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Management of Water Supply in the Cultivation of Different *Agaricus bisporus* Strains

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Abstract: The objective of this work was to evaluate the water supply method in the cultivation of button mushroom. The strains used were ABI 18/02, ABI 18/04, ABI 19/03, and ABI 11/19. An analysis of the ITS 1 + 2 regions, widely used as a fungal barcode, was performed in order to assess the genetics. The compost was packed in 35 × 50 cm plastic boxes with 10.5 kg m^{-2}, and the inoculum was added (1% in relation to the compost weight). Before the addition of the casing layer, in half of the boxes, 25 L m^{-2} of water was added directly to the colonized compost, resulting in the treatment of the water added to the compost. The yield, number, and weight of the mushrooms were analyzed. A phylogenetic tree for the *A. bisporus* strains based on ITS sequences confirms a close genetic relationship among the different collections of this species, and additional molecular markers are required to distinguish genotypes related to superior agronomic traits. The water management methods presented similar yields, except for one strain (ABI 18/04). The ABI 18/02 and 11/019 strains were more efficient when considering the method of adding water to the compost. This study suggests that the treatment of water added to the substrate is an alternative tool for reducing the application time and labor involved and as a control treatment to reduce water use.

Keywords: irrigation; ITS 1+2 regions; phylogenetic tree; yield

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1. Introduction

Water stands as a fundamental element crucial for sustaining life and ecosystems, as well as driving social and economic progress [1]. Consequently, the agricultural sector comes under intense scrutiny regarding its water consumption, necessitating decisions aimed at maintaining or even enhancing productivity while reducing water usage, all while taking into account food security [2,3]. The issue of water scarcity predominantly impacts regions in the mid to low latitudes of the Northern Hemisphere and African nations, primarily due to inadequate infrastructure resources [4,5]. The United Nations (UN) has identified drinking water and sanitation as a key component of the Sustainable Development Goals, including objectives to enhance water efficiency across all sectors and to ensure universal access to clean water by 2030 [6]. Moreover, the State of Food and Agriculture Report (SOFA) in 2020 revealed a disconcerting statistic: freshwater resources per capita have diminished by over 20% within two decades [7]. This underscores two critical challenges: the burgeoning global population and the diminishing water resources.

Similar to other crops, mushroom production relies on water resources throughout various stages, starting from compost production and continuing through the mushroom

harvesting phase [8]. Because mushrooms originate from fungal hyphae, water is indispensable for microbial metabolism and cellular development, as roughly 90% of a mushroom's structure comprises water, with most of it originating from the compost and casing layer [9].

Among the stages of mushroom production, the cultivation phase, specifically phase III, which encompasses mycelium growth, casing, and harvesting, is the most time-intensive [8]. These steps significantly influence water provision and can be executed within the compost [10] or casing layer [11]. When applied to the compost, its primary function is to rectify the moisture lost during the pasteurization, conditioning, and mycelium growth phases, particularly when carried out in pasteurization tunnels, often referred to as "phases II and III together" [12].

Alternatively, when applied to the casing layer, which is the more common practice, its primary role is to sustain a humid microclimate [13]. Hence, it is recommended to use a material with high water retention capabilities [14], with peat being the current choice [15]. Ideally, during cultivation, the peat should be maintained at 80% to 90% saturation [16]. However, it is worth noting that peat's evaporation capacity significantly influences mushroom fruiting [17], necessitating regular irrigation throughout cultivation.

Another crucial aspect is selecting the mushroom strain, with genetic and agronomic characterization being of the utmost importance in relation to the fungus's water requirements for fruiting. Recent studies have demonstrated that mushroom morphology and yield can vary by up to 30% depending on the strain used [18–20]. These parameters are critical for the commercialization of the final product, especially because they impact the size, weight, and classification of the mushrooms [21,22]. Different strains may also exhibit varying degrees of disease resistance [23] and produce varying amounts of enzymes [24]. However, to date, no study has compared water demand and efficiency across different genetic materials.

