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Studies on mycorrhizal inoculation on dry matter yield and root colonization of some medicinal plants grown in stress and forest soils

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Abstract: *Five medicinal plants viz. Abelmoschatus moschatus Linn., Clitoria ternatea L., Plumbago zeylanica L., Psorolea corylifolia L. and Withania sominifera L. were grown in a polypot experiment in five soils representing coal mine soil, copper mine soil, fly ash, skeletal soil and forest soil with and without mycorrhizal inoculations in a completely randomized block design. Dry matter yield and mycorrhizal root colonization of plants varied both in uninoculated and inoculated conditions. The forest soil rendered highest dry matter due to higher yield of A. moschatus, P. zeylanica and P. corylifolia while fly ash showed lowest dry matter without any inoculants. P. cernatea were best in coal mine soil and W. sominifera in copper mine soil without mycorrhizal inoculation. The mycorrhiza was found to enhance the dry matter yield. This contributed minimum 0.19% to maximum up to 422.0% in different soils as compared to uninoculated plants. The mycorrhizal dependency was noticed maximum in plants grown in fly ash followed by coal mine soil, copper mine soil, skeletal soil and forest soil. The mycorrhizal response was increased maximum in W. sominifera due to survival in fly ash after inoculation followed by P. corylifolia and P. cernatea. Percent root colonization in inoculated plant was increased minimum of 1.10 fold to maximum of 12.0 folds in comparison to un-inoculated plants .The native mycorrhiza fungi were also observed to colonize 4.0 to 32.0% roots in plants under study. This study suggests that mycorrhizal inoculation increased the dry matter yield of medicinal plants in all soils under study. It also helps in survival of W. sominifera in fly ash.*

Key words: *Mycorrhiza, Root colonization, Dry matter yield, Inoculation, Mycorrhizal dependency, Stress soil*
PDF of full length paper is available online

Introduction

Land use change affects soil properties such as surface mining for coal or copper causes drastic land disturbance and severe soil degradation (Ussiri *et al.,* 2006). Fly ash is discharged by thermal power plants in the process of power generation. Skeletal soil is degraded pasture consist spherical nodules of iron and sodium oxides with heavy biotic pressure. These soils lack most of the physical, chemical and biological characteristics of forest soil and some unfavorable conditions for vegetation and microbial growth. The successful restoration of such site is always a big challenge for agriculturists, foresters and scientists. In present study five medicinal plants were evaluated in different stressed soils.

A major beneficial component of soil microbial community is mycorrhizal fungi, contributes to plant growth and survival by reducing stresses through symbiosis in problematic soil (Orlic *et al.,* 2005). Smith and Read (2008) also reported that vesicular arbuscular mycorrhizal fungi are ubiquitous in most soils and plays beneficial role in plant establishment and growth. The fungus is known to extend the plant root system through natural link and intimate relationship permits a more direct regulation of mycosymbionts by the plant than other soil biota. The increasing benefit of the fungi to plant health with increasing stress for more efficient supply of nutrient and water has also been reported (Miransari *et al*., 2008). The fungus contributes greatly to phosphorus uptake (Schnepf *et al*., 2009) besides the uptake of other ions. AM has been shown to effectively enhance the stomatal conductance, photosynthetic rate and water use efficiency of their host during severe stress conditions (Querejeta *et al*., 2006).

It is reported that the plant dependency to mycorrhizal fungi depends on the level of soil fertility and receptivity of soil to inoculants (Covacevich and Echeverria, 2008), which influences both the host and percent root colonization. Hence the mycotrophic plants differ in the rate of mycorrhizal formation in root responsiveness (Moretimer *et al*., 2008) Despite various reports on beneficial role of mycorrhizal fungi on plant growth the information particularly on medicinal plants under stress soil condition is scares. Due to this, the mycorrhizal potential is often ignored during establishment of vegetation on such sites. Therefore, the present investigation was undertaken to evaluate the dependency of medicinal valued plants to mycorrhizal inoculation under various stress soils and forest soil for comparison.

