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Yield of different white button strains in sugar cane by product-based composts

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The production of quality compost for *Agaricus bisporus* using alternative and local agricultural wastes beyond the search for productive strains are among the main factors related to improve yield. Thus, we evaluated the effect of compost nitrogen supplementation type using two compost formulation based on sugar cane by-products such as straw and bagasse as raw materials: (1) classic compost using chicken manure and (2) synthetic compost using soybean bran, urea and ammonium sulfate, for the cultivation of the five strains of *A. bisporus*: ABI-05/03, ABI-04/02, ABI-06/05, and ABI-09/10 ABI-09/11. We found that the classic compost obtained average temperature and mass loss of 10.56 and 13.29% higher than the synthetic compost, respectively, during the composting, pasteurization and conditioning process. The classic compost achieved greater yield in the end of 25 days of harvest by the strains ABI-05/03, ABI-06/05 and ABI-04/02 corresponding to 26.78, 25.34 and 24.71%, respectively. We concluded that the classic and synthetic, based on sugar cane straw and bagasse are suitable agricultural waste for *A. bisporus* cultivation and the classic compost obtained higher composting temperatures in relation to synthetic compost, corroborating higher yields and more defined-pattern cultivation cycle for all strains when grown in classic compost.

Key words: *Agaricus bisporus*, by-products, supplementation, classic, synthetic.

INTRODUCTION

The *Agaricus bisporus* Lange (Imbach) ("white bottom") is the most significant fungus of the edible mushrooms cultivated in the world and is estimated that its production is around 40% of the global edible mushroom production (Sanchez and Royse, 2009). Compost production can be cited as one of the most important factors among those related to high yield for *A. bisporus* cultivation, which amounts to the majority of costs in the *Agaricus* cultivation, and when summed up, they reach about 50% of the costs of cultivation (Royse and Chalupa, 2009; Royse, 2010). To make viable the mushroom cultivation using alternative raw materials, one must consider are the availability, transport, seasonality, and the costs of these materials (Stamets, 2005). Furthermore, based on the choice of materials, confirmed with the analysis of carbon and nitrogen concentrations, aiming to promote better development of the fungus, the compost is formulated with ideal carbon/nitrogen (C/N) ratio. According to Oei (2003), 40 different types of vegetable wastes are available for edible mushroom cultivation, and amongst them, about 80% can be used directly as substrate, whereas 20% can be used as nutritive

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supplement. In Brazil, 458 grass species alone were registered in São Paulo State and represent about 80% of State's flora (Pivetta, 2001). Straws (rice, wheat, oat and barley), and chicken manure and by-products such as sugar cane bagasse are the most important components used as lignocellulosic sources in composts (Andrade et al., 2008; Peil et al., 1995).

Therefore the availability of raw materials, Brazil is the world's largest sugar cane producer, highlighting the States of São Paulo, Rio de Janeiro, Minas Gerais, Pernambuco, Alagoas, and Paraíba (CONAB, 2012).

The grinding of sugar cane by the producer units at Brazil Central-Southern region account for 556.19 million tons being accumulated since the beginning of harvest until 31 January, 2011, an increase of 2.63% compared to the volume processed in the 2009/2010 harvest (UNICA, 2011).

Each ton of sugar cane generates 250 kg of bagasse as by-product, with 50% of moisture (Cortez et al., 1992). According to Ripoli and Ripoli (2004) and Copersucar (2001), the sugar cane can generate, beyond the industrialized stalks, around 15 to 30% of straw.

Therefore, the high availability of this material in Brazil is a factor that emphasizes the choice of it as raw material for the purpose of mushroom cultivation. However, it is important to investigate the effect of different strains of *A. bisporus* on the viability and potential of conversion into fungal biomass.

MATERIALS AND METHODS

The experiment was conducted from January to April, 2010 in the experimental area of Mushroom Module, located in the Department of Plant Protection, Agronomic Sciences College, UNESP, Botucatu/SP, Brazil.

