Spanish Journal of Agricultural Research
18 (2), e0902, 11 pages (2020)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2020182-16037
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Influence of substrate density and cropping conditions on the cultivation of sun mushroom

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Abstract

Aim of study: To evaluate agronomical features demanded by the sun mushroom *(Agaricus subrufescens)* in order to optimise the commercial cultivation of this worldwide demanded medicinal mushroom.

Area of study: The study was carried out in Castilla-La Mancha (Spain), the second most productive region of cultivated mushrooms in Spain.

Material and methods: In this work we summarise the results obtained while evaluating the performance of sun mushroom crops *(A. subrufescens)*. Two agronomical traits have been evaluated, the effect on the productive outputs of applying five different compost filling rates of high N substrate (yield and BE of the compost), and the influence of implementing two different conditions for the induction to fructification on the analytical properties of the harvested mushrooms. Besides, two commercial compost formulations (CM and VC) obtained from local providers have been used.

Main results: The number of sporophores harvested and the yield per unit area increased with rising density of compost load, although the biological efficiency was not significantly modified. Compost fill rate of 70 kg m^{-2} provided an average yield of 13.33 kg m^{-2} and BE=55.45 kg dt^{-1}, generally higher than those values reported in the literature. The proposed moderate slow induction provides better yields, particularly in the last flushes, and larger sporophores. Proximate analysis of harvested sporophores has not shown significant differences between treatments or factors.

Research highlights: As guidance for growers, compost fill weight between 65 and 70 kg per m^2 of productive area with a moderate slow induction to fructification is presented as the best option for commercial production under controlled environmental conditions.

Additional keywords: *Agaricus subrufescens;* medicinal mushroom; compost filling rate; agronomic performance; proximate analysis.

Abbreviations used: BE (biological efficiency); CM (Compost Manchego S.L.); DM (dry matter); dt (deciton, 100 kg); VC (Compost Villacasa S.L.).

Authors' contributions: Conceived, designed and performed the experiments: APG, DCZ. Analyzed the data: APG, JC, DCZ. Contributed reagents/materials/analysis tools: APG, JEP, MAO, DC. Drafting of the manuscript: APG, JC. Wrote the paper: APG, JC. Obtaining funding: APG, DC. All authors read and approved the final manuscript.

Citation: Pardo-Giménez, A; Carrasco, J; Pardo, JE; Álvarez-Ortí, M; Zied, DC (2020). Influence of substrate density and cropping conditions on the cultivation of sun mushroom. Spanish Journal of Agricultural Research, Volume 18, Issue 2, e0902. https://doi.org/10.5424/sjar/2020182-16037

Received: 17 Nov 2019. **Accepted:** 22 Jun 2020

Funding agencies/institutions	Project / Grant
Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil	2018/21492-2

Competing interests: The authors have declared that no competing interests exist.

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Introduction

Currently the production of edible and medicinal mushrooms tends to be continuously diversifying, with the steady development of new cultivated species. Among them, the production of the sun mushroom, *Agaricus subrufescens* Peck, has generated a significant interest worldwide in the last few years, becoming increasingly popular (Kerrigan, 2005; Colauto & Linde, 2012; Wisitrassameewong *et al.,* 2012). As a result, crop production has expanded into many countries, mainly due to its high international market price compared to other marketable mushrooms, and its exceptional medicinal and culinary properties.

Two major advantages are at disposal to the concerned consumers demanding healthier food alternatives when mushroom are introduced in their diet, the therapeutic properties of some species and an important source of high value proteins with low calorific value (Henriques *et al.,* 2008). Several studies reviewed by Wisitrassameewong *et al.* (2012) highlight the importance of *A. subrufescens* medicinal properties. Due to the noted health benefits, it has been traditionally used to treat many common diseases, with significative improvements in different aspects showing subjective perception of an improved quality of life (Firenzouli *et al.,* 2008; Wasser, 2017). Besides its medicinal interest, *A. subrufescens* poses a food of high nutritional value, rich in protein, fiber and minerals, with low lipid content (Largeteau *et al.,* 2011).

The nutritional substrates, the quality of the mycelium, the material employed as casing layer or the growth conditions required for the crop are limiting factors to standardized production. For this purpose, the optimized system for the commercial cultivation of common button mushroom has been adopted by modifying certain agronomic procedures (Pardo-Giménez *et al.,* 2014).

Regarding the substrate, the sun mushroom is usually cultivated in compost with relatively low nitrogen (N) content (1.15-1.45%) and C/N ratio around 25-27 by the end of phase II (Zied *et al.,* 2017), despite producers frequently use substrate with a formulation and composition developed for the white button mushroom, with lower C/N ratio around 17.2 (Lisiecka *et al.,* 2013; Pardo-Giménez *et al.,* 2014). The compost fill rate (understood as the selected phase II or phase III compost density disposed at the initial cropping stage expressed as weight per unit area: kg m^{-2}) used in plastic bags or bulk is another aspect to consider. It is historically accepted that during the cultivation of white button mushroom, related to the sun mushroom, yield per unit production area is directly related to the compost fill rate applied within the range of 80-100 kg per m^2 of crop area (MacCanna, 1984), meanwhile the biological efficiency (BE: kg of fresh mushroom harvested per 100 kg of compost in dry matter) does not vary significantly (Schisler, 1982; MacCanna, 1984). Although the traditional bag system used to employ load densities over 100 kg m^{-2} (MacCanna, 1984), the nowadays optimised cropping system of *A. bisporus,* with phase III compost and three flushes harvested, required 80-85 kg m^{-2} (bulk material in shelf system) (Den Ouden, 2020; Navarro *et al.,* 2020).

