

IMPORTANCE OF SUBSTRAT DIZINFECTION ON OYSTER MUSHROOM (*PLEUROTUS* SP.) CULTURE

Ficior Diana¹⁾, D. Indrea¹⁾, Al. S. Apahidean¹⁾, Maria Apahidean¹⁾, Rodica Pop¹⁾,
Z. Moldovan²⁾, D. Mănișiu¹⁾, Rodica Ganea¹⁾, I. Paven¹⁾

¹⁾ University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Mănăștur St., 400372, Cluj-Napoca, Romania; e-mail: diana_ficior@yahoo.com

²⁾ National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania

Abstract. Interest for oyster mushrooms is increasing very much in the last years due to their special taste and nutritional value. One of the most important aspects on mushroom culture is represented by substrate disinfection. The yield can be compromised if the competitive microorganisms are not removed from the cellulosic materials. The aim of these researches was to determine the most efficient method of disinfection. We have chosen five different methods of disinfection: material boiled for one hour, material boiled for 10 minutes, material scalded with boiled water (100°C), material disinfected with a fungicide (Derosal 0.01%) and material soaked in water for 24 hours with no disinfection. It has been noticed that method of disinfection affects the mycelium development, date of fructification and yield. The best yields have been obtained for variants with material scalded with boiled water (100°C), material disinfected with a fungicide (Derosal 0.01%) and material boiled for one hour. Mushrooms grown on material without disinfection have recorded very low yields.

Key words: Oyster mushroom, *Pleurotus* sp., cellulosic substrate, disinfection

INTRODUCTION

Oyster mushrooms (*Pleurotus* sp.) are one of the most appreciate mushrooms due to their high nutritional value, very good taste and medicinal value. Technology is relatively simple and materials used as substrate are relatively cheap. Oyster mushrooms can be cultivated on a wide range of cellulosic materials. To diminish the damages produced by different pathogens (bacteria, moulds or pests) on mushroom development and yield it is recommended to disinfect the substratum before spawning. Removing the competitive fungi will permit a faster and better and more uniform spawning and will assure a better resistance to future infections. In consequence the mushroom yield will be higher and qualitatively superior.

The disinfection method should destroy only the competitive fungi and not the useful micro organisms. These useful micro organisms do not compete with mushroom mycelia but they disturb the development of competitive micro organisms (Apahidean, 2006). From this point of view it is better to use for disinfection pasteurization rather than sterilization.

Disinfection can be done in different ways: thermal with water at different temperatures, with hot steam or chemical. The temperatures recommended vary very much. For example, Mateescu (1985) recommended pasteurization at 55-58°C for 6-24 hours while Mirosilava Ziombra (1999) recommends maintaining the substrate for 24 hours at 70°C and

Oei (1996) mentions sterilization as a suitable method as well. Chemical disinfection can be done with diluted solutions of fungicide (Indrea and Apahidean, 1995).

MATERIAL AND METHODS

The aim of the experiment was to determine the most efficient method of disinfection for cellulosic substrate used in oyster mushroom culture. In order to realise this aim we have chosen five methods of disinfection.

In the experiences has been used the *Pleurotus hybrid HK 35*. The fruit bodies of this strain appear in clusters and have a grey-brown colour. Comparative experiments were organized by randomized block method in three repetitions; four bags represented experimental plot.

As a cellulosic substrate it has been used corn cob. The material was shredded into pieces of 1-5 cm and moistened. The substrate was then disinfected. In the case of variant 1 and 2 materials have been boiled for one hour respectively 10 minutes and then let to cool on a platform. Material for third variant has been scalded with boiled water and let to cool down slowly. For the fourth variant the cellulosic material was soaked in a Derosal solution (0.01%) for 48 hours. Finally, the material for the fifth variant was not disinfected at all and it has been only soaked in water for 24 hours. In this way have resulted five variants:

V₁ – material boiled for one hour;

V₂ – material boiled for 10 minutes;

V₃ – material scalded with boiled water (100°C);

V₄ – material disinfected with a fungicide solution (Derosal 0.01%);

V₅ – material soaked in water for 24 hours.

Comparative experiments were organized by randomized block method in three repetitions; four bags represented experimental plot.

Boiled barley grains have been added to the substrate as a source of nitrogen. To regulate the pH of substrate it has been added CaCO₃ (4%). After cooling, the substrate has been spawned with 3% spawn and it has been filled in perforated plastic bags with 30 l volume.

During the culture the usual technology has been applied and the climatic conditions have been maintained in the optimal parameters.

The harvesting of fructifications has started in November and finished at the end of January. During this period mushrooms have been harvested in three flushes.

