Investigation on the growth parameters for Libyan wild truffles

Zohur Elfalla, Hamida B. Mirwan, Hisham Nagi El Waer, Seema R. Bajaj, Salem Shamekh

Abstract— **Desert truffles have been used as a substitute for meat in Libyan meals, recently exported to some Arab Gulf countries, but they depend entirely on wild truffles. A few truffle ascocarps have been harvested intermittently since the early nineties from the forest belonging to Experimental and Research Stations at the Faculty of Agriculture, University of Tripoli, Tripoli, Libya. This study aimed to identify the environmental and ecological factors that promote (stimulate) the natural growth of Libyan truffles. The study was conducted at the Experimental and Research Stations of the Faculty of Agriculture, University of Tripoli, Tripoli, Libya. This is the first scientific study that has been performed to determine growth promotion criteria for *Tierfezia* at the Research Station. Soil and plant samples were collected during September 2021. Soil samples were analyzed to estimate the physical properties and associated plants were identified. The results showed that the harvested truffle ascocarps grow in sandy soil with pH 8, high porosity (50.4), and electrical conductivity (2 mm hos/cm at room temperature). *Helianthiamum* spp. was identified alongside harvested truffles indicating their possible symbiotic association.**

Index Terms— **Libya, desert truffle, *Terfezia*, *Helianthemum***

I. Introduction

Truffles are the most costly edible fungi across the globe, and belong to the family *Tuberaceae*. They produce hypogeous sporocarps and grow in symbiotic association with various trees such as oak, hazel, beach and birch [1, 2]. Truffles vary in texture and colour, they can be wrinkled, bruised, smooth or reticulated, generally, they are divided into black and white truffles based on their colour. Several phases of the truffle life cycle are essential, including (spore germination, mycelial growth, contact between the mycelia and the fine roots, formation of mycorrhiza, and growth and fusion of mycelia of different mating types) [3]. On an average, the time required in case of commercial black truffles, for inoculation to fruiting, spans from many years to a decade [4]. In old times, truffle were collected and picked up annually from the wild areas, mainly at Mediterranean basin forest, and this is still the case in most developing countries. In the last century, wild production of truffles has fallen drastically, leading to the progress of truffle cultivation. Nowadays, truffle orchards contribute to around 80% of production of *Tuber melanosporum* (black diamond truffle) in France [5, 6]. Plantation of black diamond truffle has is being considered as economically important across the Mediterranean (France, Italy, and Spain) and is also gaining attention in various other continents, which show a Mediterranean-like climate; however, ascocarps of several species of truffles are being discovered in more intense environments (warmer or colder) [7]. One of the most noticeable successes of cultivation of truffle is in the boreal zone by Shamekh et al. (2014) [8] who has founded a truffle orchard at Juva in Finland, where seedling survival, growth, and development of truffle ectomycorrhiza has been studied in a boreal climate, which displays long harsh winters with low soil temperatures. Apart from this, establishment of an experimental plantation in a subtropical climate has also been carried out, which is yet to report on production. The motivation of truffle cultivation is driven by the demand in the market fresh, locally produced truffles.

Desert truffles are popular in Arab countries and are considered as a delicacy. Wild desert truffles are ectomycorrhizal hypogenous fungi and are edible in nature. They develop and grow in mutual association with roots of the desert sunflower plant (*Helianthemum* species), and are well-adapted to the extreme arid/semi-arid conditions. These truffles include the genus such as *Tirmania* and *Terfezia* which are endemic to Libya [9]. For centuries, wild desert truffles have been consumed as food and also as medicine in Libya on a traditional basis. Desert truffles fruitification is observed periodically upon receiving adequate regular rainfall and in the presence of suitable conditions of soil as well as the appropriate plant that acts as a mycorrhizal host (for example, *Helianthemum* species). In Libya, these truffles are locally known as either Terfasa or even Al-Kamaa. In Saudi Arabia and Kuwait, they are called as Al-Faga.

