ABSTRACT

Growing mushrooms is an art based on science and technology. The science of growing mushrooms is well-known and the progress recorded in the last decades is remarkable. But the art of their producing consists in ordering some variable factors with a decisive part in achieving the desired results. The space meant for growing, the raw materials used, the available biological material, the microclimate conditions ensured, the biosecurity of the technological process and last but not least the experience in producing mushrooms are among these factors. Most of those who start this activity expect fast results, obtained in a quite facile way, but the success in this field lies at the end of a rather long list regarding the minimum conditions which must be accomplished, to the astonishment of many of them.

At present, the mushroom growers all over the world substantially invest in applying new technologies which are meant to increase productivity, to improve quality and to obtain "secure" products. In Romania, the investors in this field must look for the most efficient solutions in order to attain the same objectives and, in particular to ensure their financing for a complete investment and training a responsible staff who must be aware of the exigency requirements of the technological process for producing mushrooms.

The main target of the research done was to analyze the factors which intervene during the technological process for producing mushrooms, since the raw materials were received and till the delivery of the of the packed product, ready for consuming, and to intervene in each stage by clearing risk factors which can affect both the production and the ascertainment of the optimum variants which are meant to lead to the anticipated results.

The research regarding this technology for growing *Pleurotus* mushrooms was carried out in S.C. "AGET IMPACT" S.R.L. Podoleni, Neamt, from 2002 to 2006.

The master's degree paper is presented on 316 pages and it is divided into five chapters. The paper contains 108 tables and 129 pictures and the bibliography contains a number of 227 titles quoted in the paper.

In Chapter 1 an incursion in the background of growing mushrooms is done, the evolution and dynamics of the production, the nourishing and therapeutical value of the mushrooms as well as the present stage of the research regarding the growing of mushrooms are presented. The phenology and reproduction elements, the taxa of the most spread worldwidely species of mushrooms – *Agaricus spp*. and *Pleurotus spp*. are also presented.

In comparison to other species, the *Pleurotus* mushrooms were grown much later, only

at the beginning of the 20th century. In 1910 Matruchot, a French botanist, has obtained *Pleurotus* mushrooms from spawn to the stage of carpophores forming in the laboratory. From 1916 till 1917 the controlled growing of *Pleurotus spp.* on tree stocks was carried out and in 1929 the first fructifications of *Pleurotus* were obtained on a cellulosic mixture made up of pine sawdust, malt extract and maize flour. After 1965 research regarding growing mushrooms on lignocellulosic waste was done.

In Romania, growing *Pleurotus* mushrooms started in 1972. The culture technologies for the species of *P. ostreatus, P. florida, P. cornucopiae* and *P. sajor-caju* , which were first grown between 1972-1985, were established by a group of researchers made up of Mateescu Nicolae, Ioana Tudor and Zagrean Valentin.

Mushrooms are food-stuff with a high therapeutical and nutritious value. They possess the three main properties which a food product must meet: • they are rich in proteins, vitamins, mineral salts and they have a reduced amount of lipids; • they have a delicious taste, with unique flavors; • they presents physiological functions owing to bioactive substances which they contain. Traditionally, it was found out that the mushrooms have anti-cancerous and immunological therapeutical properties which have mainly been tested in the last three decades. The most important medical effects of the mushrooms are as it follows: • imuno-controller; • antioxidant; • gene-protector; • anti-allergic; • anti-cancerous; • liver-protector; • anti-diabetes; • hypo-cholesterolic. Thus, the mushroom growers supply a valuable, simple and fresh product to the consumer.

In Chapter 2, the main growing, ecological and morphological characteristics of the most grown species of lignin cellulosic species of mushrooms are presented. The systems, methods and places used in growing *Pleurotus* mushrooms, the growing substrate and its composition as well as the stages of the technological flow for producing *Pleurotus* mushrooms are fully presented in this chapter, too.

The growing places which are meant for growing *Pleurotus* mushrooms must be thermal insulated, well ventilated, artificially illuminated with fluorescent lamps and they must maintain the humidity within the optimum parameters. The growing place must be adjusted for the intensive system so that it will allow to carry on the technological process in optimum conditions as well as the operations of hygiene, disinfection and the evacuation of the used substrate.

