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Research Article

Optimizing soybean (Glycine max (L.) (PS 1347) seed protein accumulation under lead stress through mycorrhizal fungi mediation

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Abstract
Lead is among the most toxic and harmful heavy metals to living things, including plants. It is absorbed through roots from soil and causes several detrimental impacts on plant functioning. The present study aimed to elucidate the ameliorative effects of arbuscular mycorrhizal (AM) fungi on soybean (*Glycine max* (L.) variety PS 1347) under lead (Pb) stress by investigating growth parameters, yield attributes, and seed protein characteristics. Following established legume Pb tolerance thresholds and soil Pb limits, Pb (as $Pb(NO_3)_2$) was introduced into soil-filled polybags at three concentrations: 200, 500, and 700 mg/kg of soil. In addition to inoculating polybags with AM fungi *Glomus mosseae* and *G. fasciculatum*, both individually and in combination, the fungi were utilized for seed priming Vesicular Arbuscular Mycorrhiza (VAM) powder, 25g/kg of seeds. Without AM fungi, Pb stress negatively impacted all growth parameters, yield metrics, and seed protein characteristics. At 200 and 500 mg Pb concentrations, individual *Glomus* species exhibited greater effectiveness in enhancing plant growth and yield attributes and mitigating Pb effects, while dual *Glomus* species treatment was more effective in improving the soybean growth characteristics with higher Pb concentration (700 mg). The analysis also showed that Pb toxicity in soybean plants decreased seed protein content to 7 %, which was restored by applications of *Glomus* treatments. Thus, given their pivotal role in optimizing soybean seed protein quantity and quality, these two AM fungal species are recommended for application to enhance plant performance in Pb-affected soil.

Keywords: AM fungi, *Glomus*, Lead toxicity, Seed proteins, Soybean

INTRODUCTION

Soybean, also known as *Glycine max* L., belongs to the Fabaceae family, and it is one of the world's most important crops due to its oil and protein-rich seeds that find diverse uses in various food items (Anderson *et al*., 2019). The soybean seeds contain an average protein content of 36-44%, making them a valuable source of high-quality plant protein. This protein is renowned for its nutritional value and its versatility in various industries (Małecki *et al*., 2021). However, the productivity and protein content of soybean seeds are greatly affected by environmental conditions. One notable threat is the presence of heavy metals, specifically lead (Pb), which can significantly impact the growth, yield, and accumulation of protein in the seeds (Li *et al*., 2023).

The widespread presence of lead in the environment, caused by human activities, has been extensively documented as a pollutant with detrimental effects on plant growth and development (Kumar *et al*., 2020). Lead disrupts crucial physiological processes, hindering growth and compromising the productivity of crops (Madhu and Sadagopan 2020). As a result, food security is put at risk. Furthermore, when plants absorb lead, it contaminates the food chain, posing harmful consequences for human health and the environment (Meena *et al*., 2020).

Implementing innovative strategies is crucial in addressing the challenges posed by lead stress on soybean crops and simultaneously enhancing seed protein

production. One promising approach is the symbiotic association between soybean plants and arbuscular mycorrhizal (AM) fungi (Adeyemi *et al*., 2021). These fungi are commonly present in the rhizosphere and can improve plant performance in adverse conditions. By aiding nutrient uptake, enhancing stress tolerance, and promoting overall plant growth, AM fungi offer a viable solution for mitigating the impact of lead stress on soybean crops (Dhalaria *et al*., 2020).

This research endeavours to elucidate the potential of AM fungi to optimize soybean seed protein production in the presence of lead stress, a critical and timely inquiry given the escalating challenges of soil contamination and the pressing need to enhance protein-rich crop yields sustainably.

Through a comprehensive assessment of vegetative growth, chlorophyll content, seed protein profiles, and yield components, this research aims to contribute to understanding the mechanisms by which AM fungi mediate soybean resilience to lead stress, ultimately offering a pathway toward sustainable soybean cultivation in contaminated soils.

MATERIALS AND METHODS

Location, polybags and soil

In the northern part of India's Haryana state, the experimental site used for this study is controlled by the Department of Botany at Kurukshetra University. The location's geographic coordinates are approximately N 29°.95903 and E 76° 813125. The samples of both clay and sandy soil types were taken from a depth of 12 to 15 centimetres, air-dried, ground, and then analysed for their physical and chemical characteristics (Table 1). Each 15x16-inch polybag (recyclable and UV-protected) was sterilised before being filled with 10 kg of the prepared soil.

Seed variety, inoculum and seed priming

Soybean (Glycine max (L.) seeds of PS 1347 variety were sourced from the National Seed Corporation Ltd. (NSC) in Kota, India. This particular cultivar boasts resistance to a spectrum of pathogens, including yellow mosaic virus, soybean mosaic virus, bacterial pustule, and bacterial leaf blight (BLB).

The inoculum of fungi *Glomus mosseae* and *G. fasciculatum*, comprising soil, spores, mycelium, and fragments of maize roots densely colonized by mycorrhizal fungi, was procured from the Laboratory of Plant Pathology within the Department of Botany at Kurukshetra University in Haryana, India.

To ensure the sterilization of the soybean seeds, a 5% sodium hypochlorite solution (v/v) was employed, followed by thorough rinsing with distilled water to eliminate any residual disinfectant. Subsequently, the seeds were subjected to inoculation with *G. mosseae* and *G. fasciculatum*, individually or in combination, with each seed uniformly coated with 25g of VAM powder per kg of seeds.

Field experimental setup

The experiment was conducted following a completely randomized block design of three sets. The first set served as a control group without any seed or soil treatments. In the second set, three control groups were exposed to a different level of lead (Pb) contamination. The third set encompassed nine distinct treatment conditions, incorporating both single and dual combinations of *Glomus* species along with the three Pb levels, as detailed in Table 2. Consequently, three sets, each comprising five replicates, were employed in this experimental setup.

Subsequent to seed priming, the seeds were sown in individual polybags, with an additional 15 grams of AM fungi (equivalent to approximately 40 spores per gram of soil) introduced into each polybag one week after germination had commenced. Plant thinning was carried out to ensure optimal growth conditions, maintaining a consistent five plants per polybag population.

Preparation of heavy metal solutions and plant soil treatment

Lead nitrate ($Pb(NO_3)_2$) was employed in this study at three different concentrations, i.e., 200 mg, 500 mg, and 700 mg per kilogram of polybags soil. These Pb solutions were administered at distinct stages of plant growth and development: the initial treatment occurred during the vegetative phase, the second application coincided with the blooming stage, and the final exposure occurred before pod formation. Comprehensive assessments of morpho-physiological parameters were conducted and meticulously documented throughout the experiment.

The soybean crop, subject to these controlled Pb exposures, underwent a maturation period lasting approximately 120 days. This maturation process yielded compact, determinate plants characterized by tawny pubescence and the development of bright yellow seeds. Subsequently, to facilitate protein analysis, mature seeds were collected, dried, and processed into seed

Table 1. Chemical composition of experimental soil

Soil type	Sandy loam soil
pH	7.50
EC (ds/m)	0.52
Organic carbon (%)	0.56
Available N (ppm)	64.0
Available P (ppm)	0.61
Available K (ppm)	53.0
Available S (ppm)	71.0

meals for further investigation.

Morphological and physiological parameters analysis

This study assessed various plant morphological attributes (Shoot length (cm),number of branches/plant, number of leaves/plant) to comprehensively understand the impact of experimental conditions. Shoot length was measured in centimetres using a meter scale. The quantification of branches and leaves per plant was conducted manually, starting from the base and extending to the tip of each plant. Additionally, chlorophyll and carotenoid content were quantified according to the procedures described by Arnon (1949) and Holden (1965).

