Biological Forum – An International Journal **15(1): 519-525(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Arbuscular Mycorrhizal Fungi (AMF) on Growth, Biomass and Photosynthetic Pigments of *Leuceana leucocephala* under Arsenic Soil Stress in Nursery

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(Received: 09 December 2022; Revised: 11 January 2023; Accepted: 14 January 2023; Published: 21 January 2023)
(Published by Research Trend)

ABSTRACT: The inoculation of arbuscular mycorrhizal (AM) fungus is seen as a possible biotechnological method for the environmentally friendly treatment of dangerous pollutants. A greenhouse experiment was conducted to analyse growth, biomass, chlorophyll and carotenoid content in *Leuceana leucocephala* seedlings inoculated with 2AMF (*Glomus macrocarpum* and *Glomus fasciculatum*) under arsenic stress (0, 25, 50, 100 mg/kg soil) for 3 months. Treatments was arranged in randomized complete block design. Outcomes of study shown that arsenic has impacted plants negatively whereas the plants inoculated with AMF (both) had impacted their growth positively. AMF inoculated plants shown significant increase in plant growth, biomass, chlorophyll and carotenoid content. Our results suggest that *Glomus macrocarpum* and *Glomus fasciculatum* both an effective bio-inoculant of *Leuceana leucocephala* for adverse effect under arsenic stressed soil. And *Leuceana leucocephala* showed a fair tolerance ability for arsenic soil contamination.

Keywords: AMF, *Glomus macrocarpum, Glomus fasciculatum, Leuceana leucocephala.*

INTRODUCTION

All forms of crustal rocks naturally contain arsenic (As), a deadly metalloid, especially orpiment, realgar, and other ores (Li *et al*., 2018). As a result of higher anthropogenic emission of As into the environment and its extreme toxicity to creatures and ecosystems, arsenic pollution, particularly in soil and water, has gained attention on a global scale (Moreno-Jiménez *et al*., 2012). Arsenate [As (V)] and arsenite [As(III)] are the two major inorganic category of arsenic found in soils, although a common biological methylation process can be used to convert them into many organic forms. Methylated As is said to be less hazardous than inorganic As(III) and As, primarily in the forms of pentavalent monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), and trimethylarsine oxide (TMAO) (V) (Campbell and Nordstrom 2014). Mining, metallurgy, agriculture, forestry, fossil fuel treatment facilities, urban trash, and cow rearing are the main human activities that generate arsenics (Adriano *et al*., 2001; Fitz and Wenzel 2002). The main kind of P found in soils, the phosphate anion, is similar to arsenate. When P is applied to soil, repressed As is released (Fitz and Wenzel 2002).

Arsenic affects plants by accumulating mostly in the root system and to a lesser extent in the above-ground organs (Stoeva *et al*., 2005). Arsenic prevents the growth and accumulation of fresh and dry biomass (Stoeva *et al*., 2003). The chloroplast membrane was harmed by arsenic, which also disrupted the structure of the membrane (Miteva and Merakchiyska 2002). In order to adapt to or deal with the harmful effects of heavy metal (HM) toxicity, plants develop a variety of techniques (Bilal *et al*., 2019).

One technology that successfully reduces heavy metal toxicity is phytoremediation. Just as there is always a tight relationship between the microorganisms in the rhizosphere and the plant, which results in an enhanced activity connected to soil remediation, phytoremediation cannot be done by the plant alone (Karimi *et al*., 2011; Yadav *et al*., 2010). The reduction of heavy metal phytotoxicity with the aid of arbuscular mycorrhizal fungus (AMF) is a practical and environmentally benign method (Riaz *et al.,* 2021). Through their extraradical mycelial networks, AMF might improve the absorption of nutrients and water by host plants while also shielding them from the toxicity of HM (Cui *et al*., 2019; Leyval *et al.,* 1997). The direct effects of abiotic stress on arbuscular mycorrhizal (AM) fungi are less well known, although they can mitigate the impacts of abiotic stress by increasing host plant stress tolerance (Millar and Bennett 2016). Some plant roots in As-polluted soils exhibit AM colonisation, and research suggests that AMF is frequently linked to higherplants' adaptation to As stress (Chen *et al.,* 2007; Leung *et al.,* 2006; Li *et al*., 2018; Zhang *et al.,* 2020). Recent research has demonstrated that AMF *Glomus* *macrocarpum* and *Glomus fasciculatum* increases plant growth and biomass, which reduces the impacts of arsenic poisoning (Kapoor and Bhatnagar 2007; de Souza Moreira *et al.*, 2015).