Strains and inoculum production

The strains obtained were ABI-05/03 (medium-big size, white, isolated from Cabreúva/SP), ABI-06/05 (medium-big size, white, summer strain, isolated from Piedade/SP), ABI-04/02 (medium size, white, provided by Brasmicel-Mogi das Cruzes/SP), ABI-09/10 (medium-big size, steam thin and large cap, provided from mycelium lab Hong Kong, China), and ABI-09/11(medium size, steam thin and large cap, provided from mycelium lab Hong Kong, China). They were stocked in tubes with compost-agar (CA) medium submerged in sterilized mineral oil and preserved at 4°C and stored at the Mycology Collection of Mushroom Module from FCA/UNESP, Botucatu/SP.

The inoculum preparation followed the methods proposed by Minhoni et al. (2011) using sorghum grains, 20 g kg^{-1} calcium carbonate, and 160 g kg^{-1} gypsum. They were then added into high-density polyethylene bags (HDPE), using about 1200 g of grains per bag.

The substrates were autoclaved at 121°C for 3 h and added to the plastic bags, 20 g kg^{-1}. Finally, it was incubated at 22 ± 1°C for 30 days. The substrates were already colonized by the fungus, being thus referred to as spawn, and then were ready to be inoculated into the compost.

Composts

The sugar cane bagasse (0.5 to 2 cm length) was donated by the ethanol and sugar mill "Usina Açucareira Furlan S/A". Sugar cane straw (10 to 15 cm length) was donated by the ethanol and sugar mill "Açucareira Zillo Lorenzeti". Chicken manure was purchased at a local chicken farm. Soybean, gypsum, and limestone were purchased from a local agricultural store. All raw materials used for compost production (classic and synthetic) were subjected to analysis of carbon and nitrogen concentration to elaborate the balancing and initial C:N ratio using a software developed by Mushroom Module. Soybean bran and chicken manure were analyzed for macro and micronutrients, organic matter, organic and inorganic nitrogen. Both composts were balanced to start the composting process from distinct C/N ratio, thus 28/1 was selected as the value of the initial C/N ratio (Tables 2 to 4).

Initially, the quantities of sugar cane bagasse and sugar cane straw were weighed and separated for both composts for the starting stage of pre-wetting, conducted during 6 days. From the 7th day, the composting began, marked by the addition of nitrogen supplements and the inorganic conditioners (gypsum and limestone), which was being conducted for 12 days with periodic mixing to complete homogenization of the material. On the 12th day of composting, the composts were packed in polypropylene (PP) boxes that were stacked randomly at Dalsem Mushroom Projects® chamber for pasteurization process followed by conditioning, which was controlled by the software VEC-31. Pasteurization has been carried out at 59 ±1°C for 8 h and after that conditioning has been done for 10 days at 48 ± 1°C (Table 1).

Inoculation and spawn run

After pasteurization and conditioning, all the boxes were weighed again to measure the mass loss during the FII. During the inoculation, the compost was removed from each box and manually transferred to other parcels of the same model. Each box was previously wrapped with plastic, 90-μm-thick, PP film and then the weight was adjusted to 13.0 kg. For inoculation, 15 g of inoculum was manually added to each of the five strains of *A. bisporus* that was being inoculated kg^{-1} fresh weight of compost (FWC). Once inoculated, the boxes were again randomly arranged within the chamber Dalsem to spawn run for 15 days in the absence of light, and the temperature of the compost was maintained at 25 ± 1°C with 90 ± 5% relative humidity.

