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Soybean the Main Nitrogen Source in Cultivation Substrates of Edible and Medicinal Mushrooms

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1. Introduction

The "MUSHROOM" word is used in all part of world to describe the fruiting bodies of saprophytic, mycorrhizal and parasites fungi, belonging to the order of Basidiomycetes or Ascomycetes. They can be found in soils rich in organic matter and humus, moist wood, animal waste, etc., after heavy rain (with thunderstorm or not) or a sudden change of temperature and soon after a few hours or days they disappear, leaving no sign, but vegetative mycelium.

The terminology "MUSHROOMING", or mushroom cultivation refers to the intentional and directed production of mushrooms, substituting wild collection in the fields and forests with a harvest in defined conditions of growing, resulting in strict quality control, food safety without risk of consumption of poisonous or toxic species, and with guarantees on the benefits generated by these fungi.

The cultivation of edible mushrooms is actually an alternative biotech which is fast, environmentally friendly and feasible to recycle organic byproducts from agribusiness into high nutritional and medicinal quality food both with respect to the amount of protein or minerals and selected substances with medicinal and pharmacological properties, for example the presence of β -glucans like lentinan, and thus it can contribute significantly in feeding human.

Currently the most cultivated mushroom in the world are *Agaricus bisporus* (Lange) Imbach "Champignon" or botton mushroom, *Lentinula edodes* (Berk.) Pegler "Shiitake" and *Pleurotus ostreatus* (Jacq.) P. Kumm "Oyster Mushroom" and other *Pleurotus* species (Fig. 1). Recently, extreme attention is paid to *Agaricus subrufescens* (formely *Agaricus blazei* ss. Heinemann and *Agaricus brasiliensis*), the "Almond mushroom". The interest for this Basidiomycete fungus also called the Medicinal mushroom or Sun mushroom is due to its medicinal proprieties such as the presence of glucan protein which has tumor-inhibition activity. All of

them are saprophytic fungi but the *Agaricus* species are humicolous ones whereas *Pleurotus* spp. and *L. edodes* are lignicolous ones. Consequently, the former need a composted substrate for their cultivation and the later can be grown on raw lignocellulosic materials.

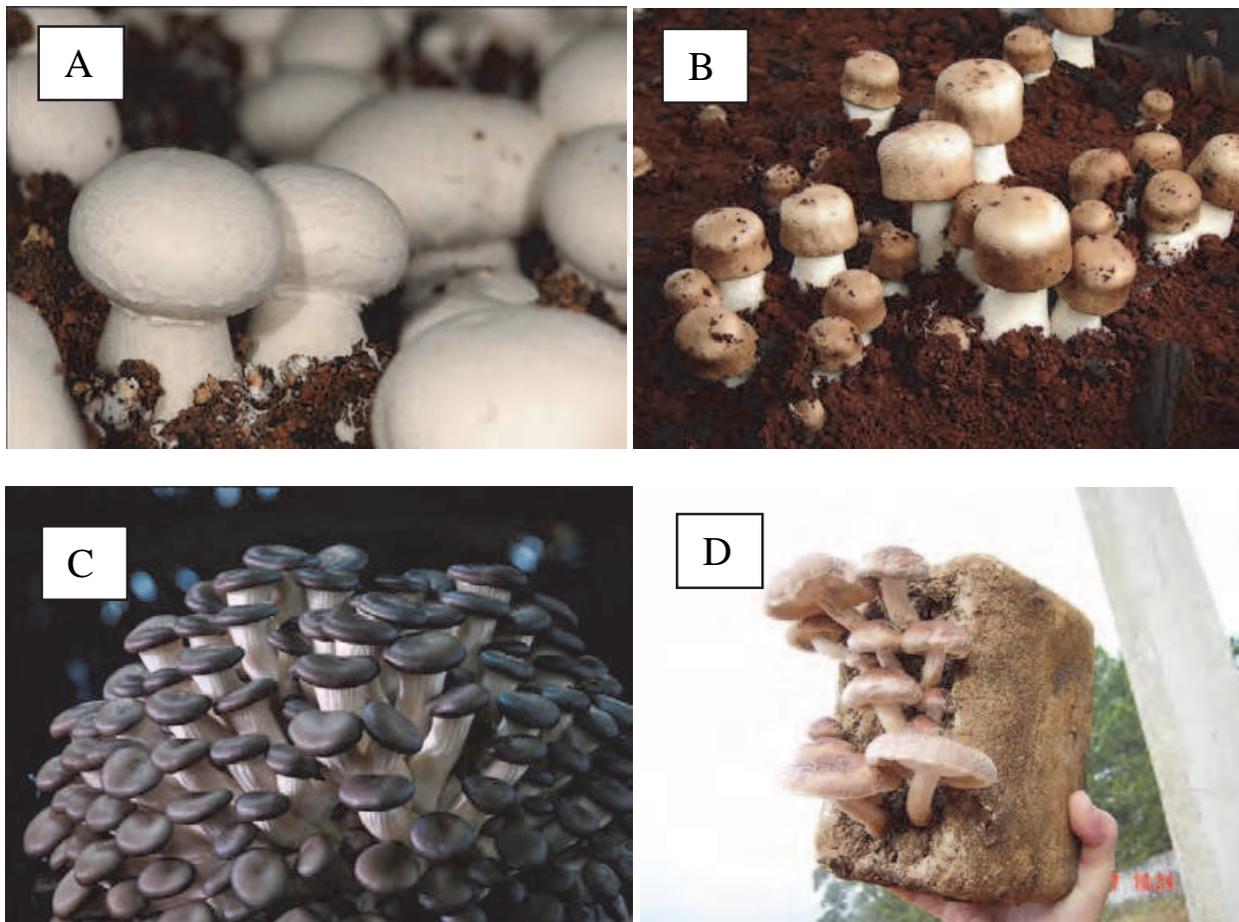


Fig. 1. A: Button mushroom, B: Almond mushroom, C: Oyster mushroom and D: Shiitake.

The production of the substrate to mushroom growth is recognized as the most critical stage of cultivation, having dramatic consequences on the yield and quality of the crop and consequently on the economic viability (Gerrits 1977, 1988; Cormican and Stauton, 1991; Dhar, 1994). The substrates are both a physical support and a source of nutrients for the mushrooms needed to complete their life cycle (vegetative and reproductive phases).

