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AM FUNGI AND MINE SPOIL CONSORTIUM: A MICROBIAL APPROACH FOR ENHANCING PROSO MILLET BIOMASS AND YIELD

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ABSTRACT

Proso millet (*Panicum miliaceum* L.) was grown with inoculation of four indigenous AM fungal species in presence of different levels of mine spoil under green house conditions. The growth parameters such as biomass production, grain yield, per cent root infection and number of viable AM fungal spores in the rhizosphere of the mycorrhizal proso millet grown with 25% mine spoil were higher over the remaining treatments. The consortium of indigenous AM fungus *Acaulospora laevis* and mine spoil (25%) have supported significant growth of Proso millet compared to same concentration of mine spoil with other indigenous AM fungal inoculation. Higher levels of mine spoil (50% and 75%) affects on the growth and development of both AM fungi and experimental plant. The present work is an attempt towards utilization of mine spoil for the augmentation of mycorrhizal plant growth and it can be considered as eco-friendly management of mine spoil.

KEYWORDS: **Mine Spoil, AM fungi, Proso millet, *Acaulospora laevis*, and Grain yield**

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INTRODUCTION

Fungi, which form symbiotic association with most of the terrestrial plants, are referred to as mycorrhizal fungi and the association itself is called as mycorrhizae. The most common mycorrhizal association found in cultivated crops throughout the world is the arbuscular mycorrhizal (AM) fungi. Mycorrhizae form the connecting link between the biotic and geochemical portions of the ecosystem[19]. The fungal mycelia found in mycorrhizae are very important factors in maintaining soil structure since; they can help to bind soil particles together. This will assist in preventing soil erosion and loss. It is also reported that, the presence of AM fungi in poor- quality soil will greatly increase the chances of plants being able to thrive in such locations upon the formation of symbiotic relationships. Recently there has been considerable interest developed in the possible utilization of AM fungi for the reclamation of degraded lands such as mined areas. Much of the interest has stemmed from the experimental evidences that, the AM fungi improve the survival and growth of seedlings by alleviating deficiencies encountered by plant species during their establishment on mine wastes[1, 6, 11, 14, 15 & 25].

In India about 6, 85,000 hectare of land is under mining, which has created widespread environmental damage, affecting not only the landscape, but also the social being[23]. During the extended period of mine spoil storage, anaerobic conditions are created in the top soil[8] along with decreased microbial activities[28]. However, arbuscular mycorrhizal fungi provide an attractive system to advance plant based environmental cleanup. During symbiotic interaction between the AM fungi and plant, the hyphal network functionally extends towards the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up heavy metals from an enlarged soil volume[24& 26]. Therefore, based on the aforementioned importance of AM fungi in the reclamation of waste lands and mine spoil, the present study was undertaken to evaluate the response of proso millet (*Panicum miliaceum* L.), planted in mine spoil amended potting mixture (Growth Media). Proso is an ancient Slav name used in Russia and Poland[2]. The proso millet has also been called as common millet, hog millet, broom corn, yellow hog and white millet. Proso millet (*Panicum miliaceum* L.), is a member of the family Poaceae. The feed value of proso millet has been studied with several classes of livestock. Luis et al., [16 & 17], investigated both broiler and turkey diets with proso millet. The turkey poults were significantly heavier at the end of the 28 day feeding period with proso than those on either corn or sorghum diets. Kies et al., [10] investigated various millet mixes in human diets and discussed their potential. The feed value of proso millet for cattle and swine is generally considered equal to that of grain sorghum and maize. Hence, the proso millet (*Panicum miliaceum* L.) was selected as an experimental plant to assess the effect of different mine spoil with mycorrhization on biomass production and yield.

