The combined effect of Arbuscular Mycorrhizal Fungi and Arsenic doses on Oxidative stress of *Leuceana leucocephala*in Nursery

Abstract

The aim of this research was to examine the effect of high levels of arsenic in soil, on *L. leucocephala* seedlings in nursery, an important small tree for forage and fodder purpose.Arbuscular mycorrhizal fungus (AMF) can shield host plants from the toxicity of arsenic (As). However, there is currently little known about how woody legumes react to As stress. Antioxidants activity to oxidative stress were evaluated in *Leuceana leucocephala*subjected to various doses of arsenic toxicity and also inoculation with arbuscular mycorrhizal fungi (AMF) in order to evaluate its effect on protection against oxidative stress and its tolerance after mycorrhizal inoculation. In order to evaluate this, MDA, H_2O_2, APX, CAT, GPX and GR. Exogenous application of arsenic results in negative effect on growth by increasing the production of Reactive oxygen species (ROS). While inoculation with Mycorrhizal fungi an enhancement in growth and other characters were noticed. This suggests that the use of these stress-relieving substances (AMF) may have reduced oxidative stress in As-stressed *L. leucocephala* seedlings. Accumulation of arsenic and phosphorus was increased by AMF inoculation in Root of seedlings.

Keywords: AMF, antioxidants, oxidative stress, Reactive oxygen species.

INTRODUCTION: All environmental and biotic stresses trigger a generalized stress response called an oxidative stress which candamage cell components and cause their dysfunction**(Demidchik 2015)**. Oxidative stress is a physiological condition which refers to "An imbalance between the generation of oxidants and their elimination systems, i.e. antioxidants, in favor of oxidants, resulting in disruption of redox signaling and control and/or molecular damage"**(Sies et al., 2017)** ,When electron loss (oxidation) outweighs electron gain (reduction), chemical (oxidative) damage to cell molecules results**(Demidchik 2015)**. Second, it is one of the "stress factors" that harms cells and sets off signaling and defensive mechanisms (much like salinity, drought, and others)**(Ma et al., 2020)**.In this definition oxidation refers to the condition in which a species losing electrons, gaining oxygen, or losing hydrogen. But if one item is oxidized, another must be decreased. Context determines the outcome and An antioxidant is any substance that, when present at low concentrations as compared with those of an oxidizable substrate, significantly delays or prevents the oxidation of that substrate**(Halliwell and Poulsen 2006)**. To keep the biological redox stable states, antioxidants may operate as oxidant scavengers**(Luo et al., 2020)**. ROS formation and detoxifying processes coexist, Because stress factors that directly produce ROS (such as transition metals, UV rays,

or ozone) also drive the production of ROS via NADH oxidases and peroxidases **(Nawkar et al., 2013; Zhang et al., 2010).**

The increased generation of reactive oxygen species (ROS) in plants is one of the main effects of abiotic and biotic stress**(Polle et al., 1993)**. The intermediates O_2, $HO^{\bullet-}$, and H_2O_2 produced by the subsequent reduction of molecular oxygen to H_2O are potentially hazardous since they are more reactive than O_2 itself**(Giannakoula et al., 2021)**.However, when under stress, ROS production increases and/or antioxidant defenses decline, disrupting the natural balance between ROS and antioxidant defense**(Scandalios, 2002; Bhosale and Shinde, 2023; Bhosale et al., 2020).**

One of the most important physiological characteristics of plants is photosynthesis, which is one of several metabolic processes. However, it has been claimed that different heavy metals have a deleterious effect on it**(Shu et al., 2012; Singh et al., 2022; Ramadhano et al., 2022).**Inhibition of photosynthetic activity by heavy metals One of the main effects of stress on plants is the inhibition of both the light and dark responses of photosynthesis, which reduces chlorophyll production and inhibits Calvin cycle activities either directly or indirectly**(Azab and Hegazy 2020; Küpper et al., 2002).** Different types of plants show different patterns of heavy metal buildup, which may affect how photosynthetic pigments are made. Heavy metal buildup in plants may interact with the machinery used for photosynthetic reactions, leading to a range of harmful consequences, including photo oxidative damage**(Giannakoula et al., 2021**).