Since Libyan wild desert truffles grow in the wild, and are routinely retailed by truffle collectors, on local markets without the control exerted by the government on the quantity and quality, there have been no official reported data available on total Libyan truffle production and consumption. Truffle market prices in the 90s were less than 10 dinars/kg (which accounts for around 2€/kg). However, recently the price reached 200 Libyan dinars/kg (about 50€/kg).

Truffle ascocarp formation is influenced by numerous parameters such as type of soil, its chemical characteristics, climatic conditions, rainfall quantity, etc. [10, 11]. Bouzadi et al. (2017) [9] reported that the pH value of 8.2-8.5 was giving the optimal conditions for truffle fructification in Hamada Al-Hamra region, Libya. In the Murcia region of Spain, the annual desert truffle production ranged from 50 Kg/ha to 170 Kg/ha where a rainfall of 350 mm to 400 mm was observed [12]. In the truffle plantations, irrigation set-up is not a must, provided that there is adequate rainfall, knowing the fact that the association of mycorrhiza is well-suited for the arid/semi-arid climatic conditions [13].

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Based on their association with annual/perennial plants, such as *Helianthemum* and *Xolantha* species, in Spain, truffles are gathered in the spring season [14]. In regions of Hamada Al-Hamra, Libya, from November until April, the fruiting season of truffles essentially correlates with the flowering period of particular genus of *Helianthemum*. Moreno et al. (2000) [15] shed light on the importance of these mycorrhizal fungi in the maintenance of arid Mediterranean shrub-lands. This was majorly by averting erosion of soil and desertification which can also be observed in Libya. This study aimed at providing a baseline data for long-term monitoring by investigating the climatic and ecological parameters that affect truffle productivity in the Experimental and Research Stations of the Faculty of Agriculture, University of Tripoli, Tripoli, Libya.

II. MATERIALS AND METHODS

A. *Analysis of soil and vegetation*

Representative volumes of samples of soil from the truffle habitat area were taken for analysis of physical and chemical properties, and samples of common plant species in the study area were collected for identification.

B. *Physical and chemical properties of soil*

The detailed analysis of soil parameters was carried out at the Soil and Water Department, Faculty of Agriculture, University of Tripoli, Libya. The textural classes, porosity, true and bulk density of the soil samples were determined according to the method of AOAC (2016) [16]. The pH and electrical conductivity of the truffle habitat soil was determined using a pH/EC meter (MBT-700, Boeco, Germany). The chemical properties were analyzed by using different methods [17].

C. *Vegetation in truffle habitat and collection of truffles*

Samples of common plant species in the study area were collected and identified. For the investigation, a representative number of ascocarps of truffles which presumably belonged to the genus *Tirmania* or *Terfezia*, which commonly grow in Libya were gathered from the area under study. The collected specimens of truffles were washed to remove the adhering soil and were then identified.

III. RESULTS AND DISCUSSION

A. *Ecology of the studied Libyan desert truffles*

Vegetation observed in the areas where truffles grow naturally in Libya are generally rare and largely restricted to areas where adequate annual precipitation is seen during Autumn and Winter; in which Helianthemum species was the dominant plant found near all the harvested truffles belonging to Terfezia species. The findings of our study agreed with these results. The identity of the plant associated with the collected truffle from the Stations was Helianthemum species as indicated by the herbarium of the Faculty of Science, University of Tripoli, Tripoli, Libya, and the truffle was Terfezia species as identified by its morphological characters (Fig. 1). As a matter of fact, truffle vegetative cells under the soil get their carbohydrate from host plants to survive by forming truffle mycorrhizae (ectomycorrhizal, endomycorrhizal and ectendomycorrhizal) in association with the root system of the Helianthemum such as H. lippii [18, 19, 20].

Tuber species generally form a symbiotic relationship with trees such as birch, pine, spruce and oak [8]; However, depending on the surrounding conditions, desert truffles of species Terfezia and Tirmania have been reported to form ecto-, ectendo- or endo-mycorrhizal association with roots of Cistaceae family, especially with the genus Helianthemum [21, 22]. Such reports have established a scientific base for the results observed in the present study, and thus validate the findings. The association of desert truffles with Helianthemum species is so well established, which encourages many researchers to study their symbiotic relationship for better understanding [23], with the ultimate goal of cultivating desert truffles with this plant.

