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Full Length Research Paper

Cultivation of an edible desert truffle (*Terfezia boudieri* Chatin)

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This research was conducted to study the fruiting of an edible truffle *Terfezia boudieri* Chatin in fields using mycorrhizal plants of *Helianthemum sessiliflorum* Desf. Pers. in two soil types (gypsy and sandy loam soils). Two cultivation methods have been tested. The first is the transplantation of inoculated plants (with *T. boudieri* ascospores) maintained in greenhouse and the second is the seedling of inoculated *H. sessiliflorum* seeds in experimental field (24 m^2). The first ascocarps was detected one year after (2006) nearby transplanted plants in gypsy soil, two fruiting bodies of *T. boudieri* were observed in both soil types near the plants obtained by direct inoculation of *H. sessiliflorum* seeds in the second year (2007). Three years after (2009), the experimental field produces two ascocarps close to transplanted plants in sand loamy soil and directly seedling plants in gypsy soil.

Key words: *Helianthemum sessiliflorum,* arid land, desert truffle, cultivation, plantation, mycorrhization, seeds.

INTRODUCTION

Edible truffles are the fructification of some Ascomycetes fungi. Their mycelia form mycorrhiza with the roots of some host plant species. These last depend on site characteristics (soil type and climatic conditions) and truffle species. These mushrooms (truffles bodies) are known with their culinary use and aromatic toast. The sell prices of one pound of *Tuber melanosporum* Vittad. and *Tuber magnatum* Pico reach $1000 and $3000 respectively, in USA (Lefevre, 2008). Prices continue to increase because the harvested truffles in natural lands are decreasing (Bunyard, 2008). Many researchers have investigated the possibilities of truffle cultivation under controlled conditions in order to satisfy human requirements. The first truffles cultivation experiment was established in France at 1970s for *Tuber melanosporum* species (Chevalier and Grente, 1979). Many cultivation tests were made in several countries around the world (Chevalier and Frochot, 1997). These tests were based on the field plantation of mycorrhizal plants. Many truffle farms were created in Italy, Spain, Finland, Australia, North America, New Zeland and China (Hall et al., 2007; Morte et al., 2008). Despite the high number of recognized truffles species, only *T. melanosporum*, *Tuber uncinatum* Chatin, *T. magnatum* and *Tuber borchii* Vitt are cultivated actually (Chevalier, 2008).

Desert truffles are common in arid and semi arid regions of the world. These fungi have been considered edible since three thousand years (Chang and Hayes, 1978; Morte et al., 2008). Truffles are excellent nutritional sources with specific good taste (Trappe, 1971; Al-Delaimy and Ali, 1970; Morte et al., 2009). In Tunisia, three fungal genera (*Terfezia*, *Tirmania* and *Picoa*) are known by their important economical income for local population and their good taste (very appreciated by local populations) (Patouillard, 1894; Malençon, 1973; Slama et al., 2006). These fungi are widely distributed in arid and desert regions, representing about three quarters of Tunisian area, when the rainfall conditions are favourable (Houerou, 1959). Slama et al. (2006) shows that *Helianthemum sessiliflorum* is the host plant of Tunisian desert truffles and that *Terfezia boudieri* is the most common and harvested fungi species. Desert truffles development is irregular and ambiguous and depends on

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Figure 1. Inoculated *H. sessiliflorum* plants with *T. boudieri* ascospores in greenhouse (plants used for transplantation).

several conditions such as soil and climatic characteristics. Only Spanish researchers have investigated some experimentation in order to cultivate them (Morte et al., 2008). This work aims to control two methods of cultivation of *T. boudieri* in the field using mycorrhization of *H. sessiliflorum* plants in two soil types. The success of such methods allows the creation of desert truffles farms and helps in the economical development of rural and local populations.

