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Production of *Agaricus bisporus* in Zaccar (Algeria)

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ABSTRACT

Mushroom production is very well suited to sustainable agriculture. Mushrooms provide additional protein, essential vitamins and minerals. The objective of the work is to promote mushroom culture through cultivation of white button mushroom (*Agaricus bisporus*) mycelium. The product obtained at the end of this operation is called "white mother" which will be used later to inoculate the fruiting substrate based on horse manure and straw. From the kits, pure cultures of mycelium were prepared on Potato dextrose agar medium, which were then inoculated into various sowing substrates under strict aseptic conditions. The results show that the cultivated fungus is sensitive to various fungal pathogens.

Keywords: *Agaricus bisporus*, fungus, colonization substrates, fruiting media

One of the consequences of the increase in the world's population is increased consumption of meat, leading to an increase in animal husbandry to meet dietary protein requirements (Kerfez, 2015). The term biotechnology includes specific techniques such as microbiology, cellular and molecular biology, genetics, chemical engineering, etc. They have in common the fact that they are involved in a biotechnological process for production of product of interest on an industrial scale (product of interest), like mushrooms (Kerfez, 2015). Mushroom cultivation has been identified as a potential sector offering various benefits such as nutrition, food security as well as improved livelihoods through income diversification and job creation. Mushrooms are still ignored products and farmers adopt this culture slowly, which induces a quantitative and qualitative insufficiency of mushrooms on the market (Imanishimwe, 2018).

In addition, mushroom cultivation and trade provide livelihoods, which can not only reduce vulnerability to poverty, but also improve the ability of a person or community to act on other economic opportunities through generating quick returns and better income. This activity can be practiced, by faith, in rural areas and in urban and peri-urban areas. It is suitable on a small or large scale, for own consumption or as an additional or main source of income (Marshall and Nair, 2009). Indeed, mushroom cultivation is compatible with other livelihood activities, as it requires minimal physical activity, inputs and financial resources. In addition, it represents an activity to be undertaken successfully (Marshall and Nair, 2009). The present work aims to determine and follow the different stages of cultivation of mushrooms at the mushroom farm located at the level of the Zaccar-Miliana caves (Ain Defla).

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MATERIALS AND METHODS

Study area

The mushroom farm is located in Miliana (Wilaya of Ain Defla, Algeria) which is built on the slopes to the south of Mount Zaccar, the highest point of the Dahra which dominates on one side the Chlef and Tipaza on the other. Our study species is *Agaricus bisporus* which is grown in this mushroom farm. The Zaccar mushroom farm is located at an altitude of 1550 m whose coordinates are 36 ° 19 2 52 3 North, 2 ° 12 2 48 3 East

Fungal products

To carry out this work, substrate is prepared for mushroom cultivation using horse manure and composted straw. Additionally, calcium carbonate is added to overcome the mineral deficiencies and to adjust the pH of the substrate. Urea and ammonium sulfate are added optionally to supplement nitrogen in the substrate. In addition, a casing layer is added as a necessary element for the cultivation of the button mushroom. The fungal culture isolation is carried out on agar medium (PDA). The mycelium is then transferred to a sterilized substrate. The product obtained at the end of this operation is called "mother white" which is used as seed for various fruiting substrates. The mother white spawn requires heat treatment to make it sterilized and homogenous for mycelial growth all through the substrate. This also enables us to study mechanism and parameters of mycelial growth in the spawn.

Compost production

It is a process of degradation and fermentation of organic matter in an aerated environment by the combined action of bacteria. It is accompanied with a rise in temperature and results in the preparation of compost. It consists of 75% horse manure (they are obtained at the horse farm of Ain Defla and Blida) and 25% straw. It produces a strong odor due to ammonia and other gases, which have a role in yield. Indeed, the compost is does not have odour of ammonia, means the deficiency of nitrogen in the manure in use and in such composts mushroom mycelium cannot grow well. For the compost, you can use chicken waste which contains a high concentration of ammonia, so when you have a low yield, you mix the two. It is necessary to have a large amount of manure, about ten tons, in order to guarantee a certain degree of temperature and ensure good fermentation. This quantity decreases by 30% after 15 days. It is composed of three zones, namely, an aerobic superficial zone; an aero-anaerobic zone; and an anaerobic (warm) interior zone (Fig. 1).

