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Ectomycorrhization of Date Palm and Carob Plants

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Strictly as per the compliance and regulations of :

Ectomycorrhization of Date Palm and Carob Plants

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Abstract - The effectiveness of plastic pots inoculation technique to infect *Ceratonia siliqua* and *phoenix dactylifera* L. seedling roots with ectomycorrhizal fungal *Pisolithus tinctorius* [(Pers) Coker & Couch] was established. Spores powders inoculum units were prepared from crushed carpophores of *Pisolithus tinctorius*. Six months after inoculation, ectomycorrhizas were established in date palm and carob seedlings roots. The ectomycorrhization of *Ceratonia siliqua* and *Phoenix dactylifera* using spores powders inoculum was performed for the first time in our experiment conditions. These ectomycorrhizas results can provide an enormous potential for the development of large scale inoculation procedures of these species seedlings in commercial nurseries. The ectomycorrhization of date palm can be exploited to fight against *Fusarium* wilt using *Pisolithus tinctorius* as biocontrol.

Keywords : pisolithus tinctorius, ceratonia siliqua, phoenix dactylifera, ectomycorrhization.

Résumé - L'efficacité de la technique d'inoculation des racines de *Ceratonia siliqua* et *Phoenix dactylifera* L. par le champignon ectomycorhizien *Pisolithus tinctorius* [(Pers) Coker & Couch], dans des pots en plastique, a été démontrée. L'inoculum en poudre a été obtenu par broyage des carpophores du *Pisolithus tinctorius*. Six mois après l'inoculation, les racines des plantules du palmier dattier et du caroubier ont formés des mycorhizes. L'ectomycorhization du caroubier et du palmier dattier en utilisant un inoculum naturel sous forme de poudre est ainsi, démontré pour la première fois dans nos conditions de travail. Ces résultats sur les ectomycorhizes peuvent fournir un énorme potentiel pour le développement à grande échelle des procédures d'inoculation de ces deux espèces dans les pépinières commerciales. En outre, l'ectomycorhization du palmier dattier pourrait être exploitée pour lutter contre la fusariose vasculaire du palmier en utilisant le *Pisolithus tinctorius* comme agent de lutte biologique.

Motsclés : pisolithus tinctorius, ceratonia siliqua, phoenix dactylifera, ectomycorhization.

I. Introduction

Ectomycorrhizal (ECM) fungi like *Pisolithus* (Alb. & Schwein) are known to enhance tree growth by increasing the uptake of nitrogen (N) (Martin 1985; Chalot and Brun 1998) and phosphorus (P) by roots in P deficient soils (Grove and al. 1994; Marc and al. 2004). In addition ECM fungi are able to mobilize nutrients from organic substrates (proteins, amino acids, chitin, phosphomonoesters and phosphodiesters) or nutrients linked to organic residues by secreting extra-cellular enzymes (Dighton 1983; Abuzinadah and Read 1986 a,b; Leake and Read 1990; Guttenberger and al. 1994). The ability to secrete extra-cellular enzymes differs with ECM fungal species and with season (Buée and al. 2005). Some authors have reported that ectomycorrhizae can also protect seedling roots against pathogens and since then, several studies have noted this protective capability of mycorrhizas (Davis and al. 1942; Chakravarty and Hwang 1991; Chakravarty et al. 1991; Duchesne 1994; Hwang and al. 1995; Morin et al. 1999). It is well known that mycorrhizal fungi create a physical barrier between roots and pathogens, exude antimicrobial metabolites and use surplus carbohydrates to reducing roots pathogenic organisms attractiveness (Machon and al. 2009).

For these reasons, research on ectomycorrhizas has evolved greatly over the last 40 years (De Roman et al. 2005).

Pisolithus tinctorius is an ectomycorrhizal fungus frequently used for inoculation in controlled mycorrhization programs (Marx and al., 1982; Burgess and al., 1995). Isolates of this fungus are some of the most commonly used in forestry, with growth stimulation reported for several tree species including Eucalypts, Pines and Acacias (Marx et al. 1977; Garbaye et al. 1988; Duponnois et Ba 1999). The common occurrence of *Pisolithus* fruiting bodies, the ability of this fungus to form ectomycorrhizae and its wide host range makes it a very interesting organism for artificial inoculation of nursery plants.