Yield attributes

Yield attributes were assessed following the protocols established by Hussain *et al*. (2019). The quantification of pods per plant involved the random selection of ten plants from each treatment, followed by the enumeration of pods (each containing at least one seed). Subsequently, the average number of pods per plant was calculated. To determine the number of seeds per pod, 25 pods were randomly selected from each treatment, and the same approach was employed to ascertain the number of seeds per plant, using ten randomly selected plants from each treatment. Seed weight was determined by cleaning and crushing the pods from each replicate, and the resulting seed weight per plant was measured in grams per plant. For the assessment of 100-seed weight, one hundred seeds were randomly chosen from each treatment and weighed individually. Twenty-five pods were randomly selected from each treatment to establish pod weight, and the average weight per pod was calculated accordingly.

Defatting of seed meal

The finely ground seed meal underwent a defatting process using hexane (1g seed meal per 10ml hexane) at a temperature of 4ºC, lasting for two hours. Following this, centrifugation was carried out for 10 minutes, leading to the separation and removal of the supernatant. This defatting process was repeated to ensure thorough removal of lipids. Subsequently, the residual seed meal, now devoid of lipids, was subjected to vacuum drying, following the procedure outlined by Singh and Matta (2008). This lipid-free seed meal was then employed in various protein characterization studies.

Protein quantification

The total seed protein content was determined using the semi-micro Kjeldahl method, following the protocol described by Welcher in 1963. In this method, seed meal was initially digested with sulfuric acid in the presence of a mixture containing copper sulfate, selenium dioxide, and potassium dichromate in a 1:2:1 ratio. Subsequently, the digestion mixture was subjected to distillation using Markham's distillation assembly, where 40% NaOH was employed to release ammonia. The liberated ammonia was then trapped in boric acid and subsequently titrated volumetrically against N/40 HCl to quantify the nitrogen content. The protein content was calculated by multiplying the nitrogen content by a factor of 6.25. To distinguish between the 7S and 11S globulin sub-fractions, the methods outlined by Thanh and Shibasaki in 1976 were employed. Furthermore, the protein concentration within the isolated sub-fractions was determined utilizing Bradford's method, as detailed by Bradford (1976).

Preparation of total seed protein extract

Extraction of defatted seed meal involved suspending 30 mg of the material in 1 ml of a 0.025M Tris-HCl buffer (pH 6.8) containing 2% sodium dodecyl sulfate (SDS). The resulting suspension was subjected to heat treatment on a water bath set at 80°C for a duration of 40 minutes. Subsequently, the mixture was centrifuged at 12,000g for 10 minutes to separate the supernatant. This supernatant was then combined with a solution comprising 10% (v/v) glycerol and 2% 2-mercaptoethanol, creating reducing conditions. The resulting mixture was further heated at 95°C for 10 minutes before loading onto gels, as previously described (Singh *et al*., 2021)

SDS-polyacrylamide gel electrophoresis

The electrophoretic separation of the total seed protein extract followed the discontinuous gel system initially devised by Davis (1964) and Ornstein (1964), which followed the formulation proposed by Laemmli (1970). This separation process was conducted on a 14% polyacrylamide SDS-gel under reducing conditions. Stacking and separation of proteins were achieved using direct currents of 18 mA and 32 mA, respectively. Subsequently, protein bands were visualized through staining with Coomassie Brilliant Blue R-250, and their respective molecular weights were determined by referencing them to standard marker proteins co-electrophoresed on the same gel.

Densitometric scanning of gel

Densitometry analysis of polypeptides was conducted using the Gelanalyzer software, a powerful tool designed to process gel images acquired through scanning, camera capture, or digital recording. This software offers a robust platform for band identification and comparative analysis, taking into account the comparative electrophoretic mobility (Rf) of the bands. Furthermore, it possesses the capability to assess bands with less distinct resolutions. Gel-Analyzer employs a sophisticated algorithm to calculate normalized coordi-

nates for the identified bands. It also conducts spectral analyses to detect similarities or variations in band components, visually presenting the results for quantitative evaluation and comprehensive band analysis. To streamline the present data presentation and facilitate clarity in the graphs, all raw values collected during the present study were scaled down by a factor of one thousand during the computational analysis.

Statistical analysis

The data were presented as means with their corresponding standard errors based on five replicates for each dataset. Statistical analysis was performed using SPSS 17.0 to assess the collected data.

RESULTS

Growth and developmental variables

The shoot length in control plants was 58.1 cm and at the first concentration level of Pb (200 mg), the shoot length decreased to 55.6 cm. However, when *Glomus* species were used individually or in combination, the shoot length reached a maximum of 57.7 cm. At 500 mg Pb concentration, the shoot length decreased to 53.4 cm, significantly less than the control. However, the shoot length improved when the plant was inoculated with *G. mosseae, G. fasciculatum*, or both AM fungi species, ranging from 55.5 cm to 55.9 cm (Table 3). Without Pb and AM fungi treatment, the number of branches per plant was 11.9 and they decreased to 9.8 at the first Pb concentration level (200 mg). With increasing Pb stress, there was generally a steady decline in the number of branches per plant. Increasing the Pb concentration 500 to 700 mg, significantly reduced the number of branches. At both Pb levels, dual seed treatment with AM fungi improved the number of leaves per plant compared to the control and individual treatment.

Photosynthetic pigments

The results suggested that AM fungi had a beneficial effect on soybean plants under Pb stress. Without AM fungi seed inoculation, Pb stress significantly decreased the concentration of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids). The lowest concentrations of photosynthetic pigments were found in the absence of AM fungi at the highest Pb concentration (700 mg), reflecting substantial damage to the plant's photosynthetic activity (Figure 1). Also, much better results were recorded with dual seed treatment of both *Glomus* species. At the highest Pb concentration of (700 mg), the presence of both AM fungi species contributed to slightly improved levels of photosynthetic pigments compared to the plants without AM fungi. However, the degree of improvement was smaller than at the lower Pb level.

Yield parameters

In the absence of Pb, the control plants exhibited optimum yield parameters. However, increased Pb concentrations from 200, 500, and 700 mg affected the yield parameters (Table 4). At 200 mg Pb, *G. mosseae* and *G. fasciculatum* treatments positively influenced the yield parameters. The number of pods per plant, number of seeds per pod, and number of seeds per plant were relatively higher when the plants were treated with both *Glomus* species. At the highest Pb concentration (700 mg), a significant reduction in the number of pods per plant, number of seeds per pod, and number of seeds per plant was observed compared to 200 and 500 mg Pb. It is significant to note that when cultivated with *Glomus* species, soybean plants showed improved growth and yield parameters (Tables 3 and 4) under Pb stress, suggesting that they thrived and flourished with

Table 2. Experimental treatment allocations (G1= *Glomus mosseae*, G2= *G. fasciculatum*)

1 C-0	**Control**	**Without any amendments (no Pb, no AM fungi)**
2 C-1	Only with Pb treatments	Pb (200, 500 and 700 mg/kg)
6 C-2		
10 C-3		
3 T-1	With AM fungi treatments	Pb 200mg + G1
4 T-2		Pb 200mg + G2
5 T-3		Pb 200mg +G1+G2
7 T-1		Pb 500mg + G1
8 T-2		Pb 500mg + G2
9 T-3		Pb 500mg + G1 + G2
11 T-1		Pb 700mg + G1
12 T-2		Pb 700mg + G2
13 T-3		Pb 700mg + G1 + G2

