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Potential for the cultivation of exotic mushroom species by exploitation of Mediterranean agricultural wastes

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ABSTRACT: Four agricultural wastes abundant in eastern Mediterranean countries, i.e. wheat straw, cotton gin-trash, peanut shells and poplar sawdust, were comparatively evaluated as substrates for the cultivation of selected strains of *Pleurotus ostreatus, P. eryngii, P. pulmonarius, Agrocybe aegerita, Lentinula edodes* and *Volvariella volvacea.* Both quantitative and qualitative parameters were examined, i.e. substrate incubation efficacy, earliness, yield, biological efficiency, basidiomata number, weight and size, etc. Wheat straw and cotton gin-trash are the most suitable substrates among those tested for *Pleurotus* spp. and *A. aegerita* (the former being more advantageous for high BE's and size of basidiomata, the latter for earliness and length of cultivation cycle), followed by poplar sawdust and peanut shells. Cotton gin-trash proved to be unsuitable *for L. edodes* which performed far better on wheat straw and poplar sawdust for all parameters examined, whereas *V. volvacea* on cotton gin-trush and wheat straw. The results, verified for *P. ostreatus* and *P. pulmonarius* strains in successive cultivation studies of five *Pleurotus* strains on cotton gin trash, are encouraging for mushroom production diversification by the exploitation of cotton gin-trash, which is cheap and abundant in the Mediterranean area, as alternative substrate for the cultivation of exotic mushroom species.

1 INTRODUCTION

Fungi of the genus *Pleurotus* have provoked great interest to the mushroom cultivation sector due to their ability to bioconvert efficiently a wide range of agro-industrial residues such as wood chips, sawdust, cannabis leaves, paddy straw, sugarcane bagasse, cotton waste, olive-oil mill wastes, orange peels, grape stalks etc. (Cho et al. 1981, Nicolini et al 1987, Bahukhandi & Munjal 1989, Negi & Gupta 1995, Ragunathan et al. 1996, Zervakis & Balis 1996, Da Serra & Kirby 1999). Although *Pleurotus* cultivation represents an excellent prospect for valorizing a wide range of ligno-cellulosic residues and by-products of agricultural activities in Mediterranean countries, wheat straw based-substrates dominate as far as their use is concerned in mushroom production of this area (Lanzi 1986, Olivier 1994). Therefore the need for exploitation of locally-abundant agricultural wastes as alternative substrates is apparent and a few relevant studies were conducted in the past with this objective (Platt et al. 1982, Zervakis & Balis 1992, Zervakis et al. 1996).

Indicative example represents the case of *Pleurotus* spp. cultivation in Greece, a relatively recent agro-industrial activity which seems to be very promising since *P. ostreatus* and *P. pulmonarius* production (the only cultivated exotic species nowadays in Greece) is steadily augmenting (Philippoussis & Zervakis 2000). Moreover there are good prospects for *P. eryngii*, well known and highly prized in the local market of wild mushrooms, to enter soon in commercial production. The increasing interest in mushroom consumption suggests that there is a market for additional mushroom species which could also support product diversification by the cultivation of exotic mushroom species such as *Lentinula edodes, Volvariella volvacea, Agrocybe aegerita* (Philippoussis & Zervakis 1998).

Numerous lignocellulosic substrates, such as barley and wheat straw, orange peels, maize straw, grape stalks, reed, rice husks and sunflower, appear to be suitable for the cultivation of *Agrocybe aegerita* (Nicolini et al. 1987, Zadrazil 1993b). Moreover several attempts have been made to cultivate *Lentiinula edodes* on substrates different than the traditional hardwood logs, which are based on wheat straw and oak sawdust (Royse & Bahler 1986, Pettipher 1988, Delpech & Olivier 1990, Kirchhoff & Lelley 1991, Zadrazil 1993a, Kalberer 1995).

Besides this, several workers have reported successful cultivation of the paddy straw mushroom *Volvariella volvacea* on various agricultural wastes, mainly on composted substrates composed of cotton or wheat straw (Chang 1974, Chang 1978, Scrase 1996). However, there are reports on the use of other agricultural wastes as well, namely corn cob, cassava peels, rice straw, sawdust, oil palm pericarp wastes (Chang 1974, Levanon et al. 1993, Fasidi 1995).

