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Research Article

The allelopathic effects of exogenous pyrogallol on antioxidant metabolism and leaf gas exchange in arsenic-stressed maize (*Zea mays* L.) seedlings

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Abstract – Pyrogallol (PG) is a polyphenol naturally occurring in the leaves and fruits of various plants and is widely utilized as an active component in pharmaceuticals. Although the allelopathic activities of phenolic compounds are well-documented, the allelopathic effects of pyrogallol under heavy metal stress remain poorly understood. This study investigated the effects of PG on oxidative stress indicators, enzymatic and non-enzymatic antioxidant responses, and leaf gas exchange parameters in maize (*Zea mays* L.) seedlings under arsenic (As) stress. The combined treatment with PG and As led to a significant 5-fold increase in arsenic accumulation compared to treatment with As alone. This application also caused excessive oxidative stress, which exceeded the antioxidant system's capacity. Although the application of PG or As alone enhanced activity of antioxidant enzymes, their combined application suppressed these enzymes, reducing total antioxidant capacity. Similarly, the combination of PG and As caused a significant decline in photosynthetic performance, further disrupting redox balance and physiological stability. These findings reveal the synergistic toxicity of PG and As, which severely impair plant metabolism. In As-contaminated soils, phenolic compounds like PG may intensify oxidative stress, influencing plant physiology, depending on the concentration. This study underscores the importance of careful management of phenolic compounds in agricultural systems exposed to heavy metal pollution.

Keywords: antioxidant capacity, heavy metals, oxidative stress, phenolic compounds, photosynthesis

Introduction

Arsenic (As) is a toxic heavy metal and a significant global concern, particularly due to its increasing prevalence in water pollution across many countries (Abedi and Mojiri 2020, Bali and Sidhu 2021). The mobilization of As is strongly influenced by geochemical, microbial, and anthropogenic activities. Plants absorb As ions from the soil in the forms of arsenate (As V) or arsenite (As III). This uptake leads to elevated levels of reactive oxygen species (ROS) in plants, including superoxide radicals, singlet oxygen, hydroxyl radicals, and hydrogen peroxide (H_2O_2), which induce oxidative stress and damage plant cells (Tripathi et al. 2012, Nahar et al. 2022). As a result, cellular macromolecules are compromised, and essential physiological processes like photosynthesis are inhibited, ultimately reducing plant growth and productivity (Khan et al. 2021, Asgher et al. 2022). Studies indicate that plants grown in arsenic-contaminated soils exhibit heightened sensitivity to this toxic element, leading to developmental disruptions and impairing their antioxidant defense systems (Srivastava et al. 2016, Asgher et al. 2021, Bali and Sidhu 2021).

Exogenous applications of phenolic acids such as salicylic acid (SA), gallic acid (GA), and caffeic acid (CfA) effectively mitigate the adverse effects of heavy metal (HM) stress in plants. For instance, foliar SA application enhances tolerance to heavy metal stress by enhancing antioxidant defenses, reducing ROS levels, and limiting metal accumulation in *Solanum tuberosum* L. under cadmium (Cd) stress and in *Melissa officinalis* L. under nickel stress (Ni) (Soltani Maivan et al. 2017). In sunflower seeds, GA pretreatment reversed Cd-induced effects, including elevated H_2O_2 levels, lipid peroxidation, and antioxidant enzyme activity, while restoring thiols, chlorophyll, and lipid content (Saidi et al. 2021). Similarly, CfA acts as a precursor for ferulic acid and melatonin, with its key enzyme, caffeic acid O-methyltransferase (COMT), showing increased expression in *Medicago sativa* L. and *Brassica napus* L. under copper (Cu), Cd, and aluminum (Al) stress (Vega et al. 2022). Additionally, phenolic acids such as catechol, benzoic acid, p-hydroxybenzoic acid,

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GA, SA, syringic acid, vanillic acid, protocatechuic acid, and gentisic acid have been confirmed to exhibit allelopathic activity (Gulzar et al. 2016). Allelopathy, first defined by the German scientist Molish (1938), refers to the interactions among plants including microorganisms. This definition was later expanded by Rice (1984) to describe allelopathy as the beneficial or detrimental effects of one organism on another through the release of organic compounds into the environment (Cheng and Cheng 2015). These organic compounds, known as allelochemicals, are crucial in natural ecosystems. Allelopathy is a widespread phenomenon that plays a vital role in understanding plant community structures, species distributions, successional dynamics, coevolutionary relationships, and biological invasions (Kalisz et al. 2021). Phenolic allelochemicals are synthesized through the shikimate pathway and serve as defensive agents against pathogens while acting as signaling molecules in plant-pathogen interactions (Misra et al. 2023). Phenolic compounds, which are classified into categories such as flavonoids, tannins, coumarins, lignans, quinones, stilbenes and curcuminoids (Kisiriko et al. 2021), are particularly notable for their strong allelopathic effects (John and Sarada 2012).

