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ORIGINAL ARTICLE

First report of European truffle ectomycorrhiza in the semi-arid climate of Saudi Arabia

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Abstract

Tuber melanosporum Vittad. (Black or Périgord truffle) is a truffle native to the Mediterranean Southern Europe, popular for its unique flavor, and has great economic importance. The present work focused on assessing the possibility of cultivating *T. melanosporum* associated with *Quercus robur* L. in the desert climate of Saudi Arabia. The plantation was initiated in November 2018 by planting 271 oak seedlings in the Al-Qassim desert area and checked for survival and ectomycorrhiza development after 1.5 years of plantation maintenance. Amongst the 271 seedlings planted, 243 plants survived two harsh seasons (2019 and 2020), and the randomly selected and tested seedlings were still mycorrhized with *T. melanosporum*. The mycorrhization level with *T. melanosporum* was between 5 and 35% of all fine roots, and the share of contaminant ectomycorrhiza was low. In comparison to other areas where *T. melanosporum* is successfully cultivated, the Al-Qassim desert area has 10–15 °C higher average summer temperatures and a low total annual precipitation, which necessitates regular irrigation of the plantation. This work opens the avenue for an adapted, yet sustainable cultivation of *T. melanosporum*-inoculated oak tree in a desert climatic condition and introduces new opportunities of the agro-forest business in Saudi Arabia and GCC region.

Keywords *Tuber melanosporum* · *Quercus robur* · Desert climate · Truffle cultivation · Ectomycorrhiza

Introduction

Truffles are popular edible fungi with potential health benefits, great nutritional properties, a distinctive flavor and texture, and bioactive properties such as antioxidant, antiviral, antimicrobial, hepato-protective, anti-mutagenic, and anti-inflammatory activities (Wang and Marcone 2011). Truffles belong to genus *Tuber* and the family *Tuberaceae* (Zambonelli et al. 2016; Kirk et al. 2008), and have the highest economic value among all the edible fungi due to their distinctive aromatic compounds (Strojnik et al. 2020; Gioacchini et al. 2005). Different truffle species reach marked prices from several hundred to several thousand euros per kg. *Tuber melanosporum* is the highest valuated and frequently cultivated species.

T. melanosporum is characterized by reddish-brown to brown peridium and black gleba with flattened prominent warts. Asci contain 1–6 ellipsoid, dark brown and spiny spores. The aroma of ripened ascocarps is usually described as wet forest aroma with a slight taste of radish and a tinge of hazelnut by truffle specialists, and is a result of combination of aroma compounds with fruity, earthy, or sulfurous odor type (Strojnik et al. 2020). It is a Mediterranean species commonly growing in mycorrhizal association with oaks and other broad-leaved trees, in a well-aerated soil with a high carbonate level (Hall et al. 2007; Montecchi and Sarasini 2000). It requires several years (2–15) for successful mutual growth of plant and fungal partner to reach the sexual maturity and potential truffle fruiting (Zambonelli, personal communication).

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Truffles are ectomycorrhizal (Rinaldi et al. 2008) or ect-endomycorrhizal (Ori et al. 2020) and can complete their life cycle only in a symbiotic relationship with a plant host (Bonito et al. 2010). The life cycle of truffles involves several stages, among which following are crucial for the truffle cultivation: spore germination and vegetative growth of the mycelium, contact of mycelium with fine roots and formation of mycorrhiza, and growth and fusion of mycelia of different mating types which is a prerequisite for production of fruiting bodies i.e. truffles under the ground (Peterson and Bonfante 1994; Rubini et al. 2011). An average time from inoculation to fruiting of commercial black truffles ranges from several years to over a decade (Oliach and Pere 2012).

Traditionally truffles were hunted in nature, mainly in Mediterranean forested areas, until recently. In the last century, natural production of truffles dropped drastically leading to the development of cultivation practices (Hall et al. 2003). Currently, over 80% of *T. melanosporum* production in France comes from truffle orchards (Mello et al. 2006; Murat 2014). Plantation of *T. melanosporum* has gained importance across the Mediterranean region (i.e., Spain, France, and Italy) and is becoming common also on other continents, mostly in areas with a Mediterranean-like climate. *T. melanosporum* plantations proved to be widely prosperous in suitable habitats around the world (Bonet et al. 2009); however, a shortage of knowledge may increase the risk of failure, which seems to be almost as frequent as successes in a plantation for truffle cultivation (Guerin-Laguette et al. 2013). In last 2 decades, truffle plantations focusing on *T. melanosporum* or *Tuber aestivum* Vittad. were set up in many countries such as New Zealand, Israel, North America, Australia, South America, and etc. (Streiblová et al. 2010; Reyna and Garcia-Barreda 2014), some of them already producing well (Grebenc's personal communication with plantations owners).

