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Response of Ectomycorrhizal Fungi on the Growth and Mineral Nutrition of *Eucalyptus* Seedlings in Bauxite Mined Soil

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Abstract: The growth and nutritional status of *Eucalyptus tereticornis* seedlings in bauxite mined soil was studied in response to inoculation of the ectomycorrhizal fungus *Pisolithus albus*. The mean dry weights of shoot and root were significantly increased by the inoculation of *P. albus* compared to uninoculated seedlings in a nursery. The ectomycorrhizal plants accumulated less aluminum in the shoots compared to nonmycorrhizal plants. The inoculation of *P. albus* altered the nutritional status of the *E. tereticornis* seedlings grown in bauxite mined soil. Uptake of calcium and potassium was significantly enhanced in the shoots of ectomycorrhizal seedlings compared to nonmycorrhizal seedlings. The roots of ectomycorrhizal seedlings showed increased uptake of calcium, potassium and phosphorus than in nonmycorrhizal roots. The total organic carbon and available phosphorus also increased in the inoculated soil compared to uninoculated soil. These results indicate that *P. albus* has the potential to protect the plants from detrimental effects in bauxite mined soil.

Key words: Ectomycorrhizal fungi · bauxite mined soil · *Eucalyptus tereticornis* · *Pisolithus albus* · Aluminum toxicity · mineral nutrition

INTRODUCTION

Degradation of forest land by way of mining leads to very serious environmental hazards. Mining, in general and open cast mining in particular may lead to severe environmental degradation. Mining activities generate a variety of wastes whose presence in soils has adverse effects on plant growth, such as low water infiltration rates, rough surfaces, poor aeration, high levels of heavy metals, low fertility, salinity and extremes of pH [1, 2]. These attributes may have deleterious effects on plant and also on soil microbial communities and must be ameliorated to ensure successful land reclamation. Under these conditions, mycorrhizal associations can improve plant establishment and growth [3-5]. Aluminum is extracted from bauxite ores through Bayer's process and its mining activity changes the soil characteristics, with negative effects on growth of plants and microbes. Emphasis has to be placed on the reclamation and rehabilitation of the mined bauxite lands once mining operations have ceased. Mycorrhizal inoculation to mine waste may be critical for the establishment of a viable, diverse and sustaining plant community. Despite the number of reports showing the contribution of mycorrhizal inoculation for rehabilitation of mining sites, little work has been done on rehabilitation of bauxite mined soils using mycorrhizal fungi [3, 4, 6]. Present study reports the response of ectomycorrhizal fungus *Pisolithus albus* on the growth and mineral nutrition of *Eucalyptus tereticornis* seedlings grown in bauxite mined soil.

MATERIALS AND METHODS

The ectomycorrhizal fungus *Pisolithus albus* was isolated from the basidiomata associated with *Eucalyptus tereticornis* in Koraput, Orissa, India. The mycelial inoculum of *P. albus* in soilrite - vermiculite carrier was prepared according to the method of Marx and Bryan [7]. Mycelial agar plugs cut from the actively growing culture were inoculated into Erlenmeyer flasks (1L capacity) containing 750 cm^3 of vermiculite moistened with 375 cm^3 of Modified Melin Norkran's liquid medium. The flasks were incubated at 25°C for 30 days. The inoculum was removed from the flasks and held with two layers of cheesecloth while being leached with cool distilled water to remove the unused nutrients. Excess water was removed by gently squeezing the inoculum wrapped in cheesecloth.

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Bauxite mined soil was collected from mining area of National Aluminum Company, Damanjodi, Orissa, India. The soil samples were air-dried, passed through a 5mm sieve and chemically characterized. The characteristic features of bauxite mined soil were: pH: 5.50, total organic carbon: 0.4% [8], total nitrogen: 0.06% [9], available phosphorus: 0.5 mg kg^{-1} [10] and aluminum: 168.2 mg g^{-1}. A nursery experiment was conducted with *E. tereticornis* plants by completely randomized design. The bauxite mined soil was mixed with vegetative inoculum of *P. albus* in the ratio of 50:1 (v/v) and filled in the pots. Aseptically grown *E. tereticornis* seedlings (4.5-4.8 cm height) were transferred to pots and grown for 15 weeks in a nursery. The plants were harvested by destructive sampling and were analyzed for their growth parameters and also for the different mineral nutrients. The shoots and roots were dried at 70°C for 48 hours and the dry weight was recorded. One gram of dried ground leaves/shoots and roots were digested with conc. HNO_3 and Perchloric acid (3:1) according to Page *et al.* [11] and the contents of Ca, Mg, K and Al were estimated using inductively coupled plasma emission spectrophotometry. The total phosphorus was estimated by the method of Kitson and Mellon [12]. The soil was analyzed for various physico-chemical characteristics after harvesting the plants. Data was subjected for statistical analysis using GraphPad prism version 4.03.

