Research Article E-ISSN: 2707-6261 RAFT Publications

Lybian Journal of Basic Sciences

Identification of some Pathogenic Organisms Involved in Rotting of the Libyan Tirmania Truffles

Mohammed Ahmed Emhmed

Department of Botany, Faculty of Science, Al-Zintan University, Al-Zintan, Libya

***Correspondence:** Mohammed Ahmed Emhmed, Department of Botany, Faculty of Science, Al-Zintan University, Al-Zintan, Libya, Email: ali.elfeituri@uob.edu.ly

Received: 17 Jun 2021 **Accepted: 22 Jul 2021** **Published: 25 Aug 2021**

DOI: https://doi.org/10.36811/ljbs.2021.110072

Citation: Mohammed Ahmed Emhmed. 2021. Identification of some Pathogenic Organisms Involved in Rotting of the Libyan Tirmania Truffles. LJBS. 5: 109-116.

Abstract

Truffles are ectomycorrhizal fungi that grow symbiotically with several trees. Truffles are highly valued edible mycorrhizae fungi in economic and gastronomic terms. Desert truffles have been traditionally used as food in Libya. The rotting of truffle due to environmental factors is very common in Libya. The mechanism of truffle rot is complex and attributed mainly due to the infection with fungi, followed by bacteria. In order to document and highlight the rotting of Libyan Desert truffle phenomenon, the existence of microorganisms in the infected fruit body of Tirmania truffles has been investigated. The investigations showed changes in physical characteristics such as color, hardness, and humidity of the studied truffle samples. Also, the results showed a diversity of pathogens involved in the deterioration of truffle includes bacteria (*Bacillus pumilus, Citrobacteryoungea*), saprophytic fungi (*Fusarium solani, Alternariachlamydosporia, and Rhizopusstolonifer*). This study concluded the importance of studying microbial flora living in truffle and their environment.

Keywords: Libyan Desert truffle; Tirmania; Rotting; Pathogens

Introduction

Truffle is reported to possess chemical (nutritional and aromatic profile) and biological properties such as antioxidant, antiviral, antimicrobial, hepato-protective, anti-mutagenic, anti-inflammatory, anti-carcinogenic, and anti-tuberculoid [1,2]. Due to this wide range of biological activities, truffle attracted considerable attention as a potential nutraceutical and medicinal agent [3,4]. The truffle extract contains different bio-actives such as carotenoids, anthocyanins, phenols, and esterified phenols, flavonoids, and ascorbic acid [5,6]. The juice prepared from the truffle prevents the formation of fibrous tissue in the post-treatment of trachoma, superior to other pharmaceutical treatments [7]. Truffles also contain an abundant quantity of protein [8], unsaturated fatty acids, vitamins (A, B, C, and D), and non-starch polysaccharides [9,10]. Under favorable environmental conditions such as water activity and temperature, a truffle prone to microbial attack leads to deterioration of the truffle. In some cases, truffle forms the host for microbial communities (bacteria and fungi) [11,12]. The soil contains a large diversity of bacteria in which some are useful and others are pathogenic [13,14]. However, only a few bacteria are found abundantly in the fruit body of truffle which indicates that the biological factor governs the transfer of specific bacteria from soil to the fruit body of truffle [15]. The bacteria in the fruit body produces volatile organic compounds which featured the truffle [16]. Some microorganism forms symbiotic association with the truffles, contribute to the growth and development of truffle fruit body [17,18], while other species of microbes are pathogenic [19]. Besides the bacteria, a diversity of pathogenic fungi contributes to the rotting process of truffles (as examples F*usarium spp*, *Rhizopus spp, and Alternaria spp*).

Fusarium solani is an important plant pathogen and soil saprophyte and associated with a variety of diseases like keratitis, onychomycosis, eumycetoma, skin lesions, and disseminated diseases [20]. An earlier report showed *Fusarium* to be the most prevalent pathogen in decaying tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) an important industrial crop [21].

Rhizopus is reported to be an important etiological agent of mucormycosis of severely habilitated human hosts. They exude phytotoxin, *rhizoxin, cyclopeptides,* and *rhizonins*. None of these metabolites are permitted in the foods even at pico- or fentogram levels, due to its toxic nature for human consumption [22. An earlier report indicated *Rhizopusoryzae*to be the decaying pathogen in potato tubers [23].