MATERIAL AND METHODS

Materials

H. sessiliflorum is a perennial dwarf shrub distributed in sandy regions of southern Tunisia. This plant species is known to be usually associated to desert truffles (Floc'h, 1983; Slama et al., 2006). *H. sessiliflorum* seeds were collected in March 2003 from Ben Guardane region (33°17'N, 10°46'E). Seeds were wiped (to remove sand from their surface) and stored for six months in the seed bank of Range Ecology Laboratory of the Institut des Régions Arides (Médenine, Tunisia) with 30% relative humidity and 20°C. *T. boudieri* fruiting bodies were collected in April, 2004 from the same region. This last is characterized by irregular rainfall and harsh dry summer period and lower mineral and organic matter contents in the soils. Ascocarps were sun dried during 2 months and stored in the said seed bank. Two soil types were used in this study. The first is sandy loam sampled from Ben Guardane (organic matter = 1.17%, K = 27.27 ppm, total phosphorus = 575 ppm, EC = 353 µs cm, pH = 7.1). The second is gypseous sampled from an experimental field of the Institut des Régions Arides (33°30'N, 10 38E; southeast Tunisia) (organic matter = 3.45%, K = 12.8 ppm, total phosphorus = 212 ppm, EC = 469 µs cm, pH = 7.7). Both soils were 2 mm-sieved and sterilized in a drying stove (180°C/30 min) before *H. sessiliflorum* cultivation. For each treatment and type of soil, 30 plastic pots (1-L volume, 7.5 cm base diameter and 12 cm top diameter) were sterilized by washing with a solution of sodium hypochlorite (12°C) and filled with the considered soil types. Mix of soil and vermiculite, with or without *T. boudieri* acospores (for inoculated or non-inoculated plants), was prepared according to Fortas and Chevalier (1992)'s method. This mix was deposited in the middle of each pot. The vermiculite provides aeration and fixes *T. boudieri* ascospores. Pots were perforated at the bottom and containing a disinfected layer of gravel to ensure the excess water drainage.

Plant inoculation and cultivation

For each pot, 0.5 g of *H. sessiliflorum* seeds was added after surface scarification using an emery paper. Mix of truffle fruit body fragments and deionized water was prepared and added to the soil and vermiculite mix according to Chevalier et al. (1973) and Chevalier and Grente (1979)'s methods. The control pots were maintained without truffle mixture. Seeds were deposited above each pot (inoculated and non-inoculated) and recovered with a thin soil layer. Inoculated and non-inoculated seedlings were maintained in growth chamber with 23±1°C temperature, 50% day and 75% night relative humidity and 16 h light/8 h dark regime with 250 µmol m^{-2} s^{-1} photosynthetic active radiations (PAR). Capillary irrigation was conducted twice a week with tap water during the experimental period. These plants were transplanted in the experimental field containing the two indicated soil types (Figure 1). Transplantations were carried out in February and April, 2005 (spring).

Direct inoculation and plantation of *Helianthemum sessiliflorum*

In the same experimental field, direct planting of *H. sessiliflorum* seeds with or without inoculation was performed using the two soil types (Figure 2). Plantations were made in November, 2004 to permit plant emergency and installation. For these plants, ascospores addition was performed every year (in November) to promote natural mycorrhization.

Experimental field properties

The experimental field is localized in the Institut des Régions Arides, Southern Tunisia. The field was divided in two parts. In the first (12 m^2), 0.5 m floor soil surface have been removed and substituted with sand-loamy soil brought from Ben Guardane

Figure 2. Direct inoculation and plantation of *H. sessiliflorum* seeds in the experimental field.

Sand-loamy soil								Gypseous soil							
C	C	C	C	I	I	I	I	I	I	I	I	C	C	C	C
Nov. S	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Nov. S	April trans	Nov. S	Nov. S	Feb. trans	Nov. S	Nov. S	Feb. trans	Nov. S	April trans
Nov. S	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Nov. S	April trans	Nov. S	April trans	April trans	Feb. trans	Nov. S	Feb. trans	Nov. S	April trans
Nov. S	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Nov. S	April trans	Nov. S	April trans	April trans	Nov. S	Nov. S	Feb. trans	Nov. S	April trans
Nov. S	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Nov. S	April trans	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Feb. trans	Nov. S	April trans
Nov. S	Nov. S	April trans	Feb. trans	Feb. trans	Nov. S	Nov. S	April trans	Nov. S	April trans	April trans	Nov. S	Nov. S	Feb. trans	Nov. S	April trans

Figure 3. Arrangement and localization of *H. sessiliflorum* plants in the experimental parcel, Nov: November, Feb: February, Trans: Transplanted plant, S. plant seedling, C: control, I: Inoculated plants.

region. The second part (12 m^2) was kept with its original gypsy soil. Each part contains 4 lines (1 x 3 m) (Figure 3) and the plantation density was about 5 plants/m^2.

Morphological characterization of mycorrhiza

In spring, the coloured roots (by fuchsine acid (CAS: 3244-88-0; Fisher Health Care) according to Phillips and Hayman (1970)'s method) were observed with Leica DMLS microscope in order to study mycorrhiza type.