Ways to determine oxidative stress: There are three possible methods for determining oxidative homeostasis and determining if equilibrium was upset and oxidative stress developed. Since ROS are unstable, they cannot be used as easily recognizable markers or stable adducts. As a result, macromolecules that have undergone oxidation and their derivatives serve as direct identifiers. As typical indicators of oxidative damage, protein carbonyls, Malondialdehyde produced by lipid radical oxidation, and 8-oxo-deoxyguanosine can all be mentioned **(Pohanka 2013; Samsel et al., 2013; Yang et al., 2012).**Biological samples may easily have Malondialdehyde and protein carbonyls measured using spectrophotometry **(Pohanka 2014)**. There are also chromatography methods for analyzing stress indicators **(Al-Rimawi 2015)**.

The expression of antioxidant enzymes—enzymes that can detoxify ROS—occurs after the onset of oxidative imbalance. These enzymes may be employed as indicators, and their abundant presence in plant tissues allows us to infer the development of oxidative stress. Superoxide dismutase is a typical enzyme with strong antioxidant properties that may be utilised to detect oxidative stress in plants **(Cui et al., 2015; Jain et al., 2015; Rady and Hemida 2015).** Other enzymes that are expressed when under stress include catalase and peroxidase(**Naz et al., 2015**)**.**

Massive metal Inactivating enzymes, blocking functional groups of metabolically significant molecules, replacing or substituting essential elements, and disrupting

membrane integrity are just a few of the physiological processes that can be altered by phytotoxicity, which is the release of reactive oxygen or nitrogen species in natural pathways like photosynthesis, the tricarboxylic acid cycle, and the Calvin cycle**(Rascio and Navari-Izzo 2011)**.

Cells are capable of storing heavy metals. Heavy metals may accumulate rapidly in both plants and microorganisms, which is advantageous for bioremediation (**Fryzova et al., 2018**). However, mycorrhiza and root-colonizing bacteria can greatly improve the bioavailability of different heavy metal ions for absorption(**Singh et al., 2003**)**.**

To combat the toxicity of heavy metals, plants have developed a variety of defense mechanisms. The main defensive tactic entails excluding or attaching the metal to a cell wall in order to prevent it from entering the cell. Antioxidants from different classes make up the secondary defense mechanism, which works to counter the increased ROS generation brought on by metals (**Rossato et al., 2012).** These antioxidants are chemicals that defend cells from the harmful effects of xenobiotics, medicines, carcinogens, and damaging radical reactions (either directly or indirectly) **(Pinho and Ladeiro 2012**).

(**Scandalios 2005)**recommended the following distribution for the primary antioxidant components: Ascorbate, glutathione, and peroxidase make up 73% of the contents of the vacuole; 17% of the contents of chloroplasts (carotenoids, -tocopherol, ascorbate, ascorbate peroxidase, glutathione, glutathione reductase, Cu/ZnSOD, monodehydroascorbate radical reductase, and dehydroas), 5% are found in the cytosol (ascorbate peroxidase, CuZn-SOD, catalase, peroxidase, glutathione, ascorbate, glutathione reductase, and monodehydroascorbate radical reductase); 4% are found in the apoplast (peroxidase and ascorbate); 1% are found in the mitochondria (catalase, glutathione, glutathione reductase and monodehydroascorbate radical reductase) and peroxisomes (catalase; Cu/Zn-SOD).

Plants may overcome the harmful effects of heavy metal exposure in modest doses by using processes of avoidance such translocation, complexation, and sequestration. However, heavy metals become extremely phytotoxic when there is an excess of metals in the soil**(Singh et al., 2016).**

The objective of this research were to examine the consequence of arsenic present in soil, on *Leuceana leucocephala* seedlings in nursery, an important small tree for forage and fodder purpose. In addition it is also used for green manure, biomass production and food for humans and pulpwood for paper industry. Arsenic's impact on the photosynthetic pigments and oxidative stress (H_2O_2, MDA, enzymatic and non-enzymatic antioxidants) of seedlings was investigated. It was chosen for this investigation, because arsenic is a metal that causes cancer, is dangerous even at low concentrations, and can build up in plants to hazardous levels. It is found in Bihar, Jharkhand, Uttar Pradesh, West Bengal, Assam, and Chhattisgarh in India along the rivers. On the other hand, phytomycoremediation is a potential approach to the heavy metal toxicity issue. Previous studies on phytoremediation demonstrated that

plants can depleting the amount of arsenic (a heavy metal) in the environment or its hazardous effects also. Many plant species had proven their eligibility to this technique. Many plant species had established their suitability for this method.

