Valorization of a wild saprophytic edible mushroom in western Algeria: characterization and nutritional value

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Abstract

This study concerned a species of wild edible mushroom, oyster mushroom collected in western Algeria forests, which is well consumed by the local population. These species were identified by macroscopic, microscopic and molecular characteristics. The mushroom's mycelium was isolated and the optimal conditions for mycelial growth were determined. Variation in different physicochemical parameters (pH, temperature, relative humidity and light) significantly influenced the mycelial growth of the mushroom on potato dextrose agar medium. Optimal mycelia growth was observed at pH 5.6, 25 °C, 100% humidity and low exposure to light. Wheat was used as spawn substrate and straw was used as fructifying substrate. The sporophores of the mushroom were obtained after one month of incubation. We cultivated this wild mushroom from its mycelium and defined the nutritional value of the obtained sporophores. The nutritional analysis was performed according to the most common analytical methods. The composition in moisture, ash, fat, protein, total carbohydrates allowed us to demonstrate that the wild edible mushroom harvested in forests of western Algeria had an important nutritional value. This wild edible mushroom was a remarkable and potential resource for development.

Keywords: Optimal growth conditions, Oyster mushroom, Sporophores, Western Algeria, Wild edible mushroom.

Introduction

Mushrooms are very important in the life of human beings. They participate in the geochemical cycles of matter in nature and they are decomposition agents which break down complex organic matter into simple substances. Also, many of them are edible and delicious and their sporophores accumulate a variety of metabolites such as phenolic compounds, polyketides, terpenes, polysaccharides and steroids. These metabolites offer medicinal effects and functional values strengthening the immune system, blood lipid control and antitumoral action **(**Arbaayah and Kalsom, 2013; Ishmael *et al.*, 2017; Mwangi *et al.*, 2022). The mycelia isolated from the wild edible mushrooms contain several qualities that improve human nutrition (Lindequist *et al.*, 2005). In addition to these, many recent studies have also shown the presence of bioactive compounds, especially antifungal constituents, in metabolites of other fungal species particularly in biocontrol agents such as species of *Trichoderma* (Khan and Javaid, 2020a; Khan *et al.*, 2021), *Aspergillus* (Khan and Javaid, 2021, 2022a) and *Penicillium* (Khan and Javaid, 2022b).

The known characteristic of edible mushrooms is that they are woody hygrophytes that like the undergrowth (Madamo *et al.*, 2017). The consumption of mushrooms is of great interest in food because of their nutritional wealth (Badalyan, 2012; Acharya *et al.*, 2016; Zięba *et al.*, 2020). There are molecules even in the mycelium that help the human diet due to their glucose content. Also the wild sporophore samples gave the highest levels of organic acids (Pinto *et al.*, 2013; Díaz-Godínez*et al.*, 2016). Sporophores of many edible mushrooms are rich in carbohydrates, proteins, polyunsaturated fatty acids, polysaccharides, dietary fibers (non-starch polysaccharides) and minerals (Peter, 2013; Zhong *et al.*, 2013; Di Anibal *et al.*, 2015; Jedidi *et al.*, 2017; Phan *et al.*, 2018; Sanem *et al.*, 2019). Also, they are used in African food fortification formulations as sources of minerals and proteins (Tounkara *et al.*, 2017). In Congo where population is mycophilic (mushroom lovers), 74 species of mushrooms are included in the diet (Madamo *et al.*, 2017).

Wild edible saprophytic edible mushrooms are badely known in Algeria. Many studies were made only on wild macrofungi and basidiomycetes in Algeria (Benazza-Bouregba, 2016; Yakhlef *et al.*, 2020; Mesfek *et al.*, 2022; Ait-Hamadouche *et al.*, 2022). Wild edible mushrooms are known by people in Europe (especially in Spain and Portugal), America and Australia (Kalač, 2009; Martins and Ferreira, 2011; Davis *et al.*, 2012; Leal *et al.*, 2013), Asia (Toshinungla *et al.*, 2016) and rarely cited in Africa except in countries such as Nigeria and South Africa (Popich, 2014), Congo (Toshinungla *et al.*, 2016), Egypt (El-Fallal*et al.*, 2017), Morocco (Ali *et al.*, 2015) and Tunisia (Ouali *et al.*, 2021). Mycelium growth and fruiting of sporophores of these edible mushrooms depend on many external physicochemical factors (Eynard, 2018).