Pyrogallol (1, 2, 3-trihydroxybenzene, PG), an important phenolic acid, is naturally found in various organisms, including fruit and vegetables. Numerous studies have documented its allelopathic activity. For instance, *Myriophyllum spicatum* L. produces PG acid, which, along with ellagic acid, GA, and catechin, inhibits the growth of the cyanobacterium *Microcystis aeruginosa* (Nakai et al. 2000). PG applied in high concentrations (e.g., 1 and 2 g L^{-1}) has also been shown to inhibit seed germination and seedling growth in *Lolium perenne* L. (Sang et al. 2024), highlighting its allelochemical potential. However, the role of PG in stress tolerance and its effects on plants under stress conditions remain largely unexplored. To overcome this knowledge deficiency, the following research questions arise: (i) What is the role of PG as a phenolic allelochemical in modulating plant responses to As-induced stress? (ii) Does PG, under arsenic stress conditions, influence antioxidant defense mechanisms and photosynthetic efficiency in plants? (iii) How does the allelopathic activity of PG interact with arsenic stress to affect plant physiological and biochemical processes? For this reason, this study comprehensively investigated the effects of PG and As applications, both individually and in combination, on maize seedlings. Gas exchange parameters, including photosynthetic rate (P_n), stomatal conductance (g_s), transpiration (E), and intercellular carbon dioxide (C_i) levels, were measured to evaluate the extent of photosynthetic impairment. Oxidative stress levels were assessed through thiobarbituric acid reactive substances (TBARS) and H_2O_2 accumulation, while the antioxidant defense system was analyzed both enzymatically including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and non-enzymatically (total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC). Additionally, As uptake was determined by inductively coupled plasma mass spectrometry (ICP-MS). This study provides the first detailed evidence of the destructive impacts of PG on photosynthesis and antioxidant status under As stress, offering new insights into its allelopathic role.

Material and methods

Plant material and treatments

Zea mays L. (cultivar, ADA 523) seeds were sourced from Sakarya Maize Research Institute. The seedlings were cultivated in a growth chamber for 21 days under controlled conditions: relative humidity of 60–65%, light intensity of 250 μmol m^{-2} s^{-1} (Demiralay 2022) a temperature of 25 ± 2 °C, and a photoperiod of 16 h light/8 h dark. When the seedlings reached the three-leaf stage, their above-ground parts were excised to ensure uniform and efficient uptake of PG by the maize plants. Following this step, the seedlings were placed in glass tubes (16 × 100 mm) containing distilled water (DW) for 1 h to reduce stress-induced damage. Pyrogallol concentration was set at 5 mM (0.63 g L^{-1}) according to previous findings that higher concentrations, such as 1 and 2 g L^{-1}, exhibit strong inhibitory effects on germination and root growth due to their allelopathic properties, while lower concentrations, such as 0.25 – 0.50 g L^{-1}, have moderate effects (Sang et al. 2024). The selected concentration aims to strike a balance between avoiding excessive toxicity and allowing measurable impacts on plant physiology. We selected 100 μM as the As stress concentration as Ghosh et al. (2016) clearly demonstrated that this concentration triggers a pronounced oxidative stress response in maize seedlings. The experimental design included four treatment groups: (i) Control group with no treatment: seedlings exposed to DW for 18 h, (ii) As-stressed group: pre-treated with DW for 6 h, followed by exposure to 100 μM As for 12 h, (iii) PG-stressed group: pre-treated with 5 mM PG for 6 h, followed by DW treatment for 12 h and (iv) PG+As group: pre-treated with 5 mM PG for 6 h, followed by treatment with 100 μM As for 12 h.

Determination of As content

For the simultaneous multielement detection of As contents in maize seedlings, an Agilent 7900 model inductively coupled plasma mass spectrometer (ICP-MS) was employed. First, 0.1 g of each sample was digested using 8 mL of 65% (v/v) HNO_3 and 1 mL of 35% (v/v) H_2O_2 in polytetrafluoroethylene vessels. These vessels were then placed in a microwave oven (Anton Paar Microwave Reaction System), with the following conditions: heating to 185 °C for 20 min, maintaining a constant temperature for 15 min, and cooling to 60 °C for 21 min. Each sample was subsequently diluted with 50 mL of DW. This prepared solution was then analyzed for As content using an ICP-MS device equipped with a concentric nebulizer, a quartz torch with a quartz injector tube, and a cyclonic spray chamber. On-line Suppl. Tab. 1 displays displays the ICP-MS working conditions.