The most significant biochemical process on earth is photosynthesis. It acts as the biggest solar battery in the world. Huge amounts of sunlight are converted through photosynthesis into electrical and subsequently chemical energy (Rahman *et al.*, 2007). The most significant photosynthetic pigment present in leaves are chloroplast (contain chlorophyll-a and chlorophyll-b). They use various wavelengths of light to carry out photosynthesis (Peng, 2000). Toxic substances interfere with the photosynthetic process by lowering the chlorophyll concentration of the leaves, which restricts photosynthesis (Bankaji *et al*., 2014).

There are some studies available that uses *Leuceana leucocephala* for phytoremediation of arsenic soil toxicity while inoculated with Mycorrhizae, more researches are needed in this field to clearly define the role of AMF. Present study investigated the role of *Glomus macrocarpum* and *Glomus fasciculatum* in growth, biomass and photosynthetic pigments of *Leuceana leucocephala* seedlings under different concentrations of soil arsenic stress in nursery conditions.

METHODOLOGY

Plant and soil. The investigation accomplished at the nursery of the Department of Forestry and NR, H.N.B. Garhwal University, Uttarakhand, India. The seedlings were grown in a nursery in a temperature of 20-35°C and relative humidity of 75-90% from July to September of 2021. *Leuceana leucocephala* seeds were taken from the university campus during the fruiting season, and they were placed subsequent to absorbing water for 24 hours. Soils were taken, from the departmental nursery. To take out non-soil components, the collected soils were then sieved through a 2 mm mesh screen and sterilized prior to use for filling polybags/pots for seedlings. Each pot contained around 2 kg of sieved soil, leaving the best 10 cm of the pots void to make watering more straightforward.

For treatment, various concentrations of As (0, 25, 50, 100mg As/kg soil) were taken utilizing Sodium Arsenate ($Na_2HASO_4.H_2O$). To establish homogeneous appropriation, arsenic were blended in water (50 ml) and mixed completely with soil. The pots was permitted to equilibrate for a time of one month by going through rehashed patterns of immersion with distilled water and air drying.

Mycorrhizal inoculums. The wet sieving and decanting technique was used to isolate AM fungus spores from soil (Grerdemann and Nicolson 1963). After separation of these mycorrhizal spores were multiplied through pot culture with *Trigonella foenum graecum* (Methi) plant for a period of 3-4 months .100 percent AMF root Colonization was noticed. The plant roots were cut into around 1cm pieces and blended in with the dirt, this was utilized as inoculum.

Experiment. The three treatments Non-Mycorrhizal (control), *Glomus macrocarpum*, and *Glomus fasciculatum*) and the four levels of arsenic in the soil (0, 25, 50, and 100 mg/kg). As a result, there will be twelve treatments (4×3), with each one replicated five times

Sample preparation and chemical analysis. Shoots and roots were collected independently. To dispose of sticking soil particles, samples were delicately washed in distilled water. In the wake of drying in oven at 60°C until weight stayed steady, the dry weight of the shoot and root were determined.

To examine the growth responses of plants inoculated with *Glomus macrocarpum* and *Glomus fasciculatum* following parameters were measured- shoot height, root length, collar diameter, number of nodules, number of leaves ,Fresh and dry weight of root and shoot and photosynthetic pigments on leaves (chlorophyll and carotenoids) after three months of growth.