During the experience several observations have been done: spawning rate, number of days from spawning to pinning and fructification, crop dynamic and yield. In the same time it has been determined the efficiency of disinfection method on pre-existing micro organism (using as a culture media Czapek-Dox, for fungi and Thornton, for bacteria) and the accumulation of fungicide in the fruit bodies.

RESULTS AND DISCUSSIONS

Spawning rate and mushroom development

During the vegetation period of mushrooms it has been observed the rate of spawning and the length of period between spawning and pinning respectively harvesting (Table 1). As it can be noticed the best rate of spawning has been obtained for variant 1 (material boiled 1 hour), 3 (material scalded with boiled water) and 4 (chemical treatment). The lowest spawning rate has been realized in the case of variant 5 (untreated substrate). The shortest

period of vegetation has been observed in the case of variant 3 (material scalded with boiled water), 4 (chemical treatment) and 1 (material boiled 1 hour). These suggest that a good method of disinfection will permit to mushroom mycelia to grow better and faster so the yield will be obtained earlier.

Table 1
Influence of disinfection method on spawning rate and mushroom development

Variant	Spawning rate	Number of days between spawning and	
		pinning	fruiting
V ₁	+++++	21.5	29.0
V ₂	+++	24.0	31.5
V ₃	+++++	18.0	25.0
V ₄	+++++	19.5	28.0
V ₅	+	22.5	31.5

After the disinfection has been taking it samples to determine the effect of different methods of sterilization on micro organisms. There have been used two different media: Czapek-Dox for fungi and Thornton for bacteria. The observations have been done after four and fourteen days from the inoculation of media. There have been identified several species of fungi (*Aspergillus niger*, *Mucor mucedo* and *Fusarium* sp.) as well as fermentative bacteria. As results from the Table 2 the variants with the lowest rate of infection were variant 1 (material boiled 1 hour) on both media followed by variant 4 (chemical disinfection) in the case of Czapek-Dox media respectively variant 3 (material scalded with boiled water) in the case of Thornton media. The highest rate of infection has been realised as we were expecting for the substrate without disinfection (variant 5).

Table 2
Effect of different methods of disinfection on microorganisms from cellulosic substrates

Variant	Czapek-Dox media		Thornton media	
	after 4 days	after 14 days	after 4 days	after 14 days
V ₁	+	++	+	++
V ₂	+++	+++	++	++++
V ₃	+++	++++	++	+++
V ₄	++	+++	+++	++++
V ₅	++++	+++++	++++	+++++

Crop dynamic

The harvest of mushrooms started, generally, after approximately one month, in the second decade of November. As results from the Figure 1 the majority of variants have recorded the highest yield on the second decade of November, except the third variant who recorded the highest yield on the first decade of November. This can be the consequence of the shorter period of vegetation realized by the mycelium grown on the material disinfected with boiled water (variant 3).

The harvest of mushrooms has been realized during three flushes. As it can be seen in the Figure 2 the highest production has been obtained during the first flush for all five variants. For the first four variants the quantity of mushrooms harvested in the first flush represents about 50% from the total and the second flush about 30%. The lowest quantity of mushrooms has been harvested in the third flush. Variant fifth has recorded a much higher yield in the first flush, about 74% from the total and lower yield on the second and third flush.