Terfezia boudieri fruiting control

The quantification of *T. boudieri* ascocarps fructification (appearance of telltale crakes in the soil during four years 2005/2006, 2006/2007, 2007/2008 and 2008/2009) was performed from January to April which corresponds to the availability period of this fungi in natural conditions.

RESULTS

Mycorrhiza type

Microscopic observation of *H. sessiliflorum* inoculated plants shows the presence of endomycorrhiza in roots. Intracellular coiled hyphae within the cortical cells and no mantle was observed in this mycorrhiza type. This observation is the same in all *H. sessiliflorum* plants (inoculated and cultivated in a growth chamber in gypsy and sand-loamy soils (Figure 4) after four years of transplantation (Figure 5) and for the direct sown in

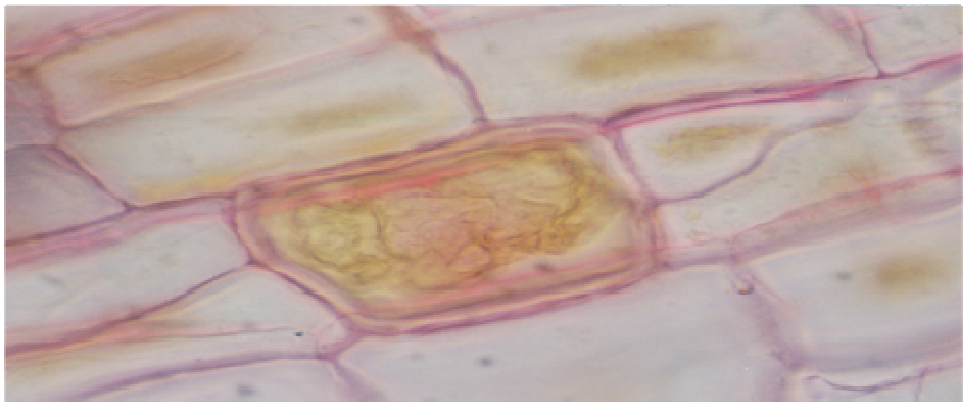

Figure 4. Microscopic observation of coloured roots of *H. sessiliflorum* inoculated plants, 5 month age and cultivated in sand loamy soil in greenhouse (1000X).

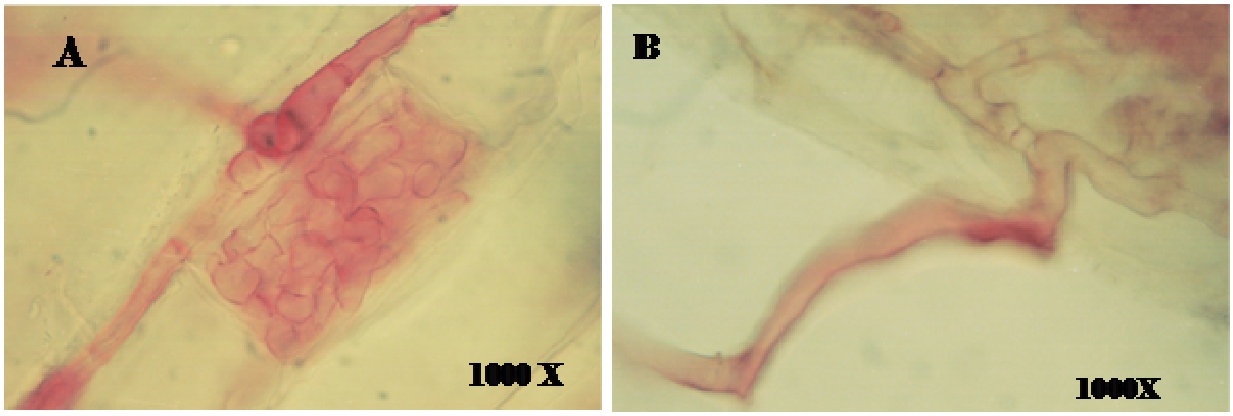

Figure 5. Microscopic observation of coloured roots of *H. sessiliflorum* inoculated plants, two years after transplantation in sand loamy (A) and gypsy soils (B).

experimental field (Figure 6).

