Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Biology

Application of Endophytic *Trichoderma harzianum* to Alleviate the Adverse Impact of Chromium in *Lupinus albus* L

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DOI: 10.36347/sajb.2023.v11i08.003 | **Received:** 12.07.2023 | **Accepted:** 16.08.2023 | **Published:** 23.08.2023

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Abstract | **Original Research Article**

The present study was conducted to understand the role of interactive usage of *Trichoderma harzianum* to alleviate the adverse impact of chromium (Cr) stress for sustainable production of the legume plants (*Lupinus albus* L.) with special reference to physiological mechanism. The exogenous application of *T. harzianum* significantly enhanced seed germination and plant growth, along with all physiological and biochemical attributes examined in lupin plant. The germination percentage of lupin seeds treated with conidia suspension of *T. harzianum* was 100% with 37% increase as compared to the control. *T. harzianum* inoculation increased seedling height (41.3%) and root length (75.6%). Treatment of *T. harzianum* also resulted in significant enhancement in the photosynthetic attributes like chlorophyll pigment synthesis (13.75% increase as compares to the control), reflecting a significant enhancement in the net photosynthetic rate. Results of our study demonstrated that *L. albus* have improved tolerance to inhibitory concentrations of Cr when inoculated with *T. harzianum*. So, we suggest the application of *T. harzianum*, which could be used to improve the quality and yield of lupin (*L. albus*) plants in Cr-contaminated areas and in fertile soils.
Keywords: Endophytic fungi; lupin plant; heavy metals; agro-ecosystems.

1. INTRODUCTION

In the modern world, several soil pollutants restrict the growth of plants. With the ongoing technological advancements in industrialization and urbanization process, release of toxic contaminants like heavy metals in the natural resources has become a severe problem worldwide. Metal toxicity affects crop yields, soil biomass and fertility. Heavy metals including chromium (Cr) are important environmental pollutants that cause toxic effects to plants (Alengebawy *et al.*, 2021); thus, lessening productivity and causing dangerous threats to the agro-ecosystems (Alengebawy *et al.*, 2021). A variety of natural and anthropogenic activities lead to Cr contamination of water and soil, potentially leading to Cr accumulation in crops and causing serious health risks in humans and animals (Singh *et al.*, 2022). Chromium toxicity is a major problem in agricultural soils that negatively affects a plant's metabolic activities. It reduces biochemical and antioxidant defense system's activities (Kumar, 2021). Chromium toxicity causes chlorosis, growth retardation, wilting of top and injury of roots (Ghori *et al.*, 2019). White lupin (*L. albus*) is an annual legume traditionally cultivated around the Mediterranean. It is used for human consumption, green manuring and as forage. The composition of the grain, especially the high protein content makes white lupin highly suitable for ruminant diets as a protein-rich product in intensive farming systems (Huyghe, 1997). Endophytic fungi are defined as organisms colonizing healthy plant tissue without causing overt symptoms of apparent injury to the host (Bills, 1996). They have been isolated from nearly all plants families growing in different climatic regions of the world (Larran *et al.*, 2002). Many commercially relevant arboreous plants, crops, and officinal herbs support communities of endophytic fungi (Redlin and Carris, 1996; Marshall *et al.*, 1996; Huang *et al.*, 2001). Although being biotrophic and consume plants nutrients, endophytic fungi may exhibit a beneficial role for plant health antagonizing pests via mycoparasitism, competition, and/or antibiosis. In addition, they may directly stimulate plant growth and immune response inducing resistance to diseases (Arnold *et al.*, 2003; Niere *et al.*, 2004; Santamaría and Bayman, 2005). The fungal genus *Trichoderma* occur as endophyte particularly in the tropical arboreous plants, and its strains often exhibit high antagonistic activity against pathogens of these plants (Brewer *et al.*, 1987; Watts *et al.*, 1988; Dunlop *et al.*, 1989). However, the importance of Trichoderma or other endophytic fungi as a part of endogenous microbial diversity and their application in inducing resistance against chromium stress has not been investigated yet. The present study aims at understanding the role of interactive usage of *Trichoderma harzianum* to alleviate the adverse impact of chromium stress for sustainable production of the legume plants; *Lupinus albus* and to broaden the scope of further research on this topic in many fields of science.

2. RESULTS

2.1. Growth and development

After 30 days of sowing, it was obviously recorded that Cr limits seed germination rate of *L. albus* seeds to about 36% (Figure 1). The maximum germination percentage was estimated under treatment with *T. harzianum.* Chromium also inhibited the elongation of *L. albus* shoots and roots, resulted in significant decline in seedlings shoots and roots lengths as compared to the control (Figure 2). Maximum significant increase in shoots and roots lengths was recorded under application of *T. harzianum*.