The cultivation of *Pleurotus* spp., *A. aegerita, L. edodes* and *V. volvacea* on agricultural wastes which are produced in large quantities in Mediterranean countries, not only reduce disposal problems caused by residues accumulation, but also provides an economically acceptable alternative for the production of high quality food and fodder through mushroom production of proteins which might contribute significantly to the increase of the farmers income.

The present work aims at supporting diversification of mushroom production by contributing to the valorization of locally-abundant substrates.

2 MATERIALS AND METHODS

2.1 Fungal material and culture media

For the needs of this study, the following eight strains, obtained from established fungal culture collections, were examined: (i) *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. (LGAM P69, isolated on *Salix* sp. in Mt. Chelmos, Peloponnese), (ii) *Pleurotus pulmonarius* (Fr.) Quel (LGAM P26, isolated on *Fagus sylvatica* in Mt. Oxia, central Greece), (iii) *Pleurotus eryngii* (D.C.: Fr.) Quel. (LGAM PI01, isolated on Ferula sp. in Andros island; AMRU RE1 and AMRU HER3, isolated on *Eryngium* sp. in Crete island), (iv) *Agrocybe aegerita* (Bring.) Sing. (SIEF 0834, originating from Guizhou, China), (v) *Lentinula edodes* (Jacq.: Fr) Kumm. (SIEF 0231, originating from Fujian, China) and (vi) *Volvariella volvacea* (Bull.: Fr.) Sing (SIEF 1318, originating from Gunagdong, China).

The culture media used for routine culture and storage purposes were Complete Yeast Medium, (Raper at al. 1972) and potato dextrose agar (PDA, Difco). Spawn was prepared on wheat grains (Puri et al. 1981).

2.2 Substrate preparation and cultivation conditions

For the production of basidiomata, the substrates from agricultural wastes namely wheat straw (WST), cotton gin-trash (CGT), peanut shells (PES) and poplar sawdust (POS), were prepared as follows: residues (torn to small particles where necessary) were soaked in water for 24 hours, the surplus water was drained off. The substrates were mixed with calcium carbonate (2 % dw) to obtain a pH value of 6-7 suitable for the growth of fungi and supplemented with wheat bran to a 90:10 ratio (w/w, in terms of dry weight), except poplar sawdust substrate that was supplemented with 30 % bran to reduce the C/N ratio. The moisture content of the sterilized substrates was measured to be around 70% and the C/N ratio in the range of 30-50/1. Three replicates for each particular substrate and for each strain examined were prepared.

Substrates were filled into polypropylene bags (each weighting 1.2 kg), sterilized twice with 24 hour interval, for one hour (by autoclaving at 15 psi, 121°C) and inoculated centrally, along the vertical axis, with 3% (w/w) wheat spawn. Incubation took place in controlled environmental chambers, in the dark, at 35°C in the case of *V. volvacea* and at 27±1°C for the rest of the species. After complete colonization of the substrate, polypropylene bags were removed and environmental conditions (temperature, relative humidity, aeration and light intensity) were altered for basidioma induction and were maintained at the appropriate levels during the entire length of the production cycle. During fructification the light intensity was 700 lux (12 h/day) by fluorescent lamps, air exchanges were frequent to maintain low CO_2 level (<1500 ppm), the temperature was set at 30°C for *V. volvacea* and 17.5±1°C for *Pleurotus* spp., *L. edodes* and *A. aegerita* and the relative humidity in all cases was maintained nearly 90%. In general, three successive flushes were collected during the cultivation cycle that lasted at least two months.