The field of biotechnology offers a great opportunity to find solutions for the accumulation of agricultural waste in the environment such as the recycling of straw that is used in the cultivation of mushrooms (Thongklang and Luangharn, 2016). China is one of the main countries producing mushrooms and is interested in their nutritional and healthy values (Sánchez, 2010; Wang *et al.,* 2014); mushroom consumption may reduce the risk of breast cancer (Li, 2014). The consumption of wild mushrooms is not widespread in Algeria and in current traditional consumption because the mushrooms are new food and linked to urban development in Algeria. Also, people ignore the importance and the nutritional value of these mushrooms. That's why we were interested to study wild edible mushroom in our country. We focused on one genus of edible mushroom harvested from western Algeria. This mushroom was identified by macroscopical, microscopical and molecular biology methods. On another side, we analyzed the nutritional value of the sporophores and performed *in vitro* experiments in which we discussed the work of authors about physicochemical conditions for mycelium growth and we presented our observations.

Materials and Methods

Morphological characterization

The wild mushroom was harvested in 2019, from Madegh forest of Oran in western Algeria. This wild mushroom was edible. Several species of equipment were used for sampling, including a digital camera and plastic food boxes to preserve the fresh sporophores of the wild mushroom and transport them quickly to the laboratory to avoid deterioration. The determination of the species was based on the macroscopic and microscopic characters and genetic characterization. The macroscopic and microscopic characteristics of this mushroom were studied according to Ge *et al.* (2015) and Halama *et al.* (2019).

Macroscopic characters were observed with the naked eye and using a Leica stereomicroscope in which we were able to observe the different parts of the sporophore (hymenophore, pileus, lamellae) and dimensions were expressed in centimeter (cm). Also, the shape and color of different parts of the mushroom including the color of the cap and the hymenophore, the type of hymenophore, the insertion of the lamellae have been described. We recorded color, shape and dimensions of the spores. The microscopical observation of the spores (color, shape) and their dimensions were carried out using the Olympus CX22 microscope. Measurement of 30 spores was made using a calibrated micrometer placed on the optical microscope. We observed the spores with the technique of scanning electron microscopy using analytical scanning electron microscope (JSM-6610LA) in which an electron beam scanned the surface of the analyzed sample. Different detectors allow the emitted particles to reconstruct an image of the sample surface.

Molecular characterization

Extraction of genomic DNA from pure mycelium was performed using a DNA kit, following the manufacturer's protocol. Internal transcribed spacer (ITS) region primers were used for the DNA amplification, reaction was carried out and the mixture contains volume as follow: 2 µL genomic DNA extracts, 6 µL 5X PCR mix, 1 µL primer forward, 1µL primer Revers, H_2O qsp 30 µL. Thermal cycler was programmed as follows: an initial denaturation step at 94 °C for 12 min followed by 35 cycles of 20 s denaturation at 96 °C , a phase hybridization at 56 °C for 20 s then an extension phase at 72 °C for 1min and final extension at 72 °C for 5 min, a negative control (H_2O) is systematically amplified under the same conditions as DNA. Sequencing of reactions were performed on a 96 capillary ABI3730XL sequencer in Biofidal Laboratory, France.

After obtaining the sequences, the identification was performed by BLAST, Basic Local Alignment and Search Tool (Stephen *et al.,* 1997) on the internet (http://www.ncbi.nlm.nih.gov) in order to compare our sequence with those present in banks then aligned with them. The given alignment is associated with a score; the BLAST always seeks to maximize the higher score, and the greater resemblance (Zheng *et al.,* 2000). The ITS sequences alignments were performed using the MUSCLE algorithm in MEGA-X. Then Phylogenetic tree was constructed on the final alignment using the Maximum likelihood program implemented in MEGA-X (Kouame *et al.,* 2018). *Morchellaelata* (HM756742.1) was used as out-group species to root the phylogeny. The nucleotide sequences of the mycelium obtained in the present study were deposited in the GenBank database (MZ928449.1).