Table 3. Morphological parameters of soybean plants grown under different Pb and seed inoculation concentrations with two *Glomus* species

Set of treatments	Lead Treatment	VAM Treatment	Shoot length (cm)	No. of branches/plant	No. of leaves/plant
1C-0	NA	NA	58.1 ± 1.60^{c}	11.9 ± 1.10^{ab}	59.8 ± 1.15^{c}
2C-1	Pb level 1	None	55.6 ± 1.56^{b}	9.8 ± 0.96^{a}	55.3 ± 1.11^{b}
3T-1	Pb level 1	G. mosseae	57.5 ± 1.65^{d}	11.4 ± 1.21^{c}	57.3 ± 1.19^{bc}
4T-2	Pb level 1	G. fasciculatum	57.7 ± 1.43^{a}	11.4 ± 1.09^{a}	57.3 ± 1.21^{d}
5T-3	Pb level 1	Both	57.7 ± 1.58^{c}	11.6 ± 1.15^{b}	57.7 ± 1.05^{a}
6C-2	Pb level 2	None	53.4 ± 1.46^{a}	8.4 ± 1.18^{bc}	52.2 ± 1.12^{b}
7T-1	Pb level 2	G. mosseae	55.5 ± 1.62^{d}	10.2 ± 1.16^{b}	52.7 ± 1.09^{a}
8T-2	Pb level 2	G. fasciculatum	55.6 ± 1.42^{a}	10.4 ± 1.25^{d}	55.4 ± 1.18^{c}
9T-3	Pb level 2	Both	55.9 ± 1.54^{b}	11.0 ± 1.10^{ab}	56.8 ± 1.15^{c}
10C-3	Pb level 3	None	51.1 ± 1.70^{e}	7.3 ± 0.98^{a}	48.6 ± 1.74^{f}
11T-1	Pb level 3	G. mosseae	52.8 ± 1.81^{e}	8.4 ± 1.05^{b}	52.0 ± 1.61^{e}
12T-2	Pb level 3	G. fasciculatum	53.0 ± 1.55^{b}	8.8 ± 0.89^{a}	52.5 ± 1.40^{d}
13T-3	Pb level 3	Both	53.9 ± 1.36^{a}	9.4 ± 0.95^{a}	53.8 ± 1.35^{d}

Each value is a mean of five replicates, ± SE and means followed by same letter/s are not significantly different at P ≤ 0.05

more effective morphological and yield attributes.

Seed protein content

The degree of Pb stress and the accumulation of seed proteins showed a negative correlation (Figure 2). *G. fasciculatum* was especially effective in ameliorating Pb level 1 and 2, leading to higher seed protein content. However, at the highest Pb concentration level (700 mg), the collective use of both *Glomus* species was more effective in restoring the seed protein content (41.56%) compared to the control plants (38.5%). This enhancement in seed protein was approximately 7%. Notably, the seed protein content was significantly higher (42.1%) without Pb stress or any AM fungi treatment. It suggests that Pb stress, at even low concentrations, had a negative impact on seed protein accumulation in soybean plants. Furthermore, the presence of AM fungi, particularly *G. fasciculatum*, or dual treatment of *Glomus* species could reduce the detrimental effects of Pb stress.

Content of globulin subfractions

With a single or dual inoculation of the *Glomus* species (Figure 3), at different Pb concentrations, glycinin and β-conglycinin content varied considerably. The β-conglycinin fraction varied from 22.1% (control) to 27.6% (dual treatment) at 200 mg Pb stress, while glycinin ranged between 24.6% (control) and 26.3% (dual treatment). In the second set, with a 500 mg Pb concentration, the recorded content of β-conglycinin and glycinin in the control plants was 21.5% and 24.3%, respectively. However, they were recorded as highest in *G. fasciculatum*-treated plants. In the last set, with 700 mg Pb concentration, the β-conglycinin and glycinin contents were highest in plants with both fungi compared to the control. In the control sets without any Pb stress or AM fungi treatment, the β-conglycinin and glycinin content were 26.7% and 27.6%, respectively.

SDS-polyacrylamide gel electrophoresis

Improved globulin sub-fractions were observed when both AM fungi were co-inoculated, as revealed by the darkness of bands on the gel (Figure 4). These intensity differences were also noticed by analysing the gel with the Gel-analyzer software. The control plants treated with 200 mg Pb, the band intensities of α and α' (of β-conglycinin) were 3.56 and 1.78, respectively (Figures 5 and 6). However, the band intensities increased with the addition of the *Glomus* species. Regarding the glycinin fraction, the band intensity of the acidic and basic subunits was 3.45 and 7.82, respectively, in the control plants treated with 200 mg Pb. Similarly, for the basic subunit, the band intensities were 8.06 (*G. mosseae*), 7.37 (*G. fasciculatum*), and 7.63 (dual treatment).

The influence of a 500 mg concentration of Pb on seed protein fractions in soybean and the counter effects of AM fungi is depicted in Figures 7 and 8. With the addition of *Glomus* species treatments (*G. mosseae*, *G. fasciculatum* and dual inoculation), the band intensity values for α' increased to 2.27, 2.63, and 3.47. The band intensities for the glycinin fraction i.e., acidic and basic subunit, also improved with and independent treatment. When both *Glomus* species were simultaneously inoculated, the intensities of the acidic and basic subunits were noted as 5.46 and 9.49, respectively.

In the third set, with the highest Pb concentration (700 mg), the band intensities of the 7S α', α, and β subunits

Table 4. Yield attributes of soybean plants grown under different concentrations of Pb and seed inoculation with two *Glomus* species

Sets of treatments	No. of Pods/ plant)	No. of seeds/ pod	No. of seeds/ plant	Seed Weight (g)	100 seed weight (g)	Pod weight (g)
1C-0	22.5 ±1.51^{cd}	3.1 ± 0.24^{c}	69.7± 2.28^{ab}	0.17 ± 0.02^{a}	17.5 ± 1.07^{a}	0.57±0.02^{a}
2C-1	18.7 ± 1.48^{c}	2.7 ± 0.22^{b}	50.5 ±2.47^{cd}	0.14 ± 0.01^{a}	14.3 ± 1.36^{b}	0.46±0.05^{b}
3T-1	22.2 ± 1.17^{a}	2.9 ± 0.28^{c}	64.4 ± 2.35^{c}	0.15 ± 0.04^{c}	15.2 ± 1.52^{d}	0.49±0.06^{b}
4T-2	22.1 ± 1.41^{c}	2.9 ± 0.25^{b}	64.1 ± 2.25^{a}	0.15 ± 0.05^{d}	15.6 ±0.49^{cd}	0.50±.08^{cd}
5T-3	22.3 ± 1.69^{e}	3.0 ± 0.21^{ab}	66.9 ± 2.37^{c}	0.16 ± 0.02^{a}	16.3 ± 1.62^{e}	0.54±0.05^{b}
6C-2	17.8 ±1.39^{bc}	2.4 ± 0.20^{a}	42.7 ± 2.11^{a}	0.12 ± 0.04^{c}	12.6 ± 1.21^{a}	0.35±0.04^{a}
7T-1	19.1 ± 1.48^{c}	2.5 ± 0.19^{ab}	47.7 ± 2.24^{a}	0.14 ± 0.06^{e}	14.7 ± 1.31^{b}	0.41±0.09^{d}
8T-2	19.2 ± 1.23^{a}	2.6 ± 0.16^{a}	49.9 ±2.32^{ab}	0.15 ± 0.01^{a}	15.1 ±1.54^{cd}	0.46±.05^{ab}
9T-3	20.6 ± 1.34^{b}	2.8 ± 0.14^{a}	57.7 ±2.41^{cd}	0.15 ± 0.03^{b}	15.7 ± 1.40^{c}	0.49±0.07^{c}
10C-3	16.4 ±1.60^{de}	1.9 ± 0.26^{bc}	31.1 ± 2.49^{d}	0.11 ± 0.05^{d}	11.8 ±1.35^{bc}	0.27±0.06^{b}
11T-1	17.3 ±1.52^{cd}	2.2 ± 0.22^{b}	38.0 ± 2.72^{e}	0.12 ± 0.02$_{a}$	12.7 ± 1.67^{e}	0.32±0.08^{c}
12T-2	17.4 ± 1.79^{e}	2.2 ± 0.29^{d}	38.3 ± 2.40^{c}	0.12 ± 0.04^{c}	12.8 ± 1.46^{c}	0.33±0.09^{d}
13T-3	17.9 ± 1.42^{c}	2.2 ± 0.31^{d}	40.1 ± 2.75^{e}	0.13 ± 0.03^{b}	13.1 ± 1.69^{e}	0.35±0.10^{e}