Alternaria produces more than 30 toxins, some of them showed severe effects on human beings are *alternariol* (AOH), *alternariol monomethyl* ether (AME), altenuene (ALT), alter toxins, II, III (ATX-I, -II and -III), and *tenuazonic acid* (TeA) [24]. Exposure to toxins has been linked to a variety of adverse health effects. The Alternariol has been reported to involve in the etiology of human *oesophageal* cancer. Therefore, it is very important to conduct an extensive study specialized in the fungus living in truffles and their environment. An earlier report indicated *Alternaria solani* to be a cause of defoliation of potato plants [25].

The rotting can be transmitted from the infected part to the non-infected parts of the truffle fruiting body through the pathogenic fungi [26]. The presence of truffle rot is common to all truffle-growing regions around the world. The rotting of truffles causes a change in their color, foul smell, softening of the tissue's surface wetness [26]. Different environmental parameters are involved in truffle rotting such as the presence of a microbial pathogen, exposure of truffle to the soil surface, invertebrate herbivores, water activity, and temperature of the soil. Among these parameters, exposure of truffles to soil surface dramatically increases the risk of truffle rot, larger truffles have a greater risk of exposure to the soil surface and hence more chances of susceptibility [26]. In 2015, an outbreak of Salmonella enteritis that resulted in 159 illnesses was linked to truffle oil served at a single restaurant in Washington D.C.

Although the phenomenon of truffle rotting has been known in ancient times among the peoples who were consuming the truffles, its official documentation was only made relatively late [26]. The phenomenon of truffle rotting is very common in Libya but the literature on the same is scanty. There are only a few methods to investigate the diseases that affect the large economic fungi (truffles and mushrooms), have been documented in the scientific literature. The Libyan soil is the richest source for the cultivation of desert truffles. There are many species grown in Libya, among these most common species are *Terfezia* and *Tirmania*. These species are known to consume freshly and can be preserved by drying for consumption in other seasons [9]. In a previous study, the characterization of natural habitats and diversity of Libyan desert truffles *Terfezia* and *Termania* was highlighted [9]. The present study was undertaken to investigate the pathogenic organisms involved in the rotting of the Libyan *Tirmania truffles* and their effect on physical characteristics. Therefore, this study was aimed to investigate pathogenic microbes involved in the rotting of *Tirmania truffle* and its detrimental effect on physical characteristics.

Materials and Methods

Media components and chemicals

Potato dextrose agar was procured from Thomas Baker (Chemicals) Pvt.Ltd.Czapekdox agar was purchased from Thermo Fisher Scientific Oxoid Ltd. Basingstoke, Hampshire England. The nutrient agar and nutrient broth were procured from PRO-Analise Ltd, Brazil.

Collection of Infected Samples

The *Tirmania* truffle samples were obtained directly from the reaping sites of truffles near the city of Al-Zintan. The infected fruits of the truffle sample (white truffle) were isolated from the healthy fruits.

Initial Screening

Infected samples of truffle were categorized into three groups; first: for investigation of the physical changes of texture, smell, and color (compared with the healthy fruits). Second: for detection of a pathogenic fungus, and third for detection of pathogenic bacteria.

Detection of Pathogenic Fungi

The representative samples of truffle (50 g) fruit including infected and non-infected parts from the outer shell to a pulp was mixed with sterile distilled water (100 ml). The mixture was shaken gently for 3 min to ensure the uniform mixing of studded pathogenic fungi. Further, this mixture (1 ml) was spread with a sterile glass spreader on the sterile Petri plates containing potato dextrose agar (PDA) and incubated at 25 °C for 5 days. After incubation, the plates were observed for the growth, and based on the color of fungal colonies and their morphology, the single fungal culture was isolated and purified. The purified fungal cultures were transferred to fresh PDA plates and incubated at 25 °C for 5 days and stored in the refrigerator until further use.

The pure isolated culture was transferred to microscopic slides and a single drop of methyl cotton blue dye was added to it and warm on the flame for few seconds to ensure the reaction of a fungus with dye. The slides were observed under a light microscope (Model WetzlarVB 356 Hund Wetzlar, Ltd. Germany). The fungi were identified based on morphological characteristics.