Physicochemical characterization of the mycelium

In order to obtain the mycelium from mushroom sample, a fragment of the sporophore was disinfected with 70% ethanol then was placed on a Petri dishes containing PDA medium supplemented with actidione (0.5 g L^{-1}) and gentamycine (2 mL L^{-1}). The Petri dishes were incubated at 25 °C until the obtention of a white mycelium (Suruga *et al.,* 2020). A fragment of obtained mycelium was controlled regularly with optical microscope and observed with Congo red stain (10%). The diameter of the hyphae was measured (20 hyphae per treatment) using the optical microscope.

The study of physicochemical parameters aimed to reveal the optimal conditions for the mycelial growth of wild edible mushrooms. Ha and

Chun-Li (2015) described manipulations to monitor mycelial growth. In this study, the influence of 4 parameters was tested separately *viz.* temperature, pH, relative humidity and the duration of exposure to an artificial light (500 Lux in intensity).

Eight different temperatures were used to determine the optimal temperature for the mycelial growth of the mushroom: 15, 17, 19, 21, 23, 25, 28 and 30 °C. The influence of pH on the mycelial growth was studied on the buffered PDA agar medium at the following values: 4, 5, 5.6, 6, 7 and 8. In order to determine the optimum relative humidity level for the mycelial growth of the mushroom, six different values were tested: 14, 50, 74, 80, 95 and 100%. For the study of the effect of light on the mycelial growth of the mushroom, an intensity light of 500 Lux was exposed for 5, 10, 15, 20 and 24 h on inoculated Petri dishes of PDA. For all the parameters tested, mycelial fragment of 1cm in diameter was taken from a 10 days' preculture and placed in the center of the PDA agar in Petri dishes. After 10 days of incubation in each physicochemical condition, the mycelial growth was evaluated by measuring the diameter of the fungal colony in centimeters. A quadriplicate was used for each test.

Sporophores production and nutritional value

The first step after obtaining pure mycelium of the edible mushroom was to growing it in jars on wheat grains (spawn substrate), which were incubated at 25 °C for 15 days. Wheat straw was used as a fruiting substrate. The straw was at first cooked in tap water for 2 h, then it was placed in plastic bags (10 L) and autoclaved at 120 °C for 30 min. The sterilized straw bags were inoculated with wheat grains invaded by the mycelium and incubated at 25 °C for 27 days. After mycelial invasion, the bags were placed in growth room at 18 °C, 390 ppm of CO_2 and more than 65% relative humidity, controlled with APOMOS6 analyzer (Rahal *et al.*, 2021). A light intensity of 500 Lux was exposed during five weeks. Once the fresh sporophores were obtained, they were harvested and used for the next tests (Alejandro *et al.*, 2010).

The fresh sporophores of the wild edible mushroom were analyzed for their nutritional components (moisture, ash, proteins, fat, and carbohydrates) using different methods. All the analyzes were carried out in triplicate. In addition to the nutritional value, the condition of the mushroom (fresh, dry or freeze-dried), the color and the smell of the mushroom should be taken into account.

The principle of the method for determination of the moisture content is based on the weight loss of the sample, when the mushroom was placed in an oven at 105 °C overnight until constant weight. The ash content was determined by incineration of the sample then the sporophores of mushrooms were placed at 550 °C in a muffle furnace for 24 h. After cooling, they were placed in a desiccator.

