Ecology and distribution of desert Truffles in Algeria.

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Research Article

Keywords: Distribution, pedoclimatic characteristics, mycorrhizal association, Terfezia Tirmania, Picoa

Posted Date: May 3rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-248631/v1

Abstract

This first study focuses on geographical distribution of desert truffles in Algeria, their diversity and their ecological requirements during growing seasons according to field surveys carried out during thirty years (1986–2016). Investigations in 61 productive sites of desert truffles spread over several bioclimatic areas revealed the presence of nine species to genera *Terfezia, Tirmania* and *Picoa* and their host plants *Helianthemum* spp. This study complements our earlier work on characterization of *Terfezia* and *Picoa* samples harvested throughout Algeria by macro-and micromorphological studies and by molecular phylogeny. Pedoclimatic factors were evaluated, host plants species were identified and mycorrhizal relationships of these fungi species under field conditions were examined. Results revealed that good natural yield desert truffles species is closely linked to intensity and distribution of rainfall, the temperature, frequency of storms during ascomata growing seasons. Nine desert truffles species grow on sandy loam soil and form with annual or perennial *Helianthemum* spp. endomycorrhizae on calcareous soil and also ectomycorrhizae without mantle on acidic soil in *Helianthemum guttatum..*

Introduction

Desert truffles are edible mushrooms (Ascomycota) which include several species of the genera *Terfezia, Tirmania* and *Picoa* producing hypogeous ascomata and growing especially in arid and semi-arid areas (Fortas and Chevalier 1992; Alsheikh 1994; Khabar et al. 2001; Slama et al. 2006; Mandeel and Laith, 2007; Ammarellou and Sarem 2008; Kagan-Zur and Roth-Bejerano 2008; Al-Thani 2010; Sbissi et al. 2011; Jamali and Banihashemi 2012; Al-Qarawi and Mridha 2012; Akyüz et al. 2012; Türkoğlu and Castellano 2014; Kagan-Zur and Akyuz 2014; Akyüz et al. 2015; Owaid 2016, 2018 ; Bawadekji et al. 2016**;** Berkami et al. 2017; Bouzadi et al. 2017; Zitouni-Haouar et al. 2018; Hashem et al. 2018) but can be found also in other habitats as in temperate forests (Janex-Favre et al. 1988; Honrubia et al. 1992), in mediterranean scrubland (Riousset et al. 2004) and in a wide range of habitats, such as temperate deciduous forests, conifer forests, prairies, or even heath lands (Moreno et al. 2014) .These edible mushrooms are most appreciated for their nutritional and economic value, but also for their properties in traditional medicine particularly by local populations of North Africa, the Middle East and the Arabian Peninsula. Most desert truffles establish important mycorrhizal symbioses with different host plants, most often Cistaceae family with including different annual and perennial species of the genus *Cistus* and more especially *Helianthemum* spp. (Awameh and Alsheikh 1979, Díez et al. 2002; Kagan-Zur and Roth-Bejerano 2008; Slama et al. 2010; Morte et al. 2008; Loizides et al. 2011; Bordallo et al. 2013; .Zitouni-Haouar et al. 2014). However, somes species are found under oaks and pine trees (Díez et al. 2002; Chevalier 2014). This mycorrhizal association plays an important role in the maintenance of Mediterraneen shubs-lands by preventing erosion and desertification. (Honrubia et al. 1992) mainly in the arid and semi-arid regions of North Africa, the Near and Middle East and the Arabian Peninsula. Although desert truffles are widely distributed in several countries and their genera and species differ from continent to continent many aspects of their ecology are similar, as are their uses by geographically distant, nomadic but culturally unrelated desert peoples (Trappe 1979). In Algeria, three genera *Terfezia, Tirmania* and *Picoa* were collected in different bioclimatic regions near annual or perennial *Helianthemum* spp. (Chatin 1891 a, b; Maire, 1907; Duggar and Pinoy 1907; Fortas 1990; Fortas and Chevalier 1992; Chafi et al. 2004; Bradai et al. 2014; Zitouni et al. 2014, 2015, 2018). However, their development is irregular and their abundance depends on the distribution and intensity of rainfall, physicochemical composition of soil and host plants..This study focuses maintly on geographical distribution of desert truffles in Algeria, their diversity and their ecological requirements during growing seasons according to field surveys carried out during thirty years (1986-2016) in several bioclimatic zones.

Materials And Methods

Prospecting and sampling areas of desert truffles

Algeria is a country in the subtropical zone of North Africa and its climate is very different in three geographical regions. It is of Mediterranean (Csa climate class according to Köppen–Geiger classification, Peel et al. 2007) on all the northern fringe (4 % of the territory) which includes the littoral and the tellien Atlas (hot and dry summers, wet and cool winters), arid on the Highlands in the center of the country (Bsk climate class according to Köppen–Geiger classification, Peel et al. 2007) and desert as soon as one crosses the chain of the Saharan atlas (87% of the national territory) (BWh climate class according to Köppen–Geiger classification, Peel et al. 2007). In Algeria, rainfall is characterized by a very significant spatio-temporal variability. The North and Middle curbs receive an average annual rainfall of between 400 to 800 mm. They are therefore more watered than the rest of the country. In the Highlands and in the Saharan Atlas annual rainfull do not exceed the amount 200 to 400 mm. In the pre-Saharan zone, the average annual rainfall is between 100 mm and 150 mm and in Sahara precipitation is extremely rare below 100 mm in almost 90 % of its area (National Meteorological Office 2012). The field survey was initiated during the growing season in several producing sites of desert truffles spread over three geographical zones located in the northwestern and northeastern areas of coastal strip, in various bioclimates existing in the High steppe Plains (Highlands) and in Saharan areas (Fig. 6). High steppe Plains constitute a geographical unit whose limits are defined by only bioclimatic criteria. All sites prospected are located in different bioclimatic zones: subhumid, semi-arid, arid (medium and inferior arid) and Saharan. The classification of these bioclimates is made through 3 main criteria: average annual rainfall, average daily minimum temperature of coldest month and average daily maximum of coldest month (Le-Houérou et al. 1977; Le-Houérou 1995; Benabadji and Bouazza 2000; Medjerab and Henia 2005; Bensenane et al. 2013). Surveys were carried out at various sites from January to March during thirty growing season of desert truffles to collect ascomata samples and to note some ecological factors of these hypogeous fungi. The location of studied sites was based on our investigations (Fortas 1990; Fortas and Chevalier 1992; Chafi and Fortas 1999; Chafi et al. 2004; Dib-Bellahouel and Fortas 2011; Zitouni et al. 2014, 2015, 2018) with the effective help of the nomadic population, local inhabitants and seasonal collectors of these mushrooms, especially in the wide steppic and Saharan regions. Literature devoted to Algerian desert truffles (Chatin 1891 a, b; Maire 1907) also guided us to localize some producing sites in High steppe Plains and Saharan areas although some of them have undergone various anthropogenic factors (overgrazing, land clearing, reforestation, urbanization,...etc.) thus causing their gradual disappearance.

Sampling procedure

Ascomata sampling belonging to genera *Terfezia*, *Tirmania* and *Picoa* sp. were often harvested in spring from March to April and exceptionally from January to April in Saharan areas during the good growing seasons. They were collected at depth of 10 cm near annual or perennial *Helianthemum* spp. in a wide variety of habitats characterized by xerophilous vegetation dominated by *Helianthemum* spp. (Table 1).The presence of ascomata is identified by uprisings and soil cracks caused by increasing fruiting. The top of soil is removed and a stick orrudimentary tool is used to extract ascomata (Fig. 1a; Fig. 2a,b; Fig. 3a,b; Fig. 4a,b; Fig. 5a,b). Samples collected were stored at 4 °C for five days to study the macroscopic characteristics. Some ascomata samples were sun-dried during 2 months and stored at room temperature in the collection of LBMB research laboratory, at the University of Oran 1 (Algeria). *Helianthemum* spp. host plants were carefully removed from the substrate and the majority of soil was manually removed from the root systems (Table 1). Roots samples are washed with tap water to remove soil particles and fixed in FAA (formalin, glacial acetic acid and ethanol). Soil samples for analysis were collected from

30 desert truffles producing sites located in different bioclimatic zones; they were carried out at a depth of 10 cm from the soil, after the ascoma havest. The selection of sites was based on our surveys (2 sites in subhumid areas; 2 sites in semi-arid to temperate winter; 2 sites in medium arid areas to temperate winter; 12 sites in medium arid areas to cold winter; 6 sites in inferior arid; 6 sites in hyper-arid areas). These selected sites were usually known for their good production of desert truffles. Soil sampling was performed by combining elements from five different spots in each site.

Statistical analyses

Statistical analyses were performed using the SPSS software program (SPSS 23.0). The data were analyzed by One-way analysis of variance (ANOVA). To detect the statistical significance of differences ($p< 0.05$) between means, the Tukey test was performed.

Climatic parameters

Data for climatic parameters from 1986 to 2016 were collected from different national meteorological stations located in several Algerian provinces ("Wilayas"): National Office of Meteorology (ONM), Agrometerological Department of the National Institute of Soils, Irrigation and Drainage of Algiers (INSID) and National Hydraulic Resources Agency (INRH).

Study of collected samples

Soil samples were dried at room temperature and sieved (2 mm diameter). Physicochemical analyses of soil samples were performed in regional laboratories of National Institute of Soils, Irrigation and Drainage of differents provinces ("Wilayas") (Oran, Mostaganem, Alger, Matmar-Relizane, Tiaret, Adrar and Bechar). The first phenotypic and genotypic characterization of several samples of *Terfezia* spp., *Tirmania* spp. and *Picoa* spp. has been studied with this Algerian collection in our earlier work. Macro- and micromorphological studies and phylogenetic analyzes based on the sequencing of the ITS intergenic spaces of the rDNA were carried out on 73 samples of desert truffles from this collection (Zitouni-Haouar 2016). The second macro- and micromorphological studies as well as phylogenetic analysis using ITS sequences and 28S rDNA were conducted on thirty-one *Terfezia* samples from this Algerian collection and two samples newly collected in Spain. This study was to characterize the diversity of *Terfezia* in North Africa. (Zitouni-Haouar et al. 2018). The third macro-micromorphological and phylogenetic study was conducted on thirty-four *Picoa* taxa from this Algerian collection and several taxa from different countries located around the Mediterranean basin. These analyses improve our understanding of the biogeographical and phylogenetic relationships between different lineages of *Picoa* (Zitouni-Haouar et al. 2015). Macromorphological characteristics were recorded from fresh ascocarps (size, colour, gleba, peridium thickness). Shape, sizes of asci and ascospores were determined from rehydrated sections of dried ascocarps using Melzer's reagent and observed with an Olympus CX22 microscope. Ascospore ornamentation was examined and photographed with a scanning electron microscopy.

Results

Distribution

Desert truffles species belonging to *Terfezia*, *Tirmania* and *Picoa* grow in Algeria between 23-36 °N latitude. They are distributed in 61 natural producing sites spread over nineteen provinces ("Wilayas") characterized by different bioclimatic zones subhumid and semi-arid in coastal areas, arid in High Steppe Plains and in hyper-arid in Sahara .The distribution of desert truffles in 61 producing sites in Algeria is illustrated in Fig. 6 and ecological data of collected desert truffles samples are listed in Table 1. Morphological and phylogenetic characteristics of desert truffles samples collected in several bioclimatic zones in Algeria made possible to identify three *Terfezia* species (*T. arenaria*, *T. boudieri*, *T. claveryi*), two *Tirmania* species (*T. pinoyi*, *T. nivea*) and two *Picoa* species (*Picoa juniperi* Vittad. and *Picoa lefebvrei* (Pat.)= *Phaeangium lefebvrei* (Pat.). This phylogenetic analysis based on the ITS sequences of several taxa of *Terfezia* and *Tirmania* revealed a remarkable genetic variability noted particularly in *T. claveryi* including for some specimens collected from the same station (Zitouni-Haouar 2016). Results of the complementary study based on morphological and phylogenetic analyses of large ribosomal subunit (28S rDNA) and internal transcribed spacer (ITS rDNA) of *Terfezia* samples revealed the presence of five distinct *Terfezia* species: *T. arenaria*, *T. boudieri, T. claveryi, T. eliocrocae (*reported for the first time from North Africa) and a new species *T. crassiverrucosa* sp. nov., described and characterized by its phylogenetic position and unique combination of morphological characters. Variability in spore ornamentation was the most important morphological feature for discriminating between three *Terfezia* species: *T. claveryi, T. boudieri* and *T. arenaria* are clearly discriminated from each other by their typical ascospore spherical with truncated warts in *T. arenaria,* round-tipped warts in *T. boudieri* and reticulated in *T. claveryi*. However some morphological similarities recorded in the same type of ornamentation of *T. claveryi, Terfezia eliocrocae* and *Terfezia crassiverrucosa* ascospores complicate their identification and serious lead to misidentification (Zitouni-Haouar et al. 2018). The characteristics of spores and peridium have proven to be the most reliable micromorphological characteristics for identification of *Terfezia* species.

