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Review Article

Attacks on the precious truffles by useful and harmful invadors

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Abstract

Truffles are ectomycorrhizal mushrooms that grow in specific climates. They are rare and famous for their secondary metabolites and their promising biological activities. Truffles are considered from the most expensive macrofungi all over the world. Understanding the rare and uncommon types of interaction between truffles and their different invadors is of high significance. However, studies describing such relation are very limited. In this review, we describe general description and ecology of truffles. Examples on microbes attracted to colonize fruiting bodies of truffles were mentioned. Finally, factors causing truffles damage such as pathogenic fungal attacks, truffles rot, and insects' invasion were highlighted.

Keyword: truffles; disease; biological activities; fungi; insects

Introduction

Truffles are the fruiting bodies of edible fungi. These are known to possess high economic value among mushrooms mainly for their characteristic aroma and flavour profile (**Wang and Marcone, 2011**). Their flavours are celebrated in many European cuisines (**Zambonelli et al., 2016**). Mainly, over the past two decades, various species of truffles cultivated in different regions around the globe have been investigated for their nutritional and therapeutic potential (**Wang and Marcone, 2011**). The ever-growing human demands for new products, inclination towards natural products, and increasing health concerns have driven research in this field. Truffle extracts have been studied for the presence of bioactive components, and their manifestations of antioxidant, anti-infammatory, and anti-microbial properties in various in vitro model systems (**Beara et al., 2014**). Antimicrobial activities of desert ectomycorrhizal mushrooms (Truffles) have been investigated decades ago by many researchers who studied extracts of Terfezia claveryi which exhibited antiviral activity. (**Al-Marzooky, 1981; Hussain and Al-Ruqaie, 1999**). Ectomycorrhizal mushrooms (Truffles) contain a variety of biochemical components, including steroids, pheromones, flavonoide, anthocyanine, carotenoide, oligosaccharide and volatile organic compounds such as dimethyl-sulfide and bisulfide, butanedione, butyrate, hexenone, ethyl-, methyl and propylphenole and methylthiomethane. Till now, limited studies were conducted on truffles though they represent unexplored sources with potential therapeutic activities due to containing, antiviral, antioxidant, antimicrobial, hepatoprotective, immune-modulating, anticarcinogenic and sedative bioactive compounds (**Hannan et al., 1989; Gajos et al., 2014; Elkhateeb et al., 2019**). Truffle extracts showed Antiviral activity as reported by **Hussan and Al-Ruqaie**, **(1999**). These bioactives are present in trace amounts and their concentrations vary with species and location of cultivation (**Al-Laith, 2010**). Some truffle fungi may also benefit their plant host in other ways as some volatile organic compounds released by Tuber may negatively impact plant growth (**Splivallo et al., 2007**). Truffles associated with a host plant in order to produce mature fruiting bodies and complete their life cycle (**Payen et al., 2014**).

Truffles description and ecology

Truffles are symbiotic, ectomycorrhizal fungi that grow in the specific climates over a wide range of host plants such as oak, hazel and form hypogenous fruiting bodies. Truffles are a hypogenous fungi usually harvested by trained truffle dogs. Truffles live in symbiosis with plant host roots in order to accomplish their life cycle. Some species, such as, the white truffle, and the black truffle, are highly appreciated in many countries because of their special taste and smell (**Mello et al., 2006; Elkhateeb et al., 2019; Thomas et al., 2019**).