The (Hiscox and Israelstam 1979) technique was used to estimate photosynthetic pigments. Samples of leaves were put in tubes with dimethyl sulfoxide (DMSO). The entire combination was then let to sit at 65°C until the leaf tissue decolorized. By measuring the absorbance at 645 nm and 663 nm and expressing the results as mg g^{-1} fresh weight, the amount of chlorophyll in the plant sample will be estimated. Carotenoids measured at 480 nm and 510 nm absorbance.

Statistical analysis. The information were measurably analyzed using the SPSS program. When a significance ($P < 0.05$) treatment effect was found, the mean values were analyzed utilizing the Duncan's different examination test.

RESULTS

Effect of arsenic in plants physical parameters. An effective symbiosis between AMF and plant roots was seen in a microscopic analysis. All inoculated plants were colonized by AMF, though no mycorrhizal contamination were seen in roots of control plants (non-mycorrhizal). Mycorrhizal colonization reduced with increasing arsenic stress level in soil.

The effects of AMF on the morphological parameters of seedlings of *Leuceana leucocephala* under four levels of arsenic stress—0, 25, 50, and 100 mg/kg soil are shown in Fig. 1. Data shown that there is a significant increase in all parameters- Root length, Shoot length, Collar diameter, number of nodules, number of leaves comparing with non- inoculated seedlings. In comparison to *Glomus macrocarpum* and control plants, seedlings inoculated with *Glomus fasciculatum* exhibited the highest values for nearly all growth parameters.

Glomus fasciculatum inoculated plants have longer roots than *Glomus macrocarpum* inoculated plants at all arsenic stress levels (0, 25, 50, and 100 mg/kg soil), notwithstanding that both mycorrhizal plants experience a huge decrease in root development as arsenic concentration increases (Fig. 1A). The shoot height and collar diameter is diminishing with rising arsenic stress in soil, while *Glomus fasciculatum* has higher results in the both in comparison with *Glomus macrocarpum* and control plants (Fig. 1B and D).

Number of leaves are more in *Glomus fasciculatum* in every one of the three arsenic levels (0, 25, 100 mg/kg soil) than Glomus macrocarpum and control, however at 50 mg/kg soil As level *Glomus macrocarpum* showed bigger numbers than *Glomus fasciculatum* (Fig. 1E). The number of nodules are more in *Glomus fasciculatum* at 0 and 25 mg/kg As stress than *Glomus macrocarpum*. Where the most elevated values in number of nodules was seen in *Glomus macrocarpum* at 50 and 100mg/kg As stress in soil contrasted with *Glomus fasciculatum* inoculated seedlings (Fig. 1C).

Regardless of the degree of arsenic stress at any level and for all parameters examined, the values were comparable for control and mycorrhizal plants.

Effect of arsenic on Biomass. The presence of AMF inoculation and As level had a substantial impact on total biomass of seedlings. *Leuceana leucocephala* root and shoot biomass measured after 3 months of growth was significantly lower at the highest As level (100 mg/kg soil) than it was at the lowest As level (0 mg/kg) for all plants (control and mycorrhizal both). Comparatively to mycorrhizal plants, non-mycorrhizal plants were more susceptible to the addition of Pb. At all arsenic stress concentrations (0, 25, 50, and 100 mg/kg soil), *Glomus fasciculatum* seedlings have more root and shoot biomass than *Glomus macrocarpum* and control seedlings (Table 2).