The sun mushroom commercial cultivation is commonly developed indoors in climate-controlled facilities or greenhouses, following the cultivation standards established for *A. biporus*, with the exception of that relating to cultivation temperature (Largeteau *et al.,* 2011). A temperature range between 21 and 30ºC is used for the mycelium growth of the almond mushroom, between 21 and 25ºC for the primordia formation and between 23 and 27ºC for fruit body development (Lisiecka *et al.,* 2013). It is worth noting that different methods of inducing primordia and for the management of the crop cycle in controlled environmental conditions have been reported (Largeteau *et al.,* 2011; Zied *et al.,* 2017).

The aim of this study was to investigate the influence of the compost fill rate while using compost with high N content on the productive outputs and the analytical properties of the sun mushroom sporocarps harvested while evaluating two different agronomic managements for the induction to fructification. Ultimately the goal of the work was to establish appropriate agronomic standards for the commercial cultivation of *A. subrufescens* under controlled environmental conditions, which eventually will favour the diversification of the business for the producers of edible mushrooms.

Material and methods

Substrates used

Two commercial phase II composts based on wheat straw and chicken manure, provided by Compost Villacasa S.L. (VC, Casasimarro, Cuenca, Spain) and Compost Manchego S.L. (CM, Villanueva de la Jara, Cuenca, Spain) were used as substrates for the *A. subrufescens* production. Euroveen® (Euroveen BV, Grubbenvorst, The Netherlands), which is a peat-based commercial mixture of Dutch origin, was used as casing layer.

To determine the physical, chemical and biological characteristics of composts, the following measurements were taken: moisture content, pH, total N content, organic matter and ash, C/N ratio, crude fiber, crude fat, N-free extracts, cellulose, hemicellulose, lignin and neutral detergent-solubles. The methodology previously described by Pardo-Giménez *et al.* (2016) was used. Composts characteristics are shown in Table 1.

Table 1. Chemical and physical characterisation of the compost employed in the trials from commercial compost yards: Compost Villacasa S.L. (VC) and Compost Manchego S.L. (CM).

Variables	Compost VC	Compost CM
pH (1:5, w/v)	7.44	7.63
Moisture (g kg^{-1})	654.0	664.7
Total nitrogen (g kg^{-1})	21.3	19.7
Protein (g kg^{-1})	132.9	123.1
Ash (g kg^{-1})	224.6	237.2
Organic matter (g kg^{-1})	775.4	762.8
C/N	21.2	22.5
Crude fiber (g kg^{-1})	292.8	330.0
Crude fat (g kg^{-1})	3.3	2.5
N-free extract (g kg^{-1})	346.4	307.2
Hemicellulose (g kg^{-1})	106.8	123.4
Cellulose (g kg^{-1})	190.3	204.8
Lignin (g kg^{-1})	226.7	208.8
Neutral detergent-solubles (g kg^{-1})	251.6	225.8

Spawn

Strain ABL99/30 (Mycotec of the Modulo de Cogumelos, FCA-UNESP, Brazil) was selected for the experiment. This strain, collected in Piedade (São Paulo, Brazil) in 1999, is characterized as a producer of medium to small size fruit bodies with dense texture, high yield and early cropping, reduced time to first flush, and slightly low fructification temperature (Zied *et al.,* 2014). According to the code employed by the Germplasms of *Agarics* in Bordeaux (CGAB, INRA-Bordeaux, France), this strain corresponds to CA-561 (Llarena-Hernández *et al.,* 2014).

Spawn was prepared according to the following steps: selection of mushroom, production of subculture, production of parent spawn, and production of grain spawn (Zied *et al.,* 2017). Compost was inoculated with grain spawn at a rate of 12 g kg^{-1} of fresh weight of compost.

Research layout

The experimental design used was a 5x2x2 equilibrated factorial plan with six replicates (randomized blocks with three factorial factors). Factor 1, with five levels, corresponded to the compost filling weight per unit area (50, 55, 60, 65 and 70 kg m^{-2}). Factor 2, with two levels, corresponded to compost origin (two commercial composts). Factor 3, with two levels corresponded to two different conditions for the induction to fructification (two twin growing rooms subjected to fast or moderately slow cooling down respectively). A total of 120 trays (between 7.25 and 10.15 kg compost, 1450 cm^2 each one) were used, that were positioned at two levels on both sides of the two growth chambers selected.

Crop cycle and production parameters

A. subrufescens trials were carried out in two twin experimental growing rooms, equipped with a humidification system, a heating/cooling system, and internal air circulation/external ventilation. Trays were cased 19 days after spawning. The thickness of the casing was 5 cm. Casing was deeply ruffled 8 days after applying it, when the mycelia appeared on the surface. A day later, the environmental temperature, relative humidity, and carbon dioxide level were decreased, with illumination (150 lux, 12 h d^{-1}) provided to induce fruiting.