2. Methodology:

2.1. Plant material and Culture: *L.leucocephala*seedlings were cultivatedwith different concentration of Arsenic (0,25,50 and 100 mg/kg soil As) at nursery of HNB Garhwal University of Srinagar Garhwal Uttarakhand during 2021. Plants were grown in polybags containing sterilized mixture of 1:4 (soil: fine sand). For mycorrhizal treatment two most available AMF fungi *Glomus macrocarpum* and *Glomus fasciculatum*had been used to inoculate seedlings. Inoculum of these AMF were produced in before plant cultivation in the nursery through pot culture.

2.2. Chlorophyll and Carotenoids: The leaves were gathered after 90 days of growth following the application of various arsenic toxicity dosages. Fresh leaf material (10 mg) was inserted in a 2 ml eppendroff tube with warmed DMSO and incubated at 65℃ for 30 minutes to estimate the amount of photosynthetic pigment. Using a spectrophotometer, the absorbance of chlorophyll was measured at 645 and 663 nm, and the total chlorophyll was calculated. At wavelengths of 480 nm and 510 nm, carotenoids' absorbance was measured**(Hiscox and Israelstam 1979)**.

2.3. Hydrogen peroxide:10 mg of leaf sample was centrifuged at 1000 rpm for 20–25 minutes after being homogenized with 0.1% TCA. Potassium iodide and 10 mm phosphate buffer were vigorously mixed in with the obtained enzyme. After one hour of incubation, the reaction mixture's absorbance at 390 nm was measured**(Velikova, Yordanov, and Edreva 2000)**.

2.3. Lipid peroxidation: It is measured as MDA, 0.G mg of leaf sample was homogenized in buffer having 0.1 mm EDTA. This homogenate centrifuged at 12000 rpm for 20 minute.20 % TCA (having 0.5% TBA) and once more centrifuged for 10 minutes at 10000rpm. Absorbance was measured at 532 nm and 600nm**(Heath and Packer 1968)**. The results are shown as µmol MDA g^{-1} FW.

2.4. Enzymatic antioxidants estimation: Leaf samples were taken, cut into little pieces, placed in a mortar, and homogenized with 80% methanol. Centrifugation was then performed for 25 minutes at 15,000 rpm, while adding 0.5M phosphate buffer and 0.2Mm EDTA, crude extract was produced.Catalase estimated By detecting the reduction in absorbance at 240 nm in a reaction medium containing 50 mM potassium phosphate buffer (pH 7.2) and 20 mM H_20_2, the activity of CAT was evaluated in homogenates**(Aebi 1984)**.In accordance with **(Nakano and Asada 1981)** descriptions, APOX activity was confirmed right away in fresh extracts. Using a 1 ml reaction mixture with 20 mM potassium phosphate buffer (pH 7.0), 20 mM hydrogen peroxide, 0.25 mM ascorbic acid, and 0.2 mM EDTA. Ascorbate underwent hydrogen peroxide-dependent oxidation, which was followed by a drop in absorbance at 290

nm.Using **(Egley et al., 1983; Sharma et al., 2017)** the increase in absorbance brought on by the oxidation of Guaiacol to tetra Guaiacol at 470 nm was determined. The reaction mixture included 20mM of pH 7 phosphate buffer. 1 mM hydrogen peroxide, 0.1 mM EDTA, and 0.05% Guaiacol with enzyme extract.Glutathionereductaseassay conducted by usingSolution containing enzyme Extract, 20mM phosphate buffer (pH 7),0.2 mM EDTA, 0.12 NADPH, 0.5mM glutathione disulphide. The increase in absorbance was measured at 412 nm using method of**(Foyer and Halliwell 1976)**.

3. Results

Concentration influenced the effects of the heavy metal (arsenic) on plants growth and presence of AMF in soil enhance the growth. However combination of both significantly increases the growth as compared to control.