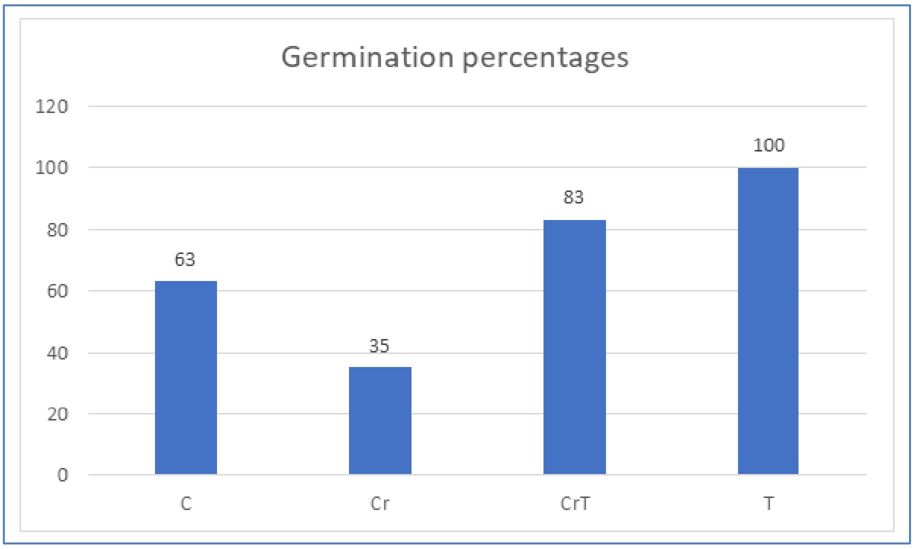

Figure 1: Germination (%) of L. albus seeds under different treatments
C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

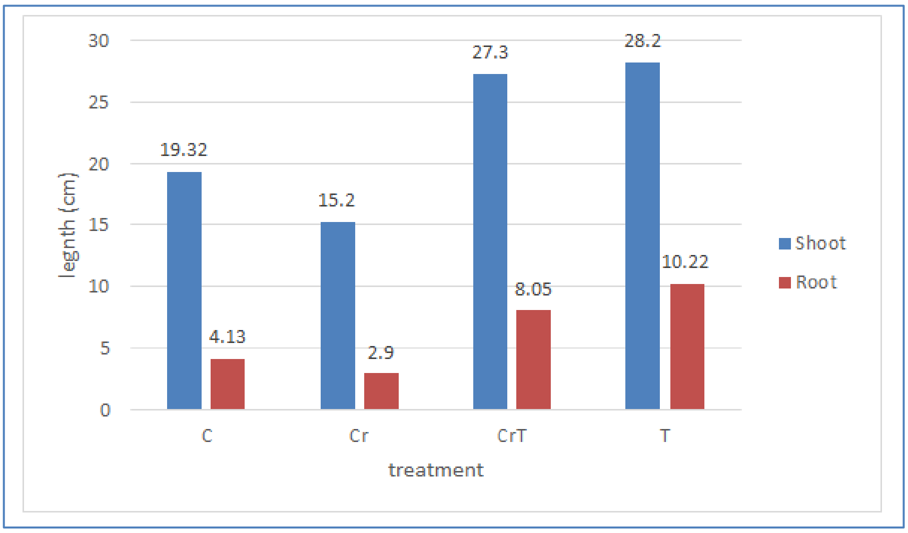

Figure 2: Variations in shoot and root lengths (cm) of L. albus seedlings under different treatments
C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

Different growth parameters were used in our study to calculate indices of Cr tolerance in each treatment. It is notable that for the plants in the Cr treatments, the index of tolerance was greater than 50%, which is considered to be the minimum desired biomass production for the plants growing in a metal-contaminated site. The stress tolerance index of *L. albus* for growth parameters under different applied treatments has its lower values with under chromium stress while it increased in the *T. harzianum* treatments (Table 1).

Table 1: Tolerance index values for deferent growth parameter of L. albus L. stressed seedlings.

Treatments	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
Cr	68	79.65	86.91	98.46	87.5	87.84
CrT	230.43	145.43	101.78	125.97	112.5	100.90
T	183.67	141.32	122.62	138.33	125	114.41

Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

2.2. Physiological processes

Chlorophyll pigments show remarkable decrease in treatments under Cr stress, as compared to control (Figure 3). However, treatment incorporated Cr with *T. harzianum* resulted in a slight increase in chlorophyll pigments but was still less than that estimated in the control (17.6% less as compared to the control). The highest increase (11.64 μ mol/g) was attained under treatment of *T. harzianum* alone (11.8% of control).

Chromium also alter the content of essential mineral nutrients. Na and Mg contents in lupin plant were significantly decreased under chromium stress in all treatments as compared to the control. It is notable that adding *T. harzianum* increases the content of mineral elements detected in the present study, as compared to the control (Figure 4). It is worthy mentioned that both K and Ca show significant increase under Cr stress.

