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Importance of *Picoa* spp. as Desert Truffles Fungi

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Birds' truffles; *Picoa* spp. have been the topic of few reviews, they are considered between desert truffles which are a valuable food and used as medicine in Arabian Gulf countries including Saudi Arabia and several other countries of the world. Recently, many researchers have demonstrated that desert truffles including bird's truffles are source of important nutritional elements. Also, present biological activities such as antibacterial and antioxidant activities, have a kind of medicinal properties and used in folk medicine. Meanwhile, birds' truffles were not investigated for its "cultivation", ecology, taxonomy, physiology, edibility, medicinal properties as well as antimicrobial activities. Rare studies of bird truffles, could be found in literatures, concerning geographic distribution and molecular studies which were achieved to elucidate the ambiguity of the classification of bird's truffles; this with the help of the advanced recent techniques in molecular biology. In this present article, we compile recent data on the importance of *Picoa* spp. as desert truffles fungi. The *Picoa* can play a significant role in biological control agents, provide food for birds, decomposition of the fruit-bodies of *Picoa* in the soils can improve the physical, chemical and biological conditions of the soils that will improve their ecological conditions.

Keywords: Desert truffles; *Picoa* spp.; Importance; Saudi Arabia.

Picoa spp. called "Birds' truffles" are considered as desert truffles. It is valuable as food and used as medicine in Arabian Gulf countries including Saudi Arabia and several other countries of the world. Truffles, in general, have been considered for centuries as valuable food due to their high nutritional value[1]. As it is well known, desert truffles are a natural source for several chemical components such as proteins, amino acids, vitamins, flavor compounds, sterols, terpenes, fatty acids; they are also rich in minerals and carbohydrates[2,3,4,5]. Truffles were usually used to make typical food in several cultures, while desert truffles are highly valued by people principally in Middle East and north of Africa[6-7]. In addition, desert truffles were widely used in traditional medicine due to their pharmaceutical properties and medicinal value. *Picoa* spp. cited in all of the countries of Mediterranean basin including Saudi Arabia and Iran. It can be found in a gypsiferous and calcareous gravelly deserts soil and the fruiting bodies appears from January to April in Kuwait[8], while in Saudi Arabia appears from November to March[9], these periods is directly related to climatic conditions. During the fruiting season, Ascomata of *Picoa* spp. can indirectly noticed by remarking the surface of the soil which appears cracked or convex, this modification of soil surface and the presence of *Helianthemeum* spp. confirm that the ascomata of *Picoa* spp. are in

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the remarked place. In addition, the mutual relationship between desert truffles and *Helianthemum* sp. had been proved, via Isotopes tracer $^{14}CO_2$ aided research[10]. In comparison with desert truffles, birds' truffles have not been sufficiently studied; few research works concerning *Picoa* spp. were highlight ecological importance, distribution as well as the taxonomy. In this present article, we compile recent data on the importance of *Picoa* spp. as desert truffles fungi.

Occurrence of desert truffles

The desert truffles grows principally in countries around the Mediterranean basin, they have been reported by many scientists in countries of Southern Europe including Spain and Portugal[11-12], France[13], Italy and France[14], Hungary[15], Turkey[16], they have been also reported in North Africa that extends from Mauritania to Egypt[17,18,19,20,21], Middle East[22,23,24,25] and Qatar[26]. However, some species of desert truffles were also found in Botswana, South Africa[7,27,28] and in North America[29]. Regarding the morphology of desert truffles, several types have been collected in Africa and in the Middle East region. They have been evaluated by numerous scientists. Some wild macro-fungi species were reported in Saudi Arabia, Bahrain, and Egypt such as *Terfezia claveryi* and *Tirmania nivea*[4,5,30,31,32]. Others types of desert truffles, such as *Choiromyces echinulatus, Terfezia pfeilii* and *Kalaharituber pfeilii* have been also reported in South Africa[33]. Furthermore, various types were found in Tunisia such as *Picoa juniperi, Picoa lefebvrei* and *Terfezia* sp.[34]. Moreover, five types of desert truffles were found and identified in different parts of Iran such as *T. claveryi, T. nivea, Tirmania pinoyi, P. juniperi* and *P. lefebvrei*[35].