For the production of substrate, various materials (mostly agricultural residues and agro-industrial by-products) can be used, according to the location of the culture, the type, quality, distance and frequency of availability and finally the cost of these materials. According to many researchers the material used to produce the substrate may be classified as: bulky (make up about 60-85% of the total volume of the substrate, being formed with materials largely of cellulose, hemicellulose and lignin); concentrates (make up about 15-35% of the total volume of the substrate, and be materials with high contents of protein, nitrogen, fat and carbohydrates) and conditioners (make up about 5-10% of the total volume of the substrate, the base materials being a source of calcium). Table 1 shows the main types of materials used for production of mushroom cultivation substrates in Brazil and Europe.

Materials	Brazil	Materials	Europe
Bulky	Sugarcane bagasse, wheat, oats, rice, braquiária, coast cross and tifton straw, horse manure, etc.	Wheat straw, barley straw, horse manure, sawdust, grape stalks, kenaf fibre, etc.	
Concentrated	Soybean meal, chicken manure, cereal meals, cottonseed meal, urea, ammonium, superphosphate, etc.	Chicken manure, urea, ammonium sulphate, grape pomace, cereal meals and brans, feather flour, etc.	
Conditioners	Calcitic lime, calcium carbonate and gypsum.	Gypsum and calcium carbonate.	

Table 1. Classification and types of materials used in mushroom cultivation.

Standards of mushroom cultivation substrates are: absence of pests and diseases, specific availability of nutrients for the cultivated fungi at the exclusion of other competing fungi, pH between 6 to 7.5, and a bulk density allowing gas exchanges and avoiding excess of moisture content. The use of concentrated materials in growing mushroom is intended to correct the C/N ratio (carbon / nitrogen). Each mushroom has an optimum of C/N ratio for growth, allowing getting the highest yield in a short period of production.

Mushroom	C/N ratio			Reference
	Minimum	Maximum	Optimum	
<i>Agaricus subrufescens</i>	16/1	33/1	27/1	Eira (2003); Kopytoswky-Filho (2004)
<i>Agaricus bisporus</i>	16/1	22/1	19/1	Sharma and Kilpatrick (2000)
<i>Agaricus bitorquis</i>	16/1	22/1	19/1	Oie (2003); Dhar (1994)
<i>Agaricus brunescens</i>	16/1	21/1	19/1	Cies (2009)
<i>Pleurotus ostreatus</i>	40/1	90/1	45-55/1	Heltay <i>et al.</i> (1960)
<i>Pleurotus ostreatus f. florida</i>	40/1	150/1	45-55/1	Heltay <i>et al.</i> (1960); Kaul <i>et al.</i> (1981)
<i>Pleurotus sajor-caju</i>	40/1	150/1	45-55/1	Gramss (1979); Poppe (1995)
<i>Pleurotus flabellatus</i>	40/1	100/1	45-60/1	Chang-Ho <i>et al.</i> (1979)
<i>Pleurotus cornucopiae</i>	40/1	97/1	45-55/1	Chang and Miles (1989)
<i>Pleurotus eryngii</i>	40/1	70/1	45-55/1	Chang and Miles (1989)
<i>Lentinula edodes</i> (sawdust)	25/1	55/1	30-35/1	Zied <i>et al.</i> (2009)

Table 2. Examples of C/N ratio being desirable to obtain highest yield in different species of mushrooms.

Due to its high protein content, soybean meal is a good source of nitrogen that reduces the levels of carbon in the substrate mainly because to the use of bulk materials. Several advantages are observed in the use of soybean with the production of substrates in Brazil and other American countries, such as: a protein source of high quality, without the presence of heavy metals, found during all seasons, which takes up little space (yet without the need to be stored) and a relatively low price. In Europe where soybean meal is less available as local production, nitrogen in raw ingredients is generally obtained from other sources.

In addition to the use of soybean meal as concentrated material in the formulation of the cultivation substrates it can be added as supplement at the time of inoculation of the substrate with the fungi to be cultivated or later during cultivation. Supplements used both in America and Europe are most of the time manufactured products containing denatured soybean meal and other organic protein sources enriched with minerals. These supplements are frequently used for cultivation of *Agaricus* species.

The Table 2 shows the minimum, maximum and optimum C/N ratio, that some mushrooms need at the time of inoculation. The differences in optimum C/N between *Agaricus* species and the others are consequences of their ecological differences: *Agaricus* species need nitrogen rich humus like substrates obtained by composting, the other species are adapted to lignocellulosic material degradation. The use of soybean meal in the cultivation of both groups of mushrooms as concentrated material or supplement is reviewed in the following parts.

2. In cultivation of *Agaricus* species

2.1 Use as concentrated ingredient for substrate preparation

Substrates for *Agaricus* species obtained after a composting process generally composed of two phases. After a pre-wetting period, ingredients are mixed and Phase I occurs for 1 to 2 weeks either outdoor in long rectangular stacks, or indoor with air flow and temperature controls. Phase I is a biological and chemical process with the breakdown of available organic materials for energy and its incorporation into microbial biomass being the crucial mechanism.

The use of soybean meal as the main source of organic nitrogen in the cultivation substrate of *Agaricus* species is illustrated with *A. subrufescens* for which it has been extensively tested by the scientific community (Eira, 2003). Soybean meal replaces partly chicken manure that is not used for *A. subrufescens* whereas it is commonly used for *A. bisporus*.

Table 3 shows a series of formulations using soybean meal and their respective levels of yield in the end of mushroom cycle and the C/N ratios at the end of the composting process. Some authors used values between 28-23/1 and nitrogen content between 1.15 to 1.45% (Kopytoswky-Filho and Minhoni, 2004) and other 22-17/1 and nitrogen content between 1.7-2.6% (Andrade et al., 2007). The consequences on the yields were not clear. The highest yield was obtained in a study where soybean meal was not used (formulation 4).