MATERIALS AND METHODS

(i) Collection of germplasm, mine spoil and Plant growth conditions

Proso millet (*Panicum miliaceum* L.) seeds were procured from Hanumanamatti Agricultural research station, (University of Agricultural Sciences Dharwad), Ranebennur (Haveri district), Karnataka (India). Seeds were surface sterilized by keeping them in 2% mercuric chloride ($HgCl_2$) solution for 2-3 min and then rinsed with distilled water. Four surface sterilized seeds were sown in each earthen pots (diameter, diameter and height = 15cmX15cmX20cm). The growth media (GM) was a mixture of soil and sand (3:1 v/v). Mine spoil (MS) was collected from two year old dumps of iron mining areas of Sandoor (Bellary district in Karnataka, India). This mine spoil was amended with the potting mixture (growth

media) in different gradients. The experiment was conducted in polyhouse /green house conditions. Seedlings in each pot were watered twice in week and 10 ml of nutrient solution (Hoagland solution without 'P') was given at every fortnight till harvest.

(ii) Experimental Design

The experiment was a completely randomized block design of five treatments with triplicates per treatment. The following treatments were maintained separately for each AM fungal inoculation:

T1: Mine spoil and potting mixture (75:25) with AMF.
T2: Mine spoil and potting mixture (50:50) with AMF.
T3: Mine spoil and potting mixture (25:75) with AMF.
T4: Mine spoil and potting mixture (10:90) with AMF.
T5: Mine spoil and potting mixture (0:100) with AMF.

All the above treatments were maintained without any AM fungal inoculation as control (Non-Mycorrhizal treatment). Four indigenous AM fungal species *Acaulospora laevis* (ALVS), *Glomus fasciculatum* (LFSC), *Sclerocystis dussii* (SDSS) and *Glomus bagyarajii* (LBRJ) were used as inocula. The inocula were prepared at Microbiology Laboratory, P.G. Department of Studies in Botany, Karnatak University, Dharwad (INDIA), by using *Sorghum* (host plant). AM fungal inoculum containing chopped root bits of host plant, hyphal segments and spores were placed 5 cm below the soil surface in each experimental pots.

(iii) Plant analysis

Plants were harvested 90 days after sowing and triplicates of each treatment were harvested to determine dry weight, number of grains per plant and 100 grains weight. Sub samples of fresh roots were stained with tryphan blue and percent mycorrhizal colonization (PMC) was quantified as described by Trouvelot *et al*., [30]; Phillips and Hayman[22] and Mc Gonigle[18]. The mycorrhizal spore number (MSN) was counted as illustrated by Gerdemann and Nicolson[5], in the rhizosphere of the experimental plant.

(iv) Statistical analysis

All the data were subjected to a Statistical one way analysis of variance (ANOVA) [27] using the SPSS software student version 18. Treatment means were compared by the Duncan's multiple range test (DMRT) at P=0.05.

RESULTS

1. Effect of mine spoil on Root colonization by AM fungi and spore number

Overall AM fungal colonization was higher in the lateral roots of the proso millet (*Panicum miliaceum* L.), regardless of the AM fungal inoculum compared to non-mycorrhizal plants. The plants inoculated with AM fungus *Acaulospora laevis* (ALVS) showed greater per cent mycorrhizal colonization (PMC) (Table 3) and it was followed by *Glomus fasciculatum* (LFSC). Similarly, more number of viable AM fungal spores from the rhizosphere of proso millet inoculated with *Acaulospora laevis* and 25% mine spoil were recorded over the other AM fungi inoculated plants with same concentration of mine spoil in the experimental pots. There was the least per cent mycorrhizal colonization and numbers of AM fungal spores were measured in the rhizosphere of plants grown with 75% mine spoil in the experimental pots. With respect to mycorrhization, proso millet inoculated with *Glomus bagayarajii* had shown lesser values for mycorrhizal colonization and AM fungal spores in the rhizosphere.