Macro- micromorphological characteristics of nine desert truffles species:

Terfezia arenaria (Moris) Trappe (1971) (Fig. 2 c-e) ascomata (3.5-11.8 cm diameter) globose to subglobose often pedunculated, sometimes lobed, fresh weight 5.5 - 140 g ; peridium (0.4–2.5 mm thick) pinkish to dark brown at maturity; gleba solid, fleshy, light pink to light brown contains many pockets of fertile tissue separated by light yellow sterile veins.; asci (57 × 82.5 µm) globose to subglobose, hyaline, eight spored; ascospores (20.1-29 µm diam.) globose and ornemented with truncated warts up to 6-7 µm tall.

Terfezia boudieri Chatin (1891) (Fig. 1b-d) ascomata (3.5–7.4 cm diameter) broad, subglobose often lobed, fresh weight 5-152 g ; peridium (0.4-1 mm thick) light brown to dark brown, smooth; gleba whitish to red brown at maturity, with brown fertile pockets separated by pale sterile veins; asci (52–58×5-80 µm), 5-8 spored, hyaline, globose to subglobose; Ascospores globose, 20-28,4 µm including ornamentation, hyaline or yellow in the immature state and dark brown at maturity, ornamented with round-tipped warts up to 3.2-5.9 µm.

Terfezia claveryi Chatin (1891) (Fig. 3 c-f) ascomata (4.9–7.5 cm diameter) subglobose; fresh weighing 6-152 g ; peridium (0.33-0.46 mm thick) red brown to dark brown at maturity; gleba light brown, spongy in the immature state becoming solid with age; asci (61-68×45.5-56 µm) subglobose, 6-8 spored; ascospores (17-22 µm) globose, hyaline to light brown ornamented with very tight irregular reticulum.

Terfezia eliocrocae Bordallo, Morte & Honrubia (Bordallo et al. 2013) reported for the first time from North Africa (Zitouni-Haouar et al. 2018) can be misidentified as *T. claveryi* but the large epispore reticulum and the pseudoparenchymatic peridium separates this species from the *T. claveryi* group; peridium thin (0.1 mm thick), smooth, light brownish yellow to brown mottled with black patches; asci eight spored; ascospores globose (15) 16–19 (20) µm broad including ornamentation with prominent irregular reticulum, the reticular wall is 0.5–1 µm thick.

Terfezia crassiverrucosa Zitouni-Haouar, G. Moreno, Manjón, Fortas, & Carlavilla, sp. nov. (Zitouni-Haouer et al., 2018) clearly differs from its closest relatives *Terfezia* species with warty-reticulate spores; peridium is smooth, light to dark brownish, 100–200 µm thick with emergent hyphae 6–10 µm broad at septa, pseudoparenchymatous, composed of subglobose, polygonal to irregularly oblong cells (9)12.5–38 × 15–60(62) µm, with cell walls 1–2 µm thick; asci (4) 6–8– spored, hyaline, often ellipsoid to ovoid or at times subglobose, 51–60 × 60–81 µm, non stalked; ascospores globose measuring 22 µm in diameter including ornamentation consisting in more or less broad flat truncate to round-tipped warts (0.5)1–1.5 × 2–4.5(6) µm, and relatively elongated rounded elements measuring 1–1.5 × 1.5–3 µm.; the prominent crowded ornamentation hides a fine reticulum on the spore surface formed between the warts.Two species of *Tirmania*, *Tirmania pinoyi* and *Tirmania nivea* differ from *Terfezia* species by their amyloid mature asci (turn green to blue in Melzer's reagent) and their smooth ascospores.

Tirmania pinoyi (Maire) Malençon (1973) (Fig. 4 c,d) ascomata (3.5-10 cm diameter), subglobose, irregular, sometimes pyriform, fresh weighting 15-580 g; peridium (0.4-0.6 mm thick) whitish to yellowish to light brown at maturity; gleba white to light brown, fleshy, spongy; asci (51-60×70-95 µm) octospored, subglobose, ellipsoid, thin-walled gives a positive reaction with Meltzer's reagent; ascospores (17-22 µm) globose, hyaline, their surface is smooth but inner side of wall finely roughened.

Tirmania *nivea* (Desf. : Fr.) Trappe (1971) (Fig. 5 c, d) ascomata (4–9 cm) in diameter may reach 10 –12 cm, subglobose, pear shaped or irregular shape, fresh weighing 10-250 g; peridium (0.5-1.3 mm thick) yellowish white to brownish orange at maturity; gleba white to yellowish, fleshy, spongy; asci (40-50 × 67.5-89 µm) octospored, ovoid, pyriform with short pedicel, thin walled gives a positive reaction with Meltzer "s reagent; Ascopores (13-15 × 16-19 µm) smooth, ellipsoid, hyaline.

Two lineages of *Picoa* (Zitouni-Haouar et al. 2015), the Lineage II of *Picoa* (= *P. juniperi* Vittad.*)* with verrucose spores is harvested near *H. lippii* in the same habitats as *T. claveryi* and rarely in Saharan area. The Lineage VI of *Picoa* (= *Picoa lefebvrei* (Pat.) = *Phaeangium lefebvrei* (Pat.) with smooth spores. Phylogenetic analyzes of the ITS rDNA sequences of several *Picoa* taxa originating from Algeria and from different countries located around the Mediterranean basin have revealed a high degree of genetic variability throughout the genus and a strong taxonomic affinity between the two species of *Picoa*.

Habitats

In Algeria, favorable ecosystems where desert truffles proliferate in good conditions are usually small beds of wadis and depressions where rainwater stagnated for some time**.** Desert truffles develop in small meadows called "SEHB" during sufficiently rainy years. They are also harvested in the valleys and sometimes high up on some hills such as for example sites 13 and 14 in High steppe Plains and 44 and 45 in Sahara areas (Table 1; Fig. 6).*T. arenaria* is locally called "terfes" or "terfas". This species often rare in Algeria is harvested near *Helianthemum guttatum* on acid sandy soil in forests ecosystems in subhumid coastal areas and in semi-arid areas to temperate winter and sometimes in medium arid areas to cold winter.on calcareous alkaline sandy soil (Table 1; Fig. 6). *T. boudieri* is locally known as "terfess lakhal" (black terfez), this species relatively common grows on calcareous alkaline sandy soil in semi-arid to temperate winter near *Helianthemum ledifolium* in forests ecosystems, in medium and inferior arid areas near *H. salicifolium, H. hirtum* and *H. lippii* and Saharan areas near *H. lippii* (Table 1; Fig. 6). *T. claveryi* Chatin is commonly called « terfess lahmar » (red terfez), this species is grows on calcareous alkaline sandy soil in medium arid areas near *H. hirtum* or *H. lippii* and rarely near *H. salicifolium,* in inferior arid areas near *H. hirtum* or *H. lippii* and in Saharan areas near *H. lippii. T. claveryi* grows in medium arid zones often mixed with *Tirmania* *pinoyi.* This species is rare in Saharan areas (Table 1; Fig. 6). *Terfezia eliocrocae* Bordallo, Morte & Honrubia (Bordallo et al. 2013) and *Terfezia crassiverrucosa* Zitouni-Haouar, G. Moreno, Manjón, Fortas, & Carlavilla, sp. nov. (Zitouni- Haouer et al. 2018).These species were harvested in the same producing sites of *T. claveryi*; they grow in sandy alkaline soil in medium arid areas in High steppe Plains near *H. hirtum* or *H. salicifolium. Terfezia eliocrocae* is rarely harvested in Saharan areas near *H. lippii* (Zitouni et al. 2018) (Table 1; Fig. 6). *Tirmania* sp. or "big white terfass" of Southern Algeria (Chatin 1892) is locally called "belhourech" or "terfess labyadh" and also as "bribri" when calcium carbonate crystals ($CaCO_3$) contained in the ascomata shine. Two *Tirmania* species, *Tirmania pinoyi* and *Tirmania nivea* are commonly widespread in Saharan areas and harvested near *H. lippii* on calcareous alkaline sandy soil. *T. pinoyi* is rarely mixed with *T. claveryi* in medium arid areas in High steppe Plains (Table 1; Fig. 6). *Picoa sp.* locally called "Jaouber"; two lineages of *Picoa* are harvested in Algeria (Zitouni-Haouer et al. 2015). The Lineage II of *Picoa* (= *P. juniperi)* with verrucose spores grows near *H. lippii* in the same habitats as *T. claveryi* and rarely in Saharan area (Table 1; Fig. 6). The Lineage VI of *Picoa* (= *Picoa lefebvrei* (Pat.) = *Phaeangium lefebvrei* (Pat.) with smooth spores is associated with *H. salicifolium* and *H. hirtum* in High steppe Plains. *Picao* species grow on sandy or sandy-limestone and alkaline soils, alone or in groups of several small ascomata, at a depth of 2 to 5 cm from the soil. Their harvest always precedes the growth of *Terfezia* and *Tirmania* ascomata and announces a good harvest season (Zitouni –Haouer et al. 2015).

Table 1 Informations on desert truffles species collected in Algeria

Desert truffle species collected	Producing site	Host plant	Bioclimatic zone
Terfezia arenaria (Moris) Trappe	1,2 3 13, 14, 31, 32	*Helianthemum guttatum* *H.guttatum* *H. guttatum*	Subhumid to temperate winter Semi-arid to temperate winter Medium arid to cold winter
Terfezia boudieri Chatin	30 4, 5 15 22, 23, 24, 25 7, 8, 9, 10, 11 26, 27, 28 33, 34 35, 36, 37, 38 57	*H. hirtum* *Helianthemum ledifolium* *Helianthemum* sp. *H. lippii*, *H. hirtum* *H.. hirtum, H. salicifolium* *H.. hirtum* *Helianthemum* sp. *H. lippii* *H. lippii*	Medium arid to cold winter Semi-arid to temperate winter Inferior arid Medium arid to cold winter Medium arid to cold winter Medium arid to cold winter Medium arid to cold winter Inferior arid Saharan or desert
Terfezia claveryi Chatin	6 7, 8, 9, 10, 11 12 15 16, 17,18 ,19 20, 21 22, 23, 24, 25, 35, 36, 37, 38 39, 40 50, 51, 53	*H. lippii* *H. hirtum,* *H.salicifolium* *H. hirtum* *Helianthemum* sp. *H. lippii , H. hirtum* *Helianthemum* sp. *H. lippii*	Medium arid to temperate winter Medium arid to cold winter Medium arid to cold winter Inferior arid Inferior arid Inferior arid Saharan Saharan