Casing, induction, and harvest

After 20 days of mycelium running, we proceeded to cover the compost with a previously prepared casing layer. The procedure was done in an environment covered with natural ventilation and concrete floor. The top layer consisted of 70% of land bank (Oxisol clay-sandy) and 30% of coal (fragments of 0.5 to 2 cm). The pH of the soil was adjusted to 7.0 with limestone, according to methodology used by Andrade et al. (2008). Casing was pasteurized with the use of moist heat (steam) inside the chamber Dalsem, at a temperature of 60 ± 1°C for 10 h. After 15 days of incubation for colonization of the casing layer, water was added manually to the surface of the blocks using watering can and continuous forced aeration of 100 m^3 h^{-1} air was began, with renewal of outside air, keeping the temperature air at 19 ± 1°C and relative humidity of 80 to 90%. Crops were picked by hand in the stadium next to the rupture of the veil. The mushrooms were picked with a slight twist of the base of the stalk, and a knife was used to cut the bases of the stalk with accumulatio of hyphae and casing

Table 1. Procedures to compost (classic and synthetic) preparation.

Accumulated period (days)	Procedures	Stage	Phase
-7	Wetting the sugar cane straw	Wetting	Pre wetting
-4	Mix of straw + bagasse, wetting	Wetting	
0	Adding nitrogen supplements + $CaCO_3$ + 50% of $CaSO_4$; mix	Composting start	FI*
3	Eventual moisture correction + mix	1º mix	
5	Eventual moisture correction + mix	2º mix	
7	Eventual moisture correction + mix	3º mix	
10	Eventual moisture correction + mix; Adding 50% $CaSO_4$	4º mix	
11	Adding compost to plastic box and start pasteurization	Filling pasteurization chamber	FII**

*FI - Composting; ** Pasteurization and conditioning.

Table 2. Raw materials utilized in the preparation of the synthetic and classic composts.

Raw material	Synthetic compost		Classic compost		C:N ratio
	DW (kg)	DW (%)	DW (kg)	DW (%)	
Sugarcane bagasse	200.20	39.8	110.00	22.9	141:1
Sugarcane straw	217.50	43.2	187.50	39.0	101.2:1
Chicken manure			150.00	31.2	8:1
Soybean bran	34.00	6.8			7.70:1
Urea (45% N)	7.50	1.5			0.6:1
Ammonium sulphate (20% N + 12% S)	7.50	1.5			0.3:1
Lime ($CaCO_3$)	12.00	2.4	11.00	2.3	-
Gypsum ($CaSO_4$)	24.00	4.8	22.00	4.6	-

Initial C:N ratio was estimated using software developed by Mushroom module.

Table 3. Average level of carbon (C), nitrogen (N), organic matter (OM), C:N ratio, organic nitrogen (N org.) and inorganic nitrogen (N inorg.) of nitrogen supplements utilized in the composts formulation.

Material	N	C	OM	Dry weight	C:N ratio	N org.	N inorg.
	kg					%	
Classic compost							
Chicken manure	6.30	49.16	83.55	150.00	8:1	7.62	Nd
Synthetic compost							
Soybean bran	2.41	16.83	30.29	34.00	7.70:1	4.20	Nd
Urea (45% N)	3.38	1.65	-	7.50	1:45	Nd	3.38
Ammonium sulfate (20% N + 12% S)	1.50	-	-	7.50	1:20	Nd	1.50

layer.

Variables analyzed

Carbon, nitrogen, C/N ratio, organic matter, pH, moisture and temperature

Throughout the final Phases I and II, the samples were collected in triplicate for each 200 g of the substrate for determination of carbon, nitrogen, C:N ratio, organic matter, pH, and moisture, which was performed at Laboratory of Fertilizers and Correction - FCA/UNESP, as well as losses of dry fresh weight, according to the methodology adopted by Lanarv (1988). To evaluate the composting temperatures were used at three areas in the compost pile, labeled as Points A (at the 1.60 m high), B (located at 1.00 m height) and C (located at 0.40 m height). Each point used three equidistant points, and the average temperature was expressed in (°C).

Yield

Yield (Y) was expressed as fresh weight of mushrooms (FWM) / (FWC) × 100. The FWM was determined at the end of the harvesting, and FWC was determined at the end of Phase II.

Table 4. Analysis of macro and micronutrients, pH and C:N ratio of the soybean bran and chicken manure.