Fig. 1. The various areas of the compost

Oxygenation phase

The first step in the preparation of compost is oxidative fermentation. This process is carried out by the use of a special machine, manufactured in Miliana by Mr. KHELIFI Abdelkader. This machine therefore provides oxygenation and aeration to the compost by mixing the three zones of manure. It also ensures humidification by adding water for 1-4 days (Fig. 2) which helps the fermentation process. In addition, after 7 days the Gibbs (calcium) is added in order to regulate the humidity level. The same process is repeated on the 11th day and then on the 13th day. Without this oxygenation process, the microbes will not be able to continue their activities, and as a result the temperature drops.

Pasteurization phase

During the second phase, the transfer of the compost to the pasteurization chamber (figure 6) is done by a specific technique and tool (Fig. 3). In order to continue the compost fermentation process, this chamber is equipped with a device that regulates the temperature, provided with probes that measure the temperature and a small fan on the top for ventilation.

1-water to moisten the compost; 2-Steam for oxygenation.

Fig. 2. Compost oxygenation machine

After transferring it to this closed chamber its temperature rises to 60-68 ° C. So it's a "stinging temperature" that lasts 6 to 10 hours. This process eliminates parasites (bacteria and mold) without adding chemicals. After 10 hours, the fan begins to operate in order to decrease the temperature, it takes a maximum of 6 days to reach 25 to 30 ° C and then be ready for larding (Fig. 5).

Fig. 3. Tool for processing compost into the pasteurization chamber

Preparation of the mycelium

This manipulation is simple to perform, the main difficulty is to succeed in maintaining maximum sterility during each step of the process. The process is based on a technique of taking a piece of flesh from inside the fungus aseptically and placing it on a sterile substrate (agar medium) to produce mycelium. The best is to choose a young mushroom (still having the cap closed) and vigorous because the future harvests will depend on this individual.

Seeding

This phase was observed in the caves where the temperature is low, around 17 to 18 ° C, (10 ° C slows down the growth of the fungus cap), the neutral pH = 7.8 and the humidity relative is sufficient RH = 95% (important factor in the production of mycelium) (Fig.

a. Pasteurization chamber

b. Device which regulates the temperature

1-Tool that transfers the compost;
2- Fan; 3- Probes.
c. room probes and fan

Fig. 4. Pasteurization chamber

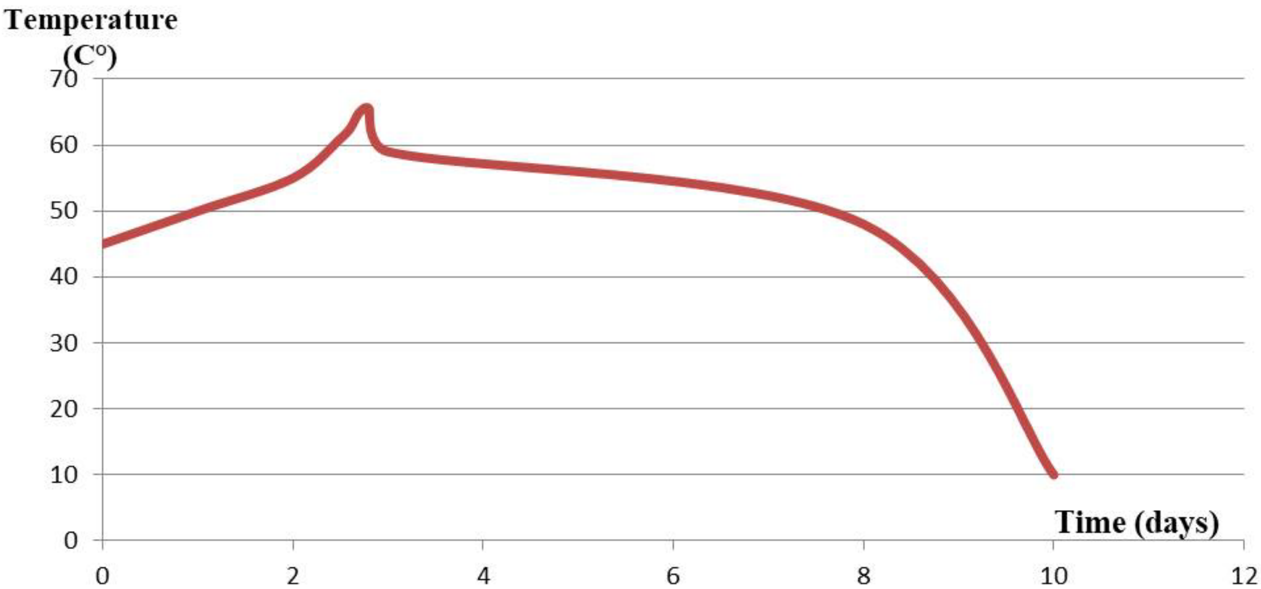

Fig. 5. Curve represents the temperature of the compost as a function of time (days) in the pasteurization phase

6). The white is added and mixed homogeneously with the surface layer of the compost prepared in 15 kg covered sachets (Fig. 7); this operation is called seeding or larding. In general, it takes two weeks for the substrate layer to be sufficiently colonized by the mycelium.

