J. Mater. Environ. Sci. 2 (4) (2011) 345-350
ISSN : 2528-2028

Ectomycorrhization of date palm

F. Zegaye [*], A. Khalid, A. Hasnaoui, H. Caid Serghini, A. El Amrani

Laboratory of plants and microorganisms Biology, Department of Biology, Faculty of Sciences, University Mohamed I, Oujda Morocco

Received in 15 june 2011, Revised 12 Sept 2011, Accepted 12 sept 2011.
* Corresponding author's e-mail: bvfarah@yahoo.fr

Abstract

Seedlings roots of *Phoenix dactylifera* L. growing in plastic pots inoculated with *Pisolithus tinctorius* [(Pers) Coker & Couch] spores have developed ectomycorrhizae. Spores powders inoculum was prepared from crushed carpophores of *Pisolithus tinctorius*. Six months after inoculation, ectomycorrhizas were established in date palm seedlings roots.
The ectomycorrhization of *Phoenix dactylifera* plants using spores powders inoculum was performed for the first time in our experiment conditions. This ectomycorrhizas result can provide an enormous potential for the development of large scale inoculation procedures of this species seedlings in commercial nurseries.

Key Words: Ectomycorrhization, *Pisolithus tinctorius*, spores powders inoculum, *Phoenix dactylifera*.

Introduction

Ectomycorrhizal (ECM) fungi like *Pisolithus* are known to enhance tree growth by increasing the uptake of nitrogen (N) [1, 2] and phosphorus (P) by roots in P deficient soils [3, 4]. 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 [5-9]. The ability to secrete extra-cellular enzymes differs with ECM fungal species and with season [10]. Some authors have reported that ectomycorrhizae can also protect seedling roots against pathogens [11-16]. 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 [17]. For these reasons, research on ectomycorrhizas has evolved greatly over the last 40 years [18].
Pisolithus tinctorius is an ectomycorrhizal fungus frequently used for inoculation in controlled mycorrhization programs [19, 20]. 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 [21-23]. 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.
The date palm *Phoenix dactylifera* L. belonging to the Arecaceae family represents an important economical and ecological culture for many countries in the North Africa and in the Arabian Golf. Somme reports [24, 25] have shown that roots of *P. dactylifera* are commonly colonised with arbuscular mycorrhizal fungi (AMF) in

controlled conditions. However, there are no studies using ectomycorrhizal fungi as inoculum.
In the aim to improve interest species culture as date palm (*Phoenix dactylifera*) we have used *Pisolithus tinctorius* basidiocarps to mycorrhizing this species.
In this paper we report for the first time the mycorrhization of *Phoenix dactylifera* by *Pisolithus* spores inoculum.

Material and methods

Inocula preparation:
P. tinctorius basidiocarps (Fig. 1.a) used in this study were collected during the spring season (April 2009) in *Eucalyptus gomphocephala* plantation (Fig. 1.b) in eastern region of Morocco. *Pisolithus* basidiocarps harvested were dried at 35°C for 72 h [26], and crushed to produce spores powder. Initial fungal spore concentration was measured with a haematocytometer and mixed with sterile peat before being sown by the pre-germinated seeds. One gram of *P. tinctorius* powder spores contains 1.10^8 spores.

Plant materiel and seed germination: Phoenix dactylifera seedlings were obtained from seeds produced by 'Boufaggous' during 2009 date palm production season in Figuig (South East of Morocco). Boufaggous is a high quality variety grown in Morocco.
Boufaggous seeds were first surface sterilized with 10% sodium hypochlorite for 5 min and rinsed four times in sterile distilled water. They were then soaked in boiling water and allowed to cool in tap water for 48 h, before being transferred to germinate in autoclaved peat. The pre-germination is conducted in darkness at 30°C for 16 days.
Ectomycorrhizal synthesis using natural inoculums: Two cultures were led: a control culture (not inoculated by mycorrhizal fungus powder) and a culture test (inoculated at a rate of 70.10^8 spores of dry carpophores /g of autoclaved peat). 20 seedlings were used for each treatment and the experiment was repeated two times. The Plastic pots (500 mL) were filled with 300 g of autoclaved peat mixed to fruiting bodies powder (70.10^8 spores/g of autoclaved peat). Then pre-germinated seed of date palm were inserted into the substrate (one per plastic pot). Plastic pots, open at the bottom, were set in a saucer of water to ensure substrate humidity. The seedlings were placed in a growth chamber maintained at 28 ± 2°C with 16 h photoperiod.