2.3 Cultural characters assessment

Comparative evaluation of the agricultural wastes as substrates for the cultivation of the above mentioned genera was based on: (i) cultural parameters: average values of observations with respect to the length of each phase of the production cycle (incubation, primordia formation, harvesting) and earliness, defined as the time elapsed between the day of inoculation and the day of the first harvest; (ii) on quantitative and qualitative production characters: the yield, the biological efficiency (BE), calculated from the weight of fruiting bodies as percentages of the dry weight of the substrate, the number, average weight and size of fruit bodies. *V. volvacea* mushrooms were harvested in the so-called "egg" stage, i.e. before rupture of the universal veil. Data were statistically analyzed using the Least Significant Difference (LSD) Test (p: 0.05).

3 RESULTS

3.1 The effect ofsubstrates to incubation length and earliness

Four different lignocellulosic agro-industrial residues, i.e. wheat straw (WST), cotton gin-trash (CGT), peanut shells (PES) and poplar sawdust (POS) were examined for their efficiency to support mushroom production of the following strains: *P. ostreatus* P69, *P. pulmonarius* P26, *P. eryngiiP\0\,A. aegerita* 0834, *L. edodes* 0231 and *V. volvacea* 1318.

The effect of different substrates on incubation phase, basidioma induction period and earliness, presented as a total of these two phases, is presented in Figure 1. Mycelium growth and basidioma formation was completed very soon for *P. pulmonarius* and *P. ostreatus* on WST and CGT; *P. pulmonarius* demonstrated the shortest pre-harvest period among all *Pleurotus* strains tested.

Earliness was not favored by POS in both strains and by PES in *P. pulmonarius.* In both sub-

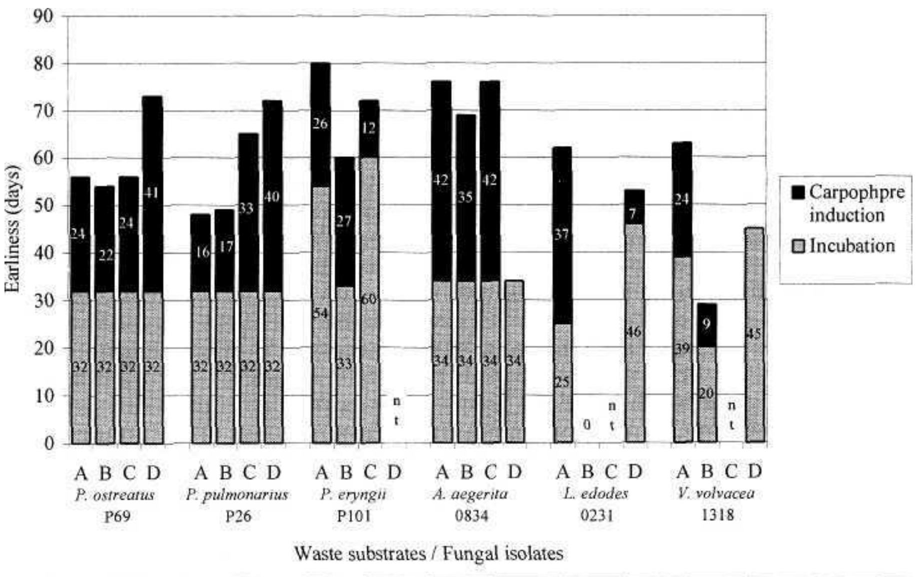

Figure 1: Period required for colonization and basidiomata formation (earliness) in different agricultural wastes. (A: wheat straw; B: cotton gin trash; C: peanut shells and D: poplar sawdust, n.t: not tested).

strates (WST and CGT), *P. eryngii* fructification process started considerably later than in *P. pulmonarius* and *P. ostreatus.* Among the other wastes, CGT proved to favor earliness since basidiomata were produced 12 and 20 days sooner than on PES and WST respectively.

Although *A. aegerita* strain presented fast mycelium growth rates on all substrates studied, basidiomata production started quite later. Apart from this, CGT promoted faster colonization and fruiting periods among all wastes. POS, although fully colonized by the *A. aegerita* mycelium, failed to induce basidiomata.

CGT promoted also earliness of *V. volvacea,* and fructification started one month earlier than on WST. POS proved to be unsuitable for the cultivation of *V. volvacea.*

Earliness data indicated that *L. edodes* performed far better on POS and on WST, while CGT appeared not to support growth and fructification of this particular species.