For the 0 mg/kg soil arsenic treatment level, there was no arsenic buildup seen. Non-mycorrhizal plants treated with 100 mg/kg soil As showed the maximum arsenic buildup in shoots. Adding mycorrhizal fungi to seedlings of *Leuceana leucocephala* marginally reduces the amount of arsenic in their shoots (**Figure 1**). *G. macrocarpum* inoculated seedlings had the lowest levels of arsenic (3.6, 5.3, and 7.2 mg/g DW), followed by *Glomus fasciculatum* (4.2, 6.2, and 8.6 mg/g DW) at 25, 50, and 100 mg/kg soil. When comparing the arsenic accumulation at a level of 100 mg/kg soil arsenic treatment, *Glomus macrocarpum* seedlings had a drop of 26.5% and *Glomus fasciculatum* seedlings saw a decrease of 6% of Non- Mycorrhizal seedlings.

Arsenic concentration effects by increasing its concentration in the roots of seedlings when the soil's arsenic content rises from 0-100 mg/kg soil. When compared to uninoculated seedlings, AMF colonisation significantly lowered the content of the arsenic **(Figure 1).** *G.fasciculatum* seedlings (12.97 mg/g DW) collected the most root arsenic, followed by *Glomusmacrocarpum* (11.97 mg/g DW) and non-mycorrhizal seedlings (11.6 mg/g DW).

The phosphorus concentration in the shoot and root of *Leuceana leucocephala* seedlings was significantly affected ($p < 0.01$)by arsenic, AMF treatments and their interactions.Arsenic content in the soil decreases the amount of phosphorus in seedling leaves. Phosphorus content is increased in AMF-inoculated plants compared to non-mycorrhizal seedlings. Shoot phosphorus content in *Glomus macrocarpum* (7.8 mg/g DW) responded better in mycorrhizal seedlings than *Glomus fasciculatum* (7.6 mg/g DW). However, the findings of both mycorrhizal inoculations for phosphorus content in *Leuceana leucocephala* seedlings were quite comparable.At the highest arsenic treatment dose, *Glomus fasciculatum* (12.79 mg/g DW) and *Glomusmacrocarpum* (12.38 mg/g FW) seedlings accumulated the most phosphorus in their roots.