In this paper we report for the first time the mycorrhization of *Phoenix dactylifera* and *Ceratonia siliqua* by *Pisolithus* inoculum. The development of these ectomycorrhizal associations can provide an enormous potential for the development of large scale inoculation procedures of these species seedlings in commercial nursery. In view of limited studies on antifungal action of endomycorrhizal fungi to defy *Phoenix dactylifera* wilt (Bayoud) caused by *Fusarium oxysporum albedinis fsp.* (F.O.A) (Jaiti and al. 2007; Oihabi 1991), and no studies using ectomycorrhizal fungus, the present study try to explore *Pisolithus tinctorius* as biocontrol agent against F.O.A

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II. Materiel and Methods

a) Fungus inoculum

Pisolithus tinctorius is the cosmopolitan basidiocarps in warm temperate regions (Martin et al. 2002) and forms ectomycorrhizal associations with a broad variety of forested plants including: Myrtaceae, Mimosaceae, Pinaceae, Fagaceae, Cistaceae, Diptero-carpaceae and Caesalpiniaceae (Moyersoen et al. 2004).

Pisolithus tinctorius basidiocarps (fig1.a) used in this study were collected during the spring season (April 2009) in *Eucalyptus gomphocephala* plantation (fig1.b) in eastern region of Morocco. *Pisolithus* basidiocarps harvested were dried at 35°C for 72 hours (Rincon et al. 2005), and crushed to produce spores powder. Initial fungal spores concentration was measured with a haematocytometer and mixed with sterile peat before being sown by the germinated seeds. One gram of *Pisolithus tinctorius* powder spores contained $1x10^8$ spores.

b) Host plants and seeds germination

Before use, all seeds were first surface sterilized with 10% sodium hypochlorite for 5 min and rinsed four times in sterile distilled water.

i. *Date palm seeds*

Phoenix dactylifera L. seedlings were obtained from seeds produced by 'Boufaggous' during 2009 date palm production season in Figuig (South East of Morocco). Boufeggous is a high quality variety grown in Morocco. It is mainly attacked by *Fusarium oxysporum fsp. Albedinis* (F.A.O) responsible for date palm wilt (Bayoud) disease (Hakkou and Bouakka 2002) and Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), causing extensive losses in storage (Azelmat et al. 2006).

Boufaggous seeds were first soaked in boiling water and then allowed to cool in tap water for 48 hours before being transferred to germination in autoclaved peat. Seeds were incubated in darkness at 30 °C for 16 days. The germinated seeds were sown in plastic pots (V: 500 ml. 14cm diameter; 10cm height) containing 300g of autoclaved peat.

ii. *Carob seeds*

Ceratonia siliqua seeds used in this study were collected manually from street trees in Oujda city (Eastern Morocco). Only, intact seeds of *Ceratonia siliqua* were selected to dip with boiling water. After that, seeds were allowed to cool in tap water for 72 hours and were then allowed to germinate in sterile peat in darkness. After 11 days, seeds rate germination was 95%.

All germinated seeds were then transferred in plastic pots (V: 500ml. 14cm diameter; 10cm height) containing a sterilized peat (300g) and inoculated by 7g powder spores in a growth chamber maintained at 28 ± 2°C with 16 h photoperiod. The pots open at the bottom were placed in containers support, filled with water which level was adjusted to ensure substrate a constant humidity.

c) Ectomycorrhizal synthesis

i. *Using natural inoculum*

These systems are routinely used for synthesis of ectomycorrhizae (Mulette 1976). It consists of a 14 cm diameter plastic pot filled with autoclaved peat mixed to fruiting bodies powder ($70x10^8$ spores/g of autoclaved peat). Sterile date palm and Carob seedlings destined to inoculation were then, inserted into the substrate. Plastic pots were set in a saucer of water to ensure substrate humidity.

ii. *Using mycelia inoculum*

We have previously tested the compatibility of *Pisolithus tinctorius* isolate, *in vitro* with *Eucalyptus turquata.* Plugs of fungus cultures were taken aseptically with a sterile cork borer (10mm in diameter) from the actively growing mycelium front and subcultured in modified Melin Norkrans (MNM) agar slant surface (Marx 1969) in glass test tubes. *Eucalyptus turquata* seedlings were placed then with fungus inoculum of *Pisolithus tinctorius*. After six weeks incubation, roots seedlings were harvested, washed gently with tap water, colored (Philips and Hayman 1970) and observed under microscope (fig 2).

d) Microscopic studies

Six months after inoculation, date palm and carob seedlings roots were rinsed under water, incised and colored before being examined using an Olympus optical microscope. A randomly samples of short roots was cleared in 10% KOH for 30min at 90°C and stained for 15 min with Trypan Blue (0.1 % in lactoglycerol). Tinted short roots were mounted on microscope to check ectomycorrhizas (ECM) presence and mycelia structure. Observations of control (fig.3) mycelium structure collected in margin of a rapid growth culture on MNM agar medium have been made.

e) Data collection and mycorrhizal measurement

The experimental protocol used in this study is completely random using five repetitions. And the percentage of mycorrhized plants was determined according to the method established by Maroneck and al. (1982).