Hosts plants of desert truffles

Desert trufûes have a mycorrhizal association with numerous annual and perennial xerophytic host plants that belongs to the Cistaceae family. They form mycorrhizae mainly on roots of different species of the genus *Helianthemum* sp.[10], or *Cistus* sp. and *Helianthemum* sp.[36]. The type of this association between desert truffles and *Helianthemum* sp. is function to culture conditions[36], and function to phosphorus content culture medium in sterile conditions; an ectomycorrhiza in high phosphorus level and ectendomycorrhiza in culture lacking phosphorus or an endomycorrhiza present in deficient level of available phosphorus[37]. Interestingly, scientists have successfully obtained an endomycorrhiza between *Helianthemim* spp with *Terfezia* and *Tirmania* spp.[38,39] which were called few years later as "helianthemoid" mycorrhizae specific to this type of mycorrhizal association which is firstly described by Alsheikh[40]. The plasticity of desert truffles to form different mycorrhizal association types have been demonstrated recently by Zitouni-Haouar *et al.*[41] when they inoculated in *in vivo* conditions six *Cistaceae* species with three *Terfezia* species: *Terfezia leptoderma, Terfezia boudieri,* and *T. claveryi*. In fact, typical endomycorrhizae was formed in *Helianthemum ledifolium, Helianthemum lippii* and *Fumana procumbens* whereas an ectomycorrhiza was synthesized in *Cistus* species (*C. albidus, C. incanus, C. salvifolius*) and *Pinus halepensis* (Aleppo pine) in green house conditions.

Ecological importance

This association plays an important role in the maintenance of Mediterranean shrub lands and grasslands, furthermore, they help prevent erosion and desertification[42], and have an ecological and economical interest not only because they can be an alternative agriculture yield in arid and semi-arid lands but also because of their high price on the market[43], also can have positive effect on sustainability and biodiversity[44] and may play a considerable role in eco-tourism in arid and semi-arid regions.

Cultivation and conservation of desert truffles

Cultivation of truffles in arid and semi-arid lands was studied by different authors, but certain were focused their work on cultivation and fruiting of *T. boudieri* Chatin in semi controlled conditions (green house) and *in situ*, by inoculating of *Helianthemum sessiliflorum* Desf. Pers. using two type of soil; gypsy and sandy soil[45], the results of this research are encouraging to turn toward "cultivation" of desert truffles. Cultivation of desert truffles may represent *ex situ* conservation[46], despite a specific cares should be followed in this activities. Evaluation of soil and environmental characteristics of the plantation site are crucial, especially in semi-arid areas where climate conditions are critical[47,48]. *Ex situ* conservation of desert truffles can constitute a new branch of

ecotourism for local population in Middle East and north of Africa. In this scope Saudi Wildlife Authority which manages several natural reserves in Saudi Arabia takes, every year, an action to organize desert truffles collecting inside the natural reserve of Harrat Al Harrah in the northern region of Saudi Arabia, in a way to preserve fungal, flora and fauna of this reserve[49].

Few studies have been conducted to characterize the mycorrhizae of desert truffles cultivated with their host plants under semi-controlled conditions in green house. In fact, the first attempt to cultivate desert truffles in the western desert in vivo was performed in Iraq[10]. Actually, they simulated desert condition to cultivate desert truffles and investigate their relationship with *Helianthemum* herbs. Interestingly, Alrawi and co-workers have demonstrated that some critical factors are essential to improve desert truffles production such as soil composition and texture, moisture contents, temperature, as well as the type of water and vegetal flora. Moreover, they have also tested the effect of irrigation on the growth of desert truffles, surprisingly, irrigation test was succeeded and all irrigated areas have produced desert truffles. On the other hand, the research of at Arar city constitute an installation of a system of drip irrigation in a specific locality; these localities produce, naturally, desert truffles. Irrigation of these selected plots in autumn has a positive effect, qualitatively an quantitatively, on the production of desert truffles ascomata[50]. Additionally, the importance of irrigation factor for successful cultivation has been reported, also, by Morte *et al*.,[51]. Others, demonstrated that irrigation should be applied twice during the season, one time at the end of the summer especially when rainfall is less than 150 mm and the second time during the fruiting season in dry period of the year[52]. Several *Helianthemum* species such as *H. salicifolium* and *H. ledifolium* have been inoculated by different species of desert truffles in the conditions of green house[10].