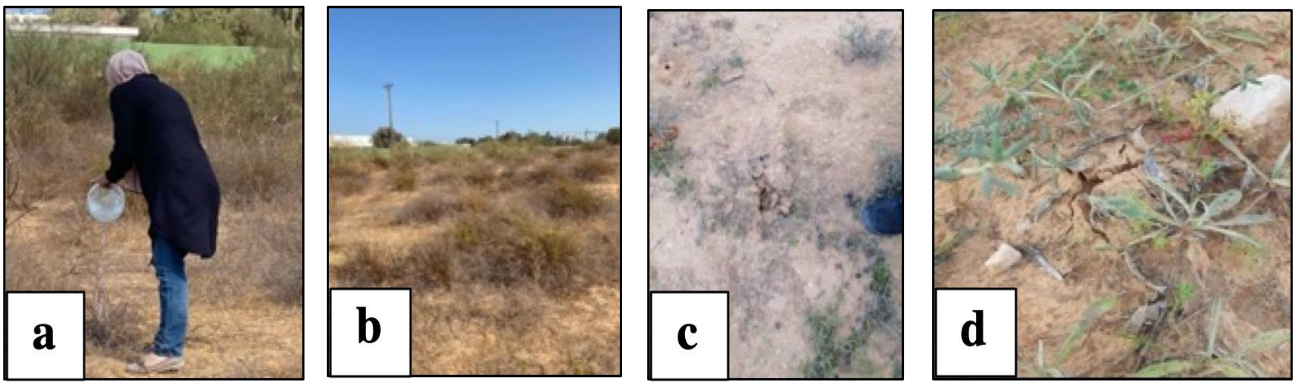

Fig. 1: Habitat of the collected Libyan wild truffles (Terfezia); (a) researcher irrigating the area; (b) the potential host plant Helianthemum lippii; (c) & (d) cracks being seen in the soil upon growth of truffle ascocarps

B. *Particle size distribution*

The analysis of soil showed that the amount of silt, sand and clay of studied truffle habitat soil samples on an average were found to be 8.4, 89.7 and 1.9%, respectively (Table 1). Therefore, the textural class of the harvested truffle habitat soil was classified as sandy soil using the textural triangle [24], while the texture of the soil for Swedish *Tuber aestivum* was silty to sandy. On the other hand, the nature of French soils has been reported to be more towards clayey [25]. Our results are in unison with the results stated by Bouzadi et al. (2017) [9]. In a study, the authors compared two types of soil, viz. gypseous and sandy loam for studying the growth, colonization of roots and status of nutrients after inoculating *H. sessiliflorum* Desf. seedlings with ascospores of the ectomycorrhizal fungus *Terfezia boudieri* Chatin. Even though the plants showed better growth on gypseous soil, mycorrhizal colonization was observed to be better in sandy loam soil (89%) than in gypseous soil (52%). This substantiates the results of soil analysis observed in the present study; other studies also support such findings [22].

C. True and bulk density

Table 1 shows the physical characteristics of the studied soil samples. The true and bulk densities of soil samples that were assessed in this study were observed to be 2.7 and 1.4 gm/cm3, respectively. These results were comparable with a previous report by [8] where true density was reported to vary from 2.65 gm/cm3 for mineral particles, to 0.2 gm/cm3 for organic matter.

Table 1: Physical properties of truffle habitat soil

Property	***Quantity***
Sand	*89.7%*
Silt	*8.4%*
Clay	*1.9%*
Field capacity	*10.4*
Porosity	*50.4%*
True density	*2.7 gm/cm^3*
Bulk density	*1.4 gm/cm^3*

D. Porosity and field capacity

In the present study, we observed high porosity value (50.4%) for truffle habitat soil samples, whereas the field capacity was seen to be substantially low (10.4%). These results were anticipated due to the textural class of the soil i.e. sandy. The sandy soil as shown in Table 1 has low silt and clay content. This criterion minimizes the ability of sandy soil to retain water when compared with that of silty clay or clay loam soils. The high value of macro porosity seems to point at the crucial role from the viewpoint of providing adequate air for optimal truffle growth and reproduction. A higher porosity value of up to 45% has been reported in an earlier study [9], where two wild Libyan desert truffles viz. Terfezia and Tirmania were identified and characterized. In addition, plants of Helianthemum species (H. lippii and H. kahiricum) were observed to be the dominant plants that were seen to establish a mycorrhizal association with these truffles [9], thus pointing at the possible role of soil porosity in plant and mycorrhizal growth.