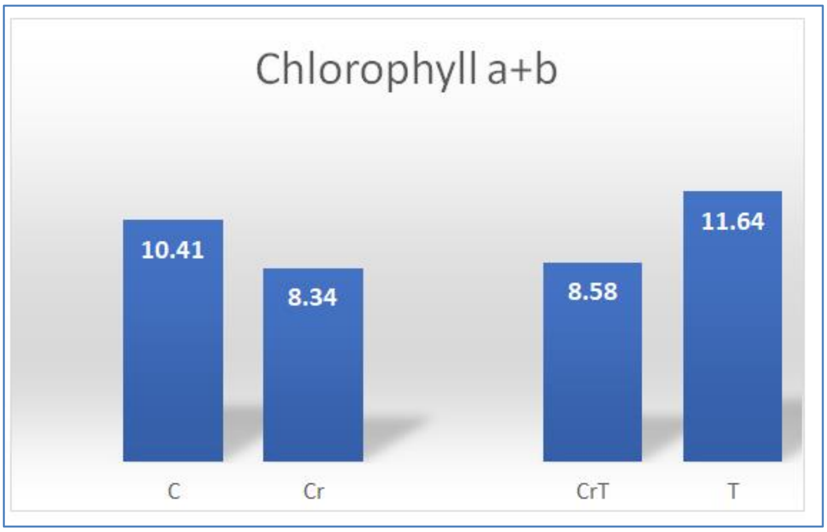

Figure 3: Variations in Chlorophyll content (μ mol/g) in shoots of lupinus albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

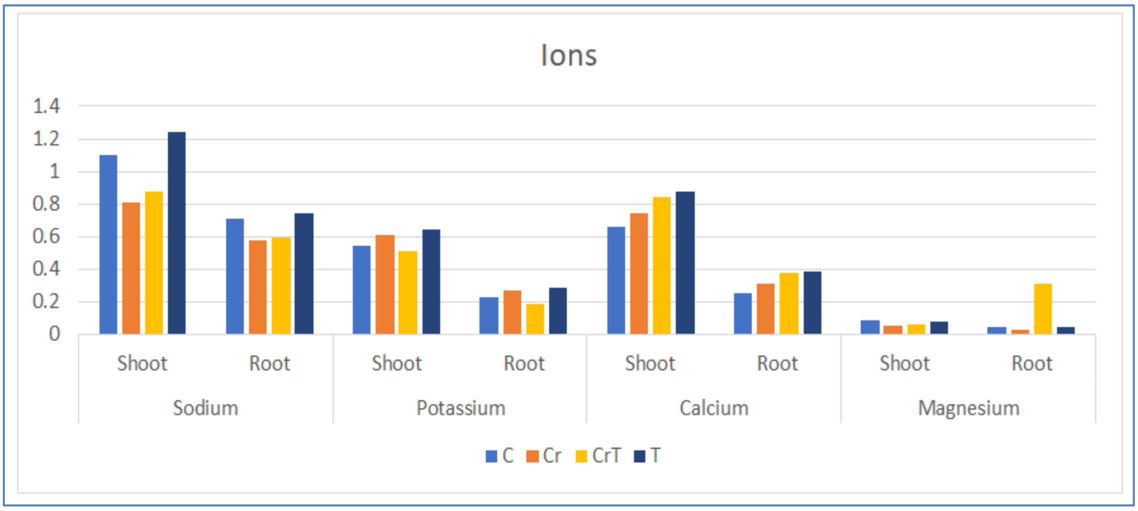

Figure 4: Variations in different ions (Sodium, Potassium, Calcium and Magnesium) (g/Kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

2.3. Enzymes and other compounds

Results recorded in the present study showed that there is significant reduction in Glycine betaine (GB) content in shoots of lupin plant under chromium stress (Figure 5). Significant differences in GB content between Cr-only, and Cr+ *T. harzianum* groups were recorded. The results also showed a significant reduction in the proline content under chromium stress (Figure 6). It is worthy mentioned that application of *T. harzianum*, increase both GB and proline contents in *L. albus* as compared to the control.

