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Physiological and biochemical bases of AMF-mediated antimony stress tolerance in *Linum usitatissimum*: enhancing growth, phytochemical production, and oxidative damage resilience

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Abstract: Antimony (Sb) pollution from industrial activities poses a severe global threat, particularly impacting valuable medicinal crops like linseed, which are highly sensitive to heavy metals. This study reveals the remarkable potential of arbuscular mycorrhizal fungi (AMF) as a sustainable solution to this challenge. Our research demonstrates that while Sb stress significantly impairs linseed growth and photosynthesis, it also triggers oxidative damage. AMF improved photosynthetic performance and water status, and notably enhanced the biosynthesis of crucial

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phytochemicals like phenolics, flavonoids, and citric acid. These compounds are vital for both plant defence and human health. Furthermore, AMF promoted the accumulation of essential detoxifying agents, leading to a better redox balance and significantly reducing Sb uptake and translocation by 47%. This dual action not only bolsters the plant's tolerance to Sb but also enhances its medicinal value by boosting health-promoting bioactive metabolites. These promising findings underscore AMF's dual role: a powerful tool for phytoremediation and a natural enhancer of phytochemical quality. Arbuscular mycorrhizal fungi provide a sustainable, nature-inspired approach to safely cultivate medicinal plants in environments contaminated with heavy metals, underscoring the vital role of plant-microbe interactions in alleviating environmental stresses.

Keywords: medicinal plants; symbiosis; heavy metal; redox homeostasis; detoxification; physiological parameters

Heavy metal contamination, such as antimony (Sb), significantly affects agriculture, which lowers crop quality and productivity (Periferakis et al. 2022). Rarely, heavy metals are not considered vital nutrients, and they were recently added to the list of critical raw materials (CRM) (Tzamos et al. 2020). It also shares biochemical properties with arsenic and bismuth due to their placement in the same group on the periodic table. As metalloids, they exhibit multiple oxidation states (Adeyem et al. 2020). It naturally occurs in soils, sedimentary rocks, and water, with concentrations ranging from 0.3–8.6 mg/kg, 0.15–2 mg/kg, and 1 µg/mL, respectively (Filella et al. 2002). Although Sb is naturally present at low concentrations, the buildup of this pollutant in soil, water, and plants results from industrial operations and intensive farming methods (Nishad and Bhaskarapillai 2021). For example, Sb's presence in soil due to human activities like mining, smelting, and the disposal of electronic waste has led to an increasing worry about Sb toxicity in plants (Duan et al. 2023).

Due to the similar physicochemical properties of heavy metals and essential microelements, plasma membrane transporters in plant roots facilitate their uptake (Al-Raddadi et al. 2025). In this regard, Sb is easily taken up by plants, harming their growth and health (Cai et al. 2016). Consequently, accumulation can harm plant health, including reduced growth, decreased photosynthetic activity, and impaired nutrient uptake (Zhou et al. 2022). Sb uptake hindered plant growth by reducing photosynthesis and nutrient uptake and altering the synthesis and production of metabolites (Zhou et al. 2022). Exposure to Sb significantly reduced plants' net photosynthetic rate (P_n), leaf pigment levels, and the quantum yield of PSII electron transfer (Zhou et al. 2018). High ted Sb levels in plant cells interact with the sulfhydryl groups of chloroplast proteins, disrupting chloroplast structure and function (Luo et al. 2021). At oxidative stress levels, the high uptake of Sb by plants also prompts oxidative shock, resulting from the overproduction of reactive oxygen species (ROS) (Peško et al. 2016), which damages plant lipids, proteins, and membranes (Ortega et al. 2017). Consequently, an increase in lipid peroxidation levels was linked to the rise in ROS levels by Sb, indicating the onset of oxidative damage. In fact, the enzymatic antioxidant systems behave differently when Sb builds up. In fact, the presence of Sb in plant leaves changed the levels of various antioxidant enzymes, including ascorbate (ASC), peroxidase (APX), glutathione (GSH), reductase (GR), catalase (CAT), peroxidase (POX), dehydro-ascorbate reductase (DHAR) and glutathione reductase (GPX) (Khamis et al. 2023).

Sustainable agriculture requires methods to lessen the effects of Sb toxicity in plants (Haider et al. 2024). Various techniques were employed to do both the immobilisation and mobilisation of Sb. Compared to alternative techniques, the phytoremediation method employing the growth-promoting organisms was the most environmentally friendly (Gao et al. 2011). Among them, arbuscular mycorrhizal fungi (AMF) can remediate heavy metal toxicity. Over the past two decades, research has explored the possibility of using AMF in heavy metal remediation (Alotaibi et al. 2021). AMF are crucial for enhancing plant growth, photosynthesis, and metabolism (Albqmi et al. 2024). They significantly contribute to heavy metal toxicity, a rising environmental concern (Wei et al. 2016, Albqmi et al. 2024). Specifically, AMF counteract Sb toxicity by reducing harmful reactive oxygen species formation (Zhou et al. 2022, Albqmi et al. 2024) and preserving vital photosynthetic functions (Zhou et al. 2022, Albqmi et al. 2024). This ROS detoxification mechanism was linked with increased antioxidant defence system by Sb (Zhou et al. 2022, Albqmi et al. 2024). This safeguards the

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plant's energy production. Additionally, AMF can alter heavy metals such as Sb uptake and distribution by immobilising it in fungal structures, complexing it in the rhizosphere, or influencing its bioavailability through root exudates (Zhou et al. 2022). AMF's effectiveness against Sb stress is influenced by specific plant-fungus combinations and Sb concentrations (Zhou et al. 2018). Furthermore, it has been observed that symbiotic relationships between AMF and plants might develop in situations where Sb exposure is excessive. AMF encourages plant growth by enhancing the uptake of essential mineral elements, altering the root structure of the host plant, and boosting resistance to heavy metals (Zhou et al. 2022). AMF improved plant growth, photosynthesis rate and metabolism (Albqmi et al. 2024). It has been observed that AMF enhances the antioxidant defence mechanisms of host plants growing under Sb stress. The colonisation by AMF triggers modifications in the plants' antioxidant systems, potentially mitigating the oxidative stress induced by Sb exposure (Mi et al. 2023). This was shown by the increased accumulation of antioxidant metabolites, including phenols and flavonoids, as well as the activity of the enzymes superoxide dismutase, APX, and CAT in the roots and shoots of mycorrhizal-treated plants (Albqmi et al. 2024). This increase in antioxidant activities may also lessen the stress that plants experience when they react to oxidative damage caused by pollutants by increasing their production of ROS (Kaur et al. 2018). AMF can affect the bioavailability of a wide range of elements and also sequester heavy metals such as lead, chromium, cadmium, nickel, and zinc through the release of an AMF protein (glomalin) in root tissues (Zhou et al. 2022). Strong evidence also suggests that AMF can promote the development and uptake of heavy metals by host plants (Jiang et al. 2021).

Flax (*Linum usitatissimum* L.) is a flexible crop with many uses. Though it originated in southwest Asia and the Mediterranean region, its ability to adapt to various environmental conditions has made it easier to cultivate throughout the Middle East, India, Canada, and several European nations (Kiryluk and Kostecka 2020). Linum is the biggest genus in the subfamily Linoideae, including over 300 species. It is used for its fibre, oil, and seeds that can be harvested. Flax fibres are widely employed in many industrial purposes, such as making bedsheets, dressing linens, twine, and ropes (Stavropoulos et al. 2023). Moreover, these fibres are also essential to the paper industry since they are used, most famously, in creating banknotes (Adorian et al. 2022). It is important to note that flax seeds are a rich source of nutrients because they contain bioactive components (Adorian et al. 2022).

Despite the substantial work done on the toxicity of several heavy metals on plants such as linseed (Zainab et al. 2020), there is a lack of studies investigating the impact of Sb on linseed growth and performance, particularly regarding its physiological and biochemical analyses. Given that AMF have proven to increase resistance to various abiotic stressors, such as salt in linseed (Neetu et al. 2012). The objective of the current study was to explore the potential of AMF inoculation as a promising technique to enhance the physiological and biochemical responses of linseed plants exposed to Sb contamination.