Given the growing concern regarding water usage in *A. bisporus* cultivation, it is crucial to establish appropriate management practices to minimize consumption while enhancing environmental and social efficiency. Consequently, this study aimed to assess the water supply methods in button mushroom cultivation, employing various strains.

2. Materials and Methods

The experiment was carried out at the facilities of the Centro de Estudos em Cogumelos (CECOG) at the Faculdade de Ciências Agrárias e Tecnológicas (UNESP, Dracena Campus, Dracena, São Paulo, Brazil). The strains used in the experiment were ABI 18/02, ABI 18/04, ABI 19/03, and ABI 11/19, which are deposited in the public collection of CECOG.

2.1. Genetic Characterization of the A. bisporus Strains

In order to assess the genetic differences among the ABI 19/03, ABI 18/04, ABI 18/02, and ABI 11/19 *A. bisporus* strains, an analysis of the ITS 1+2 (internal transcribed spacers 1 and 2) regions, situated between the small subunit ribosomal RNA (rRNA) and large subunit rRNA genes, was performed. The analysis of this DNA fragment is adopted as a primary fungal barcode for the rapid identification of fungal specimens in biodiversity and ecological studies [25,26]. Approximately 100 mg of the pure culture from different *A. bisporus* strains was used for the DNA isolation with the DNeasy Plant Mini Kit (Qiagen), following the manufacturer's protocol. The amplification of ITS 1+2 was carried out in PCR reactions using 50 ng of DNA, the universal ITS 1+2 primers ITS5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) [27], and the Taq DNA Polymerase master mix (Cellco). The aliquots of PCR reactions were analyzed with 1.5% agarose gel electrophoresis for 35 min at 130 V to confirm the presence of amplicons. The amplicons were purified using the QIAquick PCR Purification kit (Qiagen) and cloned into pGEM-T Easy vectors (Promega) in a 5:1 insert:vector ratio. Chemocompetent *Escherichia coli* Stellar cells were transformed with the ligation reactions. Bacterial cultures were plated in selective LB media containing ampicillin and incubated at 37 °C for 16 h.

Positive clones were confirmed by the digestion of the plasmid DNA with the endonuclease EcoRI (New England Biolabs). Four clones from each *A. bisporus* strain were sequenced using the universal primers pUC/M13F (5′-CGCCAGGGTTTTCCCAGTCACGAC-3′) and pUC/M13R (5′-CAGGAAACAGCTATGAC-3′) using the Sanger method with an ABI 3730 DNA Analyzer (Applied Biosystems) at the Centro de Pesquisa sobre o Genoma Humano e Células-Tronco da Universidade de São Paulo (CEGH-USP, São Paulo, Brazil).

The consensus sequences produced for each *A. bisporus* strain were deposited in the NCBI nucleotide database under the accessions: OQ414664 (*A. bisporus* ABI 19/03), OQ414665 (*A. bisporus* ABI 18/04), OQ414666 (*A. bisporus* ABI 18/02), and OQ414667 (*A. bisporus* ABI 11/19). Phylogenetic and molecular evolutionary analyses were conducted using the software MEGA11: Molecular Evolutionary Genetics Analysis version 11 [28]. A multiple alignment using the MUSCLE tool was performed for ITS sequences from the four *A. bisporus* strains investigated in this work and the other 137 sequences available in the NCBI nucleotide database for this species. A bootstrap consensus tree was inferred from 100 replicates using the maximum likelihood method and Tamura's 3-parameter model [29]. A discrete Gamma distribution was used to model the evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.2221)).

2.2. Spawn and Compost Production

The methodology proposed by Zied et al. [30] was used to produce spawn. The inoculum was produced from sorghum grains (*Sorghum bicolor*), boiled for approximately 40 min in water. After this step, excess water was drained and 2% calcium carbonate was added to the grains, based on their wet weight, to provide calcium and raise the pH [31]. After homogenization, the grains were placed in autoclavable plastic bags and sterilized for a period of 4 h at 121 °C. After cooling, the bags were inoculated under aseptic conditions in a laminar flow chamber and then incubated at 25 °C for 15 days.