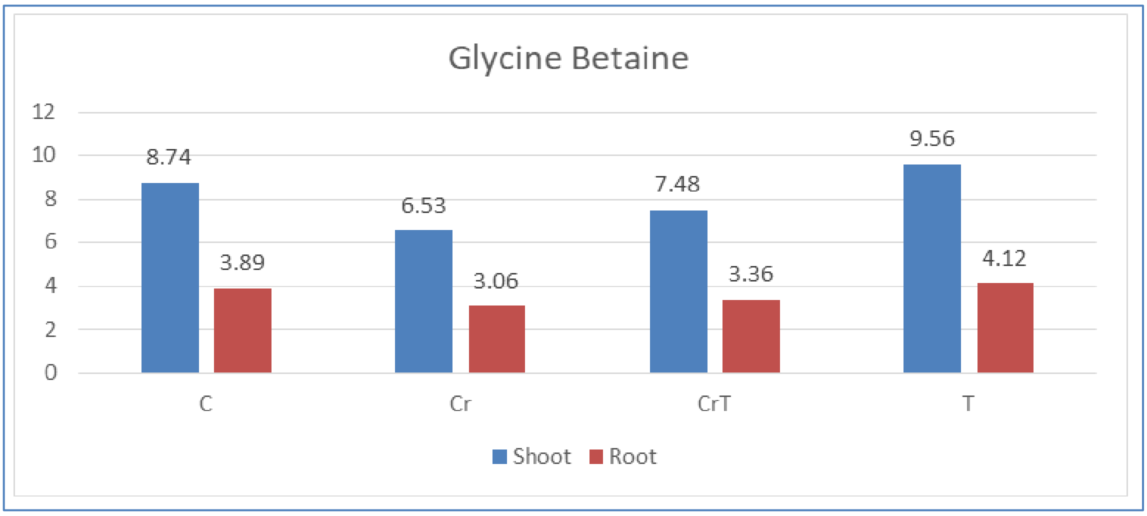

Figure 5: Variations in Glycine Betaine (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

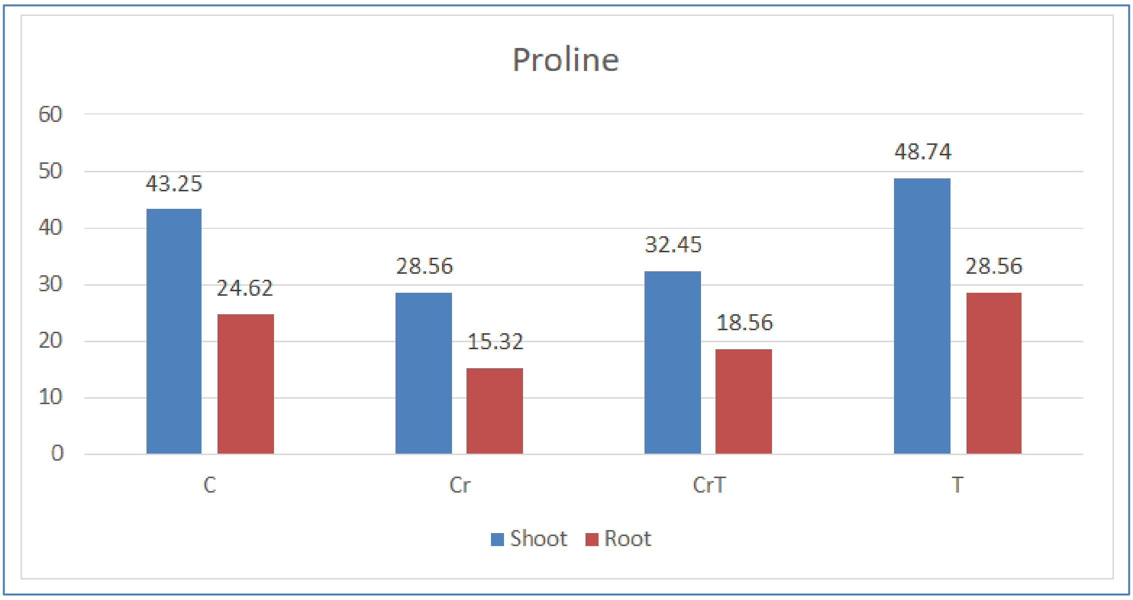

Figure 6: Variations in Proline (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

A significant reduction was detected in polyphenol oxidase under Cr. stress (20.94%, 25.49 for both shoot and root respectively) as shown in Figure 7. Activities of polyphenol oxidase showed that treatment with *T. harzianum* was effective in increasing enzyme activities (Figure 7). The highest increase of polyphenol oxidase activities as compared to the control was achieved with *T. harzianum* treatment.

Data also showed significant reduction in ascorbate peroxidase and guaiacol peroxidase activity under Cr stress in both shoot (37.35%, 32.28%) and root (43.31%, 28.79%) respectively as compared to control (Figure 8 and 9). The application of *T. harzianum* considerably increase in both ascorbate peroxidase and guaiacol peroxidase activity as compared to control it attained the highest values in shoot (13.25%, 5.51%) and root (8.66%, 9.84%) respectively as compared to control.

Generally, Cr consistently decreased catalase activity in treatments of the present study except the treatment with *T. harzianum* , where it attained the maximum amount of enzyme (543mg kg−1) (Figure 10).

