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Growth stimulation of *Gazania splendens* plants grown under lead and cadmium stress conditions using mycorrhiza fungus

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Abstract: A pot experiment was conducted at the Horticultural Research Institute Antonides in Alexandria (2022-2023 seasons) to evaluate the effect of arbuscular mycorrhizal fungi (AMF) on *Gazania splendens* under cadmium (2, 4, 8 ppm) and lead (150, 200, 400 ppm) stress conditions. A randomized block design was used, comprising thirteen treatments with three replications each. Results showed that the highest concentrations of Cd (8 ppm) and Pb (400 ppm) significantly inhibited vegetative growth, root development, and floral traits, while increasing metal accumulation in leaves and reducing carotene and proline content. In contrast, AMF inoculation (200 g/plant) significantly mitigated heavy metal stress, improving all studied growth and physiological parameters. AMF-treated plants showed lower heavy metal uptake and higher proline levels compared to non-inoculated stressed plants. The study demonstrates that AMF is an effective biotechnological tool for enhancing the tolerance of *Gazania splendens* in heavy metal-contaminated soils.

1. Introduction

Ornamental plants are among the natural elements that have a significant impact on human life, as they play an important role in psychological, aesthetic, cultural, health, and economic conditions (Pittenger *et al*., 2001). The genus Gazania comprises 17 species and belongs to the family Asteraceae, specifically within the subfamily Cichorioideae and the tribe Arctotideae. Traditionally, *Gazania splendens* Moore has been used to relieve toothache, earache, urethral stricture, and to prevent miscarriage. *Gazania krebsiana* is valued as a soil stabilizer and is commonly used as a border plant. Additionally, an infusion prepared from the roots of *Gazania rigens* var. uniflora was traditionally

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used by young Zulu men in a magical context during courtship rituals (Reddy *et al.*, 2014). *Gazania splendens L.,* of the Asteraceae family, is a perennial herb that flowers in spring and summer and grows up to 15 cm in height. Recognized as a ground cover, its blooms open during the day and close at night. It is propagated by seeds or by dividing offshoots from the plant's base. Versatile and decorative, it works well as a potted plant or planted directly in soil (Sardoei and Shahdadneghad, 2014). Gazania plants are propagated by seed or division of old plants because they produce numerous offshoots within the same year and can be grown in pots or planted directly in the ground. Despite the effectiveness of chemical fertilizers in improving plant growth, it has been scientifically demonstrated that these substances are harmful to the environment and human health. Modern agricultural policies seek to provide nutrients that promote plant growth, limit environmental harm, and boost plant resilience to adverse conditions (Al-Taey *et al.,* 2019). In this context, growth stimulants - including amino acids, organic acids, plant hormones, plant extracts, and biofertilizers - have been widely adopted. These bio stimulants have proven effective in enhancing both growth and productivity in a wide range of horticultural crops (Al-Taey *et al.,* 2018).

In recent years, heavy metal (HM) contamination has become a widespread issue in both natural and anthropogenically impacted ecosystems, raising serious health and environmental concerns. These challenges have led to stricter regulations and increased interest in remediation methods. One such approach gaining traction is phytoremediation, which utilizes plants and their symbiotic organisms. Despite growing interest, the gap between fundamental mycorrhizal research and its practical applications remain significant (Crișan *et al.*, 2024).

Heavy metals (HMs) are among the most persistent environmental stressors and are known to reduce key soil ecosystem services globally (Rillig *et al.*, 2023). The most commonly encountered HM contaminants worldwide include arsenic (As), mercury (Hg), and lead (Pb) (FAO, 2021). In agricultural soils, arsenic (As), copper (Cu), and nickel (Ni) are prevalent (Tóth *et al.*, 2016), whereas urban soils are often contaminated with elevated levels of Cu, chromium (Cr), Ni, Pb, and zinc (Zn) (Binner *et al.*, 2023). Environmentally friendly or «green» solutions to HM pollution receive the highest levels of public support (Riaz *et al.*, 2022).

Broadly defined, HMs are metals and metalloids with high elemental densities and associated toxicities. However, some scholars have proposed narrower definitions or suggested using alternative terms such as "potentially toxic elements (PTEs)" (Ali and Khan, 2018; Pourret and Hursthouse, 2019).

The interaction between HMs and plants typically begins at the cell wall, which serves both as a barrier and a target. Functional groups in cell wall components can bind HMs, either detoxifying them or disrupting cell wall synthesis (Parrotta *et al.*, 2015). Among these components, pectin moieties play a significant role due to their chelating capacity, while lignin biosynthesis is often enhanced under HM stress (Kosakivska *et al.*, 2021). HM uptake by plants can occur passively through underground or aerial organs or actively via ion channels and transporters. Once absorbed, HMs can be transported systemically through the xylem and phloem, reaching various plant tissues (Ghuge *et al.*, 2023).