The mushroom growth cycles were carried out according to two fruiting induction regimes. After incubation and pre-fructification with a compost temperature of 28ºC, two different set of climatic conditions were introduced and applied for successive flushes of mushrooms. A rapid induction that we might call "aggressive", adapted from Kopytowski Filho & Minhoni (2007) and Zied *et al.* (2017), and a moderately slow one, adapted from Kopytowski Filho *et al.* (2008), were evaluated in order to reduce the energy cost associated with the significant decrease in temperature required for induction, reducing the size gap of the temperature range applied. The mushroom crop cycle was carried out according to Pardo-Giménez *et al.* (2014), the growing standards are summarized in Fig. 1. The total growth cycle lasted 83 days and four mushroom flushes were harvested.

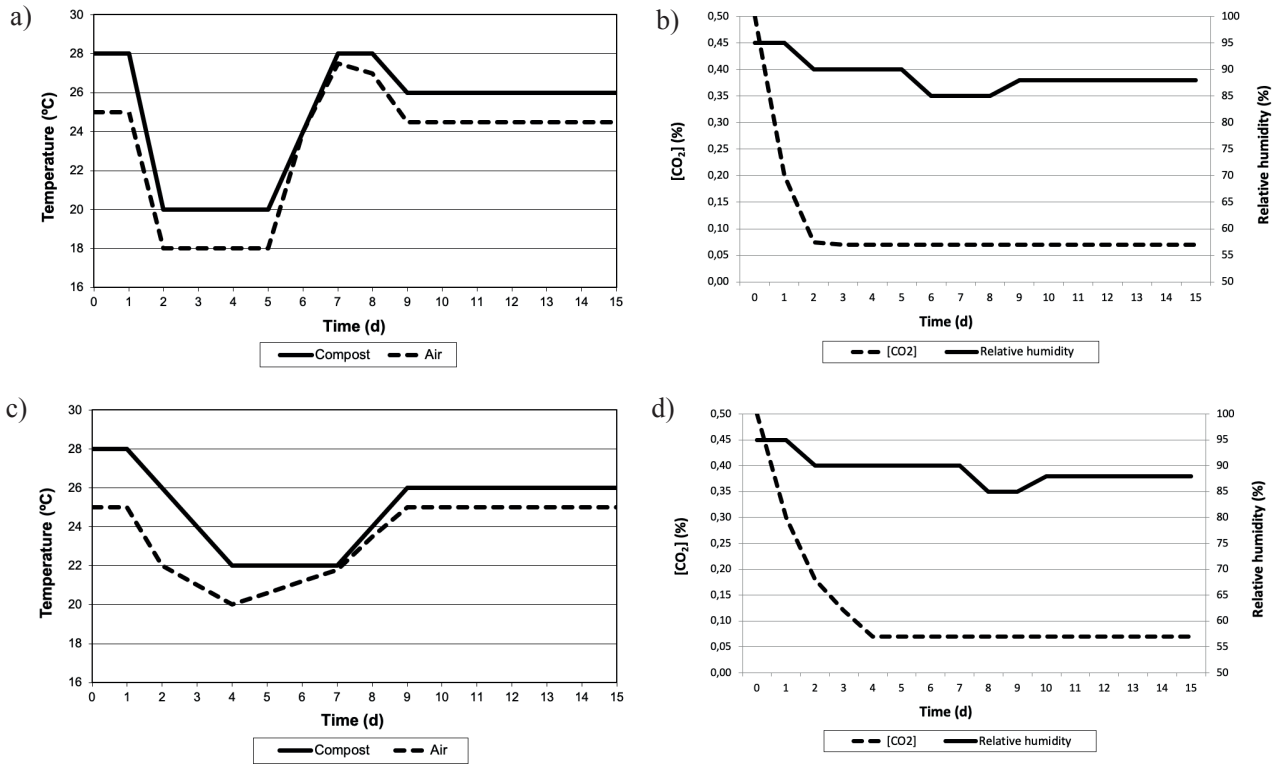

Figure 1. Environmental growing conditions including temperature of compost and air, percentage of CO_2 and relative humidity (%) for: a, b) rapid induction; c, d) moderately slow induction (days after the ruffling point).

The main productive outputs (number of mushrooms, yield per unit of area, BE, weight per unit, and dry matter (DM) content) were evaluated according to Pardo-Giménez *et al.* (2016). To assess the productive outputs for comparative purposes, fruit bodies weight before stipe trimming and the total number of mushrooms picked from each tray (crop area of 1450 cm^2) were recorded daily.

The method used to determine the DM and moisture content of the mushrooms consisted of measuring the loss of weight after oven drying at 105ºC for at least 72 h (Lau, 1982). Mushroom protein content was calculated by multiplying the total N content, obtained by the Kjeldahl method (CEN, 2001; FOSS, 2003), by a conversion factor of 4.38 (Miles & Chang, 1997). To determine ash content, sporocarps were reduced to ash at 540°C, for at least 6 h, to constant weight (Lau, 1982; Sullivan, 1993). Crude fat (ether extract) was estimated gravimetrically by filter bag technique after petroleum ether extraction of the dried sample in an extraction system Ankom XT10 (ANKOM, 2009). To determine the content of crude fiber, the Weende technique adapted to the filter bag technique was applied, using an Ankom 220 fiber analyser (ANKOM, 2008). Total carbohydrate content was calculated by subtracting the sum of the crude protein, total fat, water and ash from the total weight of the mushrooms (Sullivan, 1993). Available carbohydrate content (N-free), fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism, was calculated by subtracting the crude fiber from the total carbohydrate content (Lau, 1982). The energy value of mushrooms was estimated from the relative content of protein (Nx4.38), fat and carbohydrates using specific modified Atwater factors 3.75, 8.37 and 4.20 kcal g^{-1} of each component, respectively (Lau, 1982).