Microscopic studies: Six months after inoculation, date palm seedlings roots were rinsed, incised and colored. 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 for ectomycorrhizas presence and mycelia structure. Observations of control mycelium structure collected in margin of a rapid growth culture on modified Melin-Norkrans (MNM) medium [27] have been made (Fig. 2).

Results and discussion

Sampling of plants and evaluation of root ectomycorrhizas: Twenty four weeks post inoculation, *Pisolithus tinctorius* mycelia characteristics were noted in date Palm roots systems (Fig. 3a). Microscopic observations revealed the presence of *P. tinctorius* mycelia derived from germinated spores (Fig. 4a, b). 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 (Fig. 3b, c, d) characterized by labyrinthine branching. A fungal mantle around roots and emerged hyphae outwards roots were also observed (Fig. 5). This complex hyphal is considered to increase the fungal surface in contact with the cells roots and explored soil area. Previous studies have suggested that ectomycorrhizal organs arise spontaneously when hyphae of ectomycorrhizal fungi come into contact with compatible and uncolonised young lateral rootlets [28]. After this, the hyphae have contacted the root surface, which is associated with a switch of the hyphal growth pattern from an apical dominated to a multibranched and multiple apices mode [29], they penetrate the rootlets intercellularly. In this context, it has been revealed that a densely interwoven, two-dimensional fungal tissue composed of so-called palmettes develops between the epidermal and cortical cells and forms the Hartig net [30].
The principal aim of this study was to induce to *P. dactylifera* seedlings roots ectomycorrhizae on the controlled conditions. The mycorrhizal fungus *P. tinctorius* was selected because it is ubiquitous soil and it is very effective in nursery systems.

In our study, ectomycorrhizal plants frequency after inoculation by *P. tinctorius* was 20%. As a result, *Phoenix dactylifera* were ectomycorrhized for the first time in our experience conditions. Examination of control date palm plants root systems showed that they are free from mycelia contamination (Fig. 6).

Previous research has focused on date palm endomycorrhization. Thus, it was demonstrated [25] that the inoculation of date palm seedlings with *Glomus mosseae* reduces the Fusarium disease severity. With a same aim, antagonism between *Fusarium oxysporum* fsp *albedinis* and other micro-organisms was studied. Therefore, arbuscular mycorrhizal fungi (AMF) (*Glomus monosporus*, *Glomus deserticola*, *Glomus clarum* and a complex of native AMF coming from the Aoufous date palm grove in the south of Morocco) have been shown to protect date palm seedlings against bayoud disease [24] .