3.2 Mushroom yield and substrate evaluation

P. ostreatus and *P. pulmonarius* basidiomata were produced in higher abundancy in CGT and WST substrates, followed with great difference by POS and PES (Table 1). The same former two substrates gave high mushroom number in *A. aegerita* too, where for the rest of the agricultural wastes similar results were obtained. In *P. eryngii* no significant difference was recorded among the substrates tested since only a few basidiomata were produced. Similarly, low numbers were obtained for *L edodes* and *V. volvacea,* from all substrates tested: the higher number was recorded in wheat straw for the former and in cotton gin-trash for the later.

It is evident from the results presented in Table 1 that better average yields and BEs where obtained when *Pleurotus* spp. where cultivated on WST and CGT, the first favoring most *P. pulmonarius* and the second *P. eryngii* strains. In both substrates *P. ostreatus* produced equally high yields of mushrooms. Although PES and POS supported fructification, significantly lower yields were obtained for all *Pleurotus* species, and in particular PES presented the least BE from all the substrates studied.

WST and CGT recorded higher BEs in *A. aegerita.* WST and POS appeared to equally affect basidioma production in *L. edodes,* since there is no significant difference in yields obtained; whereas CGT and to a lesser degree WST supported mushroom production in *V. volvacea.*

As regards the average mushroom weight and size, it must be indicated that POS and PES produced less but heavier mushrooms than WST and CGT in *P. pulmonarius* and *P. ostreatus* strains, while size and weight of *P. eryngii* individual basidiomata where equally promoted by those two substrates followed by PES.

3.3 Production characters ofPleurotus spp. cultivated on cotton gin trash.

The quantitative and qualitative production caracters of five isolates of *Pleurotus* spp. i.e. *P. ostreatus* P69, *P. pulmonarius* P26, *P. eryngii* P101, *P. eryngii* RE1 and *P. eryngii* HERS, cultivated in a successive experiment on cotton gin trash are presented in Table 2.

Basidiomata appeared first in *P. pulmonarius* P26 followed after five days by the *P. ostreatus* P69. The total mushroom yield of *P. pulmonarius* and *P. ostreatus* isolates reached 295.94g and 213.5Ig, while the BEs were 103.19 and 59.96 respectively. In both species three flushes were harvested, giving small to medium size of mushrooms in respect to their average weight.

Although mycelium growth period lasted only three weeks, basidiomata production from *P. eryngii* strains initiated considerably later (15 days) than the rest of the *Pleurotus* strains examined. Among them, strain P101 appeared to be the "faster fruiter". All *P. eryngii* strains presented remarkably shorter fructification periods and only one flush was harvested during the entire production cycle which lasted 2.5 months. This resulted in significantly lower total yields, but the mushrooms produced were much larger, especially these of by *P. eryngii* HER3.

4 DISCUSSION

The scope of the present study was to examine the potential exploitation of selected agricultural wastes (WST, CGT, PES, POS), as substrates for the cultivation of six exotic mushroom species i.e. *P. ostreatus, P. pulmonarius, P eryngii, A. aegerita, L. edodes* and *V. volvacea.* These wastes, abundant in eastern Mediterranean area but rarely used in the mushroom industry, are common substrates for the cultivation of edible fungi world wide (Chang 1974, Pettipher 1988, Zadrazil 1994).

The valorization of the substrate incubation efficacy showed the suitability of all the substrates studied to be colonized by all fungal strains with the exception of *L. edodes* on CGT. CGT and to a lesser degree WST supported shorter incubation times for *Pleurotus* spp., *A. ae-*

Table 1. Mushroom production parameters of *Pleurotus* spp., *Agrocybe aegerita, Lentinula edodes* and *Volvariella volvacea* strains on four substrates composed of agricultural wastes.