E. pH value and chemical conductivity

pH and EC values of truffle soil habitats were determined using MBT-700 (pH/ORP/EC, Germany). The pH value of studied soil appears to be suitable (8.2) for truffle growing, which is comparable with findings reported by [9]. Relatively high pH, generally above 7.5 (which varies as per the truffle species) and similar texture of habitat soil as has been observed in this study are also required for growing other cultivated truffles such as many Tuber species [2, 25, 26]. Lands with appropriate parameters of soil (for instance, texture of clay-loamy nature with basic pH of around 8.5) can possibly be more rigorously propagated and maintained for organized cultivation in the future, at the same time giving attention to other environmental properties.

In the present study, the electrical conductivity of soil was seen to be 0.20 mm hos/cm at ambient temperature (25 °C). Generally, this is considered as a lower value than that of good production of crop on most of the soils [27].

F. Mineral elemental analysis of soil

The results of the analysis of the chemical properties of the soil samples collected form the study area illustrated in Table 2, the mineral content showed high calcium concentration, followed by potassium and Nitrogen at 128, 123 and 20, respectively. The higher content of calcium of soil (128 ppm) was perhaps due to the calcite occurrence, which seems to be crucial in delivering proper habitat for optimal truffles growth and reproduction. Earlier studies stated that content of calcium of habitat of truffles ranges from 21.3 to 292.5 ppm [28]. It is well-known that calcium plays a crucial role in preserving soil pH value. In our study, nitrogen and phosphorus contents of soil were observed to be low (20 and 27 ppm) (Table 2).

Table 2. Chemical properties of truffle habitat soil

Element	**Concentration (mg/kg)**
Calcium	128
Magnesium	16

Sodium	9.2
Potassium	123
Total nitrogen	20
Total phosphorous	27.04

G. Truffle sample morphology

Although truffle collectors have been using dogs to find truffles in most European countries such as Finland and Italy, however, Libyan truffle collectors never use dogs to find natural matured truffles. In Libya, matured truffles rise up and telltale cracks the soil surface, this indicating the presence of a truffle underneath it. All truffles ascocarps collected during the last seasons were morphologically identified as Terfezia (called as red/black truffle) which is depicted in Figure 2, which is a common species of Terfizia that grows naturally in Libya. Terfezia has been growing wildly in many regions of Libya, although the valleys and foothills of the mountains of Libya are rich in precious truffles, the most famous of them is the Al-Hamada Al-Hamra area, about 500 km southwest of the capital, Tripoli. The phylogenetic analysis of the genomic rDNA ITS of the next seasons harvested truffles will be investigated in order to identify all possible species of Terfezia.

Fig 2: Pictures of the harvested Terfezia which were used in the present study (Picture courtesy of Dr. Hisham Nagi El Waer)

IV. CONCLUSION

The present study aimed at investigating the physical and chemical properties of the soil which Libyan wild desert truffle growing at the Experimental and Research Stations at the Faculty of Agriculture, University of Tripoli, Tripoli, Libya. The characters of the soil was indicated as sandy alkaline soil with pH 8.2, with high calcium concentration and High value of porosity but low field capacity. in addition, the symbiotically associated plant was successfully identified as Helianthemum species. Based on the soil analysis results and a thorough literature search, an appropriate correlation between the findings of the host plant (Helianthemum) and the occurrence of the desert truffle Terfiza species was established. This study could be considered as a base for future research on growing desert truffles for economic gains, as well as identifying further findings of tubers at a genetic level, so that a deeper understanding of (host plant-truffle) association is achieved.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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