Boating

When the surface substrate is completely invaded by the mature mycelium, it is mandatory to add and cover the sown compost with a cover layer 3 to 5 cm thick (figure 10) (casing layer which is imported from Spain).

Objectives of this step are to provide the necessary microorganisms and the necessary humidity level to encourage the mycelium to produce a good harvest, to protect the mycelium against incidents that may occur during watering directly, to contribute 70% to the good yield and production of the button mushroom and to serve as a water reserve for the compost to keep it moist. The temperature is maintained at 17 °C, humidity at 80-95% and CO_2 percentage at 900 - 1000 ppm.

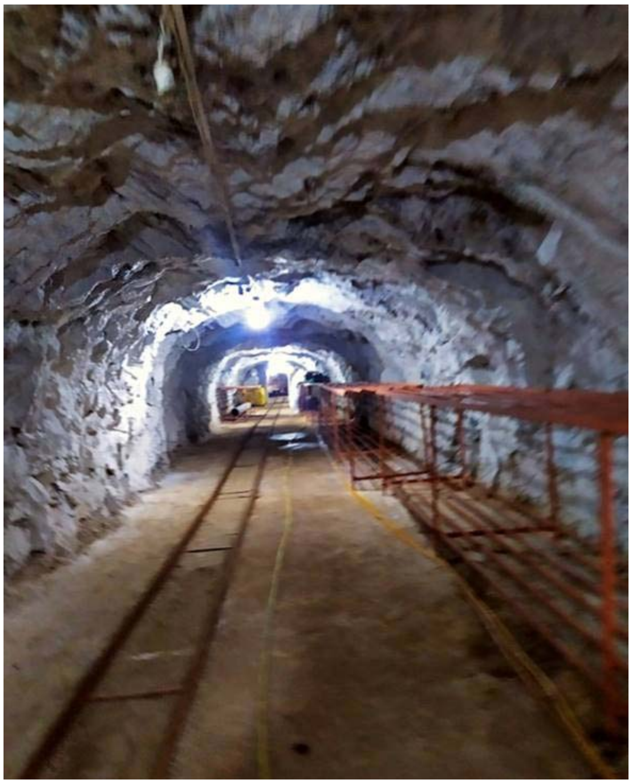

Fig. 6. The Zaccar-Miliana caves

Fig. 7. Seeding of the mycelium

Fig. 8. Substrate invaded by mycelium

Harvesting

When the substrate is completely colonized, it is necessary to lay out a casing layer and then place it again in incubation condition until the mycelium "pierces" this layer (1 to 3 weeks). It is then necessary to place this substrate in fruiting condition while respecting the parameters of the species (15 °C - 20 °C, humidity and good ventilation). Indeed, it will be possible to pick the mushrooms by performing a slight twisting movement, so that they come off easily. The mushrooms will grow in several waves called "flocks". After each flight the substrate must be cleaned and all the mushrooms must be harvested. In addition, some grow in large groups under which, after harvesting, a kind of "rock" remains which must be removed from the substrate (risk of rotting). Then you have to replace the casing layer and spray a little sterile water on the surface, then wait until the next harvest. After three harvests, the substrate will be worn out and can be used as fertilizer in gardens. It is mandatory to put "fly and flying insect catch rollers" in the caves (Fig. 9) in order to get rid of them because they negatively affect the cultivation and quality of the mushrooms. At the end of the harvest, it is necessary to disinfect the caves with formalin taking into consideration the prevention methods (mask, protective gloves, etc.).