The *Pleurotus* mushrooms, on the basis of their enzyme composition, have the ability to use the three categories of policarbohydrates which are present in the biomass of the vegetal materials that is cellulose, hemicellulose and lignine. Thus, these mushrooms can be grown on a large variety of substrates which are worldwidely available, substrates which have as a basis cellulosic waste proceeded from agriculture and silviculture. In Romania, most of the substrate recipes are based on corn cobs, cereal straw and deciduous tree sawdust.

The disinfection of the growing substrate represents the most important stage in the technological process for producing *Pleurotus* mushrooms. The quality of the cellulosic substrate at the moment of spawning is determined by its selectivity and it appears as a feature of the nutritive material for which the optimum conditions necessary to a fast and efficient colonization by the mushroom growing are created.

Creating selectivity is achieved by the pasteurization of nutritive substrate which has the purpose to eliminate the competitors (disease and pests) out of its mass and the purpose to protect and stimulate the useful micro-flora, present in the substrate, which competes with the harmful micro-organisms. Moreover, these useful micro-organisms have the ability to decompose the hemicellulose which *Pleurotus* mushrooms can't directly consume. Thus, the disinfection of the lignin cellulosic substrate should be done by pasteurization and not by sterilization. The sterilized growing substrate represents an easy target to the harmful micro-organisms in the air, water, soil etc. and the infections with different mould can be massive.

The cultivation systems differ depending on the species and the nature of the used growing substrate, thus having two growing systems: growing on broken up cellulosic substrates and growing on logs. The selecting of cultivation methods is done depending on the mushroom's variety, market demands and farmers' preferences the most recommended being the cultivation methods on logs, shelves, boxes and polypropylene containers or polyethylene bags.

The main growing characteristics of the *Pleurotus spp*. mushrooms, aspects regarding the harvesting and selling of mushrooms, the identification of the potential hazards associated with mushroom production and processing as well as some elements of economic efficiency of growing are also presenting.

In chapter 3 the aim and the objectives of the research, the material and working method, the conditions in which the experiments took place are presented. The main elements involved in working out the technology for producing mushrooms were the objectives of the research theme:

- Growing space and the conditions which must be provided by it in order to carry on the technological process in optimum conditions;

- Nutritive substrates, the influence of its quality and composition on mushroom production;

- Growing *Pleurotus* mushrooms on different broken up cellulosic substrates made up of corn cobs, wheat straw and beech sawdust;

- Growing *Pleurotus* mushrooms on wooden substrate, made up of beech and poplar logs;

- Enriching cellulosic substrate by means of some protean substances;

- Disinfecting cellulosic substrate and the influence of different used disinfection methods on production;

- The quality of the biological material used in growing, the spawn rate used for spawning and their influence on production;

- The amount of substrate used for filling containers and its influence on the spreading capability of the spawn;

- The evolution in growing of four *Pleurotus* hybrids and the production obtained on different types of substrate.

The biological material used in these experiments was made up of four *Pleurotus* hybrids: HK35 - produced in Hungary, H 3033, H 421 and HK35 produced in Romania. The comparative experiments were organized by using the method of randomized block in three repetitions, three growing cycles per year.

The growing technology which was applied in order to achieve the desired objectives has taken into account two major aspects concerning the international and European legislation implementation relating to food production:

♦ applying the guide of good management methods for the safety of growing, harvesting and packing of fresh mushrooms;

♦ integrated control of the disease and pests in growing mushrooms.

Chapter 4 includes the experimental part in which I have presented the results of my own research. The following experiments have been organized:

Experiment 1 (2004-2005). *The growing space and its influence on the production of Pleurotus mushrooms*. Three spaces were used for this experiment: a mushroom house – a specially arranged space for growing *Pleurotus* mushrooms throughout the year and in this space six growing cycles per year are achieved and other two provisionally arranged spaces – a storehouse and a cellar, spaces in which three growing cycles per year can be achieved.

The results obtained demonstrate that the growing space influences the mushroom production through the microclimate conditions which it must ensure throughout the production cycle. For growing mushrooms in an intensive system, the growing space must be thus measured as to ensure the necessary spaces for the performing of technological flux in optimum conditions, of hygiene, disinfection and the evacuating of used substrate operations as well. The average production obtained in the mushroom-house was 24,1 kilos mushrooms for 100 kilos substrate (three growing cycles), this production being very significant in comparison to the average production obtained in the storehouse and cellar – 20,0 kilos, respectively 19,05 kilos, for 100 kilos substrate (three growing cycles).