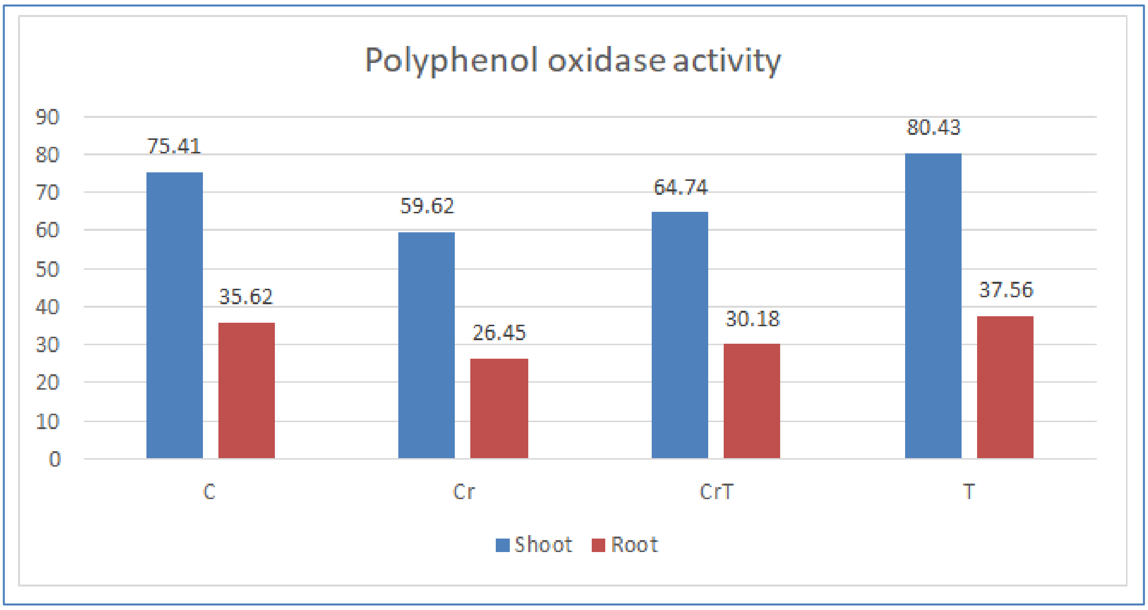

Figure 7: Variations in polyphenol oxidase (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

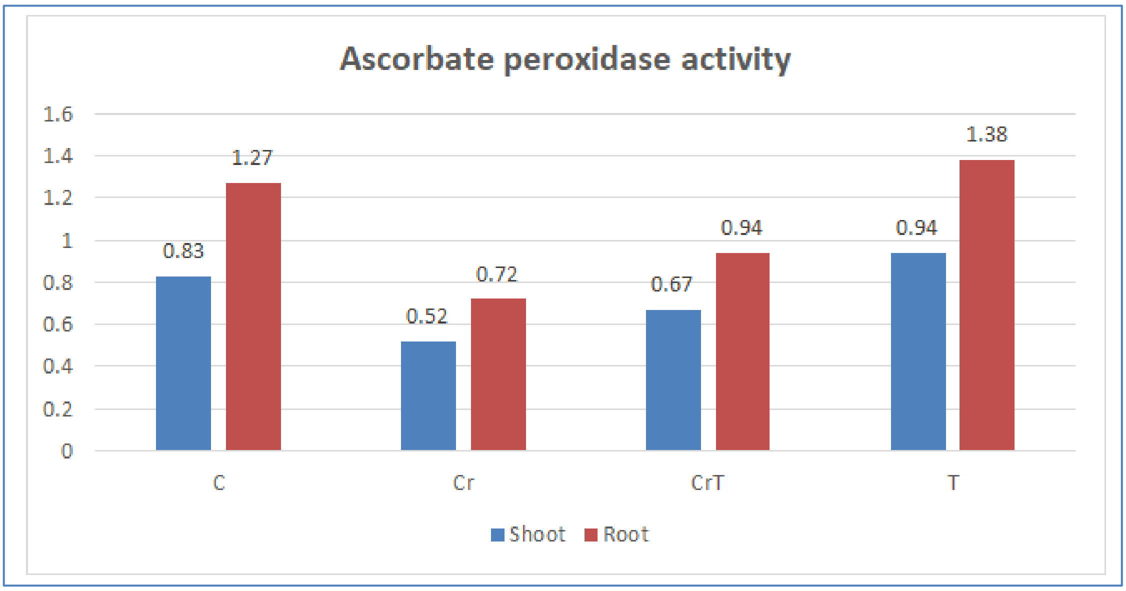

Figure 8: Variations in ascorbate peroxidase activity (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