2. Effect of AM fungi and mine spoil on plant growth

The plant growth parameters such as shoot length and root length were measured. The experimental results showed significantly increased shoot and root length in all the

mycorrhizae inoculated plants over the control. It was observed that, mycorrhizal plants grown under 25% mine spoil with different AM fungi inoculation have brought significant results over the remaining mine spoil amendments. But the extent of increased shoot and root length varied with each AM fungus. The higher shoot and root length was recorded in plants grown with inoculation of AM fungus *Acaulospora laevis* at 25% mine (Table 1). The least increased plant height was observed with inoculation of AM fungus *Glomus bagyarajii*. In general biomass production was more in mycorrhizal *Panicum miliaceum* L. (proso millet), compared to non-mycorrhizal plants. The greater values for biomass production was recorded in plants treated with AM fungus *Acaulospora laevis* and minimum value was measured in proso millet inoculated with *Glomus bagyarajii*. The mycorrhizal proso millet grown with different concentrations of mine spoil have showed varied rate of biomass production. The maximum biomass production rate was recorded in mycorrhizal plants grown in potting mixture amended with 25% mine spoil over the non-mycorrhizal plants. The results also revealed that, least biomass production in mycorrhizal plants grown under 50% and 75% mine spoil (Table-2). The same trend was observed for all the AM fungal inoculation. Particularly, the plants inoculated with AM fungus *Acaulospora laevis* at 25% mine was found to be superior over the remaining AM fungi inoculated plants at the same concentration of mine spoil. The deleterious effect on plant was observed with 50% and 75% mine spoil amendment in the experimental pots.

Table 1: ***Effect of different concentrations of mine spoil and AM fungi on height of Panicum miliaceum* L.**

Treatments	*ALVS*		*LFSC*		*SDSS*		*LBRJ*		NM	
	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)	SL (Cm)	RL (Cm)
75 MS : 25 GM	61.33 ±0.57d	18.33 ±0.57d	59.33 ±0.88d	17.33 ±0.33d	55.33 ±0.88e	14.42 ±0.11e	52.66 ±1.20e	12.35 ±0.04e	44.00 ±1.15e	10.70 ±0.25c
50 MS : 50 GM	72.66 ±0.57c	20.66 ±0.57c	63.66 ±0.66c	20.33 ±0.66c	61.00 ±1.15d	18.63 ±0.06c	56.66 ±0.33d	17.13 ±0.05c	51.00 ±1.15d	16.33 ±0.33b
25 MS: 75 GM	108.66 ±0.57a	31.66 ±0.57a	105 ±0.57a	30.33 ±1.33a	86.03 ±0.49a	28.6 ±0.05a	79.33 ±0.88a	25.11 ±0.02a	78.00 ±1.00a	20.16 ±0.60a[4]
10 MS : 90 GM	75.33 ±0.57b[1]	24.66 ±0.57b[2]	84.00 ±1.00b	23.66 ±0.33b[3]	75.23 ±0.39b	20.13 ±0.01b	64.33 ±0.66b	18.09 ±0.03b	63.60 ±0.66b	19.66 ±0.66a[4]
00 MS : 100 GM	74.33 ±0.57b[1]	21.33 ±0.57b[2]	66.00 ±0.57c	21.33 ±0.88b[3]	65.86 ±0.69c	17.18 ±0.08d	59.90 ±0.10c	16.92 ±0.03d	56.86 ±2.48c	16.76 ±0.05b

Note: MS: Mine Spoil, GM: Growth Media, SL: Shoot Lenght, RL: Root Lenght, ALVS: Acaulospora laevis, LFSC: Glomus fasciculatum, SDSS: Sclerocystis dussii, LBRJ: Glomus bagyarajii and NM: Non-Mycorrhizal. 1,2,3,4, Each value represents the mean of three determinations. Mean values followed by the same letter within a column do not differ significantly at P = 0.05 according to DMRT.