Terfezia eliocrocae Bordallo, Morte & Honrubia	49 10	*H. lippii* *H. hirtum, H. salicifolium*	Saharan Medium arid to cold winter
Terfezia crassiverrucosa Zitouni-Haouar, G. Moreno, Manjón, Fortas, & Carlavilla, sp. nov	31, 32	*H. hirtum, H. salicifolium*	Medium arid to cold winter
Tirmania pinoyi (Maire) Malençon	6 16, 17, 18 ,19, 20, 21, 22, 23, 24, 25 29 35, 36, 37, 38 8,7 41,42, 43, 44, 45, 46, 47,48, 52, 55, 56, 51, 53, 54, 57, 58, 59	*H. lippii* *H. lippii, H. hirtum* *H. lippii* *Helianthemum* sp. *H. hirtum* *H. lippii*	Medium arid to temperate winter Medium arid to cold winter Medium arid to cold winter Inferior arid Medium arid to cold winter Saharan
Tirmania nivea (Desf. ; Fr.) Trappe	39, 40, 42, 43, 44, 45, 46, 47,48, 52, 55, 56, 51, 53, 54, 57, 58, 59, 60, 61	*H. lippii*	Saharan
Picoa juniperi Vittad.	53	*H. lippii*	Saharan
Picoa lefebvrei (Pat.) **= *Phaeangium lefebvrei*** (Pat.)	8, 11 34 16, 17, 18, 19, 20, 21	*H. hirtum., H.salicifolium* *H.lippi* *H. lippii*	Medium arid to cold winter Inferior arid Medium arid to cold winter

Climatic parameters Development of desert truffles ascomata requires specific climatic conditions during their life cycle: favorable temperature, sufficient rainfall well distributed as well as thunderstorms. Climatic parameters of studied sites (rainfall, temperature and storms) collected each year during 30 years (1986–2016) in meteorological stations allow us to know favorable climatic conditions to ascomata development and their good growing seasons. *T. arenaria* grows in the northeastern coastal sites near *H. guttatum* in subhumid bioclimatic area, characterized by a mild and wet climate in winter, average of temperatures around 11-12 °C in January and around 25-26 °C in August. The rains occur between October and April. Ascomata development of this rare species requires a total amount rainfall between 350-400 mm well distributed from September to April. They are harvested in April-May generally in small quantities each year because these sites are more watered than all others productive sites of desert truffles. *T. arenaria* grows also near the same host plant in medium arid area in High Steppe Plains.

T. boudieri grows also in northwestern coastal sites near *H. ledifolium* characterized by semi-arid bioclimate with a mild winter and average annual temperature 18.3 °C. Ascomata live cycle and good growing seasons require a total amount of rainfall between 450-500 mm well distributed from September to April. Desert truffles species (*Terfezia, Tirmania, Picoa*) (Table 1) grow in medium arid areas in the High Steppe Plains characterized by low and irregular rainfall (200-400 mm per year); temperature often reaches less than 0 °C in winter and exceeds in summer 30 °C and even 40 °C. These desert truffles require for their ascomata development and their good havest a total amount rainfall of 175 to 250 mm (Fig. 7a) exceptionally 350-450 mm well distributed between September to March as was the case in 1990-1991, 2008-2009 (Fig. 7b). Rainfall in September would be useless especially in periods of drought, if they were not followed by rain showers in October or November. There is no ascomata production if precipitations have not reached this amount of rainfall. Temperature during ascomata development is 24-25 °C in September, 10-15 °C in November–December and 18-20 °C in March-April (Fig. 7c). In arid areas, havesting desert truffles take place in April-May. Some desert truffles species (Table 1) grow in inferior arid sites (pre-Saharan zone) characterized by the weakness of rainfall (100-150mm) and in hyperarid bioclimatic areas (Saharan) characterized by precipitations less than 100 mm per year, very irregular sometimes exceptional during the year and between years. The average seasonal temperature is 15 to 28 °C in winter and reaches 40-45 °C in summer and even 50 °C. Good growing seasons of desert truffles in pre-Saharan sites require precipitation 130-245 mm well distributed from September to October. While in hyperarid productive sites they require 90-100 mm exceptionally more 100mm (Fig. 8 a). An appreciable quantity of stormy rains must necessarily occur in mid-August during the mid-period of the **"**smaïms**"** for good development of ascomata. These rainfull would be useless after a period of drought, if they were not followed by rain showers in October or November (Fig. 8b), temperature is 15-20 °C (Fig. 8c). *Tirmania* species.are generally harvested from February to April. Exceptionally during very rainy season as for example 1993-1994, 2008-2009, 2014-2015, their harvesting takes place in January and lasts more than four months. Good productive seasons of desert truffles are generally characterized by stormy rainfall well distributed during ascomata life cycle. So we followed the number of storms during growing seasons of desert truffles from 2004 to 2012 in arid and hyper-arid zones. The data reveal their frequency from mid-August to October especially in hyperarid areas and from September to April in arid areas.

Characteristics of desert truffle soils

The results of physicochemical analyses of soil samples from sites in different bioclimatic zones are presented in Table 2 and the soil texture shown in Fig. 9. Soil analyses of sites producing desert truffles revealed heterogeneous soil characteristics between sites located in different bioclimatic zones. Indeed, ANOVA showed significant variation in values of most soil parameters between the investigated sites, fine and coarse silt ($p<0.05$), fine sand and Mg^{++} ($p<0.01$); total nitrogen ($p<0.001$); pH**,** total carbon, total carbon ratio and $CaCO_3$ ($p<0.0001$). No significant value was observed on clay coarse sand, organic matter, electrical conductivity, Ca^{++}, K^{+}, phosphorus. Tukey's post-hoc showed that these sites are different according to soil parameters that would explain the heterogeneous distribution of desert truffles in Algeria. The physical soil analysis revealed that all habitats of desert truffles have soils of sandy texture. Clay and silt are present in soils but sand is largely dominant. The contents of fine and coarse sand are between (50.98 ± 9.17–79.93 ± 8.68 %) and (20.26 ±7.96-31.16 ± 6.84 %) respectively. According to textural triangle (Schoeneberger et al. 2012), desert truffles grow in sandy loam soils. All habitats of desert truffles have non-saline soils because electrical conductivity values are low (0.23 ±0.02- 0.76 ±0.077 mS/cm**).** Overall, chemical characteristics of soil showed various areas seem to be suitable as desert truffle habitats. In subhumid coastal areas, the habitats of desert truffles were characterized by soils having acidic pH (pH= 6.18 ± 0.52), very low level of calcium carbonate (0.0023 ± 0.0024 %), high contents of organic matter (1.12 ± 0.79 %) and nitrogen (2.50 ± 1.42 %) and a low level of phosphorus (33.9 ± 30.20 ppm). In semi arid and medium arid areas, desert truffles habitats were generally characterized by soils having alkaline pH (7.87 ±0.53–8.8 ± 0.00), high contents of organic matter (1.42 ±2.27 – 3.37 ±000 %) and calcium carbonate (18.49 ± 6.67-38.4 ± 0.00 %), low level of phosphorus (25 ±0.033-111.2 ±101.69 ppm)**.** In inferior arid areas and Saharan (hyperarid) zones, the habitats of desert truffles were characterized by soils having alkaline pH (8.32 ± 0.53-8.41 ± 0.25), a moderate level of calcium carbonate (10.39 ±8.27– 12.34 ±7.60 %), low content of organic matter (0.06 ±0.03–0.80 ±1.16 %), low contents of nitrogen (0.19 ±0.13- 0.65 ±0.37 %) and phosphorus (38.75 ±33.33– 64.00 ±6.00 ppm) All desert truffles soils are relatively well supplied in magnesium and potassium.

Table 2 Means (± standard deviation) of physicochemical analyses of soils in desert truffles producing sites located in different bioclimatic zones

Soil Parameter	Subhumid	Semi-arid to temperate winter	Medium arid to temperate winter	Medium arid to cold winter	Inferior arid	Hyper-arid	One- Way Anova
Clay < 2µm (%)	ND	4.012 ±0.00	4.28 ±3.32	7.92 ±6.3	5.11 ± 08	6.54 ±1.59	0.725ns
Fine silt (%)	ND	19.03 ±0.00	4.98 ±1.64	13.40 ±6.46	7.98 ±4.65	13.52 ±8.00	3.046*
Coarse silt (%)	ND	12.07 ±0,00	ND	7.59 ±3.09	11.6 ±1.57	ND	4.87*
Fine sand (%)	ND	5.01 ±0.00	70.13 ±1.17	50.98 ±9.17	6.09 ±22.81	79.93 ±8.68	5.80**
Coarse sand (%)	ND	26.02 ±0.00	20.60 ±0.50	20.26 ±7.96	31.16 ±6.84	ND	2.39ns
pH	6.18 ±0.52a	8.8 ±0.00b	8.11 ±0.26b	7.87 ±0.53b	8.32 ±0.53b	8.41 ±0.25b	20.09****
Organic matter (%)	1.12 ±0.79a	3.37 ±0.00a	1.93 ±1.4a	1.42 ±2.27a	0.80 ±1.16a	0.06 ±0.03a	1.44ns
Total nitrogen (%)	2.50 ±1.42	ND	0.055 ±0.0325	0.218 ±0.21	0.23 ±0.17	0.38 ±0.125	8.588***
Total carbon (%)	ND	1.96 ±0.00	2.64 ±0.97	0.98 ±0.61	0.152 ±0.13	0.039 ±0.029	15.01****
C/N	ND	ND	17.78 ±1.42	9.69 ±0.44	ND	7.3 ±0.10	123.23****
Electrical conductivity (mS/cm)	0.23 ±0.02a	0.42 ±0.00ab	0.53 ±0.23ab	0.46 ±0.29ab	0.53 ±0.28ab	0.76 ±0.077bc	2.72ns
Total	0.0023±0.0024a	38.4 ±0.00b	ND	18.49 ±6.67ec	12.34 ±7.60ae	10.39 ±8.27ae	15.04****

$CaCO_3$ (%)							
Ca^{++} (ppm)	ND	140 ±0	ND	257.2 ±205.6	226.6±16	282.6 ±20.6	9.8 ns
Mg^{++}(ppm)	3.48 ±3.72	ND	ND	36.67 ±22.56	131.04 ±78.48	12.12 ±2.88	109.56**
K^{+} (ppm)	113.49 ±112.32	ND	ND	52.26 ±110.76	55.77 ±35.1	0.663 ±0.156	34.32 ns
Phosphorus (ppm)	33.9 ±30.20ac	111.2 ±101.6a	50.05 ±49,9ac	25 ±0.033ac	38.75 ±33.3bc	64.0 ±6,0ac	2.68ns

Means in the same column with different letters are significantly different from each other (p<0.05) according to the Tukey test. Significance levels: ns indicates no significance, * p<0.05, **p<0.01, *** p<0.001, ****p<0.0001; ND not determined

The symbiotic plants are the third main factor for the geographic distribution of desert truffles. In subhumid bioclimate and on acidic sandy loam soils *H. guttatum* (L. Foureau) associated with *Terfezia arenaria* form ectomycorrhiza without mantle fungal hyphae intrude between cortical cells to form the Hartig net, we did not observe any typical fungal mantle (Fig. 10 a,b). While on alkaline sandy loam soil, in semi-arid and in medium arid bioclimate, *H. guttatum* (L. Foureau) associated with *Terfezia arenaria* form typical endomycorrhizae without mantle (Fig. 11 a,b). In arid and hyper-arid bioclimate and on alkaline sandy loam soil *H. ledifolium* (L.) Mill., *H. salicifolium* (L.) Mill., *H. lippii* (L.) Dum., *H. hirtum* (L.) Mill. associated with *Terfezia*, *Tirmania* and *Picoa* species form typical endomycorrhizae without mantle (Fig. 11c,d). The morphology of intracellular hyphae is different in endomycorrhizae of *H. lippii* associated with *Tirmania pinoyi* and *Tirmania nivea* in Saharan sites (Fig. 11e,f). In the northern regions and sometimes in the Highland Steppe Plains *Terfezia* species are commonly associated with annual *Helianthemum* sp. while in arid and hyperarid areas *Tirmania* sp. are closely related to perennial *Helianthemum* sp. In these endomycorrhizas, the fungal hyphae completely invaded the cortical cells of *Helianthemum* sp. roots and formed coils or “pelotons” with some coralloid ramifications. The central cylinder is not colonized by the fungal hyphae.