Materials and Methods

Representative soil samples of coal mine soil, Korba; copper mine soil, Malajkhand; fly ash, National Thermal Power Corporation, Korba; skeletal soil, Bilaspur and forest soil of Sal (*Shorea robusta* Gaten) dominated forest area Katghora (Korba) were selected for the study. The samples represented State of Chhatishgarh and Madhya Pradesh, experience 1100-1600 mm annual rainfall.

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Chemical and physical characteristic of soil samples were analyzed before use in experiment. Nitrogen content was determined by Auto Kjeltech, 2300, phosphorus and sulfur as per Jackson (1958) methods while potassium by flame photometer. Organic matter content of soil was estimated by Black (1965) and pH measured from soil solution at 1: 2.5 soil water ratio by pH meter. Copper content of soil samples determined through atomic absorption spectrophotometer (AAS) GBC 1030 by following Liang and Karamanos (1993) method. Clay content was measured by hydrometer techniques (Bauyoucens, 1927). The properties of fly ash were obtained from NTPC authority. Mycorrhizal status in different soil were estimated as per Gerdemann and Nicolson (1963) technique and counted through nematode counting technique under stereomicroscope.

Each soil types were filled in polyethylene bags of 3.5 kg capacity and five medicinal species *i.e. Abelmoschatus maschatus* L (Muskdana) (M1), *Clitoria ternatea* L. (Aparajita) (M2), *Plumbago zaylanica* L. (Chitrak) (M3), *Psoralea corylifolia* L. (Bawchi) (M4) and *Withania sominifera* L. (Ashwagandha) (M5) were grown in the experiment. The seeds of each species were germinated in sand mixture (2:1 by volume) which was autoclaved at 120°C for 60 min twice before it is used for germination. 10 days after germination seedling were transplanted to bags.

25 g mycorrhizal incoulum was placed in planting hole made before transplantation. The inoculum load estimated 0.17x 10^4 propagules in the form of chlamydospore of *Glomus intraradics* Schenck Smith, *G. mosse* Gerdemann and Trappe and *Acaulospora scrobiculata* (Trappe) Mortan and 80% infected roots of trap host (*Panicum maximum*). Experiment was arranged in a randomized factorial block design with triplicate. Each block contained 20 plants per replicate. Control plants without mycorrhizal inoculation were also maintained separately for comparison.

15 plants were selected randomly after 160 days age from each treatment representing 5 from replicate, uprooted and roots were washed carefully. The whole plant was cut into pieces of 10 cm size and fresh weight was taken. Dry matter yield (DMY) was estimated by keeping the plants in oven at 80°C for 64 hr then weighed again to find out dry matter yield.

Percent root colonization was determined by cleaning the roots in 10% KOH followed by staining with 0.01% trypan blue (Phillips and Hayman, 1970). Gridline intersect method of Giovanneti and Mosse (1980) was followed for estimating root infection percentage. The plant dependency to mycorrhizal inoculation was calculated as: DMY of inoculated plant - DMY of uninoculated plant / DMY of uninoculated x 100. Data were subjected to analysis of variance and least significant differences using SPSS computer software.

Results and Discussion

The physiochemical properties of soils under study are given in Table 1. Coal and copper mine soils were sandy, fly ash contained high silt, while skeletal and forest soils had sandy loam texture. Mined materials were almost similar to copper mine soil except sulfur and copper contents, which were high in coal and copper mine soil respectively. Skeletal soil contained spherical nodules of iron and sodium oxides, while fly ash contained more aluminium and iron, which created an alkaline pH. The other soils were acidic. Stressed soils were deficient in organic matter, major nutrients and mycorrhizal population as compared to forest soil.