Each value is a mean of five replicates, ± SE and means followed by same letter/s are not significantly different at P ≤ 0.05

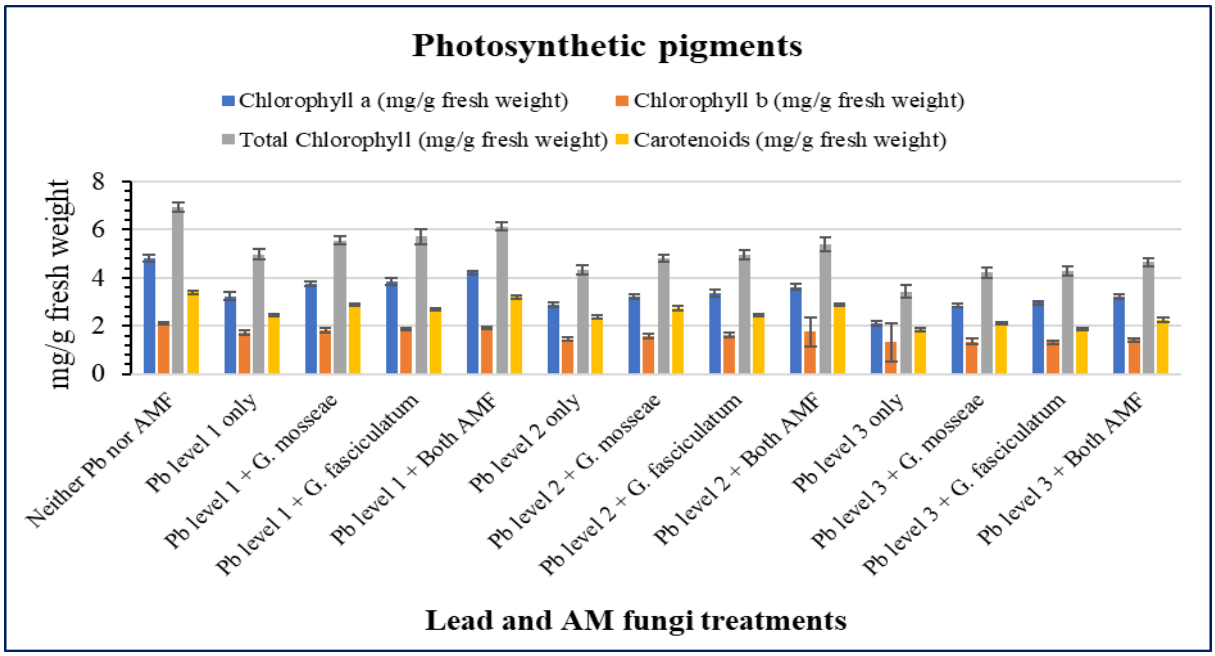

Fig. 1. *Photosynthetic pigments of soybean plants grown under different concentrations of Pb and seed inoculation with two Glomus species*

were measured as 1.62, 2.15, and 2.87, respectively (Figures 9 and 10). Co-inoculation of both *Glomus* species resulted in band intensities of 3.22 (α'), 4.99 (α), and 5.27 (β). Regarding the glycinin subunits, the acidic and basic had intensities of 2.67 and 5.68, respectively, in control plants. Both subfractions (11S and 7S) displayed almost identical degrees of enhancement in band intensity with AM fungi treatment. *G. fasciculatum* and co-inoculation of both AM fungi performed better under the Pb stress.

DISCUSSION

The present study for our work shows seed priming and inoculation of AM fungi significantly enhanced soybeans' growth and morphological characteristics, including shoot length, number of branches and leaves per plant. However, the increased supply of Pb in the soil negatively impacted growth and developmental parameters, particularly at higher concentrations. In present work, the shoot length decreased to 53.4 cm,

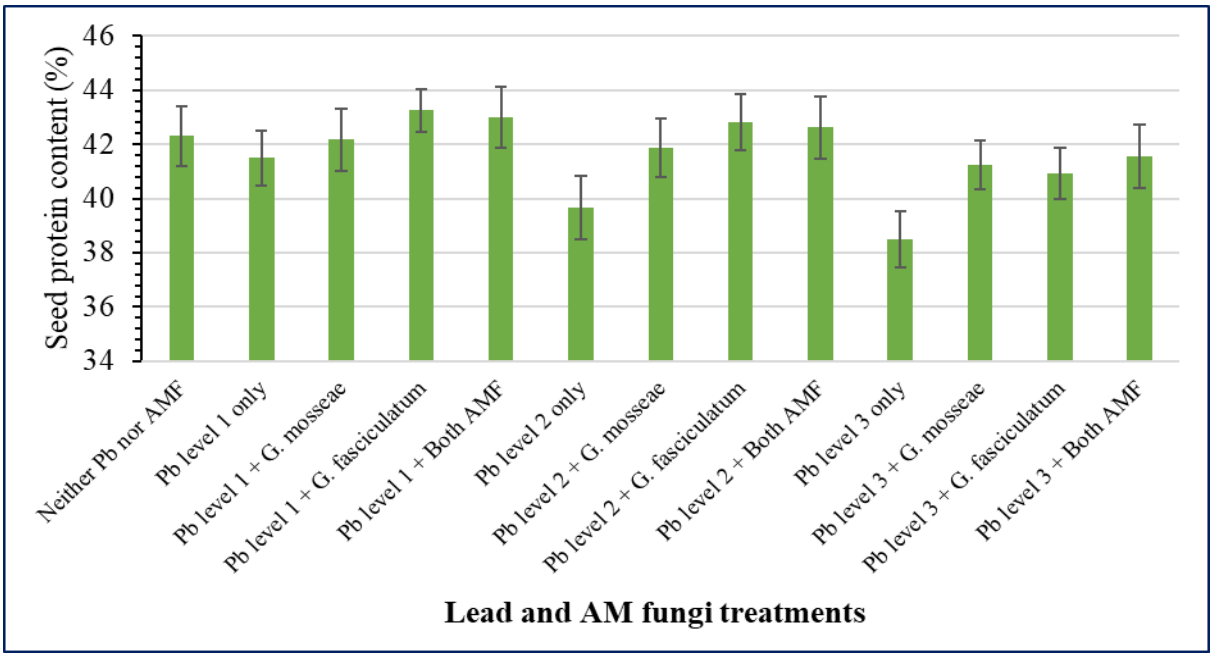

Fig. 2. *Seed proteins content (%) of soybean plants grown under different concentrations of Pb and seed inoculation with two Glomus species*