III. Results and Discussion

a) Sampling of plants and evaluation of root ectomycorrhizas

Six months after inoculation, seedling roots were washed free of substrate and ectomycorrhizas were identified according to coloration methods (trypan blue). Each seedling root was cut into 2-3 cm segments

(Rincon et al. 2005) to evaluate the possible presence of ectomycorrhizas and frequency of ectomycorrhized plants. Twenty four weeks post inoculation, *Pisolithus tinctorius* mycelia characteristics were revealed in Carob (fig.4) and date Palme (fig.5) roots systems. Microscopic observations revealed the presence of *Pisolithus tinctorius* mycelia derived from germinated spores (fig.6). The ectomycorrhizae interface cell was composed by both *Pisolithus* mycelia and host cells wall. We have also noted that the mycelium grew only between cells cortex showing a typical Hartig net hyphae (fig.5b, c, d) characterized by labyrinthine branching. A fungal mantel around roots and emerged hyphae outwards roots were also observed (fig.7). This complex hyphal is considered to increase the fungal surface area in contact with the cells roots and explored soil area.

In this study ectomycorrhizal plants frequency after inoculation by *Pisolithus tinctorius* in this study was 6.66% for date palm and 10% for *Ceratonia Siliqua*. Thus, *Phoenix dactylifera* and *Ceratonia siliqua* were ectomycorrhized for the first time in our experience conditions.

Examination of control plants root systems was shown that they are free from mycelia contamination (fig.8).

If there are several works on endomycorrhization of date palm (Jaiti et al. 2007) and carob plants (Cruz et al. 2004) in controlled conditions, there are no results using the ectomycorrhizal fungi as inoculum. In the date palm *Fusarium Oxysporum Albedinis* interaction, little is known concerning the contribution of mycorrhizas to Bayoud disease control. Oihabi (1991) has shown that the inoculation of date palm seedlings with *Glomus mosseae* reduces the disease severity. In this context, antagonism between F.A.O and other micro-organisms was studied. El Hassni and al. (2005) have shown some isolates of *Bacillus spp., Pseudomonas spp.,* and *Rahnella aquatilis* are able to enhance defence reactions of date palm without causing any seedlings mortality. In other study, Machón and al. (2009) have demonstrated that inoculation of *Pinus pinea* seedlings by the ectomycorrhizal fungi *Laccaria laccata* in nurseries reduces both damage intensity and seedlings mortality caused by *Fusarium* damping-off. In the present study, ectomycorrhized roots are characterised by the presence of fungal sheath. This mantle adheres to the root surface and consists of aggregated hyphae (Ammarellou et al. 2007). Barker and al. (1998) have reported that these hyphae are responsible for the mineral nutrition and water uptake of the symbiotic tissues. Cruz and al. (2004) have demonstrated that *Ceratonia siliqua* colonized roots by *Glomus intradices* improves the nitrogen nutrition of plants, generally when growing at low levels of nutrients.

In the perspectives of using controlled ectomycorrhization to induce date palm plants resistance against the *Fusarium* wilt, the antagonism between *fusarium oxysporum albedinis fsp.* (F.A.O) and our *Pisolithus tinctorius* isolate, in vitro, were already begun in our laboratory (data not shown). In addition, the controlled ectomycorrhization of carob plants was performed for the first time.

Our results on ectomycorrhization allowing biologists a very useful tool for studies on resistance to wilt date Palm and a rigorous way to defy biotic and abiotic constraints.

Figure 1 : Pisolithus tinctorius basidiocarps (a) associated with Eucalypts plant (arrow) (b). Scale bar = 33000 μm.