Terfezia boudieri fructification

The first fruiting body of *T. boudieri* was detected one year after the experimentation date (March 2006) nearby transplanted plants in the gypsy soil (Figure 7). In March 2007, two ascocarps were assembled in both soil types near the plants obtained from direct inoculated seeds (Figure 8). Two years after plantation, plant density was highly increased by the emergency of new young *Helianthemum* and some other plant species. These last seem to have negative effect on mycorrhiza between *H. sessiliflorum* and *T. boudieri (*limit *Helianthmum* growth and fungi fructification). *T. boudieri* fruiting body was also founded in March, 2008 nearby transplanted plants in gypsy soil. In March, 2009 two ascocarps were collected close to transplanted plants in the sand loamy soil and sowed plants in gypsy soil (Figure 9).

DISCUSSION

Endomycorrhizae, observed in the root system of *H. sessiliflorum* inoculated by *T. boudieri*, proves the existence of symbiotic association between them. The endomycorrhizae type is the same for the tested cultivation methods on the two soil types. According to some authors, *Helianthemum* species can develop ectomycorrhiza without mantle with different truffle species (Chevalier et al., 1984; Cano et al., 1991). However, many other authors have observed endomycorrhiza without either Hartig net or mantle and with undifferentiated intracellular hyphae in roots of *Helianthemum* species (Awameh, 1981; Alsheikh, 1984; Dexheimer et al., 1985; Fortas and Chevalier, 1992; Kagan- Zur et al., 1999; Zaretsky et al., 2006). According to Morte et al. (2008), mycorrhizae structure changes only with the synthesis conditions. In natural conditions, root forms endomycorrhiza in their cortical cells (Gutiérrez et al., 2003). Similar observations were given

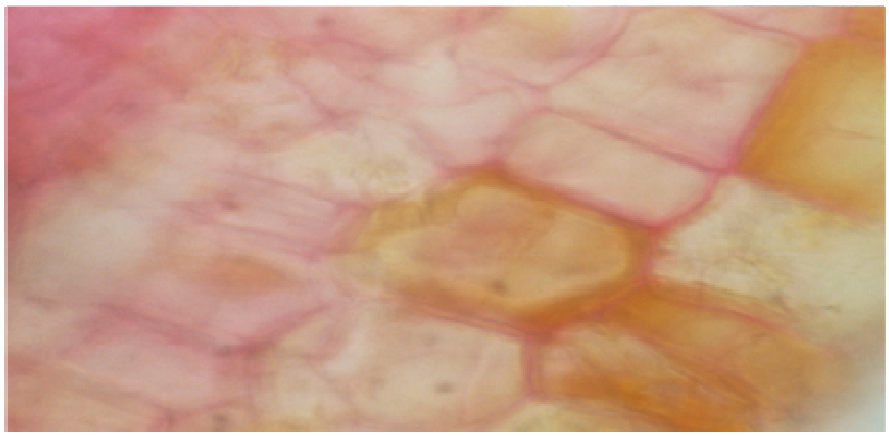

Figure 6. Microscopic observation of coloured roots of *H. sessiliflorum* inoculated plants, one year after direct inoculation in gypsy soil.

Figure 7. First *T. boudieri* fructification observed in March, 2006 nearby transplanted plants in gypsy soil.

Figure 8. Location of *T. boudieri* ascocarps developed in sand loamy and gypsy soils. near direct inoculated plants in March 2007.