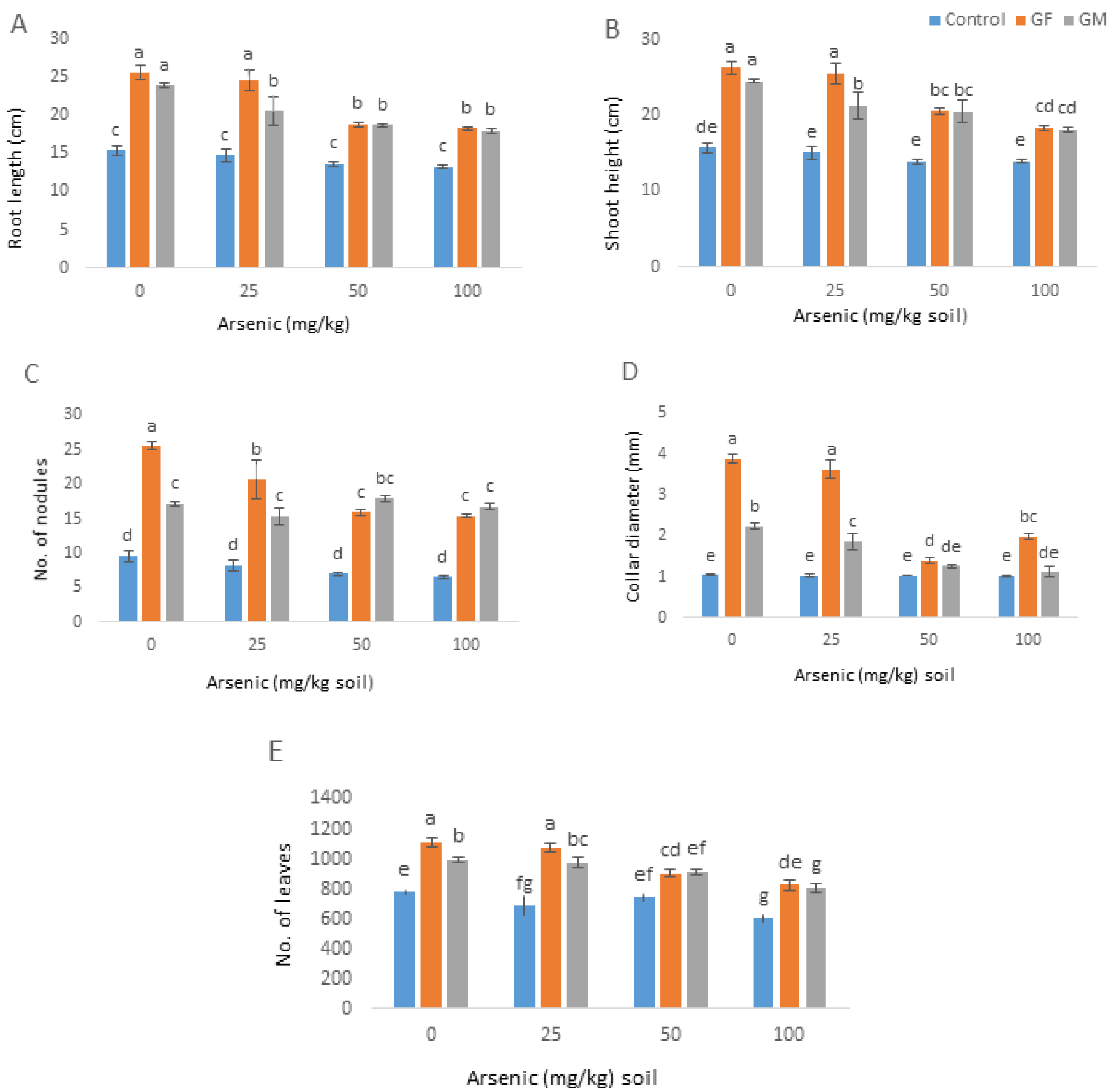


Fig. 1. Effect of As addition levels and AMF treatments on *Leuceana leucocephala* seedlings (A) Root length (B) shoot height; (C) Nodule number; (D) Collar diameter; and (E) Number of leaves. The data is shown as mean±SE (n = 5). According to the Duncan's multiple comparison test, bars with different lettering indicate significant difference among treatments (p 0.05); where (GM) stands for *Glomus macrocarpum* and (GF) for *Glomus fasciculatum.*