In a more detailed study, Kopytoswky-Filho and Minhoni (2004) tested different proportions of soybean meal and urea for obtaining C/N ratio of 37/1 at the beginning of Phase I composting. The highest yield (10.1%) was obtained with a ratio 1.5/1 (soybean meal/urea), while the ratio 4/1 and 1/1.5 resulted in a yield of 8.13 and 7.29%, respectively (Kopytoswky Filho and Minhoni, 2004) showing that a high quantity of soybean is not favorable to the yield.

However, the bulky material used in the production of substrate also influences the yield. Table 4 shows the chemical analysis and yield of mushrooms in an experiment conducted with three different composts with the same amount of soybean added during Phase I composting. It is noteworthy that addition of organic nitrogen source (soybean) and the relationship between organic and inorganic N, other factors also influence the quality of compost, such as: size of the particular material, moisture, density of compost, method of composting used, ammonia concentration in the final compost, etc. (Cormican and Stauton, 1991; Dhar, 1994)

Formulation	Materials	C/N (Phase II)	N (%)	Yield	Reference
1	Sugarcane bagasse, Coast-cross straw, soybean meal, urea, calcitic lime and gypsum.	23.7/1	-	12.9- 16.2%	Zied <i>et al.</i> (2010)
2	Sparagus straw, cottonseed hull, soybean cake and gypsum.	-	1.47	9.8 kg of fresh mushroom per m ²	Wang <i>et al.</i> (2010)
3	Sugarcane bagasse, brachiaria sp. grass, coast-cross grass, soybean bran, urea, ammonium sulfate and gypsum.	-	-	7.1 kg of fresh mushroom per m ²	Colauto <i>et al.</i> (2010)
4	Sugarcane, coast-cross, wheat bran, limestone, gypsum, superphosphate and ammonia.	-	1.1	16.3% (mushroom fresh weight/compost fresh weight)	Siqueira <i>et al.</i> (2009)
5	Sugarcane bagasse, oat straw, soybean, urea, gypsum and calcitic lime.	25.6/1	-	8.7-12.8%	Zied <i>et al.</i> (2009)
6	Sugarcane, coast-cross, soybean meal, gypsum and limestone.	18/1	2.28	10.1% (mushroom fresh weight/compost fresh weight)	Andrade <i>et al.</i> (2007)
7	Sugarcane bagasse, braquiária straw, soybean meal, urea, gypsum and limestone.	27-33/1	1.15	7.29-10.01% (mushroom fresh weight/compost fresh weight)	Kopytoswky-Filho and Minhoni (2004)

Table 3. Example of materials used in production of the substrate, its C/N ratio, N content (%) and yield observed by the authors.

Compost	N	P ₂ O ₅	K ₂ O	Ca	Mg	S	MO	C	Na	Cu	Fe	Mn	Zn	C/N	pH	Yield
	mg kg ⁻¹ dry matter														%	
1	1.81	1	1.09	5.15	0.48	1.36	64	35.6	440	0	500	260	20	20/1	6.25	10.77
2	1.76	0.66	1.04	5.44	0.48	1.26	68	37.8	440	0	400	160	18	21/1	7.04	10.72
3	1.6	0.64	1.31	5.20	1.68	1.40	65	36.2	1160	172	400	306	212	23/1	6.94	9.13

Compost 1. Sugar cane bagasse, Massai straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.

Compost 2. Sugar cane bagasse, oat straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.

Compost 3. Sugar cane bagasse, Aruana straw, soybean meal, urea, ammonia, superphosphate, limestone and gypsum.

Table 4. Chemical analysis of three composts (Phase II) and yield of mushrooms (%).

Even if the different data presented here came from different works, they tend to show that the performance of soybean used with nitrogen source during Phase I of composting, for having a positive effect on mushroom yield with *A. subrufescens* is not completely defined.

2.2 Use as supplement in compost

At the end of composting, the nitrogen content and the value of C/N may be corrected by a supplementation performed either at spawning (inoculation with the mycelium) or at casing (addition of a layer of peat, clay and gypsum at the surface of the culture for inducing the fruiting). Supplementation of compost with soybean based products is common in the cultivation of *A. bisporus* with the aim to increase the nutritional value of mushrooms, which directly consume them, in order to enhance performance, but without affecting quality. Yields generally increase by 5-20%, and occasionally by more.

This technique emerged in the 1960s (Lemke, 1963; Schisler and Sinden, 1962; Sinden and Schisler, 1962). Outstanding aspects to be considered include, on the one hand, the types of nutrients required and the most suitable time for them to be applied without forgetting, on the other hand, economic costs and profits (Randle, 1985). In their study into the economic aspects of supplementing compost, Randle and Smith (1986) estimated that the cost of supplementation is covered by a 1.5 kg m⁻² increase in yield.

Cereal grains and oilseeds, widely used as mushroom compost supplements, contain varying amounts of the three basic nutritional requirements: carbohydrates, proteins and fats (Randle, 1985). Oil-extracted seed meals and whole-seed meals rich in protein and lipids have proved most consistent in increasing mushroom yields, irrespectively of being added to compost at spawning or at casing (Randle, 1985). Thus, the majority of modern supplements are based on protein-rich vegetable-based raw materials. The products generally consist of soybeans or soybean by-products or other vegetable by-products. Soya meal, maize gluten, potato protein and feather meal are particularly suitable (Gerrits, 1985). In practice, soya meal is the most commonly used. Many products consist of finely textured grain by-products, while others are larger particles or cracked full-fat soybeans (Dahlberg, 1990). The use of a cracked soybean has the added advantage that the protein, fat and carbohydrate contents remain in a natural nutritional balance. The grain by-product supplements generally consist of defatted soybean meal or corn gluten meal (Dahlberg, 1993). However, the need for micronutrient technology that can continue to stimulate yields, but improve quality, shelf life and hopefully disease control, is beginning to be discussed (Wheeler and Wach, 2006; Peeters, 2008).

