Effect of Ectomycorrhizal on Growth and Mineral Nutrition of *Acacia saligna* (Labill.) Seedlings

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Seeds of *Acacia saligna* were inoculated with Ectomycorrhizal (ECM) and germinated in seedbeds furnigated with methyl bromide. Rock phosphate was added to evaluate the effect of mycorrhizae on increasing the available phosphorus to the plants. Growth and mineral content were determined after two growing seasons. The obtained results indicated that addition of mycorrhizae to the soil increased the growth, and that better growth was obtained by adding the rock phosphate to the soil. The N, P and K content of inoculated seedlings was 3-5 times higher than the control. Thus, the addition of rock phosphate in the presence of mycorrhizae resulted in more accumulation of the N, P and K in the plants.

Keywords: *Acacia saligna*; Ectomycorrhizal; Growth; Mineral Nutrition.

Ectomycorrhizal fungi are beneficial to the growth and development of trees. Inoculated trees have a much larger physiologically active root fungus surface area for nutrient and water absorption than mycorrhizae free trees. They also absorb and accumulate in the fungus mantles nitrogen, phosphorus, potassium and calcium more rapidly and for longer periods of time than nonmycorrhizal feeder roots (Marx. 1975 and 1977). Ectomycorrhizae appear to increase tolerance of trees to drought, high soil temperatures, soil toxins and extremes of soil pH caused by high levels of sulfur or aluminum. Ectomycorrhizae functions as biological deterrents to infection of feeder roots by root pathogens.

The effects of ECM on the growth of trees have been studied by many investigators (e.g. Marx 1979, Marx and Brvan 1975. Marks and Foster 1973, Garbaye *et al*. 1988, Mitchell 1987).

Ectomycorrhizae commonly are associated with areas of high organic matter accumulation (Heinrich *et al* 1988). Moreover P in this material is likely to make an important contribution to the overall supply of this nutrient to trees in the field (Heinrich and Patrick, 1985, 1988).

Inoculation of *Eucalypts pilularis* seedlings with *Pisolithus tinctorius* with subsequent development of ectomycorrhizae led to improved acquisition of P. Dry weight gain was increased by inoculation where growth was limited by P supply, (Heinrich *et al* 1988).

Thomson (1989) mentioned that a fibrillar substance is present between fungal hyphae and the root hair surface. It is known that root hairs of some species exude a considerable quantity of material (Head, 1964); which may be used as a carbon source by ectomycorrhizal fungi.

Acacia saligna (Labill.) H.L. or the Port Jackson willow is very adaptable and fast growing

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evergreen shrub or tree native to Western Australia (Midgley and Turnball 2003). It is a dense with several stems, thorn less and spreading shrub or with a single stem, growing to a height of about 9 meters. The bark of this tree is smooth and grey to red-brown in small branches and dark grey and fissured in older trees(Van, and Wyk 2005). *Acacia saligna* wood possesses sappy wood, light and it is not popular for firewood. Wood of this species has been used to make particle board and the wood is also used as fuel, charcoal, for making stakes for vines and agricultural implements (Michelides 2001).

Acacia saligna can be used for multiple purposes, as it grows under a wide range of soil conditions into a woody shrub or tree. It has been used for tanning, revegetation, animal fodder, mine site rehabilitation, firewood, mulch, agroforestry and as a decorative plant. This study describes the effect of ectomycorrhizae produced from the inoculation with the gasteromycete *Pisolithus tinctorius* (Pers.) on the ability of *Acacia saligna* seedlings to acquire P and increasing growth.

MATERIALS AND METHODS

This study was carried out at the nursery of Department of Botany and Microbiology, King Saud University, Riyadh. Concrete basins of $1m^3$ size (1x1x1m). were filled with medium textured soil up to 20 cm from the rim. Table (1) shows the chemical analysis of the soil. Seeds were collected which is adjacent to the nursery. Seed beds were sterilized with methyl bromide (40 g per m^2 of soil surface for 48 hr under plastic. Healthy seeds were surface sterilized by using 1% sodium hypochlorite for 10 min then vigorously rinsed with sterilized double distilled water (DDW). Seeds were mixed with spores(one g, of spores for each seed bed) (Fig. 1, 2) to compare their growth with enonmycorrhizal seedlings. Seedlings were thinned after 4 months at distances of 10 cm approximately. Rock phosphate was added to the basins in amounts zero, 8 and 16 gram of rock phosphate per basin.

At the end of the experiment after two years plants were sampled to measure the growth characteristics the survival percent, height, stem diameter and total dry weight were determined. Leaf samples were taken to determine their NPK content in saturation extract were determined by the method of (Jackson 1973, Toth *et al.*, 1948), and the total amount per plant was calculated. The available P in soil was determined at the first and second season.

The plant height were measured by using a meter scale and stem diameter by Vernier caliper after removal from the soil. The total fresh weight of plant were recorded, then placed in an oven run at 65°C for 72 h. These dried plants were weighed to record the total dry weight of plant.

Statistical analysis

The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's multiple-range test at $P < 0.05$ % level.