The expense registered in those three growing spaces was different, as it follows: in the mushroom-house, the expense for 100 kilos substrate amounted to 23,4 € whereas in the storehouse and cellar, the expense was more reduced (17,0 € respectively 16,2 € for 100 kilos substrate. The income registered for 100 kilos substrate (three growing cycles) was, in average of 42,6 € in the mushroom-house (the price per kilo varying in accordance with the period for turning to profit), whereas in those two provisionally arranged spaces, the income was 30,2 €, respectively 28,8 €.

Experiment 2 (2004-2005). *The cellulosic nutritive substrate and the influence of the substrate composition on the amount and quality of the Pleurotus mushrooms*. Three different cellulosic materials were used in this experiment: corn cobs, wheat straw and beech sawdust which were used in order to obtain nine substrates recipes, representing the experimental variants: V_1 - corn cobs (100%); V_2 - wheat straw (100%); V_3 - beech sawdust (100%); V_4 - 50% corn cobs + 50% straw; V_5 - 50% corn cobs + 50% sawdust; V_6 - 50% straw + 50% sawdust; V_7 - 75% corn cobs + 25% straw; V_8 - 75% straw + 25% corncobs; V_9 - 35% corn cobs + 35% straw + + 30% sawdust. In the case of experimenting nine recipes of substrate, the most increase average production was obtained for the substrate variant made up of 75% corn cobs and 25% wheat straw (23,2 kilos mushrooms/100 kilos substrate). The maximum biological efficiency was registered in the case of the same variant of substrate (66,28%), and the minimum one in the case of the variant made up of beech sawdust (20,8%).

The lowest productions were obtained when the recipe made up 100% of sawdust (10,4 kilos mushrooms/100 kilos substrate) was used. Medium weight/bunch, the highest, was obtained for the substrate variant made up of 75% corn cobs and 25% wheat straw (150 grams/bunch). The highest number of bunches (medium), was obtained for the substrate variant made up of 50% corn cobs and 50% wheat straw (21 bunches/briquette).

Experiment 3 (2004-2005). *Remarks regarding the growing of Pleurotus mushrooms on substrates made up of corn cobs, straw and beech sawdust*. For the third experiment, four types of substrates made up of the most frequently used materials were used, that is: V_1 - corn cobs 100%; V_2 - wheat straw 100%; V_3 - beech sawdust 100%; V_4 - 50% corn cobs + 50% straw. The spawn rate was 1% of HK35 hybrid. The average biological efficiency, the highest (66,48%), was recorded for the substrate type number 4, made up of 50% corn cobs and 50% straw and the lowest (28,2%) in the case of the type number 3 made up of beech sawdust 100%. The highest values as far for the diameter of the cap and the weight of the carpophores were recorded on the substrate made of 100% corn cobs (7,5 cm in diameter, 27,5 g/carpophor). The heavier the carpophores are, the fewer their number within a fructification bunch is.

The highest growing speed (mm/day) was recorded for the type made up of 100% corn cobs. The first two flushes are significant. As a percentage, the average production recorded for the first two harvests represents about 60%, respectively 30% of the total production obtained for a growing cycle.

Experiment 4 (2002-2006). *The experimental growing of Pleurotus mushrooms on wooden substrate*. This system of growing was tested for five years, that is 2002-2006, in a roof-covered space in which the necessary microclimate conditions were ensured: temperature, humidity, lighting, ventilation. The inoculation of the logs was done in January-February 2002, the first harvest being recorded in February 2003. Stocks of two ligneous species were used: poplar, lightwood species and beech, hardwood species.

The growing system of *Pleurotus* mushrooms on wooden logs of different essences is recommended to the outdoor places, without any investments for ensuring the microclimate conditions, the productions being obtained on stages within time periods from 2 to 4 years. The efficiency obtained on the substrate made up of beech wood was of 36,08%. For the substrate made up of poplar wood, the efficiency was of 30,34%. In the first two years, 75%, respectively 85% out of the total production recorded during those four years of growing, was obtained. In relation to the morpho-growing characters of the carpophores an increasing of both of the cap diameter (8,8-10,6 cm in diameter), and of the average weight of a carpophore (47,1-62,5 g) was noticed, the average number of carpophores being of 3-4/bunch.