The compost was obtained from Compobras® company (Castro, Paraná State, Brazil) and was composed of wheat straw, chicken manure, gypsum, and limestone. During the composting phase I, the materials were moistened for 5 days, then it was kept in a bunker for 5 days, then transferred to a second bunker where it remained for another 4 days, and finally, to a third bunker for another 3 days. In phase II, the substrate was pasteurized for 18 h at a temperature of 59 ± 1 °C and conditioning was carried out for 5 days at a temperature of 47 ± 2 °C. The C/N ratio of the compost at the end of phase II was 19/1, the moisture was 65%, the pH was 7.2, and the organic matter content was 256 g kg^{-1}.

2.3. Experimental Design

The experimental design used was a 4 × 2 equilibrated factorial plan with four replicates (randomized blocks with two factorial factors). Factor 1, with four levels, corresponded to the selected strains. Factor 2, with two levels, corresponded to the irrigation method (with and without irrigation of phase III compost).

2.4. Cultivation Crop

The compost was packed in 40 × 30 cm plastic boxes (0.12 m^2), with 2.5 kg of substrate each (10.5 kg m^{-2}), and the inoculum was added at a rate of 1% in relation to the compost weight, considering an experimental pilot model. The boxes were taken to the production chamber at a temperature of 25 °C and relative humidity above 85%. After the colonization of the compost, which took place in 15 days, and before the addition of the casing layer, in half of the boxes, 25 L of water per m^2 was added directly to the colonized compost, resulting in the treatment of the water added to the compost.

The casing layer, based on peat from the company MaisTerra® (Castro, Paraná State, Brazil), was added to the boxes at a height of 3 cm. When the mycelium also colonized the casing, the induction of the primordia was started, gradually reducing the temperature to 19 °C. Irrigation in the period of the casing colonization and the harvest phase was carried out when a reduction in the humidity of the casing was observed. For this procedure, a

0.5-inch PVC pipe was used, sealed with a cap and with four nebulizer micro sprinkler nozzles attached to a hose (Figure 1). The flow of the nozzles was measured every day before the irrigation activity of the boxes, totaling an average of 3.5 L/min.

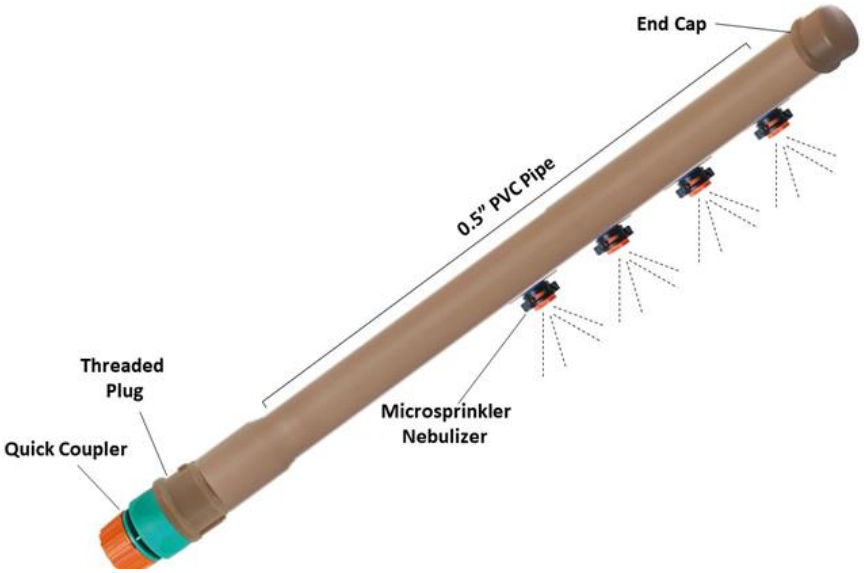

Figure 1. Tool used to irrigate the casing layer using micro sprinkling.