Statistics

ANOVA was used to statistically analyze the data recorded and the Tukey-HSD test employed to establish significant differences between means (p=0.05). Simple regression analysis to explore the relationships between the values of the total yield of mushrooms per unit area, the biological efficiency and the filling weight of compost were performed. All calculations were carried out using Statgraphics software (Statistical Graphics Corp., Princeton, NJ, USA).

Results

Table 2 summarises the results of the main mushroom productive outputs recorded varying compost fill rate (in the range of 50-70 kg m^{-2}) and the crop management for the induction to fructification (rapid induction or moderately slow induction). The CM substrate subjected to moderate slow induction and with a compost fill rate of 70 kg m^{-2} showed the best performance in terms of the number of

Table 2. The results obtained for the main productive outputs assessed in the mushrooms while varying compost fill rate in compost (within the range of 50-70 kg m^{-2}) and the management for the induction to fructification (rapid induction or moderately slow induction).

Substrate	Mushrooms (No. m^{-2})	Yield (kg m^{-2})		Unitary weight (g)	Biological efficiency (kg dt^{-1} compost)					Production rate (kg dt^{-1} d^{-1})	DM (%)
		Gross	Net		1st flush	2nd flush	3rd flush	4th flush	Total		
VC-50-RI	446 c	6.40 c	5.08 c	14.94 b	0.55 b	12.15 ab	14.03	9.97	36.70	0.46	12.46
VC-55-RI	613 abc	9.05 abc	7.25 abc	15.42 b	5.90 ab	17.82 ab	11.52	11.90	47.17	0.59	12.23
VC-60-RI	675 abc	10.67 abc	8.47 abc	16.13 b	10.55 ab	16.45 ab	14.88	9.08	50.95	0.63	11.93
VC-65-RI	758ab	12.18 ab	9.76 abc	16.22 b	13.88 a	15.80 ab	13.54	10.50	53.72	0.67	11.75
VC-70-RI	842 a	12.96 ab	10.32 ab	15.38 b	10.62 ab	17.64 ab	15.42	9.40	53.08	0.68	12.03
CM-50-RI	504 bc	8.26 bc	6.53 bc	16.34 b	5.18 ab	19.33 ab	16.02	8.35	48.85	0.60	12.12
CM-55-RI	634 abc	9.86 abc	7.86 abc	15.60 b	8.06 ab	21.84 a	9.96	12.97	53.00	0.66	11.66
CM-60-RI	715 abc	10.70 abc	8.42 abc	15.00 b	9.40 ab	19.6 ab	12.63	11.05	52.73	0.65	12.03
CM-65-RI	634 abc	9.86 abc	7.79 abc	15.71 b	7.88 ab	16.47 ab	11.18	9.32	44.83	0.56	11.74
CM-70-RI	768 ab	12.23 ab	9.70 abc	16.10 b	7.33 ab	13.95 ab	15.87	14.45	51.63	0.64	12.16
VC-50-MI	508 bc	9.95 abc	8.10 abc	19.98 ab	10.00 ab	19.17 ab	9.93	17.97	57.07	0.66	12.04
VC-55-MI	550 abc	10.80 abc	8.74 abc	19.73 ab	13.52 a	16.62 ab	11.53	14.63	56.30	0.65	11.50
VC-60-MI	624 abc	11.00 abc	8.99 abc	17.32 ab	5.68 ab	12.33 ab	19.50	15.00	52.55	0.62	12.21
VC-65-MI	435 c	9.45 abc	7.80 abc	23.46 a	8.03 ab	14.55 ab	9.05	10.03	41.67	0.49	12.31
VC-70-MI	764 ab	13.50 ab	10.90 ab	17.52 ab	5.70 ab	12.85 ab	22.85	13.88	55.28	0.65	11.64
CM-50-MI	476 bc	8.29 abc	6.69 abc	17.58 ab	4.47 ab	8.85 b	17.627	18.08	49.03	0.57	11.85
CM-55-MI	526 bc	10.30 abc	8.35 abc	20.07 ab	5.50 ab	14.32 ab	14.70	20.87	55.37	0.64	12.30
CM-60-MI	554 abc	10.10 abc	8.14 abc	17.99 ab	6.95 ab	13.52 ab	16.58	12.73	49.78	0.58	12.01
CM-65-MI	771 ab	13.70 ab	10.96 ab	18.67 ab	10.08 ab	16.10 ab	25.50	10.67	62.33	0.73	11.98
CM-70-MI	838 a	14.64 a	11.86 a	17.19 ab	8.23 ab	19.03 ab	19.95	14.62	61.82	0.72	11.93
Mean	632	10.69	8.58	17.32	7.88	15.92	15.11	12.77	51.69	0.62	11.99

VC: compost Villacasa; CM: compost Manchego; 50-70 kg m^{-2}: filling weight; RI: rapid induction; MI: moderately slow induction. DM: dry matter. Values followed by a different letter within a column are significantly different at 5% level according to Tukey's HSD test.

mushrooms harvested (838 mushrooms m^{-2}), gross yield (14.64 kg m^{-2}) and net yield (11.86 kg m^{-2}).