Detection of Pathogenic Bacteria

The infected part of the truffle was aseptically transferred to sterile nutrient broth with a sterile inoculating loop and incubated under 37 °C for 24 h. The 24 h old broth was streaked on Petri plates containing nutrient agar and incubated under 37 °C for 24 h. The plates were observed for the growth in the Petri plates. The bacterial colonies were isolated and purified by enrichment technique. The obtained single colonies of bacteria were subjected to Gram staining and observed under an optical microscope. Further, these bacterial samples were subjected to genus and species identification.

Results and Discussion

Changes in physical characteristics: The infected samples of truffles showed visual color changes (darker) as compared to healthy samples (Figs 1(A, B, and C). Apparent examination of the infected truffle showed a change in the texture of fruit body, softer and hydrated than the healthy samples. Some water droplets were filtered through the infected samples when pressed gently. The infected samples are also characterized by a distinctive smell due to the production of some volatile compounds by bacterial activities correspond to Splivallo and his team (*16*). They also showed bad taste like rancid fat, due to the oxidation of unsaturated fatty acids into peroxide derivatives which cause some of the poisoning cases, along with other physical changes such as color, hardness, and humidity.

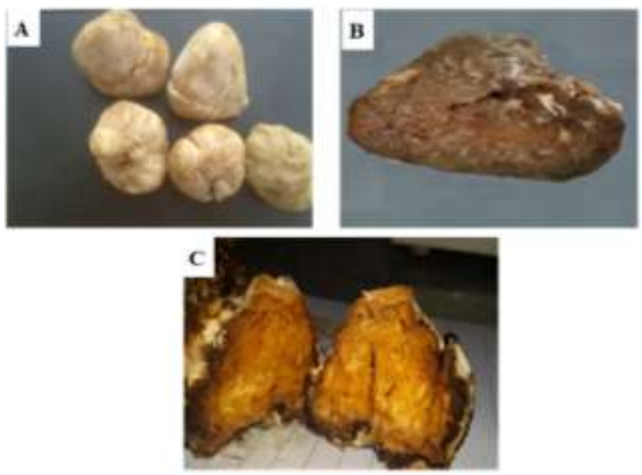

Figure 1: Morphology of *Tirmania* truffles (A) Healthy truffle (B) and (C) Infected truffles.

Detection of pathogenic fungi: Six pathogenic fungi were isolated and purified from the infected truffle sample. Among the six fungal samples, only three different species were identified as *Fusarium solani* (Fig. 2A), *Rhizopus stolonifer* (Fig. 2B*), Alternaria chlamydospores* (Fig.2C), while the other three species found to be inactive due to the temperature drop below 0 °C. These types of saprophytic fungi have been reported to spread in the food samples and cause degradation of organic components with the use of some digestive enzymes. Fungi are also reported to produce some secondary metabolites for instance *mycotoxins* lead to acute and chronic illness effects in animal and human beings [24,27].

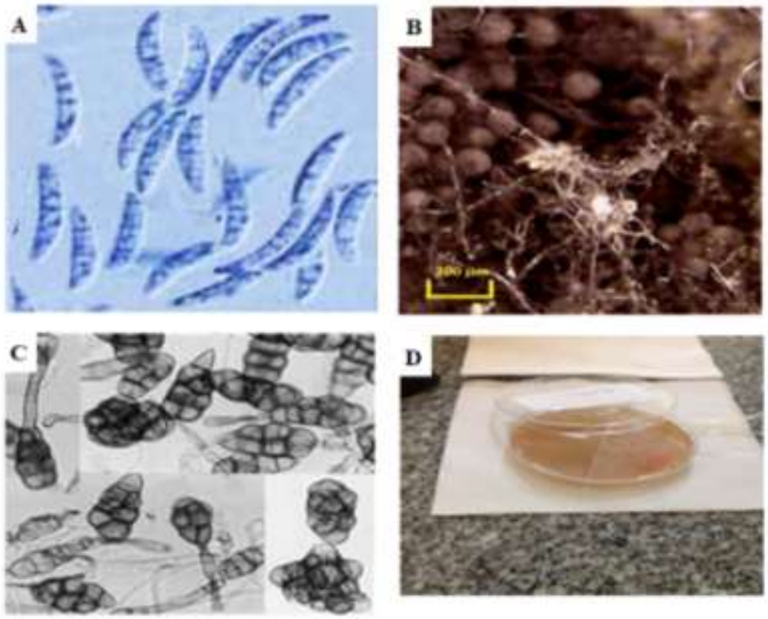

Figure 2: Microscopy of truffle infecting (a) *Fusarium solani* (b) *Rhizopusstolonifer* (c) *Alternaria chlamydosporialani*(D) Fungal culture.