Supplement can be added at two times: at spawning or at casing after an incubation of the inoculated compost. The results of addition at casing as opposed to spawning are better but this one is only possible when incubation is performed in bulk before filling trays or other containers and casing. This type of incubation is called Phase 3 and is applied only in some countries in large facilities. Applying supplement at spawning appears to be the stage that offers the best operational advantage if added with the inoculum. In this case, however, compost selectivity may be affected by the increased risk of competing fungi appearing (Gerrits, 1985). The optimum quantity of supplement depends on a number of factors, first on the thickness of the compost layer. In general, the best results are obtained with least risk if 1 kg m⁻² is used. This applies to products with a protein content of no more than 50%. If the protein content is much higher, it is better to limit the quantity to 0.5-0.7 kg m⁻² (Gerrits, 1985).

The increase in temperature immediately after supplementing due to the high metabolic activity of the fungus and other microorganisms in compost should be controlled and supplements treated to delay the immediate availability of nutrients are needed for successful supplementation at spawning. The first references of treating mushroom compost supplements with formaldehyde, which had been previously applied in animal feed, correspond to Carrol and Schisler (1976), and led to the so-called delayed-release nutrients. These authors assumed that as formaldehyde reduced the solubility and denatured the proteins of supplements, it inhibited their utilization by weed moulds so that the mushroom mycelium, when it was dominant in the compost at 2 weeks post-spawning, could utilize the slowly-available lipo-protein supplement which was then effective through the whole cropping period. While preparing their product, cotton-seed meal was blended with peanut oil, spray-dried and then treated with 10% formaldehyde to denature the protein, making it less soluble and less readily available.

Gerrits (1986) evaluated the supplementation at spawning and at casing with soybean meal treated with solutions of formaldehyde in water (formalin) at different concentrations. It was proved that supplementation with soybean meal treated with 0.2% formalin at casing gives positive response; while at spawning it is advisable to apply soybean-meal treated with 0.6% formalin, in order to offer a better protection. The mushroom yield increase achieved by supplementation at spawning is just a half of that achieved by supplementation at casing. For *Agaricus bitorquis* (Quel.) Sacc, a mushroom replacing *A. bisporus* for cultivation at high temperature, Saharan and Guleria (2001b) treated different supplements with various concentrations (0.2, 0.3 and 0.5%) of formaldehyde and the maximum increase (37.4%) in mushroom yield was recorded in compost supplemented with 0.3% of soybean cake treated with formaldehyde.

Delayed release supplement gives up its nutrients right through out the life of the crop. And today numerous studies support the positive effect of post-composting supplementation with soybean-based supplements in *Agaricus bisporus* (J.E. Lange) Imbach all over the world. For example, we can find references to its use in Germany (Lemke, 1963; Lelley, 1984), UK (Randle et al., 1983; Randle, 1985; Randle and Smith, 1986), Canada (Rinker, 1991), the Netherlands (Gerrits, 1983, 1985, 1986, 1989; Gerrits and Amsing, 1996; Resink and de Leeuw, 1993; Peeters, 2008), USA (Schisler and Sinden, 1962; Sinden and Schisler, 1962; Schisler, 1970, 1971, 1979; Schroeder and Schisler, 1981; Abell, 1988; Dahlberg, 1990, 1993; Wach and Wheeler, 1998), Ukraine (Petrenko and Bisko, 2004), India (Garcha et al., 1987; Gupta y Vijay, 1992), Italy (Lanzi, 1985), Belgium (Pitblado, 1993) and France (Vedie, 1990; Vedie and Retailliau, 1992; Desrumaux et al., 1999). However, due to the toxicity of formaldehyde other kinds of treatments resulting in protein tanning have to be investigated, specifically for organic production of mushrooms.

Various soybean products and commercial supplements based on treated soybeans may be used alone or in mixtures with other nitrogen rich components. Depending on the types of soybean supplements and mixtures added to the compost the consequences on the yield may vary. Petrenko and Bisko (2004) explored the influence of soybean extrudate, soybean meal and protein soybean concentrate on yield of *A. bisporus* under commercial conditions. Soybean extrudate produced a statistically significant increase (37%). Concentrations from 0.7 to 1% (compost wet wt. basis) produced the greatest response. Supplementation with 2% rate (compost wet wt. basis) of soybean cake provided a 21.9% increase in yield over control for *A. bitorquis* production (Saharan and Guleria, 1993).

Dogan et al. (2000) investigated the effect of corn flour, wheat flour, hen grain, soybean meal and sunflower seed hulls added to synthetic compost on mycelium growth, yields and early ripe of *A. bitorquis*. The growth period of mycelium was shortened and amount of yield also was increased. Saharan and Guleria (2001a) supplemented wheat straw compost with oil seed cakes viz; cotton seed, groundnut, mustard, soybean and till each at three different rates (1.0, 1.5 and 2.0%) on wet weight basis of compost at spawning. Supplementation with soybean cake (1.5%) resulted in maximum increase (38.7%) in yield over control.

In our currently experiments conducted at the Centro de Investigación, Experimentación y Servicios del Champiñón (Cuenca, Spain), supplementation of commercial compost at spawning with three different delayed-release nutrients has shown a significant increase in biological efficiency (4.0-7.1%) and protein content of mushrooms (13.8-16.6%). Also, earliness and unitary weight of mushrooms were positively affected (Table 5).

Supplement and dose	Biological efficiency (kg 100kg ⁻¹ compost)	Earliness (days from casing)	Mushroom unitary wt (g)	Protein (Nx4.38) (g kg ⁻¹)
Unsupplemented control	81.42 b	21.6	8.22	219.0 b
Promycel [®] Gold (10 g kg ⁻¹)	84.68 a	21.4	8.98	249.2 a
Champfood [®] S (10 g kg ⁻¹)	87.18 a	21.2	8.69	250.1 a
Calprozime [®] (5 g kg ⁻¹)	84.78 a	21.2	8.44	255.4 a

(*) Values followed by a different letter within a column are significantly different at 5% level according to Tukey's HSD test.

Table 5. Production parameters with respect to various supplementations applied to commercial compost in *Agaricus bisporus* cultivation (*)

Finally, studies adding soybean meal and other commercial supplement (Champfood) at spawning and before casing were also done by Kopytoswky-Filho et al. (2008) in cultivation of *A. subrufescens*. The authors began the process of composting with a mixture of sugarcane bagasse, braquiária straw, soybean meal, urea, limestone and gypsum, with C/N ratio of 37/1. According to the results, the supplementation at spawning and before casing did not differ statistically. The yield values at spawning were 9.1, 9.6 and 8.6 kg of fresh mushroom per m² and before casing were 5.6, 6.1 and 9.3 kg of fresh mushroom per m², respectively, for the control (no supplement), supplemented with soybean and use of a commercial supplement.