Determination of oxidative stress parameters

The amount of lipid peroxidation was measured in terms of TBARS content (Heath and Packer 1968). Leaf sample (0.1 g) was homogenized in 0.1% trichloroacetic acid (TCA), centrifuged, and 1 mL of supernatant was mixed with 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95 °C for 30 min, cooled, and absorbance was measured at 532 nm with nonspecific absorbance at 600 nm subtracted. TBARS content was calculated using a molar absorption coefficient of 155 mM^{-1} cm^{-1}.

The H_2O_2 assay was performed according to the method described by Velikova et al. (2000). Leaf tissue (0.1 g) was homogenized in 0.1% TCA and centrifuged. From the resulting supernatant, 1 mL was mixed with 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (KI). The absorbance was measured at 390 nm. The H_2O_2 content was quantified using a standard curve prepared with known H_2O_2 concentrations ranging from 0 to 100 µM.

Determination of enzymatic antioxidant activities

The plant leaf sample (0.5 g) was ground in liquid nitrogen and homogenized in 5 mL of extraction buffer (50 mM K_2HPO_4, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7 and 0.1% polyvinylpolypyrrolidone (PVPP)). The homogenate was centrifuged at 20000 g for 20 min at 4 °C, and the resulting supernatant was used for enzymatic activity analysis.

The SOD (EC 1.15.1.1) activity was determined using the method described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), 50 µL enzyme extract, and 2 µM riboflavin. An enzyme-free mixture (blank) was also prepared as a control. Upon illumination, riboflavin generated superoxide anions that reacted with NBT to produce formazan, a blue-colored complex. The reduction in formazan formation was proportional to the SOD content, and a 50% decrease in formazan formation was defined as one unit of SOD activity. The reaction was initiated and terminated by turning the light on and off. The mixture was exposed to white light at an intensity of 375 µmol m^{-2} s^{-1} for 10 min, after which absorbance at 560 nm was recorded. SOD activity was calculated using the following formula:

(Absorbance of blank - Absorbance of sample)/Absorbance of blank/(%50 × reaction mixture volume).

The CAT (EC 1.11.1.6) activity was determined according to Aebi (1984). 1 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 30 mM H_2O_2, and 20 µL enzyme extract was measured at 240 nm for 5 min. The activity was calculated using an extinction coefficient of 39.4 mM^{-1} cm^{-1} for H_2O_2.

The APX (EC 1.11.1.11) activity was measured based on the decrease in absorbance at 290 nm (Nakano and Asada 1987). The assay was performed using a 1 mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 250 µM ascorbate (ASC), 5 mM H_2O_2, and 20 µL enzyme extract. The activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} for ASC at 290 nm.

Protein content was determined in antioxidant enzyme extracts according to the method of Bradford (1976). A standard curve was generated using bovine serum albumin (BSA) standards in the range of 0-100 µg ml^{-1}. The absorbance of the complex formed between Coomassie Brilliant Blue G-250 dye and the protein was measured at 595 nm. Protein concentration was calculated in mg and used to express enzymatic activities.

Determination of antioxidant capacity

The powdered dry maize leaf sample 1 g was mixed with methanol (20 mL) and incubated at 25 ± 1 °C for 24 h to ensure optimal extraction of polar phytochemicals. The mixture was then passed through Whatman No. 1 filter paper to remove particulate matter. The filtrate was concentrated under reduced pressure using a rotary evaporator set at 40 °C until a semi-solid extract was obtained. The obtained crude extracts were stored at –20 °C in amber vials until further use. Antioxidant properties of the extracts were evaluated through the determination of total phenolic and flavonoid contents. In addition, their reducing capacity was assessed using FRAP and CUPRAC assays. To identify and quantify specific phytochemical constituents, high-performance liquid chromatography (HPLC) analysis was performed.

Determination of gas exchange parameters

The LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Inc., Lincoln, NE, USA) was used to measure the P_n, E, g_s, and C_i in *Zea mays*. Measurements were taken from the uppermost fully expanded third leaf of five plants per group, with ten readings recorded at 5-second intervals. Conditions included a light intensity of 250 µmol m^{-2} s^{-1} (Demiralay 2022), 25 °C block and leaf temperatures, and 60% relative humidity. CO_2 level was set to 400 µmol mol^{-1} and allowed to equilibrate for 30 min before measurement.

Statistical analysis

All experiments were conducted in five replicates. The results were calculated using the SPSS (version 27 for IBM SPSS Statistic) program with One-way ANOVA, and significant differences were determined by Duncan's multiple range tests, with $P < 0.05$ considered significant.