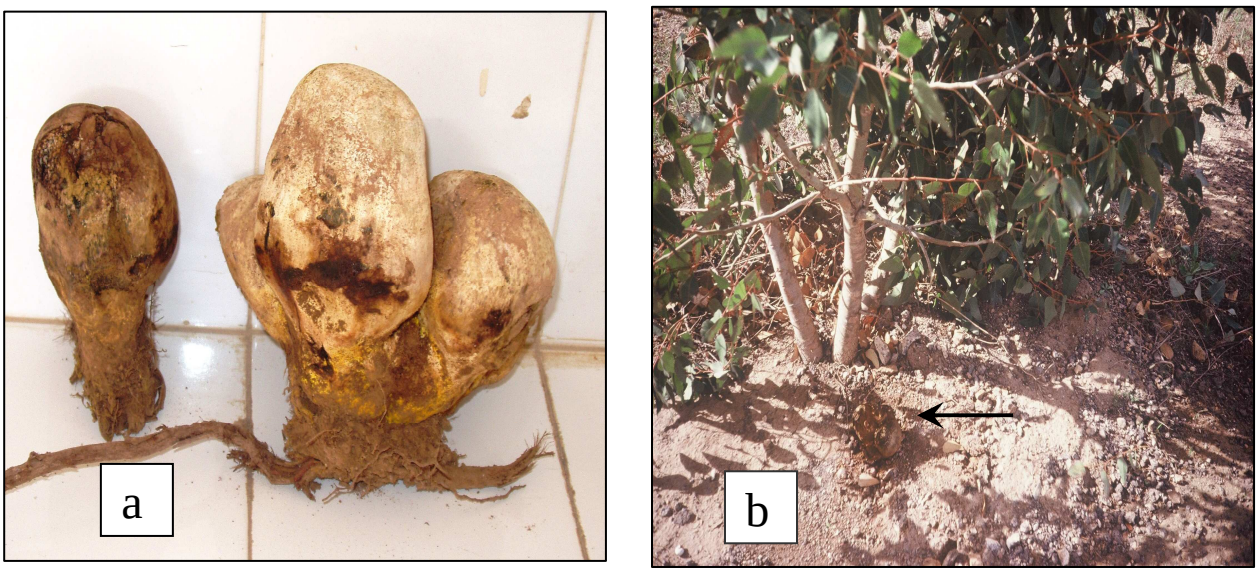


Fig.1. *Pisolithus tinctorius* basidiocarps (a) associated with Eucalypts plant (arrow) (b). Scale bar = 33000 µm.

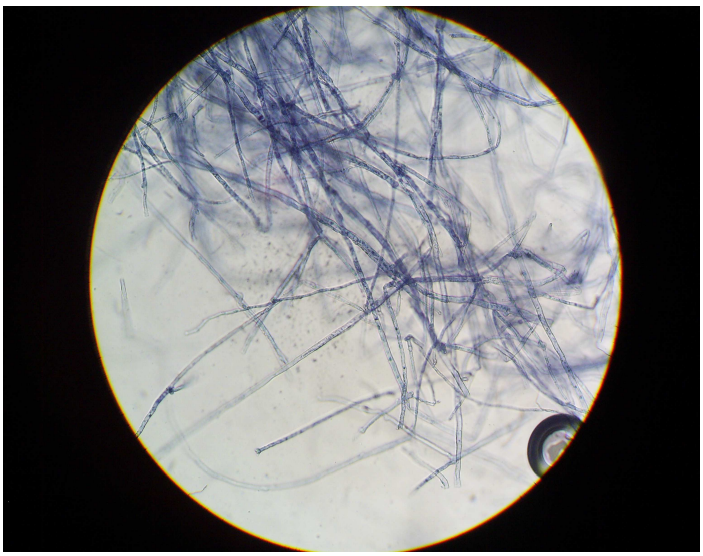
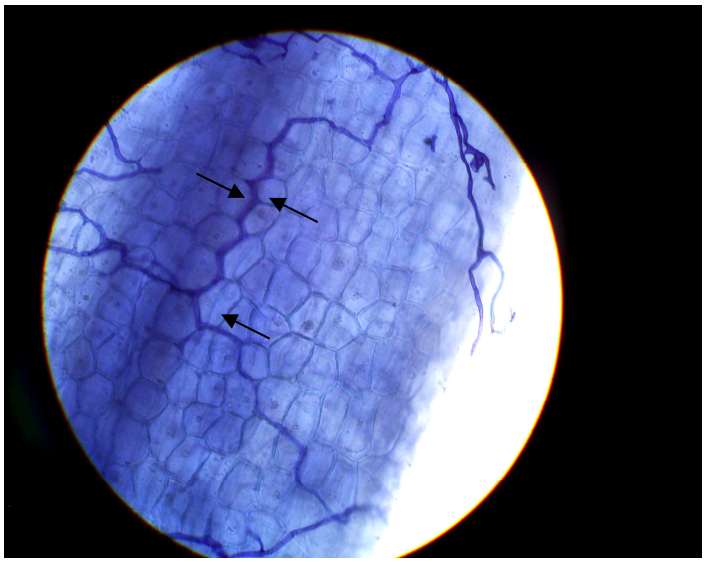
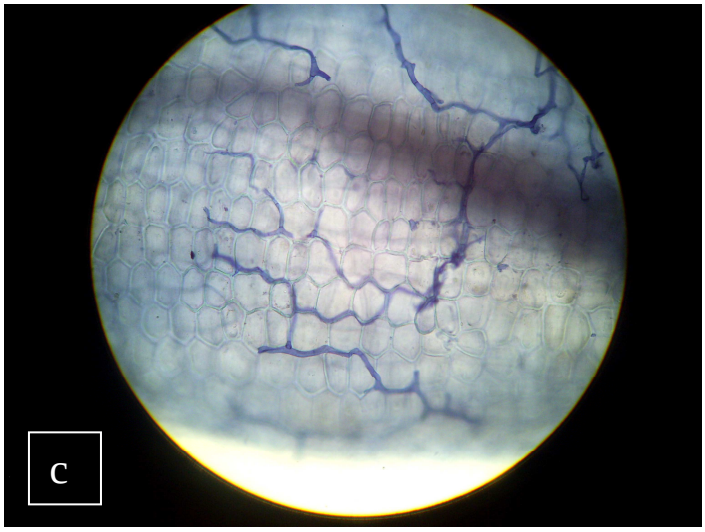