In the case of *A subrufescens,* the substrates of high N content (21.3 and 19.7 g kg^{-1}) and C/N ratio of 21.2 and 22.5 (Table 1) used during our trials, reflect that out of the 4 flushes harvested, the 2nd and 3rd have been the most productive, with a 4th flush more productive than the first one (Tables 2 & 3).

The flush distribution showed an irregular behaviour in terms of BE, resulting in poorly defined flushes, with non-significant differences of total BE between treatments, probably associated to the different activity of the evaluated blocks due to the distinctive filling rate applied since the strain used, 99/30, has showed flushes well distributed during cultivation at 27°C (Zied *et al.,* 2011). The best results in terms of BE were provided by the CM substrate with moderate slow induction and load densities of 65 kg m^{-2} (BE 62.33 kg dt^{-1}) and 70 kg m^{-2} (BE 61.82 kg dt^{-1}). Comparing both commercial substrates assayed, as compiled in Table 1, the content in crude fiber of compost CM was 12.6% higher than VC compost, with larger fraction of cellulose (12.3%) and hemicellulose (20.5%), and a lower fraction of lignin (20.9%) in respect to VC compost (10.7%, 19% and 22.7% respectively). The production rate has exhibited no significant differences between treatments, with values between 0.46 and 0.72 kg dt^{-1} d^{-1}) nor in the DM content measured in the basidiomes with values between 11.50 and 12.46%.

Regarding the different factors considered (Table 3), no significant differences were observed between the two commercial substrates used for none of the production parameters quantified. The fact that non-significant differences among the different compost kind used have been noted, together with the good agronomic behaviour in both of them (mean BE 51.69 kg dt^{-1}), confirms that the use of the formulation of the compost studied is suitable for the production of *A. subrufescens* under the growing conditions employed.

On the other hand, a significant increase in the number of mushrooms harvested and the yield per unit area (gross and net) has been observed as the compost filling weight increases, although the size (unit weight) and DM content of the sporophores have not been significantly influenced, nor the BE or the production rate. Values of 13.33 kg m^{-2} of gross yield and BE=55.45 kg dt^{-1} have been recorded for the compost fill rate of 70 kg m^{-2}.

Within the range of 50-70 kg m^{-2}, a moderately strong positive correlation (slope=0.230; r=0.512) was noticed

Table 3. The results obtained for the main productive outputs assessed in mushrooms for the different factors considered in the experimental design: compost formulation employed, Villacasa (CV) and Manchego (CM); compost fill rate of compost; conditions for the induction to fructification (rapid or moderately slow).

	Mushrooms (No. m^{-2})	Yield (kg m^{-2})		Unitary weight (g)	Biological efficiency (kg dt^{-1} compost)					Production rate (kg dt^{-1} d^{-1})	DM (%)
		Gross	Net		1st flush	2nd flush	3rd flush	4th flush	Total		
Compost VC	621	10.59	8.54	17.61	8.44	15.54	14.23	12.24	50.45	0.61	12.01
Compost CM	642	10.79	8.63	17.03	7.31	16.30	16.00	13.31	52.94	0.63	11.98
Filling wt 50 kg m^{-2}	484 c	8.22 c	6.60 c	17.21	5.05 b	14.88	14.40	13.59	47.91	0.57	12.12
Filling wt 55 kg m^{-2}	581 bc	10.00 bc	8.05 bc	17.70	8.24 ab	17.65	11.93	15.09	52.96	0.64	11.92
Filling wt 60 kg m^{-2}	642 b	10.62 b	8.50 b	16.61	8.15 ab	15.48	15.90	11.97	51.50	0.62	12.05
Filling wt 65 kg m^{-2}	650 b	11.30 ab	9.08 ab	18.52	9.97 a	15.73	14.82	10.13	50.64	0.61	11.94
Filling wt 70 kg m^{-2}	803 a	13.33 a	10.69 a	16.55	7.97 ab	15.87	18.52	13.09	55.45	0.67	11.94
Rapid induction	659	10.22	8.12 b	15.68 b	7.94	17.11 a	13.51 b	10.70 b	49.27	0.61	12.01
Moderate slow ind.	605	11.17	9.05 a	18.95 a	7.82	14.73 b	16.72 a	14.85 a	54.12	0.63	11.97
Mean	632	10.69	8.58	17.32	7.88	15.92	15.11	12.77	51.69	0.62	11.99

DM: dry matter. For each factor within a column, values followed by a different letter are significantly different at 5% level according to Tukey's HSD test.

between the total yield of mushrooms per unit area (kg m^{-2}) and the filling weight of compost (Fig. 2a), with statistically significant relation at a 95.0% confidence level ($p<0.05$). Therefore, the mushroom yield obtained is correlated with the compost filling weight applied, increasing gradually from 50 to 70 kg m^{-2}.

However, the correlation is very weak (slope=0.255; r=0.135) in the case of BE (Fig. 2b), without statistically significant relation at a confidence level of 95.0% or higher ($p=0.1412$). Therefore, there is not a statistically significant correlation between the BE of the compost (kg dt^{-1} compost) and the filling weight applied.

The proximate analysis of the harvested sporophores (Table 4) did not show any significant differences between treatments or factors. The harvested basidiomes contained: protein, 282.7 g kg^{-1}; crude fat, 13.1 g kg^{-1}; total carbohydrates, 634.8 g kg^{-1}; available carbohydrates, 576.2 g kg^{-1}; crude fiber, 58.6 g kg^{-1}; ash, 69.4 g kg^{-1}; energy value, 359 kcal per 100 g (mean values, dry wt. basis).

