić, Đ., Kevrešan, S., Popović, M., Štajner, D., Popović, B., Kiprovski, B., et al. (2012): Cholic acid changes defense response to oxidative stress in soybean induced by *Aspergillus niger*. – Central European Journal of Biology 7(1): 132-137. DOI: 10.2478/s11535-011-0104-y

[15] Mandal, S., Mitra, A., Mallick, N. (2008): Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiol.* – Molecular Plant Pathology 72: 56-61. DOI: 10.1016/j.pmpp.2008.04.002

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098

[16] Misra, H. P., Fridovich, I. (1972): The role of superoxide anion in the autooxidation of epinephrine and a simple measurement for superoxide dismutase. – Journal of Biological Chemistry 247: 3170-3175. DOI: 10.1016/S0021-9258(19)45228-9
[17] Morkunas, I., Gmerek, J. (2007): The possible involvement of peroxidase in defense of yellow lupine embryo axes against *Fusarium oxysporum*. – Journal of Plant Physiology 164: 185-194. DOI: 10.1016/j.jplph.2005.11.005
[18] Ponzio, C., Weldegergis, B. T., Dicke, M., Gols, R. (2016): Compatible and incompatible pathogen-plant interactions differentially affect plant volatile emissions and the attraction of parasitoid wasps. – Functional Ecology 30: 1779-1789. DOI: 10.1111/1365-2435.12689
[19] Shimizu, T., Jikumaru, Y., Okada, A., Okada, K., Koga, J., Umemura, K., et al. (2008): Effects of a bile acid elicitor, cholic acid, on the biosynthesis of diterpenoid phytoalexins in suspension-cultured rice cells. – Phytochemistry 69: 973-981. DOI: 10.1016/j.phytochem.2007.10.005
[20] Shinya, T., Miyamoto, K., Uchida, K., Hoho, Y., Yumoto, E., Okada, K., Yamane, H., Galis, I. (2022): Chitooligosaccharide elicitor and oxylipins synergistically elevate phytoalexin production in rice. – Plant Molecular Biology 109: 595-609. DOI: https://doi.org/10.1007/s11103-021-01217-w
[21] Thakur, M., Sohal, B. S. (2013): Role of elicitors in inducing resistance in plants against pathogen infection: a review. – ISRN Biochemistry 2013: 762412. DOI: 10.1155/2013/762412
[22] Volkov, V., Lobanov, A., Voronkov, M., Baygildiev, T., Misin, V., Tsivileva, O. (2023): Kinetics and mechanism of epinephrine autoxidation in the presence of plant superoxide inhibitors: a new look at the methodology of using a model system in biological and chemical research. – Antioxidants 12(8): 1530. DOI: 10.3390/antiox12081530
[23] Zarattini, M., Launay, A., Farjad, M., Wénès, E., Taconnat, L., Boutet, S., et al. (2017): The bile acid deoxycholate elicits defences in *Arabidopsis* and reduces bacterial infection. – Molecular Plant Pathology 18(4): 540-554. DOI: 10.1111/mpp.12416
[24] Zhu, X. X.; Brown, G. R. (1990): A simple HPLC method for the analysis of bile acids. – Analytical Letters 11: 2011-2018. DOI: 10.1080/00032719008052545

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2403_40874098