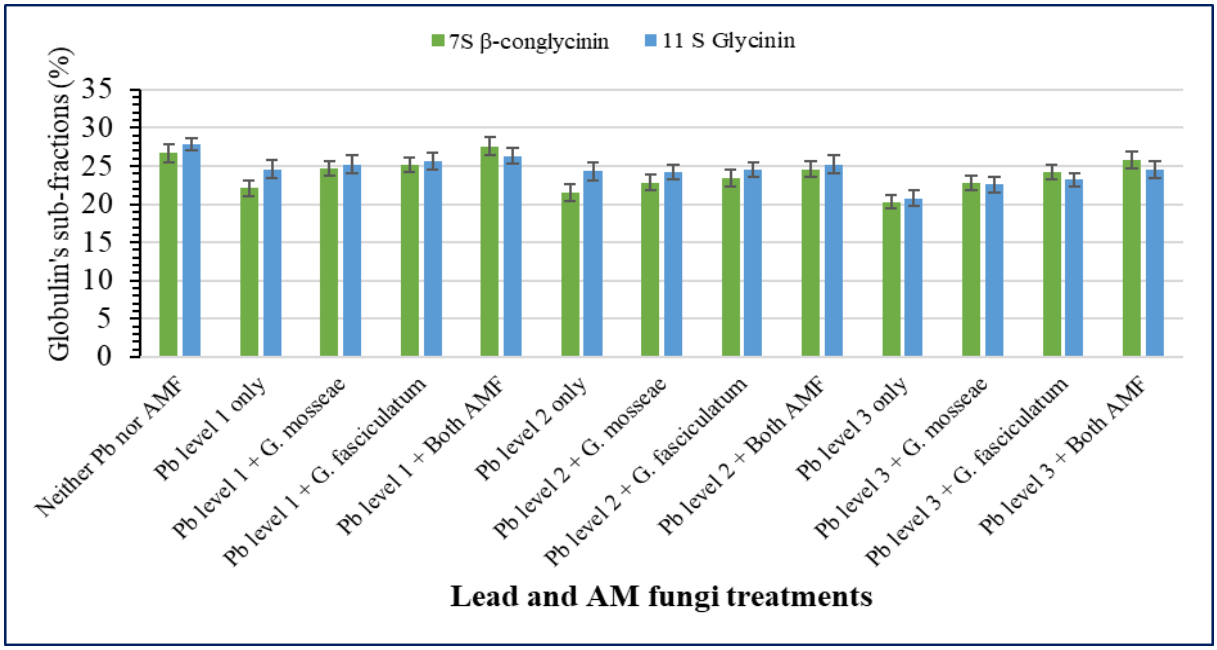

Fig. 3. *Globulins sub-fractions in seeds of soybean plants grown under different concentrations of Pb and seed inoculation with two Glomus species*

significantly less than the control 58.1 cm at a 500 mg Pb concentration, which improved with *G. fasciculatum* to 55.9 cm. It has been demonstrated that AM fungi improve the growth and yield parameters of wheat (Raghib *et al*., 2020), faba bean (Pereira *et al*., 2019), chickpea (Kumar and Naik, 2020), carrot (Yadav *et al*., 2021), soybean (Amit *et al*., 2024), etc. Arbuscular mycorrhizal (AM) fungi have been found to enhance plant growth and yields under heavy metal stress by facilitating nutrient uptake (Miransari, 2017). AM fungi are crucial bioagents that produce fungal structures, such as arbuscules, which facilitate the exchange of inorganic chemicals and minerals and serve as a biological filter for heavy metals, avoiding their accumulation in the plant system (Sharma *et al*., 2023). Studies conducted by Diagne *et al.* (2020) have highlighted the role of AM fungi in reducing heavy metal toxicity and enhancing plant growth and morphology.

Regarding photosynthetic pigments, AMF enhanced chlorophyll a, b, total chlorophyll, and carotenoid content under Pb stress conditions. *G. fasciculatum* and *G. mosseae* treatments showed promising effects on photosynthetic pigments, indicating improved photosynthetic activity. Such positive enhancement in photosynthe-

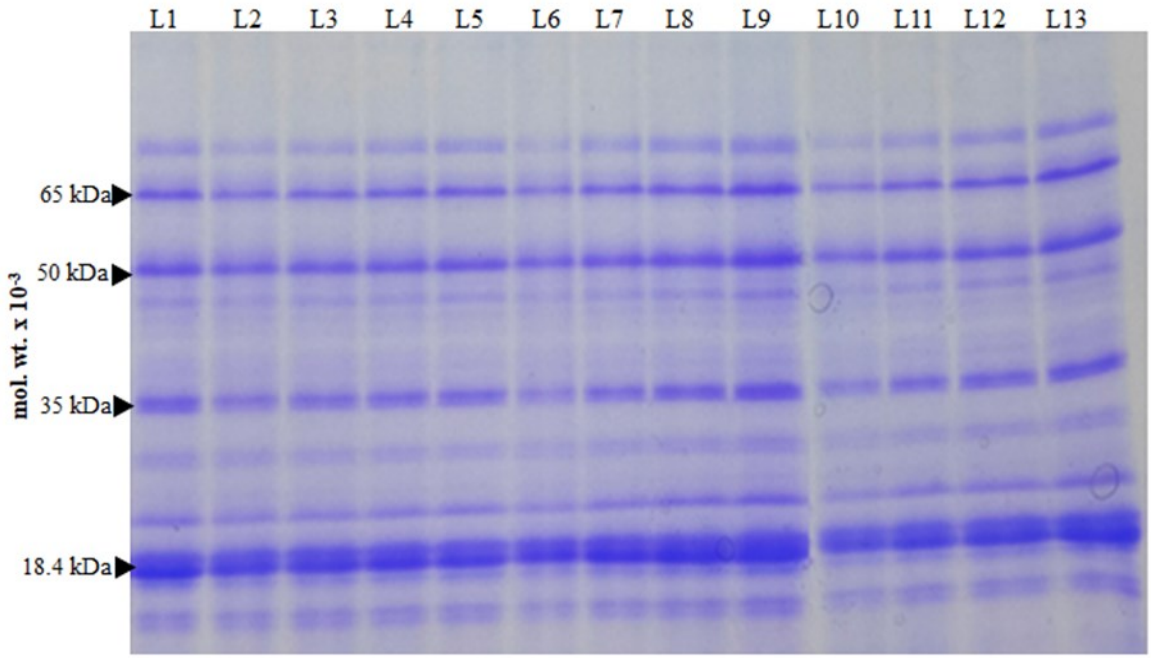

Fig. 4. *SDS-polyacrylamide gel electrophoresis of AM fungi inoculated soybean seeds at different concentrations of Pb below*

Lane on SDS-gel	Treatment sets	Lead and AM fungi treatments	
L1	1C-0	No Pb	No AM fungi
L2	2C-1	200 mg Pb	None
L3	3T-1		*G. mosseae*
L4	4T-2		*G. fasciculatum*
L5	5T-3		Both
L6	6C-2	500 mg Pb	None
L7	7T-1		*G. mosseae*
L8	8T-2		*G. fasciculatum*
L9	9T-3		Both
L10	10C-3	700 mg Pb	None
L11	11T-1		*G. mosseae*
L12	12T-2		*G. fasciculatum*
L13	13T-3		Both