Cadmium (Cd) is considered one of the most phytotoxic HMs, as it significantly impairs plant growth and posing severe health risks through its accumulation in the food chain (Shah and Dubey, 1998). Excessive Cd in soil is known to cause a range of stress symptoms in plants, such as reduced root elongation, nutrient imbalances, and disrupted carbohydrate metabolism, ultimately leading to reduced biomass (Moya *et al.*, 1993). Lead (Pb) is another prevalent environmental contaminant and is one of the most abundant metals in the Earth's crust. Even at low concentrations, Pb can hinder critical plant processes, including seed germination, photosynthesis, mitosis, water uptake, mineral absorption, and enzymatic activity (Patra *et al.*, 2004). Pb exposure also disrupts nitrogen metabolism, carbohydrate utilization, and water relations in plants (Hamid *et al.*, 2010). Additionally, Pb contamination alters soil microbial communities, degrades soil fertility, and contributes to reduced plant productivity (Majer *et al.*, 2002).

The main objective of this study is to investigate the effect of mycorrhizal fungi in stimulating the growth of *Gazania splendens* plants cultivated under lead and cadmium stress conditions.

2. Materials and Methods

Plant material and experimental conditions

A pot experiment was carried out at the

Horticultural Research Institute - Antonides, Alexandria, during the 2022 and 2023 growing seasons to evaluate the effect of mycorrhizal application on the growth of *Gazania* plants under lead and cadmium stress conditions. To ensure the purity and accurate identification of the mycorrhizal species, the inoculum was sourced from the Namha Microbiology Laboratory, rather than using a commercial mixture.

Planting was conducted on April 15 in both growing seasons. Pots with a diameter of 16 cm were filled with 1.5 kg of soil whose chemical characterization, performed during both growing seasons, is reported in Table 1. According to the experimental treatments, cadmium at concentrations of 2, 4, or 8 ppm or lead at concentrations of 150 or 400 ppm, either alone or in combination with the addition of 200 g of mycorrhiza, were thoroughly mixed with the soil, as reported in Table 2.

A total of thirteen treatments were arranged in a Randomized Complete Block Design (RCBD) with three replicates.

Plant growth and flowering

Vegetative growth parameters were recorded throughout the experiment. Plant height was measured from the base of the stem at soil level to the shoot tip using a tape measure. The number of leaves per plant was measured by counting all fully expanded leaves on the selected plants, and the number of branches per plant was also recorded. Stem diameter was measured at the midpoint of the main stem. Fresh weight of the shoot system was measured by averaging the weights of five plants per experimental unit.

Root characteristics were evaluated by measuring root length, fresh weight, and dry weight. The roots were carefully harvested, and fresh weight was recorded immediately, while dry weight was determined after drying at 105°C for 72 hours until a constant weight.

Flower characteristics were recorded, including the number of flowers per plant, flower diameter, flower fresh weight, flower duration (in days from opening to senescence), and days to flowering for each treatment.

Chlorophyll analysis

Chlorophyll content was measured at the end of the vegetative growth cycle, defined as the stage when plants reached full vegetative development prior to flowering. Total chlorophyll content in SPAD units was measured in fully expanded healthy leaves from each replicate using a Minolta SPAD meter following the method of Jemison and William (2006).

Chemical analysis

Leaf samples were oven-dried at 50°C to a constant dry weight prior to chemical analysis. For the determination of cadmium (Cd) and lead (Pb) concentrations, leaves were dried at 78°C, ground into a homogeneous powder, and digested using nitric acid (HNO_3) at 180°C under 20 atm pressure for 40 minutes using a microwave digestion system

Table 2 - Experimental treatments applied to *Gazania splendens* under cadmium and lead stress

Treatment code	Description
C	Control (plants grown in untreated soil without Cd, Pb, or mycorrhizal inoculation) [z]
Cd2	Cd at 2 ppm
Cd4	Cd at 4 ppm
Cd8	Cd at 8 ppm
Pb150	Pb at 150 ppm
Pb200	Pb at 200 ppm
Pb400	Pb at 400 ppm
Cd2+M	Cd at 2 ppm + 200 g mycorrhiza
Cd4+M	Cd at 4 ppm + 200 g mycorrhiza
Cd8+M	Cd at 8 ppm + 200 g mycorrhiza
Pb150+M	Pb at 150 ppm + 200 g mycorrhiza
Pb200+M	Pb at 200 ppm + 200 g mycorrhiza
Pb400+M	Pb at 400 ppm + 200 g mycorrhiza

[z] The control treatment consisted of plants grown under the same conditions but without heavy metal addition or mycorrhizal inoculation.