Fig.2. Microscopic observation of the *Pisolithus tinctorius* mycelia developed in MNM medium (X100). Scale bar = 500 µm.

ISSN : 2528-2028





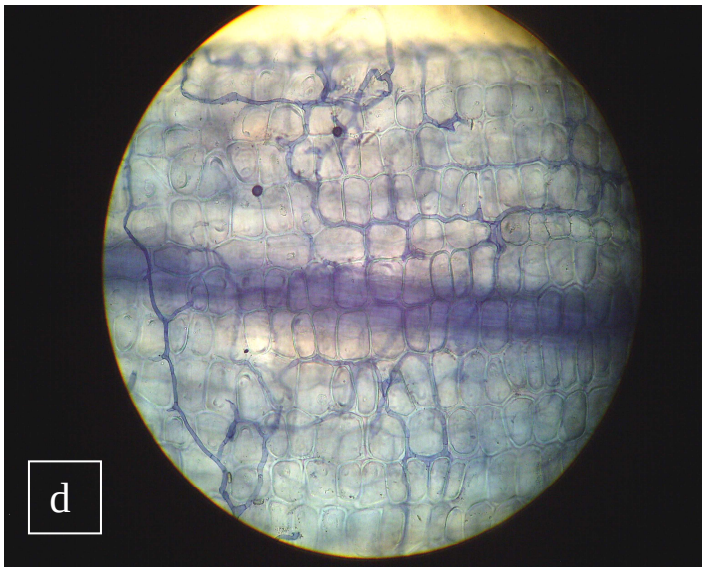


Fig.3. Lateral root of *Phoenix dactylifera* colonised bay *Pisolithus tinctorius* Hyphae (a) (arrows) (X100). Scale bar = 200 µm. The mycorrhizal fungus grows by branching hyphae and continues to elongate only between cortical cells interfaces (arrows) (b, c and d) (X400). Scale bar = 100 µm.