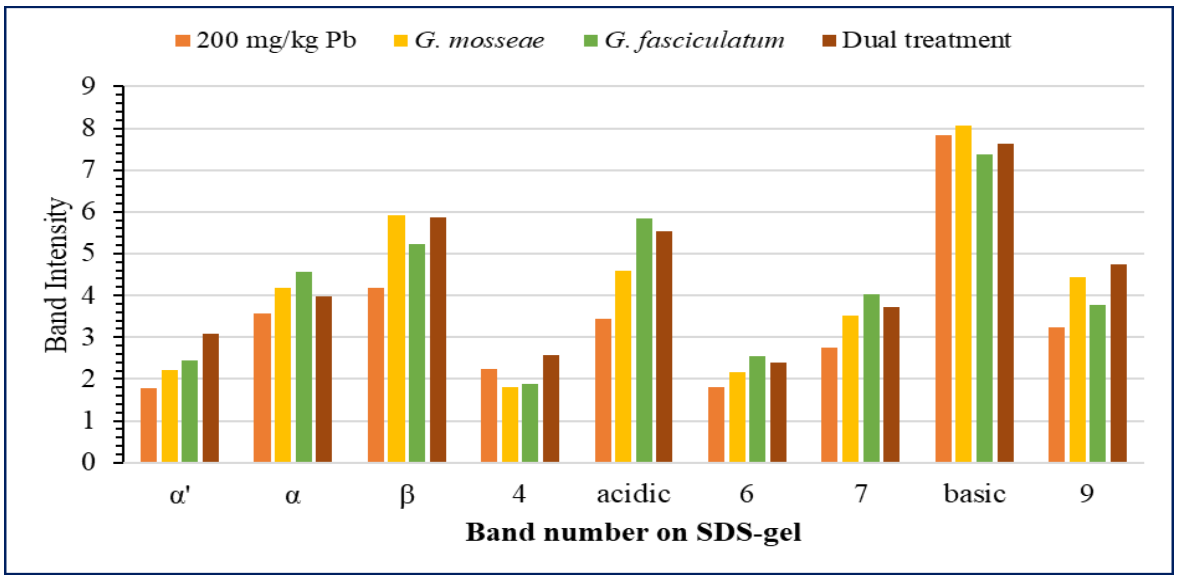

Fig. 5. *Changing band intensity in Glomus species inoculated soybean seeds at 200 mg Pb concentration*

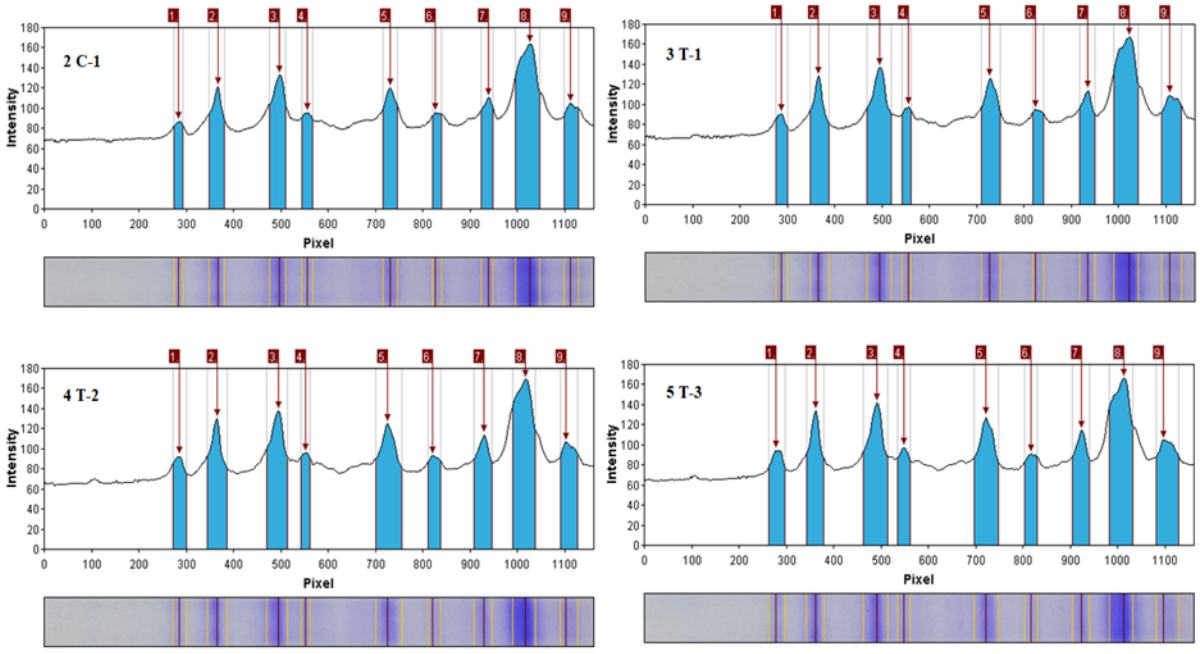

Fig. 6. *Band intensities (recorded with Gel-analyzer) at 200 mg of Pb without and with G. mosseae and G. fasciculatum treatments* (2C1- Control 1, 3T-1 treatment with *G.Mossese*, 4T-2 treatment with *G. fasciculatum and 5T-3 with dual Glomus application*)

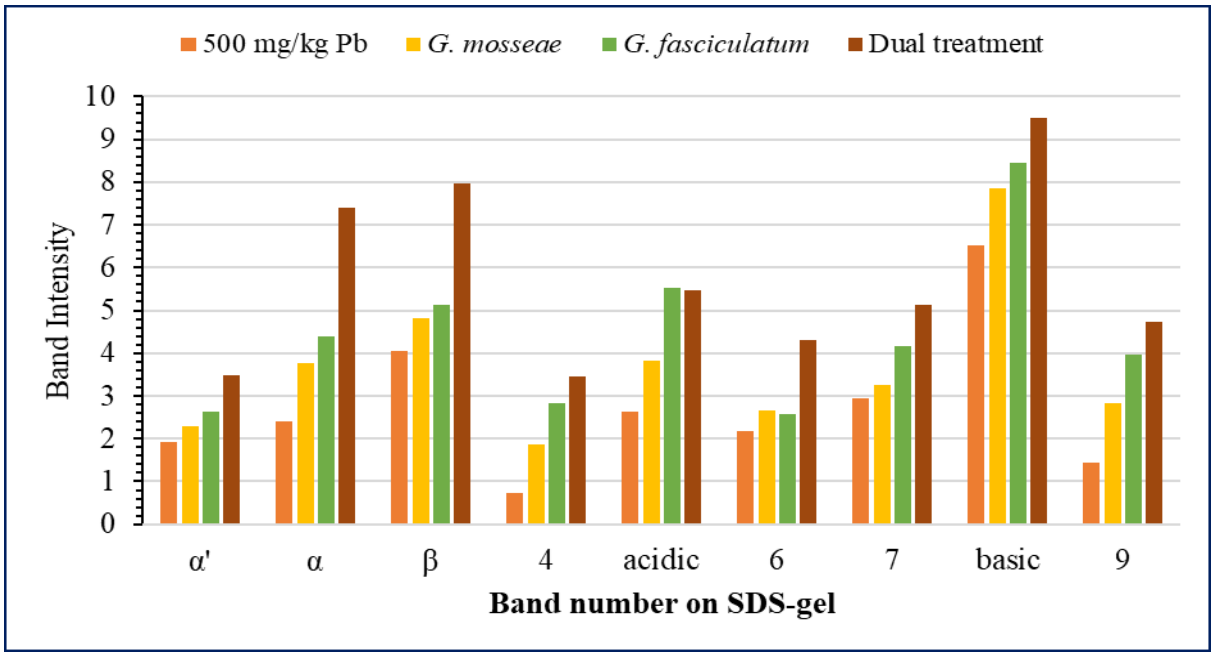

Fig. 7. *Changing band intensity in Glomus species inoculated soybean seeds at 500 mg Pb concentration*

sis with AM fungi has also been reported in many different crops like wheat (Raghib *et al*., 2020), rice (Tisarum *et al*., 2019), barley (Khan, Shah and Tian 2022), maize (Saboor *et al*., 2021) etc. The present study revealed the potential benefits of AM fungi in improving crop production under heavy metal stress. A significant improvement was noticed in the number of seeds per plant 57.7 and 40.1, under both Pb concentrations 500 (42.7) and 700 mg (31.1) by collective use of both *Glomus* species. Moreover, the different AM fungi species may have varying effects on plant growth and yield under heavy metal stress, highlighting the need for further research in this area. However, it was important to note that AM fungi's optimal species and application method may vary depending on the plant species and environmental conditions.