RESULTS

The ectomycorrhizal plants showed better growth and survival when compared to nonmycorrhizal plants in bauxite mined soil. *P. albus* colonized 57.8% of the lateral roots. The mean plant height of ectomycorrhizal plants was significantl higher than the nonmycorrhizal plants. The dry weights of shoot and root were significantly more in ectomycorrhizal plants than nonmycorrhizal plants. The shoot/ root ratio was significantly lower for the ectomycorrhizal plants compared to nonmycorrhizal plants (Table 1). The presence of ectomycorrhizal fungi significantly altered aluminum concentrations. Ectomycorrhizal plants had lesser shoot aluminum concentration when compared to nonmycorrhizal plants. The roots of ectomycorrhizal plants accumulated less of aluminum than the roots of nonmycorrhizal plants. The inoculation of *P. albus* led to the alterations in the contents of nutrient elements. Uptake of calcium and potassium was significantly enhanced in the shoots of ectomycorrhizal plants compared to nonmycorrhizal plants while there was no significant change observed in the magnesium and phosphorus content of the plant shoots. The roots of ectomycorrhizal plants showed increased uptake of calcium and potassium than nonmycorrhizal plants but the results were not significant (Table 2).

Table 1: Effect of Pisolithus albus on the growth of Eucalyptus tereticornis plants grown in bauxite mined soil

	Measured parameters per plant				
Soil inoculation	Shoot height (cms)	Shoot dry wt. (gms)	Root dry wt. (gms)	Shoot/ root ratio	Mycorrhizal colonization (%)
Nonmycorrhizal	17.3	0.56	0.13	4.38*	0.0
Mycorrhizal	26.6*	1.10*	0.33*	3.31	57.8*

*Significant at $P<0.05$ as determined by t-test

Table 2: Effect of Pisolithus albus on the mineral content of Eucalyptus tereticornis plants grown in bauxite mined soil

	Elemental level (mg g^{-1} dry wt.)					
Soil inoculation	Plant tissue	Ca	Mg	K	P	Al
Nonmycorrhizal	Shoot	2.46	1.50	7.37	0.37	0.31*
	Root	2.54	1.58	3.80	0.34	25.0*
Mycorrhizal	Shoot	3.48*	1.46	8.46*	0.37	0.21
	Root	3.61	2.18	4.21	0.41	9.43

*Significant between mycorrhizal and nonmycorrhizal shoot and root respectively at $P<0.05$

Table 3: Effect of ectomycorrhizal plants of Eucalyptus tereticornis on the soil characteristics of bauxite mined soil

Soil characteristics	Initial soil	Nonmycorrhizal	Mycorrhizal
pH of soil	5.50c	5.85a	5.62b
Total organic C (%)	0.40c	2.72b	4.19a
Total N (%)	0.06a	0.07a	0.06a
Total P (mg kg^{-1})	469.30a	457.20a	458.60a
Available P (mg kg^{-1})	0.49c	1.74b	2.54a
Mg (mg g^{-1})	0.88b	1.99a	3.34a
K (mg g^{-1})	1.40a	0.99b	1.24ab
Ca (mg g^{-1})	0.62b	1.41a	1.68a
Al (mg g^{-1})	168.20a	109.80c	125.60b

Mean values sharing a common letter within a row are not significant at $P<0.05$

The soil was analyzed after harvesting the plants for its elemental composition. The total organic carbon content of the soil was increased 10 times higher in *P. albus* inoculated soil compared to initial soil. Similarly, inoculated soil was found to be rich in available phosphorus where it increased significantly compared to nonmycorrhizal soil. The aluminum content was more in ectomycorrhiza inoculated soil than in nonmycorrhizal soil. The levels of Ca, Mg and K were also increased due to inoculation of *P. albus* (Table 3).