Constituent	Soybean bran	Chicken manure
	%	
N	7.10	4.20
Ca	0.37	18.6
Organic matter	89.1	55.7
C	49.5	32.77
	mg kg^{-1}	
Na	59	3881
Fe	244	4851
Mn	73	358
Zn	57	370
C:N	7.7	7.8
pH	5.9	7.81

Table 5. Parameters observed during pre wetting, composting, pasteurization and conditioning.

Parameter	Day						
	Pre wetting			FI		FII	
	-4	-3	-1	4	6	9	24
	Synthetic compost						
Temperature (°C)	25	38	29	64	59	63	25
Moisture (%)	75.95	78.16	80.49	77.4	75.32	73.56	66.7
pH	6.27	6.13	6.25	7.32	6.4	6.5	6.61
Organic matter (%)	86.94	88.2	86.94	73.98	73.08	57.06	48.96
Carbon (%)	48.3	49	48.3	41.1	40.6	31.7	30.2
Nitrogen (%)	0.56	0.97	1.5	1.47	1.7	1.72	1.78
C:N ratio	86	50	32	28	24	19	16
	Classic compost						
Temperature (°C)	38.3	39.7	33.5	67.7	62.7	67.5	25
Moisture, %	77.48	80.63	79.16	73.11	76.13	74.97	68.1
pH	6.38	6.07	6.23	6.93	6.88	7.1	6.8
Organic matter (%)	86.94	87.84	88.92	73.08	66.06	55.08	54.36
Carbon (%)	48.3	48.8	49.4	40.6	36.7	30.6	27.2
Nitrogen (%)	0.49	0.7	1.11	1.47	1.6	1.77	1.81
C:N ratio	99	70	45	28	23	18	17

Experimental design

The experimental design was completely randomized factorial of 5 × 2, corresponding to five strains and two types of nitrogen supplementation, totaling 100 boxes, 10 treatments with 10repetitions. Each replicate corresponded to a box containing 13 kg of compost. Data were subjected to analysis of variance. Means were compared by Tukey test (5%) using statistic program ASSISTAT 7.5 Beta.

RESULTS AND DISCUSSION

Relative to compost

The beginning of the composting process, marked by the addition of nitrogen supplements, shows a tendency toward higher temperatures for the classic compost with the addition of chicken manure (Table 5). During the addition of supplements in the composts, it was observed previously that the distribution of chicken manure in the classic compost, the 7th day of composting, was carried out more efficiently because of the greater quantity of material, chicken manure, rather than the amount of soybean bran added in the synthetic compost (Tables 2 and 3). Throughout the process, classic and synthetic composts had average temperatures of 62.6 and 56.2°C, respectively, where the classic compost gained 10.56% average temperature higher than the synthetic compost (Figure 1).

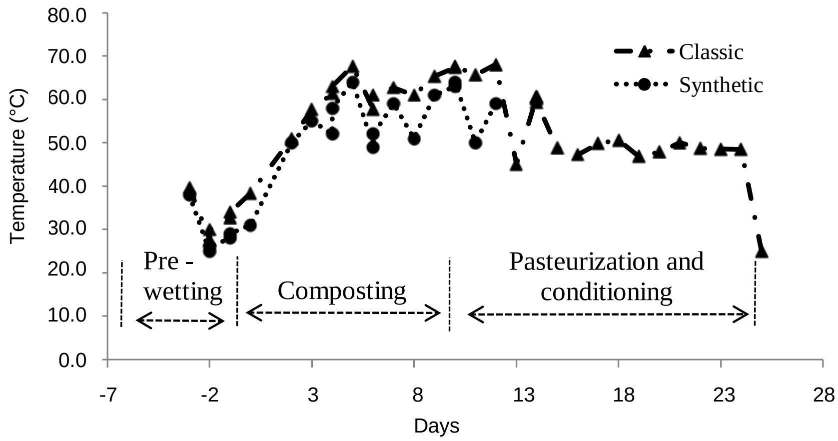

Figure 1. Temperature dynamic (°C) of the classic and synthetic composts during the periods of prewetting and composting.