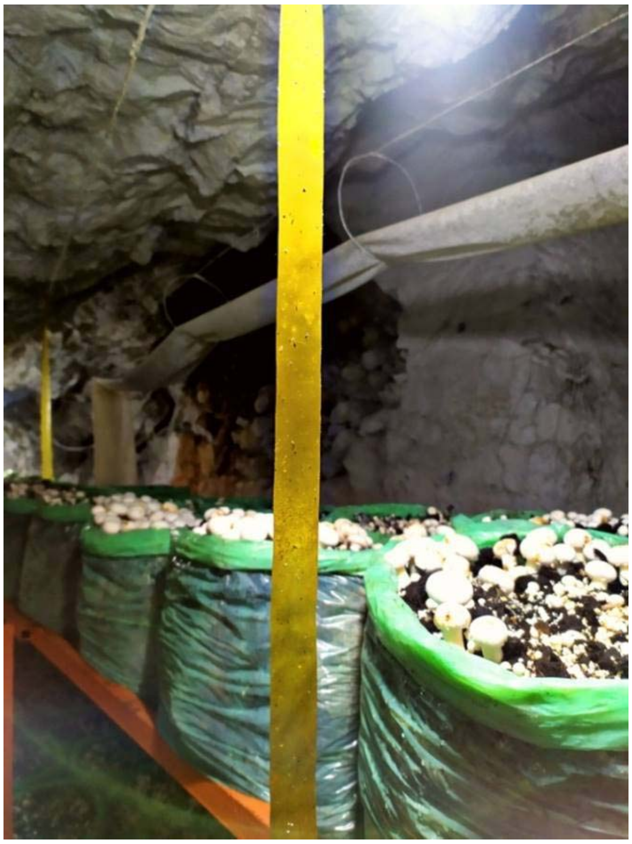

Fig. 9. Flytrap rollers and flying insect

RESULTS AND DISCUSSION

In the harvest phase, only man can judge the maturity of the mushroom and pick it at the right time, according to their shapes, sizes and colors. In this phase, we found white mushrooms (Fig. 10) with a round cap and a smooth stem, more or less cylindrical thanks to a good casing layer.

Other types of mushroom are cultivated with the same process, they are the brown button mushroom, button mushroom with yellow to brown spots at the cap and sometimes up to the stems (Fig. 11). Unlike the white, the brown fungus is considered hardy and not very susceptible to attacks from other fungi.

Fig. 10. Mature button mushroom ready to be picked

In compost alone, *A.bisporus* could not form fruit bodies. This is the reason why the compost should be covered with a layer composed of a mixture of different varieties of peat. This layer, we call it casing earth (Kerfez, 2015). The casing must be free from pathogens (Flegg and Smith, 1982). It should have the correct structure, pH 7.8 and a capacity to retain a large amount of water. Bacteria that live in the casing soil stimulate the mycelium to form fruiting bodies. Therefore, good casing soil is essential for mushroom cultivation (Fritsche, 1978). Certain biotic or abiotic conditions can be harmful for *A.bisporus*, and result in either a reduction in crop yield or in the production of poor quality mushrooms, unsuitable for marketing (Largeteau, 2007).

Two abiotic conditions are responsible for a drop in quality: Too much humidity which causes browning of the cap of the sporophores and a high concentration of CO_2 during the fruiting period will promote the development of the foot at the expense of the cap, a deformation of the carpophore as well as a greater potential for contaminant. The room must therefore be well ventilated during fruiting if this is possible (Largeteau, 2007).

Various biotic agents (bacteria, fungi (molds), insects, etc.) are responsible for the drop in production

a. Brown button mushroom

b. Button mushrooms with brown spots on the cap and feet

c. Button mushroom with a slightly longer stalk

Fig. 11. Other types of button mushroom

and / or loss of quality in commercial crops of *A.bisporus*. Most of the pathologies concern the sporophore formed or in the course of formation, but the mycelium can also be damaged (Largeteau, 2007) among these biotic agents. Bacteria cause bacterial stain or bacteriosis; which is the most common of the cultivated mushroom and, every year, it causes significant economic losses by degrading the quality of the crops. Symptoms appear more often on fruit bodies that remain moist for long periods of time. The most common symptom is the appearance of pale yellow spots on the top of the hat that turn golden yellow then yellow, brownish or chocolate brown. The foot can also be affected. Sometimes the hats take on an unappealing coloring, deteriorate quickly, and change color after harvest.

The causative pathogen is *Pseudomonas tolaasii*, which is an aerobic, Gram-negative, non-sporulating, rod-shaped bacterium which measures approximately 0.5 by 1 to 2 µm. It is fluorescent, oxidase negative and arginine dihydrolase negative. It metabolizes L-arginine and L-arabinose and does not grow below 4 ° C. The bacteria are found naturally in peat and lime used for casing and can be easily isolated from compost after pasteurization. Between crop cycles, it probably survives on the surface of mushroom structures, in debris, on tools and on equipment. The bacteria can be easily spread from crop to crop by handling, harvesting equipment, insects, mites, water drops and fungus spores. Once the disease has set in, watering quickly spreads the bacteria. Usually the incidence of the disease is particularly high in the first flight. As the crop ages, there are fewer fungi and more air circulates around the fruit bodies, resulting in better drying after watering and lower disease incidence. The bacterial spot is controlled by modifying the environmental conditions. High levels of relative humidity and humidity on the surface of fruit bodies promote symptom expression. When the fungi remain moist for more than two to three hours, the spot grows quickly. Increased ventilation after watering helps the crop to dry out. Maintaining a stable difference of 1 to 1.5 ° C between psychrometer readings significantly decreases the incidence of the problem. When the spot is a problem, avoid watering the crop on two consecutive days and one or two days before harvest (Wicht *et al.,* 1971). Fungus *Verticillium* can attack at different stages of the crop, and depending on the weather, its consequences can be seen one way or another. If the infection occurs during the formation of the primordia, they can be severely affected, with serious, incurable deformities. If it comes to a more advanced stage, gray or brown circular shaped spots may appear on the fruit bodies and deformities such as the wider base or twisted hat (Quesada Moya, 2020).