Truffles were rarely cultivated outside the Mediterranean or close-to-the Mediterranean climates so far, despite ascocarps of various truffle species being found also in more extreme (warmer and colder) environments (Grebenc et al. 2019). The most prominent success is cultivation of truffles in boreal zone by Shamekh et al. (2014) who established a plantation in Juva, Finland, to study seedling survival, growth, and truffle ectomycorrhiza development in a boreal climate characterized by long winters and low winter soil temperatures. Several other trials, such as an experimental plantation in subtropical climate (Sulzbacher et al. 2019) were set, butare still waiting for productionto the best of our knowledge. The only successful plantation in extremely dry climate was recently established in South Africa (Hall et al. 2017; Zambonelli's personal communications). This drive for cultivation outside traditional truffle areas is related to market demands for fresh and locally produced truffles. For example, truffles are a popular delicacy in Arab countries. Saudi Arabia already grows desert truffles such as *Tirmania nivea* (Desf.) Trappe and *Tirmania pinoyi* (Maire) Malençon. The country has a demand of over 50 tons of truffles per year. Khanaqa (2006) investigated the potentials and limitations of desert truffle production in Saudi Arabia. Unfortunately, desert truffles reach lower market prices than European temperate climate true truffles (75–100 € kg^{-1} for desert truffles versus 600–1000 € kg^{-1} for a wholesale of *T. melanosporum*) and also lower protein content and aromas (Harki et al. 2006; Bouzadi et al. 2017). To fill the market demand, the knowledge transfer and studying the cultivation of true truffles such as *T. melanosporum* in the desert climate is of great importance.

To test the principle of bringing knowledge on truffle cultivation and establish a truffle plantation outside the Mediterranean areas, we planted the first truffle orchard in central Saudi Arabia, located in a semi-arid desert environment with harsh and long summers. Our motivation was not only to grow truffles in the sand dunes area, but also to bring the knowledge on truffle cultivation to the local community. We focused on establishing a fully equipped plantation of *T. melanosporum* inoculated oaks, and on testing the effect of a combination of orchard adaptations and management methods on the survival and growth of oak seedlings to overcome the environmental restrictions. Finally, we aimed at testing the survival and potential growth of truffle ectomycorrhiza in this semi-arid region with extreme environment.

Materials and methods

Geographical location and ecological characteristics of the truffle orchard

The orchard was established in Qassem (also called Al-Qassim; 25°48′23″ N 42°52′24″ E), Saudi Arabia, in November 2018. The area is located 600–750 m above sea level. It has a typical desert climate with virtually no rainfall during most of the year. The Köppen–Geiger climate classification is BWh (arid hot climate) with average annual air temperature of 24.1 °C and average annual rainfall of 216 mm. Winters are cool with infrequent rain, and summers are hot and less humid (https://en.climate-data.org/). Soil samples for physical and chemical analyses were collected prior to planting, irrigation, and fertilization but after the soil preparation, using a soil borer at a 0–20 cm depth from three locations in the plantation. The soil was analyzed for pH, electrical conductivity, and total soluble solids, and chemical parameters (chloride, sulphate, sodium, potassium, calcium, magnesium, carbonate, nitrate, phosphorus, and boron) using methods in USDA (The United States Department of Agriculture) Handbook no. 60 (Burt 2009). An exchangeable sodium percentage and a sodium absorption ratiowere calculated. The soil analysis was carried out by theIndependent Laboratories and Materials Testing Company, Riyadh, Saudi Arabia.

Cultivation of oak seedlings inoculated with T. melanosporum

Two hundred and seventy-one oak seedlings (*Quercus robur* L.) were inoculated under controlled conditions by Pépinières Robin (Saint Laurent Du Cros, France) 1 year prior to the plantation and transported to Saudi Arabia according to phytosanitary regulations of the destination country. The deciduous oak species *Q. robur* was selected as a host plant due to its broad ecological niche, high tolerance to soil conditions including broad pH range, and a tolerance to range of soils, from humid to xeric (Ducousso and Bordacs 2004). *Tuber melanosporum* Vittad. ectomycorrhizal partner was selected for inoculation.

The truffle orchard was planted at the beginning of the cold period in November 2018. The orchard area under cultivation is half hectare and the soil had been previously used as agricultural land to produce various agricultural products such as crops, grass, and vegetables prior to the cultivation of oak seedlings. Oak seedlings were planted in rows at a 3 m distance between plants and 4 m distance between rows (Fig. 1) to ensure easy access to harrowing machines (Shamekh et al. 2014). The planting scheme was selected to ensure optimal irrigation and growth conditions under arid and hot climate. The soil between the rows was regularly ploughed, harrowed, or weeded by labors to prevent growth of competing vegetation (competition for water and nutrients) and keep the root growing zone in a pioneer stage optimal for growth of *T. melanosporum* mycelia (García-Montero et al. 2006). All seedlings were checked once every 4 months for survival, growth, and visual damage.

Each seedling was irrigated using a dripping irrigation system (Fig. 1). A soil moisture sensor meter (Dr Meter S20)

Fig. 1 The first truffle plantation in the Qassem (Saudi Arabia), where *Tuber melanosporum* Vittad. inoculated *Quercus robur* L. seedlings were planted in a 3 m×4 m planting scheme

was used to determine the moisture content of soil. Irrigation was planned based on the moisture content of soil. The water requirements were determined after draught indices published in Wang et al. (2015). During summer, each seedling received an average of 1.5 L of water twice a week, whereas in wet period, the amount of water was reduced to 1 L per seedling twice a week. Better soil moisture retention was observed in autumn and winter. Iron fertilizer FerroActive iron chelate 6% EDDHA (ethylenediamine-*N*,*N*′-bis,2-hydroxyphenylacetic acid) (Deretil Agronutritional, Barcelona, Spain) 150 g per 200 L, and the "ACM micro nutrients solution" (Agro Consulting Ltd., Middlesex, NJ, USA) 20 mg per 5 L were added to irrigation water to support the growth of the plant.