After 18 days of casing, harvesting started and lasted for 20 days. The mushrooms were harvested at their optimal commercial development stage, corresponding to morphogenetic stage 3 (closed cup) [14].

A methodology adapted from Navarro et al. [11] was used to determine the moisture content of the compost and casing layer. Briefly, 75 g samples of each treatment were collected every 7 days of cultivation and dried in an oven with air circulation at 105 °C until they reached a constant weight.

2.5. Agronomic Traits

The mushrooms picked were counted and weighed. With these data, the yield, number, and weight of the mushrooms were analyzed [32]. The yield was determined by dividing the weight of the harvested mushrooms by the fresh weight of the substrate and multiplying the result by 100 to obtain a percentage. To calculate the biological efficiency (BE), the weight of the harvested mushrooms was divided by the dry weight of the compost, and the result was multiplied by 100 to obtain a percentage. The precocity was determined by dividing the harvest time into two periods (yield in the first period/total yield harvested), and the result was multiplied by 100 to obtain a percentage. The number of mushrooms harvested per box was determined by counting throughout the entire crop cycle. The average weight was calculated by dividing the total weight of the harvested mushrooms by the number of mushrooms.

To calculate the amount of water used in irrigation, the water flow of the equipment was measured with a test tube for two cycles of 1 min. The time spent irrigating the crop was also valuated.

2.6. Statistical Analysis

The data were analyzed using an ANOVA test and the means were compared using the Scott–Knott test at a 5% probability with the Sisvar 5.6 program (Lavras, Minas Gerais State, Brazil).

3. Results

3.1. ITS Sequencing of A. bisporus Strains

A high level of identity (99.2%) was observed among the consensus sequences obtained from the ITS sequencing for *A. bisporus* strains ABI 18/04, ABI 19/03, ABI 18/02, and ABI 11/19. Indeed, only 6 in 763 nucleotide positions were variable among the different strains (Table 1).

Table 1. Variable positions in the ITS sequences for *A. bisporus* strains ABI 18/04, ABI 19/03, ABI 18/02, and ABI 11/19. The numbers in the rows indicate the position in the multiple alignment of the ITS sequences. The letters represent nucleotides, with Y = C or T and R = A or G.

Strain	93	252	314	563	604	735
ABI 18/04	C	R	A	C	C	Y
ABI 19/03	Y	A	A	T	T	T
ABI 18/02	C	A	A	C	C	T
ABI 11/19	Y	A	R	T	T	T

A phylogenetic tree inferred from the ITS sequences for the *A. bisporus* strains also confirms a close genetic relationship among the different collections of this species (Figure 2). Similarly, previous results for the ITS-2 and 28S ribosomal DNA sequences for the 16 samples of *Agaricus* suggest that species belonging to this genus are closely related [33]. Therefore, further studies are required to investigate other genomic regions related to superior agronomic traits.

Some molecular markers and quantitative trait loci (QTL) for important agronomic traits were characterized for a substantial collection of *A. bisporus* strains over the last decade [34–37]. Most of them are related with disease resistance and yield, including bruising (discoloration after damaging the cap skin), earliness (first day picking), firmness, cap color, compost colonization, and scaling of mushroom caps [34,35]. In addition to these data, a recent report evaluated the genetic diversity of 170 accessions of *A. bisporus* by analyzing 111 single-nucleotide polymorphism (SNP) markers [37] based on the previously published KASP (Kompetitive Allele-Specific PCR) markers [36]. Together, these results represent valuable genetic resources that are potentially useful for breeding, enabling the development of strains with superior agronomic traits. The contribution of this work is to select strains with a lower water demand and high productivity for future studies, which will be essential for food generation and social distribution, especially in regions with scarce natural resources.