Our research significantly advances understanding of plant-microbe interactions under stress by offering a uniquely comprehensive and novel approach. Unlike previous studies that often focus on limited parameters, our objectives integrate an unparalleled range of detailed measurements, encompassing Sb uptake, growing substrate pH, citric and phenolic acid levels, and multiple oxidative stress markers. Crucially, we move beyond general detoxification indicators to specifically investigate the mechanisms of improved resilience through key enzymes and compounds like metallothionein, glutathione (GSH), and GSH-s-transferase (GST). This holistic approach aims to precisely unveil how AMF enhances plant resilience by promoting nutrient uptake, reducing oxidative stress, and boosting overall plant health under challenging conditions.

MATERIAL AND METHODS

Growing substrate treatments and plant cultivation. A growing substrate (a mix of 30% Tref EGO substrates (Moerdijk, the Netherlands) and sterilised 70% sand). There were two groups of growing substrates. The first group of growing substrates was inoculated with *Rhizophagus irregularis* MUCL 41833 (65 spores/g of wet growing substrate), and the second group was uninoculated. The initial growing substrate contained 13.1 mg/g of organic carbon (C), 16.3 mg/g of nitrate-N, and 9.7 mg/g of P/g. The growing substrate water content was 47%, growing substrate pH was 7.56, and electrical conductivity (EC) was 3.4 dS/m. For heavy metal treatment, KSb (OH) was applied (1 500 mg/kg growing substrate) to one set of the growing substrate, while another set (control) received

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0 mg/kg growing substrate of KSb (OH). *Linum usitatissimum* seeds were obtained from the Agriculture Research Centre in Giza, Egypt. They were surface-sterilised by immersion in 5% sodium hypochlorite (household bleach) for 10–15 min. They were then thoroughly rinsed [5 times] with sterile distilled water to remove all traces of sterilising agents before use. Then they were sown in pots (12 cm in diameter × 20 cm in depth) – five pots (each a biological replicate) per treatment. Plants were grown under controlled growth conditions in a climate-controlled chamber (24/18 °C with a 16/8 h day/night photoperiod and 290 µmol/m^2/s photosynthetically active radiation (PAR) and 60% humidity. After 11 weeks, the rhizosphere of the growing substrate was collected, and plants and seeds were harvested and immediately stored at –80 °C

Mycorrhizal parameters. Plant roots were subsequently stained with trypan blue in lactoglycerol (0.05%) to determine the colonisation rate. Hyphal length was measured (Andrade et al. 1997), and the abundance of arbuscules was quantified by the number of arbuscules per cm root.

Growth and photosynthetic parameters. Net photosynthetic rate (A) and stomatal conductance (g_s) were measured using a LI-COR LI-6400XT system (Lincoln, USA). Measurements were taken on fully expanded leaves between [e.g., 09:00 and 12:00] under controlled chamber conditions (e.g., 800 µmol/m^2/s PPFD, 400 µmol/mol CO_2, 25 °C, 60–70% RH). Readings were recorded once stable, with 3–5 biological replicates per treatment. Photosynthetic pigments and carotenoids, chlorophyll *a*, *b*, and total carotenoids, were quantified spectrophotometrically (Wellburn 1994). Fresh leaf samples (e.g., 0.1 g) were extracted in (e.g., 80% acetone) in the dark at 4 °C until colourless (Alotaibi et al. 2021). Extracts were centrifuged, and absorbance was measured at 663 nm (chl *a*), 646 nm (chl *b*), and 470 nm (carotenoids). Pigment concentrations were calculated and expressed as mg/g FW (fresh weight).

Oxidative stress. Malondialdehyde (MDA) content, a widely used indicator of lipid peroxidation, was quantified to assess oxidative stress comprehensively (Taulavuori et al. 2001). Plant samples were extracted in 0.1% trichloroacetic acid (TCA), and the MDA-TBA (thiobarbituric acid) adduct was measured spectrophotometrically at 532 nm (Versieren et al. 2017). Complementing this, hydrogen peroxide (H_2O_2) content, a crucial ROS signalling molecule, was determined using the sensitive xylenol orange technique after initial extraction in trichloroacetic acid (Rahman et al. 2023).

Furthermore, the activities of key enzymes involved in photorespiration were measured. Hydroxypyruvate reductase (HDR) activity was estimated by monitoring the oxidation of NADH at 340 nm, a direct measure of its catalytic function in the photorespiratory cycle. Similarly, glycolate oxidase (GO) activity, which catalyses the initial step of photorespiration, was determined by measuring the formation of a glyoxylate complex with phenylhydrazine (Novitskaya et al. 2002). This method quantifies the product of GO activity.

Finally, the glycine/serine (G/S) ratio, a critical metabolic indicator reflecting photorespiratory flux and carbon partitioning, was precisely determined. Serine and glycine were separated using a Waters Acquity UPLC-tqd system, employing a BEH amide 2.150 column for robust chromatographic resolution and tandem mass spectrometry for accurate quantification.

Defense system

Antioxidant metabolites. Ferric reducing/antioxidant power (FRAP) was applied to measure antioxidant capacity using Trolox as the standard. Polyphenols and flavonoids were extracted in 0.3 g FW and then were determined using a Folin-Ciocalteu assay according to Zhang et al. (2006) and Hozzein et al. (2019) and the $AlCl_3$ method, respectively. Tocopherols were extracted in hexane using dimethyl tocol as internal standard, and measured by HPLC (Shimadzu, Hertogenbosch, the Netherlands). HPLC analysis detected reduced GSH and ASC (AbdElgawad et al. 2022).

Antioxidant enzymes. The buffer consisted of 0.05 mol/L potassium phosphate buffer (pH 7.0, prepared from KH_2PO_4 and KH_2PO_4) plus 10% (*w*/*v*) PVPP, and phenylmethylsulfonyl fluoride (0.0501 mol/L) and ASC (0.0501 mol/L) – the activities of CAT, POX, APX, GPX, GR, DHAR, and MDHAR. By estimating the inhibition of the reduction of nitro-blue tetrazolium at 560 nm, SOD activity was calculated (Dhindsa et al. 1981, Zinta et al. 2016, AbdElgawad et al. 2022, El-Sawah et al. 2023). The assessment of pyrogallol's oxidation leads to measuring POX's activity. Besides, the CAT activity was measured by the reduction of H_2O_2 at 240 nm. APX, MDHAR, DHAR, and GR activities were evaluated (Naudts et al. 2013, Hadwan 2018).

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Detoxification-related parameters. Glutathione S-transferase (GST), metallothionein (MTC), and phytochelatins (PCs) were measured. GST activity was spectrophotometrically determined by monitoring the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm (Poimenova et al. 2024). Briefly, enzyme extracts were prepared from plant tissue by homogenisation in a 50 mmol/L potassium phosphate buffer (pH 7.0). The increase in absorbance, indicative of the formation of the S-2,4-dinitrophenylglutathione conjugate, was continuously measured, and activity was expressed as nmol CDNB-GSH conjugate formed per minute per milligram of protein. MTC levels were quantified electrochemically using the differential pulse voltammetry (DPV) technique (Poimenova et al. 2024). Plant samples were extracted and subjected to a heat denaturation step to remove interfering proteins. The resulting MTC-rich supernatant was then analysed *via* DPV, with the peak current corresponding to the sulfhydryl groups within the protein used for quantification against a standard curve. Results were expressed as μg MTC per gram fresh weight. PCs levels, crucial heavy metal chelators, were measured calorimetrically at 412 nm using Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB). This method quantifies free thiols present in PCs. Plant samples were extracted in metaphosphoric acid to preserve thiol integrity, and the reaction with DTNB was immediately measured spectrophotometrically, with results expressed as μmol PCs equivalents per gram fresh weight.

Statistical analysis. Data from five biological replicates are presented as means ± standard error (SE). Statistical comparisons of treatment means were performed using Duncan's multiple range test ($P \leq 0.05$) *via* SPSS software (version 25.0, New York, USA). Biplots, generated with Seaborn (Stanford University, USA) and Matplotlib (Pennsylvania, USA), visualised parameter loadings and treatment centroids, highlighting multivariate relationships among the measured traits.

RESULTS

AMF related parameters

Significant decreases in AMF colonisation, hyphal length, and arbuscule count were reported (Table 1). Under Sb stress, colonisation, hyphal length, and arbuscule count decreased by 50, 40, and 4%, respectively, compared to the AMF treatment alone (Table 1).