Morphological analysis of ectomycorrhiza

Ten percent among survived seedlings (15 seedlings in total) were randomly chosen after 18 months from the time of plantation for sampling of fine roots and ectomycorrhiza. To limit the damage to seedling's root system, a single side root was carefully searched for and dug out with a spatula at about 0–15 cm depth. The side root was carefully cut close to the main root and followed away from the main root until completely excavated. Each side root was placed in sealed plastic bag and kept refrigerated until further processing in laboratory. In laboratory, all side root samples were carefully rinsed with sterile distilled water to remove the attached soil particles and observed for the presence of ectomycorrhiza under Olympus SZX12 stereomicroscope (magnification 3.5×–144×). Root samples were cut into 2 cm pieces and subsequently these pieces were randomly selected for count of non-mycorrhizal fine roots (mainly represented by old ectomycorrhizal root tips or non-mycorrhized root tips) and identification and count of vital ectomycorrhizae was carried out following method developed and applied by Shamekh et al. (2014). Share of vital ectomycorrhiza of *T. melanosporum* and the contaminant fungi were identified based on morphological criteria (Agerer and Rambold 2004–2017; Águeda et al. 2010; Zambonelli et al. 1995) using Olympus GX light microscope.

Results and discussion

Examining the survival of oak seedlings

The first challenge in truffle cultivation in suboptimal conditions is ensuring the survival of planted seedling. The survival was monitored once every four months in the first 1.5 years of planting. Amongst 271 seedlings, 243 survived, giving the mortality of about 10%, predominantly due to drying of complete seedlings or extensive root system damages due to herbivores attacks (Table 1).

The mortality of 10% is rather high in comparison to plantation setting in optimal (Mediterranean) conditions where roughly 1% was noted by Olivera et al. (2014) or up to 4% in sub Mediterranean conditions in Slovenia (Grebenc personal communication: Volk, Beznik). The mortality observed in anarid hot climate of Saudi Arabia represents a considerable extra cost of the overall plantation financial plan and should be reduced in future. Since inadequate irrigations at plantation edges in dry period and pest attacks in wetter times of the year were most common causes of death of seedlings, truffle growers in dry/desert areas should primarily focus on these threats. The climate in Saudi Arabia differs a lot from the areas where *T. melanosporum* grows naturally (Spain, France, Italy) or was recently been successfully cultivated (Australia, Chile, New Zealand, South Africa, USA, Croatia) (Fig. 2). The similardry condition, although not so extreme, are present in a truffle orchard established in South Africa in Groenfontien where the first truffles were collected in 2018 (Miroset al. 2016; Zambonelli, personal communication).

Average and maximum monthly temperatures were the most outstanding deviations of the Saudi Arabia *T. melanosporum* plantation conditions, especially in summer. Highest summer temperature recorded on the Saudi Arabia *T. melanosporum* plantation reached peak temperature 43 °C and summer averages above 30 °C (Fig. 2a, b), which is 10–15 degrees higher than in areas with natural *T. melanosporum* sites or areas with producing plantations (Thomas 2014). Another discrepancy is a low total annual precipitation with slightly above 200 mm, which is well below the minimum from native *T. melanosporum* sites in the Mediterranean with average 600–900 mm annually (Giovannetti et al. 1994). Furthermore, the duration or period without any rain in the Saudi Arabia *T. melanosporum* plantation on an average lasts for up to 3 months (Fig. 2c) which may in long-term reduce, if not completely eliminate, the survival of mycelium and production of truffles (Büntgen et al. 2012).

To overcome such unfavorable conditions, a drip irrigation system was established which is known to increase drought resistance and reduced shoot height (Arend et al. 2011). These adapted irrigation practices increased total root tips. Olivera et al. (2011) confirmed that a low dose of irrigation increased total root tips and *T. melanosporum* ectomycorrhizae on the truffle-oak plantation. Not included in this study, but worth mentioning are also various mulching approaches such as covering the topsoil surface with straw, grass, and stones to reduce water evaporation and weed growth. These earlier studies support our hypothesis that European oak seedlings can survive in hot and dry climate as long as appropriate management practices are applied.

Soil condition is another factor that may affect the survival of seedlings, of black truffle ectomycorrhiza and ultimately, lead to the production of truffles. Physical–chemical properties of orchard soil (Table 2) differ from optimal *T. melanosporum* soil in lower calcium content and a pH value at the lower optimal values (Jaillard et al. 2016).