Mushroom species	Isolates	Waste substrates	Yield (g)	Biological efficiency*	Fruit-bodies number*	Average weight* (g)
		Wheat straw	223.70**	64.59 b	24b,c	9.78 a
Pleurotus ostreatus	P69	Cotton gin-trash	234.76	65.47 b	28 c	8.53 a
		Peanut shells	56.73	13.57 a	5 a	9.96 a
		Poplar sawdust	124.70	33.37 a	13a,b	11.74 a
		Wheat straw	265.20	76.57 c	31 b	9.05 b
Pleurotus pulmonarius	P26	Cotton gin-trash	215.22	60.03 b	33 b	7.11 b
		Peanut shells	59.07	14.13 a	6 a	9.45 a
		Poplar sawdust	102.85	27.87 a	11 a	9.68 a
		Wheat straw	81.40	23.50 a,b	4 a	20.69 a
Pleurotus eryngii	PI 01	Cotton gin-trash	112.17	31.32b	6 a	20.01 a
		Peanut shells	64.03	15.32 a	4 a	17.35 a
		Poplar sawdust	***	***	***	***
		Wheat straw	164.55	47.51 b	120 b	1.34 b
Agrocybe aegerita	0834	Cotton gin-trash	109.19	30.46 b	104 b	1.03b
		Peanut shells	29.91	7.15 a	21 a	1.77b
		Poplar sawdust	0.00	0.00 a	0 a	0.00 a
		Wheat straw	110.14	16.75 b	4 b	30.32 c
Lentinula edodes	0231	Cotton gin-trash	0.00	0.00 a	0 a	0.00 a
		Peanut shells	***	***	***	***
		Poplar sawdust	46.54	12.78 b	2 b	18.19b
		Wheat straw	10.27	3.39 b	2 a	5.44 b
Volvariella volvacea	1318	Cotton gin-trash	35.07	8.64 c	8 b	4.56 b
		Peanut shells	***	***	***	***
		Poplar sawdust	0.00	0.00 a	0 a	0.00 a

' Values (means of three replicates) not sharing common letters are significantly different at PO.05.
** Means of three replicates.
*** Not tested.

Table 2. Cultivation parameters of *Pleurotus* spp. cultivated on cotton gin-trash.

Pleurotus species	Isolates	Incubation efficacy (days)	Earliness (days)	Flushes harvested	Yield (g)	Biological efficiency (%)	Average weight (g)
P. ostreatus	P69	17*	32	3	213.51	59.96	7.84
P. pulmonarius	P26	19	27	3	295.94	103.19	7.43
P. eryngii	P101	21	40	2	86.52	30.17	15.58
P. eryngii	RE1	21	45	1	18.25	6.36	9.33
P. eryngii	HERS	21	43	1	28.51	9.93	30.88

; Means of three replicates

gerita and *V. vohacea* and WST and POS for *L. edodes.* In general, the shorter incubation phase plays an important role in mushroom cultivation as it is related to longer fructification periods. However, for *V. vohacea* it has to be noted that incubation phase was much longer than respected values reported in literature (Chang et al. 1981). This may be attributed to the use of non composted substrates in the present experiments, substrate preparation method adopted on conventional cultivation (Scrase 1996) and to unfavorable conditions during the cultivation process.

The results of the present study are in accordance with the earliness values previously reported for *P. eryngii*, *P. ostreatus* and *P. pulmonarius* cultivated on various types of wastes (Zervakis & Balis 1992, Zervakis et al. 1996). The positive effect of cotton waste on early initiation of fruiting in *Pleurotus ostreatus* has been reported by Platt et al. (1982), and this was further supported by the data of this work which demonstrated that CGT was the fastest fruiting-promoting substrate for the cultivation *of Pleurotus, Agrocybe* and *Volvariella* species. Especially *V. volvacea* and *P. eryngii* isolates produced basidiomata 20 and 30 days respectively earlier than they did on WST (i.e. the substrate that is conventionally used for their cultivation in commercial scale). Although CGT supported early fruiting in *V. volvacea* in comparison to the other substrates studied, earliness values are not in agreement with those reported in other works by 15 to 25 days (Khan et al. 1991). *A. aegerita* and *V. volvacea* failed to fruit on POS, and these results are in agreement to those previously reported by Zadrazil (1993) for the former, and by Fasidi (1996) for the latter. In addition, *A. aegerita* presented similar incubation periods in all substrates tested, however CGT supported earlier fruiting than WST and PES. Earliness values recorded here for *L. edodes* (53 days on POS and 62 days on WST) were lower or comparable to those quoted from similar experimental trials or from established mushroom production schemes (Pettipher 1988, Olivier 1994).