Table 2: ***Effect of different concentrations of mine spoil and AM fungi on biomass production of Panicum miliaceum*** **L.**

Treatments	*ALVS*		*LFSC*		*SDSS*		*LBRJ*		NM	
	DWR	DWS	DWR	DWS	DWR	DWS	DWR	DWS	DWR	DWS
75 MS : 25 GM	0.23 ±0.01e	1.21 ±0.01e	0.13 ±0.00e	0.96 ±0.00e	0.09 ±0.00c	0.33 ±0.00e	0.09 ±0.00c	0.95 ±0.01e	0.03 ±0.00e	0.49 ±0.00e
50 MS : 50 GM	0.26 ±0.01d	1.45 ±0.01d	0.17 ±0.00d	1.48 ±0.01d	0.12 ±0.00b	0.63 ±0.01d	0.12 ±0.01c	1.6 ±0.01d	0.09 ±0.00d	0.87 ±0.00d
25 MS: 75 GM	1.36 ±0.00a	5.82 ±0.00a	0.97 ±0.00a	4.66 ±0.01a	1.20 ±0.10a	4.09 ±0.02a	0.95 ±0.01a	2.53 ±0.01a	0.36 ±0.01a	2.41 ±0.02a
10 MS : 90 GM	0.52 ±0.00b	3.83 ±0.00b	0.51 ±0.01b	3.29 ±0.15b	0.20 ±0.00b[1]	1.80 ±0.01b	0.18 ±0.00b[2]	1.77 ±0.01b	0.24 ±0.00b	1.75 ±0.00b
00 MS : 100 GM	0.46 ±0.00c	2.23 ±0.05c	0.47 ±0.00c	2.09 ±0.03c	0.19 ±0.00b[1]	1.69 ±0.01c	0.16 ±0.01b[2]	1.67 ±0.01c	0.14 ±0.00c	1.66 ±0.00c

Table 3: ***Effect of different concentrations of mine spoil and AM fungi on mycorrhizal status in the roots and rhizosphere of Panicum miliaceum*** **L.**

Treatments	*ALVS*		*LFSC*		*SDSS*		*LBRJ*		NM	
	PMC	MSN	PMC	MSN	PMC	MSN	PMC	MSN	PMC	MSN
75 MS : 25 GM	37.33 ±0.57e	65.33 ±1.52d	37.00 ±1.00e	57.00 ±1.15e	37.33 ±0.88e	54.00 ±0.57d	37.33 ±0.88e	51.33 ±0.88d	0.00 ±0.00	0.00 ±0.00
50 MS : 50 GM	48.33 ±1.15d	79.00 ±1.73c	46.66 ±1.85d	62.66 ±1.66d	47.00 ±1.15d	58.00 ±0.57c	45.00 ±1.00d	57.66 ±0.66c	0.00 ±0.00	0.00 ±0.00
25 MS: 75 GM	95.33 ±1.15a	158.33 ±1.15a	86.00 ±1.00a	108.33 ±0.66c	83.00 ±1.52a	96.00 ±1.73a	77.00 ±1.00a	84.33 ±0.88a	0.00 ±0.00	0.00 ±0.00
10 MS : 90 GM	83.33 ±3.21b	141.66 ±1.52b[1]	78.00 ±1.00b	127.00 ±1.15a	77.66 ±2.40b	86.66 ±0.88b[2]	68.33 ±0.66b	76.33 ±1.66b[3]	0.00 ±0.00	0.00 ±0.00
00 MS : 100 GM	79.33 ±1.52c	139.33 ±1.15b[1]	66.33 ±1.20c	121.66 ±1.66b	62.33 ±0.66c	88.33 ±0.66b[2]	57.66 ±2.18c	77.66 ±1.33b[3]	0.00 ±0.00	0.00 ±0.00

Note: MS: Mine Spoil, GM: Growth Media, PMC: Per cent Mycorrhizal Colonization, MSN: Mycorrhizal Spore Number per 25 g soil ALVS: Acaulospora laevis, LFSC: Glomus fasciculatum, SDSS: Sclerocystis dussii, LBRJ: Glomus bagyarajii and NM: Non-Mycorrhizal. Each value represents the mean of three determinations. 1, 2,3, Mean values followed by the same letter within a column do not differ significantly at P = 0.05 according to DMRT.