The amount of protein from fresh sporophores was calculated by the determination of total nitrogen, according to the Kjeldhal method and using the conversion factor (N×4.38)**.** In order to highlight the nitrogen, we used a flask in a mineralizer at 105 °C. Pale green color indicated the end of the mineralization. After cooling, the analysis was completed in the Kjeldhal apparatus (Manzi *et al.*, 1999). The fat content is determined using hexane as solvent in a Soxhlet extractor. After evaporation of the hexane and drying of the capsule in an oven at 105 °C for 30 minutes, the difference of weight gave the fat content of the sample (Beluhan and Ranogajec, 2011). For the value of carbohydrates, the Phenol-Sulfuric Acid method was used for the determination of sugars in mushroom sample. It was a simple and rapid colorimetric method (Nielsen, 2017).

Statistical analysis

Values are statistically analyzed by variance (one way ANOVA), using Graph Pad Prism 7 where SDs are significantly different at $P \leq 0.05$.

Results

Mushroom characteristics

This is the first study on a wild sapropytic edible mushroom in western Algeria (Fig. 1). The sporophores were harvested in Madegh forest (Oran in western Algeria) growing on wood substrate. The characterization of the mushroom species was based on the macroscopic characters and the biotope. Sporophores were found in nature growing in groups (Fig. 2A). The hemispherical cap, reached from 6–15 cm in diameter, was in the shape of oyster shell, with a stipe at the base of the cap. The cuticle was smooth and brilliant initially yellowish beige (the young sporophores), then pale gray (the old specimens). The gills were decurrent more or less tight, descending throughout the stip. They tended to narrow towards the margin (Fig. 2B). The stipe was very short and whitish. The flesh was white, thick and fibrillated. Its smell was pleasant with white spore print. The sporophores were growing from fall to winter on hardwood stumps, in compact clumps.

Micromorphological characteristics of the mushroom spores were performed using scanning electron microscopy and showed that the spores had the shape of an elliptical cylinder, and reached up to 50µm in diameter (Fig. 2C).

Molecular biology techniques were used on the mycelium of the mushroom, by using the sequencing of the ITS region of the DNA which is the most commonly used molecular marker for fungal species (Carlson *et al.*, 2014). When we compared the sequence of MZ928449.1 *Pleurotus ostreatus* with other similar sequences found in GenBank, BLAST Nuclioted allowed us to find similar regions between several sequences and to

perform an alignment of these homologous regions in order to find functional relationships between these sequences, so the sequence of the *P. ostreatus* strain MZ928449.1 was identical to the sequence of *P. ostreatus* NR163515.1 with a very high score of 789 and with an identification percentage equal to 99.09%. The principle of BLASTnucleotid is to consider the higher score and the higher similarity (Fig. 3).

The influences of pH, temperature, Relative humidity and light were investigated on the mycelium growth of *P. ostreatus* (Fig. 5). All experiments were performed into quadriplicate. The results were expressed as histograms and data evaluation was obtained for mycelial growth during 10 days. Analyses of variance were used according to Bartlett's test by GraphPad Prism 7 software (highly significant at $P \leq 0.05$). The results showed that the variations of different physicochemical parameters influenced significantly the mycelial growth. During the experiment, the pH was between 4 and 8. The mycelial strain studied grew slightly in the presence of pH 4 and 7 (acid to neutral); no growth was observed at pH 8. Mycelial growth of *P. ostreatus* at 20 and 25 °C was very significantly higher than at 15 °C *(*$P \leq 0.0001$); whereas there was no growth at 10 and 30 °C. Relative humidity (RH) was a factor that affected very significantly ($P \leq 0.05$). The results showed that there was no difference between humidity levels at 74, 80 and 95%. The optimal growth of *P. ostreatus* mycelial strain was defined at pH 5.6, 25 °C and 100% humidity. The analysis of variance performed on the mycelial growth of *P. ostreatus* by Bartlett's test revealed that the light factor very significantly ($P \leq 0.05$) affects the mycelial growth.