***Picoa* spp.**

Desert truffles include several valuable hypogeous macrofungus species; few scientists have been interested in discovering and studying new desert truffles species, especially the genus *Picoa* spp. which belong to Ascomycota (Pyronemataceae, Pezizales). Several *Picoa* species have been reported in arid and semi-arid ecosystem especially in Tunisia, Saudi Arabia, and Iran. Table1 present few examples of *Picoa* species reported in Africa and Middle East.

Actually, *P. juniperi* and *P. lefebvrei* are not, traditionally, the most highly appreciated desert truffles in Middle East and north of Africa. Because fruit-bodies of *Picoa* are very small and are not collected for this reason, in spite of their edibility. A recent field study[58], showed that drip irrigation of naturally producing plot of *Picoa* spp. in Muayala Natural Reserve near Arar city at the north-east of Saudi Arabia, has permit to obtain for the first time of relatively big ascomata of *Picoa* spp. with a diameter reach to 7.5 cm. The presence of *Phaeangium lefebvrei* in the northern area of Saudi Arabia has been confirmed by[57]. Also, others studies revealed for the first time the presence of *P. lefebvrei* in the eastern area of northern borders province in Saudi Arabia[9].

Classification

Classification of desert truffles including birds' truffles was based on morphological characters such as morphology of ascomata, spore, peridium, sporocarp odor, as well as color of the gleba. Classification of *Phaeangium* was the subject of different opinion. In taxonomical point of view, *Picoa lefebvrei* was firstly descried and grouped in the genus *Phaeangium*; *P. lefebvrei* Pat.[59]. Current name: *Picoa lefebvrei* (Pat.) Maire[60]. Also, the same name was adopted by[61,62], other synonym: *Terfezia schweinfurthii* Henn. after Gücin *et al*.[55]. While *Phaeangium* was placed temporarily in Peronymataceae[63]. Classification based on morphological features is common and

Table 1. Examples of *Picoa* species reported in Africa and Middle East

Truffles	Countries	References
Picoa juniperi	Tunisia	[34]
Picoa lefebvrei		
Picoa lefebvrei	Turkey	[53]
Picoa juniperi		
Picoa Vittad.		[55]
Picoa lefebvrei	Saudi Arabia	[9]
"*Phaeangium lefebvrei*"		[57]
Picoa lefebvrei	Iran	[54]
Picoa spp.		[56]

used till these days. Spores in *Phaeangium* are ornamented at maturity and a have tomentose peridium, whereas *Picoa juniperi* has smooth spores and no tomentum. *Phaeangium* and its single species, *P. lefebvrei*, are re-described and placed in the family Pyronemataceae, tribe Mycolachneae[22]. Actually, classification basing on morphological characters is a complementary approach but, at the same time, it is difficult and not sufficient to differentiate two close species. This may be attributed to the effect environmental factors and various ecological habitat conditions on morphological characters[64].

Recent studies have been carried out to revise the classification of *Picoa* using molecular methods; the obtained results showed that most morphological character used in classifications of *Picoa* were not sufficient to study phylogenetic relationship and don't provide phylogenetic Information, whereas molecular markers especially Internal transcribed spacer (ITS), were principally phylogenetically informative and useful to separate the different lineages in *Picoa*, also in addition, geographical and ecological, rather than morphological data, are most useful character for separation of these lineages[65]. Other phylogenetic analyses demonstrate that *Picoa lefebvrei* belongs to the *Geopora–Tricharina* clade of *Pyronemataceae* family. In fact *Phaeangium lefebvrei* was placed in *Picoa* Vittad. due to its close genetic relationship with *P. juniperi* Vittad.[65,66].