Table 1 - Chemical properties of the used sandy soil used during the two 2022 and 2023 growing seasons

Season	pH	EC dS/m	Water soluble cations (meq/l)				Water soluble anions (meq/l)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2022	7.83	1.50	3.1	3.0	6.4	1.2	3.3	6.8	2.5
2023	7.91	1.53	3.3	3.4	6.6	1.3	3.5	6.6	2.3

(Mars-5, Candela, USA). The concentrations of Cd and Pb were subsequently measured spectrophotometrically using inductively coupled plasma (ICP) spectrometry (OPTIMA 2000 DV, Perkin-Elmer, USA).

Proline content (mg/g) in the leaves was determined according to the method described by Bates *et al.* (1973).

Carotene extraction was conducted based on the procedure outlined by Hagos *et al*. (2022) with minor modifications. Specifically, β-carotene was extracted by soaking 0.5 g of leaf tissue in 10 mL of acetone at room temperature in the dark to ensure complete extraction. The solution was magnetically stirred for 30 minutes.

Statistical analysis

All collected data were subjected to statistical analysis using the MSTAT software package to perform analysis of variance (ANOVA) as described by Gomez and Gomez (1984). Differences among treatment means were evaluated using the Least Significant Difference (LSD) test at a 0.05 level of significance.

3. Results

Vegetative growth

Results presented in Tables 3 and 4 indicate that the application of mycorrhizal fungi improved the growth of *G. splendens* plants under cadmium and lead stress conditions. However, increasing concentrations of cadmium and lead decreased all vegetative growth parameters of *G. splendens* as compared to the control treatment, which recorded the highest values for all traits. The control treatment showed the highest values for all measured traits across the two examined seasons, including plant height (36.9 and 39.1 cm), number of leaves (111.6 and 112.7), number of branches (16.5 and 17.4), stem diameter (1.1 and 1.2 cm), total chlorophyll content (58.8 and 62.4 SPAD), and fresh weight (372.1 and 405.5 g). These were followed by plants treated with Cd (2 ppm) combined with 200 g of mycorrhiza, which exhibited slightly lower values of plant height (33.9 and 36.0 cm), number of leaves (100.4 and 101.4), number of branches (15.2 and 16.0), stem diameter (0.9 and 0.9 cm), total chlorophyll content (58.5 and 60.7 SPAD), and fresh weight (372.1 and 383.8 g). Similarly, plants treated with Pb (150 ppm) plus 200 g of mycorrhiza showed intermediate values: plant height (31.3 and 32.3 cm), number of leaves (92.4 and 93.3), number of branches (13.9 and 14.6), stem diameter (0.8 and 0.9 cm), total chlorophyll content (53.8 and 57.2 SPAD), and fresh weight (362.3 and 373.1 g). In contrast, plants exposed to the highest Pb concentration (400 ppm) recorded the lowest values across all parameters, including plant height (22.0 and 23.3 cm), number of leaves (57.1 and 59.3), number of branches (9.9 and 10.3), stem diameter (0.5 and 0.6 cm), total chlorophyll content (33.1 and 36.8 SPAD),

Table 3 - Effects of lead, cadmium, and mycorrhizal application on plant height, leaf number, and branch number of *G. splendens* during the 2022 (1st) and 2023 (2nd) growing seasons

Treatment	Plant height (cm)		Total leaves/plant (n)		Total branches/plant (n)	
	1st	2nd	1st	2nd	1st	2nd
Control	36.88	39.10	111.58	112.70	16.52	17.35
Cd2	29.51	28.15	82.13	82.95	13.87	14.61
Cd4	26.56	25.34	71.91	74.65	11.90	12.49
Cd8	23.90	21.28	65.52	67.19	10.94	11.40
Pb150	26.15	27.77	76.60	80.84	13.22	13.88
Pb200	24.43	25.90	62.24	61.46	12.16	12.77
Pb400	21.99	23.31	57.12	59.31	9.85	10.34
Cd2+M	33.93	35.97	100.43	101.43	15.20	15.96
Cd4+M	30.19	32.25	90.38	91.29	13.68	14.36
Cd8+M	25.88	26.67	83.24	84.08	11.31	11.93
Pb150+M	31.33	32.33	92.39	93.31	13.87	14.61
Pb200+M	27.54	28.37	83.15	83.98	12.38	12.50
Pb400+M	23.48	24.14	72.55	73.61	10.71	11.24
$LSD_{(0.05)}$	0.43	0.45	1.27	1.27	0.11	0.12