Calcium content of the orchard soil in this work was lower in comparison to the calcium content of soil samples analyzed from native *T. melanosporum* sites (Wedén et al. 2004; Jaillard et al. 2016). The magnesium content of analyzed soils was also low, and in addition the Saudi Arabia *T. melanosporum* plantation soil showed potassium: magnesium ratio < 2 (1.92) which is below optimal (Wedén et al. 2004). This may be due to the ecological differences between these two sites. Hence, liming of soil is needed to increase the pH, calcium content and to shift the potassium: magnesium ratio to favorable level. High alkaline soils are suitable for the growth of *T. melanosporum*, and pH 7.9 is considered as optimum for the cultivation of *T. melanosporum* (Wang and Marcone 2011) which is higher than optimum for most of ectomycorrhizal fungi ranging from pH 4–6 (Erland et al. 1990). To reach optimum pH conditions in the Saudi Arabia *T. melanosporum* plantation soil, liming and periodical monitoring of those key soil parameters is proposed.

Table 1 Causes for loss of 28 out of 271 *Quercus robur* L. seedlings inoculated with *Tuber melanosporum* Vittad. in the first 1.5 years of the plantation in Qassem (Saudi Arabia)

Cause of death	Time/season of death (descriptive)	Number/share of dead seedling
Corner effect (dried due to insufficient irrigation)	During the dry period, most of the seedlings were located at line edges with insufficient/inadequate irrigation	13/46%
Root damage (herbivores grazing root systems)	In first half year after planting (corresponding to winter)	8/29%
Root damage (insect grazing on fine roots)	No specific time	5/18%
Other/unknown reasons	Spring	2/7%
Total share of all planted seedlings		28/10.3%

Main causes were estimated and a season in which mortality due a specific reason happened is given

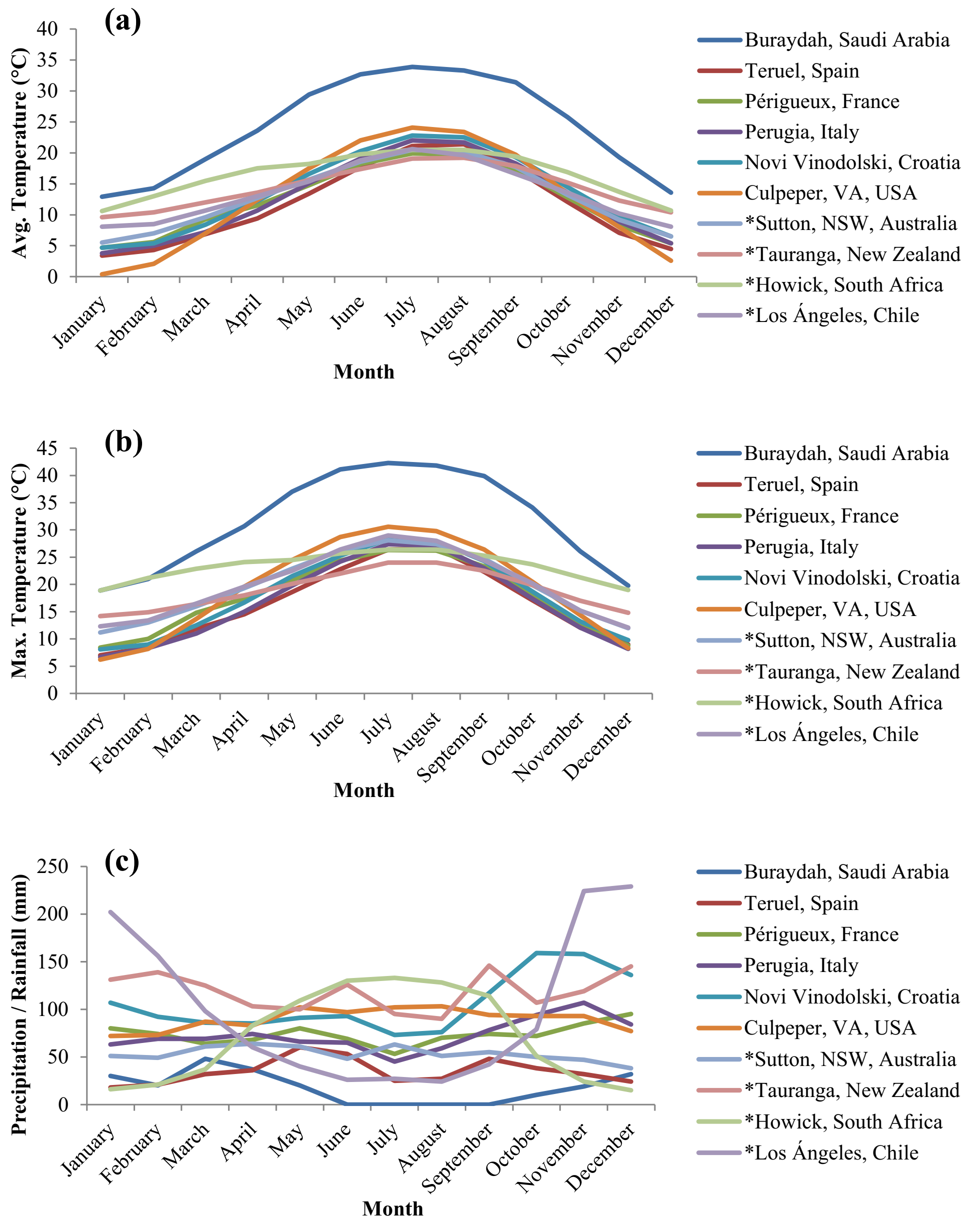

Fig. 2 Yearly data of **a** average temperature, **b** maximum temperature, and **c** rainfall for the truffle plantation in the Qassem (Saudi Arabia)