DISCUSSION

The inoculation of *E. tereticornis* seedlings with ectomycorrhizal fungus *P. albus* resulted in an overall highly positive effect on the plant growth, nutrient absorption and on the efficiency with which the absorbed nutrients were used to produce plant biomass. *Pisolithus albus* was very efficient in colonizing the roots of *E. tereticornis* seedlings grown in bauxite-mined soil. The results showed better growth and survival of *E. tereticornis* seedlings inoculated with *P. albus* compared to uninoculated plants. Mycorrhizal plants had a greater nutrient utilization index for dry matter production than nonmycorrhizal plants. Because of the low fertility of mine spoils and the poor capacity of mining sites to retain nutrients, high efficiency in converting scarce resources to biomass may be an additional advantage for plants during the growth in bauxite mined soils. This together with improved nutrient absorption may explain the general success of mycorrhizal plants in rehabilitation programs [3, 13]. Lunt and Hedger [5] reported that inoculation of ectomycorrhizal fungi did not benefit oak seedlings when grown in mine spoil due to its poor physical properties, whereas the growth was stimulated by these fungi when the mine spoil was organically enriched. Totola and Borges [6] reported that the plants of *Cedrella fissilis* and *Anadenanthera peregrina* did not responded to different amendments in bauxite spoil unless they were mycorrhizal. Reddell *et al.* [4] reported that inoculation of seedlings of *Eucalyptus miniata* with spores of ectomycorrhizal fungi increased both growth and leaf phosphorus concentration grown on a waste rock dumps.

Bauxite mined soil contains Aluminum (Al) which is toxic especially for plants grown in moderately to highly acidic soils [14]. Due to acidic nature of bauxite mined soil (pH 5.5) used in this study, Al toxicity might also be a limiting factor for the growth of plants. Al influences the plant nutrient status generally is manifested by imbalances or deficiencies of calcium, phosphorus, magnesium and some other minerals [15]. The ability of ectomycorrhizal fungi to reduced Al toxicity on different plant species were reported by Cumming and Weinstein [16] and Schier and Mc Quattie [17] who concluded that mycorrhizas can protect trees from detrimental effects of this metal. Binding of Al to the fungal cell walls or sequestration of Al into fungal cell vacuoles [17] might have reduced the flux of Al into the root cortex. Further, Al can be detoxified by complexation with low molecular weight organic anions [18]. Ectomycorrhizal seedlings have been found to excrete more oxalate than nonmycorrhizal tree seedlings in response to Al in axenic conditions [19]. In this study, the level of calcium and magnesium were also altered in tissues of inoculated plants compared to uninoculated plants. Differences in tissue concentration of Ca, Mg and Al between nonmycorrhizal and ectomycorrhizal seedlings can result from exclusion of Al and improved uptake of Ca and Mg. Though Ca content in shoots is increased but Mg content did not differ significantly in this study. Scholl *et al.* [20] also showed that ectomycorrhizal colonization did not affect the uptake of Ca and Mg with Al in solution. The level of aluminum present in uninoculated plant roots was significantly higher than inoculated plant roots indicating that mycorrhizal fungi might play an important role in protecting the plants from aluminum toxicity.

The physico-chemical properties of the soil after harvesting the plants were compared with the initial physico-chemical properties of the bauxite mined soil and the results showed improvement of organic carbon and available phosphorous status of *P. albus* inoculated soil than uninoculated soil. The soil inoculated with *P. albus* had more aluminum than uninoculated soil probably the fungus had chelated aluminum in the soil by secreting organic acids rather than accumulating it in the plant roots as evident from the root analysis that nonmycorrhizal plant roots accumulated more aluminum than ectomycorrhizal roots. These results are in accordance with the hypothesis that the ectomycorrhizal fungi modified the rhizosphere by complexing the heavy metals and thus the plant is protected from the detrimental effects of the toxic metal. In this respect, it can be considered that an ectomycorrhizal pioneering fungus in the mycorrhizal colonization of the host plant at the first stages of growth is the most critical in a forestation and reclamation of contaminated soils. From these results, it could be concluded that inoculation of *P. albus* to *E. tereticornis* plants grown in bauxite mined soil improved the growth and survival of plants. The fungus also improved the mineral nutrition of the plants grown in bauxite mined soil compared to uninoculated plants. These results suggest that *P. albus* could serve as a fungus of choice in reclamation of bauxite mined/ aluminum contaminated soils.

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