Host plants

Helianthemum sp. is single host plant of desert truffles in Algeria. Our multi field investigations revealed that *Terfezia*, *Tirmania* and *Picoa* species are not associated with other genera of Cistaceae in high density near the lodging of desert truffles such as *Cistus salvifolius, Cistus heterophyllus and Cistus sericeus* accompanied annual *Helianthemum* or other families of plants accompanied *H. hirtum* and *H. lippii* as Plantaginaceae (*Plantago amplexicaule*, *Plantago albicans*, *Plantago ovata, Plantago ciliata, Plantago psyllium*), Asteraceae (*Artemisia herba-alba, Atractylis serratuloïdes, Atractylis humilus, Atractylis corduus, Atractylis cancellata, Rhantherium suaveolens*), Geraniaceae (*Erodium hirtum, Erodium bipinnatum, Erodium guttatum, Erodium triangulare*), Poaceae (*Stipa tenacissima*, *Cynodon dactylon*) and Chenopodiaceae (*Atriplex halimus, Arthrophytum scoparium, Anabasis* *aretioîdes*) (Chafi and Fortas 1999; Chafi et al. 2004). All these different natural plants accompanied *Helianthemum* sp. form arbuscular mycorrhizas (Fig. 12).

Discussion

Field studies have enabled us to map 61 desert truffles productiong sites and locate the distribution of nine species belonging to genera *Terfezia* (*T. arenaria, T. boudieri, T. claveryi, Terfezia eliocrocae, Terfezia crassiverrucosa*), *Tirmania* (*T. pinoyi, T. nivea*) and and two lineages *Picoa* (Lineage II = *P. juniperi* and Lineage VI = *Picoa lefebvrei*) and their host plants *Helianthemum* spp. *Terfezia arenaria* and *T. boudieri* associated with annual *Helianthemum* spp. are distributed in coastal suhumid, coastal semi-arid and medium arid sites whereas *Terfezia claveryi*, *Tirmania pinoyi, T. nivea* and two lineages *Picoa* rather xerothermophilous are encountered in arid and hyper-arid bioclimate rarely near annual *Helianthemum* spp. and commontly near perennial *Helianthemum* spp. (*H. hirtum and H. lippii.*). *Tirmania pinoyi* and *T. nivea* are especially predominant in hyper-arid areas associated only with *H. lippii.* Consequently, desert truffles distribution belonging to genera *Terfezia* and *Tirmania* seems to be linked to bioclimate, soil and host plants. As for two lineages II and VI of *Picoa*, the study of biogeographical and phylogenetic relationships between different lineages of *Picoa* (Zitouni-Haouer et al. 2015) revealed that morphological criteria cannot be reliable used to discriminate between these two lineages since the most frequent lineage in Algeria displayed either smooth or warty spores. The authors suggested that ecological characteristics and putative host plants of the genus *Helianthemum* instead of morphological criteria would be the most useful characters to separate these lineages of *Picoa*. Similar distribution of desert truffles has been reported by many authors in Europe countries (Honrubia et al. 1992; Moreno et al. 1986, 2000; Kovacs et al. 2011; Bordallo et al. 2013; Chevalier 2014; Bordallo and Rodriguez 2014, Bordallo et al. 2015, 2018), in Northern African countries (Fortas and Chevalier 1992; Khabar et al. 2001; Slama et al. 2006; Sbissi et al. 2011; Bradai et al. 2014, 2015, Berkami et al. 2017; Bouzadi et al. 2017; Zitouni-Haouar et al. 2015, 2018) and Middle East and Arabian Peninsula countries (Alsheikh and Trappe 1983; Bokhary 1987; Pacioni and El-Kholy 1994; Mandeel and Laith 2007; Ammarellou and Sarem 2008; Kagan-Zur and Roth-Bejerano 2008; Barseghyan and Wasser 2010; Al-Thani 2010; Jamali and Banihashemi 2012; Al-Qarawi and Mridha 2012; Akyuz et al. 2015; Zamborelli et al. 2014; Türkoğlu and Castellano 2014, Kagan-Zur and Akyuz 2014; Owaid 2016, 2018; Bawadekji et al. 2016; Hashem et al. 2018). However, although few species of desert truffles grow in southern European countries, most of these hypogeous mushrooms are xerothermophilous and widespread in arid and hyper-arid ecosystems in Northern Africa, Middle East and Arabian Peninsula. Northern Africa countries are biotopes very favorable to their proliferation (Chatin 1891a, b) and their production are most abundant in arid and Saharan areas. Good natural production of desert truffles is closely linked to distribution and quantity of rainfall, especially in autumn, as well as the temperature during ascomata life cycle. In fact, ascomata growth requires well distributed rainfall from September to April with maximum from October to November. Although the amount rainfall varies considerably from one year to the next, total amount rainfall during good growing season is from 350 to 500 mm in coastal sites with subhumid or semi-arid bioclimates; it is 175 to 250 mm exceptionally 350 to 450 mm in High Steppe Plains and 90 to 100 mm exceptionally more than 100 mm in the Saharan sites. Low and erratic rainfull during autumn period affects biological cycle of the fungus and therefore ascomata production. Temperature during growing season is from 15 to 22 °C with an average 11-15 °C in autumn (October-November), 10-12 °C in winter (December - February) and around 20-22 °C in spring (March-April). Autumn rainfall would induce the beginning of biological fungus cycle while low intensity spring precipitation are essential for maturation of immature ascomata which abundantly absorbing water through their mycelium, rapidly increase in size causing after a dry period a slight bulging and cracking of soil surface near of their host plants indicating the presence of ascomata.Some authors reported that a good growing season and good desert truffles production require an annual rainfall which ranges of 70 to 120 mm in North African countries and 100 to 350 mm in southern Europe countries (Morte et al. 2010). Sufficient spring precipitation could complement autumn rainfall and partially correct production if the amount of previous precipitation was insufficient (Morte al. 2008). However, all rainfall data in literature reported that desert truffles development in arid, semi-arid or hyper-arid regions occurs after rainfall heralding the end of winter and beginning of spring, with a specific temporal distribution of precipitation amounts along the year. During growing season, rainfall distribution significantly affect desert truffles life cycle and therefore their yields (Alsheikh and Trappe 1983; Bokhary 1987; Chafi et al. 2004; Mandeel and Al-Laith 2007; Kagan-Zur and Roth-Bejerano 2008; Morte et al. 2010; Bradai et al. 2014, 2015). In Algeria, thunderstorms are beneficial for ascomata growth but their true effect is unknown. According to testimonies of nomadic populations and desert truffle pickers, ascomata development is influenced by thunderstorms number particularly thunderbolts. In fact, stormy rains in autumn and spring announce a good havest season of these hypogeous mushrooms. In other countries, truffle gatherers and Bedouins associate size and abundant production of desert truffles with thunderstorms and winter lightning that hit the ground, followed by strong "thunderclaps" (Kagan-Zur 2001; Feeney 2002; Mandeel and Al-Laith 2007; Shavit 2008; Kagan-Zur and Roth-Bejerano 2008; Al Thani 2010). According to some truffles havesters spring thunderstorms and spring temperatures were important for 9.1 % and 25 % (Akyüz et al. 2017a). Pradel (1914) suggested that the electric discharges accompanying thunderstorms have a role in fructiferous initiation, inducing the passage from vegetative to reproductive phase. In Algeria, desert truffles thrive especially on sandy loam soils but each soil type has some difference in chemical characteristics. Similar soil texture observed in different desert truffles sites is also required by *Tuber* species as *Tuber aestivum* in Eastern France and in Gotland, Sweden (Weden et al. 2004). *Terfezia arenaria* is ecologically distinctive species adapted to acid soil and calcareous soil. In fact, this species grows near *H. guttatum* in subhumid bioclimate on acidic sandy loam soil with high level of organic matter and near the same plant host in arid bioclimate (semi-arid and medium arid) on calcareous sandy loam soil with moderate level organic matter. This species is harvested on calcareous soil (Fortas et Chevalier 1992), on acidic soils in northeastern Algerian coastal dunes (Dafri and Beddiar 2017a), in Morocco (Khabar et al. 1994), in Sardinia-Italy (Janex-Favre et al. 1988) and Spain (Diez et al. 2002, Morte et al. 2009). *Terfezia leptoderma* is also collected on acid soil in Marocco (Mamora forest) under *Helianthemum guttatum* and under pine (*Pinus pinaster var. atlantica* (Khabar 2014) and on calcareous soil in Southern France (Riousset et al. 2004). In arid and Saharan sites, most desert truffles grow on sandy loam soil, moderately calcareous, slightly alkaline with relatively low organic matter, high in magnesium, well supplied with potassium and low in phosphorus. These soils are mainly soils of wadi beds and spreading zones, where only alluvial deposits are brought by rainwater. Soil particle size is characterized by an alternation of fine and coarse deposits where sandy horizons based on fine sand, remain predominant; the clay horizons being thin (Dutil, 1971). Low level in organic matter is explained by the favorable conditions for rapid mineralization of organic matter under effect of high temperatures in these regions (Pouget 1980).Similar pedological characteristics of desert truffle soils have been r eported in studies in Europe (Moreno et al. 1986; Bratek et al. 1996; Riousset et al. 2004; Kovács et al. 2007; Morte et al. 2009; Bonifacio and Morte 2014), in Northern Africa (Fortas et Chevalier 1992; Slama et al. 2010; Bradai et al. 2014, 2015; Khabar 2014, Bermaki et al. 2017; Bouzadi et al. 2017), in Middle East and Arabian Peninsula (Awameh and Alsheikh 1979; Pacioni and El-Kholy 1994; Hussain and Al-Ruqaie 1999; Jamali and Banihashemi 2012; Akyüz et al. 2017b). In Algeria, *Terfezia, Tirmania* and *Picoa* species are closely attached and loyal to single host plant *Helianthemum* spp. in natural conditions. According to Molina et al. (1992), this case of natural narrow specificity concerns at the same time certain mycorrhizal fungi species and some plants likely to develop an exacerbated particularism. The host specialization and soil pH can play an important role in the distribution pattern of desert truffle species (Diez et al. 2002). Under natural conditions, *T. arenaria* has great plasticity to form with *H. guttatum* ectomycorrhizae without mantle on acidic sandy soils and endomycorrhizae without mantle on calcareous sandy soils with low level of assimilable phosphate. This case is similar to those association *H. sessiliflorum (=Helianthemum lippii - Terfezia boudieri* which produces endomycorrhizas in the Northern Sahara in Tunisia (Slama et al. 2010) whereas the same partners form ectomycorrhizas in Israel (Roth-Bejerano et al. 1990, Zaretsky et al. 2006). Although these two regions are arid, there are probably some differences between the two habitats that influenced mycorrhizae architecture (Roth-Bejerano et al. 2014). Fortas and Chevalier (1992) obtained in *H. guttatum* plants associated with *Terfezia arenaria*, *Terfezia claveryi* and *Tirmania pinoyi* ectomycorrhizae without mantle under greenhouse conditions in natural substrate with high phosphate concentrations. Others rechearchers are observed in *H. guttatum* naturally associated with *T. arenaria,* ectomycorrhiza with Hartig net and external hyphae not organized in real sheath, on acid sandy loam soil; these same partners produce ectomycorrhizae with sheath/mantle under greenhouse conditions (Dafri and Beddiar 2017b) on alkaline sandy soil containing Olsen P= 0 mg.Kg−1 (Bonifacio and Morte 2014). In Algeria, all annual and perennial *Helianthemum* spp. associated naturally with nine desert truffles species (*Terfezia, Tirmania, Picoa)* on calcareous sandy loam soil form unique type of endomycorrhizae without mantle. The same endomycorrhizal morphology is observed in *Helianthemum ledifolium* roots and those of two perennial Cistaceae (*Helianthemum lippii, Fumana procumbens*) inoculated with *Terfezia leptoderma, T. boudieri, T. claveryi* under greenhouse conditions, on soil originating from desert truffle natural habitat in Algeria (Zitouni-Haouer et al. 2014). These endomycorrhizae are morphologically similar to those *H. sessiliflorum* colonized naturally by desert truffles and under greenhouse conditions on soil originating from desert truffle natural habitat in southern Tunisia (Slama et al. 2010). In the literature, some authors recorded in Northern Africa other plants typical of the steppe regions associated with *Terfezia* and *Tirmania* spp. as *Plantago albicans* and *Artemisia herba alba* Asso.(Duggar and Pinoy 1907; Maire and Werner 1937; Malençon 1973), *Atractylis serratuloïdes* Sieb. ex Cass. and Endl. *(*Duggar and Pinoy 1906-1907, *Erodium* sp. (Maire 1907; Maire and Werner 1937). However, no desert truffle was collected under these plants accompanying commonly *Helianthemum* spp. These plants form all arbuscular mycorrhizae (Fig. 12). Our field surveys have also been confirmed by many nomads and seasonal harvesters of desert truffles. Under natural conditions, development and establishment of mycorrhiza in annual and perennial *Helianthemum* spp. seems to be influenced by some abiotic factors such as bioclimate and physicochemical features of soil in which desert truffles species are growing. Fungus efficiency to produce the endo- or ectomycorrhizal association is determined by soil pH (acid or alkaline) and bioclimate. In fact, *H. guttatum -T. arenaria* couple forms ectomycorrhizae on acidic sandy soil in sites with subhumid bioclimate whereas all others desert truffles species (*Terfezia, Tirmania*, *Picoa*) grow near annual and perennial *Helianthemum* (*H. guttatum*, *H. ledifolium*, *H. salicifolium, H. hirtum, H. lippii)* form endomycorrhiza on calcareous sandy soils in sites with semi-arid , arid and hyper-arid bioclimate. Fungal species and *Helianthemum* species do not influenced mycorrhizal morphology. However, morphology and physiology of these host plants are adapted to harsh climatic conditions especially in arid and hyper-arid areas (Ozenda 2004) and their endomycorrhizal association with these hypogeous mushrooms allows them to resist in hostile ecosystems. In fact, Malençon (1973) reported that harsh habitats of *Terfezia* and other hypogeous fungi in Northern Africa required adaptations to survive adverse weather conditions. High temperatures and drought for many consecutive months significantly limit the growth and survival of mycelia in the soil. Desert truffles overcome these environmental challenges by forming mycorrhizae with annual and perennial Cistaceae species.