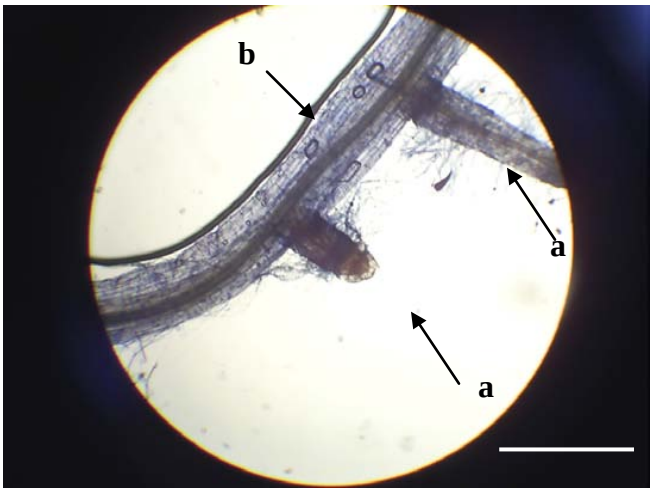
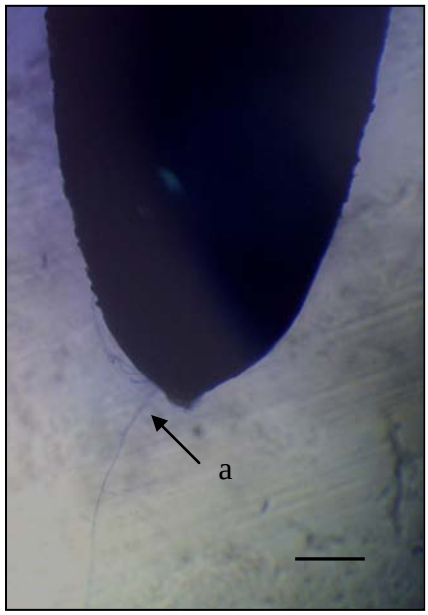


Figure 2 : Eucalypts root ectomycorrhized by *Pisolithus tinctorius* mycelia obtained in glass test tube (X 100). Arrow a: Lateral root; Arrow b: Tap root .Scale bar = 500 μm.



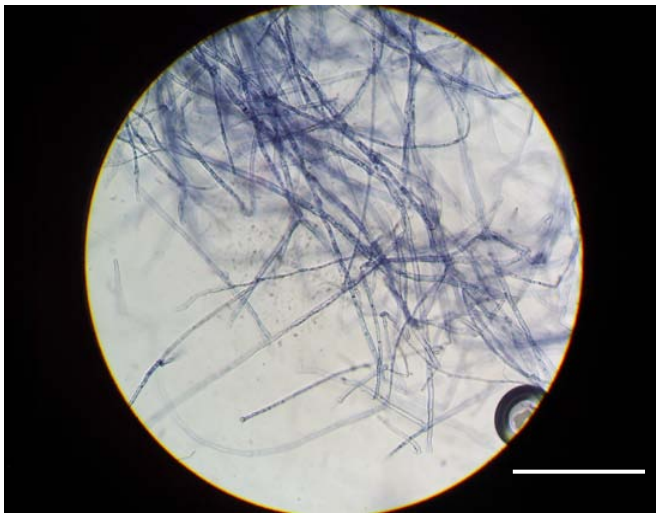


Figure 3 : Microscopic observation of the *Pisolithus tinctorius* mycelia developed in MNM medium (X100). Scale bar = 500 μm.

Figure 4 : *Ceratonia siliqua* lateral root forming a mycorrhizas with *Pisolithus tinctorius*. Fungus hyphae (a) (arrow) grow around the root to form a fungus mantle (b) (arrows) (X100). Scale bar = 200 μm