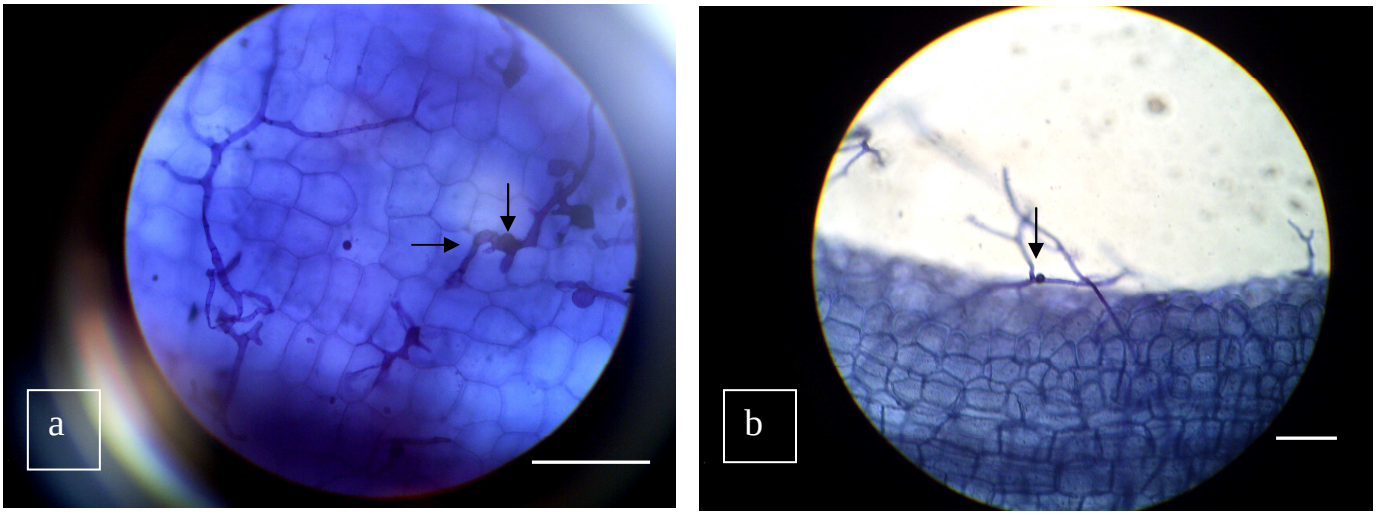


Fig.4. *Pisolithus tinctorius* mycelia derived from germinated spores (arrow), in root, grew only between cortical cells wall (a). Scale bar = 100 µm. A *Pisolithus tinctorius* spore can also germinate on the exterior of root and emits mycelia which penetrate between cortical cells (arrow) (b). Scale bar = 2000 µm.

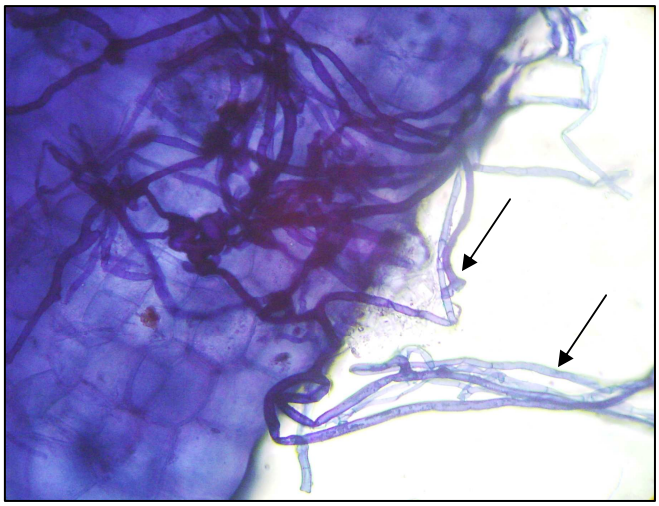


Fig.5. *Pisolithus tinctorius* hyphal emergence to outside host roots (arrows) (X400). Scale bar = 50 µm

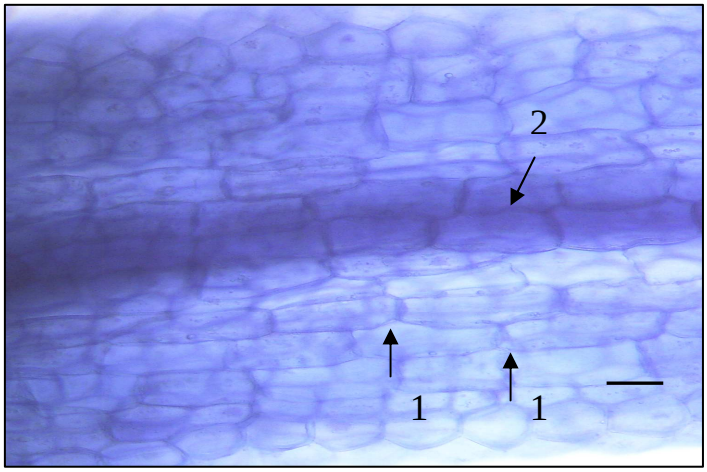


Fig.6. Control plants root free from mycelia contamination. Scale bar = 50 µm. Arrows 1: Cortical cells; Arrows 2: Central cylinder.

Conclusion

The ectomycorrhizal symbiosis with *Pisolithus tinctorius* in axenic and non axenic conditions has never been assessed with phoenix dactylifera plants. The *P. tinctorius* basidiocarps are adapted to the environmental conditions encountered in arid regions. Its ecological characteristics and its beneficial effects on the plant could be very useful in controlled mycorrhizal associations of date palm plants.
In conclusion, it appears that the controlled mycorrhization of *P. dactylifera* could be a beneficial tool in improving the survival of date palm species and consequently for the reafforestation of oasis date palm destroyed by Fusarium disease. In the date palm-*Fusarium oxysporum* fsp *albedinis* interaction little is known concerning the contribution of mycorrhizas to Bayoud disease control. Our results on ectomycorrhization allowing biologists a very useful tool for studies on resistance to wilt date Palm.