Sporophores production and nutritional value

After 1 month of cultivation, strong mycelial growth of *P. ostreatus* was recorded on the straw bags. The appearance of the primordial was noted at the beginning of the second month (5^{th} week). The sporophores of the mushroom took between 28 and 36 days (Fig. 6). The influence of the substrate on the development of the mycelium was studied; growth was obtained in 15 days. Wheat was found to be the best substrate for mycelium growth because this cereal provides optimal conditions for the development of *P. ostreatus*. The appearance of primordium was observed on a few jars of wheat at 25 °C**.** The development of the primordia until the maturation of the sporophores took 5 to 7 days. In order to determine the nutritional value of the sporophores, we look for the moisture, ash, fat, protein and total carbohydrate (Table 1). All experiments were performed in quadruplicate for each value. Results were expressed as mean ± standard deviation. Moisture, ash, fat, protein and total carbohydrates were calculated. This composition was significant ($P \leq 0.05$). It is notable that nutritional composition of *P. ostreatus* sporophores was highly significant for the following parameters: moisture (77.25%), ash (6.43%), fat (4.33%), protein (27.78%) and carbohydrates (41.29%).

Discussion

Pleurotus ostreatus belongs to the order of Agaricales; it is a monophyletic group that constitutes the largest group of Agaricomycetidae and includes almost all edible fungi (Chaboud, 2013). The ITS sequence is the most widely used DNA region for fungal phylogeny due to its high degree of variation (Khan and Javaid, 2020b, 2023). There are more than 150,000 fungal ITS sequences referenced in databases (Damon *et al.,* 2010). The molecular method of identification consists of comparing the ITS sequence of our isolated strain with other similar sequences found in GenBank, and then evaluating the phylogenetic or taxonomic criteria. By comparing some Indian species of *P. ostreatus* studied according to Kotadiya *et al.* (2021) and according to Siregar and Idris (2018), the macroscopic characteristics of the wild edible mushroom collected in western Algeria were not different. Scanning electron microscopy allowed us to compare the structure of the sample with that shown by Buchalo *et al.* (2009). The results allowed us to confirm the identity of *P. ostreatus*.

Maurya *et al.* (2019) studied the mycelial growth on different solid media; potato dextrose agar medium allowed maximum mycelial growth of *P. ostreatus* and was found to be significantly superior to other media tested. It was similar to what we obtained. According to Emayavarman and Singh (2021), the variations of different physicochemical parameters (pH, temperature, relative humidity and light) influenced the mycelial growth of *P. ostreatus*. A different result was reported by Ibekwe *et al.* (2008) in which the optimal mycelia growth was observed at pH 6.4 for *P. ostreatus* on malt extract broth medium. Mondal *et al.* (2010) recorded optimal growth of *P. florida* at 22 to 27 °C). Similar findings were reported by Fletcher (2019) who indicated that the optimum temperature for mycelium growth of *P. ostreatus* was obtained at 22 °C. Fan *et al.* (2000) studied the mathematical relationship between the effect of air humidity and substrate humidity and observed little effect of varying air humidity and a faster at 80% substrate humidity. Okwujiako (2001) considered light as a factor that weakens mycelial growth, although Nakano *et al.* (2010) found that blue light suppressed the growth of *P. ostreatus* mycelia. Siwulski *et al.* (2012) used a light intensity of 500–700 Lux and observed that light has a significant impact on mycelia performance. Another finding showed that irradiation with red and green light stimulated vegetative growth of *P. ostreatus* mycelium and

shortened the time to substrate colonization and fruiting (Poyedinok *et al.*, 2003).

Our results showed that the mycelial growth preferred low exposure to light. For *P. ostreatus* cultivation, light is used to initiate sporophores' production (Tesfaw *et al.*, 2015). Similar studies have been published by Sher *et al.* (2011) on the cultivation of *P. ostreatus* in Pakistan and another study in Mexico City by Sánchez (2010) in which they described the possibility of small- and large-scale production of *P. ostreatus*. In our study, we obtained soporophores by using white light of 500 Lux.