The higher composting temperatures in classic compost could be justified by chemical, physical, and biological factors, being the first of all the quantity and type of nitrogen supplementation (Tables 2 to 4). Increase in temperature after the stage of addition of nitrogen supplements is an indicator for a rapid and exothermic microbial activity within the compost layers, which might be a critical stage for decomposition of carbohydrates necessary to produce a selective substrate environment for mushroom growth (Baysal, 1999; Yalinkilic et al., 1994). Colak (2004) evaluated the temperature profiles of three different compost formulas for *A. bisporus* cultivation, based on wheat straw differing in three type of supplementation (Formula I, wheat bran 19.7%; Formula II, pigeon manure 18.7%; Formula III, chopped corn stem 19.33%), observed correlation between the highest temperature peak during composting process (Formulas I, II, and III, 53.0, 57.3 and 52.7°C, respectively) and highest average yield (Formulas I, II, and III, 2952, 4114, and 2428 g/kg, respectively); therefore, the compost Formula II using manure as supplement has demonstrated higher yield than the compost Formulas I and III using wheat bran and chopped corn stem, respectively, as supplement, corroborating with the results obtained in this work. Adams and Frostik (2008) evaluated several parameters during the composting process and using a commercial high-quality compost from a mushroom farm in UK as sample, based on wheat straw, chicken manure and gypsum, evaluated compost samples, through each of the composting stages as Phase I (day 1; uncovered windrow) and end of composting Phase I (day 8; covered windrow), establishing the temperatures of 75 and 69°C as ideal composting temperatures for each phase, respectively. Peil et al. (1996) evaluated the composting temperature of the composts based on rice straw, wheat straw, and horse manure in specific days of composting, days 4, 8, 11, 14, 17, and 21, observed that the compost based on rice straw obtained the higher composting temperatures during the process compared to the other composts, with temperatures of 67, 61, 60, 64, 55, and 50°C, respectively, on the days mentioned; nevertheless, it had higher mushroom average weight and higher number of mushroom flushes during harvest. In this work, we suggest that the higher quantities of organic matter and organic nitrogen available and the amount of nitrogen supplement added to the classic compost causing greater homogeneity of the materials due to more effective distribution of supplements may have contributed to the increase in microbial activity of the composting process, generating exceeding temperatures for the classic compost and larger weight loss.

Observing the parameters during the composting process, except for temperature and fresh weight loss during FII, it was noted that the values obtained from F-test by analysis of variance did not show statistically significant differences between them in relation to classical and synthetic composts (Table 6). The similarity among the values can be justified by the fact that both composts had the same bulky materials in its composition, differing only in the amount of nitrogen supplementation; nevertheless, the classic and synthetic composts had similar C/N ratios at the end of FII, 17 and 16, respectively (Tables 2, 3 and 5). According to Flegg and Randle (1980) and Randle and Smith (1986), the natural variability of the composting process hinders the establishment of significant differences in experiments with mushroom cultivation in composts, because the results lead to a high standard error, and usually, the average is not statistically different from each other.

To evaluate the weight loss of the composts, it was observed statistical significant difference in the values of F in relation to the interaction of the composts (classic and synthetic), being 102.8345, significant at 1% (Tukey). The average weight loss for the classic compost was 13.29% higher for the synthetic compost (Figure 2, and

Table 6. F values obtained in variance analysis, using the test Tukey 5%, the levels of carbon, nitrogen, pH, organic matter, moisture e temperature during the phases FI e FII of the classic and synthetic composts.