2.3 Use for new cultivation substrates

Soybean based supplement are also precious compounds for the development of new ways to prepare the cultivation substrates. Royse et al. (2008) evaluated the effect of adding a delayed release nutrient (SoyPlus[®], 4% dry wt) to colonized mushroom compost for the

production of a second crop of mushrooms. Re-casing compost after re-supplementing represents a potential opportunity for growers to increase revenues and reduce costs associated with preparation and disposal of compost. According with the authors, the ability to double crop mushroom compost would provide growers a chance to increase yields by 40% or more. In mushroom production on non-composted substrates, Till (1962) developed what is commonly known as the Till substrate which consisted primarily of straw and several additives (ground straw, white peat, calcium carbonate, cottonseed meal and soybean meal). After autoclaving, the substrate was inoculated under sterile conditions. This process was never adopted because of high operating costs.

Gibbons et al. (1991) evaluated synthetic compost formulations containing varying amounts of soybean meal as nitrogen source, with mushroom yields between 155.8 and 182.7 g kg⁻¹ (fresh wt.). Sanchez and Royse (2001) showed that a pasteurized, non-composted substrate consisting of oak sawdust, millet, rye, peat, alfalfa meal, soybean flour, wheat bran, and calcium carbonate was suitable for the production of brown Portobello, a variety of the common cultivated mushroom *A. bisporus*. Biological efficiency ranged from a low of 30.1% (when wheat straw was substituted for sawdust) to 77.1% for the basal mixture.

Findings of Bechara (2007), developing an alternative commercial *A. bisporus* mushroom production system using grain-based substrates, suggested a promising alternative to commercial compost-based system and its environmental problems. In this research, the highest yield was observed for a millet/5% soybean substrate with an additional amendment of 5% delayed-release supplement which produced 21.3 kg/m² with a BE of 273%. Mamiro et al. (2007) and Mamiro and Royse (2008) grown *A. bisporus* on non-composted substrate containing ground soybean (4%), spent mushroom compost, and mixtures of them, non-supplemented or supplemented with different nutrients, obtaining yields comparable to non-supplemented Phase II compost. Results confirm the possibility of producing mushrooms on non-composted substrates. Soybean meal has also been used in spawn production (Stoller, 1974).

3. In *Pleurotus* spp. and *Lentinula edodes* growth

Several kinds of lignocellulosic residues may be used for lignicolous mushroom cultivation, like wheat straw, corn, cotton, coconut, crushed sugar-cane and sawdust. In favorable environments (temperature, relative humidity, luminosity) they produce lignocellulase enzymes, mainly laccases, Mn-peroxidases, cellulases and hemicellulases which convert these lignocellulosic residues into food. However, the addition of supplements to these substrates, such as wheat bran, rice and soybean is usually recommended, in order to obtain a satisfactory development (Melo de Carvalho et al., 2010). Although available commercial supplements were initially developed specifically for use with humicolous *Agaricus* species, researchers found that many of these supplements were effective in stimulating yields of some specialty mushrooms such as *Pleurotus* spp and *L. edodes*. In this way, an opportunity exists for commercial development of nutrients specifically designed for lignicolous mushroom cultivation (Royse et al., 1991).

However, as in the case of *A. bisporus*, the practice of supplementation with materials rich in nitrogen and carbohydrates is not without risks. Among the most important is the ability to promote the development of competitor moulds and cause dangerous increases in the temperature of the substrate, not always easy to control if there is no adequate climatic control systems. These problems diminish in any case where the practice of

supplementation is carried out with the so-called delayed-release nutrients, available after the mycelium has fully colonized the substrate (Muez y Pardo, 2001).

Several trends are evident for *Pleurotus* spp.: decrease in time to harvest, decrease in disease incidence under proper conditions, increase in yield and quality and increase in number of production cycles per room per year (Betterley, 1989).

3.1 Soybean as supplement to substrates based on cereal straws and other crops by-products

Lot of attention was paid on the cultivation of *Pleurotus ostreatus* (Jacq.) P. Kumm. In Italy, Ferri (1985) proposed, among other formulations, a mixture of wheat straw (90%) and soybean meal (10%). Royse and Schisler (1987a) cultivated *P. ostreatus* on a pasteurized mixture of chopped wheat straw (70%) and milled corncobs (30%) non supplemented and supplemented with two levels of delayed-release nutrient. Yields increased 2.3 and 3.2 fold on substrate containing 16% and 32% (dry weight basis) of delayed-release nutrients, respectively. Larger mushrooms were produced on substrate containing higher levels of delayed-release nutrient. Mushrooms were harvested 12 to 14 days earlier from supplemented substrates.

Gea et al. (2009) obtained significant increases of 21.6% in the value of the biological efficiency when supplemented a wheat straw based commercial substrate with an additive containing denatured soybean meal and other organic protein sources. Biological efficiency values of 70.6 kg 100 kg⁻¹ substrate (dry wt.) were reached.

Recently, Jafarpour et al. (2010) evaluated combination usage of substrates including wood chips, boll, sugar beet pellet pulp and palm fiber along with wheat bran, rice bran, soya cake powder, soya cake powder + rice bran, and carrot pulp as supplements. The least growth period (30.3 d) belonged to sugar beet pulp enriched with soya cake powder. In addition, the highest biological efficiency (158.9%) was found on boll substrate enriched with a mixture of soya cake powder and rice bran supplements, with an increase of 80% compared to control.

Experiments to study the feasibility of reusing the spent oyster mushroom substrate in new production cycles had provided, as a result of supplementation, increases of biological efficiency between 51 and 70%, depending on the base substrate used and supplementation applied, as listed in Table 6 and Fig. 2. As shown in Fig. 3, supplementation also produced increases in dry matter content of fruit bodies (Picornell, 2010; Pardo-Giménez et al., 2011).