The protein contents quantified were between 257.4 and 314.6 g kg^{-1}, with a mineral content ranged between 65.1 and 73.9 g kg^{-1} and low energy value, with average of 359 kcal per 100 g of DM. The analysis shows that while employing different commercial substrates designed for button mushroom cultivation, with four different filling weights, or inducing rapid or moderately slow fructification, the quality and nutritional value of the harvested sporophores did not show significant differences.

Discussion

The performance of sun mushroom crops when cultivated using different compost fill rate with high N content compost and two different approaches for the induction to fructification has been evaluated. The agronomical characteristics investigated facilitate the adaptation of an optimised button mushroom process to the medicinal *A. subrufescens* cropping system.

The yield and biological efficiency obtained were generally higher than those previously reported. Zied *et al.* (2011) tested different strains of *A. subrufescens* on composts manufactured from wheat straw and chicken manure; the strain tested here ABL 99/30, showed potential for commercial production in temperate countries. Kopytowski-Filho & Minhoni (2007) evaluated the behaviour of the strain ABL 99/30 over three different compost formulations, recording BE values between 21.1 and 34.9 kg dt^{-1}. It was also the most productive cultivar (171 g kg^{-1}) among the 12 strains assayed by Llarena-Hernández *et al.* (2011) in wheat straw+chicken manure compost formulation and in substrates based on sugar cane bagasse and different types of straw with yields (expressed as mushroom fresh weight divided by the compost fresh weight multiplied by 100 and expressed as a percentage) between 16.29 and 20.17% (Zied *et al.,* 2012a) and between 15.34 and 20.37 % (Zied *et al.,* 2014). Productivity values of 96.3 g kg^{-1} (Llarena-Hernández *et al.,* 2013) and 75.7 g kg^{-1} (Llarena Hernández *et al.,* 2014) have been also reported with strain CA-561. Evaluating different casing materials, using this same substrate (formulated from wheat straw and chicken manure), produced yields between 12.17 and 17.07%, with biological efficiencies between 34.49 and 48.37 kg dt^{-1} were obtained (Zied *et al.,* 2012b). According to Zied *et al.* (2014) the number of harvested sun mushrooms is negatively correlated with the water-holding capacity and positively correlated with the total density and porosity of the casing layer. Pardo-Giménez *et al.* (2014) analysed the effect of different casing layers over several strains of *A. subrufescens*,

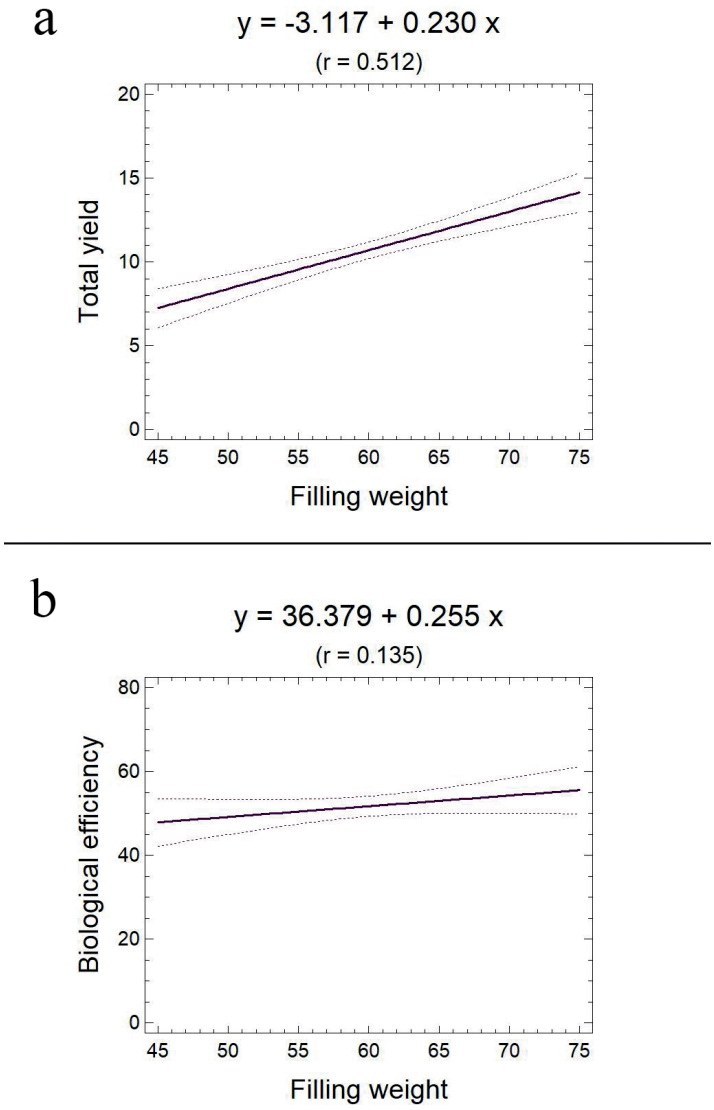

Figure 2. Relationship between the production values and the filling weight of compost (kg m^{-2}): a) production values expressed as total yield of mushrooms (kg m^{-2}); b) production values expressed as biological efficiency (kg dt^{-1}) (mean values plotted, the reproducibility is expressed in arrows by confidence limits of the results for a confidence level of 95 %).

using compost based on wheat straw and chicken manure; a highest BE of 27.57 kg dt^{-1} was achieved for the strain ABL99/30 with a peat-based casing, that was higher than when mineral casing soil was used.