Microbial-mycorrhizal interaction in the field between desert truffles and microflore are poor studied. Rougieux (1963) reported the interaction between *T. boudieri* and *Azotobacter* chroococcum and the microflora near *T. boudieri* sporocarps was much higher than in soil 50 cm. *Terfezia boudieri* Chatin activity against associated microflora reported by Dib and Fortas (2014) revealed also that *T. boudieri* stimulate soil microflora in contact with their ascomata. Number of microorganisms (bacteria, fungi, actinomycetes) is higher in contact with *T. boudieri* ascoma than at 10 cm deep. Microorganisms are represented by free nitrogen fixing bacteria (*Azotobacter*) ($2.2\times10^1 \pm0.8\times10^1$ MPN/g), ammonifying bacteria ($4\times10^3 \pm1.0\times10^1$ MPN/g), nitric bacteria (between $6.8\times10^3 \pm1.0\times10^3$ and $0.6\times10^1 \pm1.0\times10^1$ MPN/g), and nitrous bacteria (between $4\times10^1 \pm1.0\times10^1$ and $0.3\times10^1 \pm1.0 \times10^1$ MPN/g). However, the relationships between environmental factors and mycorrhizal morphology are complex; they seem to depend on the bioclimate, the pH and chemical characteristics of the soil. Some researchers attributed these differences in mycorrhizal architecture to levels of phosphorus (Fortas and Chevalier 1992), iron or nitrate (Kagan-Zur et al. 2008), auxin–phosphate interaction in root development (Zaretsky et al. 2006), culture hyphae under drought stress (Navarro-Ródenas et al. 2013), microclimatic culture conditions (Gutiérrez et al. 2003). Whereas, other authors attributed these changes of mycorrhizal types to biotic factors such as the fungus species (Chevalier et al. 1984; Dexheimer et al. 1985) or the common mycorrhizal type of host plant species involved in mycorrhizal association (Kovács et al. 2003).

Declarations

Ethics approval and Consent for publication

Authors declare Compliance with ethical standards and approve the publication.

Conflict of interest

Authors declare no conflict of interest.

Availability of data and materials (data transparency)

The work was carried out in the Laboratory LBMB : Laboratoire de Biologie des microorganismes et de Biotechnologie, at University Oran 1. Field trips were made mainly by Prof. Z. Fortas, and some outings in the presence of Prof. S. Dib-Bellahouel.

Funding

Funding of field trips and scientific equipment and chemicals were assumed by the University of Oran from 1986 to 1999 and from 2000 to 2016. Funding was carried out with the budget allocated every 3 years to LBMB, approved by the Ministry of higher education and research. The physico-chemical analyzes were carried out free of charge by the Soil Analysis Laboratories indicated in the text, the meteorological data provided to us free of charge by the national meteorological stations indicated in the text.

Author’s contributions

Fortas Z conceived the study; Fortas Z and Dib- Bellahouel S provided materials; Fortas Z and Dib- Bellahouel S carried out the experiments, Fortas Z , Dib- Bellahouel and Chevalier G. analysed the data; Fortas Z wrote the manuscript, with contributions from the other authors.

Acknowledgements

We thank M. Boumediene Boutarfa , Department of Geology , Faculty of Earth Sciences , Oran 2 University , Algeria for his help in illustrations of distribution of desert truffles and of triangle texture soil.

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Figures

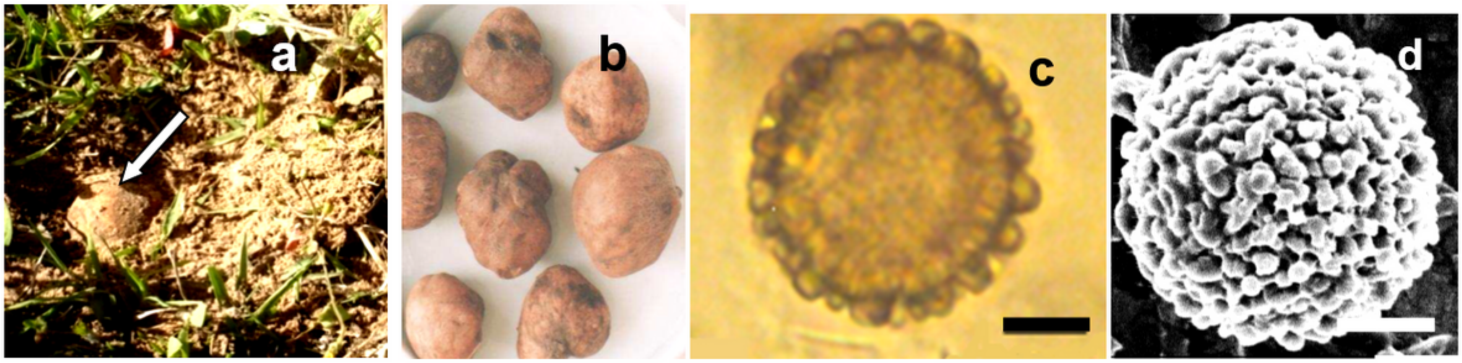

Figure 1

Terfezia boudieri in the field (site 4, Stidia forest). a ascoma near H. ledifolium in the field with covering soil removed by hand ; b ascomata; c ascospore ; d ascospore in scanning electron micrograph. Scale bars 10 μm in (c) and (d)

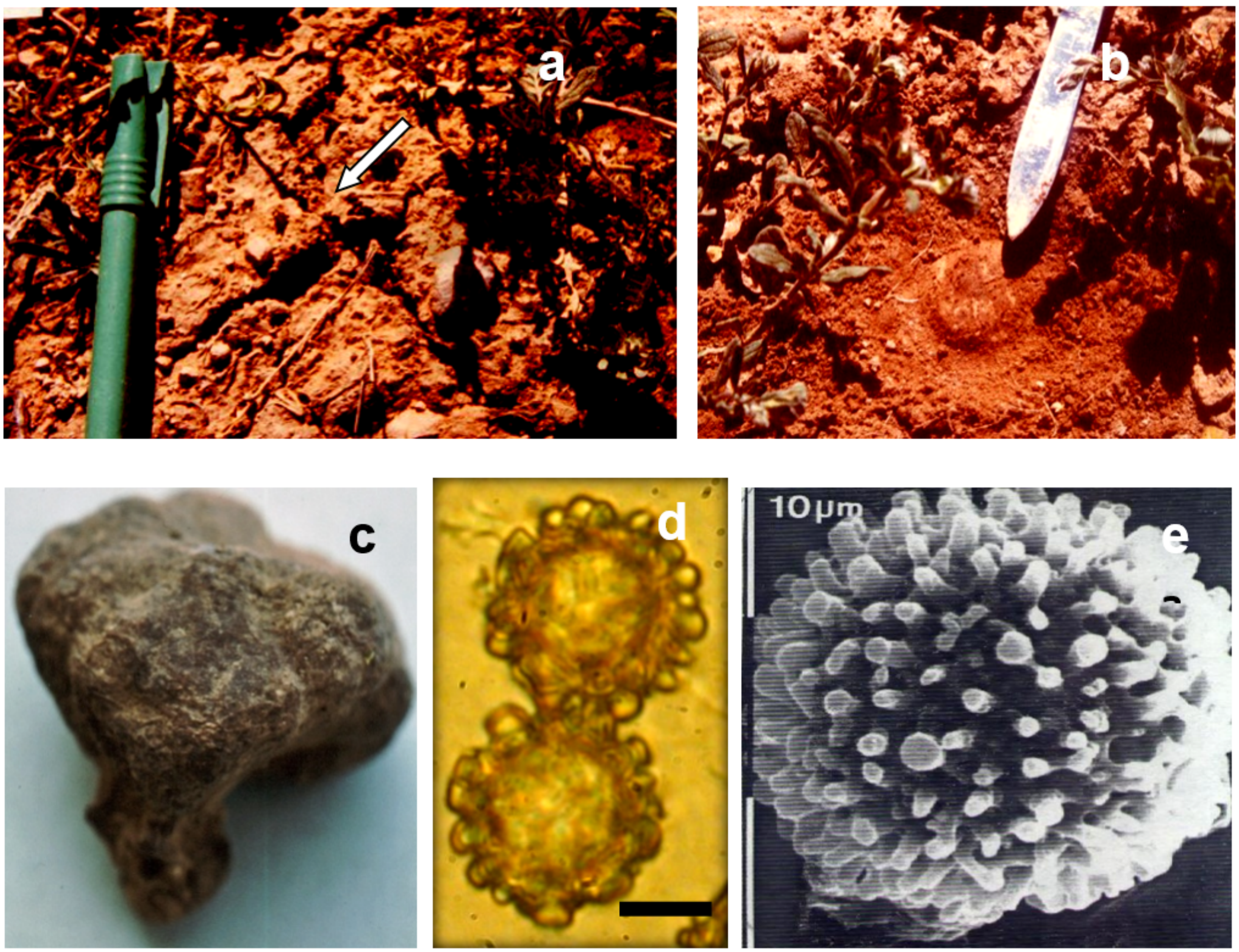

Figure 2

Terfezia arenaria in the field (site 13, Djebel Ben Kaddour). a Cracks on surface of the soil mound caused by increasing fruiting of T. arenaria near H. guttatum (arrow) ; b ascoma in the field with covering top soil removed by hand; c ascoma ; d globose ascospores showing ornamentation ; e Ascospore in scanning electron microscopy. Scale bars 10 µm in (c) and (d)