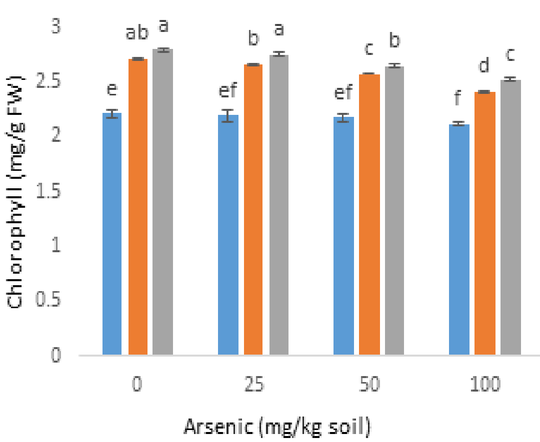
Table 1: Arsenic stress levels (As), mycorrhizal treatments (AMF), and interactions of both (As×AMF) on characteristics of *Leuceana leucocephala* seedlings were analyzed using a two-way ANOVA.

Parameters	Arsenic	AMF	As x AMF
Root length (cm)	**	**	ns
Shoot height (cm)	**	**	**
Collar diameter(mm)	**	**	**
Number of nodules	**	**	**
Number of leaves	**	**	**
Root fresh wt. (gm)	**	**	**
Root dry wt. (gm)	**	**	**
Shoot fresh wt. (gm)	**	**	**
Shoot dry wt. (gm)	**	**	**
Chlorophyll (mg/g fw)	**	**	**
Carotenoids (mg/g fw)	**	**	**
Where ns, not significant;** $p < 0.01$,* $p < 0.05$.			

Table 2: Fresh and Dry weight of Root and Shoot of *Leuceana leucocephala* seedlings in all arsenic stress level and mycorrhizal treatments *Glomus fasciculatum* (GM) and *Glomus macrocarpum* (GF).

Root weight (gm)						
As (mg/kg)	**Control**		**GM**		**GF**	
	FRESH WT.	**DRY WT.**	**FRESH WT.**	**DRY WT.**	**FRESH WT.**	**DRY WT.**
0	0.118±0.004d	0.062±0.003c	1.302±0.028a	0.661±0.032a	0.682±0.039b	0.203±0.023b
25	0.108±0.002d	0.058±0.001c	1.269±0.161a	0.67±0.013c	0.279±0.069d	0.088±0.031c
50	0.107±0.001d	0.058±0.001c	0.591±0.018bc	0.223±0.017b	0.204±0.019d	0.06±0.003c
100	0.105±0d	0.054±0.001c	0.499±0.065c	0.075±0.001c	0.109±0.012d	0.054±0.001c
Shoot weight (gm)						
As (mg/kg)	**Control**		**GM**		**GF**	
	FRESH WT.	**DRY WT.**	**FRESH WT.**	**DRY WT.**	**FRESH WT.**	**DRY WT.**
0	0.141±0.003d	0.07±0.002c	1.983±0.173a	1.105±0.112a	1.783±0.173a	1.105±0.112a
25	0.131±0.002d	0.068±0.001c	1.874±0.134a	0.944±0.195a	1.229±0.33b	0.475±0.111b
50	0.114±0.002d	0.063±0.002c	1.22±0.018b	0.618±0.02b	0.978±0.033bc	0.436±0.031b
100	0.114±0.003d	0.048±0.002c	1.105±0.037bc	0.6±0.01b	0.828±0.019c	0.136±0.003c