Table 4 - Effect of lead, cadmium, and mycorrhizal application on stem diameter, total chlorophyll, and total fresh weight of *G. splendens* during the 2022 (1st) and 2023 (2nd) growing seasons

Treatment	Stem diameter (cm)		Total chlorophyll (SPAD)		Fresh weight (g)	
	1st	2nd	1st	2nd	1st	2nd
Control	1.10	1.17	58.81	62.35	372.05	405.54
Cd2	0.71	0.75	42.12	46.33	297.64	324.43
Cd4	0.64	0.67	37.91	41.70	267.88	291.99
Cd8	0.57	0.61	32.25	35.47	241.09	262.79
Pb150	0.65	0.69	40.91	45.46	273.83	298.48
Pb200	0.58	0.62	36.46	40.51	246.45	268.63
Pb400	0.52	0.56	33.14	36.82	221.80	241.77
Cd2+M	0.88	0.93	58.50	60.69	372.08	383.77
Cd4+M	0.79	0.84	52.65	57.92	359.85	364.99
Cd8+M	0.72	0.76	47.39	52.12	301.36	312.49
Pb150+M	0.81	0.86	53.82	57.20	362.29	373.10
Pb200+M	0.73	0.77	48.44	53.28	328.06	335.79
Pb400+M	0.66	0.70	43.59	47.95	277.25	302.21
$LSD_{(0.05)}$	0.01	0.01	12.36	13.59	3.17	3.46

and fresh weight (221.8 and 241.8 g), consistently observed across both seasons examined. These results confirm that mycorrhizal inoculation helps mitigate the adverse effects of heavy metal stress, particularly at lower Cd and Pb concentrations.

Root characters

The results presented in Table 5 revealed that the root characteristics of *G. splendens* plants grown under cadmium (Cd) and lead (Pb) stress were notably improved when treated with mycorrhizal fungi. However, increasing metal concentrations led to a progressive decline in all root parameters, with the control showing the highest values. The most notable improvements with 200 g of AMF were observed when combined with moderate levels of Cd (2 ppm) and Pb (150 ppm), whereas the highest Pb concentration (400 ppm) resulted in the poorest root development across both seasons.

Specifically, control p showed the highest values for root length (1.07 and 1.19 cm), root dry weight (44.54 and 49.43 g), and root fresh weight (11.14 and

Table 5 - Effect of lead, cadmium, and mycorrhizal application on root length, root fresh weight, and root dry weight of *G. splendens* during the 2022 (1st) and 2023 (2nd) growing seasons

Treatment	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	1st	2nd	1st	2nd	1st	2nd
Control	1.07	1.19	44.54	49.43	11.14	12.36
Cd2	0.857	0.953	40.08	44.49	10.02	11.12
Cd4	0.690	0.760	35.27	39.15	8.82	9.79
Cd8	0.540	0.600	29.98	33.28	7.50	8.32
Pb150	0.933	1.03	36.88	40.94	9.21	10.24
Pb200	0.770	0.857	32.45	36.02	8.12	9.01
Pb400	0.520	0.583	27.58	30.62	6.90	7.66
Cd2+M	0.963	1.07	41.30	45.84	10.33	11.46
Cd4+M	0.747	0.827	36.35	40.35	9.09	10.09
Cd8+M	0.600	0.660	30.89	34.29	7.72	8.58
Pb150+M	0.840	0.933	42.65	47.34	10.66	11.84
Pb200+M	0.670	0.743	37.53	41.66	9.38	10.41
Pb400+M	0.540	0.597	31.90	35.41	7.98	8.85
$LSD_{(0.05)}$	0.01	0.01	0.17	0.19	0.04	0.05

12.36 g) in the two growing seasons. This was followed by plants treated with Cd (2 ppm) combined with200 g of mycorrhiza, which showed root length values of 0.963 and 1.07 cm, root dry weight of 41.30 and 45.84 g, and root fresh weight of 10.33 and 11.46 g. Similarly, Pb (150 ppm) + 200 g mycorrhiza treatment resulted in intermediate values for root length (0.840 and 0.933 cm), root dry weight (42.65 and 47.34 g), and root fresh weight (10.66 and 11.84 g). In contrast, plants exposed to the highest Pb concentration (400 ppm) resulted in the lowest values for all root parameters, including root length (0.520 and 0.583 cm), root dry weight (27.58 and 30.62 g), and root fresh weight (6.90 and 7.66 g), consistently in both seasons.