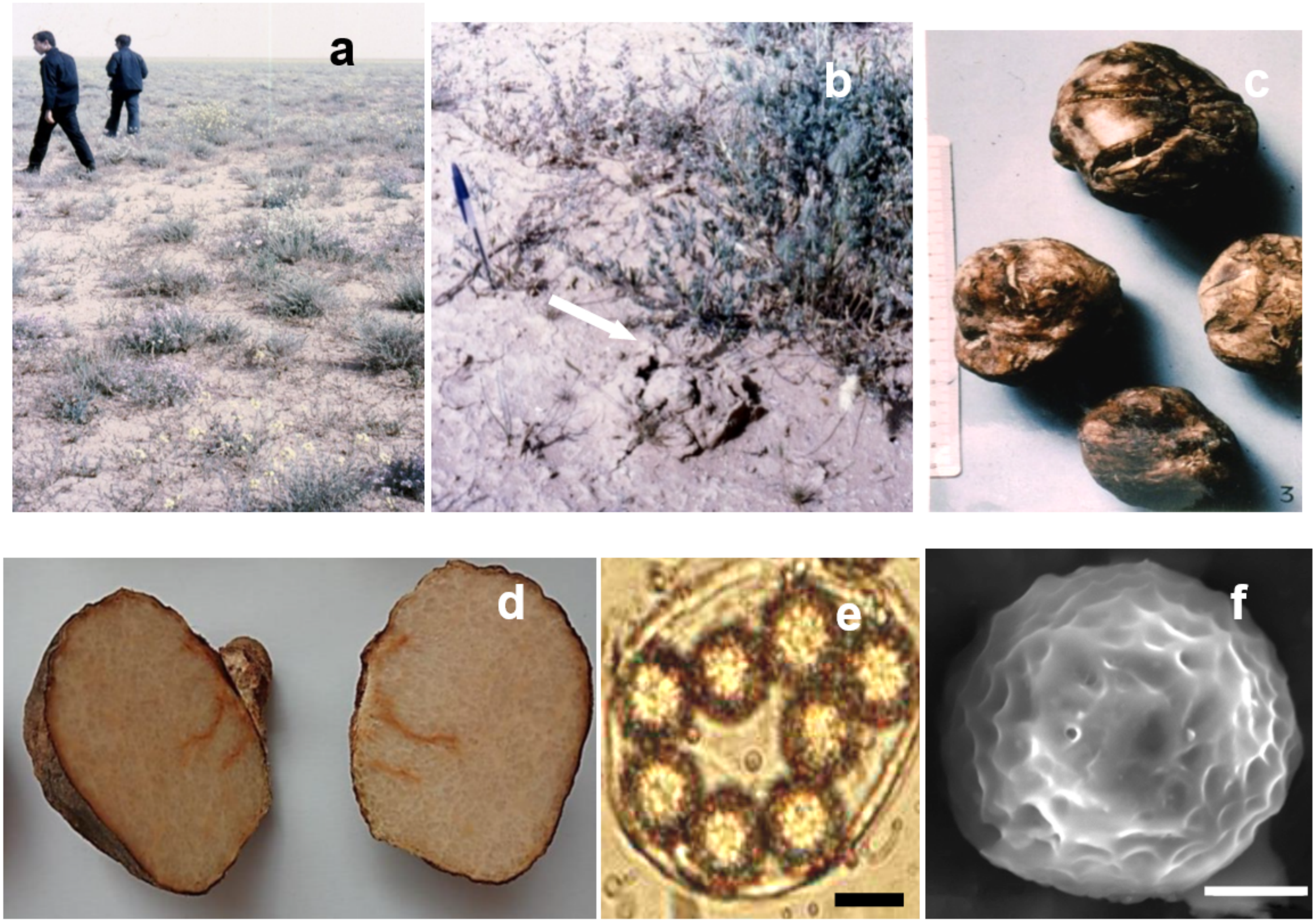

Figure 3

Terfezia claveryi in the field (site 18, Mecheria). a Terfezia claveryi collection site showing dominance of Helianthemum lippii ; b ascoma emerging from surface of the soil mound near H. lippii (arrow) ; c ascomata ; d section of ascoma; e ascus and ascospores ; f globose spore ornamented with very tight irregular reticulum in scanning electron microscopy. Scale bars 20 µm in (e), 5 µm in (f)

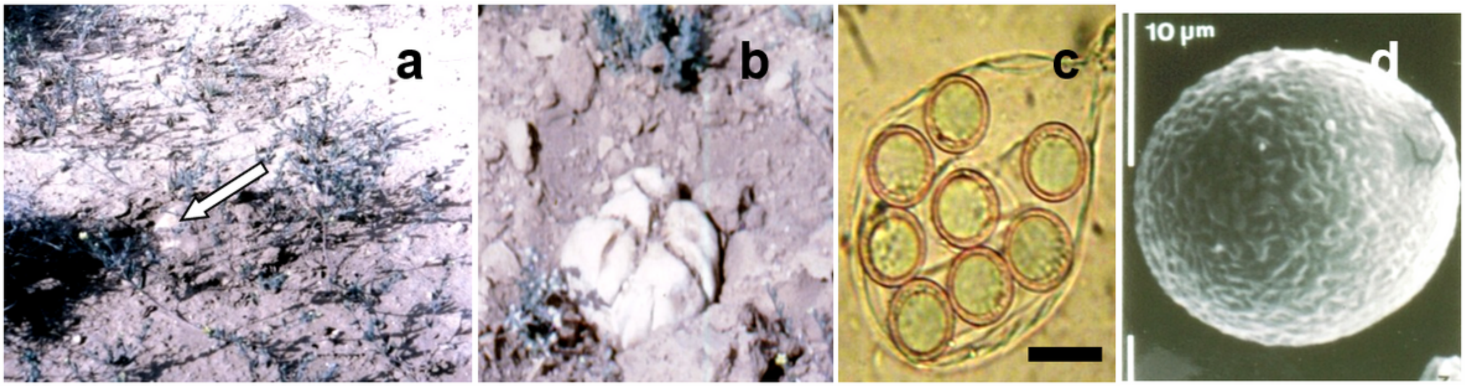

Figure 4

Tirmania pinoyi in the field (site 2 , Forthassa). a ascoma emerging from surface of the soil mound near H. lippii (arrow) ; b ascoma in the field with covering top soil removed by hand ; c ascus and ascospores ; d globose ascospores in scanning electron microscopy showing their smooth surface and inner side of wall finely. Scale bars 20 µm in (c), 10 µm in (d)

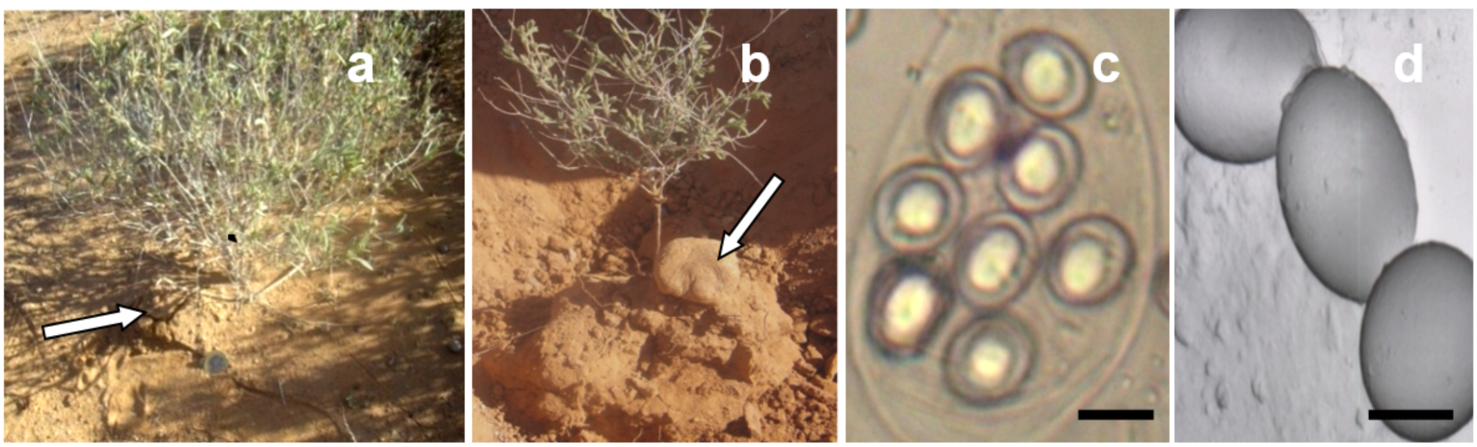

Figure 5

Tirmania nivea in the field (site 53, Hassi Manda) a Uprising and soil cracks caused by increasing fruiting of T. nivea near H. lippii (arrow); b ascoma in the field with covering top soil removed by hand ; c ascus and ascospores ; d ellipsoid smooth ascospores in scanning electron microscopy. Scale bars 20 µm in (c) and 10 µm in (d)