Experiment 5 (2004-2005). Enriching cellulosic substrate with some protean materials. Wheat bran, sunflower extraction residues and malt in different percentages have been used in this experiment, the variants being as it follows: V_1 - 0% protean addition; V_2 - 1% protean addition; V_3 - 3% and V_4 - 5% protean addition in comparison to the wet nutritive substrate. The nutritive substrate was made up of 50% wheat straw and 50% corn cobs.

The protean materials added to the substrate bring production gains due to the increasing of total nitrogen. A too high percentage of protean additions lead to the overheating of substrate and the inhibition of the spawn, compromising the crop. A direct-proportional increasing of the production with the percentage of the added protean material was found.

The differences of production recorded in the case of the substrate types for which the protean addition was of 3%, respectively 5% being insignificant, the use of the rate of 3% protean addition is recommended, thus the risk of increasing temperature in the nutritive substrate being eliminated. The best results were obtained in the case of using sunflower extraction residues as a nutritive supplement, the production gain obtained in comparison to the „control" being of 4,2% (3% protean addition). For the types of substrate to which wheat bran, respectively malt, were added, the production gain recorded in comparison to the „control" were of 3,5%, respectively 2,9% (3% protean addition). Besides the increasing of mushroom production, one could notice that the adding of protean substances to the nutritive substrate influences in a positive way the time length of the growing cycle, bringing about the reducing of number for production days.

Experiment 6 (2004-2005). *The disinfection of the substrate*. For thermal disinfection a pool having a capacity of 4,5m^3 fitted with a heatproof lied at the upper side was used. The following variants have been used: V_1 - without thermal treatment; V_2 - boiling for an hour; V_3 - steam injected 60°C - 3 hours; V_4 - immersion in hot water - 55-60°C -1 hour. In the case of the chemical disinfection the experimental variants were as it follows: V_1 - without chemical disinfection; V_2 - Virkon - 0,01%; V_3 - Bavistin - 0,02 %;V_4 - Topsin + Ridomil- 0,01%.

Hydrothermal disinfection (with hot water at 60°C, for an hour) represents the safest way of pasteurization for the cellulosic material, in terms in which performing installations (tunnels for pasteurization in controlled atmosphere) are not ensured. The best productions (25,7 kilos mushrooms to 100 kilos substrate) were obtained in the case of using the hydrothermal method for pasteurization (immersion in hot water, at 60°C).

The lowest productions were obtained by sterilizing cellulosic materials (15,8 kilos mushrooms for 100 kilos substrate), sterilization which destroyed all the useful micro-organisms that existed in the substrate, thus an extremely favorable medium was created to the infecting with germs especially moulds (*Trichoderma*).

The chemical disinfection, by means of some disinfecting substances, which belong to the new generation that have killing virus, bactericide and fungicide action , represents a method which takes place with no thermic consumption and it leads to good results of production. Using Virkon (0,01%), as a disinfectant of the cellulosic material, lead to the recording of an average production of 22,4 kilos mushrooms to 100 kilos substrate. The best type of chemical disinfection was the one in which the Bavistin fungicide-0,02% was used, in the case of which an average production of 23,9 kilos mushroom to 100 kilos of nutritive substrate was obtained. The average production recorded in the variant in which the Topsin + Ridomil fungicides -1,01% were used was of 23,5 kilos mushrooms to 100 kilos substrate.

Experiment 7 (2004-2005). *The influence of the spawn rate used for the inoculating of nutritive substrate on production and time length of the production cycle*. The substrate made up of 50% wheat straw and 50% corn cobs was pasteurized (hydrothermal method) and then spawned with different spawn rates of HK35 hybrid, produced in Hungary, the rate recommended by producer being of 1kilo/100 kilos substrate. The experimental variants were as it follows: V_1 - 0,5% spawn; V_2 - 1% spawn; V_3 - 1,5% spawn; V_4 - 2% spawn.

Using a spawn rate of 1% led to getting a high average production (25,9%), the expense registered being of 4 €/100 kilos substrate. In the case of using a spawn rate of 1,5%, the average production which was obtained increased to 26,3 kilos mushrooms for 100 kilos substrate. When doubling the spawn rate, the average production recorded was of 27,5 kilos mushrooms to 100 kilos substrate (the increase being of only 1,6%), meanwhile the afferent expense for the spawn/100 kilos substrate doubled.