Regarding yields, all *Pleurotus* species presented significantly higher yields on WST and CGT in contrast to POS and PES, while size and weight of individual basidiomata were equally favored by those two substrates. WST favored most *P. eryngii* and CGT *P. pulmonarius* yields, while in *P. ostreatus* no significant difference was recorded among these two substrates. However, the respective values of BEs are relatively superior than other previously recorded for *P. ostreatus* on WST (Bahukhandi & Munjal 1989), similar to those reported for *P. ostreatus* cultivated on cotton wastes (Platt et al. 1982) and slightly lower than those reported by Zervakis & Balis (1992) for all *Pleurotus* species tested on WST. Bahukhandi & Munjal (1989) determined that PES substrate was equally well performing as CGT, which is in contrast to the results obtained in the present work. These controversial results could be mainly attributed to the different strains and the variety in cultural parameters used. Among the other agricultural wastes tested, CGT promoted high yields in *A. aegerita,* and it was followed by WST. It must be noted that these two substrates presented significantly higher yields from those recorded in relevant studies (Zadrazil 1994).

L. edodes performed far better on WST for all parameters examined, followed by POS, whereas CGT proved to be an unsuitable substrate. The values of BEs, 16.75% and 12.78% respectively, are lower than those reported on an 1:1 mixture of cotton and wheat straw (Levanon et al. 1992), and on supplemented oak bark and WST mixture (Olivier 1994). These discrepancies could be attributed to the differences of the substrate and the short production cycle adopted in the present experiment (120 days from spawning to the end of the cycle, a short incubation phase, and a harvesting period of 60 days only), while the strain used was not an "early fruiter". It is well known that the BE of *L. edodes* depends on the length of spawn run, the length of harvest and the strain used (Royse & Bahler 1986, Kawai et al. 1995). In most of the previous experiments cited illumination was provided during the incubation phase in contrast to the present study; light exerts a beneficial effect on the fungus mycelium during the incubation period (Leatham & Stahmann 1987)

For *V. volvacea* CGT was superior than WST. Although the final product possessed high qualitative and organoleptic properties (color, flavor, shape and taste), BE's were inferior than respective values reported by Chang (1980), although there are similar cultivation studies on various substrates presenting BEs in the range of 0.9 to 19% (Khan et al. 1991). This can be attributed to the differences of the substrate formula and the preparation technique, the strain used and the cultivation conditions. As a consequence, to evaluate specific agricultural wastes suitability, additional experiments have to be conducted using other *V. volvacea* strains cultiyated on composted substrates as well.

However, the results concerning the quantitative and qualitative production characters of *Pleurotus* species on CGT, confirmed our findings from the first series of experiments regarding its suitability for the cultivation of the *Pleurotus* spp. tested, i.e. *P. ostreatus, P. pulmonarius* and *P. eryngii*. Especially for *P. ostreatus* and *P. pulmonarius,* earliness and BE values demonstrated that this residue (abundant in eastern Mediterranean countries), is a promising alternative substrate for commercial production. It is interesting to note that the experimental values of BE on CGT (59.96%-103.19%) for both *P. ostreatus* and *P. pulmonarius* are superior of the respective values (50%-75%) of the commercial production on straw-based substrates (Olivier 1994).

5 CONCLUSIONS

The present study demonstrates that wheat straw and cotton gin-trash are the most suitable substrates among those tested for *Pleurotus* spp. and *A. aegerita* (as both quantitative and qualitative data are concerned), followed by poplar sawdust and peanut shells. *L. edodes* performed far better on wheat straw and poplar sawdust for all parameters examined, whereas fruiting of *V. volvacea* was favored on cotton gin-trash and wheat straw. In general, poplar sawdust and peanut shells are less suitable than the other substrates examined for all species tested.