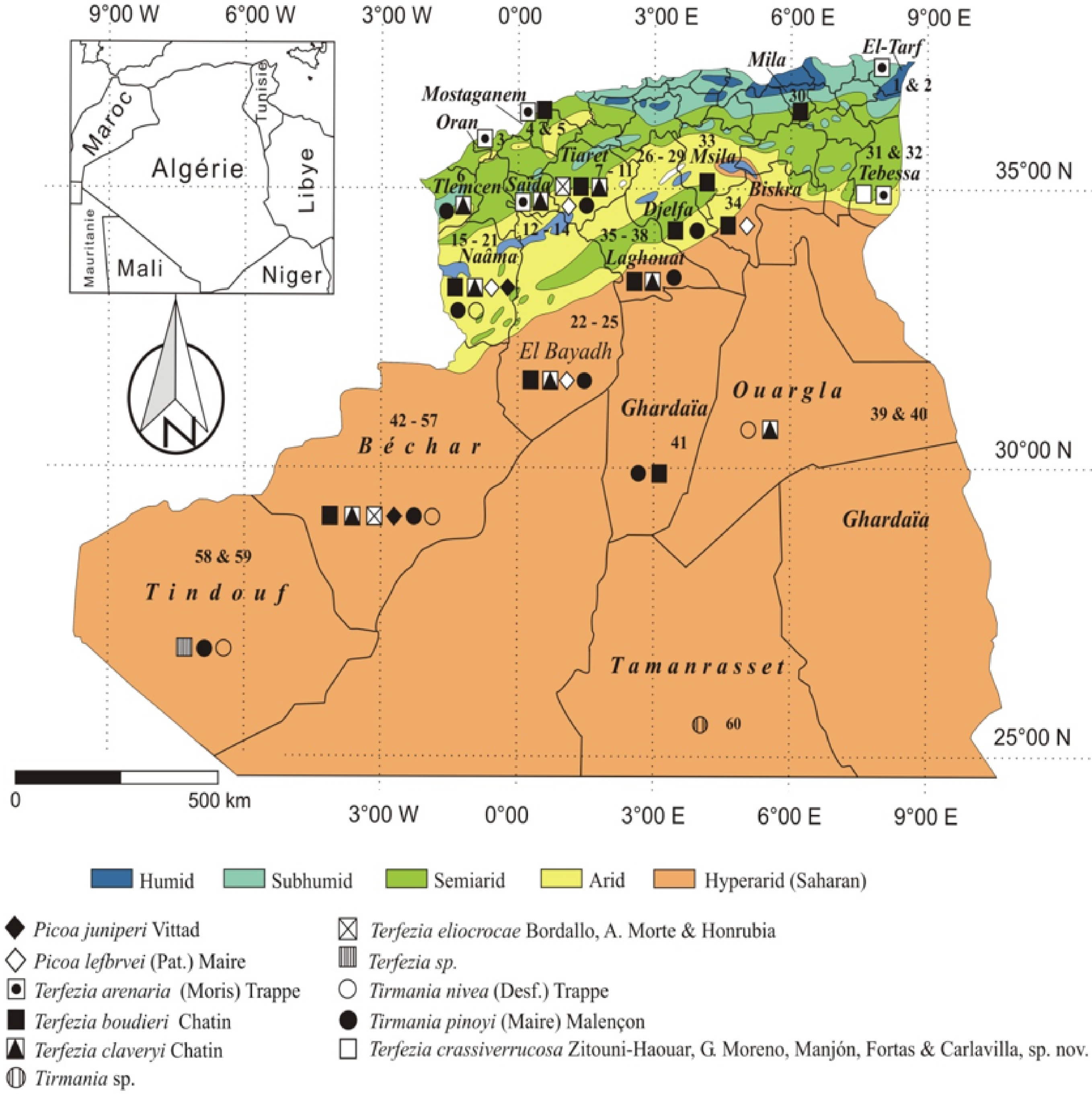

Figure 6

Distribution of desert truffles species in different bioclimatic areas in Algeria (illustration by Boumediene Bouterfa, University of Oran 2, Department of Geography) 1 Mridima; 2 Park National d'El Kala; 3 Forêt de Madegh; 4 Forêt de Stidia; 5 Kharouba; 6 El-Aricha; 7 Faraa; 8 Benhamed; 9 Sidi Bou Zebboudj; 10 Bouchouat; 11 Sidi Bouddoudj; 12 Aïn Sekhouna; 13 Djebel Ben Kaddour; 14 Zeriguet; 15 Tiout; 16 Mekalis; 17 Oulalak; 18 Mecheria; 19 Mekman Ben Amar; 20 Ain Ben Khellil; 21 Forthassa; 22 Bougtoub; 23 Mosbah; 24 Bordj El May; 25 Kef El Ahmar; 26 Moudjbara; 27 Charef (« gotaya »); 28 Hassi Bahbah; 29 El Abiodh Sidi cheikh; 30 Sidi Khalifa; 31 Hammamet; 32 Cheria; 33 Ain Oughrab (Djebbel Messaad); 34 Col de Sfa; 35 Aflou; 36 Brida; 37 Hadj El Mecheri; 38 El Beidha; 39 Oued M'ya; 40 Oued Righ; 41 El –Goléa; 42 Lahmar; 43 Benzireg; 44 Ouriah (Mougheul); 45 Oumchgag (Mougheul); 46 Oued Namous; 47 Oum Lardjem (Beni Ounif); 48 Garet sid elhadj (Beni Ounif); 49 Beni Abbès; 50 Igli; 51 Talhat Lemhara

(Tabalbala,); 52 Beni Ounif; 53 Hassi Manda (Tabalbala); 54 El Taoueze (Tabalbala); 55 Martouma (Taghit); 56 Mtilih (Taghit); 57 Oued Daoura (Tabelbala); 58 Région Lakhal; 59 Oum El Assal; 60 Oued Terroumout (Hoggar), 61 Oued Issemenan (Hoggar).

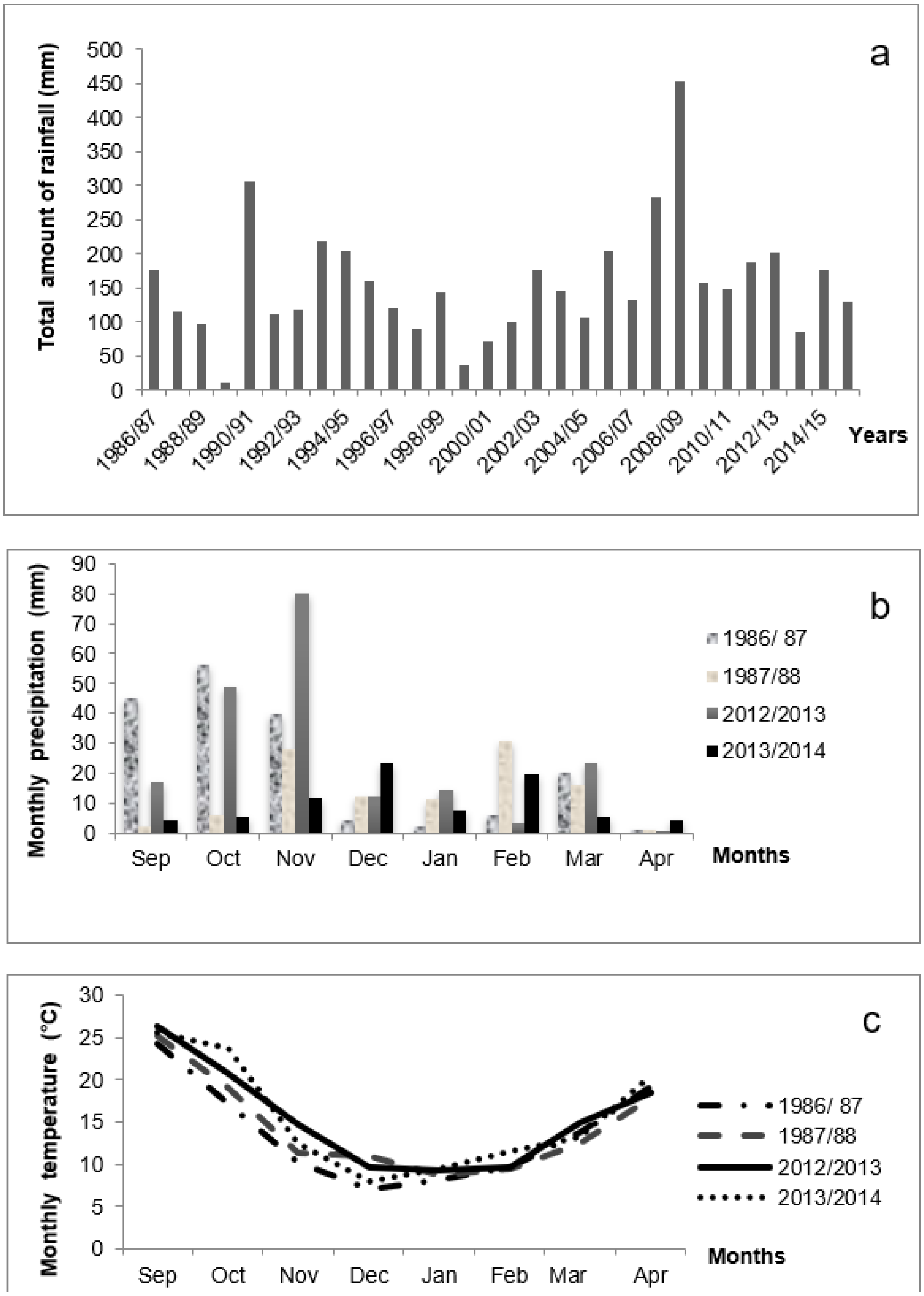

Figure 7

Variations of rainfall and of temperature during growing seasons of ascomata in a producing desert truffle site in the High Steppe Plains (arid areas). a Variation of total amount of rain from 1986-1987 to 2014-2015; b montly variations in rainfall and c temperature showing two good growing seasons 1986-1987 and 2012-2013

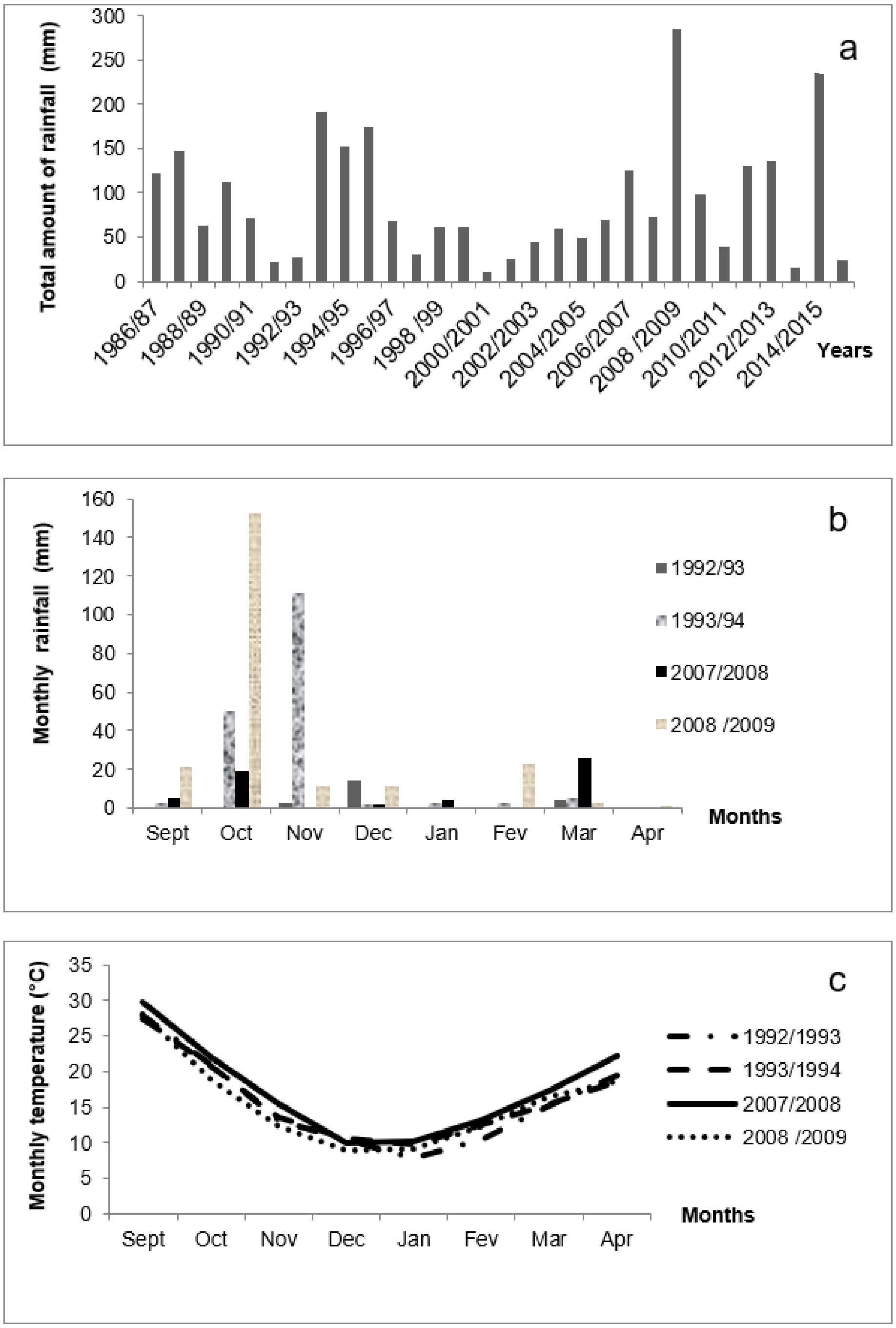

Figure 8

Variations of rainfall and of temperature during growing seasons of ascomata in a producing desert truffles site in Saharan areas. a Variation of total amount of rain from 1986-1987 to 2014-2015; b montly variation in rainfall and c temperature showing two good growing seasons 1993-1994 and 2008-2009

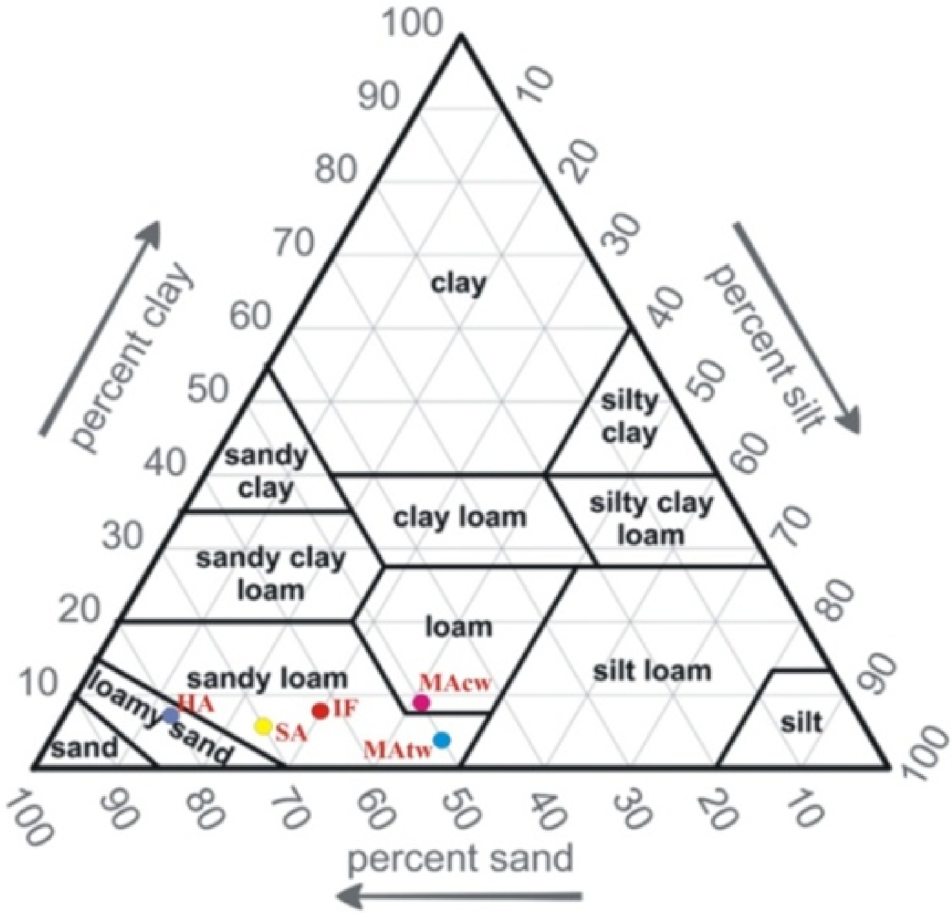

Figure 9

Diagram of soil texture in desert truffles producing sites located in different bioclimatic zones. SA Semi- arid ; MAtw Medium Arid to temperate winter ; MAcw Medium arid to cold winter; IF Inferior arid; HA Hyperarid or Saharan.