Antimony uptake

The result showed that Sb treatment increased the Sb concentration in the plants' shoots (Table 2). However, when combined with AMF treatment, a significant decrease in Sb concentration was observed, with a reduction rate of 47% in the shoots of linseed compared to the levels seen with Sb treatment alone.

Growing substrate phenolics and organic acids

Growing substrates treated with AMF and Sb, either separately or in combination, showed considerably higher levels of total phenols and citric acid than the control. The total phenols and citric acid were detected in growing substrate contaminated with Sb and treated with AMF, showing noticeably higher quantities than in the control. In response to the Sb concentration in the growing substrate, the total phenol content rose by 45%, demonstrating a greater level by 53% when Sb was combined with AMF. Likewise, citric acid concentrations increased by 43% in response to Sb concentrations in the growing substrate, indicating a higher level by 53% when Sb was coupled with AMF (Table 2).

Table 1. Effects of arbuscular mycorrhizal fungi (AMF) and antimony (Sb, 1 500 mg/kg growing substrate) on AMF colonisation, hyphal length, and number of their arbuscules

	–AMF		+AMF	
	control	Sb	control	Sb
Colonisation (% root)	0	0	55.67 ± 6.55^a	27.50 ± 1.69^b
Hyphal length (cm)	0	0	20.76 ± 2.05^a	12.39 ± 1.88^b
Number of arbuscules (No./cm root)	0	0	3.60 ± 0.59^a	3.44 ± 0.40^a
Colonisation (% root)	0	0	55.67 ± 6.55^a	27.50 ± 1.69^b

Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

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Table 2. Effects of arbuscular mycorrhizal fungi (AMF) and antimony (Sb, 1 500 mg/kg growing substrate) on the levels of Sb in the plant and growing substrate, as well as phenolic and citric acid in the growing substrate

	–AMF		+AMF	
	control	Sb	control	Sb
Antimony in plant (ug/g dry weight)	0.00 ± 0.00^{c}	566 ± 128^{a}	0.00 ± 0.00^{c}	566 ± 128^{a}
Antimony (mg/kg growing substrate)	0.00 ± 0.00^{c}	61.4 ± 0.17^{b}	0.00 ± 0.00^{c}	61.4 ± 0.17^{b}
Phenol (mg/kg growing substrate)	41.9 ± 1.90^{d}	76.9 ± 11.27^{b}	41.9 ± 1.90^{d}	76.9 ± 11.27^{b}
Citric/growing substrate (mg/kg growing substrate)	2.44 ± 0.32^{c}	4.20 ± 0.61^{b}	2.44 ± 0.32^{c}	4.20 ± 0.61^{b}

Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

Plant growth and photosynthesis-related parameters

The application of AMF to linseed plants did not significantly change ($P < 0.05$) the fresh and dry biomass contents. On the other hand, compared to the control, the Sb treatment dramatically decreased plant biomass, resulting in a 44% and 73% decrease in fresh and dry weights, respectively. Nevertheless, when AMF was given to Sb stress plants, the biomass decreases were only 25% and 37%, respectively (Figure 1).

Several photosynthesis parameters were also evaluated under AMF, Sb, and their combined application (Figures 2 and 3). Sb treatment alone negatively reduced photosynthetic rate, chlorophyll *a*, chl *b*, total *chl* and CF by 30, 37, 17, 51 and 28%, respectively, compared to the control. Compared to the control, there was a significant decrease in chlorophyll *b* and a considerable rise in carotenoids. However, the AMF treatment alone did not result in any appreciable changes observed in CF, gas exchange, or chl *a*. When compared to Sb treatment alone, the combination application of AMF and Sb did not affect gas exchange or CF. Nevertheless, the quantities of chlorophyll *a* and *b* were further lowered by this combination than by the drops observed with the separate treatments. Notably, the combined treatment markedly increased photosynthetic rates and carotenoids.

Photorespiration

Indeed, the photorespiration is a significant source of reactive oxygen species (Figure 4), particularly in stressful situations, many modifications to two essential photorespiration enzymes, namely HPR and GO show that the activities of both enzymes were enhanced by 87% and 67% for GO and HPR, respectively in linseed plants exposed to Sb treatment. This was significantly reduced to 74% in AMF and Sb-treated linseed plants for GO. This, on the other hand, was raised to 83% in HPR. AMF alone induces the activities of HPR and GO by only 40%

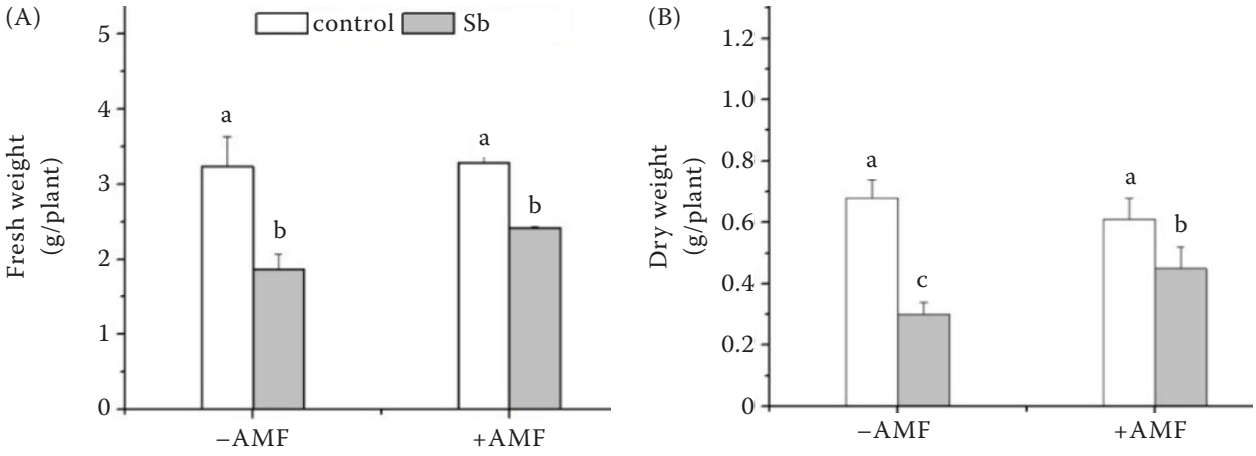

Figure 1. Effects of arbuscular mycorrhizal fungi (AMF) and antimony (Sb) on fresh and dry weight in AMF-treated and non-treated linseed plants under varying Sb stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

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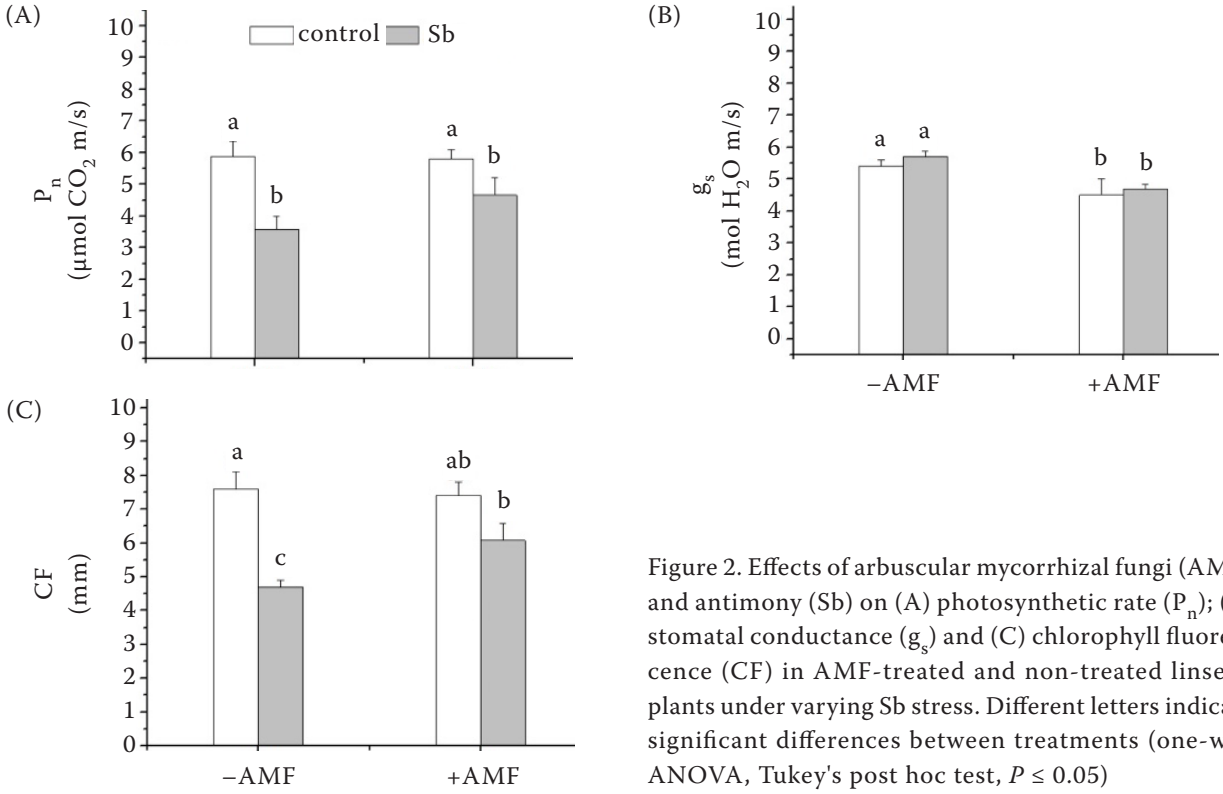

Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) and antimony (Sb) on (A) photosynthetic rate (P_n); (B) stomatal conductance (g_s) and (C) chlorophyll fluorescence (CF) in AMF-treated and non-treated linseed plants under varying Sb stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

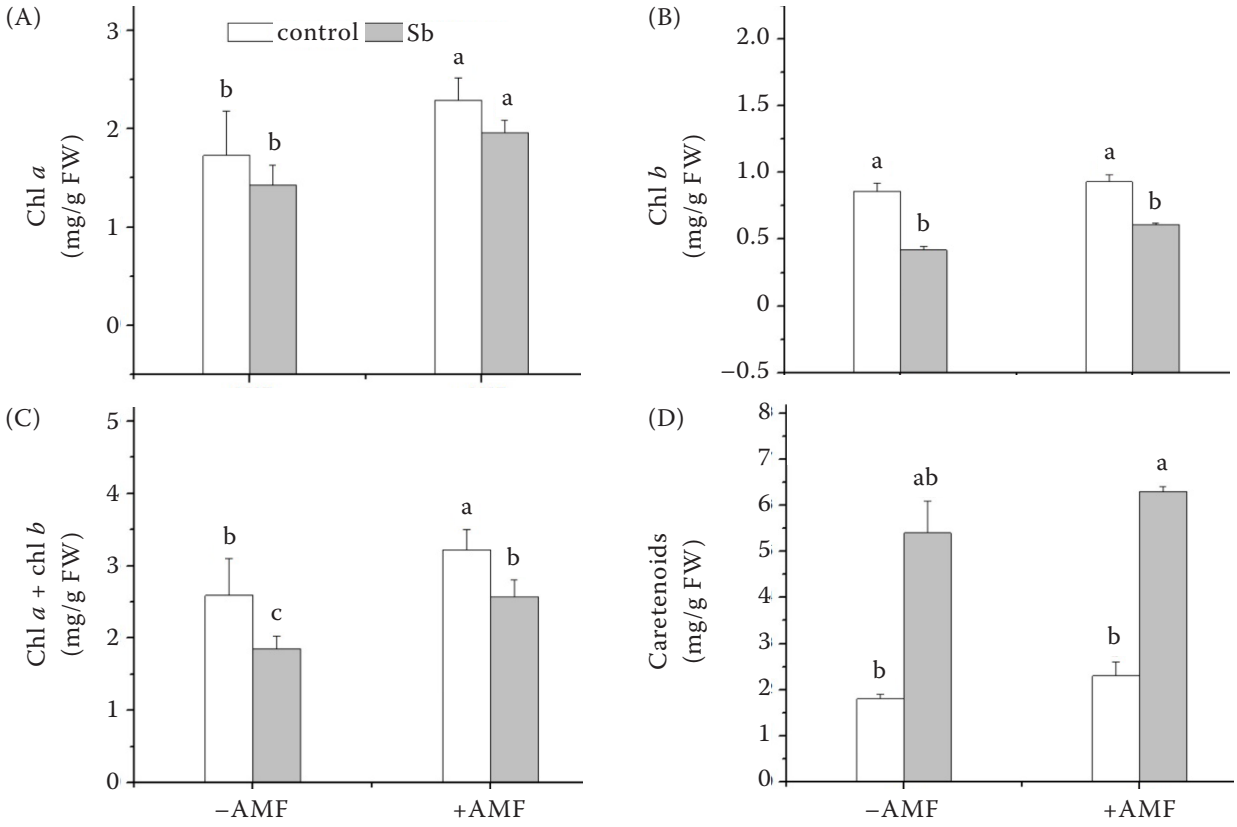

Figure 3. Leaf pigments in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under varying antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$). FW – fresh weight

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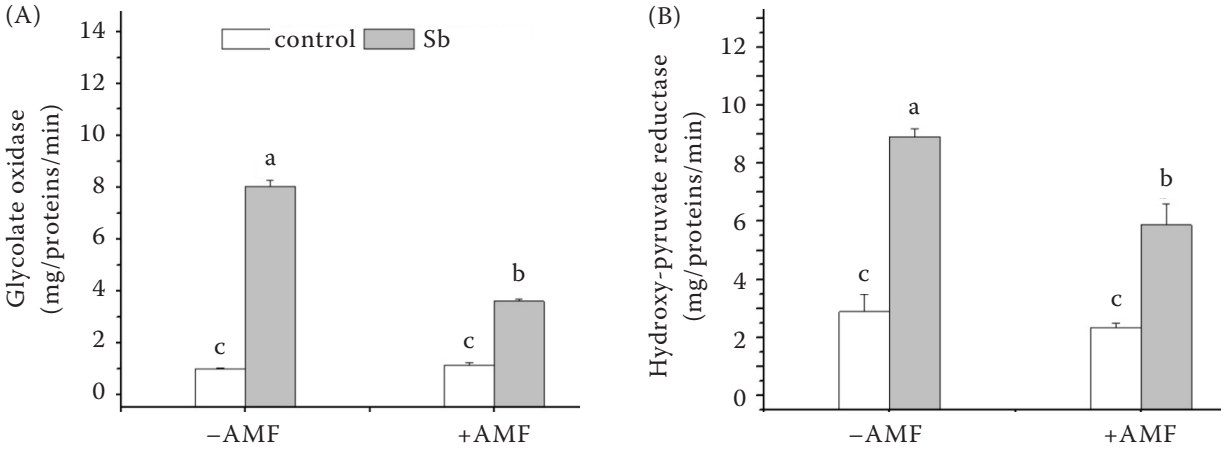

Figure 4. Photorespiration related enzymes (glycolate oxidase and hydroxy-pyruvate reductase) activity in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under varying antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

in GO and 2% in HPR. Likewise, the glycine/serine ratio increased by 44% in plants exposed to Sb treatment and 23% when Sb was combined with AMF.

Oxidative damage

Figures 5 and 6 illustrate how oxidative stress parameters vary in response to Sb, AMF, and their combination treatments. SB treatment induced accumulation of higher concentrations of H_2O_2, MDA and PO in shoots by 61, 0.61 and 0.29%, respectively compared to control plants. Moreover, the same treatment led to a significant increase in glycine/serine ratio. When Sb and AMF were coupled, PO levels remained unchanged, but H_2O_2, MDA, and Gly/ser ratio levels significantly decreased in comparison to Sb alone.

Redox homeostasis

Antioxidant metabolites. The contents of total phenols, flavonoids, total antioxidant activity (TAC) and tocopherols were all altered by the Sb, AMF and combined treatments in the leaves (TAC) and tocopherols by 46, 54, 82 and 63%, respectively, compared to the control (Figure 7). No significant change was recorded in TAC and total tocopherols in the plant inoculated with AMF, except in total flavonoids and phenols, which rose by 29% and 39%, respectively. When AMF-inoculated plants were subjected to Sb doses, the total phenols, flavonoids, total antioxidant activity, and tocopherols reached their highest content compared to other treatments.