All the experiments related above prove the interest of supplement based on soybean or other nitrogen sources for the cultivation of *P. ostreatus* in substrates that are naturally poor in this compound. Numerous similar positive effects have been obtained for the cultivation of other *Pleurotus* species (Fig. 4).

Naraian et al. (2009) evaluated different supplements in *Pleurotus florida* cultivation. Corn cob was employed as basal substrate while eight different additives such as urea, ammonium sulphate, gram flour, soybean meal, ground nut cake and molasses were used as supplements. The biological efficiencies in every supplemented set were increased over un-supplemented control set. The cotton seed cake was found the best supplement producing 93.75% biological efficiency while soybean meal was the second best additive producing 93.00% yield.

Zadrazil (1980) measured the effects of supplementing the straw substrate of *Pleurotus sajor caju* (Fr.) Singer with ammonium nitrate, alfalfa and soybean meal on the decomposition speed of the substrate, the yield of fruiting bodies and their nitrogen content. The yield of

Substrate formulation	Supplementation (20 g kg ⁻¹)	Biological efficiency (kg/100 kg substrate)
Wheat straw CaSO ₄ (50 g kg ⁻¹)	Nonsupplemented	43.4 bcd
	Promycel [®] 600	73.3 a
	Champfood [®]	65.6 a
	Calprozime [®]	72.3 a
Wheat straw + Spent oyster mushroom substrate (1:1, w/w) CaSO ₄ (50 g kg ⁻¹) CaCO ₃ (10 g kg ⁻¹)	Nonsupplemented	28.7 d
	Promycel [®] 600	42.8 bcd
	Champfood [®]	45.8 bc
	Calprozime [®]	48.9 b
Wheat straw based commercial control	Nonsupplemented	48.5 b

(*) Values followed by a different letter are significantly different at 5% level according to Tukey's HSD test.

Table 6. Results obtained for the biological efficiency assessed in oyster mushrooms originating from the various supplementations applied to two substrate formulations (*)

fruiting bodies and their nitrogen content increased more with addition of alfalfa and soybean meal than with ammonium nitrate supplementation. The highest nitrogen content in fruiting bodies (8.90%) was found using wheat straw substrate after supplementation with 30% of soybean meal (8.90%). Moreover, the yield coefficient (Yield of fruiting bodies/% Loss of organic matter) was higher in supplemented substrates (maximum 0.25) than in the nonsupplemented control (wheat straw, 0.11).

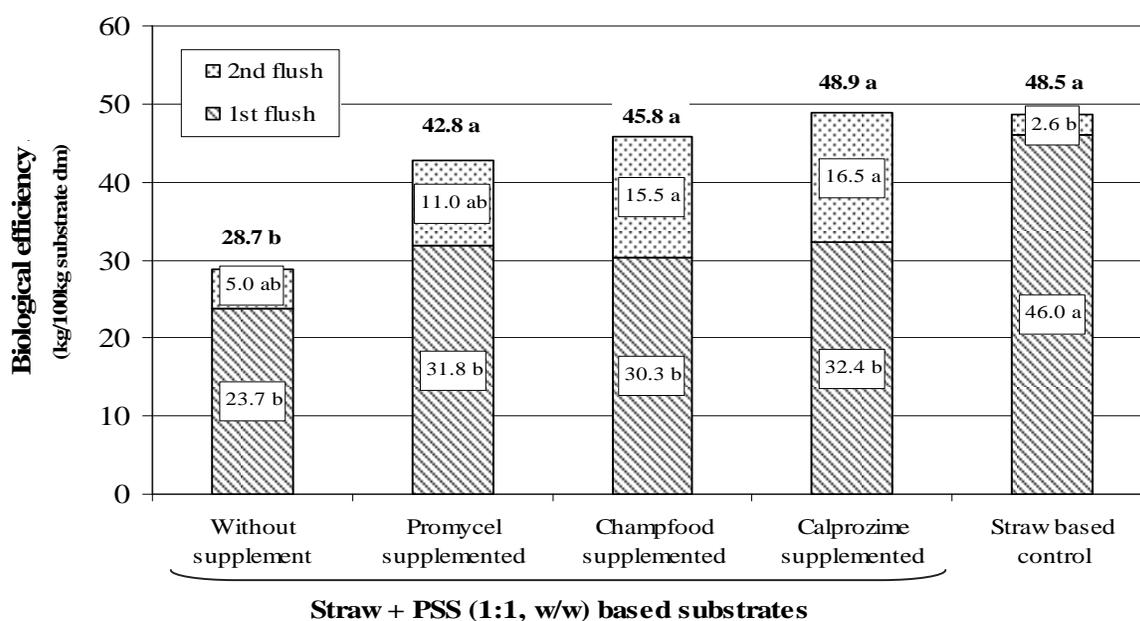


Fig. 2. Results obtained for the biological efficiency assessed in oyster mushrooms originating from the various supplementations applied to wheat straw + *Pleurotus* spent substrate (PSS) formulation