Growth and morphological identification of truffle ectomycorrhiza

One side root from 15 randomly chosen vital oak seedlings, including more than 400 root tips, were analyzed for presence and abundance of ectomycorrhiza. Morphological traits of well-formed *T. melanosporum* ectomycorrhiza on oak roots are showed in Fig. 3. All analyzed seedling showed presence of a typical *T. melanosporum* ectomycorrhiza (Fig. 3a–d) (Agerer and Rambold 2004–2017), and four among them also showed contaminant ectomycorrhiza (Table 3). The current truffle orchardhad been previously used as agricultural land to produce various agricultural products such as crops, grass, and vegetablesprior to the cultivation of oak seedlings, thus growth of other potential competing ectomycorrhizal propagules in this soil were minimal (Hall et al. 1998).

Ectomycorrhiza was present along the whole length of the side roots, namely the part mycorrhized in a greenhouse prior to planting and also new root growth in the plantation, which indicates survival and successful growth of *T. melanosporum* mycelium in the suboptimal plantation conditions. Simple ectomycorrhiza were straight and club-shaped with yellowish-brown color. Cystidia were present in a medial position, bristle-like, awl-shaped, and septate with a yellowish color (Fig. 3a, b). The outer mantle surface was puzzle like, cells varying from ellipsoid

Table 2 Physical and chemical properties of the Qassem (Saudi Arabia) *Tuber melanosporum* plantation soil based on the three samples analyzed as one composite sample

Physical/chemical parameter	Composite sample data
pH	7.4
Electrical conductivity (Micro S/cm)	9090
Total soluble salts (mg/L)	5636
Chloride (mg/L)	1524
Sulphate (mg/L)	1520
Sodium (mg/L)	715
Potassium (mg/L)	117
Calcium (mg/L)	3006
Magnesium (mg/L)	61
Carbonate (%)	1.4
Nitrate (mg/L)	134
Phosphorous (mg/L)	2.0
Boron (mg/L)	0.7
Exchangeable sodium percentage (%)	3.83
Sodium absorption ratio (%)	3.54

Most relevant nutrients, pH, and electrical conductivity were measured in water solution of the soils

and isodiametric to irregular rectangular and elongated constituted by epidermoid cells (Fig. 3c, d).

Similar morphological characteristics were reported earlier for *T. melanosporum* ectomycorrhiza cultivated on different host plants (Marozzi et al. 2017; Granetti 2005; Zambonelli et al. 1995). The possibility of growth of *T. melanosporum* ectomycorrhiza in different habitat was explored and results indicated that the cultivation method and management practices used in the present study helped the oak seedlings to grow in the desert climate of Saudi Arabia successfully. We have confirmed that European truffle *T. melanosporum* can survive under Saudi Arabia climate and soil conditions with adequate management practices, despite significant differences in environmental conditions compared to native *Tuber melanosporum* sites.

The restrictions caused by high temperatures of soil and air during most time of the year were overcome with proper orchard management and European oak can be cultivated in desert climate. This scientific work resulted in the adaptation of European truffle cultivationin Saudi Arabia and GCC region to establish sustainable forest industry.