Dry matter yields of plants exhibited significant difference with various soils both without and with mycorrhizal inoculation (Table 2, 3). Plants of forest soil rendered highest dry matter (90.10 g pl^{-1}) followed by coal mine (74.83 g pl^{-1}), copper mine (64.05 g pl^{-1}), skeletal soil (41.15 g pl^{-1}) and fly ash (16.35 g pl^{-1}) (Table 2). *A. moschatus, P. zeylanica* and *P. corylifolia* yielded highest dry matter in forest soil, *P. cernatea* in coal mine, *W. sominifera* in copper mine soil but *A. moschatus* and *W. sominifera* failed to survive in fly ash without mycorrhizal inoculation. Mycorrhizal dependency of plants was observed maximum in *W. sominifera* followed by *P. corylifolia* > *P. cerneatea* > *P. zeylanica* > *A. moschatus* (Table 4). Similarly plants of fly ash were showed maximum dependency followed by coal mine soil, copper mine soil, skeletal soil and forest soil.

Percent root colonization differed significantly with soils and plants (Table 3). Indigenous endophytes which were low in soil found effective in colonizing up to 22.13% roots in plants of coal mine and minimum 4.40% root colonization in fly ash. The root infection was examined maximum in *A. moschatus* and minimum in

Table - 1: Physiochemical properties of different soils under study

Attributes	Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil
Clay (%)	6.50	10.80	00.00	16.39	23.69
pH	5.69	6.45	8.13	7.50	6.70
Nitrogen (kg ha^{-1})	46.40	62.45	00.00	104.89	310.10
Phosphorus(kg ha^{-1})	4.70	5.66	1.59	16.48	25.11
Potassium(kg ha^{-1})	56.31	180.00	36.40	114.72	240.38
Soil Organic Matter (%)	0.62	1.12	00.00	2.19	3.05
Copper (ppm)	57.00	813.00	00.00	24.00	32.00
Sulfur (ppm)	242.15	86.00	00.00	19.20	28.30
Al & Fe oxides (%)	00.00	00.00	34.51	00.00	00.00
Mycorrhizal population (No. spore $soil^{-100g}$)	10.00	12.00	00.00	28.00	67.00

Table - 2: Dry matter yield (DMY) of plants grown in different soils

Soils(s) / Species(m)	Without mycorrhiza (I_0)					Mean I_0 x M	With mycorrhiza (I)					Mean I x M
	Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil		Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil	
A. moschatus (M1)	144.20	170.50	00.00	110.70	218.70	128.80	171.84	181.20	00.00	114.30	231.90	139.84
P. cernatea (M2)	143.00	19.20	15.50	25.28	83.62	57.32	215.30	28.74	20.00	26.27	83.78	74.93
P. zeylanica (M3)	48.90	65.20	44.76	48.94	84.46	58.52	61.49	83.70	46.69	51.63	88.15	66.30
P. corylifolia (M4)	27.53	31.92	21.50	13.83	50.46	29.05	37.85	42.15	27.70	16.79	60.73	36.89
W. sominifera (M5)	10.54	33.12	00.00	6.95	13.27	12.78	18.43	43.85	4.22	12.64	17.97	19.42
Mean (I_0xS)	74.83	64.05	16.35	41.15	90.10		100.80	75.92	19.72	44.43	96.51	
CD (p = 0.05)	I_0xM	I_0xS	I_0xSxM					IxM	IxS	IxSxM		
	9.67	9.67	5.35					17.53	17.53	9.69		