Results are encouraging for the exploitation of cotton gin-trash, a residue cheap and abundant in the Mediterranean area, as alternative substrate for the cultivation of exotic mushroom species, especially for *P. ostreatus, P. pulmonarius* and *Agrocybe aegerita.*

REFERENCES

Bahukhandi, D. & Munjal R. L. 1989. Cultivation of *Pleurotus* species on different agricultural residues. *Indian Phytopathology* 42 (4): 492-495.

Chang, S.T. 1974. Biological and commercial aspects of straw mushroom *(Volvariella volvacea)* cultivation. . In *Proceedings of the LXth International Scientific Congress on the Cultivation of Edible Fungi* 9: 157-165. Taipei: Japan Science Press.

Chang, S.T. 1978. Cultivation of *Volvariella volvacea* from cotton waste composts. In *Proceedings of the Xth International Congress on the Science and Cultivation of Edible Fungi* 10: 609-618. France.

Chang, S.T. 1980. Cultivation of *Volvariella* mushroom in South East Asia. *Mushroom News-letter for the tropics* 1(1): 5-10.

Chang, S.T., Miles, P.O. & Wai, C.C. 1981. A study of monosporous isolates of *Volvariella volvacea.* In *Proceedings of the XIth International Scientific Congress on the Cultivation of Edible Fungi* 11: 603-621. Australia.

Cho, K.Y., Nair, N.G., Bruniges, P.A. & New, P.B. 1981. The use of cotton seed hulls for the cultivation of *Pleurotus sajor-caju* in Australia. In *Proceedings of the Xlth International Scientific Congress on the Cultivation of Edible Fungi* 11: 679-690. Australia.

Da Serra, M. & Kirby, R. 1999. Development of *Pleurotus* (oyster) mushroom production in South Africa using alien wood species as lignocellulose substrate. *The International Journal of Mushroom Sciences 2* (3): 49-55.

Deplech, P. & Olivier, J.M. 1990. Champignon parfume (ou shii-take) une methode francaise pour sa culture. *P.H.M. Revue Horticole* 305: 25-30.

Kalberer, P.P. 1995. An investigation of the incubation phase of a shiitake *(Lentinus edodes)* culture. In T. Elliot (ed.), *Science and Cultivation of Edible Fungi,* 14: 375-383. Rotterdam: Balkema.

Kawai, G., Kobayashi, H., Fukushima, Y. & Ohsaki, K. 1995. Liquid culture induces early fruiting in shiitake *(Lentinula edodes).* In T. Elliot (ed.), *Science and Cultivation of Edible Fungi,* 14: 825-832. Rotterdam: Balkema.

Khan, S.M., Haq, R. & Dogar, M.A. 1991. Some studies on the cultivation of Chinese-mushroom *(Volvariella volvacea* (Fr.) Singer) on sugarcane industrial by-products. In Maher (ed.), *Science and Cultivation of Edible Fungi,* 13: 579-584. Rotterdam: Balkema.

Kirchhoff, B. & Leliey, J. 1991. Investigations of shiitake *(Lentinus edodes* (Berk.) Sing.) bag-log cultivation to increase the yield in Germany. In Maher (ed.), *Science and Cultivation of Edible Fungi* 13: 509-516. Rotterdam: Balkema

Lanzi, G. 1986. The cultivation of the oyster mushroom *(Pleurotus ostreatus)* in Italy. In *Proceedings of* *the International Symbosium on Scientific and Technical Aspects of Cultivating Edible Fungi,* pp. 443-447. Pennsylvania: Elsevier.

Leatham, F.G. & Stahmann, A.M. 1987. Effect of light and aeration on fruiting of *Lentinula edodes. Transactions of the British Mycological Society* 88 (1): 9-20.

Levanon, D., Rothschild, N., Danai, O. & Masaphy, S. 1993. Bulk treatment of substrate for the cultivation of shiitake mushrooms *(Lentinus edodes)* on straw. *Bioresource Technology* 45: 63-64.

Negi, P.S. & Gupta, R.C. 1995. *Cannabis sativa* (Bhang) leaves a suitable substrate to cultivate *Pleurotus sajor-caju. Indian Journal of Mycology and Plant Pathology* 25 (3): 304-305.