Results

As uptake

The As uptake did not differ significantly between the control and PG application. However, the As uptake was significantly increased compared to control ($P < 0.05$) with the application of As alone, showing a 1.5-fold increase. Meanwhile, the PG+As application significantly increased

($P < 0.05$) As uptake by 5-fold compared to the application of As alone (Tab. 1).

Tab. 1. Effects of exogenous pyrogallol (PG) and arsenic (As) alone or in combination (PG+As), on As uptake in maize seedlings. Results are shown as means ± standard errors of five replicates. Means followed by different letters are significantly different according to Duncan's test at $P < 0.05$.

Treatment groups	As content ($\mu g\ g^{-1}$)
Control	152.6 ± 22^{c}
5 mM PG	163.1 ± 10^{c}
100 μM As	237.5 ± 54^{b}
PG+As	1193 ± 76^{a}

Oxidative stress parameters

The TBARS content significantly increased ($P < 0.05$) with the application of PG or As, alone and the PG+As combination compared to the control group (Fig. 1A). The TBARS content with the application of PG or As alone was 1.3-fold higher than in the control group. Furthermore, the PG+As combination increased the TBARS content by 1.8-fold compared to the application of As alone.

The H_2O_2 content significantly increased ($P < 0.05$) with PG or As alone compared to the control (Fig. 1B), showing a 2.5-fold increase with the application of PG alone and a 2.3-fold increase with the application of As alone. Consistent with the lipid peroxidation results, the PG+As combination significantly increased ($P < 0.05$) the H_2O_2 content 1.5-fold compared to the application of As alone.

Activities of antioxidant enzymes

SOD, CAT and APX activities significantly changed ($P < 0.05$) with the application of PG or As alone and PG+As combination. SOD activity increased by 2.9-fold and 1.9-fold with the application of PG or As alone, respectively (Fig. 2A). However, the PG+As combination significantly decreased ($P < 0.05$) SOD activity 1.4-fold, 4.1-fold, and 2.7-fold, respectively, compared to the application of control or PG, or As alone.

CAT activity significantly increased ($P < 0.05$) with the application of PG or As alone, resulting in 2.4-fold and 2.2-fold increases, respectively, compared to the control (Fig. 2B). However, the PG+As combination caused a significant decrease ($P < 0.05$), reducing CAT activity 1.9-fold and 1.7-fold compared to the application of PG or As alone.

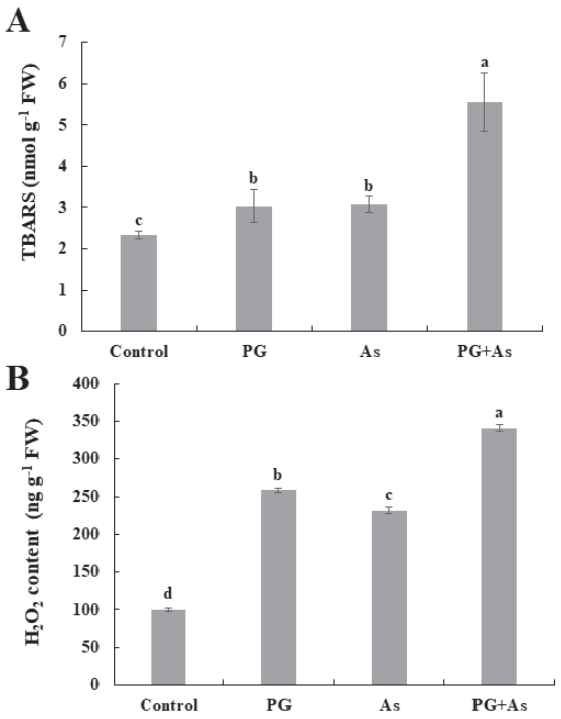

Fig. 1. Effects of exogenous pyrogallol (5 mM PG) and arsenic (100 μM As) alone or in combination (PG+As), on oxidative stress of maize seedlings: A – thiobarbituric acid reactive substances (TBARS) and B – hydrogen peroxide (H_2O_2) contents. Results are shown as means ± standard errors of five replicates. The difference between the means marked with the different letters on the columns is significant ($P < 0.05$).