Not: Fig A was used from (https://www.researchgate.net/figure/Morphologyo-observations-of-Fusarium-solani-hyphae-under-light-microscope-100-A_fig5_3037382193).

Fig C was used from: https://www.mycobank.org/name/Alternaria%20chlamydospora.

Detection of Pathogenic Bacteria

Besides the presence of fungi, results also showed the presence of Gram-positive (Bacillus pumilus) (Fig. 3A) and Gram-negative bacteria (*Citrobacteryoungae*) (Fig. 3B). These bacteria are common in the soil and roots of some plants *(28).* These pathogenic bacteria produce secondary metabolites and cause toxic effects such as neonatal sepsis and meningitis. It also causes sporadic pneumonia in neonates and immune-compromised individuals (*29*). Literature reports indicated the presence of Bacillus pupils as a pathogenic agent in the decaying potato tubers (*29, 30*). *Citrobacteryoungae* is an enteric pathogen and causes intra-abdominal infections in immune-suppressed individuals. The sampling area of the study was pastoral where sheep and goats were grazing. Therefore, *Citrobacteryoungae* found to be dominant in infected truffle samples. There was the occurrence of some yeast species (*Candida*) in the studied infected truffle samples (data not shown). These results show the urgent need to carry out an environmental microbiological study to determine the types of bacteria and yeasts in the truffles and their impact on public health.

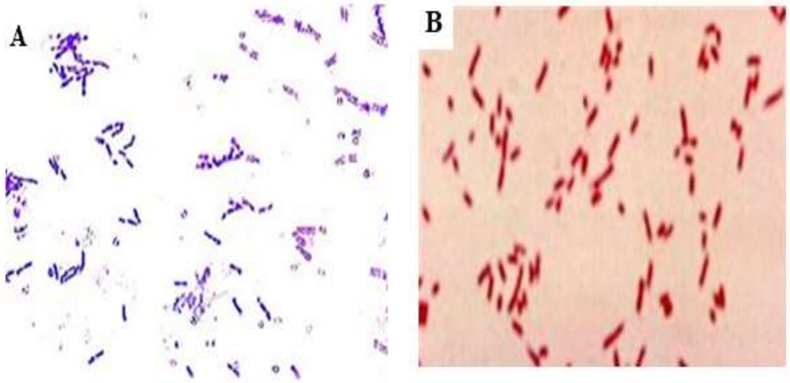

Figure 3: Microscopy of (A) *Bacillus pumilus* (B) *Citrobacteryoungea.*

Conclusion

This study concluded the following points:

To the best of our knowledge, this is the first report on the identification of pathogenic microbes involved in the rotting of *Tirmania* truffle in Libya. The necessity of directing the attention of researchers to study the possibility of poisoning or serious diseases among consumers may be caused due to deterioration of truffles. All of that leads towards the expansion of this study to be an environmental study that takes account of all pathogenic species may living in the environment of truffles and their role in the rotting process of the truffles fruit body.

References

1. A. Casarica et al., A purified extract from brown truffles of the species Terfezia claveryi chatin and its antimicrobial activity. Farmacia 64, 298 (2016).
2. C. Noël-Suberville, C. Cruz, J. Guinberteau, M. Montury, Correlation between fatty acid content and aromatic compound release in fresh blewit (Lepista nuda). Journal of Agricultural and Food Chemistry 44, 1180 (1996).
3. W. Attia, R. El-Naggar, A. Bawadekji, M. Al Ali, Evaluation of some in vitro anti-carcinogenic activities of polysaccharides extracted from Ascomata of the desert truffle Terfezia claveryi Chatin. J Appl Environ Biol Sci 8, 152 (2018).
4. X. Yan, Y. Wang, X. Sang, L. Fan, Nutritional value, chemical composition and antioxidant activity of three Tuber species from China. AMB Express 7, 1 (2017).
5. T. Guo, L. Wei, J. Sun, C.-l. Hou, L. Fan, Antioxidant activities of extract and fractions from Tuber indicum Cooke & Massee. Food Chemistry 127, 1634 (2011).
6. M. A. Murcia et al., Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. Journal of food protection 65, 1614 (2002).
7. M. Alhussaini, A. Saadabi, K. Hashim, A. Al-Ghanayem, Efficacy of the desert truffle Terfezia claveryi to cure trachoma disease with special emphasis on its antibacterial bioactivity. Trends Med Res 11, 28 (2016).
8. E. Danell, D. Eaker, Amino acid and total protein content of the edible mushroom Cantharellus cibarius (Fries). Journal of the Science of Food and Agriculture 60, 333 (1992).
9. M. Bouzadi et al., Characterization of natural habitats and diversity of Libyan desert truffles. 3