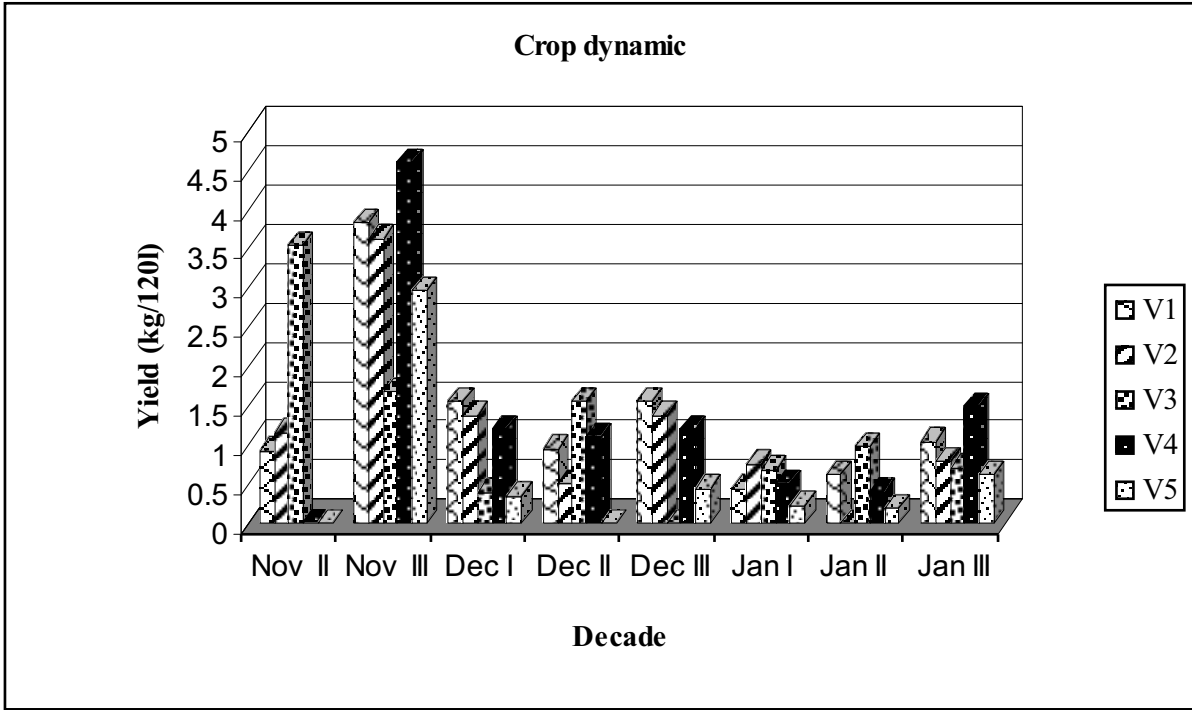


Figure 1. Crop dynamic for Pleurotus mushrooms grown on materials disinfected using different methods

This situation can be the results of the negative influence of competitive micro organism that have developed in the substrate with no disinfection on mushroom mycelia development.

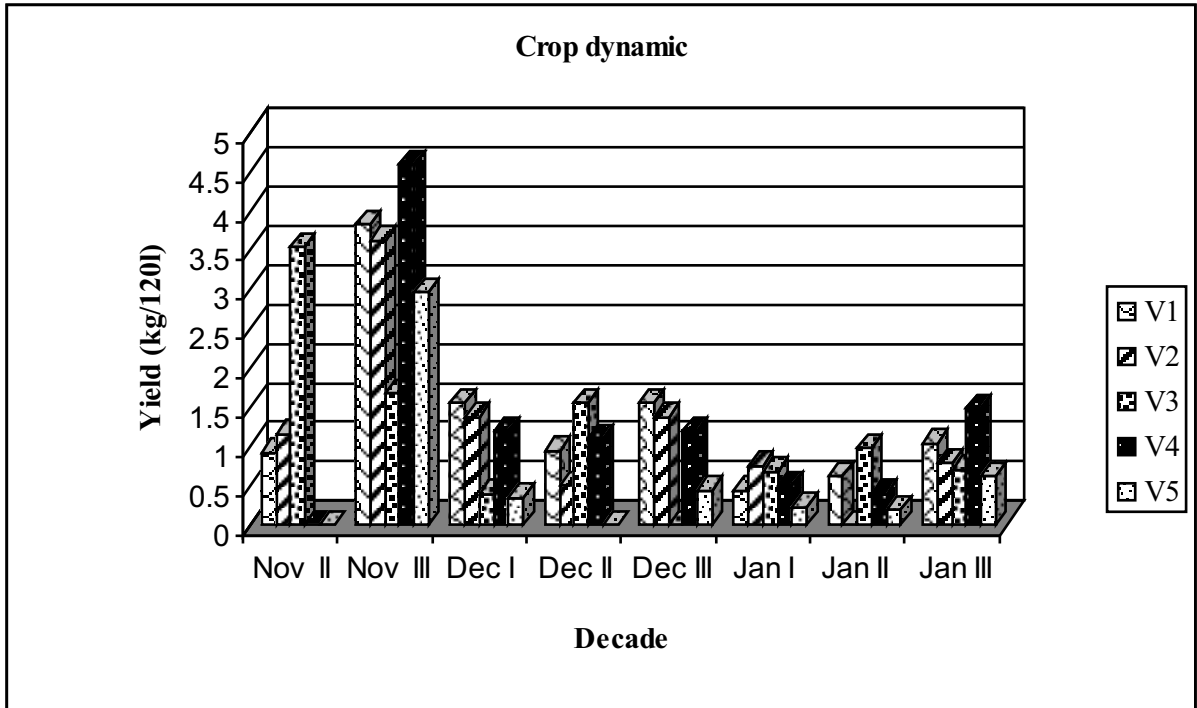


Figure 2. Repartition of mushroom yield on the flushes

Mushroom yield

Analysing the influence of disinfection method on the mushroom yield (Table 3) it can be noticed that variants 3 (material scalded with boiled water) and 4 (material disinfected with fungicide) have recorded better yields than control (variant 1 – material boiled 1 hour): 10.47 kg/120 l respectively 10.41 kg/120 l but the differences have not been assured statistically. This slightly increase of production can be due to a better protection of useful micro organism realised in the case of these method of disinfection comparative with boiling the materials. The lowest yield (5.18 kg/120 l) has been recorded for the fifth variant (material without disinfection), the yield difference given the control being very significant negative. This very low production (almost half comparative with the boiled material, chemical treated material and scalded with hot water material) suggest that disinfection of cellulosic materials is absolutely necessary to obtain high yields and in consequence to have a higher profit. It is also interesting to remark that chemical disinfection of materials assure very high products being in the same time less expensive and much easier to realise. Other easier and cheaper alternative to boiling the material is scalding with hot water.