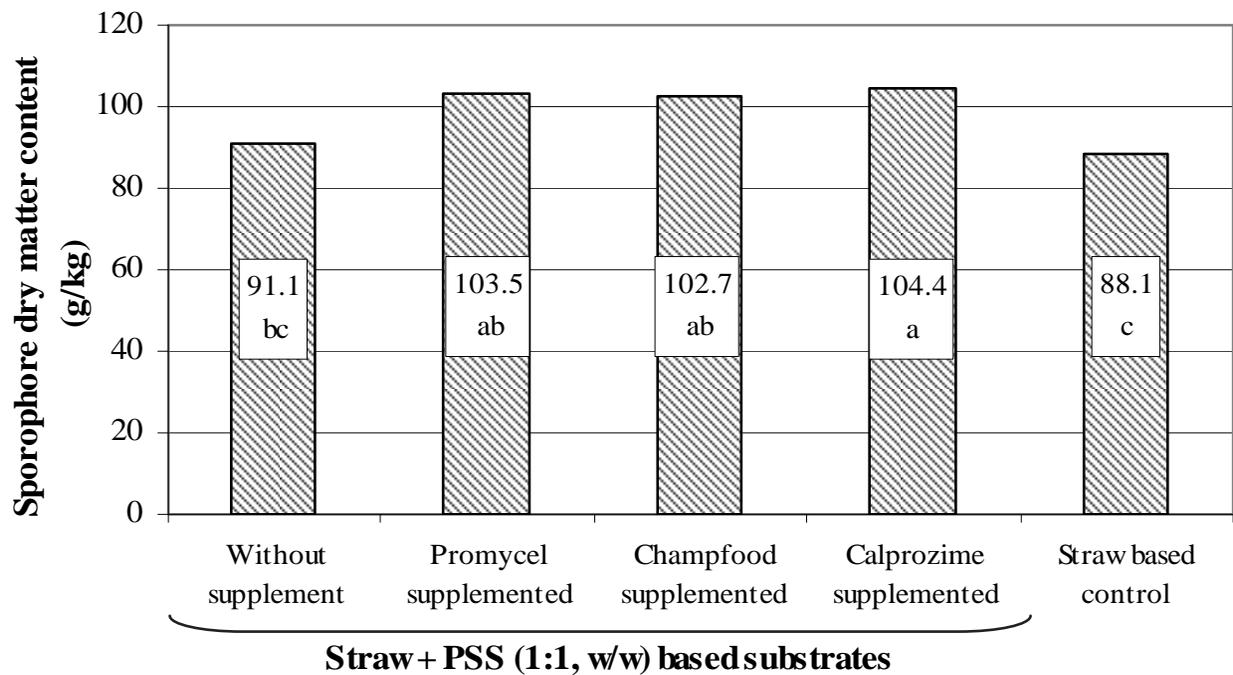


Fig. 3. Results obtained for the dry matter content of oyster mushrooms originating from the various supplementations applied to wheat straw + *Pleurotus spent substrate* (PSS) formulation

Royse and Schisler (1987a) cultivated *Pleurotus sajor-caju* on a pasteurized mixture of chopped wheat straw (70%) and milled corncobs (30%) nonsupplemented and supplemented with two levels of delayed-release nutrient. Yields increased 2.3 and 3.2 fold on substrate containing 16% and 32% (dry weight basis) delayed-release nutrient additions, respectively. For mushroom size, a differential response was observed for genotype and delayed-release nutrient. Smaller mushrooms were produced with higher levels of nutrient. Mushrooms were harvested 3 to 4 days earlier from supplemented substrate. The same authors (Royse and Schisler, 1987b) observed values of biological efficiency of 121% using high supplementation rates (63%, dry wt.) of a delayed release supplement (SpawnMate®) when Benomyl® (a fungicide) was applied during the substrate soaking process used for water absorption by wheat straw.

Royse and Bahler (1988) evaluated the combination of alfalfa hay with wheat straw and supplementation with delayed-release nutrient, with a significant increase of total yield and biological efficiency of *P. sajor-caju*. As substrate nitrogen content increased, biological efficiency increased. Highest yield (93.1% biological efficiency) was obtained from a mixture of straw and hay (80:20, w/w) supplemented at spawning with SpawnMate II® (3.5%, wet wt.). Later, Royse et al. (1991) cultivated *Pleurotus sajor-caju* grown on chopped, pasteurized wheat straw non-supplemented and supplemented with formaldehyde-treated soybean, commercial delayed-release nutrient (SpawnMate® II SE) or vegetable oil. Yield was 2.1-fold higher for substrate supplemented (12% dry wt.) with low-volume formaldehyde-treated soybean as compared to non-supplemented substrate. Mushroom yield from substrate supplemented with commercial nutrient was 1.7-fold higher than yield from non-supplemented substrate. As the supplement level increased, the mushroom yield response increased. The yield ranged from 3.56 kg/m² for non-supplemented substrate to 7.36 kg/m² for substrate supplemented (12% dry wt.) with formaldehyde-treated soybean.



Fig. 4. A: *P. ostreatus* var Florida; B: *P. pulmonarios*; C: *P. ostratus* and D: Overview of the cultivation of *Pleurotus* ssp.

The same author obtained high yields of *P. sajor-caju* (79.4% biological efficiency) by supplementing a spent shiitake basal medium with 12% of ground soybean and 1% CaCO_3 (Royse, 1992). Bano et al. (1993), supplementing the rice straw substrate colonized by the mushroom *Pleurotus sajor-caju* with powdered oil seed cakes (mustard, niger, sunflower, cotton, and soybean), observed increases of mushroom yields between 50 and 100%, compared to the unsupplemented substrate.

Royse y Zaki (1991) observed that supplementation of pasteurized wheat straw with two commercial nutrient supplements (Spawn Mate II[®] and Fast Break[®]) alone or in combination stimulated yield and biological efficiency (BE) of *Pleurotus flabellatus* (Berk & Broome) Sacc. A combination of both supplements at 84g each per kg dry wheat straw gave a yield of 6.7 kg/m² and a BE of 77.7%. Substrates supplemented only with Spawn Mate[®] (168 g/kg dry substrate) produced a yield of 5.8 kg/m² and a BE of 67.3%. Non supplemented substrate produced a yield of 1.7 kg/m² and a BE of 22.8%.

Royse (2002) evaluated the effect of spawning rate and supplementation level in the cultivation of *Pleurotus cornucopiae* (Paulet) Rolland. The substrate (a mixture of pasteurized cottonseed hulls, chopped wheat straw, and ground limestone) was spawned at various levels (1.25%, 2.5%, 3.75%, or 5% wet wt.) and not supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 3%, 6%, 9%, or

12%). Maximum yield (weight of fresh mushrooms harvested at maturity) was obtained at 3.75–5% spawn level and 6% S-41 supplement. As supplement levels exceeded 6%, yields declined significantly.

In another series of experiments, Royse et al. (2004) found a cost effective alternative substrate. For this, *P. cornucopiae* was grown on: (1) chopped, pasteurized switch grass (99%) with 1% ground limestone and (2) a mixture of pasteurized cottonseed hulls (75% dry wt.), 24% chopped wheat straw, and 1% ground limestone (all ingredients wt./wt.). The substrates were spawned at various levels (2.5%, 3.75% or 5% wet wt., crop I) and non-supplemented or supplemented with commercial delayed release nutrient (Campbell's S-41) at various levels (0%, 1.5%, 3%, 4.5%, 6%, 7.5% and 9% dry wt., crop II). As in Royse (2002), maximum yield was obtained on cottonseed hull/wheat straw substrate at a 3.75-5% spawn level and 6% S-41 supplement. On switch grass substrate, increasing spawn levels and supplement levels stimulated yields in a linear fashion. However, maximum yields were only 46% or less for those of similar treatments on cottonseed hull/wheat straw substrate.