3.2. Agronomic Traits

The first harvest (earliness) occurred after 18 days, after the addition of the casing layer, for the treatment with added water for the strains ABI18/02 and ABI11/19, and in the control treatment for the strain ABI19/03. For the remaining treatments, the first harvest occurred after 19 days.

The harvest period lasted 20 days totaling three flushes and 38 days of the harvest phase. The first flush presented the highest yields considering all strains (Table 2). In the analysis of all of the different strains, it became apparent that the first flush yielded the highest results. These values can be found in Table 2, indicating an average of 14.74% for yields and 42.12% for biological efficiency (BE). Nevertheless, the precocity remained unaffected by variations in the strains or the methods employed for water addition, as demonstrated in Table S1.

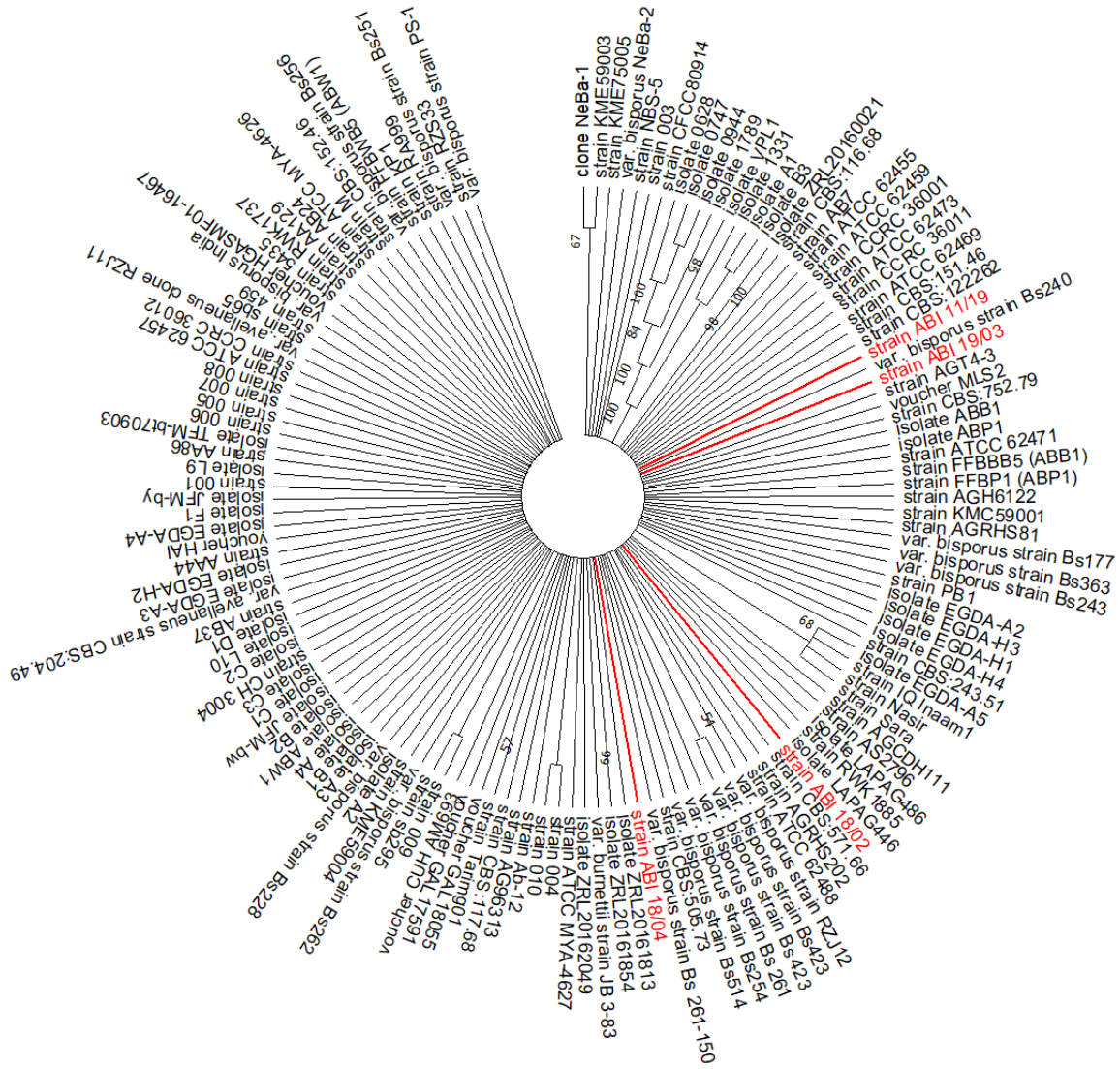

Figure 2. A bootstrap consensus tree (100 replicates) inferred from the ITS sequences for 141 *A. bisporus* strains. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. *A. bisporus* strains characterized in this work are highlighted in red.

The ABI 19/03 strain was the only one to present a statistical difference in the yield and BE, both in relation to the water supply method and the strains used, highlighting the water applied to the colonized compost. Concerning the strain, this obtained the lowest yield. Regarding the number of fruitbodies per box and the unitary weight of the mushrooms harvested in the first flush, larger mushrooms were observed for the ABI 18/04 (added water to the colonized compost) and ABI 19/03 (control) strains, however, with smaller amounts.