Enzymatic antioxidants and ascorbate-glutathione cycle enzymes. To investigate the effects of AMF and Sb on linseed plants in further detail, several metabolites, including non-enzymatic (AsA and GSH) and enzymatic antioxidant (DHAR, MDHAR, GR, APX, GPX, SOD, CAT, and POX), were examined (Figures 8–11). A species change could be observed in GR, DHAR, MDHAR, GR, APX, and GPX activity. When linseed plants were exposed to Sb treatment, a significant increase was recorded by 34, 64, 73, 81, and 58% in DHAR, MDHAR, APX, GPX and GR activity, relative to their respective controls. This increase declined when the Sb treatment was combined with AMF inoculation. Likewise, the content of GSH and ASC

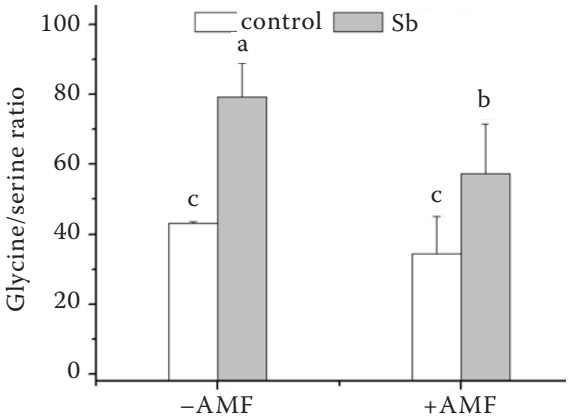

Figure 5. The glycine/serine (G/S) ratio in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under varying antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

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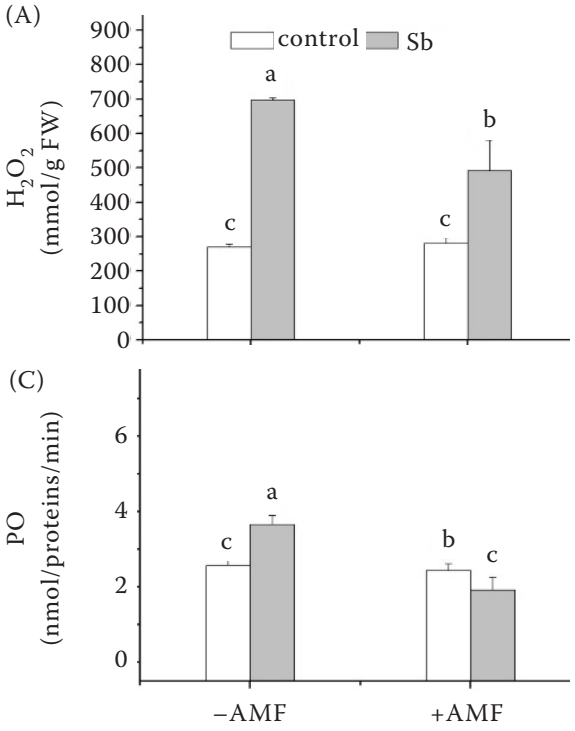

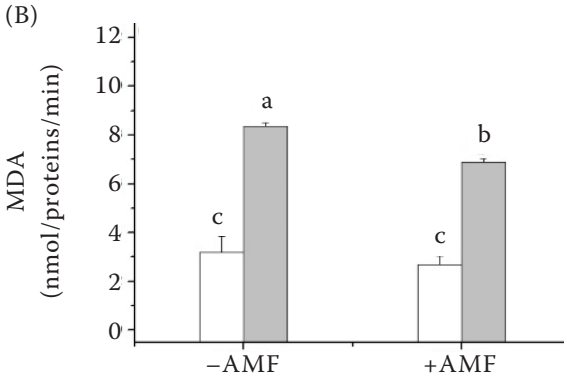

Figure 6. The levels of hydrogen peroxide (H_2O_2); malondialdehyde (MDA), and peroxidase (PO), key indicators of oxidative stress, in hydrogen peroxide (H_2O_2),-treated and non-treated linseed plants under varying antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$). FW – fresh weight

increased by 63% and 53% respectively, in response to Sb concentration. Conversely, when Sb and AMF were administered, their level dropped. Figure 8 showed that ASC/TASC declined with treatments, with the lowest ratio in AMF and Sb combined treatment. The GSH/TGSH ratio increased in response to Sb alone and Sb

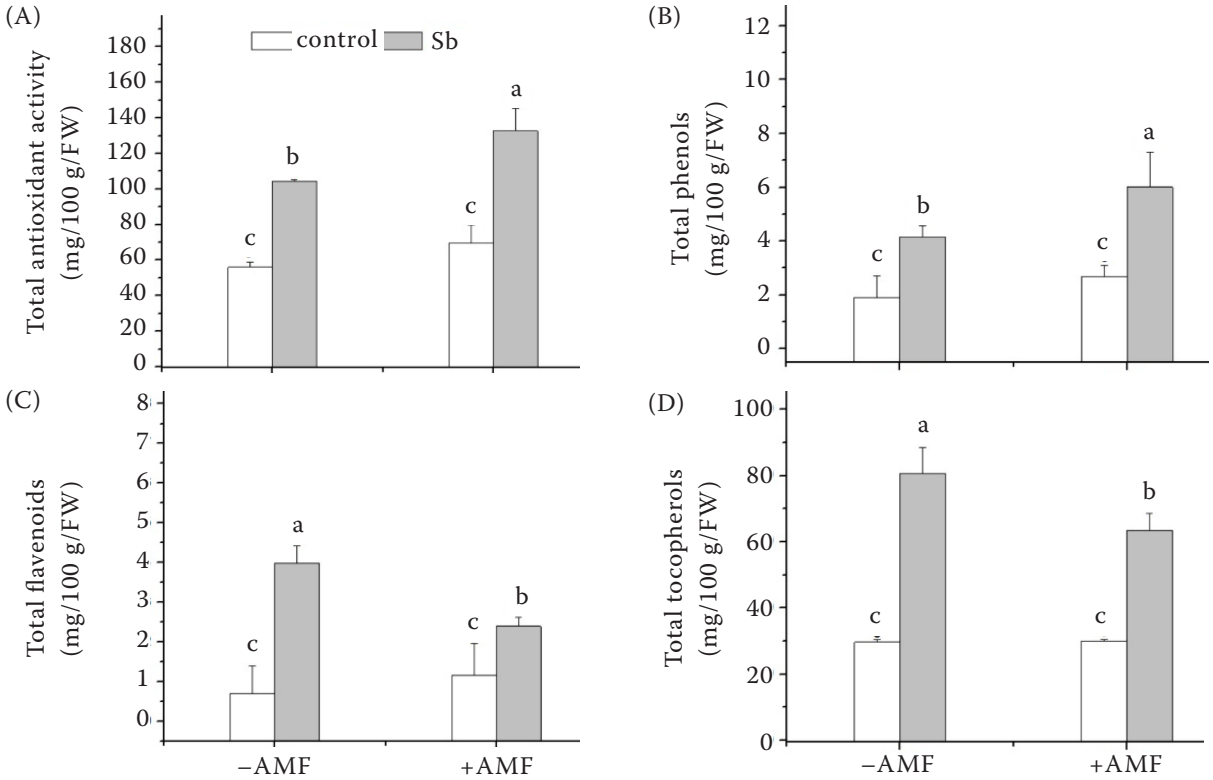

Figure 7. Under antimony (Sb) stress, the total phenols, flavonoids, tocopherol content, and total antioxidant activity were measured in hydrogen peroxide (H_2O_2),-treated and non-treated linseed plants. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$). FW – fresh weight