CD = Critical difference

Table - 3: Percentage root infection of plants grown in different soils

Soils(s) / Species(m)	Without mycorrhiza (I_0)					Mean I_0 x M	With mycorrhiza (I)					Mean I x M
	Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil		Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil	
A. moschatus (M1)	32.00	28.00	00.00	12.00	19.00	18.13	78.00	62.00	00.00	40.00	71.00	55.33
P. cernatea (M2)	27.00	9.00	7.00	28.00	12.00	16.67	66.00	29.00	28.00	36.00	51.00	42.13
P. zeylanica (M3)	17.00	20.00	4.00	12.00	10.00	12.60	54.00	50.00	19.00	31.00	25.00	35.93
P. corylifolia (M4)	15.00	14.00	11.00	20.00	27.00	17.40	38.00	27.00	44.00	22.00	53.00	36.83
W. sominifera (M5)	19.00	23.00	00.00	10.00	10.00	12.23	33.00	51.00	12.00	22.00	30.00	29.60
Mean (I_0xS)	22.13	18.60	4.40	16.40	15.60		54.00	44.07	20.67	30.20	45.87	
CD (p=0.05)	I_0xM	I_0xS	I_0xSxM					IxM	IxS	IxSxM		
	4.30	4.30	2.32					3.21	3.21	1.16		

CD = Critical difference

Table - 4: Percent mycorrhizal dependency of plants on dry matter yield

Soil(s) Species(m)	Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil	Mean
A. moschatus (M1)	19.16	6.27	00.00	3.25	6.03	8.68
P. cernatea (M2)	51.25	49.68	29.03	3.91	0.19	26.81
P. zeylanica (M3)	25.74	28.37	4.31	5.49	4.36	13.65
P. corylifolia (M4)	37.48	32.04	28.83	21.40	20.32	28.01
W. sominifera (M5)	74.85	32.39	422.00	81.87	35.41	129.30
Mean	41.69	29.75	121.04	23.18	13.26	- - -

Table - 5: Improvement (in fold) in root infection through mycorrhizal inoculation over uninoculated plants

Soil(s) Species(m)	Coal mine soil	Copper mine soil	Fly ash	Skeletal soil	Forest soil	Mean
A. moschatus (M1)	1.43	1.21	00.00	2.33	2.73	1.92
P. cernatea (M2)	2.44	3.22	4.00	1.28	4.25	3.03
P. zeylanica (M3)	3.17	2.50	4.75	2.58	2.50	3.10
P. corylifolia (M4)	2.53	1.92	4.00	1.10	1.96	2.30
W. sominifera (M5)	1.73	2.21	12.00	2.20	3.00	4.22
Mean	2.26	2.21	6.18	1.89	2.88	- - -

W. sominifera by native mycorrhiza. The colonization was increased through the inoculation of mycorrhiza maximum in coal mine followed with forest soil, copper mine soil, skeletal soil and fly ash (Table 3). The level of colonization was maximum of 78.0% in *A. moschatus* in coal mine and minimum of 12.0% in *W. sominifera* in fly ash. But the net improvement in percent root colonization was noticed maximum 12.0 folds in *W. sominifera* and minimum of 1.10 folds in *P. corylifolia* over the infection by native mycorrhiza (Table 5).

In present study, soils of stressed area and forest have shown variations in physiochemical properties due to the presence of high sulfur in coal mine, copper in copper mine, aluminium and iron oxides in fly ash and iron and sodium in skeletal soil. Forest soil, which consisted higher organic matter, nutrients and mycorrhizal propagules due to the presence of vegetations and less soil disturbances resulted into higher dry matter yield without any inoculants as compared to other soils. While in case of mined soil, where soil consisted low fertility, reduced microbial activity, low organic matter and lack of vegetation adversely affected the mycorrhizal development and vegetational establishment (Ussiri *et al*., 2006). However, in comparison to others, coal mine soil and copper mine soil rendered higher dry matter of medicinal plants next after forest soil with native mycorrhizal fungi. Fly ash and skeletal soil consisted aluminium and iron and sodium and iron respectively, no and poor organic matter might created adversity for mycorrhizal development and plant growth. Due to toxicity of fly ash *A. moschatus* and *W. sominifera* were failed to survive. These plants rendered low yield in skeletal soil. Brian *et al*. (2006) and Goransson *et al*. (2008) has also reported toxic influences of copper, aluminium and alkalinity on mycorrhizal development and plants health.