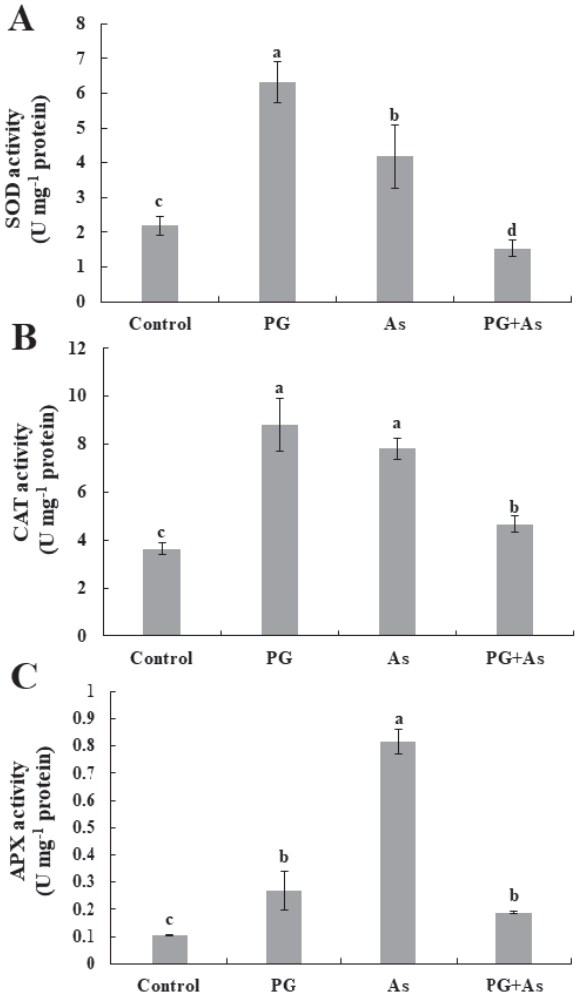

Fig. 2. Effects of exogenous pyrogallol (5 mM PG) and arsenic (100 μM As) alone, or in combination (PG+As) on enzyme activities of maize seedlings: A - superoxide dismutase (SOD), B - catalase (CAT), C - ascorbate peroxidase (APX). Results are shown as means ± standard errors of five replicates. The difference between the means marked with the different letters on the columns is significant ($P < 0.05$).

APX activity significantly increased ($P < 0.05$) with the application of PG or As alone, increasing 2.6-fold and 7.9-fold, respectively, compared to the control (Fig. 2C). However, the application of PG alone and PG+As combination caused a 3-fold and 4.3-fold significant reduction ($P < 0.05$) in APX activity compared to the application of As alone.

Antioxidant capacity

TPC significantly increased ($P < 0.05$) with the application of PG or As alone, showing a 1.4-fold increase, compared to the control. However, the PG+As combination caused a significant decrease ($P < 0.05$), reducing the TPC 1.3-fold, 1.8-fold, and 1.8-fold, compared to the application control or PG or As alone, respectively (Tab. 2).

There was no statistically significant difference in TFC between the application of control, PG, or As alone. However, the PG+As combination significantly decreased ($P < 0.05$) the TFC, 1.6-fold, compared to the application of As alone (Tab. 2).

The FRAP values significantly increased ($P < 0.05$) in the application of PG or As alone, with both increasing 1-fold, compared to the control. However, the PG+As combination significantly decreased ($P < 0.05$) the FRAP value, resulting in 1.4-fold, 1.5-fold and 1.5-fold reductions compared to the application of control or PG or As alone, respectively (Tab. 2).

The CUPRAC values significantly increased ($P < 0.05$) with the application of PG or As alone, reducing the CUPRAC 1.3-fold and 1.2-fold compared to the control. However, the PG+As combination significantly decreased ($P < 0.05$) the CUPRAC value, resulting in 1.9-fold, 2.5-fold and 2-fold decreases compared to the application of control or PG or As alone, respectively (Tab. 2).

Gas exchange parameters

Gas exchange parameters, including P_n, E, C_i, and g_s, significantly declined ($P < 0.05$) with the application of PG

Tab. 2. Effects of exogenous pyrogallol (PG) and arsenic (As) alone or in combination (PG+As) on total phenolics content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) in maize seedlings. Results are shown as means ± standard errors of five replicates. Means followed by different letters are significantly different according to Duncan's test at $P < 0.05$.

Treatments	Total phenolics content (mg GAE g^{-1} FW)	Total flavonoid content (mg QE g^{-1} FW)	FRAP (μmol Fe g^{-1} FW)	CUPRAC (mmol TEAC g^{-1} FW)
Control	3.36 ± 0.3^{b}	6.41 ± 0.25^{a}	4.72 ± 0.05^{b}	0.04 ± 0.006^{c}
5 mM PG	4.70 ± 0.3^{a}	6.43 ± 0.7^{a}	4.93 ± 0.05^{a}	0.06 ± 0.0003^{a}
100 μM As	4.60 ± 0.5^{a}	6.55 ± 0.17^{a}	4.88 ± 0.02^{a}	0.05 ± 0.001^{b}
PG+As	2.58 ± 0.09^{c}	4.11 ± 0.08^{b}	3.30 ± 0.09^{c}	0.02 ± 0.001^{d}