The present results also demonstrated the harmful effects of Pb on soybean yield parameters. This agrees with studies by Cid, Pignata and Rodriguez (2020) that have reported the negative effects of Pb on soybean

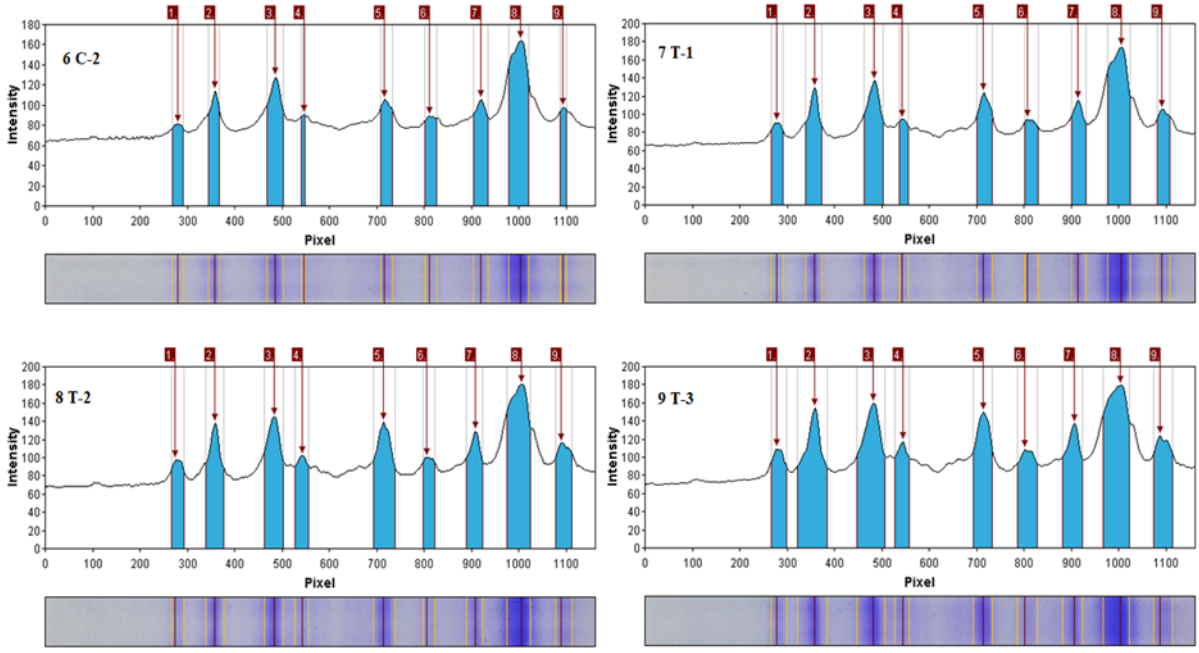

Fig. 8.*Band intensities (recorded with Gel-analyzer) at 500 mg of Pb without and with G. mosseae and G. fasciculatum treatments.* (6C2- Control 2, 7T-1 treatment with *G.Mossese*, 8T-2 treatment with *G. fasciculatum and 9T-3 with dual Glomus application*)

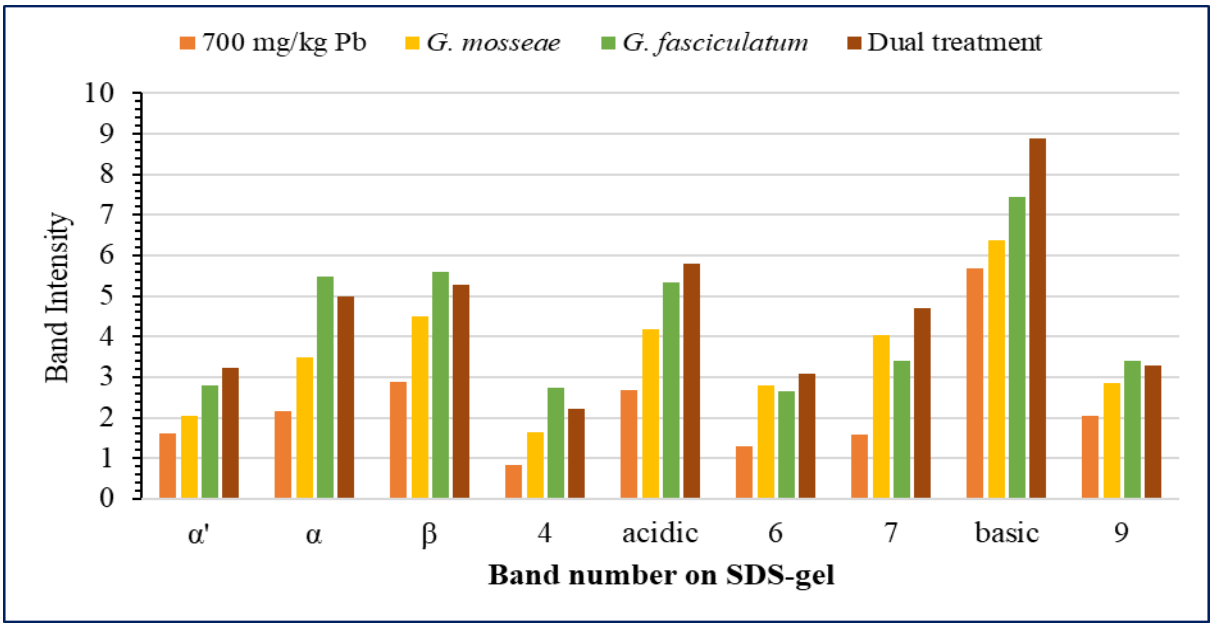

Fig. 9. *Changing band intensity in Glomus species inoculated soybean seeds at 700 mg Pb concentration*

plant growth and yield . The negative effects of Pb on soybean yield parameters can be attributed to the adverse effects of Pb on plant metabolism, including photosynthesis, respiration, and nutrient uptake (Kohli *et al*., 2020). The present analysis reported an improvement in yield parameters on inoculation of AM fungi in soybean growing under heavy metal stress. Thus, the present finding will have important implications for the sustainable production of soybean in Pb-polluted soils. They are consistent with earlier studies that reported arbuscular mycorrhizal fungi's beneficial effects on plant growth under heavy metal stress (Adeyemi *et al*., 2021)

Further, it was examined how varied Pb concentrations affected the amount of seed protein in soybean plants and the possible stress-mitigating role of *Glomus* species. The findings suggested a correlation between seed protein accumulation and the degree of heavy metal stress. The study observed that the Pb concentration increased from 500 mg to 700 mg, causing a