Variation factor	F value
C:N ratio during FI to FII	0.1846[ns]
Carbon during FI to FII	0.0025[ns]
Nitrogen during FI to FII	0.1669[ns]
pH during FI to FII	0.3765[ns]
Organic matter during FI to FII	0.0025[ns]
Moisture during FI to FII	0.0155[ns]
Temperature during FI[a]	10.5603*
Fresh weight loss during FII[b]	102.8345**

**, Significant at 1%; ns, non significant; FI[a], Temperature was evaluated during the composting phase due pasteurization and conditioning have been realized at a equalized temperature chamber for success of the process; FII[b], In order to evaluate the fresh weight loss, in the end of composting phase the composts were shared, added in plastic heat resistant boxes, weighed to start the pasteurization and conditioning phase and at the end weighed again.

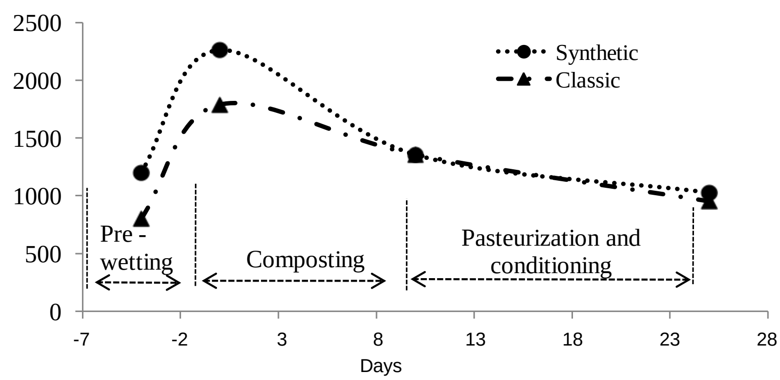

Figure 2. Fresh weight dynamic dispersion of the classic and synthetic composts during the periods of prewetting and composting.

Table 7).

Relative to production

The evaluation of variables of production occurred in 25 days after starting mushroom harvest and was marked by three production flushes. The behavior of *A. bisporus* strains varied with the production flushes according to the effect of compost type, or strain as the interaction between these two factors. There was a tendency to yield total weight of the mushrooms and biological efficiency superior to the classic compost (Table 7 and Figure 3). At the end of 25 days of production, the higher yields were observed in strains ABI-05/03, ABI-06/05, and ABI-04/02 (26.78, 25.34 and 24.71%, respectively) cultivated in classic compost, which did not differ statistically among themselves, followed by ABI-09/10 and ABI-09/11 (21.56 and 20.43%, respectively). Through the analysis of variance, a F value of 97.62 was obtained for the compost and a F value of 3.85 for compost × strain interaction, both significant at 1% probability by Tukey test. The behavior of the yield of strains ABI-09/10, ABI-09/11, ABI-04/02, ABI-05/03, and ABI-06/05 produced in the synthetic compost were 17.69, 15.70, 15.70, 15.47, and 13.88%, respectively. There was no statistically significant difference among the strains cultivated in synthetic compost. Observing the data obtained from the average mean of treatments during production, the classic compost had yield and total FWM being 33.96% higher than those for the synthetic compost. As argued previously, the biological, chemical and physical

Table 7. F values obtained for yield (for 25 days of production) of five strains of *A. bisporus* (ABI-05/03, ABI-04/02, ABI-06/05, ABI-09/10 e ABI-09/11) cultivated in the classic and synthetic composts.

Variation factor	Yield
Strain (S)	1.4898^{ns}
Compost (C)	97.6232**
S × C	3.8591**