Acknowledgement: We thank Malika Abid and Atiqua Mihamou for excellent assistance.

References

1. Martin, F., *FEBS Letters*, 182 (1985) 350.
2. Chalot, M., Bran, A., *FMES Microbiology Review*, 22 (1998) 21.
3. Grove, T.S., Malajczuk, N., Mingqin, G., Daping, X., Chonglu, Z., Proceedings of an International Symposium and Workshop, Kaiping, China, ACIAR Proceedings N° 62 (1994) 101.
4. Marc, D., Antoine, G., Gilles, C., Yves, P., *European Journal of Soil Biology*, 40 (2004) 105.
5. Dighton, J., *Plant and Soil*, 71 (1983) 455.
6. (a) Abuzinadah, R.A., Read, D.J., *New Phytologist*, 103 (1986) 481. (b) Abuzinadah, R.A., Read, D.J., *New Phytologist*, 103 (1986) 507.
7. Leake, J.R., Read, D.J., *New Phytologist*, 116 (1990) 123.
8. Read, D.J., *Experientia*, 47 (1991) 376.
9. Guttenberger, M., Compart, U., Spagele, S., Hampp, R., *Plant Physiology, (Supplement)* 105 (1994) 869.
10. Buée, M., Vairelles, D., Garbaye, J., *Mycorrhiza*, 15 (2005) 235.
11. Chakravarty, P., Hwang, S.F., *European Journal of Forest Pathology*, 21 (1991) 97.
12. Chakravarty, P., Peterson, R.L., Ellis, B.E., *Journal of Phytopathology*, 132 (1991) 207.
13. Duchesne, L.C., *In: Mycorrhizae and Plant Health. Pfleger, F.L., Linderman and R.G., eds. APS Press, St. Paul, Minnesota, USA*, (1994) 27.
14. Hwang, S., Chakravarty, P., Chang, K., *Phytoprotection*, 76 (1995) 57.
15. Morin, C., Samson, J., Dessureault, M., *Canadian Journal of Botany*, 77 (1999) 169.
16. Machon, P., Santamaria, O., Pajares, J.A., Alves-Santos, F.M., Diez, J.J. *Symbiosis*, 42 (2006) 153.
17. Machón, P., Pajares, J.A., Diez, J.J., Alves-Santos, F.M., *Symbiosis*, 49 (2009) 101.
18. De Roman, M., Claveria, V., De Miguel, A.M., *Mycol. Res*, 109 (2005) 1063.
19. 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., *For. Sci*, 28 (1982) 373.
20. Burgess, T., Malajczuk, N., Dell, B., *Mycol. Res*, 99 (1995) 1.
21. Marx, D.H.; *Can J Bot*, 23 (1977) 217.
22. Garbaye, J., Delwaulle, J.C., Diagana, D., *For Ecol Manage*, 24 (1988) 151.
23. Duponnois, R., Ba, A.A., *For Ecol Manage*, 119 (1999) 209.
24. Jaiti, F., Meddich, A., El Hadrami, I., *Physiological and Molecular Plant Pathology*, 71 (2007) 166.
25. Oihabi, A., *Doctorat d'Etat, Universite Cadi Ayyad, Faculte des Sciences Semlalia-Marrakech, Maroc* (1991) 110p.
26. Rincon, A., Parladé, J., Pera, J., *Ann. For. Sci*, 62 (2005) 817.
27. Marx, D.H., *Phytopathology*, 59 (1969) 153.
28. Brunner, I., *Urban & Fischer Verlag*, 4 (2001) 13.
29. Brunner, I., Scheidegger, C., *New Phytologist*, 120 (1992) 359.
30. Scheidegger, C., Brunner, I., Electron microscopy of ectomycorrhiza: Methods, applications, and findings. Mycorrhiza: Structure, Function, Molecular Biology, and Biotechnology (eds. A. Varma & B. Hock), *Springer*, (1999) 205.