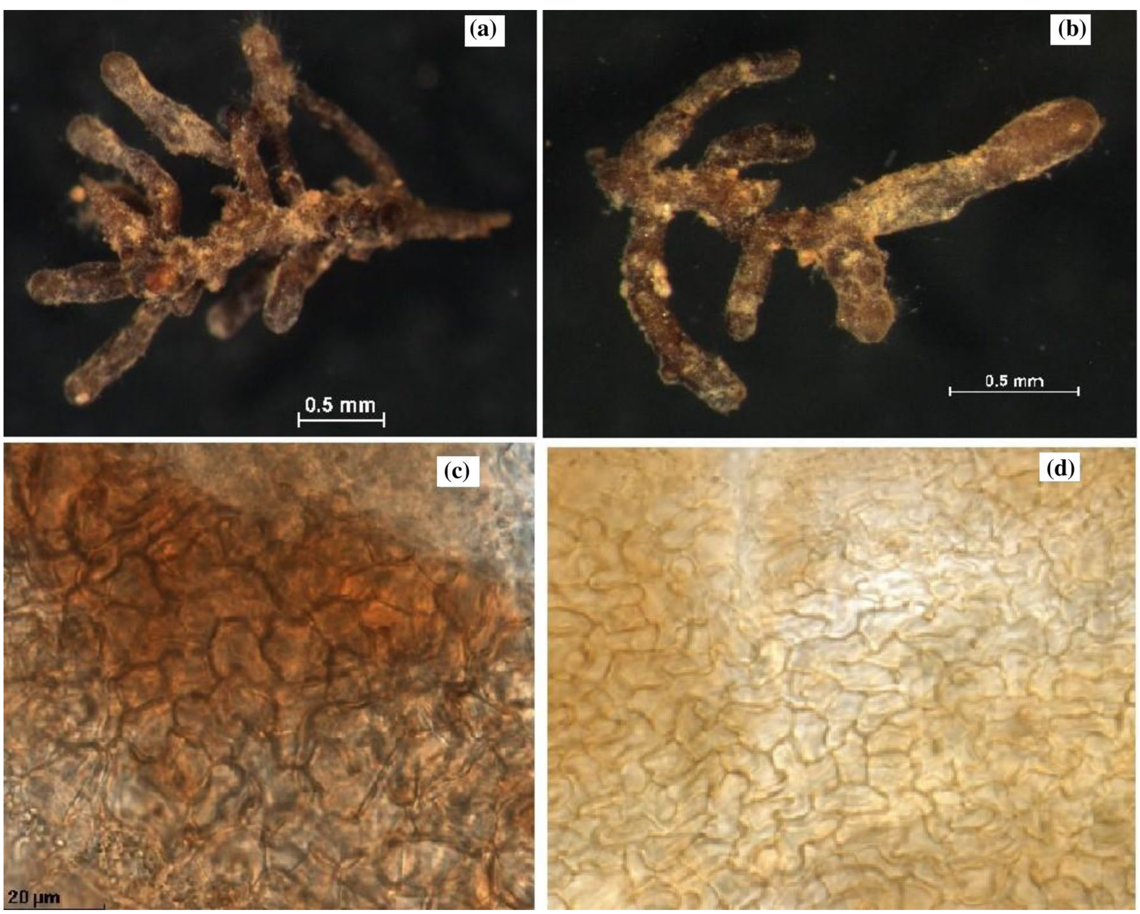

Fig. 3 *Tuber melanosporum* Vittad. ectomycorrhiza on selected fine roots of inoculated *Quercus robur* L. seedlings collected in the Qassem (Saudi Arabia) truffle plantation 1.5 years after the establishment of the plantation. **a**, **b** Monopodial-pinnate branched ectomycorrhiza with infrequent cystidia; **c**, **d** outer ectomycorrhiza mantle layers with pseudoparenchymatous cell arranged in a puzzle-like manner (type Q *sensu*Agerer and Rambold 2004–2017) (Photo a, b, and c by T. Grebenc; photo d by A. Zambonelli)

Table 3 Share of vital ectomycorrhizae of *Tuber melanosporum* and contaminant fungi on fine roots of 15 randomly selected oak seedlings from the Qassem (Saudi Arabia) truffle plantation, sampled 1.5 years after the establishment of the plantation

Share of *Tuber melanosporum* ectomycorrhiza	Share of contaminant ectomycorrhiza
5.75	0
31.75	0
17	4.25
11	0.75
13.75	0
14.5	0
31	0
20	3.5
33.75	0
37	0
8.5	0
19.25	0
28.25	0
18.5	11.25
5.75	0

This study was followed by a special practice training course for native Saudian young men in truffle orchard managementduring the year 2019 and 2020. Cultivation of oak seedlings inoculated with truffles results in increasing green areas and brings new economic opportunities for the Saudian rural areas. Truffle cultivation in Saudi Arabia and in similar tropical regions is a non-traditional agricultural practice, but it can be adopted as a profitable agricultural and sustainable agro-industry which uses less water than agricultural crops. This work will provide leadership to the Saudian farmers and landowners in the cultivation of high-value European truffles which will strengthen rural economy and support Saudi vision 2030 by enhancing food and water security.

Conclusion

This work evaluated the possibility of growing oak seedlings inoculated with *T. melanosporum* in the desert climate of Saudi Arabia. The first European truffle orchard for *T. melanosporum* was established in Saudi Arabia in 2018 to study oak seedlings survival, growth, and truffle ectomycorrhizae development in a semi-arid environment with long summers and high soil temperatures. Various plantation and irrigation practices were used to promote the growth of the seedlings. The studied orchard in an arid region of Saudi Arabia suggests that the truffle cultivation is possible in extreme desert conditions. Although the obtained results are promising, truffle cultivation technique could be further improved with appropriate mulching, soil liming and with the selection of plant and truffle proveniences looking for genotypes more adapted to extreme dry-hot conditions. This work opens up an avenue to produce *T. melanosporum* and other truffle species in Saudi Arabia as well as adds to the employment of young individuals who can be trained in the practices associated with the growth and maintenance of oak orchards and truffles. The establishment of this orchard and possible cultivation of truffles is expected to open a new avenue with economic safety. The truffle orchard is the first *T. melanosporum* orchard in the Gulf Cooperation Council (GCC) countries.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

References

Agerer R, Rambold G (2004) DEEMY—an information system for characterization and determination of ectomycorrhizae. München, Germany. http://www.deemy.de Accessed on 7 July 2020

Águeda B, Torián LF, de Miguel AM, Peña FM (2010) Ectomycorrhizal status of a mature productive black truffle plantation. For Syst 19:89–97. https://doi.org/10.5424/fs/2010191-01170