3.2 Soybean as supplement to substrates based on sawdust

With *Pleurotus eryngii* (DR.) Quél., Royse (1999) conducted several experiments to determine the effect of supplementing cottonseed hulls and oak sawdust with brewer's grain and Spawmate II-SE[®] (SM) a commercial delayed-release nutrient recommended for oyster mushroom production. In general, as percentage of SM increased, mushroom yield and biological efficiency increased. Cottonseed hulls based substrate supplemented with 9% of SM resulted in a 3-fold increase in yield over 3% supplementation.

Rodríguez Estrada and Royse (2007) performed experiments to determine effects of supplementation of cottonseed hull/sawdust substrate with Mn, Cu, and whole ground soybean (4%, 8% and 12%) on yield, mushroom size, and bacterial blotch resistance of two commercial strains of *Pleurotus eryngii*. Mushroom yields were significantly higher from substrates containing Mn at 50 µg/g and soybean at 8% and 12% supplementation compared to the basal substrate. As the level of soybean addition to substrate increased, yield also increased. Rodríguez Estrada et al. (2009) noticed that improved yield and biological efficiency (BE) of *P. eryngii* var. *eryngii* were achieved by supplementation of substrate with a commercial delayed-release nutrient and use of a casing overlay. Yield increases of 4% were achieved from cased substrates that were supplemented at time of casing with a corn and soybean delayed-release nutrient (4% dry wt.).

Species of *Eucalyptus* (*urophylla*, *grandis*, *camaldulensis* and *saligna*) and *Quercus* (*acutissima*, *dentata*, *serrata* and *mongolica*) are commonly used in the cultivation of Shiitake (*L. edodes*) for production of the substrate, which are additioned with meals (soybean, wheat, rice, cotton, corn, etc.) or bran (Table 7) in order to improve the properties of the substrate, mainly due to the increased content of nitrogen and carbohydrates available, resulting in fast spawn run and reduction the production phase, when compared with cultivation on logs (Rinker, 1991; Luo, 2004; Minhoni et al., 2007) (Fig. 5).

But high levels of nitrogen may result in a less dense mycelium growth and facilitate the presence of *Trichoderma*, the main competitor causing troubles in cultures of Shiitake. Thus the balance of nitrogen and carbon should be performed to establish a C/N ratio of 35-55/1 during mycelial growth, taking into account that in the time of fruiting it will be between 55-80/1. The recommended pH values for mycelial growth are 5.5 to 6.5, so in the time of fruiting it will be between 4.5 to 5.5 (Oei, 2003; Chen 2005; Zied et al., 2009a). Table 7 shows various formulations used in several countries.

Sawdust	Meal	Grain	Others	Author
80% Quercus	10% wheat	10% maize		Royse et al., 1985
80% Quercus	10% wheat	10% millet		Rinker, 1991
72%(1)	26% wheat		2% carbonate	Ghang, Miles, 1989
94%	3-4% rice		1% carbonate	Oie, 2003
	1% wheat or maize			
80% Eucalyptus	20% wheat		1 a 2% carbonate	Sant`anna, 1998
70-78%(2)	20% maaize		2% de carbonate	Kalberer, 2000

(1) Dalberia sisso, Acacia arabica and Populos alba; (2) 70% Quercus, 20% Faia and 10% Bordo.

Table 7. Formulations examples of the substrates for the cultivation of *L. edodes*.

The yield achieved in the cultivation of *L. edodes* is between 15 to 30% (mushroom fresh weight/compost fresh weight) and the production time is around 90 to 180 days of cultivation. This technology by adding various types of meals in sawdust offers major advantages when compared to traditional cultivation on logs, such as use of various types of waste (sawdust, sugarcane bagasse, etc.), constant production throughout the year, reduction of production time and ease of management (since each block has about 2-3 kg). But some disadvantages are also observed, as higher investment in cultivation (production of the substrate and chambers of growing semi-controlled), high energy consumption and need for skilled labor (Badham, 1988; Pettipher, 1988).



Fig. 5. Cultivation of Shiitake in sawdust, supplemented with soybean (right); and traditional cultivation in logs of *Eucalyptus* (left)

4. Conclusions

The use of soybean meal other by-products from soybean transformation in cultivation of edible and medicinal mushrooms is possible, viable and represents a good source of organic N (to be used as concentrated material or supplement). For any application it is important to know exactly the physiological behavior and the nutrition of the mushroom. It is noteworthy that the numerous advantages of its use as: a protein source of high quality, without the presence of heavy metals, found during all seasons, which takes up little space (yet without the need to be stored), a relatively low price, increase the nutritional value of mushrooms and the yield values by 5-20% and occasionally by more.

5. References

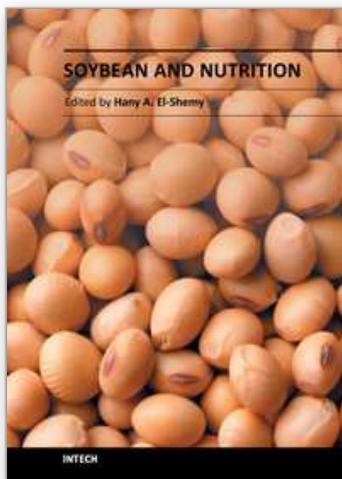
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Worldwide, soybean seed proteins represent a major source of amino acids for human and animal nutrition. Soybean seeds are an important and economical source of protein in the diet of many developed and developing countries. Soy is a complete protein and soy-foods are rich in vitamins and minerals. Soybean protein provides all the essential amino acids in the amounts needed for human health. Recent research suggests that soy may also lower risk of prostate, colon and breast cancers as well as osteoporosis and other bone health problems and alleviate hot flashes associated with menopause. This volume is expected to be useful for student, researchers and public who are interested in soybean.

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