Chemical composition of *Picoa*

Chemical composition and nutritional value of several desert truffles and *Picoa* spp. were studied and evaluated by numerous reviewers and researchers[67,68,69,70]. Precisely, other authors[68] have studied the composition of desert truffles "birds' truffle" *Phaeangium lefebvrei* Pat.; they reported that fruit-bodies are rich in minerals and contain 23% total protein (% dry weight), 18% total carbohydrates, 1% crude fat and 3% total crude fibers, and a total of 26 amino and 11 fatty acids, this can give us an indication to take care of these neglected desert truffle fungi which can participate in high quality of human nutrition.

Importance of *Picoa* spp.

Species belonging to *Picoa* spp. are not extremely appreciated by local people in Middle East and Africa, in spite of their nutritional value[2], antioxidant activity[71] and medicinal properties[72] but also for their important role in prevention of erosion and desertification. It has good chemical composition suitable for human health like other types of desert truffles. In comparison to desert truffles, the *Picoa* spp. does not have tremendous values and economic importance. The interesting study is, which confirm the importance of birds' truffles; *P. lefebvrei* and *P.juniperi* as antibacterial agent against the development or the inhibition of several bacteria; *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans*, *Preteus vulgaris*, *Salmonella typhi*, and same effects were observed against the yeast; *Candida tropicalis* and also against the dermatophyte *Trichophyton* sp.[72]. The *Picoa* can play a significant role in biological control agents, which may reduce the harmful organisms in the soils. Also, can provide food for birds, the fruiting bodies which appear partially on the surface of the soil are eaten by birds[8,73]. They were sought out by migratory birds and have been used by Bedouin as bait in bird traps[74]. Interestingly, *P. lefebvrei* were generally used in folk medicine to treat people infectious eye diseases[71]. By decomposition of the *Picoa* in the soils, it may improve physical, chemical and biological conditions of the soils that directly or indirectly improve the ecological conditions of the soils and environment.

CONCLUSION

Further studies are needed to develop molecular probes for desert truffles including birds' truffles identification, and more researches are also required to study their biodiversity and to understand in a better way the physiology of these appreciated fungi in Middle East and north of Africa. In additions, international legislations need to be developed to regulate trading of desert truffles. These multiple actions may advance our scientific knowledge on desert truffles in arid and semi-arid ecosystems, and can play as a crucial element to improve natural production including preservation in their habitat. Also, these actions will indirectly help local population to realize the concept of eco-tourism. A special attention need to be focused on developing natural production, and why not to move to the "production of desert

truffles" via controlled mycorrhization of annual or perennial associative desert plant, to meet the increasing demand of these highly valued hypogeous fungi.