The optimum amount of spawn for spawning substrate is of 1-1,5% (imported spawn), respectively 3% in the case of using autochthonous spawn. Higher rates cannot be justified economically, rising these rates does not lead to significant gains of production. In addition to other factors, using a high quality spawn, in optimum doses, ensures an efficient and rapid incubation and it can shorten the time length of the production cycle.

Experiment 8 (2004-2005). *The influence of the substrate quantity used for the filling containers over the spreading ability of the spawn and on mushroom production.*

The substrate made up of 50% wheat straw and 50% corn cobs was pasteurized using the disinfection method with hot water at 60°C, inoculated with 1% spawn, the used hybrid being HK35. The spawned substrate was put into transparent polyethylene bags of different sizes. The bags were previously perforated, the orifices of 2 cm diameter, the distance between them being of 15 cm. The bags dimensions were: 25x50 cm; 35x70 cm; 40x70cm; 50x70cm. The experimental variants were as it follows: V_1 - 5 kg nutritive substrate/bag; V_2 - 10 kg nutritive substrate/bag; V_3 - 15 kg nutritive substrate/bag, V_4 - 20 kg nutritive substrate/bag.

The amount of nutritive substrate distributed/container influences both the spreading capacity of the spawn and the production obtained/container. The more reduced the substrate quantity is, the faster the incubation ends, thus the risk of infestation with germs being eliminated. The number of days necessary to the incubation increases direct proportionally with the substrate quantity distributed/container. The optimum quantity of spawned substrate distributed in a container is from 8 to 10 kilos.

Experiment 9 (2004-2006). *The evolution in comparative growing of four Pleurotus hybrids*. The experimental variants were represented by the four hybrids used for inoculation: V_1 - H 3033, V_2 - H 421, V_3 - HK35 autochthonous, V_4 - HK35 produced in Hungary. The used growing substrates were as it follows: wheat straw, corn cobs, beech sawdust, corn cobs + wheat straw.

The low temperature (10-12°C) lead to a slight spreading of the spawn, the growth of some competitors of the spawn (moulds) being thus stimulated. The highest increase was recorded in the case of the spawn of HK35 hybrid produced in Hungary, at an environmental temperature of 25°C. As far for the earliness of the appearance of the primordias, it was established that the low temperatures during the incubation delay the appearance of the fructification primordias with 6-12 days, compared to the conditions of optimum temperature (22-24°C).

Regarding the spreading of the spawn into the nutritive substrate the highest speed of growing was recorded in the case of the HK35 hybrid, inoculated on millet support. A faster colonization of the substrate which was spawned with this hybrid is mainly due to the much more inoculation points/100 g spawn, in comparison to the spawn produce don wheat or barley support. After 7-10 days after spawning, the spawn spread into the nutritive mixture mass in a percentage of 90-100%.

The time length of a growing cycle (three flushes) was from 45 to 48 days at HK35 proceeded from Hungary and 51-54 days at H421. The lowest number of carpophores/kilo substrate was obtained at HK35 inoculated on millet support but their dimensions and weight were bigger than in the case of other hybrids. As far for the production, the lowest crop, in the case of all hybrids which were observed, was recorded on the substrate made up of beech sawdust (12,9-15,8%).

HK35 inoculated on millet support proved to be the most productive, for all tested substrates, having a production between 15,8% and 24,6% compared to the wet substrates, for three flushes. This hybrid was more productive on the substrate made up of 50% wheat straw and 50% corn cobs (24,6%).

The highest biological efficiency (68,18%) was obtained in the case of the HK35 hybrid proceeded from Hungary and grown of corn cobs. The most reduced values of the biological efficiency were recorded in the case of growing the four hybrids on a substrate made up exclusively of beech sawdust.

In *Chapter 5* the conclusions of the performed research and recommendation are presented. After analyzing our own research, we can conclude that the obtained productions, that is the mushrooms packed and displayed on the shelves in supermarkets, are the results of interaction of all factors which intervene in the technological process of producing mushrooms, beginning with vegetal materials in the field and ending with the quality of the packing in which the mushrooms are introduced after harvesting. The human factor constantly intervenes and this happens in every stage of the technological process.