Effect of As on Chlorophyll and Carotenoids content –The mean chlorophyll and carotenoids content in the leaves of *Leuceana leucocephala* seedlings was significantly affected by arsenic concentration (0, 25, 50 and 100 mg/kg) in soil. Increasing arsenic stress in soil reduces chlorophyll and carotenoids in both mycorrhizal and non-mycorrhizal plants. Lower arsenic concentration shown marginal impact than higher arsenic stress conditions. The lowest mean chlorophyll and carotenoid content was noticed in 100 mg/kg As stress which is- 2.118±0.012, 2.41±0.009, 2.523±0.023 and 0.299±0.001, 0.36±0.001, 0.39±0.001 chlorophyll and carotenoid content in control, *Glomus macrocarpum* and *Glomus fasciculatum* respectively. Whereas all mycorrhizal plants shown positive responses in all arsenic stress levels. *Glomus fasciculatum* has highest growth than *Glomus macrocarpum* and control (Fig. 2).



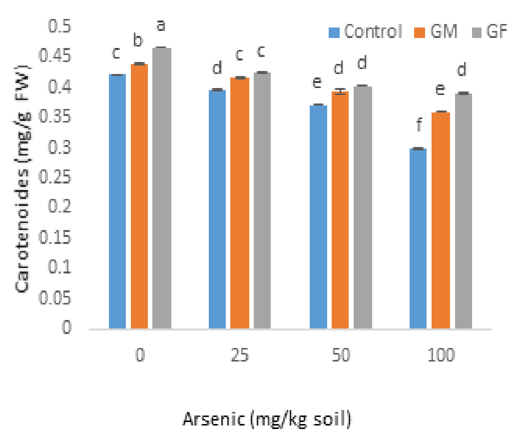


Fig. 2. Effect of As addition levels and AMF treatments on *Leuceana leucocephala* seedlings chlorophyll and carotenoids content. The data is shown as mean±SE (n = 5). According to the Duncan's multiple comparison test, bars with different lettering indicate significant difference among treatments (p 0.05); where (GM) stands for *Glomus macrocarpum* and (GF) for *Glomus fasciculatum.*

DISCUSSION

Schneider *et al.* (2013) investigated that *Leuceana leucocephala* plants infected with *Glomus clarum* had better development and are less susceptible to different soil arsenic concentrations. Zhan *et al*. (2016) results revealed that *Glomus mosseae* (GM), inoculum increased *Leuceana leucocephala* growth and P, S absorption, whereas Diversisporaspurcum (DS) inoculum did not, while grown in farmland surrounding a Pb and Zn smelter. Lins *et al*. (2006) conducted study in a mining region in the Brazilian state of Bahia, enhancing the mycorrhization's detrimental effects on *Leuceana leucocephala* seedlings. When grown in soil that included up to 50% contaminated soil, inoculated plants had larger height, leaf count and shoot and root dry mass than the non-inoculated plants. Garcia *et al.* (2020) a 60 days analysis in greenhouse of *Leuceana leucocephala* plants inoculated with 3 AMF species under different concentration of Mn stressed soil shown that inoculated plants were less impacted by the soil Mn increases, supporting the phytoprotective effect that the AMF has been working to induce. Schneider *et al.* (2017) *Leuceana leucocephala* plants have exhibited a variety of positive impacts on growth and an increase in stress-related enzymes when infected with various isolates of AM fungi and subjected to increasing concentrations of As.AMF *Glomus deserticola* when inoculated with liricidiasepium and *Leuceana leucocephala* capable of reducing the impact of eroded land and drought effect on their growth Fagbola *et al.* (2001). Moreira *et al*. (2010) studied effect of AMF on 4 leguminous species including *Leuceana leucocephala* for low fertility soil for 4 months and concluded that all AMF plants showing an increase in nodules, shoot height, root and shoot dry matter and nutrient content of plants.

In general in *Leuceana leucocephala* plants inoculated with AMF, significantly increases growth and enhanced the establishment and growth conditions for seedlings (Kumar *et al.,* 2017). Other leguminous species with AMF also tried for Arsenic contaminated soil has shown positive responses in root colonization, growth, biomass and As and P accumulation Rangel *et al*. (2014).