3. Effect of AM fungi and mine spoil on grain yield

The effect of four AM fungal species and different levels of mine spoil applications on grain yield was assessed. The experimental mycorrhizal plants have showed positive results over the non-mycorrhizal plants. The mycorrhizal *Panicum miliaceum* L. (proso millet) grown with different levels of mine spoil have also resulted in varied values for number of grains and weight of 100 grains. While the highest grain yield and 100 grains weight was measured in mycorrhizal plants grown under 25% mine spoil in the potting mixture over the other levels of mine spoil. Especially, more yield and 100 grains weight was recorded in plants inoculated with AM fungus *Acaulospora laevis* in the pots containing 25% mine, intermediate with AM fungus *Glomus fasciculatum* at same concentration of mine spoil. The lesser number of grains and 100 grains weight was recorded with 50% and 75% mine spoil in the potting mixture irrespective of AM fungal inoculation. The experimental results also revealed that, very least number of grains and 100 grains weight was recorded

with the plants inoculated with AM fungus *Glomus bagyarajii* at 75% mine spoil (Fig. 1& Fig. 2).

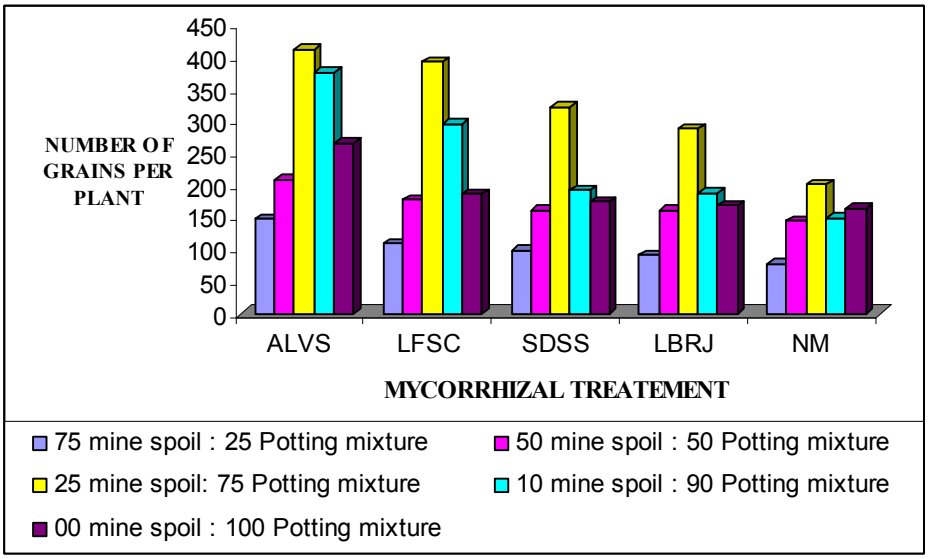

Figure 1:*Effect of different AM fungi and varied concentrations of mine spoil on number of grains per plant of Panicum miliaceum L. (Proso millet).*