Arend M, Kuster T, Günthardt-goerg MS, Dobbertin M (2011) Research paper : part of a special section on adaptations of forest ecosystems to air pollution and climate change Provenance-specific growth responses to drought and air warming in three European oak species (*Quercus robur, Q. petraea and Q. pubescen*). Tree Physiol 31:287–297. https://doi.org/10.1093/treephys/tpr004

Bonet JA, Oliach D, Fischer C, Olivera A, de Aragon JM, Colinas C (2009) Cultivation methods of the black truffle, the most profitable mediterranean non-wood forest product; a state of the art review. In: EFI proceedings, Palahi M, Birot Y, Bravo F, Gorriz E (eds) Modelling, valuing and managing mediterranean forest ecosystems for non-timber goods and services, vol 57, pp 57–71

Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Mol Ecol 19:4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bouzadi M, Grebenc T, Turunen O, Kraigher H, Taib H, Alafai A, Sbissi I, Assad MEH, Bedade D, Shamekh S (2017) Characterization of natural habitats and diversity of Libyan desert truffles. 3 Biotech 7(5):328. https://doi.org/10.1007/s13205-017-0949-5

Büntgen U, Egli S, Camarero JJ, Fischer EM, Stobbe U, Kauserud H, Tegel W, Sproll L, Stenseth NC (2012) Drought-induced decline in Mediterranean truffle harvest. Nat Clim Chang 2(12):827–829

Burt R (2009) Soil survey field and laboratory methods manual, United States Department of Agriculture, Soil Survey Staff, Natural Resource Conservation Services

Ducousso A, Bordacs S (2004) EUFORGEN technical guidelines for genetic conservation and use for pedunculate and sessile oaks (*Quercus robur* and *Q. petraea*). International Plant Genetic Resources Institute, Rome

Erland S, Söderström B, Andersson S (1990) Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. II. Growth rates in pure culture at different pH values compared to growth rates in symbiosis with the host plant. New Phytol 115(4):683–688

García-Montero LG, Casermeiro MA, Hernando J, Hernando I (2006) Soil factors that influence the fruiting of *Tuber melanosporum* (black truffle). Soil Res 44(8):731–738

Gioacchini AM, Menotta M, Bertini L, Rossi I, Zeppa S, Zambonelli A, Piccoli G, Stocchi V (2005) Solid-phase microextraction gas chromatography/mass spectrometry: a new method for species identification of truffles. Rapid Commun Mass Spectrom Int J Devoted Rapid Dissem Minute Res Mass Spectrom 19(17):2365–2370. https://doi.org/10.1002/rcm.2031

Giovannetti G, Roth-Bejerano N, Zanini E, Kagan-Zur V (1994) Truffles and their cultivation. Hortic Rev 16:71–107

Granetti B (2005) Miglioramento e Rinnovamento Delle Tartufaie. In: Granetti B, De Angelis A, Materozzi G (eds) Umbria Terra Di Tartufi. Umbriagraf, Terni

Grebenc T, Wei J, Unuk T, Sulzbacher MA, Jabeen S, Khalid AN, Karadelev M (2019) Hypogeous fungi (truffles) diversity and cultivation at the upper timber line. In: PAM 2019 book of abstracts. International conference polar and alpine microbiology, Hamilton, New Zealand, p 16. https://www.confer.nz/pam2019/wp-content/uploads/2019/02/Pam-2019-book-of-abstracts.pdf

Guerin-Laguette A, Cummings N, Hesom-Williams N, Butler R, Wang Y (2013) Mycorrhiza analyses in New Zealand truffières reveal frequent but variable persistence of *Tuber melanosporum* in coexistence with other truffle species. Mycorrhiza 23:87–98. https://doi.org/10.1007/s00572-012-0450-2

Hall IR, Zambonelli A, Primavera F (1998) Ectomycorrhizal fungi with edible fruiting bodies 3. *Tuber magnatum* Tuberaceae. Econ Bot 52:192–200. https://doi.org/10.1007/BF02861209

Hall IR, Yun W, Amicucci A (2003) Cultivation of edible ectomycorrhizal mushrooms. Trends Biotechnol 21:433–438

Hall IR, Brown G, Zambonelli A (2007) Taming the truffle. The history, lore, and science of the ultimate mushroom. Timber Press, Portland, p 304

Hall IR, Fitzpatrick N, Miros P, Zambonelli A (2017) Counter-season cultivation of truffles in the southern hemisphere: an update. Ital J Mycol 46:21–36. https://doi.org/10.6092/issn.2531-7342/6794

Harki E, Bouya D, Dargent R (2006) Maturation-associated alterations of the biochemical characteristics of the black truffle *Tuber melanosporum* Vitt. Food Chem 99(2):394–400. https://doi.org/10.1016/j.foodchem.2005.08.030

Jaillard B, Oliach D, Sourzat P, Colinas C (2016) Soil characteristics of *Tuber melanosporum* habitat. True truffle (*Tuber* spp.) in the world. Springer, Cham, pp 169–190

Khanaqa A (2006) Truffle production in the Kingdom of Saudi Arabia—potential and limitation. J Appl Bot Food Qual 80:14–18

Kirk PM, Cannon PF, Stalpers J, Minter DW (2008) Dictionary of the Fungi, 10th edn. CABI, Wallingford