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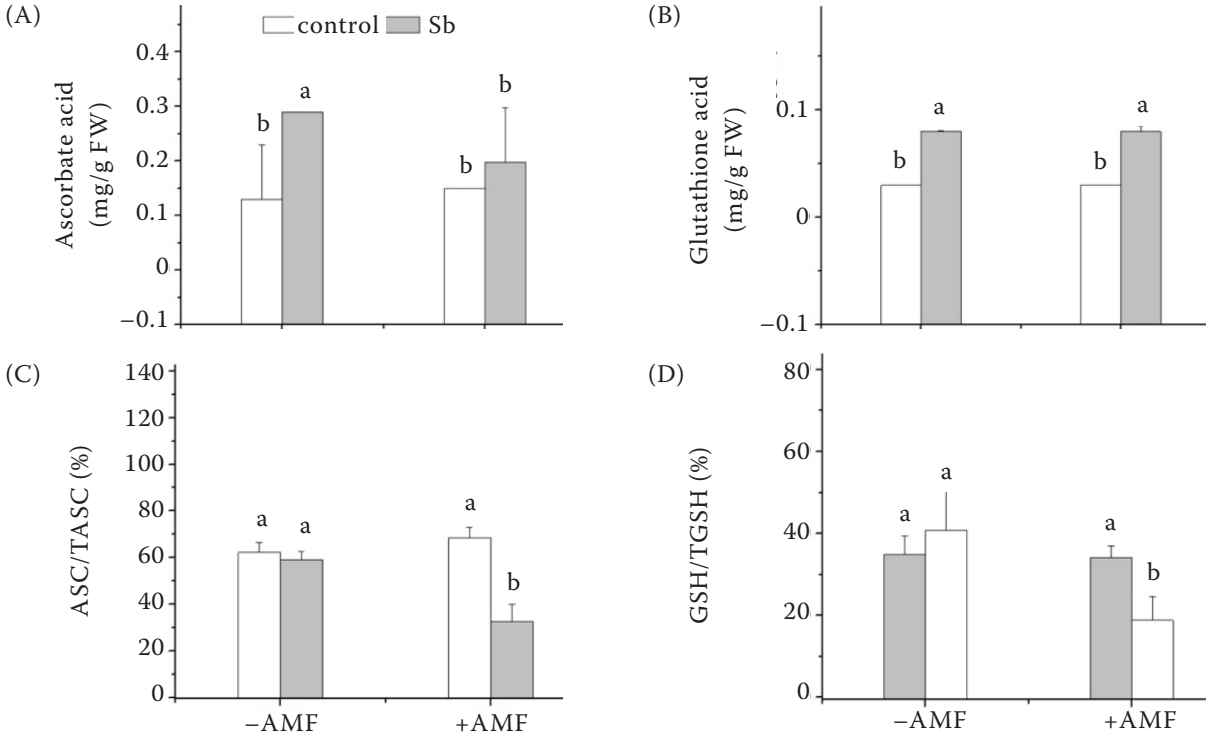

Figure 8. The levels of ascorbate and glutathione and their ratios in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$). FW – fresh weight

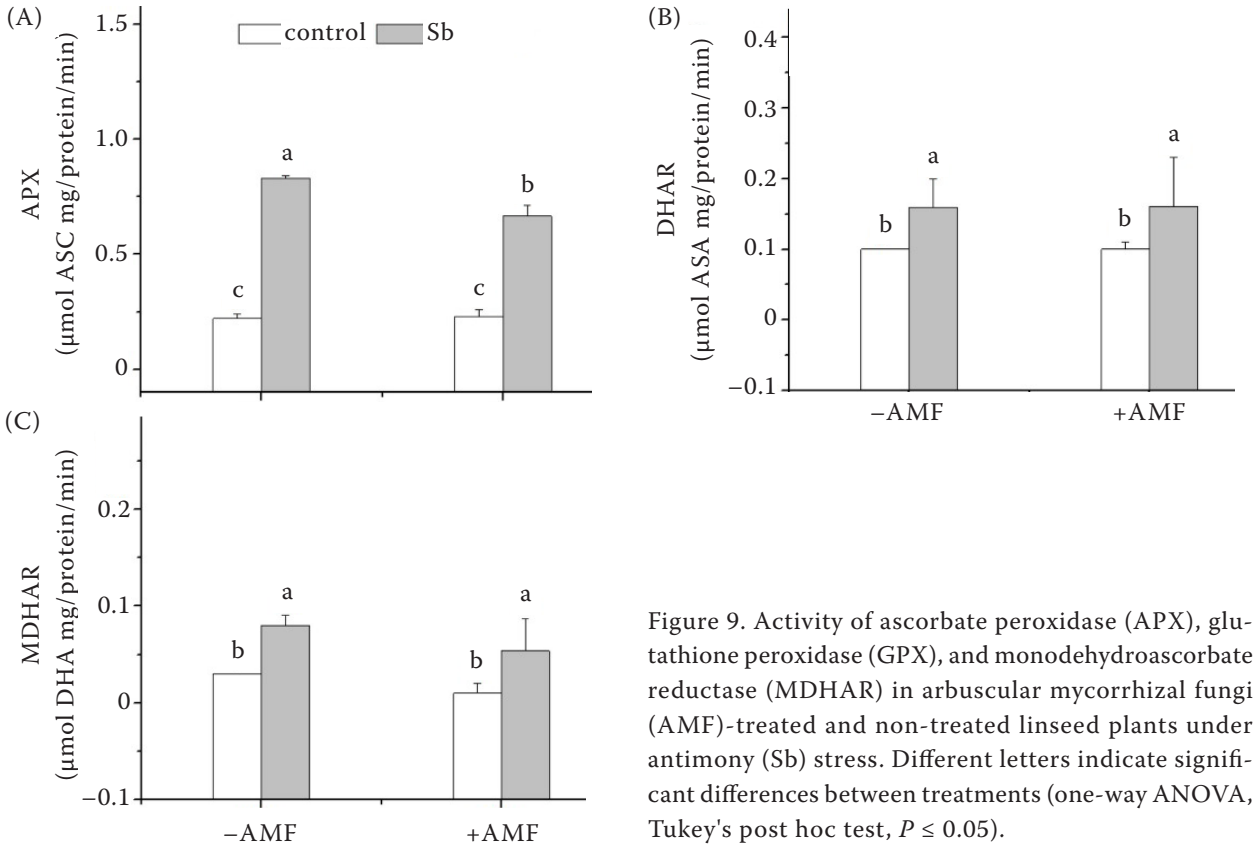

Figure 9. Activity of ascorbate peroxidase (APX), glutathione peroxidase (GPX), and monodehydroascorbate reductase (MDHAR) in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under antimony (Sb) stress. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$).

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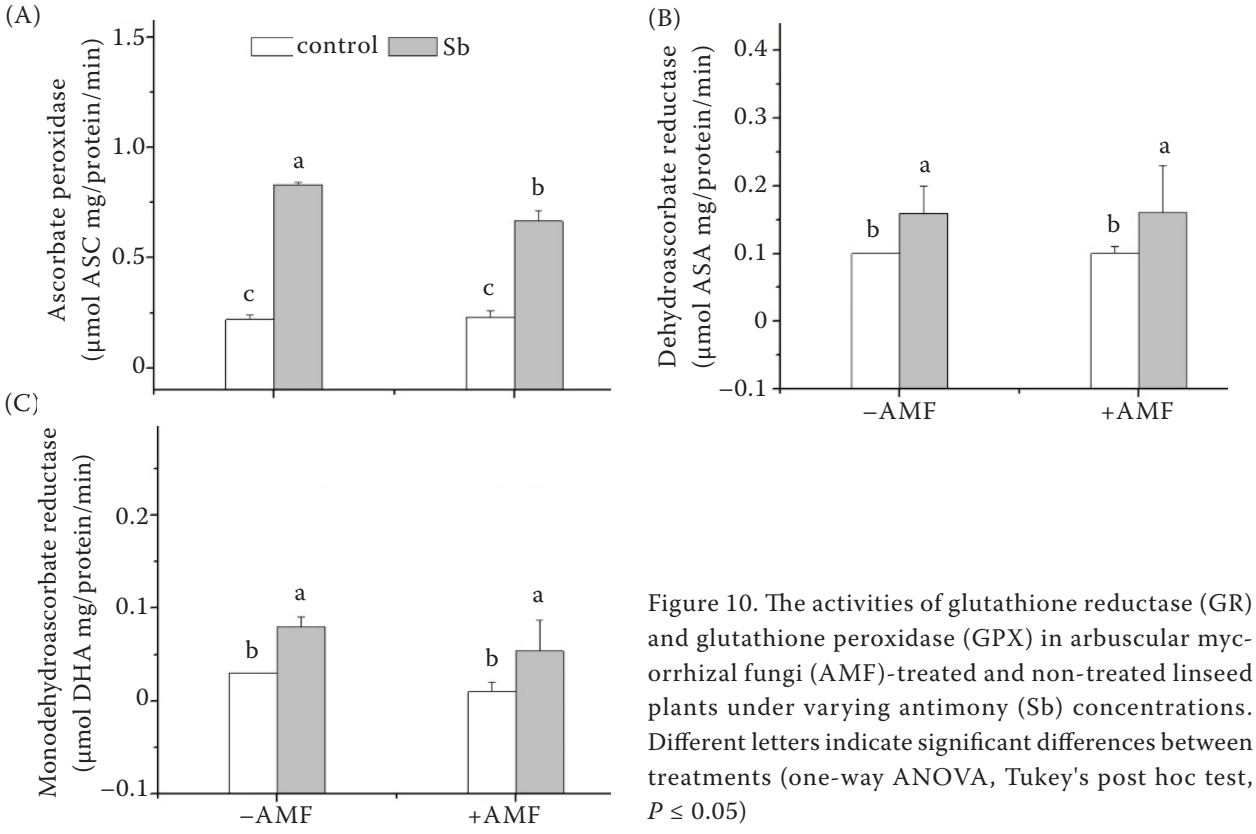