Flower characters

The results presented in Table 6 demonstrate the impact of cadmium (Cd) and lead (Pb) stress on the flower characters of *Gazania splendens* when treated with mycorrhizal fungi. An increase in the concentration of Cd and Pb led to a noticeable decline in all studied floral traits compared to the control treatment, which consistently recorded the highest values.

In the control treatment, the highest values were observed for the number of flowers per plant (16.84 and 18.35), flower diameter (13.20 and 14.65 cm), flower fresh weight (4.64 and 4.88 g), and flower duration (10.77 and 11.63 days). This treatment also recorded the shortest time to flowering (72.58 and 75.56 days) during both seasons. Following the control, the treatment with Pb (150 ppm) + 200 g mycorrhiza yielded comparably favorable results: number of flowers per plant (16.50 and 17.98), flower diameter (6.84 and 7.58 cm), flower fresh weight (3.13 and 3.29 g), flower longevity (9.05 and 9.77 days), and days to flowering (82.50 and 84.66), respectively, for the two seasons.

Chemical composition

Results in Table 7 show the chemical composition of *G. splendens* plants under cadmium and lead stress, with mycorrhizal fungi treatment. The data demonstrates that as cadmium and lead concentrations increased, carotene content decreased, while higher metal accumulation in the leaves led to higher levels of proline, cadmium, and lead compared to the control. For example, the control exhibited the lowest values of proline (0.517 and 0.563 mg/g), lead (3.02 and 2.90 mg/l), and cadmium (0.58 and 0.53 mg/l), as well as the highest carotene values (0.945 and 1.07 mg/100g fresh weight). The next best result was observed in the Pb (150 ppm) + 200 g mycorrhiza treatment (0.870 and 0.933 mg/100g fresh weight). These results underscore that the control consistently recorded the lowest stress-related values in both seasons, clearly linking increased metal stress to altered chemical composition in *G. splendens*.

Table 6 - Effect of lead, cadmium, and mycorrhizal application on some flower characters of *G. splendens* during the 2022 (1st) and 2023 (2nd) growing seasons

Treatments	No. of flower/plant		Flower diameter (cm)		Flower weight (g)		Flower duration (days)		No. of day to flowering	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	16.84	18.35	13.20	14.65	4.64	4.88	10.77	11.63	72.58	75.56
Cd2	16.32	17.24	6.15	6.83	2.48	2.53	8.15	8.33	76.80	80.57
Cd4	15.23	16.60	5.51	5.56	2.27	2.30	6.74	6.36	83.04	84.18
Cd8	14.97	15.81	4.96	5.50	2.08	2.12	5.35	5.94	87.41	97.02
Pb150	15.52	16.92	6.30	6.66	2.50	2.67	7.69	8.09	81.40	84.80
Pb200	13.71	14.30	4.72	5.13	2.32	2.49	6.12	6.49	83.42	86.26
Pb400	12.47	12.78	3.19	3.99	2.16	2.31	4.76	5.30	87.65	92.30
Cd2+M	16.16	17.61	6.48	7.18	2.75	2.82	8.80	8.99	80.64	82.51
Cd4+M	14.54	15.94	4.47	5.81	2.52	2.55	6.91	6.54	85.27	86.42
Cd8+M	13.67	14.20	3.83	4.70	2.31	2.36	4.52	4.11	89.12	90.80
Pb150+M	16.50	17.98	6.84	7.58	3.13	3.29	9.05	9.77	82.50	84.66
Pb200+M	15.85	16.28	5.20	5.61	2.98	3.12	7.60	7.29	84.89	85.22
Pb400+M	13.62	14.64	4.30	4.66	2.82	2.97	5.17	5.82	86.35	89.18
$LSD_{(0.05)}$	0.25	0.27	0.27	0.30	0.01	0.01	0.07	0.07	1.64	1.83

Table 7 - Effect of lead, cadmium, and mycorrhizal application on chemical composition of *G. splendens* during the 2022 (1st) and 2023 (2nd) growing seasons

Treatment	Carotene (mg/ 100g f.w.)		Proline (mg/g)		Pb (mg/l)		Cd (mg/l)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0.945	1.07	0.517	0.563	3.02	2.90	0.58	0.53
Cd2	0.756	0.780	0.620	0.670	8.74	7.78	1.16	1.20
Cd4	0.605	0.638	0.770	0.840	9.71	8.64	1.29	1.34
Cd8	0.484	0.510	0.967	1.05	10.79	9.60	1.61	1.79
Pb150	0.730	0.763	0.557	0.610	12.15	12.65	1.00	1.11
Pb200	0.544	0.580	0.693	0.760	13.17	13.50	1.16	1.23
Pb400	0.420	0.435	0.870	0.947	15.86	15.00	1.39	1.45
Cd2+M	0.783	0.840	0.540	0.587	5.32	5.44	0.99	1.09
Cd4+M	0.626	0.670	0.670	0.730	6.05	6.40	1.09	1.22
Cd8+M	0.501	0.540	0.810	0.843	7.55	6.72	1.37	1.52
Pb150+M	0.870	0.933	0.483	0.530	10.92	9.72	0.87	0.97
Pb200+M	0.696	0.747	0.603	0.660	12.14	10.80	0.97	1.07
Pb400+M	0.556	0.600	0.757	0.769	13.48	12.00	1.11	1.20
$LSD_{(0.05)}$	0.004	0.01	0.02	0.02	0.09	0.08	0.02	0.02