Since the early cultivation of sun mushroom in Brazil, the same compost formulation designed for the growth of *A. bisporus* has been used to grow *A. subrufescens* (Mantovani *et al.,* 2007). Dias *et al.* (2014), with a compost formulation for *A. bisporus*, based on wheat straw and chicken manure, reported BE values between 10.81 and 37.51 kg dt^{-1}. In Europe, a number of researchers have also conducted trials using the same compost initially developed for the production of *A. bisporus* and obtained high yields (Llarena-Hernández *et al.,* 2013, 2014; Zied *et al.,* 2017).

Our yield results are consistent with those of Pardo-Giménez *et al.* (2014), who obtained higher absolute values per unit area with filling densities of 70 kg m^{-2} (yield= 10.61-11.23 kg m^{-2}; BE= 48.74-51.71 kg dt^{-1} compost) than with 60 kg m^{-2} (yield= 9.06-10.91 kg m^{-2}; BE= 46.10-58.52 kg dt^{-1}compost) although the BE was lower, without significant differences in either case. Conversely, previous work by Eira *et al.* (2005) reported that a substrate density of 20 kg m^{-2} provided higher productivity than with 40 and 60 kg m^{-2}, although the authors specified that these results were obtained under low technological growing rooms or high incidence of pests and diseases.

According to Eira (2003), the compost fill rate of compost per surface unit of sun mushroom must be lower in an uncontrolled environment (20-30 kg m^{-2}) while higher

Table 4. Proximate analysis of mushrooms harvested from the different substrates employed (Villacasa (CV) or Manchego (CM)) with the agronomical traits under study, compost fill rate of compost (kg m^{-2}) and conditions for the induction to fructification (rapid or moderately slow).

Substrate	Water (g kg^{-1})	Crude protein (N×4.38, g kg^{-1} DM)	Crude fat (g kg^{-1} DM)	Total carbohydrates (g kg^{-1} DM)	N-free extract (g kg^{-1} DM)	Crude fiber (g kg^{-1} DM)	Ash (g kg^{-1} DM)	Energy value (kcal/100g DM)
VC-50-RI	875.5	313.4	15.9	596.8	543.9	53.0	73.9	359
VC-55-RI	877.8	288.3	10.8	628.9	571.4	57.6	72.0	357
VC-60-RI	880.7	283.1	11.2	637.4	581.9	55.6	68.3	360
VC-65-RI	882.5	257.4	12.0	665.5	604.0	61.5	65.1	360
VC-70-RI	879.7	278.6	12.4	640.6	581.4	59.3	68.4	359
CM-50-RI	878.9	285.9	10.1	633.5	575.7	57.8	70.6	357
CM-55-RI	883.4	274.5	12.6	642.1	582.0	60.1	70.8	358
CM-60-RI	879.7	276.9	12.6	642.5	585.5	57.0	68.0	360
CM-65-RI	882.6	276.4	12.6	641.0	578.6	62.3	70.1	357
CM-70-RI	878.5	264.7	15.7	651.1	592.9	58.2	68.7	361
VC-50-MI	879.6	274.4	14.0	641.0	582.0	59.1	70.6	359
VC-55-MI	885.1	269.6	14.8	646.5	586.2	60.4	69.1	359
VC-60-MI	877.9	314.6	13.9	602.0	545.8	56.2	69.4	359
VC-65-MI	876.9	274.4	11.7	647.6	592.5	55.0	66.3	361
VC-70-MI	883.7	298.0	12.8	615.6	551.6	64.0	73.7	354
CM-50-MI	881.5	293.8	12.8	624.3	563.3	61.1	69.0	357
CM-55-MI	877.0	296.8	12.6	622.9	568.5	54.5	67.7	360
CM-60-MI	879.9	282.2	13.2	636.4	578.9	57.5	68.2	360
CM-65-MI	880.3	265.6	13.8	651.7	588.5	63.3	68.9	358
CM-70-MI	880.8	285.0	16.3	628.5	569.8	58.8	70.2	359
Mean	880.1	282.7	13.1	634.8	576.2	58.6	69.4	359

DM: dry matter; VC: compost Villacasa; CM: compost Manchego; 50 to 70: filling weight; RI: rapid induction; MI: moderately slow induction.

under controlled environmental conditions (60 kg m^{-2}), since the control of compost activity and temperature with high loading densities is more difficult, particularly with low-tech growing facilities. Uncontrolled compost activity can be optimised employing bed cooling systems, that directly cools the compost in each mushroom bed within high-tech facilities (Buth, 2017).

However, it should be considered that compost filling rates that are too low result in loss of activity during the colonisation of the compost and the first flush, thus lengthen the incubation process and hindering the development of the second and subsequent flushes. Relatively low compost fill rates and the concurrent loss of compost activity also limit the possibility of applying irrigation under optimum conditions, resulting in compost that is too dry, with second and subsequent flushes that lack sufficient water to produce weighty mushrooms (Van Gerwen, 2019).