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REFERENCES

1. Bokhary, H.A., Suleiman, A.A.A., Basalah, M.O., Parvez, S. Chemical Composition of Desert Truffles from Saudi Arabia. *Canadian Institute of Food Science and Technology Journal*, 1987; **20**(5): 336-341.
2. Bokhary, H.A., Parvez, S. Chemical composition of desert truffles *Terfezia claveryi*. *Journal of Food Composition and Analysis*, 1993; **6**(3): 285-293.
3. Kalaè, P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, 2009; **113**(1): 9-16.
4. Omer, E.A., Smith, D. L., Wood, K. V., El-Menshawi, B. S. The volatiles of desert truffle: *Tirmania nivea*. *Plant Foods for Human Nutrition*, 1994; **45**(3): 247-249.
5. Hussain, G., Al-Ruqaie, I.M. Occurrence, chemical composition, and nutritional value of truffles: an overview. *Pak. J. Biol. Sci*., 1999; **2**(2): 510-514.
6. El Enshasy, H., Elsayed, E.A., Aziz, R., Wadaan, M.A. Mushrooms and truffles: historical biofactories for complementary medicine in Africa and in the Middle East. *Evidence-Based Complementary and Alternative Medicine*, 2013; 1-10. http://dx.doi.org/10.1155/2013/620451.
7. Trappe, J.M., Claridge, A.W., Arora, D., Smit, W.A. Desert truffles of the African Kalahari: Ecology, Ethnomycology, and Taxonomy. *Economic Botany*, 2008; **62**(3): 521-529.
8. Alsheikh, AM., Trappe, J.M. Taxonomy of *Phaeangium lefebvrei*, a desert truffle eaten by birds. *Canadian Journal of Botany*, 1983; **61**(7): 1919-1925. DOI: 10.1139/b83-204.
9. Bawadekji, A., Gargano, M.L., Saitta, A., Venturella, G. A new record of the desert truffle *Picoa lefebvrei* in Saudi Arabia. *Mycotaxon*, 2012; **122**(1): 243-247.
10. Alrawi, A.M., Mohy Aldin, M. New mycorrhizal identification, truffles cultivation, and truffles radiation preservation. *Radiation Physics and Chemistry*, 1979; **14**(3): 759-767. DOI:10.1016/0146-5724(79)90111-0.
11. Moreno, G., Diez J., Manjón J.L. *Picoa lefebvrei* and *Tirmania nivea*, two rare hypogeous fungi from Spain. *Mycological Research*, 2000; **104**(03): 378-381.
12. http://www.trufamania.com/desert-truffles.htm
13. Riousset, L., Riousset, G., Jalade, M., Chevalier, G. Prima raccolta in Francia di *Phaeangium lefebvrei* Patouillard. *Il Fungo*, 1989; **3**: 9-11.
14. Janex-Favre, M.C., Parguey-Leduc, A., Riousset, L. L'ascocarpe hypoge d'une terfez française (Terfezia leptoderma Tul., Tuberales, Discomycetes). *Bull. Soc. Mycol. France*, 1988; **104**: 145–178.
15. Ł awrynowicz, M., Markowiæ, M., Milenkoviæ, M., Ivanæeviæ, B. *Terfezia terfezioides* - a new hypogeous fungus for Balkan Peninsula. *Acta Mycologica*, 1997; **32**(2): 233-238.
16. Gücin, F., Dülger, B. The researches on the keme truffle (*Terfezia boudieri* Chatin) that is edible and showing antimicrobial activities. *Ekoloji*, 1997; **23**: 27-33.
17. Malençon, G. Champignons hypogés du nord de l'Afrique I. Ascomycetes. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 1973; **7**(2): 261-279.
18. Bokhary, H.A. Desert truffles 'Al-Kamah' of the kingdom of Saudi Arabia. 1. Occurrence, identification and distribution. *Arab Gulf J. Scient. Res. Agric. Biol. Sci*., 1987; **B5**(2): 245-255.
19. Khabar, L., Najim, L., Janex-Favre, M.C., Parguey-Leduc, A. Contribution à l'étude de la flore mycologique du Maroc. Les truffes marocaines (Discomycètes). *Bulletin trimestriel de la Société mycologique de France*, 2001; **117**(3): 213-229.
20. Slama, A., Fortas, Z., Neffati, M., Khabar, L., Boudabous A. Etude taxinomique de quelques Ascomycota hypogés (Terfeziaceae) de la Tunisie méridionale. *Bulletin trimestriel de la Société mycologique de France*, 2006; **122**(2-3): 187-195.
21. Fortas, Z. Diversité des espèces de terfez (truffes de sables) des zones arides algériennes. Oral Presentation at *International Conference EcoSys-09*, University of Ouargla, Algeria, 13–15 December 2009.
22. Alsheikh, AM., Trappe, J.M. Desert truffles: the genus *Tirmania*. *Transactions of the British Mycological Society*, 1983; **81**(1): 83-90.
23. Al-Ruqaie, I.M. Effect of different treatment

processes and preservation methods on the quality of truffles: I. Conventional methods (drying/freezing). *Journal of Food Processing and Preservation*, 2006; **30**(3): 335-351. DOI: 10.1111/j.1745-4549.2006.00069.x.