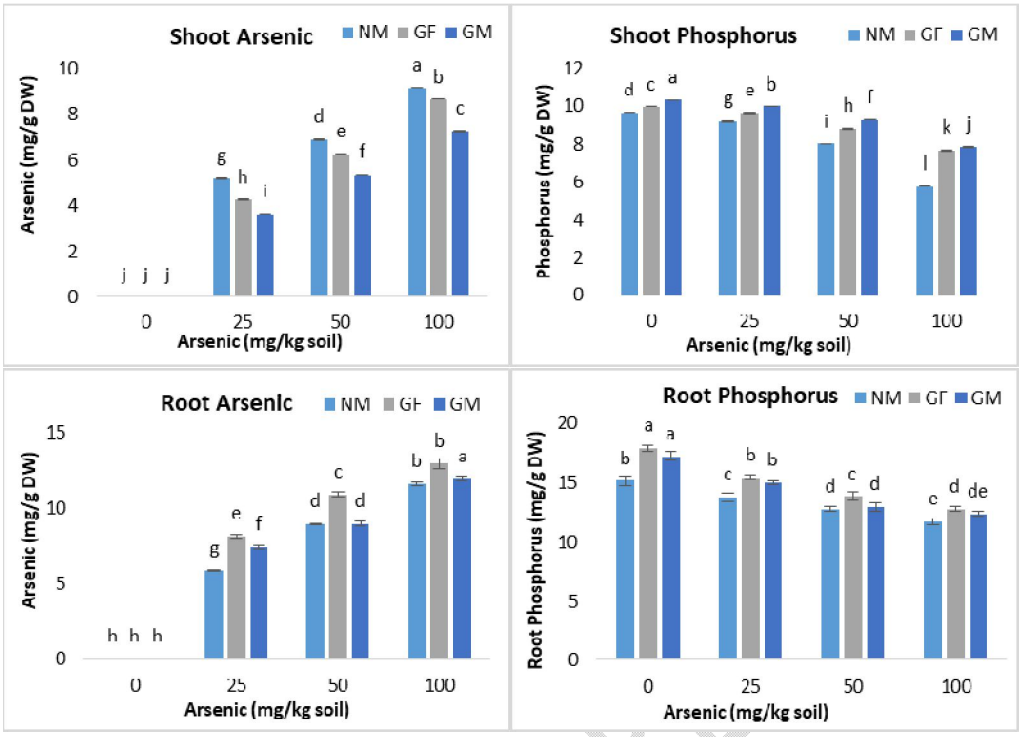

Figure 1 Effect of As addition levels and AMF treatments on *Leuceana leucocephala* seedlings Arsenic and Phosphorus content. The data is shown as mean±SE (n = 5). According to the Duncan's multiple comparison test, bars with different lettering indicate significant difference among treatments (p 0.05); where (GM) stands for *Glomus macrocarpum*, (GF) for *Glomus fasciculatum* and (NM) for non-mycorrhizal

The Hydrogen peroxide was significantly affected ($p < 0.01$) by both treatments (As and AMF) and their interactions **(Table 3).** Increasing arsenic toxicity increases the H_2O_2 content in leaves of *Leuceana leucocephala* seedlings compared to control (0 mg/kg soil As). Both AMF inoculations decrease H_2O_2 content in all arsenic concentration levels. Whereas *Glomus macrocarpum* (2.24, 2.8, 4.63 and 6.27 µmol/g fw) had lower H_2O_2 content than *Glomus fasciculatum* (2.8, 5.05, 7.2 and 9.45 µmol/g fw) seedlings**(Table 1)**.

Lipid peroxidation (measured as the concentration of MDA) was significantly accumulated by seedlings exposed to arsenic. For non- mycorrhizal seedlings there is a 50% (9.03- 13.5 µmol/g fw) increase in MDA accumulation noticed from 0-100 mg/kg soil arsenic. In mycorrhizal seedlings *Glomus macrocarpum* had lowest accumulation than *Glomus fasciculation* and non-mycorrhizal. The MDA concentration was 17%-34% decreased at all arsenic treatments than non-mycorrhizal seedlings.

The enzymatic antioxidants reactions were controlled by concentration of arsenic toxicity in soil. The lowest catalase concentration was recorded in 0 mg/kg As, non-mycorrhizal seedlings which was 10.03 mmol H_2O_2/min/mg protein and highest at

100 mg/kg As with *Glomus fasciculatum* inoculation, indicating increase in antioxidant due to stress (**Table 2**).

Table 1 Effect of Arsenic (As) and AMF [*Glomus fasciculatum* (GF), *Glomus macrocarpum* (GM) and non-mycorrhizal (NM)] on MDA, $H_2O_2$and Catalaseon *Leuceana leucocephala* seedlings. According to Duncan's multiple comparison test, Data is shown as mean±SE (n=5), different lettering Followed by them represents significant difference among treatments (p 0.05).

AMF Treatments	**Arsenic Treatments** (mg/kg soil)	**MDA** (µmol/g fw)	**H_2O_2** (µmol /g fw)	**Catalase** (mmolH_2O_2/min /mg protein)
NM	0	10.669±0.16e	3.621±0.058i	10.034±0.106h
	25	11.872±0.192d	5.917±0.089f	13.162±0.197g
	50	13.89±0.224c	8.265±0.124c	15.658±0.253e
	100	16.469±0.247a	10.084±0.106a	17.139±0.277d
GF	0	9.403±0.152f	2.81±0.042j	14.586±0.236f
	25	11.208±0.118e	5.059±0.082g	15.957±0.239e
	50	13.295±0.215c	7.27±0.109d	17.214±0.278d
	100	15.562±0.233b	9.457±0.153b	20.549±0.308b
GM	0	9.034±0.146f	2.245±0.034k	16.294±0.244e
	25	8.854±0.133f	2.882±0.047j	17.674±0.285d
	50	10.877±0.163e	4.636±0.049h	19.672±0.207c
	100	13.59±0.219c	6.276±0.094e	22.618±0.365a

Table 2 Effect of Arsenic (As) and AMF [*Glomus fasciculatum* (GF), *Glomus macrocarpum* (GM) and non-mycorrhizal (NM)] onAPX, GPX and GR on *Leuceana leucocephala* seedlings. According to Duncan's multiple comparison test, Data is shown as mean±SE (n=5), different lettering Followed by them represents significant difference among treatments (p 0.05).