Table 3

Influence of disinfection method on Oyster mushroom yield

Variant	Yield (kg/120 l)	%	Difference	Signification of difference
V ₁	10.10	100.0	0.00	-
V ₂	7.77	76.9	-2.33	oo
V ₃	10.47	103.7	+0.37	-
V ₄	10.41	103.1	+0.31	-
V ₅	5.18	51.1	-4.92	ooo

DL = 1%

1.59

DL = 5%

2.31

DL = 0.1%

3.46

Even if the fungicide solution used was much diluted we wanted to find if there is fungicide accumulation in mushrooms. In order to find the effect of fungicide on mushroom fruit bodies there have been taken samples and analyzed about fungicide accumulation. Using a spectrometer has been determine that the quantities of carbendazim (active substance from Derosal) in the mushroom fruit bodies was very low (0.5-0.8 µg/kg) much lower than legal limits. So, chemical treatment of cellulosic substrates for mushroom cultivation can be a good alternative to pasteurization or sterilization.

CONCLUSIONS

As a result of this experiment the following conclusion can be inferred:

The disinfection method will affect the spawning rate and mycelium development reducing or increasing the period of vegetation.

Disinfection is necessary in order to reduce the number of micro organisms in the substrate.

The highest percent of mushrooms are harvested at the beginning of the period, in the first flush. In the case of mushrooms grown on substrate without disinfection the first flush represents the majority of production, the competitive micro organisms affecting the mushroom yield on the next flushes.

The highest and earlier yields of mushrooms have been recorded for the materials disinfected by scalding with hot water, chemical treated respectively boiled 1 hour.

Good alternatives to the disinfection by boiling the materials are scalding with hot water (100°C) and chemical disinfection with diluted fungicide solutions. These alternative methods assure high productions and are easier to realize and much cheaper.

There is no significant accumulation of fungicide in mushroom fructifications when chemical disinfection with diluted fungicide is used. If the material is not disinfected mushroom fructifications appears later and production is significant lower.

REFERENCES

1. Apahidean, Al. S., 2006, Ciuperci cultivate și din flora spontană, Ed. AcademicPres, Cluj-Napoca.
2. Indrea, D., Al. S. Apahidean, 1995, Ghid practice pentru cultura legumelor, Ed. Ceres, București.
3. Mateescu, N., 1985, Cultura bureților, Ed. Ceres, București.
4. Oei, P., Mushroom cultivation, Leiden, The Netherlands, 1996.
5. Miroslawa Ziombra, Influence of Substrate Pasteurisation methods on the yielding of some *Pleurotus* cultivars, 1991 Research Institute of Vegetable Crops, vol. 51, 35-40.

REZUMAT

IMPORTANȚA DEZINFECTĂRII SUBSTRATULUI ÎN CULTURA BUREȚILOR DE FAG (*PLEUROTUS* SP.)

În ultimii ani interesul pentru bureții de fag este în continuă creștere datorită gustului lor deosebit și a valorii nutritive. Unul dintre cei mai importanți factori din cultura bureților de fag îl reprezintă dezinfecția substratului. Producția poate fi compromisă dacă microorganismele competitive nu sunt înlăturate din substratul celulozic. Obiectivul acestor cercetări a fost stabilirea celei mai eficiente metode de dezinfecție. Au fost alese cinci metode diferite de dezinfecție a materialului celulozic: fierbere o oră, fierbere zece minute, opărire cu apă fiartă (100°C), dezinfecție chimică cu fungicid (Derosal 0,01%) precum și material nedezinfectat, înmuiat doar în apă timp 24 de ore. S-a observat că metoda de dezinfecție influențează dezvoltarea miceliului și data apariției ciupercilor, precum și producția. Cele mai ridicate producții s-au înregistrat în cazul variantelor care au folosit ca substrat materialul dezinfectat prin opărire cu apă fiartă (100°C), cel dezinfectat chimic cu Derosal precum și în cazul materialului fiert o oră. Ciupercile cultivate pe substratul nedezinfectat au înregistrat cele mai mici producții.