Truffles belong to genus Tuber and some species are famous for their high market value. Some species of truffles, such as Tuber magnatum, Tuber melanosporum, and Tuber aestivum, are the most expensive edible fungi due to their organoleptic properties, especially the taste and unique aroma. Tuber genus belonging to the family Tuberaceae. The genus Tuber is widespread in Asia (India, China, Mongolia and Japan), Europe and North America, but to date it is almost completely absent in the southern hemisphere. (**Diaz et al., 2003; Jeandroz et al., 2008**; **Bonito et al., 2013; Daba et al., 2019**). Truffle fruit-bodies differ from mushrooms, however, in how they produce and disperse their spores. Mushrooms typically have a stem that pushes their caps up through the forest floor where they open to expose and release their spores to the air. Air movement carries many spores away. Truffles no longer have a stem robust enough to push the fruit-body up and out of the soil. Truffle spores remain enclosed within the interior of the fruit-body. As truffles mature, they produce odors that are sensed by animals, which excavate and consume them. The spores pass unharmed through the animal's digestive tract and are later dispersed when the animal defecates (**Luoma et al.**

2003; Mello et al., 2013). Truffles provide a key food resource for many forest animals. Old mycological literature often refers to "true" and "false" truffles. True truffles are formed by a phylum of fungi called Ascomycota (fungi that bear their spores in small sacs or asci), which are also referred to as ascomycetes. False truffles are formed by basidiomycetes (fungi that bear their spores on microscopic, club-like structures termed basidia); most mushroom-forming fungal species belong to this group (**Maser et al., 2008; Saidi et al., 2015**). Many species of truffles have been discovered, only few of them have a significant market value, and these include the summer/autumn truffle and the black truffle. Truffles occupy a high position in the list of the globally most expensive foods due to the gap between production (hundreds of tonnes) and market demands (**Patel et al., 2017**). **Omer et al. (1994)** reported that desert truffles, have a long history of utilization in medicine and this has mostly been recorded for the treatment of ophthalmic diseases. It should also be noted that ergosteroids have been identified in desert truffle species and these widespread fungal sterols can be transformed into vitamin D in the human body (**Doğan and Aydin, 2013**). Truffles interact with other organisms at different phases of the life cycle, and these include plants, mammals and insects (**Splivallo et al., 2011**).

Truffles attracting microbes colonizing their fruiting bodies

Truffles are well known for their attracting microbes colonizing their fruiting bodies. The identity and diversity of these microbes remain poorly investigated, because few studies have determined truffle-associated bacterial communities while considering only a small number of fruiting bodies (**Strobel and Daisy, 2003; Zacchi et al., 2003; Splivallo et al., 2019**). Only a few studies have focused on the fungal biodiversity in truffles fruiting body. **Luppi-Mosca (1973)**, identified some fungi which seem common to the truffles fruiting body. In a study of three T. aestivum Italian truffle-grounds, **Zacchi et al., (2003),** isolated several yeast species, among which, Cryptococcus strains appear to be specific to this habitat. It was reported that, Cryptococcus humicolus, present on the surface of mature truffles, may contribute to Tuber nutrition during the saprotrophic stage or facilitate fungal ascospore dispersion. **Buzzini et al. (2005)**, found that yeast isolates from Tuber magnatum and Tuber melanosporum ascocarps produced some molecules characteristic of the complex aroma of truffles. This suggested that yeasts have a balancing role in contributing to the final Tuber aroma. It was clear that, this role needs to be explored more deeply. **Murat et al., (2005)**, found that Thelephoraceae, Pezizales and Sebacinaceae were the dominant fungal taxa in the subterranean ECM community in a Tuber magnatum truffle-ground and this study provided a clear vision of the fungal community. More studies are surely needed to completely describe both the fungal biodiversity and the interactions with truffles. Microbial biodiversity of truffle-grounds affects their productivity but, at the same time, are far from knowing the mechanisms involved in this activity (**Pacioni et al., 2007; Murat et al., 2005**). Beside truffles fruiting body, **Oh et al., (2019)**, reported that 184 fungal isolates from Tricholoma matsutake fruiting body, 28 species were identified based on suitable molecular markers. Penicillium was the most frequently observed (16 species), followed by Trichoderma (4 species). Five Zygomycota species showed a high promoting effect on the growth of Trichoderma matsutake. The microfungi that promote the growth of Tricholoma matsutake can be useful for artificial cultivation.