AMF Treatments	**Arsenic Treatments** (mg/kg soil)	**APX** (mmol/min/mg protein)	**GPX** (mmol/min/mg protein)	**GR** (mmol/min/mg protein)
NM	0	1.301±0.021j	0.067±0.001i	0.058±0.001j
	25	1.83±0.03h	0.091±0.001h	0.161±0.002g
	50	2.521±0.038f	0.107±0.002g	0.222±0.003e
	100	3.761±0.056c	0.29±0.005c	0.312±0.005c
GF	0	1.621±0.024i	0.091±0.001h	0.087±0.002i
	25	2.226±0.036g	0.118±0.002g	0.191±0.003f
	50	2.774±0.042e	0.224±0.002e	0.27±0.004d
	100	4.111±0.043b	0.327±0.005b	0.367±0.006b
GM	0	1.821±0.019h	0.117±0.002g	0.122±0.002h
	25	2.507±0.038f	0.2±0.003f	0.231±0.003e
	50	3.2±0.052d	0.256±0.004d	0.314±0.005c
	100	4.352±0.065a	0.42±0.007a	0.425±0.006a

In fresh leaf material from seedlings of *Leuceana leucocephala*, which was grown in arsenic + AMF treatments demonstrated a substantial increase in APX activity. The content of APX in non-mycorrhizal seedlings increased thrice from 0-100 mg/kg soil arsenic. Mycorrhizal inoculation causes it to rise even more, with *Glomusmacrocarpum* (GM) recording the highest levels. Therefore, at 0, 25, 50, and 100 mg/kg soil As, there was a 40%, 36%, 28%, and 20% increase in GM compared to non-mycorrhizal seedlings**(Table 2).**

The GPX activity in non-mycorrhizal seedlings thrice at highest arsenic level (100 mg/kg) than control (0 mg/kg soil As). At the same time mycorrhizal inoculation make these responses much more effective highest in *Glomus macrocarpum*. Hence the value of 100, 50,25 and 0 mg/kg soil arsenic compared to non-mycorrhizal was increased by 44%,134%, 122%,104% respectively**(Table 2).**

The values of Glutathione reductase are presented in **Table 2**. The 100 mg/kg and 50 mg/kg arsenic treatment had higher responses of GR in non-mycorrhizal seedlings. However in mycorrhizal seedlings *Glomus macrocarpum* had highest GR activity than *Glomus fasciculatum* and non-mycorrhizal seedlings which showed two fold increase in their values.

Table 3 Two-way ANOVA analysis of Arsenic addition levels (As), mycorrhizal treatments (AMF) and their interactions (As × AMF) on biochemical variables of *Leuceana Leucocephala*studied

LeuceanaLeucocephala			
Parameters	**Arsenic**	**AMF**	**As x AMF**
MDA (µmol/g fw)	89.302**	36.16**	1.219**
H_2O_2 (µmol/g fw)	92.715**	46.756**	2.1**
Catalase (mmolH_2O_2/min/mg protein)	114.137**	130.315**	2.242**
APX (mmol/min/mg protein)	17.089**	1.905**	0.016**
GPX (mmol/min/mg protein)	0.184**	0.06**	0.0042**
GR (mmol/min/mg protein)	0.208**	0.036**	0.0007**
Arsenic (mg/g DW)	189.3**	7.914**	1.023**
Phosphorus (mg/g DW)	24.975**	7.598**	0.78**
Root Arsenic (mg/g DW)	300.02**	26.12**	2.91**
Root Phosphorus (mg/g DW)	69.56**	50.62**	0.56**

Where ns, not significant; ** p < 0.01, * p < 0.05.