Figure 10. The activities of glutathione reductase (GR) and glutathione peroxidase (GPX) in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under varying antimony (Sb) concentrations. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

combined with AMF as compared to control and AMF alone. POX, SOD and CAT showed a remarkable increase in their activities (by 58, 76 and 39% respectively) in Sb-stressed linseed (Figure 9). Such increases in POX, CAT, and SOD enzyme activities were decreased when both Sb and AMF were applied together.

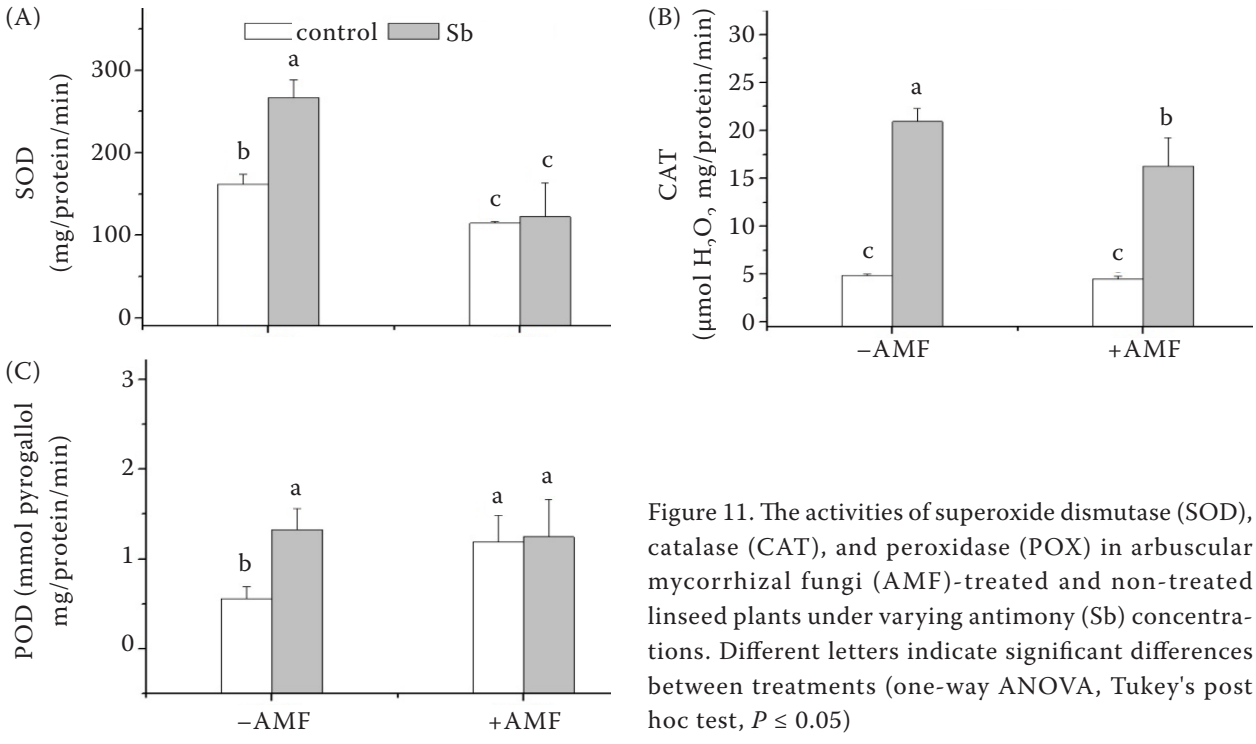

Figure 11. The activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) in arbuscular mycorrhizal fungi (AMF)-treated and non-treated linseed plants under varying antimony (Sb) concentrations. Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

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Detoxification-related parameters

The toxification-related parameters (GST, MTC, PCs, and TGSH) were analysed (Table 3). The addition of AMF did not change the content of the different parameters compared to the control. Whereas, when the plants are exposed to Sb concentration, the level of GST, MTC, PCs and TSGH increased by 52, 14, 36 and 13%, respectively, compared to their control counterparts. Under AMF treatment, a few measured parameters were altered in linseed leaves. In fact, a combination of Sb and mycorrhizal treatment increased the GST, MTC, and TSGH contents in leaves by 0.68, 0.22, and 0.26% relative to their controls, respectively. In contrast, the PC levels in the same treatment were higher than in the control but lower than in Sb alone.

DISCUSSION

There has been a global emphasis on sustainable crop production in environments contaminated with heavy metals, including Sb. In this context, AMF is a sustainable method to boost plant growth and resilience by promoting vegetative development through increased water and nutrient uptake (de Souza Buzo et al. 2023).

Interestingly, we showed that AMF reduced the uptake of Sb by 47%. In fact, AMF's ability to reduce the absorption of non-essential metals or to sequester them is principally responsible for AMF tolerance to Sb stress (Gupta et al. 2021). A similar trend was also observed by Wei et al. (2016). Also, Espinosa-Vellarino et al. (2021) examined AMF-inoculated *Cynodon dactylon* (Bermuda grass); they found reduced Sb uptake by roots (Espinosa-Vellarino et al. 2021). To understand the reduced uptake of Sb under AMF treatment, we measured phenolics and citric acid levels. The linseed rhizosphere's growing substrate had phenolics and citric acid levels under Sb and AMF treatment conditions. This observation is consistent with the results of Mohammed et al. (2023), who indicated high phenolics and citric acid levels in growing substrate of wheat and bean were observed under heavy metal stress (bismuth and arsenic) and AMF treatments (Mohammed et al. 2023). These organic acids can function as ligands that bind metals, lowering their bioavailability and toxicity through changes in growing substrate pH according to Pasricha et al. (2021). Furthermore, organic acids tend to have lower metal mobility because these acids create soluble complexes that are absorbed by the growing substrate matrix (Schwab et al. 2005).