Regarding the conditions provided for the induction of fruiting, various temperatures have been reported: 15ºC for 1-3 days (Eira, 2003), 17-19ºC for 3-5 days (Eira *et al.,* 2005), 20-22°C for 3 days (Minhoni *et al.,* 2005), below 25°C (Mendonça *et al.,* 2005), 17°C (Gregori *et al.,* 2008), 20°C (Kopytowski Filho *et al.,* 2008; Colauto *et al.,* 2010), 19°C for 4 days (Dias *et al.,* 2014), 16°C for 4-8 days (Martos *et al.,* 2017) and 18.5-21.5°C (Win & Ohga, 2018). In our case, the moderate slow induction (slow temperature decrease, compost temperature of 22°C for 3 days) has shown better performance than rapid induction (rapid temperature decrease, compost temperature of 20°C for 3 days), with better records for agronomic performance (significantly better in the case of net yield) and unit weight.

Unlike *A. bisporus*, *A subrufescens* is typically grown in substrates with relatively low N contents (1.15-1.45 %) and C/N ratios of ca 25-27 at the end of phase II (Zied *et al.,* 2017), without noting significant production losses in subsequent flushes. To overcome nutritional deficiencies, compost supplementation can be an important tool to improve the commercial yield of sun mushroom (Zied *et al.,* 2018). The *A. bisporus* mushroom crop cycle is usually limited to three flushes, with load densities of 80 kg m^{-2} reaching productions around 30 kg m^{-2}, of which approximately 25 kg m^{-2}, more than 80% of total production is achieved in the first two flushes (Carrasco *et al.,* 2016). Comparing rapid and slow induction, the BE registered for the most productive late flushes, 3rd and 4th, were significantly higher applying slow induction. *A. subrufescens* produced laccase and cellulase at measurable levels, but no manganese peroxidase nor

lignin peroxidase (Sousa *et al.,* 2016), therefore the better BE observed in compost CM could be related to the compost formulation with higher cellulose and hemicellulose fraction and lower lignin, comparing to VC compost, and the cellulolytic/ ligninolytic profile of the fungus.

In general, the gross composition of mushrooms is water (90%), protein (2-40%), fat (2-8%), carbohydrates (1-55%), fiber (3-32%) and ash (8-10%) (Firenzouli *et al.,* 2008). With regard to the composition of other edible species of fungi, *A. subrufescens* presents, in general, low water content, crude fat, crude fiber and ash, while high protein content, total carbohydrate and mean values of available carbohydrates and energy value (Chang & Miles, 2004). The protein contents quantified in the mushroom harvested here are similar to those reported by Siqueira *et al.* (2011), between 283.3 and 293.5 g kg^{-1}, and Zied *et al.* (2011), between 262 and 349 g kg^{-1}. According to data collected by Eira (2003) and Chang & Miles (2004) sun mushroom is one of the species with higher protein content among all the cultivated edible mushrooms. Fat content reported in this paper (10.1-16.3 g kg^{-1}) is, in general, of the same magnitude as those reported in the literature (Chang & Miles, 2004). Pardo-Giménez *et al.* (2013) recorded mean values of 15.9 g kg^{-1} fat content in sporocarps of *A. subrufescens* cultivated in Spain.

The harvested sporophores had an average of 58.6 g kg^{-1} of crude fiber content comparable to the fiber content reported by Eira (2003) (55.6-118.1 g kg^{-1}) and extensive to other cultivated species such as *A. bisporus* (104 g kg^{-1}), *Lentinula edodes* (80 g kg^{-1}) and *Pleurotus florida* (115 g kg^{-1}), although lower than those of *Volvariella diplasia* (174 g kg^{-1}) and *Pleurotus ostreatus* (156 g kg^{-1}). The mineral content ranged between 65.1 and 73.9 g kg^{-1}, with an average of 69.4 g kg^{-1}, which is consistent to the reported by Pardo-Giménez *et al.* (2013) (64.0-78.7 g kg^{-1}). These values are, in general, lower than those found for other cultivated mushrooms (Eira, 2003; Chang & Miles, 2004). Finally, the energy value, with an average of 359 kcal per 100g of DM is low, though similar to those values published, between 344 and 362 g kg^{-1} (Pardo-Giménez *et al.,* 2013).

According to Zied *et al.* (2014) the variation in almond mushroom yield is affected mostly by the cultivation environment, followed by the strain selected, casing layer, and compost type. Here we can conclude that commercial substrates initially developed for the production of *A. bisporus*, with high N content and relatively low C/N ratios, are suitable for the commercial production of the strain ABL 99/30 (CA-561) of *A. subrufescens*. The number of sporophores harvested and the yield per unit area moderately increase while increasing the density of compost fill rate. However, the BE was not significantly modified, which means that compost can be equally productive independently of the weight per unit area introduced but it is encouraging to introduce higher density to increase overall yield due to the long cropping period of this edible mushroom. As guidance for growers, load densities between 65 and 70 kg per m^2 of cultivated area can be recommended, meanwhile the relative cost of the substrate with respect to the global production cost may determine the choice of compost weight applied per unit area depending on the cultivation system. The proposed moderate slow induction, demanding lower temperature range and reducing the energy requirements, is presented as the best option for commercial production under controlled environmental conditions. This agronomic crop management provides better yields, particularly in the last flushes, and larger sporocarps which is a requirement from the industry. The flush distribution of highly productive at the end of the cropping cycle, together with a longer inter-flush period, comparing to other crops such as *A. bisporus*, justifies the need to set up relatively long cycles with respect to white button mushroom, in which the first flush is the most productive. However, proximate analysis of harvested sporocarps has not shown significant differences for the different treatments and factors considered in the experimental design.

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