According to Bandura *et al.* (2020), the main advantage of cereals used as spawn substrate is that they are very nutritious for mushrooms. On the other hand, growth of *Pleurotus* sp. can be evaluated on other substrates such as oil tea shells (Zhang *et al.*, 2018). According to Stanley and Awi-Waadu (2010) white corn was the most favorable substrate for mycelium extension and sporophores production. It was proved by Alvarez and Bautista (2021) that *P. ostreatus* growing on waste paper, gave better results than cereals. In Pakistan, Bajwa *et al.* (1999a, b) cultivated *P. ostreatus* on cereals residues supplemented with *Sesbania sesban* leaves and effective microorganisms (EM). We chosed wheat straw as a fruiting substrate because it was available and allowed to produce mushrooms on a large scale with economic interest as said by Abou-Fayssal *et al.* (2021). The same statement was approved by Jegadeesh *et al.* (2018). This result is comparable to that of Muswati *et al.* (2021) who showed that fructification varied between 26 and 35.5 days. The development of the primordia until the maturation of the sporophores is the same as that obtained by Fufa *et al.* (2021) finding that primordial formation was obtained between 4 to 12 days and sporophores were taken between 36 to 59 days. On the other hand, the *P. ostreatus* yield was close to that of Raman *et al.* (2021).

Regarding the nutritional value of the sporophores obtained in this study, similar results were found by Oka *et al.* (2020) for the edible mushroom *P. geesteranus*. We can say that *P. ostreatus* could play a major role in the supply of protein and carbohydrate, while it does not contain a lot of lipid (fat) that is why we can say that it is suitable for dieters. Oei (2003) stated that the nutritional content of mushrooms is comparable to dairy product and meat. He highlighted the content in moisture (90%) and dry matter (10%). Swedish *P. ostreatus* had good nutritional value as so as in our study (Grimm *et al.*, 2021).

The nutritional value of *P. ostreatus* sporophores produced in this study on straw was similar to that produced on agro-industrial wastes of palm oil fruits and cocoa almonds (Fernandes *et al.*, 2015; Silva *et al.*, 2020). Economically, the mushroom *P. ostreatus* contributes to the valorization of waste because it is cultivated on agro-industrial residues (Melanouri *et al.*, 2022). *P. ostreatus* is one of the most cultivated mushrooms in the world. Its production alone accounts for about 25% of the total cultivated mushrooms in the world (Raman *et al.*, 2021; Kazige *et al.*, 2022).

Conclusion

The identification of the harvested wild edible mushroom was based on macroscopic and microscopic characterization completed with a molecular characterization. The study of the effect of physicochemical factors (temperature, pH, relative humidity and light) on the mycelial growth of the isolated strain of mushroom indicated that there is always a value allowing the optimal growth. The results showed the nutritional value of cultivated edible mushrooms from isolated strain and their importance. It is interesting to note that they are very rich and sources of nutrients such as carbohydrates, proteins and low fat content.

Acknowledgements

Authors are grateful to the DGRSDT of Algeria for their help. They address also all acknowledgments to the anonymous reviewers for their valuable comments.

Table1: Nutritional value of the wild edible mushroom sporophores cultivated in growroom.

Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Total carbohydrates (%)
77.25± 0.75	6.43 ± 0.17	4.33 ± 0.15	27.78 ± 0.91	41.29 ± 0.78

Each value was expressed as mean ±SD

Fig. 1: The wild saprophytic edible mushroom.