In previous studies, *Leuceana leucocephala* has examined for other heavy metals and shown fair responses. Some of them mentioned here. Alkhatib *et al.* (2019) examined that various concentrations of Pb significantly affected the physiological and biochemical parameters of *Leuceana leucocephala* seedlings. While analyzing germination and growth of *Leuceana leucocephala* seedlings under lead and cadmium stress and found that Cd is more harmful compared to Pb for all measured parameters Kabira *et al.* (2018) and also by tannery effluent Karunyal *et al.* (1994).

In general chlorophyll content decreased in various stress conditions (Venkatachalam *et al.,* 2017; Perry *et al.,* 1986). In a study by Bomfim *et al.* (2021) *Leuceana leucocephala* showed that increasing concentration of iron results in reducing chlorophyll in leaves. Gupta *et al*. (2021) demonstrated that fly ash also impact the chlorophyll and carotenoids content negatively in *Leuceana leucocephala* seedlings. Inhibition of growth, the breakdown of chlorophyll, and a reduction in photosynthetic activity have all been caused by Li *et al.* (2007).

Previous studies revealed that various concentration of arsenic in soil affect the chlorophyll and carotenoids content in leaves of plants Azeem *et al.* (2017). Kumar *et al.* (2017) examines the effect of arsenic stress in various physiological parameters of *Pongamia pinnata* seedlings and concluded that at low arsenic stress no changes in chlorophyll content noticed but higher As stress reduced the chlorophyll in seedlings.

Plants inoculated with AMF shown higher chlorophyll and carotenoids content compared to non-mycorrhizal plants de Andrade *et al.* (2015). With rising Pb stress levels, *R. pseudoacacia* considerably reduced, and mycorrhizal plants had much higher Chlorophyll a, total Chlorophyll contents, and Chlorophyll a/b ratios than non-mycorrhizal plants did Yang *et al.* (2015). In a research conducted by Sepahvand *et al.* (2021) under drought conditions, it was discovered that mycorrhizal seedlings outgrew non-inoculated seedlings in terms of dry shoot weight, leaf area, seedling height, dry root weight, root length, number of secondary roots, and chlorophyll content. In a greenhouse experiment by Karimi *et al*. (2017), the effects of Pb stress were examined, and *Onopordum acanthium* was inoculated with AMF and PGPR, which reduced the toxicity of Pb to plants. In comparison to non-inoculated plants, inoculated plants with AMF and PGPR exhibited considerably greater shoot, root dry weight, chlorophyll, carotenoids and proline contents.

CONCLUSION

In the present study revealed that growth parameters and photosynthetic pigments of *Leuceana leucocephala* were reduced by arsenic stress in soil. The combination of arsenic stress and AMF (*Glomus fasciculatum* and *Glomus macrocarpum*) results in positive growth of all seedlings for all parameters. Whereas *Glomus fasciculatum* has higher responses than *Glomus macrocarpum* and control. Photosynthetic pigments were more affected by higher arsenic concentration (50 and 100 mg/kg soil), where at 25 mg/kg soil arsenic level impacts are marginal.

Phytoremediation is an effective and efficient method for getting rid of heavy metals from toxic environment. There is need to identify new hyper-accumulator plant species that are more tolerant to Arsenic and also enhancing the capacity of existing accumulators by various means.

Acknowledgement. I am highly thankful to my advisor Dr. BP Chamola, Assistant Professor Department of Forestry and Natural Resource, HNB Garhwal University, Srinagar, Uttarakhand, to support for complete this research.

Conflict of Interest. None.

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How to cite this article: Anupama R. and Chamola B.P. (2023). Effect of Arbuscular Mycorrhizal Fungi (AMF) on Growth, Biomass and Photosynthetic Pigments of *Leuceana leucocephala* under Arsenic Soil Stress in Nursery. *Biological Forum – An International Journal, 15*(1): 519-525.