24. Bawadikji, A.H. 2004. La Truffe de Désert en Syrie: Aspects Ecologiques et Economiques. *Premier Symposium Sur Les Champignons Hypogés du Bassin Méditerranéen*. Université Mohammed V, Faculté des Sciences, Rabat, Maroc. 6-8 Avril, 2004.
25. Mandeel, Q.A., Al-Laith, A.A.A. Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. *Journal of Ethnopharmacology*, 2007; **110**(1): 118-129.
26. Al-Thani, Roda F. Survey of Macrofungi (including Truffles) in Qatar. *KBM Journal of Biology*, 2010; **1**(2): 26-29. DOI: 10.5147/kbmjb.2010.0009.
27. Marasas, W.F.O., Trappe, J.M. Notes on southern African Tuberales. *Bothali*a, 1973; **11**(1&2): 139-141. DOI: 10.4102/abc.v11i1&2.1994.
28. Kagan-Zur, V., Roth-Bejerano, N., Sitrit, Y., Morte, A. Desert truffles: phylogeny, physiology, distribution and domestication. 2013 (Vol. 38). Springer, DOI 10.1007/978-3-642-40096-4.
29. Trappe, J.M., Sundberg, W.J. *Terfezia gigantea* (Tuberales) in North America. *Mycologia*, 1977; **69**: 433-437.
30. Janakat, S., Al-Fakhiri, S.M., Sallal, A.K. A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus In vitro*. *Phytotherapy Research*, 2004; **18**(10): 810-813.
31. Al-Laith, A.A.A. Antioxidant components and antioxidant/antiradical activities of desert truffle (*Tirmania nivea*) from various Middle Eastern origins. *Journal of Food Composition and Analysis*, 2010; **23**(1): 15-22.
32. Hyde, K.D., Bahkali, A.H., Moslem, M.A. Fungi—an unusual source for cosmetics. *Fungal diversity*, 2010; **43**(1): 1-9.
33. Ferdman, Y., Aviram, S., Roth-Bejerano, N., Trappe, J.M., Kagan-Zur, V. Phylogenetic studies of *Terfezia pfeilii* and *Choiromyces echinulatus* (Pezizales) support new genera for southern African truffles: Kalahari tuber and Eremiomyces. *Mycological research*, 2005; **109**(02): 237-245.
34. Sbissi, I., Neffati, M., Boudabous, A., Murat, C., Gtari, M. Phylogenetic affiliation of the desert truffles *Picoa juniperi* and *Picoa lefebvrei*. *Antonie van Leeuwenhoek*, 2010; **98**(4): 429-436.
35. Jamali, S., Banihashemi, Z. Hosts and distribution of desert truffles in Iran, based on morphological and molecular criteria. *J. Agric. Sci. Technol*., 2012; **14**: 1379-1396.
36. Gutiérrez, A., Morte, A., Honrubia, M. Morphological characterization of the mycorrhiza formed by *Helianthemum almeriense* Pau with *Terfezia claveryi* Chatin and *Picoa lefebvrei* (Pat.) Maire. *Mycorrhiza*, 2003; **13**(6): 299–307.4: 111-114.
37. Fortas, Z., Chevalier, G. Effet des conditions de culture sur la mycorhization de *l'Helianthemum guttatum* par trois espèces de terfez des genres *Terfezia* et *Tirmamia* d'Algérie. *Can. J. Bot.*, 1992; **70**: 2453–2460.
38. Awameh, M.S., Alsheikh, AM., Al-Ghawas, S. Mycorrhizal synthesis between *Helianthemum ledifolium*, *H. salcifolium* and four species of the genera *Terfezia* and *Tirmania* using ascospores and mycelial cultures obtained from Ascospore germination. In: *Proceedings of the 4th North American Conference on Mycorrhizae*, Colorado State University, Fort Collins, Colorado, 1979; pp 23–23.
39. Awameh, M.S. The response of *Helianthemum salicifolium* and *H. ledifolium* to infection by the desert truffle *Terfezia boudieri*. Mush. Sci., 1981; **11**: 843–853.
40. Alsheikh, AM. Mycorrhizae of annual *Helianthemum* species formed with desert truffles. *Proc. NACOM6*, 1984.
41. Zitouni-Haouar, FE-H., Fortas, Z., Chevalier, G. Morphological characterization of mycorrhizae formed between three Terfezia species (desert truffles) and several Cistaceae and Aleppo pine. *Mycorrhiza*, 2014; **24**(5): 397-403. DOI: 10.1007/s00572-013-0550-7.
42. Honrubia, M., Cano, A., Molina-Ninirola, C. Hypogeous fungi from southern Spanish semi-arid lands. *Persoonia-Molecular Phylogeny and Evolution of Fungi*, 1992; **14**(4): 647-653.
43. Lefevre, C. Truffles and truffle cultivation in North America. In: *Proceedings of the 3rd International Congress on Truffles*, Spoleto, 2008; pp: 25-28.
44. Al-Qarawi, A.A., Mridha M.A.U. Status and Need of Research on Desert Truffles in Saudi Arabia. *Journal of Pure and Applied Microbiology*, 2012; **6**(3): 1051-1062.
45. Slama, A., Fortas, Z., Boudabous, A., Neffati, M. Cultivation of an edible desert truffle (*Terfezia boudieri* Chatin). *African Journal of Microbiology Research*, 2010; **4**(22): 2350-2356. ISSN 1996-0808 ©2010 Academic Journals.
46. Varese, G.C., Angelini, P., Bencivenga, M.,