So mites are insects and are naturally present in compost, straw or other substrates used for growing mushrooms. Many are beneficial and do no harm to our psilocybs, but there are others that can be fatal. *Tarsonemido* is a microscopic brown mite that feeds on hyphae, causing reddish spots at the base of the fungi. Mites of the genus *Tyrophagus* feed on organic debris, are translucent in color, and damage the stems and caps of fungi. The pygmemites usually warns of the presence of *Trichoderma*, as this mite feeds on mold and can help spread infection. They appear flattened and reproduce very quickly (Quesada Moya, 2020). There are also the mushroom flies which can create contaminations during the cultivation of mushrooms which are: *Sciaridae*, *Lycoriella* and *Megaselia*. They are small insects that are usually no longer than 0.3 cm and dark in color. Their larvae are lighter in color and half the size of adults, they feed on mycelium and can transmit diseases such as *Cobweb*, *Mycogone* or *Verticillium* (Quesada Moya, 2020). Finally, there are the nematodes which can also cause contamination problems during fungal formation and are present in most agricultural substrates. They attack the mycelium, turning dark and causing malformation of fungi, or directly inhibiting their growth, so they can be extremely annoying (Quesada Moya, 2020).

Currently, of the edible macromycetes in industrial production, *A. bisporus* is the most widely cultivated in the world. It represents more than a third of the 3.2 million tonnes of edible mushrooms produced annually (Faostat, 2004), followed by shiitake (*Lentinula edodes*) and oyster mushrooms (*Pleurotus spp.*) (Chang, 1999; Beelman *et al.,* 2003). In 2004, French production was in third position in the European Union and fifth in the world after that of China and the United States. Poland is the main producer among the new members of the EU, and comes second for European production in 2004 and 2005 (Largeteau, 2007). French production is figured at 75,000 t produced in France in 2019; 300 t exported by France in 2019; 2,500 employees employed by the French sector; 250 million annual turnover; 4th place in France worldwide (Masbou, 2019). In 2016, there were still six mushroom farms in the Paris region, in Val-d'Oise, in Oise and in Yvelines. Supplier to chef Yannick Alléno, Grégory Spinelli, producer in Saint-Ouen-l'Aumône, explains that "the real Paris mushroom is cultivated in the cellar. And it does not grow in peat but on a layer of limestone, from which it draws all the minerals and which rejects less water. This is all that makes the difference with button mushrooms from Holland or Poland" (Chevallier, 2016). The mushroom loses the Parisian accent, it is still called "Paris mushroom". But its production in Ile-de-France is now anecdotal. The French remain frequent consumers of this product (Masbou, 2019).

Africa, Maghreb, Algeria

According to our extensive research, there is no information regarding the existence of cultivators of the button mushroom in Africa or the Maghreb. Indeed, the cultivator of Miliana (Wilaya of Ain Defla): the mushroom farm of Zaccar, remained the only one who developed a cave culture of this kind in Algeria.

CONCLUSION

According to our observations, the cultivation of *A.bisporus* in the Zaccar caves (Compost production, Mycelium preparation, Inoculation, Casing, Harvest) has been successful and encouraging. We note that for a good triggering of the buttons, it is necessary to take into consideration the temperature conditions between 17 ° C and 18 ° C, a humidity of around 90% as well as a high concentration of CO_2. The advantage of caves is to keep these favorable climatic conditions for its constant growth. It is possible to take into consideration the choice of efficient strains to avoid the aging of the strains, as well as the choice of casing land because the success of its culture will depend on the selection of the most adequate soil and also the influence of the soil. humidity and CO_2 concentration, which can be either a stimulant or an inhibitor. Contamination is considered to be the first enemy of button mushroom cultivation, so it must be avoided through reliable, well-ventilated infrastructures.

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