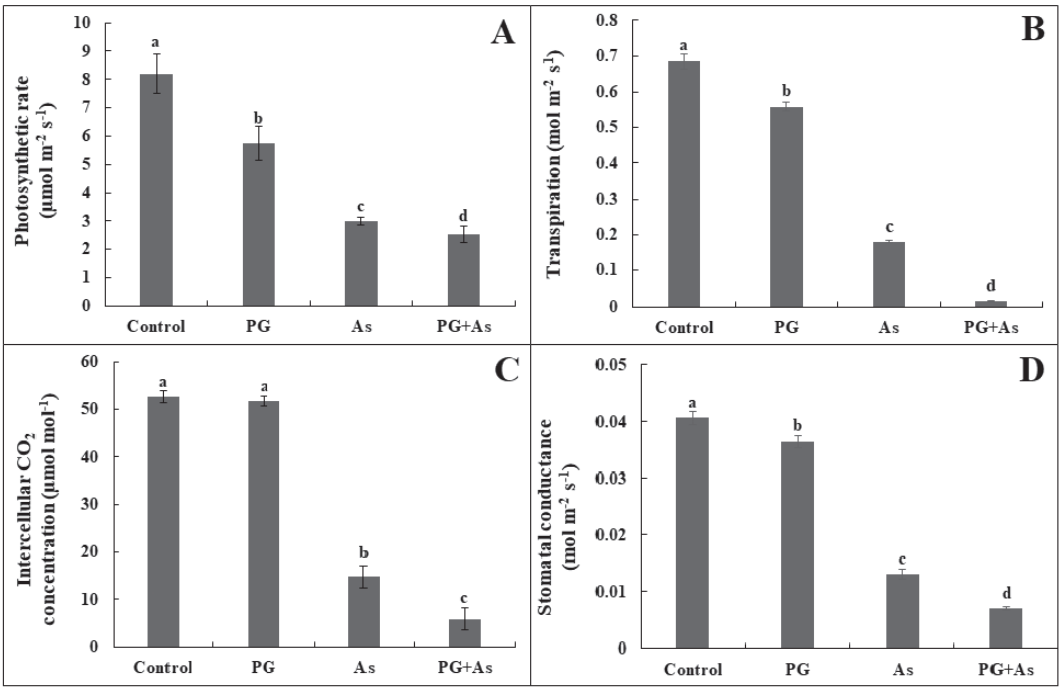

Fig. 3. Effects of exogenous pyrogallol (5 mM PG) and arsenic (100 μM As) alone, or in combination (PG+As) on photosynthetic gas exchange parameters of maize seedlings: A – photosynthetic rate (P_n), B – transpiration rate (E), C – intercellular CO_2 concentration (C_i), D – stomatal conductance (g_s). Results are shown as means ± standard errors of five replicates. The difference between the means marked with the different letters on the columns is significant ($P < 0.05$).

or As alone compared to the control. However, there was no statistically significant difference in the C_i value between the control and PG applications. In the PG application, the decreases were 1.4-fold, 1.2-fold, 1-fold, and 1.1-fold for P_n, E, C_i, and g_s, respectively. The As application significantly decreased these parameters ($P < 0.05$), resulting in 2.7-fold, 3.8-fold, 3.6-fold, and 3.1-fold reductions for P_n, E, C_i, and g_s, respectively. Furthermore, the PG+As combination significantly decreased ($P < 0.05$) all gas exchange parameters compared to the control, the application of PG, or As alone (Fig. 3). For example, compared to the control, the PG+As combination significantly decreased ($P < 0.05$) P_n, E, C_i, and g_s values 3.3-fold, 47.5-fold, 9-fold, and 5.8-fold, respectively.

Compared to the application of PG alone, the PG+As combination significantly decreased ($P < 0.05$) the P_n, E, C_i, and g_s values 2.3-fold, 38.7-fold, 8.8-fold, and 5.2-fold, respectively. Finally, compared to the application of As alone, the PG+As combination significantly decreased ($P < 0.05$) the P_n, E, C_i, and g^s values 1.2-fold, 1.3-fold, 2.5-fold, and 1.9-fold, respectively (Fig. 3).

Discussion

Allelochemicals possess a remarkable ability to disrupt plant physiological and biochemical processes, acting as both stressors and regulators in plant systems (Soares et al. 2012). Their toxic effects are highly dependent on key variables such as concentration, plant developmental stage, and environmental conditions (Won et al. 2013). By inducing oxidative stress, these compounds significantly inhibit plant growth and development (Bich and Kato-Noguchi 2012). This highlights the complicated and dynamic nature of plant responses to allelochemical-induced stress, presenting both challenges and opportunities for understanding plant-environment interactions.