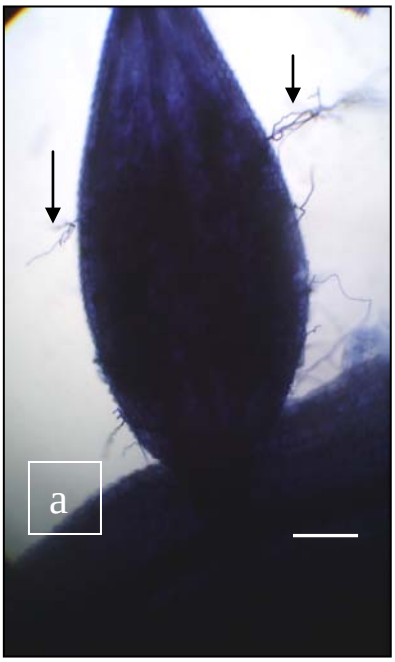
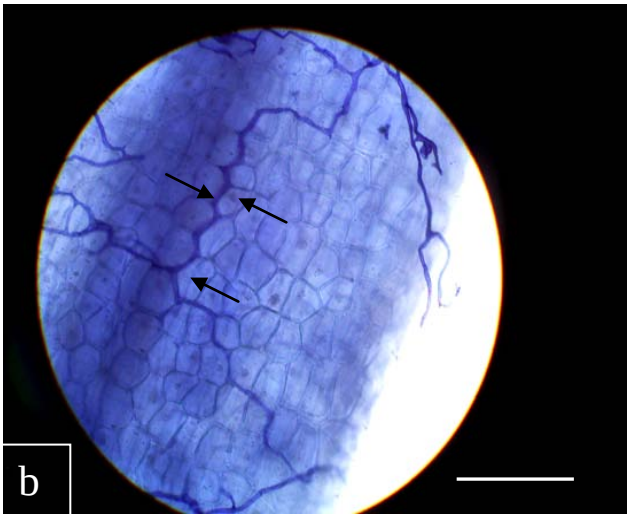
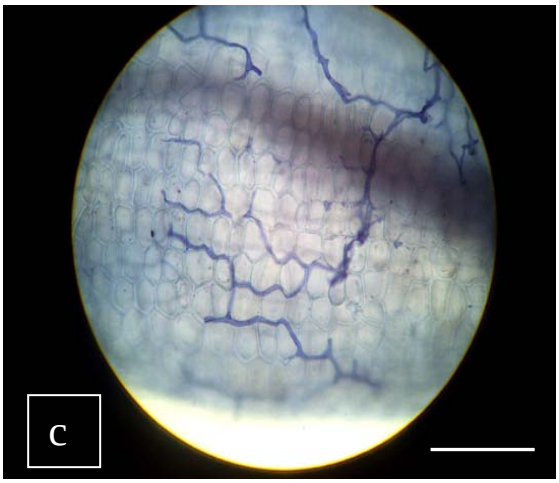
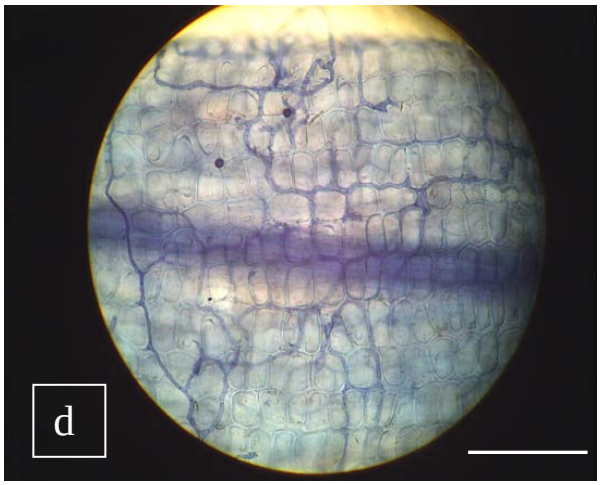


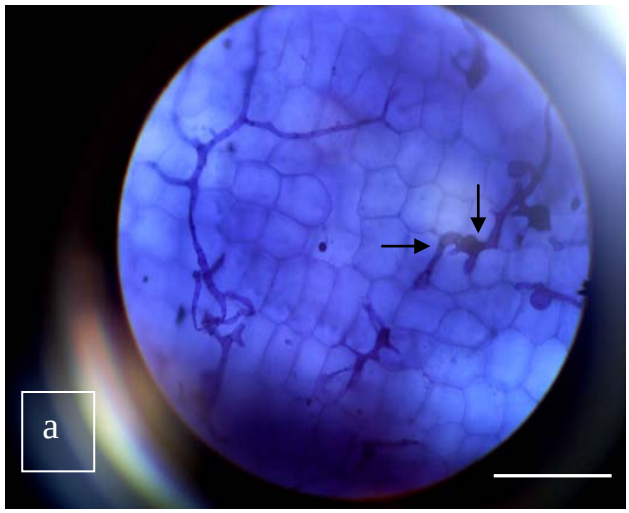
Figure 5 : Lateral root of *Phoenix dactylifera* colonised bay *Pisolithus tinctorius* hyphae (a) (arrows) (X100). Scale bar = 200 μm.

The mycorrhizal fungus grows by branching hyphae and continues to elongate only between cortical cells interfaces (arrows) (b, c and d) (X400). Scale bar = 100 μm.