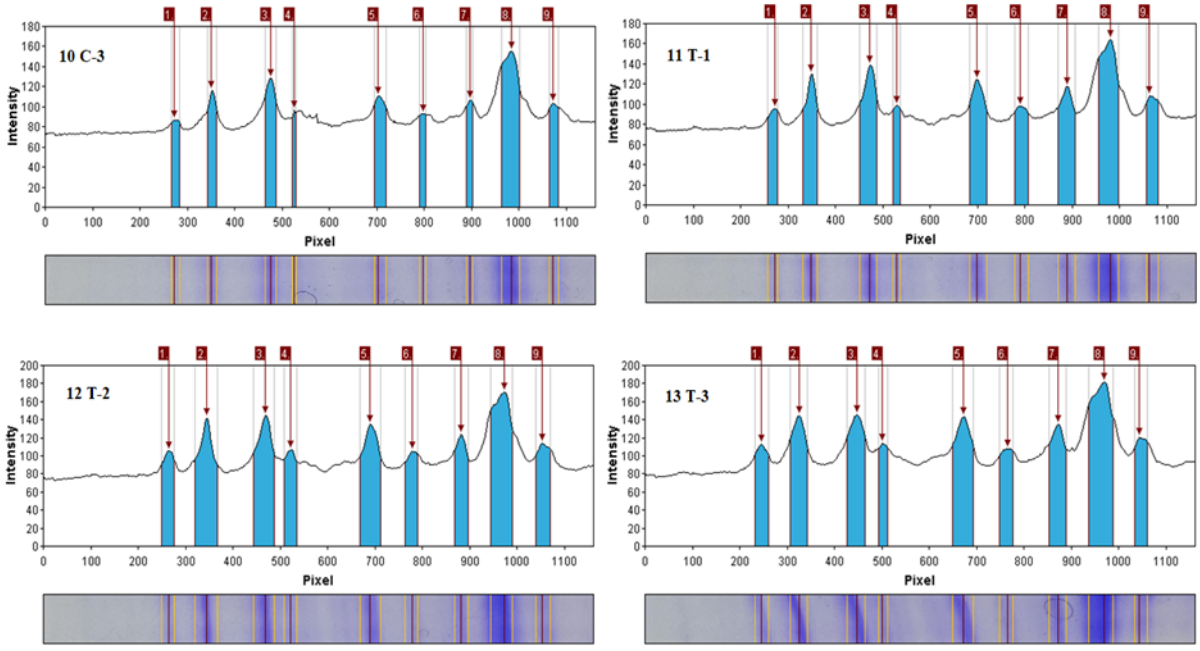

Fig. 10. *Band intensities (recorded with Gel-analyzer) at 700 mg of Pb without and with G. mosseae and G. fasciculatum treatments* (10C3- Control 3, 11T-1 treatment with *G.Mossese*, 12T-2 treatment with *G. fasciculatum and 13T-3 with dual Glomus application*)

significant decrease in seed protein content 39.6% to 38.5% compared to control 42.3%. However, it improved to 42.8% with *G. fasciculatum* and 41.5% with dual *Glomus* application. Of the two *Glomus* species, dual inoculation, positively influenced seed protein content under Pb stress. It has been found that Cd, Pb, Cr, Hg, Mn, and Co decrease dry matter, nitrogen, seed protein and production of maize plants (Ghani, 2010). Heavy metals interact with protein functional groups such as carboxyl, sulfhydryl, and amine (which are essential for protein stability and function) and this interaction causes protein damage or misfolding, hence their decreased accumulation (Tamás *et al*., 2014). Furthermore, soil contamination with heavy metals disrupts symbiotic nitrogen fixation, decreasing protein synthesis (Stambulska and Bayliak, 2020).

Mycorrhizal symbiosis helps plants absorb adequate nitrogen and phosphorus, essential for producing proteins and enzymes. Marro *et al*. (2020) reported that AM fungi increase soybean oil and seed protein content. Similarly, Golubkina *et al*. (2020) reported that rhizotrophic microbes, particularly AM fungi, led to better yield and seed protein content in chickpeas. Like previous studies, the present study also found that soybean plants inoculated with *Glomus* species have higher levels of total seed proteins than non-inoculated plants under Pb stress.

The variations in β-conglycinin and glycinin content with Pb concentration suggest that these heavy metals affect the synthesis and accumulation of seed proteins in soybean. The loss in β-conglycinin and glycinin percentage at lower Pb concentrations were minimal; however, at higher (700 mg) Pb concentration, there was a significantly reduction in both the seed fractions (20.3) and (20.8), which were improved up (25.8) and (24.5) respectively by collectively application of *Glomus* species. Similar findings have been reported in earlier studies, where heavy metals have been found to alter gene expression involved in seed protein synthesis in soybean (Bashir *et al*., 2019). AM fungi have been found to enhance the accumulation of seed protein fractions in soybean under heavy metal stress (Molina *et al*., 2020).

The SDS-PAGE analysis provided valuable insights into the qualitative and quantitative changes in seed protein fractions under Pb stress conditions. SDS-PAGE analysis on the effects of these heavy metal stress on the polypeptide patterns and changes in globulin subfractions band intensities in soybean plants, with and without AM fungi treatment revealed that Pb stress influenced the band intensities of β-conglycinin and glycinin subfractions, and the presence of *Glomus* species positively impacted seed protein accumulation. We noticed at a 700 mg Pb concentration, the band intensities of the 7S α', α, and β subunits were 1.62, 2.15, and 2.87, respectively, which was significantly increased to 3.22 (α'), 4.99 (α), and 5.27 (β) by co-inoculation with both *Glomus* species. Similarly, the 11S fractions also showed similar enhancement in band intensity with *G. fasciculatum,* proving more effec-

tive under Pb stress. *G. fasciculatum* treatment exhibited better Pb stress alleviation than *G. mosseae*, and the dual inoculation of *Glomus* species further enhanced the seed protein content. These results indicate that AM fungi, especially *G. fasciculatum,* can mitigate the negative effects of Pb stress on seed protein accumulation.

Numerous studies have reported similar effects of heavy metals and AM fungi the qualitative and quantitative alterations in soybean seed proteins (Cui *et al*., 2019; Molina *et al*., 2020; Amit and Kumar, 2023) and chickpea seed protein fractions have been seen under these heavy metal stress (Kumar and Naik, 2020; Cheema and Garg, 2022). AM fungi have also been reported to enhance the accumulations of Mg, K, Zn, Mn, and starch, resulting in increased plant resistance against abiotic stress and improved plant growth and physiology, ultimately leading to increased yield and seed protein content of many crop plants including soybean, wheat, sorghum, maize, Tomato, Sunflower etc. (Jajoo and Mathur, 2021; Wahab *et al*., 2023).

Conclusion

The present study looked into the harmful effects of lead exposure on soybean plants, specifically considering two distinct Glomus types (*G. mosseae* and *G. fasciculatum*). The findings revealed that lead negatively affected various aspects of soybean growth, physiology, yield, and seed protein characteristics. Heavy metal stress significantly reduced biomass production and photosynthetic activity, ultimately leading to an overall decline in soybean growth and yield. However, a pivotal revelation emerged when arbuscular mycorrhizal fungi (AMF) was applied. These fungal applications proved to be an effective solution to counteract the toxic effects caused by lead. It resulted in significant improvements in soybean growth and seed protein composition. Simultaneously inoculating both types of *Glomus* species could potentially be a transformative strategy, holding the key to enhancing soybean performance in lead-contaminated soils. However, further exploration is needed, focusing on adapting the use of AM fungi species to the specific dynamics of heavy metal distribution and composition in different regions. Recognizing the vital role of AM fungi in improving legume yields and mitigating metal-related challenges, the strategic approach promises to be an integral part of sustainable agricultural practices in the face of heavy metal contamination.

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Conflict of interest

The authors declare that they have no conflict of interest.

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