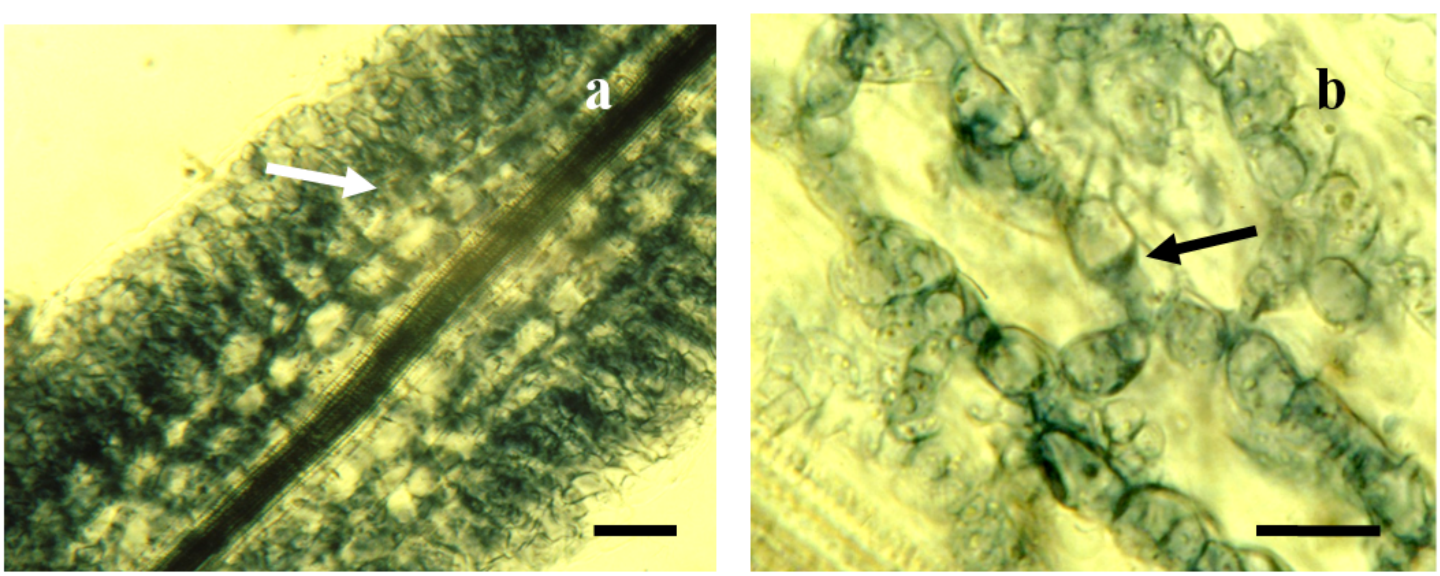

Figure 10

Helianthemum guttatum naturally ectomycorrhized with Terfezia arenaria on acidic sandy soil in northeastern sites (subhumid bioclimate). a root fragment showing intercellular hyphae forming Hartig net without mantle (arrow) ; b segmented intercellular hyphae. Scale bars = 50 µm in a; 15 µm in b

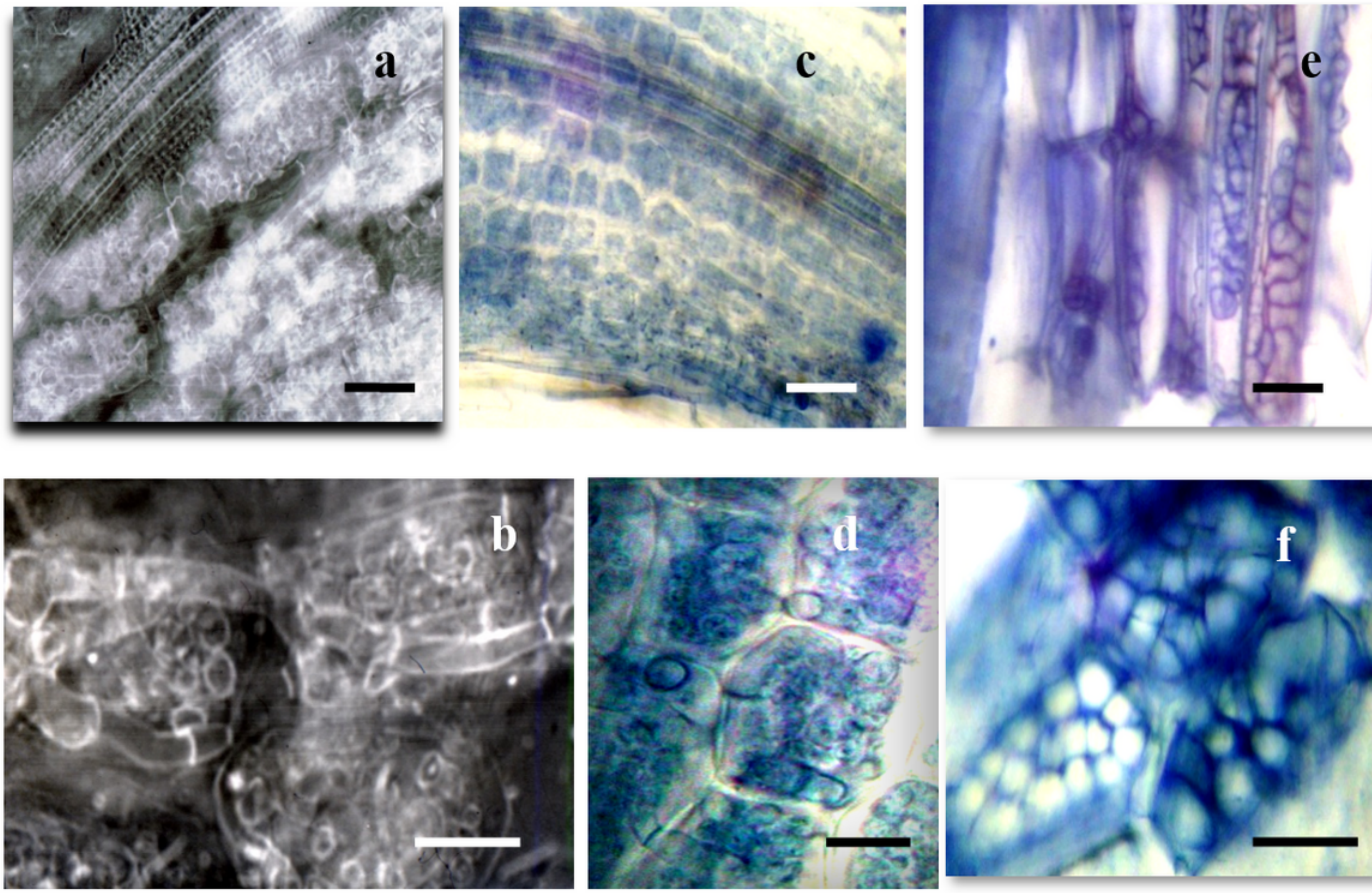

Figure 11

Root fragments of Helianthemum spp. naturally mycorrhized showing cortical cells fully colonized by intracellular hyphae of desert truffles forming typical endomycorrhizae on alkaline sandy soil. a, b association Helianthemum guttatum - Terfezia arenaria in semi- arid area ; c, d association H. hirtum - Terfezia boudieri in arid areas; e, f association H. lippii - Tirmania pinoyi in hyper-arid area . Scale bars = 20 µm in a, e; 15 µm in b, d, f ; 50 µm in c

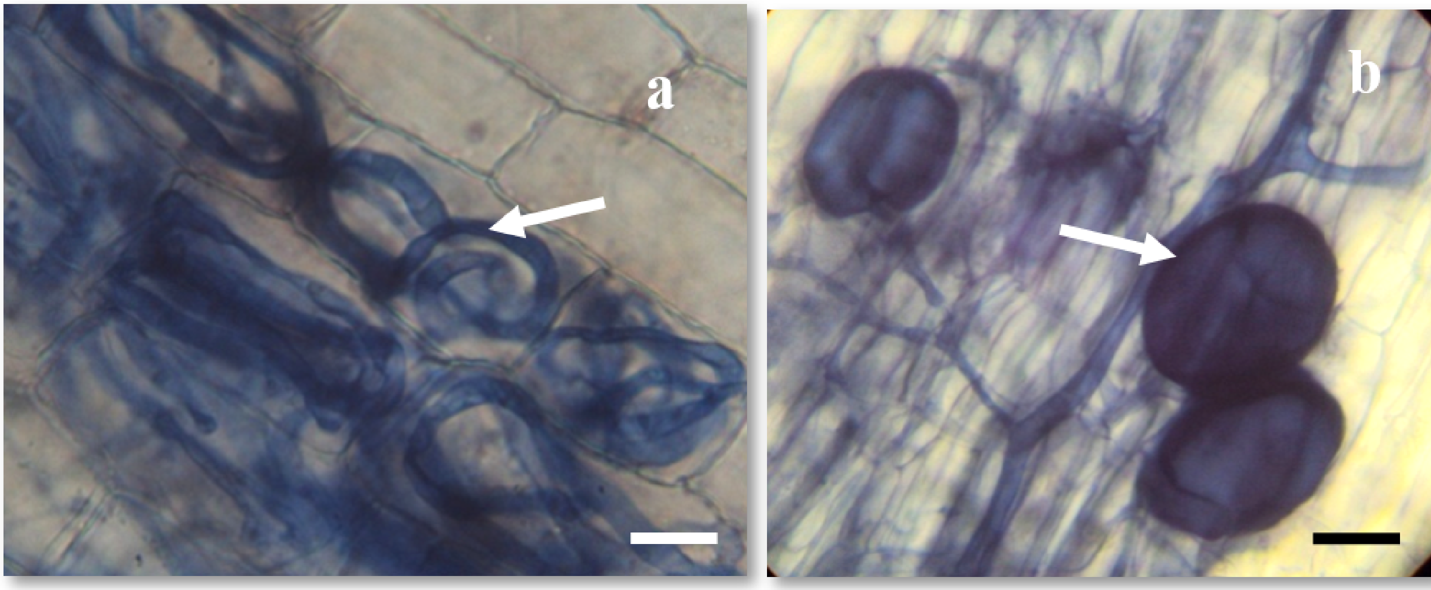

Figure 12

Arbuscular mycorrhizas in Artemisia herba alba roots, accompagned plant of H. lippii associated with T. claveryi in High steppe Plains. a arbuscular mycorrhizal fungi hyphae growing intracellularly from cell to cell to form arbuscular coils (arrow); b mycorrhiza showing ovoid vesicles (arrow); Scale bars = 20 μm in a, b