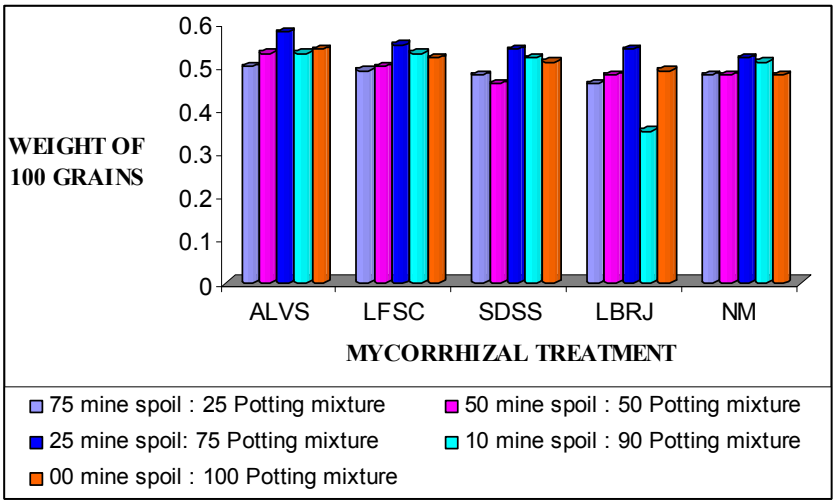

Figure 2: Effect of different AM fungi and varied concentrations of mine spoil on weight of 100 grains of *Panicum miliaceum* L. (Proso millet).

Note: ALVS: Acaulospora laevis, LFSC: Glomus fasciculatum, SDSS: Sclerocystis dussii, LBRJ: Glomus bagyarajii and NM: Non-Mycorrhizal

DISCUSSIONS

The inoculation with AM fungus *Acaulospora laevis* at 25% mine spoil showed a better growth of proso millet over the remaining treatments. These results are in accordance with those of Daft and Hacskalyo, [3] who tested the application of AM fungi, to ensure suc-

cessful reclamation of coal mined areas and improvement of the plant growth parameters. In mine wastes, AM fungi have been reported to bring an improvement of plant growth through increased uptake of mineral nutrients, particularly phosphorus. Several studies on mined areas[1&6] demonstrated the effect of AM fungi on improved plant growth. Nicholson and Mc Ginnies, [21] reported that, establishment of good plant cover can stabilize mine spoil by conducting experiments on seventeen grasses and two legumes on mine spoil stripe. Consequently improve soil conditions to promote plant succession. Mycorrhization allows the plants to have a high root: shoot ratio, causing better mineral nutrition and thereby, reinforcing the capacity to resist stress, particularly the stress of mine spoil [20 & 13]. This shows the importance of mycorrhization on mine spoil areas.

The present experimental results strongly support the contribution of earlier workers, Kadam[9] and Geeta Patil[4] who have studied independently the effect of AM fungal inoculation on different varieties foxtail millet and finger millet respectively. They have concluded that, mycorrhizal inoculation results in increased biomass production, plant height and nutrient uptake by the experimental plants compared to non-mycorrhizal foxtail millet and finger millet. Much research is currently aimed at improving quality and quantity of food grain production while at the same time ensuring proper utilization and maintaining ecological resources with safety measurements. Tobar et al., [29] have conclusively explained that, the root and shoot ratio reflects the degree of efficiency of AM fungi. The higher levels of mine spoil causes adverse effect on the plant growth, grain yield and nutrient uptake due to accumulation of heavy metals and their toxicity on AM fungal development. Zak *et al*., [31] and Lakshman, [12&13] demonstrated that, decreased number of AM fungal spores and mycorrhizal colonization was found with increase in mine spoil concentration. In the view of above discussion, it was clearly indicated that, mycorrhization will enhances the growth of the crop plants; capacity to survive and development of compatible mycorrhizal association under different levels of mine spoil.

CONCLUSION

Mycorrhizal fungal association promises the increased growth and yield of *Panicum miliaceum* L. In addition, the optimum levels of mine spoil could also responsible for augmented growth rate in mycorrhizal plants. It is evident that, the inoculation of indigenous arbuscular mycorrhizal fungus *Acaulospora laevis* to the growth media having 25% mine spoil was the best treatment for enhancing proso millet biomass and yield. From, the experimental results, it can be concluded that, optimum levels of mine spoil can be used as plant growth augmenting agent in presence of beneficial microorganisms such as AM fungi and this method of use of mine spoil for the augmentation of plant growth can be considered as eco-friendly management of mine spoil.

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