Marozzi G, Sánchez S, Benucci GMN, Bonito G, Falini LB, Albertini E, Donnini D (2017) Mycorrhization of pecan (*Caryaillinoinensis*) with black truffles: *Tuber melanosporum* and *Tuber brumal*. Mycorrhiza 27:303–309. https://doi.org/10.1007/s00572-016-0743-y

Mello A, Murat C, Bonfante P (2006) Truffles: Much more than a prized and local fungal delicacy. FEMS Microbiol Lett 260:1–8. https://doi.org/10.1111/j.1574-6968.2006.00252.x

Miros V, Miros P, Hall I, Zambonelli A (2016) Black truffle production in South Africa. Poster presented at the 8th international workshop on edible mycorrhizal mushrooms (IWEMM), 10–17 Oct 2016, Cahors, France

Montecchi A, Sarasini M (2000) Funghiipogeid'Europa. Fondazione Centro StudiMicologici dell' A.M.B, Vicenza, p 714

Murat C (2014) Forty years of inoculating seedlings with truffle fungi: past and future perspectives. Mycorrhiza 25:77–81. https://doi.org/10.1007/s00572-014-0593-4

Oliach D, Pere M (2012) Estuditècnicieconòmic del cultiu de la tòfona. Silvicultura 66:8–10 (**in Catalan**)

Olivera A, Fischer CR, Bonet JA, De Aragón JM, Oliach D, Colinas C (2011) Weed management and irrigation are key treatments in emerging black truffle (*Tuber melanosporum*) cultivation. New For 42(2):227–239. https://doi.org/10.1007/s11056-011-9249-9

Olivera A, Bonet JA, Palacio L, Liu B, Colinas C (2014) Weed control modifies *Tuber melanosporum* mycelial expansion in young oak plantations. Ann For Sci 71(4):495–504

Ori F, Leonardi M, Faccio A, Sillo F, Iotti M, Pacioni G,Balestrini R (2020) Synthesis and ultrastructural observation of arbutoid mycorrhizae of black truffles (*Tuber melanosporum* and *T. aestivum*). Mycorrhiza (submitted)

Peterson RL, Bonfante P (1994) Comparative structure of vesicular-arbuscular mycorrhizas and ectomycorrhizas. Plant Soil 159:79–88. https://doi.org/10.1007/BF00000097

Reyna S, Garcia-Barreda S (2014) Black truffle cultivation: a global reality. For Syst 23:317–328. https://doi.org/10.5424/fs/2014232-04771

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity: separating the wheat from the chaff. Fungal Divers 33:1–45

Rubini A, Belfiori B, Riccioni C, Arcioni S, Martin F, Paolocci F (2011) *Tuber melanosporum*: mating type distribution in a natural plantation and dynamics of strains of different mating types on the roots of nursery-inoculated host plants. New Phytol 189(3):723–735

Shamekh S, Grebenc T, Leisola M, Turunen O (2014) The cultivation of oak seedlings inoculated with *Tuber aestivum*Vittad. in the boreal region of Finland. Mycol Prog 13:373–380. https://doi.org/10.1007/s11557-013-0923-5

Streiblová E, Gryndlerová H, Valda S, Gryndler M (2010) *Tuber aestivum*—hypogeous fungus neglected in the Czech Republic. A review. Czech Mycol 61:163–173. https://doi.org/10.33585/cmy.61205

Strojnik L, Grebenc T, Ogrinc N (2020) Species and geographic variability in truffle aromas. Food Chem Toxicol 142:111434. https://doi.org/10.1016/j.fct.2020.111434

Sulzbacher MA, Hamann JJ, Fronza D, Jacques RJS, Giachini AJ, Grebenc T, Antoniolli ZI (2019) Fungosectomicorrízicosemplantações de nogueira-pecã e o potencial da truficultura no Brasil. CiênciaFlorestal 29(2):975–987

Thomas P (2014) An analysis of the climatic parameters needed for *Tuber melanosporum* cultivation incorporating data from six continents. Mycosphere 5(1):137–142

Wang S, Marcone MF (2011) The biochemistry and biological properties of the world's most expensive underground edible mushroom: truffles. Food Res Int 44:2567–2581. https://doi.org/10.1016/j.foodres.2011.06.008

Wang H, Rogers JC, Munroe DK (2015) Commonly used drought indices as indicators of soil moisture in China. J Hydrometeorol 16(3):1397–1408

Wedén C, Chevalier G, Danell E (2004) *Tuber aestivum* (syn.T.uncinatum) biotopes and their history on Gotland, Sweden

. Mycol Res 108:304–310. https://doi.org/10.1017/S0953756204009256

Zambonelli A, Salomoni S, Pisi A (1995) Caratterizzazioneanatomo-morfologica e micromorfologicadellemicorrize di *Tuber borchii* Vitt., *Tuber aestivum* Vitt., *Tuber mesentericum* Vitt., *Tuber brumale* Vitt. *Tuber melanosporum* Vitt. su *Pinus pinea* L. MicologiaItaliana 24(2):119–137

Zambonelli A, Iotti M, Murat C (eds) (2016) True truffle (*Tuber* spp.) in the world: soil ecology, systematics and biochemistry, vol 47. Springer, Cham