**, Significant at 1%; ns, non significant.

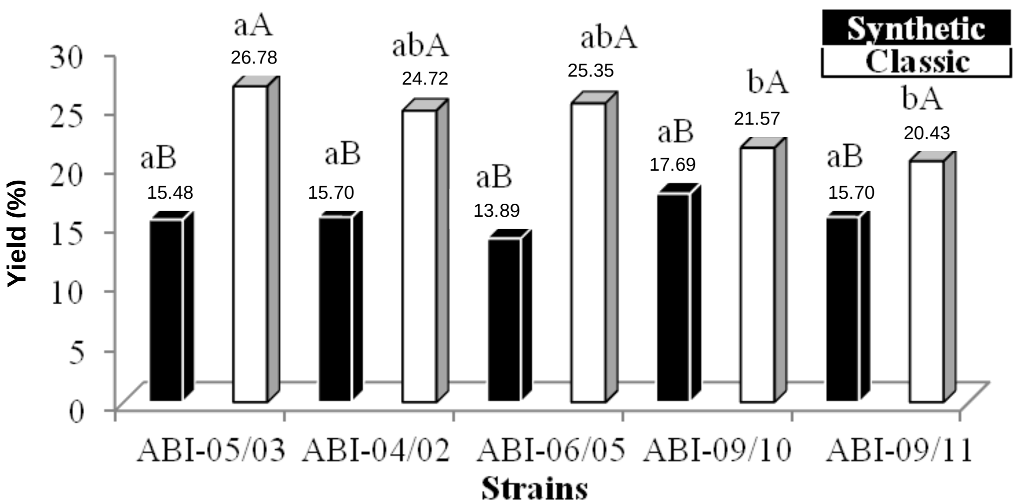

Figure 3. Accumulated yield (in percentage) of the *A. bisporus* strains (ABI-05/03, ABI-04/02, ABI-06/05, ABI-09/10 e ABI-09/11) cultivated in the classic and synthetic composts at the end of 25 days of harvest. The means followed by same letter do not differ statistically between them. Tukey Test was applied at 5% level of probability. * Lower case letters compare means between the strains in the same compost, upper case means comparing the composts to the same strain.

differences in the classic compost supplementation may have led to higher selectivity and improving yield. Comparing the results of yield achieved in this work with those of other authors, Andrade et al. (2008) evaluated the effect of three compost formulations based on straw for the production of four strains of *A. bisporus* showed significant difference to yield in relation to the type of compost as the strain used. Strains ABI-06/04, ABI-01/01, and ABI05/03 obtained the highest yield, being 18.0, 15.5, and 15.4%, respectively. In the experiment mentioned previously, the compost nitrogen supplementation was based on soybean bran and urea, similar to the supplement used in the synthetic compost; moreover, note that the greatest yields obtained by Andrade et al. (2008) are similar to the values of yield obtained in the synthetic compost.

Similar results were observed by Altieri et al. (2009) evaluating the performance of olive mill solid waste as a constituent of the substrate in commercial cultivation of *A. bisporus*, with obtained yields ranging from 21.7 to 31.0% during 2 flushes in different crops. Noble and Gaze (1996) obtained similar results by evaluating the effect of two types of composts (conventional compost and conventional compost 82% + 18% spent mushroom compost of *A. bisporus*); no significant difference was observed in the average yield, being 20.9 and 20.7%, respectively.

Weil et al. (2006), using region's traditional compost based on animal bed, evaluated the influence of several minerals such as Manganese (Mn), Molybdenum (Mo), Boron (B), Copper (Cu), Zinc (Zn), Iron (Fe) on the yield of *A. bisporus* and observed that Mn (184 mg kg^{-1}) was the only element added to the compost at spawning that produced a statistically significant increase ($p < 0.05$) in cumulative yields compared to the control, resulting in the predominant increase as a result of Mn addition during the first flush, and the cumulative yield increased greater than that for the control, 1.56 kg m^{-2} (+10,8%). The previous results corroborated with the results of this work, in which the data obtained for the macro and micronutrient analysis from chicken manure and soybean bran showed that the concentration of Mn present in chicken manure is around 490% higher than in soybean bran; therefore, such values may have significantly contributed in the production of classic compost (Table 4).

We concluded that both the classic and synthetic composts based on sugar cane straw and bagasse are suitable agricultural waste for *A. bisporus* cultivation. The classic compost obtained higher composting temperatures in relation to synthetic compost, corroborating higher yields and more defined-pattern cultivation cycle for all strains when grew in classic compost. Significant differences were observed in yield among strains of *A. bisporus* independent of the compost type cultivated.

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