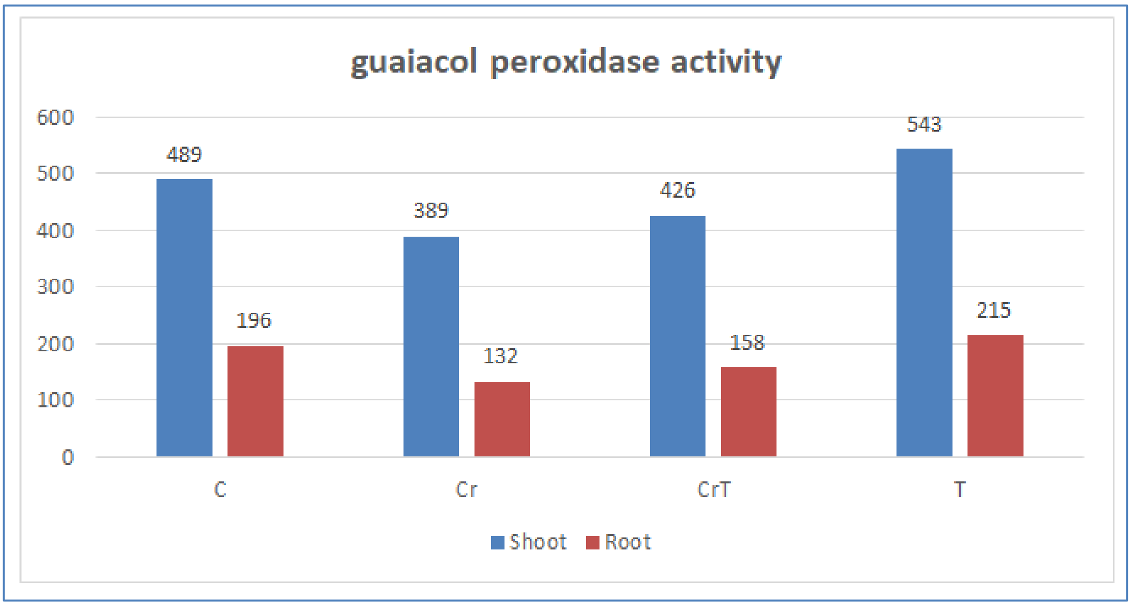

Figure 9: Variations in guaiacol peroxidase activity (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

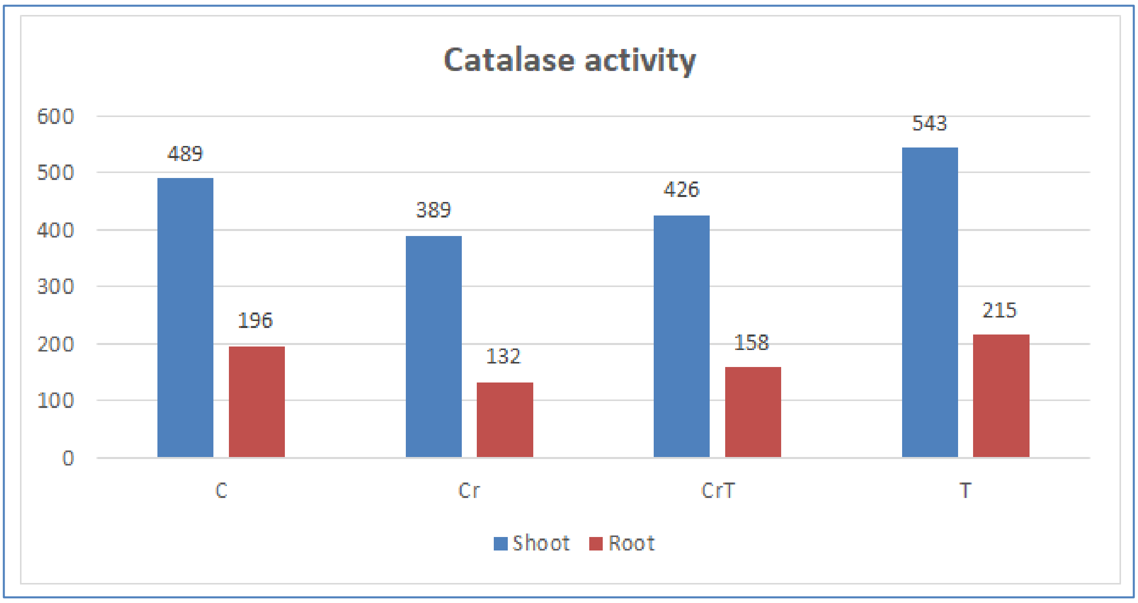

Figure 10: Variations in catalase activity (mg/ kg) in both root and shoot of L. albus seedlings under different treatments

C: control; Cr: (Chromium); CrT: (Chromium + *T. harzianum*); and T: (*T. harzianum*)