RESULTS AND DISCUSSION

Survival and growth

Table (2) shows the survival and growth of seedlings inoculated with ECM in comparison to the non-inoculated ones. Inoculated seedlings had significantly higher survival percent than the non-inoculated seedlings. However, no significant differences existed between the seedling treated with ECM and those treated with ECM and rock phosphate.

The addition of rock phosphate and ECM increased height significantly than the control treatment or those received only ECM at the end of the second growing season. Inoculated with ECM increased height, although statistically no significant, over the seedlings received ECM and the control (54.5 and 31 cm, respectively). The stem diameter and the dry weight showed similar trend like that obtained from height growth.

The improved growth of seedlings with *Pisolithus tinctorius* ectomycorrhizae was demonstrated by Marx (1977), (Oyun *et al*., 2010) and (Manoharan *et al*., 2008) who stated that certain species of ectomycorrhizal fungi were more beneficial to pines than others.

N, P and K content of seedlings

Table (3) shows the levels of N,P and K of seedlings. The nitrogen content significantly differed between the four treatments. The total amount of nitrogen per plant significantly increased in the presence of mycorrhizae as compared to the controls. The averages were 0.024

and 0.077 g/plant consequently which was three times as high. The addition of rock phosphate in the presence of mycorrhizae nearly doubled the amount of nitrogen per plant as it was 0.139 and 0.298 g N/plant respectively. The increase in the amount of nitrogen per plant may be a result to the increase in the growth and to the absorption of more nitrogen from soil by the roots.

The amount of phosphorus per plant was four times in the plants treated with mycorrhizae as those untreated (0.008 and 0.029g P/plant respectively). The addition of rock phosphate resulted in absorption of more amounts of phosphorus/plant. As the amount of added rock phosphate doubled the amount of phosphorus/ plant doubled also. This may be due to the presence of more available phosphorus where it was absorbed by the roots through the mass flow action.

The amount of potassium/plant was 5 times in the plants treated with mycorrhizae as the controls (0.021 and 0.107gK/plant respectively).

Table 1. Chemical analysis of the sandy loam soil.

P	Na	K	Ca	Mg	HCO_3	Cl	SO_4	pH	EC	$CaCO_3$	OM
1.6	2.9	0.17	3.6	1.5	1.5	3.5	3.2	8.5	0.92	2.14	1.02

Table 2. Survival and Characteristics of *Acacia saligna* seedlings for two years

Treatment	Survival (%)	Height (cm)	Stem diameter (cm)	Dry weight plant (g)
Control	41.3 c	31.0 d	0.34 c	4.75 b
ECM	81.9 b	54.5 c	0.44 c	15.30 b
ECM+ 10g P_2O_5	98.4 a	80.6 b	1.01 a	46.96 a
ECM+ 20g P_2O_5	98.4 a	98.8 a	0.75 b	67.11 a

Values followed by the same letter do not differ statistically at $P < 0.05$ (DuncanMultiple Range Test)

Table 3. Seedlings content of N, P and K at the end of the experiment

Treatment	N g/plant	P g/plant	K g/plant
Control	0.024 d	0.008 d	0.021 d
ECM	0.077 c	0.029 c	0.107 c
ECM+ 10g P_2O_5	0.139 b	0.043 b	0.166 b
ECM+ 20g P_2O_5	0.298 a	0.105 a	0.318 a

Values followed by the same letter do not differ statistically at $P < 0.05$ (Duncan Multiple Range Test)

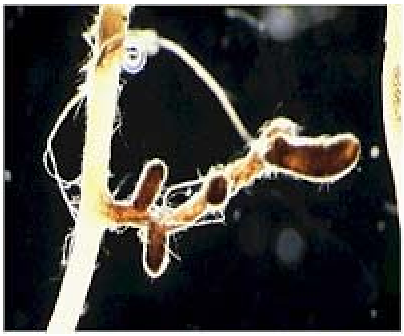

Fig. 1. Ectomycorrhizal fungi

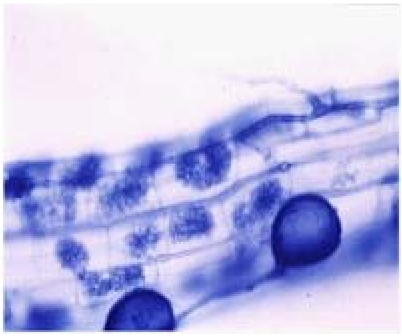

Fig. 2. *Acacia saligna* root colonised by ectomycorrhizal fungi

The addition of rock phosphate in the presence of mycorrhizae resulted in more accumulation of K in plant tissues. However, the higher amounts of rock phosphate in the presence of mycorrhizae resulted in more growth and consequently more amount of K/plant. Significant differences were found between all treatments.

It appears that the amounts of available P in the soil did not differ drastically among treatments (Table 4), although no statistical analysis could be performed on the data due to the lack of proper replication.

It is evident from this study that inoculating seedlings of *Acacia saligna* with the ectomycorrhizal fungi *Pisolithus tinctorius* promotes their growth. ECM fungi also changed the non available form of phosphorus (rock phosphate) to the available form which result in more plant growth. Seeds of *Acacia saligna* should therefore be inoculated with ECM to enhance their growth.

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