Nicolini, L., Hunolstein, von C. & Carilli, A. 1987. Solid state fermentation of orange peel and grape stalks by *Pleurotus ostreatus, Agrocybe aegerita* and *Armillariella mellea. Applied Microbiology and Biotechnology* 26: 95-98.

Fasidi, O.i. 1996. Studies on *Volvariella esculenta* (Mass) Singer: cultivation on agricultural wastes and proximate composition of stored mushrooms. *Food Chemistry* 55 (2): 161-163.

Olivier, J.M. 1994. Developments in the cultivation of specialty mushroom with emphasis on *Pleurotus* and Shiitake. *Mushroom Information* 96 (6): 5-19.

Pettipher, G.L. 1988. Cultivation of the oyster and shiitake mushrooms on lignocellulosic wastes. *Mushroom Journal* 183: 491-493.

Platt, M., Chet, I. & Henis, Y. 1982. Growth *of Pleurotus ostreatus* on cotton straw. *Mushroom Journal* 120: 425-427.

Philippoussis, A. & Zervakis, G. 1998. Production and consumption of edible mushrooms in Greece and world-wide, analysis of the current situation and perspectives for the development of mushroom cultivation (in greek, english abstract). *Geotechnical Scientific Issues 9* (1): 60-72.

Philippoussis, A. & Zervakis, 2000. Cultivation of edible mushrooms in Greece: Presentation of the current status and analysis of future trends. In L. Van Griensven (ed.), *Science and Cultivation of Edible Fungi* 15: (in press). Rotterdam: Balkema.

Puri, Y.N., Rehill, P.S. & Singh, B. 1981. Cultivation trials of *Pleurotus fossulatus. Mushroom Journal* 102:209-214.

Ragunathan, R., Gurusamy, R., Palaniswamy, M. & Swaminathan, K. 1996. Cultivation *of Pleurotus* spp. on various agro-residues. *Food Chemistry* 55 (2): 139-144.

Raper, C.A., Raper, J.R. & Miller, J. R. 1972. Genetic analysis of the life cycle *ofAgaricus bisporus. Mycologia64:* 1088-1117.

Royse, D.J. & Bahler, C.C. 1986. Effects of genotype, spawn run time and substrate formulation on biological efficiency of shiitake. *Applied and Environmental Microbiology* 52 (6): 1425-1427.

Scrase, R. 1996. Cultivating mushrooms: making composted and non-composted substrates. *Mycologist* 10(2): 52-55.

Zadrazil, F. 1993a. *Lentinula (=Lentinus)* edodes: physiology and conditions of industrial production. *Mushroom Information* 87 (6): 5-27.

Zadrazil, F. 1993b. Cultivation of *Agrocybe aegerita* (Brig.) Sing, on lignocellulose containing wastes 1. *Mushroom Information* 91 (12): 9-31.

Zadrazil, F. 1994. Cultivation of *Agrocybe aegerita* (Brig.) Sing, on lignocellulose containing wastes 2. *Mushroom Information* 92 (1): 5-22.

Zervakis, G. & Balis, C. 1992. Comparative study on the cultural characters *of Pleurotus* species under the influence of different substrates and fruiting temperatures. *Micologia Neotropical Aplicada 5:* 39-47.

Zervakis, G. & Balis, C. 1996. Bioremediation of olive oil mill wastes through the production of fungal biomass. In D. Royse (ed.), *Proceedings of the Second International Conference on Mushroom Biology and Mushroom Products.* Pennsylvania: PennState.

Zervakis, G., Philippoussis, A., loannidou, S. & Diamantopoulou, P. 2000. Influence of the air temperature and the composition of nutrient media on the linear growth rates of selected edible mushroom species. In L. Van Griensven (ed.), *Science and Cultivation of Edible Fungi 15:* (abstract, in press). Rotterdam: Balkema..

Zervakis, G., Yiatras, P. & Balis, C. 1996. Edible mushrooms from olive mill wastes. *International Biodeterioration & Biodegradation* 38: 237-243.