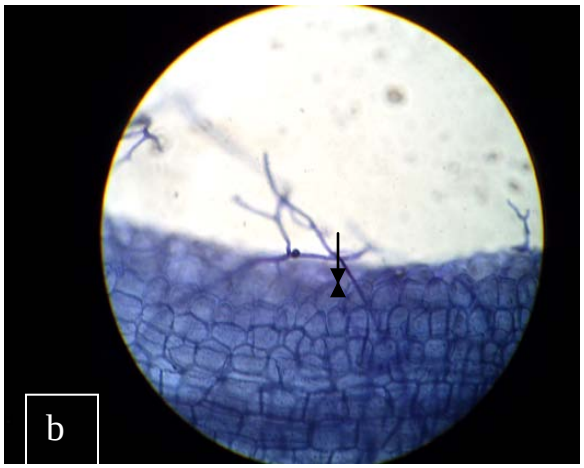
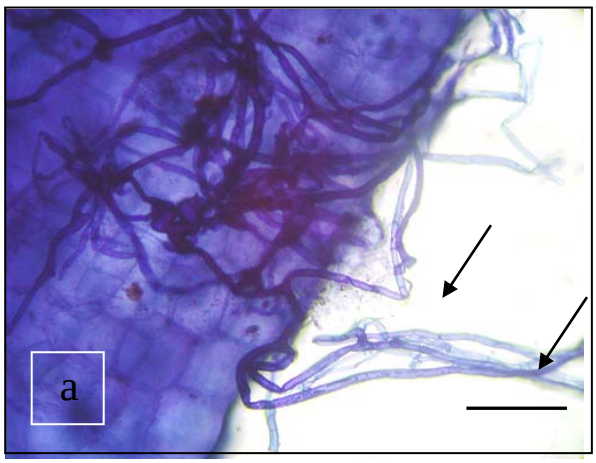


Figure 6 : Pisolithus tinctorius mycelia derived from germinated spores (arrow), in root, grew only between cortical cells wall (a). Scale bar = 100 μm. A *Pisolithus tinctorius* spore can also germinate on the exterior of root and emits mycelia which penetrate between cortical cells (arrow) (b). Scale bar = 2000 μm.



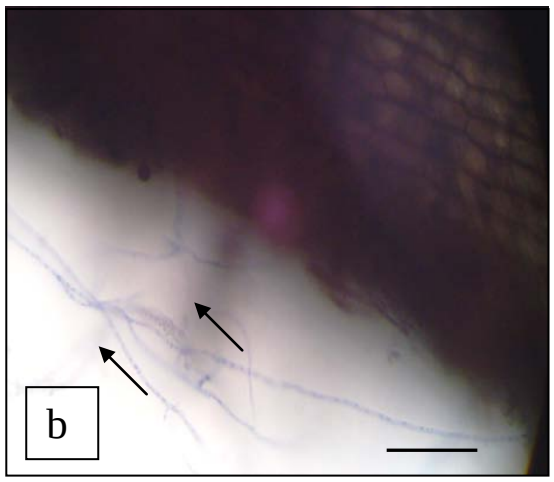
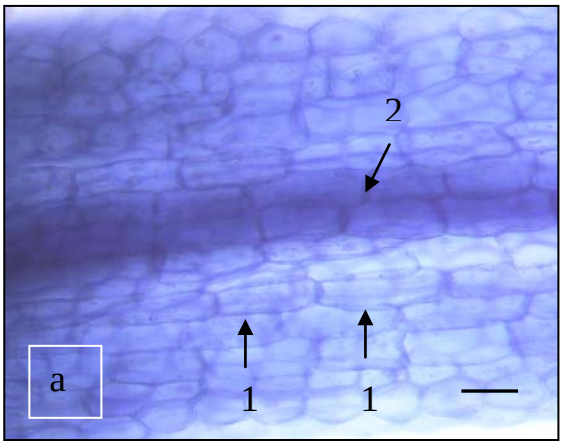


Figure 7 : Pisolithus tinctorius hyphal emergence to outside host roots. a: Date palm root (arrows) (X400). Scale bar = 50 μm; b: Carob root (arrows) (X100). Scale bar = 200μm.



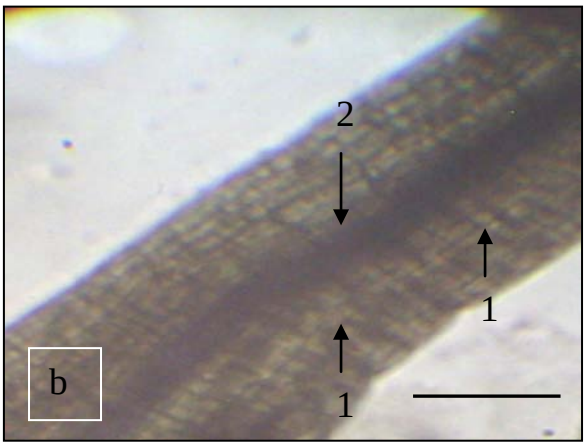


Figure 8 : Control plants root free from mycelia contamination. a: Date palm root. Scale bar = 50 μm; b: Carob root. Scale bar = 500 μm. Arrows 1: Cortical cells; Arrows 2: Central cylinder.