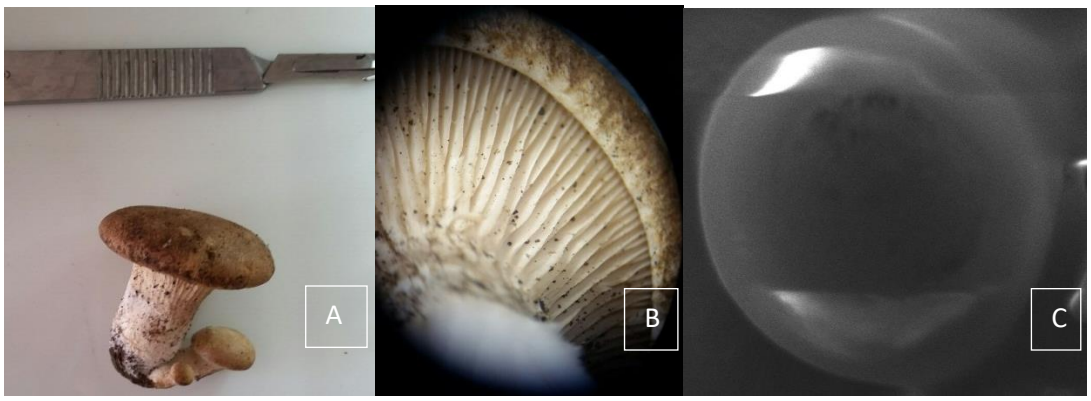

Fig. 2: Morphological characteristics of the saprophytic wild edible mushroom harvested in western Algeria. (A): entire sporophore, (B): Gils details, (C): SEM of spores.

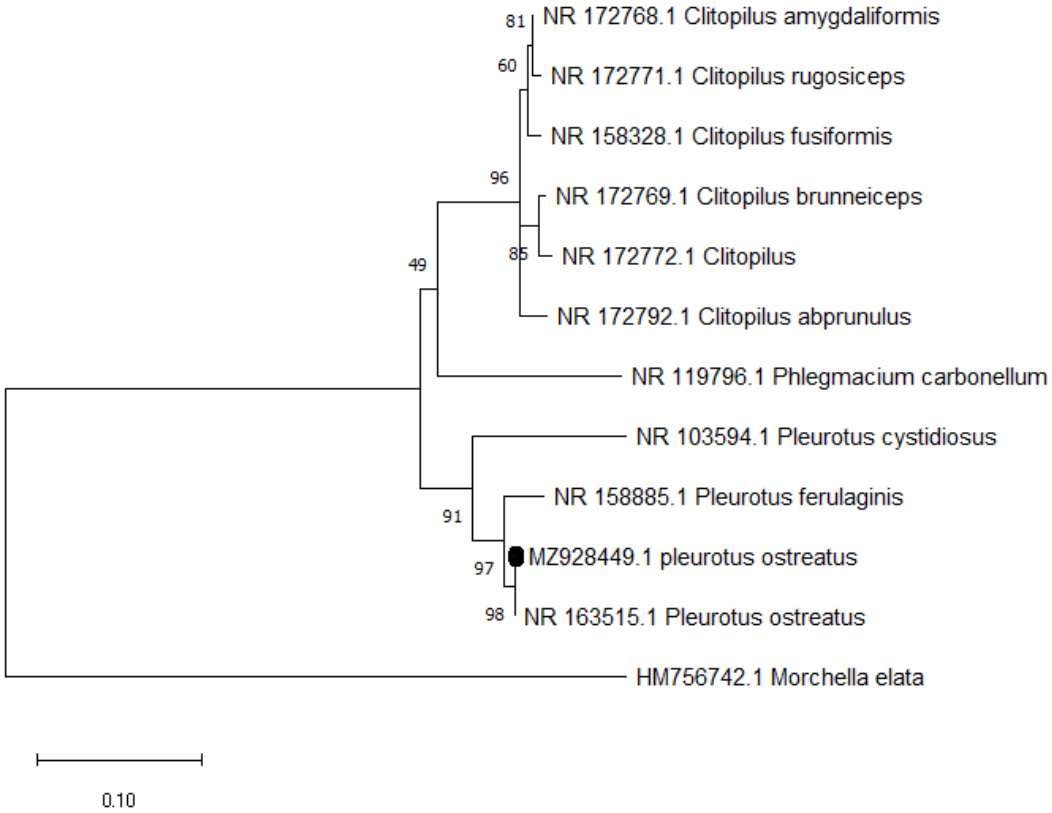

Fig. 3: Phylogenetic tree of the wild edible mushroom harvested in western Algeria (designated by a black dot). The evolutionary history was inferred by using the Maximum Likelihood method and Jukes-Cantor model (Jukes and Cantor, 1969). The tree with the highest log likelihood (-1668.28) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Jukes-Cantor model, and then selecting the topology with superior log likelihood value. This analysis involved 12 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 367 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