4. Discussion and Conclusions

As it has been clearly demonstrated that chlorophyll integrity - and thus photosynthetic efficiency - is affected by HM stress (Küpper and Spiller, 1996), chlorophyll measurements have been widely employed as reliable indicators to detect HM ion toxicity and plant damage (Chen *et al.,* 2019). Since the green tissues of land plants are the primary sites where organic compounds are synthesized to sustain plant growth and development (Lawson and Milliken, 2023), a well-balanced chlorophyll metabolism is essential throughout the plant's life cycle, highlighting the importance of related parameters. Overexcitation of the photosynthetic apparatus, chlorophyll degradation, or inhibition of its biosynthesis can impair other vital processes and have severe consequences for plant health (Hörtensteiner and Kräutler, 2011). Plants are biological solar panels that use photosynthetic pigments to absorb light in the 400-700 nm range to drive photosynthesis (McDonald, 2003). Chlorophyll molecules are bound to proteins embedded in the thylakoid membranes of the chloroplasts (Rantala *et al.,* 2020). They have a hydrophilic porphyrin head and a hydrophobic phytol tail. The pyrrole rings chelate an Mg^{2+} atom. In plants, chlorophyll a, chlorophyll b, and accessory pigments (such as carotenoids) complement each other to increase the range of light that can be absorbed. Chlorophyll a is 2-3 times more abundant than chlorophyll b (McDonald, 2003) and is the pigment essential for photochemical reactions (Björn *et al*., 2009), whereas chlorophyll b is important for the light acclimation process (Voitsekhovskaja and Tyutereva, 2015).

The effect of HMs on photosynthetic mechanisms depends on their reactivity and concentration. Replacement of Mg^{2+} in chlorophyll molecules by a heavy metal ion is one of the most important causes of metal-induced damage in plants (Küpper *et al*., 2006). Other negative effects include inactivation of photosynthetic enzymes (such as RuBisCO) and oxidative damage caused by reactive oxygen species (ROS) in chloroplasts, all of which led to reduced chlorophyll concentration, destabilization of the chloroplast membranes, and decreased photosynthetic rates (Riyazuddin *et al*., 2022) (Table 3 and 4). Heavy metal (HM) stress is known to impair root vitality and reduce plant biomass and chlorophyll content by inhibiting the uptake of essential macro- and micronutrients (Wu *et al.,* 2019; Zhao *et al.*, 2019). Consistent with our findings, *Iris wilsonii* plants inoculated with arbuscular mycorrhizal fungi (AMF) under chromium-contaminated water conditions showed improved chlorophyll content and biomass compared to non-inoculated controls (Hu *et al*., 2020 b). AMF-inoculated plants are also more effective at removing HMs from their environment due to increased biomass and can mitigate chloroplast damage by restricting HM transport to aerial parts (Janeeshma and Puthur, 2020). One plausible explanation is that plants enhance their physiological metabolism at low HM concentrations by increasing root vitality. The extensive mycelial-root network facilitates nutrient flow and helps prevent HMs from entering root cells (Alam *et al*., 2019). However, high HM concentrations can damage root cell structures, reduce vitality, and cause metabolic dysfunction (Li and Yu, 2014). AMF play a crucial role by promoting HM accumulation in roots while limiting their translocation to shoots. For example, Huang *et al*. (2017) reported that AMF symbiosis in *Phragmites australis* improved cadmium tolerance by increasing BCF (bioconcentration factor) and lowering TF (translocation factor). Previous studies have shown that longer roots in mycorrhizal plants contribute to greater HM accumulation, with a preference for accumulation in fungal hyphae rather than root cells (Wu *et al.,* 2016). This may explain the increased HM uptake observed in AMF-treated plants. The variability in Pb, Zn, Cu, and Cd accumulation across studies (e.g., Fritioff and Greger, 2006) may be due to differences in plant species and metal characteristics.