Phenolic compounds have emerged as powerful modulators of plant stress responses, owing to their pronounced allelopathic activities and multifunctional roles in enhancing stress tolerance. With over 48,000 structurally diverse molecules, polyphenols are synthesized via the shikimic acid pathway and serve as crucial secondary metabolites in plants (Stiller et al. 2021). Their redox properties allow them to function as potent antioxidants, electron donors, and radical scavengers, positioning them as central agents in mitigating oxidative stress and sustaining plant health under adverse conditions (Zagoskina et al. 2023). Especially polyphenols are recognized for their strong antioxidant properties, primarily driven by their redox potential, which allows them to function as electron donors, singlet oxygen quenchers, and radical scavengers. While these properties highlight their protective roles in plants, phenolic compounds also exhibit notable allelopathic effects in plant-plant and plant-environment interactions (Pisoschi and Pop 2015). Specifically, phenolic acids such as GA, p-hydroxybenzoic acid, CfA, cinnamic acid, syringic acid, ferulic acid, SA, and vanillic acid are known to play complex roles in regulating plant responses to abiotic stress (Mughal et al. 2024).

Many studies have highlighted PG's allelopathic activity, including its inhibitory effects on seed germination (Sang et al. 2024), specific enzyme activities, and microbial growth (Yan et al. 2010, Li et al. 2010). However, the specific mechanisms linking PG to its regulatory role in stress tolerance remain unclear. To address this knowledge gap, this study investigated the effects of PG on maize seedlings exposed to As stress. The primary aim was to determine whether PG contributes to enhanced stress tolerance and to elucidate its potential mechanisms. By focusing on the interaction between PG and As, this research has sought to provide a deeper understanding of PG's allelopathic and stress-regulatory properties.

Our findings revealed that a 5 mM PG application resulted in a dramatic 5-fold increase in As accumulation in maize tissues compared to As alone (Tab. 1). This substantial increase is indicative of severe metabolic disruptions, consistent with earlier studies that highlight As's harmful effects on plant productivity (Wu et al. 2011). The allelopathic activity of PG, previously reported to delay germination and retard development in *Lolium perenne* (Sang et al. 2024) likely aggravates developmental toxicity under As stress. The underlying mechanism of this interaction likely originates from pyrogallol's auto-oxidation, leading to an increased production of H_2O_2 and ROS (Inui et al. 2004, Upadhyay et al. 2010). The production of ROS damages the membrane, increasing its permeability and enabling more efficient As uptake. Moreover, the catalytic interactions with metal ions amplify oxidative stress, forming a toxic feedback loop that severely damages plant cells and physiological balance.

Phenolic compounds are potent inducers of oxidative stress in plants. For example, cinnamic acid has been shown to amplify oxidative damage in cucumber by inhibiting antioxidant enzymes, leading to excessive ROS production and cellular damage (Ye et al. 2006, Chai et al. 2013). Similarly, PG, despite its beneficial properties, induces oxidative stress through the generation of free radicals, which is the central mechanism of its toxicity (Upadhyay et al. 2010). Our results demonstrated that high concentrations of PG (5 mM) significantly elevated H_2O_2 and TBARS levels compared to application of PG or As alone, indicating that the PG+As combination triggers an excessive ROS burst (Fig. 1). This is consistent with the previous research findings that high concentrations of PG and related organic acids, such as syringic acid and vanillic acid, increased TBARS levels in *Pinus koraiensis* Sieb. et Zucc. and *L. perenne* L. seedlings (Liang et al. 2021, Sang et al., 2024). The synergistic toxicity of PG+As likely aggravates lipid peroxidation, further damaging cellular structures and inducing oxidative stress in maize seedlings.

The sensitivity of protective enzymes to allelochemicals is highly variable, depending on their concentration and plant species, often leading to disruptions in the precise balance between antioxidant defenses and ROS (Araniti et al. 2018, Šoln et al. 2022). Phenolic compounds, including PG, exhibit strong inhibitory effects on key enzyme activities,

interfering with metabolic pathways and amplifying oxidative stress. For instance, chlorogenic acid, CfA, and cinnamic acid derivatives target critical enzymes such as phosphorylase and ATPase, while tannic acid inhibits essential antioxidant enzymes like CAT and APX (Rice 1979, Batish et al. 2008). Our study revealed that while 5 mM PG application increased the activities of antioxidant enzymes such as SOD, CAT, and APX in maize seedlings, its combination with As resulted in a marked suppression of these enzymes (Fig. 2). This suppression highlights the toxic synergism of PG+As, which overwhelms the antioxidant defense system, leaving the plant vulnerable to oxidative damage.