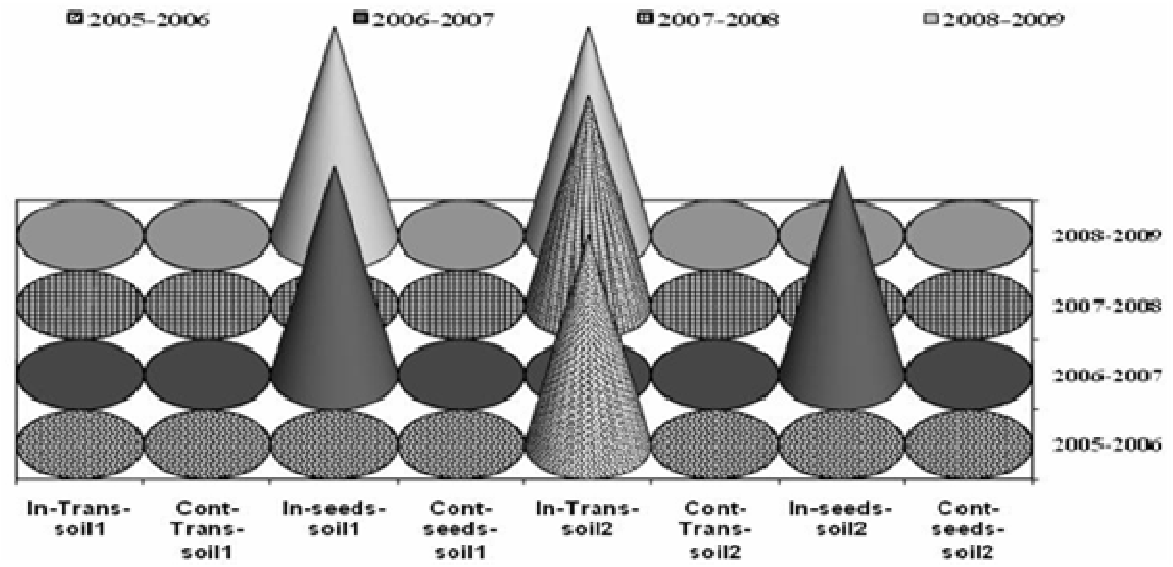

Figure 9. Impact of planting mode, inoculation and soil type on *T. boudieri* fructification. In: inoculated plants, Trans: transplanted plants, cont: control, soil 1: sand-loamy soil, Soil 2: gypsy soil.

for *Helianthemum* mycorrhiza type and showed that their morphology differs occasionally with the growing conditions (Read et al., 1977; Dexheimer et al., 1985; Fortas and Chevalier, 1992; Kagan- Zur et al., 1994; Gutiérrez et al., 2003).

Our results showed the ability to cultivate *T. boudieri* and proved that mycorrhizal *H. sessiliflorum* plants can produce *T. boudieri* ascocarps. *H. sessiliflorum* was cited by Slama et al. (2006) as the best host plant of *Terfezia* and *Tirmania* species. Earlier research have also confirmed that *Terfezia* sp. forms mycorrhizae association with Cistaceae such as *Helianthemum* (Awameh, 1981; Dexheimer et al., 1985; Roth-Bejerano et al., 1990; Morte et al., 2009). The host plant species depend on ecological and climatic conditions of the truffles sites.

This work shows that methods of plants mycorrhization (inoculation- transplantation and direct inoculation in field) allow truffle fructification. Transplantation of inoculated plants in gypsy soil seems to provide later the appearance of ascocarps (after the 4 studied years). Transplanted plants in spring period allow saving mycorrhiza type and ensure a good plant development compared to seeding. Many authors have used transplantation of mycorrhized plants to promote truffles cultivation (Hall et al., 2007; Morte et al., 2009). However, direct inoculation of plants in field had never been employed as truffle cultivation technique. Transplantation of mycorrhized plants is more interested than direct inoculation because soils contain several microorganisms which can degrade truffle's ascospores.

Morte et al. (2008) were obtained truffle fructification 23 months after planting. This period was reduced to 12 months after using an adequate agricultural management. In our study, we collect the first ascocarp one year after cultivation. *T. boudieri* is able to grow both in sand loamy and gypsy soils. Malençon (1973) explained the importance of soil texture and type in the mycelia growth and the ascocarps shape. Furthermore, Fortas and Chevalier (1992) and Slama et al. (2006) showed that some Algerian and Tunisian *Terfezia* species were harvested in sand loamy soils. Many *Terfezia* species such as *Tuber arenaria* and *Tuber leptoderma* in Kuwait (Alsheik, 1984), Morocco (Khabar et al., 2001) or Spain (Morte et al., 2008), *Tuber pfeilli* in Botswana (Taylor et al., 1995) and *Tuber terfezioides* in Hungary (Kovacs et al., 2003) grow in sandy soils.

Our data shows the possibility of use of desert truffles to rehabilitate arid lands by increasing plant performance with symbiotic relation between host plants and truffles. Improving fungi fructification (economic incomes for rural population) and reintroducing new plants (amelioration of soils quality, decreasing erosion process) can be considered as results of such technique in dry areas.

Conclusion

This work describes *T. boudieri* cultivation using *H. sessiliflorum* plants. Direct inoculation of seeds in field was assumed as the best truffle production technique. Transplantation of inoculated plants can also produce *T. boudieri* ascocarps. Fructification of this edible fungus was obtained only one year after the experimental essay. Endomycorrhizae describes the symbiotic relationship between *T. boudieri* and *H. sessiliflorum*. This type has been demonstrated in the roots of inoculated plants kept in growth chamber and in transplanted one and also for

plants inoculated directly in parcel.

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