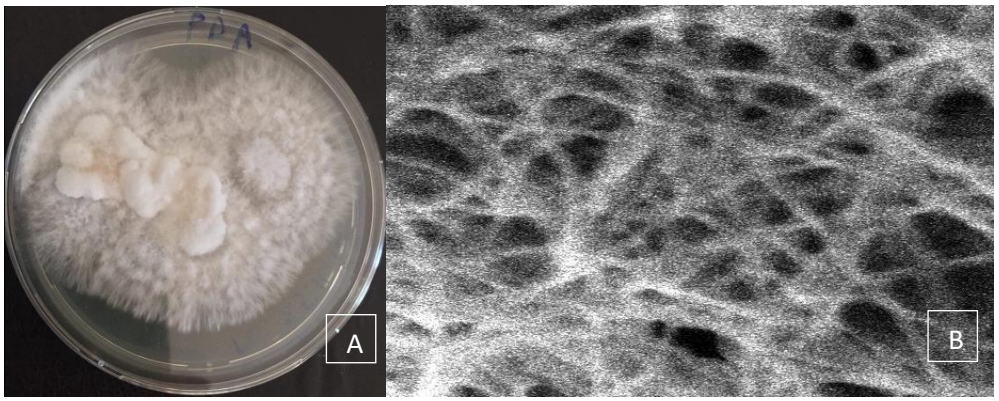

Fig. 4: Mycelium aspects of the mushroom. (A): on PDA medium in Petri dishes, (B): with scanning electron microscopy.

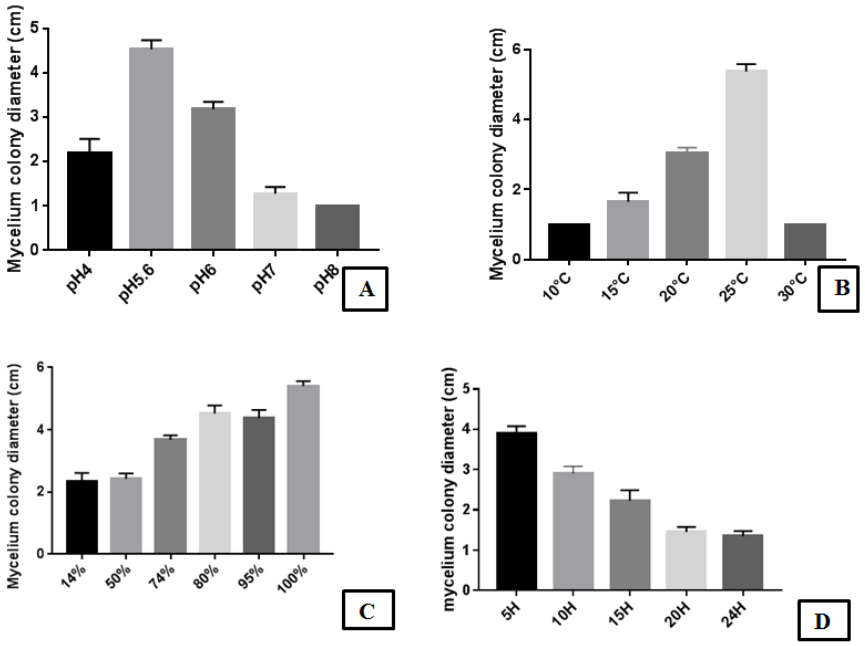

Fig. 5: Graphical representations of *Pleurotus ostreatus* mycelial growth in different physicochemical parameters. **A:** pH, **B**: Temperature, **C:** Relative humidity, **D:** Light exposure (duration).

Fig. 6: *Pleurotus ostreatus* sporophores obtained in growth room: (**A**): Primordia on wheat (**B**): Primordia on straw (**C**): Fructification in straw bags.

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