Phenolic compounds source their antioxidant capacity from their remarkable ability to neutralize ROS (Zheng and Wang 2001). Notably, this potential can be amplified through synergistic interactions with other phytochemicals, further enhancing their protective effects (Plaza et al. 2011). Our findings revealed that while PG or As applied alone significantly elevated the TPC, FRAP, and CUPRAC values in maize seedlings, the PG+As combination caused a dramatic reduction in these antioxidant parameters. This indicates that 5 mM PG application partially activates the antioxidant defense system, helping to maintain redox balance and mitigate oxidative damage. However, the PG+As combination created a toxic synergistic effect by drastically increasing ROS levels, overwhelming the plant's antioxidant capacity and causing severe suppression of its defense mechanisms (Tab. 2). These results are consistent with the findings of Tian and Li (2018), who demonstrated that phenolic compounds under stress conditions diminished antioxidant capacity in maize seedlings, reinforcing the potential risks associated with their overuse.

Photosynthesis is an indispensable physiological process vital for plant growth and survival. However, As toxicity severely compromises this process by damaging chloroplast structure, disrupting the synthesis of photosynthetic pigments, and significantly impairing the activity of photosystem I (PSI) and photosystem II (PSII). This disruption has been well documented, with As stress being shown to inhibit chlorophyll biosynthesis in various plants, including *Zea mays* L., *Trifolium pratense* L., and *Lactuca sativa* L. (Suneja 2014, Emamverdian et al. 2015, Nabi et al. 2019). Beyond As stress, phenolic allelochemicals, such as grandinol, homograndinol, CfA, coumaric acid, ferulic acid, cinnamic acid, and vanillic acid, have also demonstrated severe inhibitory effects on photosynthesis. These compounds reduce chlorophyll production and significantly lower the photosynthetic rate, ultimately stunting plant growth (Patterson 1981, Yoshida et al. 1988). In our study, both PG and As independently caused substantial reductions in key photosynthetic parameters, including P_n, E, C_i, and g_s. Remarkably, their combined application exacerbated these effects, inducing a dramatic decline in gas exchange (Fig. 3). This highlights the synergistic toxicity of PG and As, which not only disrupts core photosynthetic processes but also imposes severe metabolic constraints on maize seedlings. These findings are consistent with those of Patterson (1981), who observed that 10-30 µmol L^{-1} concentrations of phenolic acids like CfA, coumaric, ferulic, cinnamic, and vanillic acids suppressed photosynthesis and reduced chlorophyll content in soybean. The PG+As combined likely suppressed the antioxidant defense system while disrupting stomatal regulation, exacerbating water imbalance and photosynthetic inefficiency.

PG dramatically disrupts cellular redox balance under As stress, significantly impairing the fundamental antioxidant defense system and consequently triggering excessive production of ROS, intense TBARS, and cellular damage. Moreover, the potent allelopathic activity of PG synergistically interacts with As to markedly increase As uptake, leading to striking declines in critical photosynthetic parameters such as P_n, g_s, E, and C_i. This synergistic interaction clearly demonstrates that PG not only severely compromises the defense mechanisms against As stress but also undermines the overall physiological and biochemical integrity of the plants.

Conclusion

In sum, our results reveal a toxic synergy between PG and As in maize seedlings. While PG alone induces modest activation of antioxidant defenses, its combination with As leads to an increase in As accumulation up to fivefold accompanied by a burst of ROS and severe lipid peroxidation. This dual effect results in disruption of cellular redox homeostasis, significant suppression of key antioxidant enzymes, and impairment of photosynthetic efficiency, ultimately compromising both cellular integrity and metabolic function. These findings illustrate the complex, double-edged nature of phenolic allelochemicals, which can act both as mitigators and as amplifiers of stress depending on environmental conditions. This study highlights the need to carefully manage phenolic compounds in ecosystems exposed to HM pollution and the importance of precise agricultural practices to ensure sustainable crop productivity and environmental health.

Author contribution statement

Altuntaş Cansu, Aksu Kalmuk Nurşen and Gümrükçüoğlu Abidin designed the present study and have supervised this work. Altuntaş Cansu, Aksu Kalmuk Nurşen and Gümrükçüoğlu Abidin performed the experiments. The analysis and interpretation of the results were carried out by Altuntaş Cansu, Aksu Kalmuk Nurşen and Gümrükçüoğlu Abidin. The drafting of the manuscript was carried out by Altuntaş Cansu with the assistance of Aksu Kalmuk Nurşen and Gümrükçüoğlu Abidin. All the authors contributed and reviewed the results and approved the final manuscript.

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