The experimental plants showed variation in dry matter yield in various soils. *A. moschatus, P. corylifolia* and *P. zeylanica* rendered highest biomass in forest soil, *P. crenatea* in coal mine and *W. sominifera* in copper mine soils. Yang *et al*. (2008) also noticed similar variation in AMF infection on families of plants and thereby dry matter yield. Moreover, Pandeya *et al*. (2007) noticed that genetic diversity of the species as a means of adoption to differing climo-edaphic conditions. Mycorrhizal fungi were also reported to influence the yield of plants by modifying the rate of nutrient transfer, water uptake, root respiration and photosysthesis (Querejeta *et al*., 2006). The forest soil a natural home of all medicinal plants with favourable soil conditions supported higher plant growth and thereby dry yield. But in case of higher yield of *P. cernatea* and *W. sominifera* in coal mine and copper mine soil respectively might be due to the favourable soil pH as well as may be high demand of sulfur and copper by these plants respectively. Mycorrhizal association by native endophytes also played positive role in improving yield of these species in some extent by alleviating the stress effects and supply of more nutrient and water through mycelia network to roots (Schnepf and Roose, 2006).

Mycorrhizal inoculated plants improved their dry matter yield by enhanced translocation of nutrients and water from rhizosphere due to higher root colonization. The mined plants results higher response with inoculation of mycorrhiza because of more strong relationship with mycorrhiza to fulfill their phosphorus and other nutrients requirement in deficient soil and shared some of photosynthetes with fungus to maintain the symbiosis (Hobbie, 2006). The increasing dependency of plants on mycorrhiza in phosphorus and nutrient deficient soil has been also reported by Mathur *et al*. (2006) and Moretimer *et al*. (2008). In forest soil, where phosphorus and other nutrients were easily available to plants without any hazardous element the plant dependency on mycorrhiza was decreased results in a lowest response of mycorrhizal inoculation. Fly ash an inert material with no organic matter and toxic compounds and skeletal soil with high nodules of aluminium and iron oxides

created negative effects in mycorrhizal development and symbiosis thereby, lowered plant dry matter yield. Soil pH might also inhibit the mycorrhizal efficiency and plant yield in fly ash and skeletal soil as reported by Graw (2006), Bryan *et al*. (2008).

The response of mycorrhiza depends on soil characteristics, nutrient status and soil receptivity to myocorrhizal fungi. Thus variations were reported in dry matter yield and percent root colonization due to the rate of plant mycorrhizal interactions and plant genotype (Koide and Schreiner, 1992). Moreover, Yang *et al*. (2008) and Covacevich and Echeverria (2008) also observed such variations in AMF colonization and plant families due to intensity of infection and spore density. Fujiyoshi *et al*. (2006) also supported that the effect of mycorrhizal fungi on plant growth depends more on the plant species than soil developmental stage. The contribution of mycorrhiza was highest in survival of *W. sominifera* in fly ash due to reduction of stresses and creating congenial environment in rhizosphere (Orlic *et al*. 2005; Bryan *et al*., 2008). Moreover, Atul Nayyer *et al*. (2009) also noticed that AM modify soil with linking plant differently leading to species specific changes in the quality of soil and structure of the soil community.

The present study confirms that stressed soil adversely influences the mycorrhizal development and plant growth due to varied physio-chemical properties and toxic element present in soils as compared to forest soil. Mycorrhizal fungi played a major role in stress condition in the survival and establishment of plants as in case of *W. sominifera* in fly ash. Moreover, the contribution of mycorrhiza on dry matter yield of plants was increased with increasing deficiency of nutrients and toxicity. In stress soil, where indigenous mycorrhizal status was poor the apparent results were not observed but after inoculation both the percent root colonization and dry matter yield of medicinal valued plants were improved. Thus during establishment of vegetation in stress soils the potential of mycorrhizal fungi should be utilized for successful rejuvenation of the soil.

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