Tuber species may be regarded as complex microhabitats hosting varied microorganisms inside their fruiting bodies. **Perlińska-Lenart et al., (2020)**, investigated the microbial communities inhabiting Tuber aestivum, using Illumina sequencing and culture-based methods and found that identified fungi mostly belong to the phylum Basidiomycota and Ascomycota. All the specimens of Tuber aestivum were colonized by different strains of Bacillus (**Barbieri et al., 2007**). Fungal community inhabiting Tuber aestivum fruiting bodies was never shown before. The fungal microbiome should be examined in detail, and many researches should be carried out not only for Tuber aestivum but also for other Tuber spp. fruiting bodies (**Perlińska-Lenart et al., 2020**). **Pacioni et al., (2007),** showed that guest filamentous fungi were also associated to truffle ascomata, of Tuber spp., and report the morpho-molecular characterization of seven truffle-hosted mycelia isolated from healthy and intact Tuber ascomata. Some of these isolates were shown to be related to the fungal endophytes of plants.

Factors causing truffles damage

Truffles surface exposure increases the susceptibility to a wide range of disease causing agents, including insect pests, microbial pathogens, as well as weather-related factors such as drying and sunburn (Figure, 1). Also invertebrate pests found when harvesting truffles. Many of these factors may cause rot alone, or the combinations of stressors interact to reduce the truffles' natural defences to a point at which disease symptoms develop. Another factor observed in Europe is the occurrence of frost and freezing of the upper layers of soil, which when deep enough, freezes the truffle. This presents initially as patchy, pale discolouration of the truffle gleba and ultimately rotting. Given the shallow nature of the truffles in question, frosts, even mild ones, may increase the level of rot with a significant proportion of surface truffles (**Hochberg et al., 2003**; **García-Montero et al., 2004**).

Truffles rot

Truffle rot Damage of truffles resulting in truffle rot, is a significant problem. (**Eslick, 2013**). The main factors associated with truffle rot, were shown to be soil management, irrigation and fungal infection. Soil the main driver of truffle rot, exacerbated when winter temperatures and rainfall are high. Lastly, **Eslick, (2013),** study showed the occurrence of many different fungi associated with truffles showing rot symptoms. Two of these fungi, Trichothecium crotocinigenum and Acrostalagmus luteoalbus were inoculated into healthy truffles to test their pathogenicity. Only Trichothecium crotocinigenum could cause rot symptoms in healthy truffles, suggesting it is a primary cause of truffle rot in erumpent truffles, which are exposed to environmental stressors. Many different symptoms of truffle rot could be observed. Attempts to isolate fungi from a small subset of those consistently yielded Fusarium spp.

Truffles insect damage

There are two insects that cause high levels of damage to truffles, truffles fly and European truffle beetle. These truffle fly species belong to the fly family Heleomyzidae. There are many species of flies in this genus in Europe. Six have been associated with damage to black truffle in Spain. Truffles flies are well adapted to locating truffles and this ability has been used by people to harvest truffles. The flies use the scent of ripe truffles to indicate where they should lay eggs. Larvae dig after hatching to feed on truffles. (**Fortea and Santafé, 2018**). European truffle beetle, this beetle causes similar damage to truffles in Europe as Australian truffle beetle (ATB), potentially rendering individual truffles a complete loss, both larvae and adults of European truffle beetle are obligate truffle feeders and is an important pest of black truffle (Tuber melanosporum), the main culinary truffle cultivated in Australia. (**Morcillo et al., 2015**).

Figure 1: Truffles fruiting body with rot damage and insect damage

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Conclusion

Being of high pharmaceutical and nutritional values, studying truffles diseases and attacks by some invadors is extremely important. In one way, to keep this valuable rare product from being damaged. On the other way, to understand types of interaction taking place between truffles and their different invadors and their positive or harmful impacts. More studies are required in this field, especially to elucidate the variation in secondary metabolites between attacked truffles and free one. Also, to show impacts on nutritional and pharmaceutical uses of truffles.

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