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References Références Referencias

1. Abuzinadah, R.A., Read, D.J., 1986a. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. I. Utilization of peptides and proteins by ectomycorrhizal fungi. New Phytologist 103, 481–493.
2. Abuzinadah, R.A., Read, D.J., 1986b. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. III. Protein utilization by *Betula, Picea and Pinus* in mycorrhizal association with *Hebeloma crustuliniforme*. New Phytologist 103, 507–514.
3. Ammarellou, A., Saremi, H., 2007. Mycorrhiza between *Kobresia bellardii* (All.) Degel and *Terfezia boudieri* Chatin. Turk. J. Bot.32 (2008) 17-23
4. Azelmat, K., El Garrouj D., Mouhib, M., Sayah, F., 2006. Irradiation of 'Boufeggous' dates: Effects on chemical composition during storage. Post harvest Biology and Technology, 39, 217–222.

5. Barker, S.J., Tagu, D., Delp, G., 1998. Regulating of root and fungal morphogenesis in mycorrhizal symbioses. Plant Physiol 116: 1201-1207.
6. Burgess, T., Malajczuk, N., Dell, B., 1995. Variation in *Pisolithus* based on basidiome and basidiospore morphology, culture characteristics and analysis of polypeptides using 1D SDS-PAGE. Mycol. Res., 99:1-13.
7. Buée, M., Vairelles, D., Garbaye, J., 2005. Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus sylvatica*) forest subjected to two thinning regimes. Mycorrhiza 15, 235–245.
8. Chakravarty, P., Hwang, S.F., 1991. Effect of an ectomycorrhizal fungus, *Laccaria laccata*, on *Fusarium damping-off* in *Pinus banksiana* seedlings. European Journal of Forest Pathology 21: 97–106.
9. Chakravarty, P., Peterson, R.L., Ellis, B.E., 1991. Interaction between the ectomycorrhizal fungus *Paxillus involutus,* damping-off fungi and *Pinus resinosa* seedlings. Journal of Phytopathology 132: 207–218.
10. Cruz, C., Green, J.J., Watson, C.A., Wilson, F., and Martins-Loução, M.A., 2004. Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. vol. 14, N° 3, pp. 177-184.
11. Davis, W.C., Wright, E., and Hartley, C., 1942. Diseases of forest tree nursery stock. Forest Publication 9. Civilian Conservation Corps., Washington, DC. 79 p.
12. De Roman, M., Claveria, V., De Miguel, A.M., 2005. A revision of the descriptions of ectomycorrhizas published since 1961. Mycol. Res. 109 (10): 1063–1104.
13. Dighton, J., 1983. Phosphatase production by mycorrhizal fungi. Plant and Soil 71, 455–462.
14. Duchesne, L.C., 1994. Role of ectomycorrhizal fungi in biocontrol. In: Mycorrhizae and Plant Health. Pfleger, F.L., Linderman and R.G., eds. APS Press, St. Paul, Minnesota, USA, pp. 27–46.
15. Duponnois, R., Ba, A.A., 1999. Growth stimulation of *Acacia mangium Willd.* by *Pisolithus sp.* in some Senegalese soils. For Ecol Manage 119:209–215.
16. Jaiti, F., Meddich, A., El Hadrami, I., 2007. Effectiveness of arbuscular mycorrhizal fungi in the protection of date palm (*Phoenix dactylifera L.*) against bayoud disease. Physiological and Molecular Plant Pathology. 71, 166–173
17. El Hassni, M., El Hadrami, A., Daayf, F., Cherif, M., Ait Barka, E., El Hadrami, E., 2005. Biological control of bayoud disease in date palm: Selection of microorganisms inhibiting the causal agent and inducing defense reactions. Environmental and Experimental Botany 59 (2007) 224–234.
18. Garbaye, J., Delwaulle, J.C., Diagana, D., 1988. Growth response of *Eucalyptus* in the Congo to ectomycorrhizal inoculation. For Ecol Manage 24:151–157.
19. Grove, T.S., Malajczuk, N., Mingqin, G., Daping, X., Chonglu, Z., 1994. Optimising phosphorus fertiliser rates and mycorrhiza fungal inoculation for growth of *Eucalyptus grandis x E.uroPhyla* in Southern China, in: M. Brundrett, B. Dell, N. Malajczuk, G. Mingqin (Eds.), Mycorrhizas for Plantation Forestry in Asia: Proceedings of an International Symposium and Workshop, Kaiping, China, ACIAR Proceedings N° 62, pp. 101–107.
20. Guttenberger, M., Compart, U., Spagele, S., Hampp, R., 1994. Proteolytic activity in the ectomycorrhizal fungus *Amanita muscaria* and its mycorrhizas on *Betula pendula.* Plant Physiology (Supplement) 105, 869.
21. Hakkou, A., Bouakka, M., 2002. Oasis de Figuig : état actuel de la palmeraie et incidence de la fusariose vasculaire. Science et changements planétaires / Sécheresse. Volume 15, N° 2, 147-58.
22. Hwang, S., Chakravarty, P., Chang, K., 1995. The effect of two ectomycorrhizal fungi, *Paxillus involutus* and *Suillus tomentosus,* and of *Bacillus subtilis* on *Fusarium damping-off* in jack pine seedlings. Phytoprotection 76: 57–66.
23. Leake, J.R., Read, D.J., 1990. Proteinase activity in mycorrhizal fungi. II. The effects of mineral and organic nitrogen sources on induction of extracellular proteinase in *Hymenoscyphus ericae* (Read) Korf & Kernan. New Phytologist 116, 123–128.
24. Machón, P., Pajares, J.A., Diez, J.J., Alves-Santos, F.M., 2009. Influence of the ectomycorrhizal fungus *Laccaria laccata* on pre-emergence, post-emergence and late damping-off by *Fusarium oxysporum* and *F. verticillioides* on Stone pine seedlings.
25. Marc, D., Antoine G., Gilles C., Yves P., 2004. Relative infectivity of two *Pisolithus spp.* Strains inoculated to the nitrogen-fixing legume tree *Acacia crassicarpa* A.Cunn.ex Benth. in a field experiment in Madagascar. European Journal of Soil Biology 40 (2004) 105–111.
26. Martin, F., 1985. ^{15}N-NMR studies of nitrogen assimilation and amino acid biosynthesis in the ectomycorrhizal fungus *Cenococcum graniforme.* FEBS Letters 182, 350-354.
27. Martin, F., Diez J., Dell, B., Delaruelle, C., 2002. Phylogeography of the ectomycorrhizal *Pisolithus* species as inferred from nuclear ribosomal DNA ITS sequences. New Phytol 153:345–357.
28. Marx, D.H., 1969. The influence of etotrophi mycorrhizal fungi on the resistance of pine roots to pathogenic infections I. Antagonism of mycorrhizal