4. Discussion and conclusion

With the exception of a low number of plant species, practically all plants in their natural habitat have symbiotic relationships with arbuscular mycorrhizal fungi, which has a variety of advantages for the host plant. These fungi improve the plant's nutritional condition and provide it resilience to various biotic and abiotic challenges, such as salt, heavy metals, and drought**(Orłowska et al., 2012; Spagnoletti et al., 2017)**.

We have demonstrated that arsenic present in soil inhibited the mycorrhizal colonisation of *Leuceana leucocephala* seedling roots with increasing concentrations of arsenic, which increased the detrimental effects of arsenic on plant development, biomass, and oxidative stress. Such detrimental effects also described by **(Spagnoletti et al., 2017; Yizhu et al., 2020).** Currently, the majority of research point to the AMF's potential to improve plants' ability to tolerate heavy metals. For example, **Alam et al., 2019** found that catalase and chlorophyll activity was increased with application of AMF under arsenic stress compare to control.

Abiotic stressors that prevent plant development have a variety of harmful consequences on the physiological and biochemical functions of plants, with photosynthesis being the most vulnerable**(Polle et al., 1993)**. The persistence of heavy metals in the soil and water is a crucial factor in the development of plant stress**(Azab and Hegazy 2020; Pichhode and Nikhil 2016).**

The persistence of heavy metals in the soil is a crucial factor in the development of plant stress **(Azab et al., 2020).** Plants have developed a variety of defense mechanisms to deal with this form of stress, including efflux pumps, sequestration in cells and intracellular compartments, binding of heavy metals into cells, and the creation of potent ligands like phytochelation. Most plants that are resistant to heavy metals avoid the conglomeration of heavy metals in tissues **(Bates, 1981).**The oxidative metabolism results in the production of a significant number of distinct ROS, such as the superoxide anion (O_2), singlet oxygen ($_1O^2$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH^-).

The finding was confirmed by the production of H_2O_2 in our study and its distribution in leaf tissue, which helped to clarify the photoprotective mechanisms against Pb and Cu-induced oxidative stress conditions **(Jubany et al., 2010).**The majority of research to date indicate that plants exhibit increased levels of antioxidant enzymes including CAT, APX, GPX, and GR when they are under abiotic stress, and AMF inoculation also increased these levels. For example,According to **Yizhu et al., 2020**, under As (III) and As (V) stress, symbiotic relationship between AMF with plant improves antioxidant activity (CAT).In response to AMF interaction with *Triticumaestivum* under arsenic stress, SOD, GPX, CAT, Carotenoids and Proline activities increased **(Sharma et al., 2017; Zhan et al., 2018; Zhou et al.,**

2023).Mycorrhiza showed the ability to boost the efficacy of phyto-detoxification in heavily As-polluted soils. It would also be feasible to control these helpful critters so that they have the characteristics needed to be employed for agricultural remediation through the mechanisms by which AM fungus boost metal absorption and assist plants in detoxification**(Spagnoletti et al., 2017)**. Arsenic buildup in the roots of tropical leguminous plants under arsenic stress is accelerated by AMF inoculation **(Rangel et al., 2014)**.

Additionally, *Citrus aurantium* L. plants exposed to Pb and Cu showed increased antioxidant content in their leaves. Our findings imply that these tactics work to prevent oxidative damage and so safeguard the photosynthetic apparatus. Previous studies have further demonstrated that plants that respond to heavy metal stress by producing large amounts of phenolic chemicals may be excellent candidates for phytoremediation **(Vidal et al., 2020).Yang et al., 2015** Findings suggested that AMF symbiosis protected plants by elevating enzymatic activity of SOD, APX, GPX and reduced the Hydrogen peroxide and Malondialdehyde content, this way alleviated cellular oxidative stress caused by lead (heavy metal) stress. These results suggests that AMF inoculation is important tool against heavy metal stress.**Rangel et al., 2014** suggested that AMF inoculation also increases the arsenic accumulation in root.**Zhang et al., 2021** Malondialdehyde (MDA), H_2O_2, and O_2•- concentrations were decreased by AMF inoculation, whereas antioxidative enzyme activities (SOD, POD, and CAT) were enhanced in the leaves and roots of *S. viciifolia*.**Rahman et al., 2020** AMF inoculation significantly induced the biomass, superoxide dismutase and peroxidase activity, similar pattern were followed by APX, GR and CAT.

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