At the growth level, the fresh and dry weight were reduced by Sb toxicity. Sb toxicity induced shorter plants and thinner, smaller, less developed leaves (Ortega et al. 2017, Espinosa-Vellarino et al. 2021). Reduced physiological activity could explain Sb-induced growth reduction (Zhou et al. 2022). Consistently, we also observed an adverse effect of Sb on the rate of photosynthesis-related parameters, including photosynthesis rate and chlorophyll synthesis. Exposure to Sb, for example, significantly decreased the net photosynthetic rate, reduced leaf pigment content, and lowered the quantum yield of PSII electron transfer in plants, as demonstrated by Zhou et al. (2018). Sb also reduce *Chls* biosynthesis and structure (Sreekanth et al. 2013). Sb, for example, consistently reduced plant photosynthetic performance, leading to a noticeable decline in vital processes. Our observations, mirroring previous research, reveal that Sb exposure significantly slashes the net photosynthetic rate, diminishes crucial leaf pigment content, and reduces the quantum yield of PSII electron transfer (Zhou et al. 2018). Sb directly attacks chlorophyll biosynthesis and chloroplast structure (Sreekanth et al. 2013), binding to

Table 3. Effects of arbuscular mycorrhizal fungi (AMF) and antimony (Sb, 1 500 mg/kg growing substrate) on detoxification-related parameters

	–AMF		+AMF	
	control	Sb	control	Sb
Phytochelatins (mg/g fresh weight (FW))	5.11 ± 0.10^{b}	8.06 ± 0.51^{a}	5.26 ± 0.01^{b}	6.85 ± 2.00^{a}
Total glutathione TGSH (mg/g FW)	0.07 ± 0.00^{c}	0.16 ± 0.01^{b}	0.08 ± 0.00^{c}	0.23 ± 0.03^{a}
Metallothionein (MTC) (mg/g FW)	33.58 ± 2.21^{b}	39.42 ± 2.68^{b}	25.75 ± 0.50^{c}	43.58 ± 0.28^{a}
GSH-S-transferase GST (units mg/protein/min)	0.15 ± 0.01^{ab}	0.17 ± 0.01^{a}	0.03 ± 0.08^{b}	0.19 ± 0.03^{a}

Different letters indicate significant differences between treatments (one-way ANOVA, Tukey's post hoc test, $P \leq 0.05$)

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chloroplast proteins and destroying their function, impeding the plant's ability to perform photosynthesis efficiently (Luo et al. 2021). Furthermore, Sb disrupts key enzymes involved in pigment production (Garrido et al. 2021). In our study, increased Sb concentration led to a decline in linseed leaves' photochemical efficiency (CF), signalling damage to PSII reaction centres. PSII is highly vulnerable to photoinhibition, with stressors like heavy metals, flooding, and drought severely reducing its light utilisation efficiency (Zhou et al. 2022). These findings underscore how Sb profoundly compromises the very engine of plant growth.

On the other hand, when AMF and Sb are mixed, the carotenoid concentration increases sharply. This supports the findings of Yong et al. (2017) and illustrates how carotenoids function as antioxidants and photoprotectors. However, Sb stress destroys the ability of carotenoids to clear away oxygen free radicals, destabilising thylakoid membranes and harming the plants. In contrast to Zhou et al. (2018), who report reductions in the carotenoid content and carotenoid/chlorophyll ratio in response to Sb toxicity in *Acorus calamus.* The protective effects of AMF on photosynthesis and its ability to inhibit ROS generation may be the main causes of its mitigating activity. Generally, the primary source of ROS was photorespiration. We studied the impacts of antimony contamination on photorespiration in order to comprehend the downstream effects on ROS levels and production. This study investigated the photorespiration indicators (Gly/Ser ratio, HPR and GO). Additionally, the study examined the buildup of H_2O_2 in response to the toxicity of heavy metal accumulation. The outcome of this present study revealed that Sb induced the accumulation of H_2O_2 could be ascribed to the heavy metal-induced photorespiration, as it is one of the key H_2O_2 generating mechanisms. Linseed exposed to Sb stress showed increased in H_2O_2. The increase in the production of H_2O_2 induced by Sb in linseed plants is similar to that described for other plants such as *D. viscosa* plants in response to heavy metals toxicity (Espinosa-Vellarino et al. 2021). In agreement, heavy metals have been shown to cause a notable increase in H_2O_2 levels in plants. Consequently, the cell death and ion leakage were also positively increased. The results matched those of *Amaranthus retroflexus* treatments with other metals, including Cu, As, and Ni (Alsherif et al. 2022). Consequently, the cell death and ion leakage were also positively increased. The results matched those of *Amaranthus retroflexus* treatments under Cu, As, and Ni stress (Alsherif et al. 2022).

It appeared that the AMF application mitigates the negative effect of Sb in linseed by protecting the photosynthetic apparatus from ROS metabolism. It is worth noting that the addition of AMF in the rootzones of linseed exposed to Sb concentration, the level of H_2O_2 was decreased which accompanied the decline of photorespirations. We also assessed the peroxidation of membrane lipids (MDA). AMF inoculation inhibited the degree of membrane lipid peroxidation and reduced the breaking of the chloroplast membrane, hence preventing chloroplast leakage. This product's level rises and indicates the extent of membrane structure disruption, as seen in the membranes of cells and chloroplasts (Zhou et al. 2022).

As a protective mechanism, antioxidant biosynthesis is activated to scavenge H_2O_2 and reduce oxidative damage brought on by environmental stress. An antioxidant metabolites and antioxidant enzyme activity is an important factor in assessing the response to heavy metal stress (Vardhan et al. 2019). Oxidative stress was mitigated by AMF inoculation, potentially by enhancement of APX, CAT, SOD, and GR enzymes activities (Alam et al. 2023). Consistent with our results, Hu et al. (2020) also observed an increase in antioxidant enzymes following AMF inoculation in plants under Cr stress. The primary explanation was that AMF symbiosis can regulate the induction of oxidative stress-related genes (Lenoir et al. 2016). Furthermore, the capacity of antioxidant defense system can regulate their repossess to environmental stress (Chakraborty et al. 2016). Under stress, both enzymatic and nonenzymatic antioxidants control ROS and maintain cellular homeostasis, which ultimately dictates the plant's future. The ASC-GSH cycle is a significant H_2O_2 scavenging pathway that operates in the cytosol and chloroplast (Talaat 2014). The effects of Sb on ASC and GSH are somewhat consistent with findings from studies on the toxicity of Cd (Srivastava and Pandey 2014) and As (Singh et al. 2015). On the contrary, the outcome showed a decrease in GR activity induced by Sb toxicity. Similarly, Feng et al. (2009) describe the same decrease in the GR activity in fern plants exposed to Sb concentration. Thus, the defense systems of AMF plants are modified and protected against ROS burst by the augmentation of non-enzymatic antioxidants (AsA, GSH) and antioxidant enzymes (SOD, CAT, GPX, GR, APX, DHAR, and MDAR). These findings

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suggested that AMF plants are more able to sustain these antioxidant pathways during Sb stress and may be more effective at scavenging ROS.

At detoxification, plant shoot tissue induces metal chelators (MTC, PCs) and GST enzyme activity to overcome Sb toxicity. PC-metal complexes detoxify the cell compartments and reduce the ability of heavy metals to bind to the cell wall (Faizan et al. 2024). As a matter of fact, MTs are a class of proteins that also play a significant role in heavy metal detoxification. PCs and MTs can bind the heavy metals and sequester them into the vacuoles. Similar to our results, Alsherif et al. (2022) also reported high levels in both MTC and PCs in *Sesuvium portulacastrum* plants exposed to heavy metal contamination. Besides, we noticed a higher level of GST activity in response to Sb exposure. Similar to the study by Espinosa-Vellarino et al. (2020), a sharp increase in GST activity was noticed in response to Sb. Intestinally, the application of AMF with Sb increased the accumulation of the production of MTC, PCs and GST (Li et al. 2022). On the other hand, a notable rise in TAC (Jańczak-Pieniażek and Cichoński 2023). In our research, an increase in the content of total phenols, flavonoids and tocopherol was also induced due to the inoculation of AMF in Sb-stressed plants, resulting in the accumulation of heavy metals. It seemed that the AMF inoculation boosted the biosynthesis of polyphenols and flavonoids. This increase may be attributed to the activation of the phenylpropanoid pathway through increased PAL activity (Albqmi et al. 2023).

In conclusion, this study emphasises how vital AMF is in reducing the stress Sb causes in flax plants. Sb has negative effects on AMF growth, including shorter hyphal segments and fewer arbuscules; nevertheless, the presence of AMF lowers the bioavailability of Sb and promotes plant growth by increasing antioxidant enzyme activity and improving nutrient absorption. AMF also affects the chemistry of the growing substrate and the generation of organic acids, which lowers metal toxicity and improves photosynthesis. The biochemical basis behind linseed's mechanistically based ability to survive Sb toxicity and how it detoxifies and increases antioxidants through enzymatic and non-enzymatic antioxidant levels to lessen Sb oxidative stress. To improve crop productivity and environmental health, our findings call for more research on the interactions between AMF and Sb stress. They also emphasise the possibility of AMF as a long-term solution for enhancing plant resilience under heavy metal toxicity.

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