fungi to root pathogenic fungi and soil bacteria, Phytopathology 59, 153-163.
29. Marx, D.H., 1977. Tree host range and world distribution of the ectomycorrhizal fungus *Pisolithus tinctorius.* Can J Bot 23:217–223.
30. Marx, D.H., Ruehle, J.L., Kenney, D.S., Cordell, C.E., Riffle, J.W., Molina, R.J., Pawuk, W.H., Navratil, S., Tinus, R.W. & Goodwin, O.C., 1982. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on container-grown tree seedlings. For. Sci., 28:373-400.
31. Morin, C., Samson, J., and Dessureault, M., 1999. Protection of black spruce seedlings against *Cylindrocladium* root rot with ectomycorrhizal fungi. Canadian Journal of Botany 77: 169–174.
32. Moyersoen, B., Beever R.E., 2004. Abundance and characteristics of *Pisolithus* ectomycorrhizas in New Zealand geothermal areas. Mycologia, 96(6), 2004, pp. 1225–1232.
33. Mullette, K.J., 1976. *Austral. J. Bot.* 24, 193-200.
34. Oihabi, A., 1991. Etude de l'influence des mycorhizes à vésicules et arbuscules sur le bayoud et la nutrition du palmier dattier. Doctorat d'Etat, Universite Cadi Ayyad, Faculte des Sciences Semlalia- Marrakech, Maroc.110p.
35. Phillips, J.M. et Haymann, D.S., 1970. Improved proceeding for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans.Br. Mycol. Soc., 55(1): 158-161.
36. Rincon, A., Parladé, J., Pera, J., 2005. Effects of ectomycorrhizal inoculation and the type of substrate on mycorrhization growth and nutrition of containerised *Pinus pinea* L. Seedlings produced in a commercial nursery. Ann. For. Sci. 62 (2005), 817-822
37. Ying, L.C., Li H.K., Bernard, D., 2006. Inoculation of *Eucalyptus urophylla* with spores of *Scleroderma* in a nursery in south China: Comparison of field soil and potting mix. Forest Ecology and Management. 222, 439–449.