3. DISCUSSION

Growth is usually expressed as a function of genotype and environment, which is made up of external growth factors and internal growth factors. Presence of Chromium (Cr) in the external environment leads to changes in the growth and development pattern of the plant (Shanker *et al*., 2005). In the present study Cr stress reduced the germination *Lupinus albus* seeds and induced changes in seedlings' biomass. Germination percentage was reduced remarkably in response to Cr treatment, causing the greatest reduction (54.5%) of lupin seeds as compared to the control. Treatments with *Trichoderma harzianum* considerably increased the germination percentages. This is consistent with the results of Halifu *et al*., (2019), who reports that inoculation with *T. harzianum* and *T. virens* significantly promotes seedling growth and change root structure in annual seedlings of *Pinus sylvestris* var. *mongolica*. Also, Hashem *et al*., (2019), reported growth improvement of several crop plants using arbuscular mycorrhizal fungi (AMF) inoculation. Zeid, (2001) attributed the reduction of seed germination under Cr stress, to the depressive effect of Cr on the activity of amylases and on the subsequent transport of sugars to the embryo axes. Remarkable significant decline in seedlings shoot (21.3%) and root (29.8 %) lengths was detected under Cr stress as compared to the control. On the other hand, a significant increase in shoot and root lengths was observed in treatments where *T. harzianum* was added. The considerable increase (31.5% and 83.7% for shoot and root respectively as compared to the control) was detected under treatment with combination of *T. harzianum* and Cr. The present findings in our study were also in correspondence with Ayyaz *et al.*, (2021), who suggested that Cr stress induced reduction in canola plants growth attributes associated with inhibition of plant cell turgidity, cell division, biosynthesis of cell wall, cell elongation and reduced relative water content. Different growth parameters were used in our study to calculate indices of Cr tolerance in each treatment. Tolerance of Cr was influenced by the treatment. Generally, plants had higher index of tolerance when grown in the soils treated with *T. harzianum*, while the index of tolerance dropped in the same soils treated with Cr alone. Values obtained for the index of tolerance was greater than 50%, which is generally considered to be the minimum desired biomass production for the plants growing in a metal-contaminated site as mentioned by Baker *et al.*, (1994). The Cr treatment resulted in the greatest visible plant stress. Chlorosis caused by Cr was observed by many researchers (Jain *et al.*, 2000; Davies *et al.*, 2001; Bluskov, 2004). We noticed that treatment of *T. harzianum* resulted in significant enhancement in chlorophyll pigment synthesis (13.75% increase as compares to the control), reflecting a significant enhancement in the net photosynthetic rate. Several studies have shown that *Trichoderma* inoculation has significant promotion effects on plants' seedlings and crops yields, e.g., those of tomato (Bal & Altintas, 2006) and cotton (Howell *et al.*, 2000). *Trichoderma spp*. could change the pH value in the rhizosphere soil secreting organic acids to degrade minerals such as P, Fe, Mn, and Zn, and activate soil nutrients, thus promoting the uptake of nutrients by plants as well as the recycling and utilization of nutrients in the soil environment (Li *et al.*, 2015). Our results showed a significant increase in nutrient uptake for Na, K, Ca, but a considerable reduction is noticed for Mg. Yedidia *et al.*, (2001), showed that treatment with *T. harzianum* could significantly increase the nutrient conversion and absorption of P, Fe, Mn, Zn, Cu, and Na, thus promoting cucumber growth and yield. Glycine betaine (GB) is one such organic osmolyte, which plays an effective role in alleviation of heavy metal stress (Ali *et al.*, 2020). Accumulation of GB in plants increases the yields in terms of number of seeds, fruits, and flower size (Ahanger *et al.*, 2018). Singh *et al.*, (2022) study on chickpea (*Cicer arietinum* L.) reported that GB application reduced Cr accumulation and oxidative stress in the roots and leaves by increasing antioxidant enzyme activities and physical processes. Thus, the annual loss of crop yields due to heavy metal toxicity can be reduced with GB application. In the present study, results revealed that there is significant reduction in GB content in shoots of lupin plant under chromium stress. Significant differences in GB content between Cr-only, Cr+ *T. harzianum*, and *T. harzianum* groups were recorded. Application of *T. harzianum*, caused a significant increase in GB in lupin plants. Many plants accumulate higher concentration of proline against heavy metals stress (Ayyaz *et al.*, 2021). Furthermore, Singh *et al.*, (2022) reported that Cr stress also increased oxidative stress and amino acid (proline) levels in the roots and leaves of *Cicer arietinum*, ultimately reducing plant metabolism and yield. The results of the present study showed a significant reduction in the proline content under chromium stress. Reduced amounts of both GB and proline in lupin plant under Cr stress indicate the low stress tolerance of the plant against toxic elements. It is worthy mentioned that application of *T. harzianum* in Cr+ *T. harzianum* treatment, increase both GB and proline contents in *Lupinus albus*, and thus increasing its tolerance against Cr stress. In our study, data also showed significant reduction in ascorbate peroxidase, guaiacol peroxidase activity, polyphenol oxidase, and catalase activity under Cr stress in both shoot and root as compared to control. Samantaray *et al.*, (1999) used peroxidase and catalase activities as enzyme markers for identifying Cr tolerant mung bean cultivars. They found that peroxidase and catalase were higher in tolerant calluses than in non- tolerant ones. So, we can conclude that the results of our study may reflect the low tolerance of *L. albus* against Cr stress. Overall, the results of our study demonstrated that *L. albus* have improved tolerance to inhibitory concentrations of Cr when inoculated with *T. harzianum*. So, we suggest the application of *T. harzianum*, as it could improve the quality and yield of lupin plants in Cr- contaminated areas.