Buzzini, P., Donnini, D., Gargano, M.L., Maggi, O., Pecoraro, L., Persiani, A.M., Savino, E., Tigini, V., Turchetti, B., Vannacci, G., Venturella, G., Zambonelli, A. *Ex situ* conservation and exploitation of fungi in Italy. *Plant Biosyst*., 2011; **145**: 997–1005. http://dx.doi.org/10.1080/11263504.2011.633119.

47. Benucci, G.M.N., Bonito, G., Baciarelli-Falini, L., Bencivenga, M., Donnini D. Truffles, timber, food and fuel: Sustainable approaches for multi-cropping truffles and economically important plants. In: *Edible Ectomycorrhizal Mushrooms* (Zambonelli A, Bonito GM, ed.). Berlin, Heidelberg: Springer-Verlag, 2012; pp 265–280.
48. Donnini, D., Gargano, M.L., Perini, C., Savino, E., Murat, C., Di Piazza, S., Altobelli, E., Salerni E., Rubini, A., Rana, G.L., Bencivenga, M., Venanzoni, R., Zambonelli, A. Wild and cultivated mushrooms as a model of sustainable development. *Plant Biosyst*., 2013; **147**: 226–236. DOI:10.1080/11263504.2012.754386.
49. https://www.swa.gov.sa/en/protected-areas/harrat-al-harrah
50. Bawadekji, A. 2011. Success of Desert Truffles Production in Muayala Natural Reserve at Arar city KSA. *Al Riyadh Newspaper* n. 15663. http://www.alriyadh.com/2011/05/13/section.last.html.
51. Morte, A., Honrubia, M., Gutiérrez, A. Biotechnology and cultivation of desert truffles. In: *Mycorrhiza* (Springer Berlin Heidelberg), 2008; pp: 467-483. DOI: 10.1007/978-3-540-78826-3_23.
52. Morte, A., Zamora M., Gutiérrez, A., Honrubia M. Desert truffle cultivation in semiarid Mediterranean areas. In: *Mycorrhizas-Functional Processes and Ecological Impact* (Springer Berlin Heidelberg), 2009; pp: 221-233. DOI: 10.1007/978-3-540-87978-7_15.
53. Akyüz, M., Kýrbað, S., Bircan, B., Gürhan, Y. Diversity and distribution of arid-semi arid truffle (*Terfezia* and *Picoa*) in Elazýð-Malatya region of Turkey. *Mycosphere*, 2015; **6**(6): 766–783. DOI: 10.5943/mycosphere/6/6/11.
54. Jamali, S., Banihashemi, Z. Species-specific ITS primers for the identification of *Picoa juniperi* and *Picoa lefebvrei* and using nested-PCR for detection of *P. juniperi* in planta. *Molecular Biology Reports*, 2013; **40**(10): 5701-5712.
55. Gücin, F., Kaya, A., Soylu, M.K., Uzun Y. Picoa Vittad., a new truffle genus record for Turkey. *Biological Diversity and Conservation*, 2010; **3**(3): 23-25.
56. Ammarellou, A., Trappe, J.M. A first Ascomycete genus (*Picoa* sp.) record for the fungi flora of Iran. *Pakistan Journal of Biological Sciences*, 2007; **10**(10): 1772.
57. Bokhary, H.A., Parvez, S. Desert truffles 'Al-Kamah' of the kingdom of Saudi Arabia. 2. Additional Contribution. Arab *Gulf J. Scient. Res. Agric. Biol. Sci.*, 1988; **B6**(1): 103-112.
58. Bawadekji, A. 2016. *In situ* collecting of desert Truffles (Birds' truffles) *Picoa* spp. from drip irrigated plots in Muayala Natural Reserve near Arar city KSA. *https://youtu.be/aNVuoYkwPvE*