AMF can secrete active compounds like glomalin-related soil proteins (GRSP), which bind HMs in the rhizosphere, reduce their mobility, and reshape microbial communities (Ma *et al.,* 2019; Wang and Feng, 2021). GRSP, a water-insoluble glycoprotein released from mycorrhizal hyphae and spore walls, has been shown to effectively chelate heavy metals (Wu *et al*., 2014). Nafady and Elgharably (2018) confirmed that AMF inoculation significantly reduced concentrations of Cd, Pb, Zn, and Cu due to GRSP release.

Heavy metal accumulation in soil also negatively impacts microbial diversity and activity. AMF, as symbiotic microorganisms that interact directly with plant roots, play a key role in modulating the availability of mobile and immobile metal cations in the soil. Traditional remediation methods for heavy metal contamination - such as physical and chemical treatments - are often expensive and inefficient (Benavides *et al*., 2005; Sharma and Dubey, 2005). AMF reduce HM concentration through various mechanisms, including binding to their chitin-rich cell walls (Hildebrandt *et al*., 2007), secretion of glomalin (Gonzalez-Chavez *et al*., 2004), and accumulation of non-toxic metal forms in root tissues (Joner and Leyval, 1997). Increased mycorrhizal colonization of the soil boosts plant biomass and shoot growth while reducing HM concentrations in both roots and shoots (Weissenhorn *et al*., 1995). Furthermore, the root density of AMF significantly affects HM tolerance, which varies based on the mycorrhizal inoculum used. AMF are highly effective at reducing metal transport from roots to shoots, especially in the early stages of HM uptake (Tonin *et al*., 2001). Cadmium accumulation in agricultural soils poses serious risks, but symbiotic interactions between plant roots and soil microbes like AMF can help mitigate these effects. The use of mycorrhiza has shown potential in reducing heavy metal input, such as Cd, introduced through chemical fertilizers (Table 5).

These findings align with those reported by Asrar *et al*. (2012) and Vaingankar and Rodrigues (2012) who noted an increase in the number of flowers in *Pelargonium peltatum, Verbena sp., Antirrhinum majus, Chrysanthemum morifolium*, and *Tagetes erecta.* Similarly, our observations, as can be seen in Table 5, are consistent with those of Püschel *et al*. (2014), who reported a tendency for increased flower production in *Gazania rigens*. Additionally, Asrar and Elhindi (2011) found that inoculation with arbuscular mycorrhizal fungi (AMF) enhanced flower size in *Chrysanthemum morifolium* and *Tagetes erecta.*

Higher Cd concentrations in the growing medium were found to accelerate flowering in non-mycorrhizal plants but had no significant effect on the flowering time of AMF-inoculated plants. However, increasing Cd levels slightly reduced the number of flowers overall. Growth inhibition due to Cd contamination is a well-documented phenomenon in other plant species (Rivera-Becerril *et al.,* 2002; Vassilev *et al.,* 2004). If not rapidly detoxified within plant tissues, cadmium can induce oxidative stress, which may inhibit growth and ultimately lead to cell death (Schutzendubel and Polle, 2002). Dunham *et al.* (2003) also reported that under greenhouse conditions, arbuscular mycorrhizal fungi (AMF) can reduce plant growth despite improved mineral nutrition and enhanced photosynthetic activity. The benefits of mycorrhization for Cd tolerance may be less evident in pot cultures compared to field conditions. According to Douds *et al.* (1988), in nutrient-rich media, mycorrhization can inhibit root and shoot growth due to sink competition for photosynthates, while in nutrient-poor substrates, AMF helps plants access more minerals through the external hyphae, positively influencing plant growth.

Lead is generally considered to have relatively low phytotoxicity and tends to accumulate in older leaves, limiting its transport to younger tissues (Cseh *et al*., 2000). Stunting, reduced biomass, and chlorosis are common symptoms in Pb-contaminated soils (Foy *et al.*, 1978). These effects are typically due to disrupted chlorophyll synthesis, lowered photosynthetic rates, metal toxicity, nutrient antagonism, or restricted root growth (Sharma and Dubey, 2005). In this study, Pb had minimal impact on the growth and flowering of scarlet sage, likely due to its low sensitivity to Pb. Different species show variable responses to Pb, suggesting species-specific biochemical mechanisms (Begonia, 1997). Increasing Cd doses resulted in higher Cd content in shoots of both mycorrhizal and non-mycorrhizal plants. Notably, at the highest Cd concentration, AMF-inoculated plants accumulated more Cd, corroborating findings by Guo *et al.* (1996) who showed that AM fungal hyphae can absorb and transport Cd. Similarly, Pb accumulation in scarlet sage increased with rising Pb concentration. This is consistent with reports that different plant species exhibit varying Pb uptake and transport efficiencies (Begonia, 1997). Lead can damage plant cellular structures, including cell walls, membranes, mitochondria, chloroplasts, and nuclei. Salazar and Pignata (2014) noted that this disruption impairs physiological processes like protein synthesis, respiration, cell division, and photosynthesis. The transfer factor of most treatments was below 1, indicating a relatively low physiological requirement for Pb, as seen in *Salvia splendens* (El-Shanhorey and Barakat, 2020). Plants manage Pb toxicity via internal tolerance or external exclusion mechanisms. External exclusion limits Pb entry and its accumulation in organelles, while internal tolerance involves the synthesis of organic Pb compounds like cysteine, glutathione, and phytochelatins that bind Pb into less toxic forms (Pourrut *et al*., 2011; Sharma and Dubey, 2005). Metal transport from roots to shoots occurs via xylem loading and transpiration, as discussed by Blaylock and Huang (2000).