Biotech 7, 1 (2017).
10. M. A. Murcia et al., Effect of industrial processing on desert truffles Terfezia claveryi Chatin and Picoa juniperi Vittadini): proximate composition and fatty acids. Journal of the Science of Food and Agriculture 83, 535 (2003).
11. E. Barbieri et al., New evidence for bacterial diversity in the ascoma of the ectomycorrhizal fungus Tuber borchii Vittad. FEMS Microbiology Letters 247, 23 (2005).
12. G. Pacioni et al., Isolation and characterization of some mycelia inhabiting Tuber ascomata. mycological research 111, 1450 (2007).
13. L. F. Roesch et al., Pyrosequencing enumerates and contrasts soil microbial diversity. The ISME journal 1, 283 (2007).
14. P. D. Schloss, J. Handelsman, Toward a census of bacteria in soil. PLoS Comput Biol 2, e92 (2006).
15. G. M. N. Benucci, G. M. Bonito, The truffle microbiome: species and geography effects on bacteria associated with fruiting bodies of hypogeous Pezizales. Microbial ecology 72, 4 (2016).
16. R. Splivallo et al., Bacteria associated with truffle-fruiting bodies contribute to truffle aroma. Environmental Microbiology 17, 2647 (2015).
17. T. J. Aspray et al., Mycorrhization helper bacteria: a case of specificity for altering ectomycorrhiza architecture but not ectomycorrhiza formation. Mycorrhiza 16, 533 (2006).
18. P. Frey-Klett et al., Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiology and molecular biology reviews 75, 583 (2011).
19. L. P. Partida-Martinez, C. Hertweck, Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437, 884 (2005).
20. T. S. Kuruvilla, M. Dias, Fusarium Solani: a causative agent of skin and nail infections. Indian journal of dermatology 57, 308 (2012).
21. S. Jin et al., Characterization of marine Pseudomonas spp. antagonist towards three tuber-rotting fungi from Jerusalem artichoke, a new industrial crop. Industrial Crops and Products 43, 556 (2013).
22. S. Dolatabadi, G. Walther, A. G. Van Den Ende, G. De Hoog, Diversity and delimitation of Rhizopus microsporus. Fungal Diversity 64, 145 (2014).
23. A. Amadioha, Effect of infection by Rhizopus oryzae on biochemical composition of stored potato tubers. Plant Foods for Human Nutrition 53, 145 (1998).
24. V. Ostry, Alternaria mycotoxins: an overview of chemical characterization, producers, toxicity, analysis and occurrence in foodstuffs. World Mycotoxin Journal 1, 175 (2008).
25. J. Van der Waals, L. Korsten, T. Aveling, A review of early blight of potato. African plant protection 7, 91 (2001).
26. H. Eslick, Identification and Management of the Agent Causing Rot in Black Truffles: Part 2. (RIRDC, 2013).
27. J. D. Thrasher, Fungi, bacteria, nano-particulates, mycotoxins and human health in water-damaged indoor environments. J Comm Pub Health Nurs 2, 10.4172 (2016).
28. G. Dos Santos et al., Study of the Enterobacteriaceae group CESP (Citrobacter, Enterobacter, Serratia, Providencia, Morganella and Hafnia): a review. The battle against microbial pathogens: basic science, technological advances and educational programs 2, 794 (2015).
29. K. Chen, T. Chen, Y. Sue, Citrobacter youngae and Pantoea agglomerans peritonitis in a peritoneal dialysis patient. Peritoneal Dialysis International 33, 336 (2013).
30. S. E. Crawford, R. S. Daum, in Pediatric respiratory medicine. (Elsevier, 2008), pp. 501-553.