59. Patouillard, N.T. Les Terfez de la Tunisie. *Journal de Botanique* (*Morot*). 1894; **8**: 153-156.
60. Maire, R. Notes mycologiques. *Annales Mycologici*, 1906; **4**: 329-399.
61. http://www.mycobank.org/name/Phaeangium%20lefebvrei
62. http://www.gbif.org/species/5258497
63. LÆSSØE, T., HANSEN, K. Truffle trouble: what happened to the Tuberales? *Mycological Research*, 2007; III: 1075-1099. DOI:10.1016/j.mycres.2007.08.004.
64. Díez, J., Manjón, J.L., Martin, F. Molecular phylogeny of the mycorrhizal desert truffles (*Terfezia* and *Tirmania*), host specificity and edaphic tolerance. *Mycologia*, 2002; **94**(2): 247-259.
65. Zitouni-Haouar, FE-H., Alvarado, P., Sbissi, I., Boudabous, A., Fortas, Z., Moreno, G., Manjón, J.L., Gtari, M. Contrasted Genetic Diversity, Relevance of Climate and Host Plants, and Comments on the Taxonomic Problems of the Genus *Picoa* (Pyronemataceae, Pezizales). *PLoS ONE*, 2015; **10**(9):e0138513. DOI:10.1371/journal.pone.0138513.
66. Ammarellou, A., Smith, M.E., Tajick, M.A., Trappe, J.M. The phylogenetic placement of *Picoa*, with a first report on *Picoa lefebvrei* (Pat.) Maire (= *Phaeangium lefebvrei*) from Iran. *International Journal of Environmental Research*, 2011; **5**(2): 509-514.
67. Sawaya, W.N., Al-Shalhat, A., Al-Sogair, A., Al-Mohammad, M. Chemical composition and nutritive value of truffles of Saudi Arabia. *Journal of Food Science*, 1985; **50**(2): 450-453.
68. Bokhary, H.A., Parvez, S. Studies on the Chemical Composition of the Ascomycete Fungus *Phaeangium lefebvrei* Pat. *Journal King Saud Univ*., 1995; 7, Science, **2**: 215-224.
69. Wang, S., and M. F. Marcone. The biochemistry and biological properties of the world's most expensive underground edible mushroom: Truffles. *Food Research International*, 2011; **44**(9): 2567-2581.
70. Dundar, A., Farouk, O., Acay, H., Okumus V., Ozdemir S., Yildiz, A. Antioxidant properties, chemical composition and nutritional value of

Terfezia boudieri (Chatin) from Turkey. *Food Sci. Technol. Intl.*, 2012; **18**: 317-328.
71. Murcia, M.A., Martínez-Tomé, Jiménez, M., Vera, A.M., Honrubia, M., Parras, P. Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *Journal of Food Protection*, 2002; **65**(10): 1614-1622.
72. Akyüz, M., Kýrbað, S., Bircan, B. Medical Characteristics of Arid-Semi Arid Truffle (*Terfezia* and *Picoa*) in the Elazýð-Malatya region of Turkey. *Hacettepe J. Biol. Chem*., 2015; **43**(4): 301-308.
73. Castellano, M.A., Trappe, J.M., Luoma, D.L. Sequestrate fungi. In: *Biodiversity of Fungi. Inventory and Monitoring Methods* (Mueller GM, Bills GF, Foster MS, ed.). Elsevier, Amsterdam, 2004; pp 197–213.
74. https://www.anbg.gov.au/fungi/