4. MATERIALS AND METHODS

4.1. Collection and Preparation of Lupin Seeds

L. albus seeds were obtained from the Agriculture Research Center, Giza, Egypt. Before germination, the uniform seeds of lupin were surface sterilized for 2 to 3 minutes in ethanol (70%), then washed thoroughly with distilled water for at least 3 times (Abdel Latef, 2017).

4.2. Pots experiment

A completely randomized design will use for the pots experiment with five replicates. Loamy sand soil will collect from agricultural farm and will autoclave three successive times to avoid any natural infection of the soil will use in the pot experiment. Four different treatments were carried out: (control), (Cr), (Cr +*T. harzianum*), and (*T. harzianum*). The experiment was performed under greenhouse conditions (20±2ºC temperature, 75±2% relative humidity, and 14/10 light/dark photoperiod). Germination and plant growth are monitored for 30 days. Soil humidity is maintained at approx. 60% of water-holding capacity.

4.3. Determination of growth parameters

4.3.1. Germination percentage (GP) %

Germination percentage (GP) was calculated according to the general equations:

GP = number of germinated seeds/total number of seeds × 100

4.3.2. Shoot and root lengths

Fifteen plant individuals per treatment were used for determination of shoot and root lengths using measuring tape.

4.3.3. Tolerance index

Stress tolerance indices for different growth parameters are calculate using following formulae (Wilkins, 1957).

RLSTI = (Root length of stress plant / Root length of control plant) × 100.

SLSTI = (Shoot length of stress plant / Shoot length of control plant) × 100.

RFSTI = (Root fresh weight of stress plant / Root fresh weight of control plant) × 100.

SFSTI = (Shoot fresh weight of stress plant / Shoot fresh weight of control plant) × 100.

RDSTI = (Root dry weight of stress plant / Root dry weight of control plant) × 100

SDSTI = (Shoot dry weight of stress plant / Shoot dry weight of control plant) × 100

4.4. *General phytochemical and physiological analysis of L. albus under different treatments*

4.4.1. Determination of photosynthetic pigments

Chlorophyll content in leaves was estimated using dimethyl sulphoxide (DMSO), and absorbance determined spectrophotometrically according to the method described by Lichtenthaler (1987)

4.4.2. Estimation of ions

For the elemental analysis, the minerals were brought into solution by wet digestion using a mixture of nitric, sulphuric and perchloric acids (4:1:1) (Harris, 1979). Potassium and sodium were determined using flame photometer (Corning 400 uk). Calcium and Magnesium were determined using atomic absorption spectrophotometer (Shimadzu AA 6800).

4.4.3. Estimation of glycine betaine

Glycine Betaine content was determined by spectrophotometer (Beckman 640 D, USA), according to the method of Grieve and Grattan (1983).

4.3.3. Determination of proline

Determination of proline in water extract was carried out by using the acid ninhydrin method described by Bates *et al.*, (1973).

4.3.3. Extraction and assay of non-enzymatic antioxidants

Fresh leaves (0.5 g each) was homogenized in meta-phosphoric acid, 1.0 mM EDTA was added. The homogenate will centrifuge, and the resultant supernatant will use for estimation of non-enzymatic antioxidants.

4.3.3. Determination of enzymatic antioxidant

Catalase activity was determined according to (Aebi, 1984). Ascorbate peroxidase activity was assayed using a modified method of (Yoshimura *et al.,* 2002).

4.3.3. Statistical analysis

The experimental design was completely randomized with five replicates. The obtained data was given as the mean with standard deviation values by using SPSS-21 software.

CONCLUSION

In conclusion, our findings showed that

- Chromium toxicity had a negative effect on *L. albus* germination, growth, physiological and biochemical characteristics.
- Reduced amounts of both GB and proline in lupin plants under Cr stress indicate the low stress tolerance of the plant against toxic elements. So, *L. albus* could be considered as Cr stress sensitive. Further studies using field trials are needed to investigate the effects and mechanism of action of GB in *L. albus* under Cr toxicity.
- Using peroxidase and catalase activities as enzyme markers for identifying Cr tolerant species. The present study suggested that *L. albus* possesses lower antioxidative activity, indicating low Cr stress tolerance.
- Generally, lupin plants had higher index of tolerance when grown in the soils treated with *T. harzianum*, while the index of tolerance dropped in the same soils treated with Cr alone.
- Furthermore, Results of our study demonstrated that *L. albus* have improved tolerance to inhibitory effect of Cr when inoculated with *T. harzianum*. So, we suggest the application of *T. harzianum*, which could be used to improve the quality and yield of lupin (*L. albus*) plants in Cr-contaminated areas and in fertile soils.

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