Findings are in agreement with Talebi *et al*., (2014) for Gazania and Jaafari and Hadavi (2012) for *Ocimum basilicum L*., confirming the role of AMF in enhancing nutrient uptake, stress resistance, and rhizosphere microbial activity (Huang *et al*., 2018; Riaz *et al*., 2021). Despite low AMF colonization in wetlands due to limited oxygen (Calheiros *et al*., 2019), AMF have shown promise in enhancing pollutant removal in constructed wetlands (Tondera *et al*., 2021). Studies by Hu *et al.* (2020 a, b, 2021) demonstrated that AMF improves plant performance in constructed wetlands, including heavy metal (HMs) remediation. However, the role of AMF in handling combined HMs pollution in aquatic environments remains underexplored. Aeration is another method used to increase oxygen and improve pollutant removal efficiency (Feng *et al*., 2021). Gunathilakae *et al*. (2018) found that AMF had a stronger effect on removing high Cd concentrations (50 ppm) than lower concentrations, aligning with the current findings. According to Han *et al*. (2021) and Gao *et al.* (2021), AMF inoculation enhances Cd binding in cell walls by increasing polysaccharide and protein content, offering a primary barrier against Cd toxicity.

Additionally, AMF reduces the distribution of HMs in organelles, thus protecting plant structures (Huang *et al*., 2018). Considine and Foyer (2021) highlighted oxygen as a growth signal that influences root-shoot development and HMs distribution. Chen *et al.* (2004, 2017) showed that AMF alters Cd dynamics in soil and supports photosynthesis under Cd stress by enhancing nutrient uptake and antioxidant capacity (Li *et al*., 2016). Cd stress disrupts reactive oxygen species (ROS) balance, causing oxidative damage (Maiti *et al*., 2012). AMF helps mitigate this via secretion of organic acids and chelators, restricting Cd transport to shoots (Wang *et al*., 2010), and expanding root absorption area while promoting hormone production. Nonetheless, high Cd levels still impose toxicity even on AMF-inoculated plants. Zarei *et al.* (2008) found that AMF diversity decreased in zinc-contaminated soils. Tanaka and Yano (2010) reported improved nitrogen uptake in AMF-inoculated plants. Our findings show that *G. mosseae* increased nitrogen and phosphorus content in roots and leaves under Cd stress. This is likely due to AMF-mediated changes in soil microbial communities and root architecture, enhancing absorption of N, P, and minerals (Cuéllar *et al*., 2015; Cruz *et al*., 2017; Cui *et al*., 2019) further observed that Cd-tolerant genotypes restricted Cd translocation to leaves by downregulating GmHMA18 expression, reducing leaf toxicity (Table 7).

The results of this study demonstrate that arbuscular mycorrhizal fungi (AMF) inoculation positively influenced the growth and physiological performance of *G. splendens* under Cd and Pb stress. AMF-inoculated plants exhibited increased plant height, leaf number, branch number, and chlorophyll content compared with non-inoculated controls.

Moreover, nutrient analysis showed higher nitrogen (N) and phosphorus (P) contents in both roots and leaves of inoculated plants, indicating that mycorrhizal symbiosis enhanced nutrient uptake and translocation to aboveground tissues. These improvements may be attributed to AMF-mediated modifications of root morphology, increased root surface area, and changes in the rhizosphere microbial community that promote mineral acquisition. In addition, AMF contributed to the sequestration of heavy metals in the roots, limiting their translocation to shoots and thereby reducing potential toxicity. Collectively, these findings highlight the dual role of AMF in enhancing nutrient uptake and mitigating heavy metal stress, underscoring their potential for *G. splendens* cultivation in contaminated soils.

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