In the second flush, the addition of water resulted in yields ranging from 5.34% to 7.85%, along with biological efficiency (BE) values ranging from 15.25% to 22.43%. These results were lower compared to the control treatment, which achieved yields of over 11% and higher BE values of over 34% across all strains (Table 3). This suggests a recovery from the first flush. The strains did not obtain a significant effect on either yield or BE. Larger amounts of mushrooms were harvested in the control treatment, only the ABI 19/03 strain did not show a statistically significant difference in relation to the water application

method. However, this strain presented mushrooms with a high weight, mainly with micro sprinkler irrigation.

Table 2. Yield (%), biological efficiency (%), number (un), and weight of mushrooms (g) in the first flush.

1st Flush				
Irrigation/Strain	**ABI 18/02**	**ABI 18/04**	**ABI 19/03**	**ABI 11/19**
Yield (%)				
Control	17.15 ^{a}	12.15 ^{a}	7.50 ^{Bb}	14.62 ^{a}
Added water	19.9 ^{a}	11.55 ^{b}	15.09 ^{Ab}	20.00 ^{a}
	CV	25.14	Average	14.74
Biological Efficiency (%)				
Control	49.00 ^{a}	34.71 ^{a}	21.43 ^{Bb}	41.77 ^{a}
Added water	56.85 ^{a}	33.00 ^{b}	43.11 ^{Ab}	57.14 ^{a}
	CV	25.14	Average	42.12
Number of Mushrooms (un)				
Control	35.5 ^{a}	14.2 ^{b}	6.5 ^{Bb}	23.7 ^{Ba}
Added water	29.7 ^{a}	13.5 ^{b}	25.0 ^{Ab}	41.2 ^{Aa}
	CV	37.52	Average	23.6
Weight of Mushrooms (g)				
Control	13.07 ^{c}	21.38 ^{b}	29.45 ^{Aa}	16.11 ^{c}
Added water	16.77 ^{b}	23.29 ^{a}	14.68 ^{Bb}	12.73 ^{b}
	CV	22.01	Average	18.44

Different capital letters in the same column within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. Different lowercase letters in the same row within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. The absence of letters indicates that there was no statistical difference.

Table 3. Yield (%), biological efficiency (%), number (un), and weight of mushrooms (g) in the second flush.

2nd Flush				
Irrigation/Strain	**ABI 18/02**	**ABI 18/04**	**ABI 19/03**	**ABI 11/19**
Yield (%)				
Control	11.91 ^{A}	14.45 ^{A}	14.89 ^{A}	12.81 ^{A}
Added water	6.23 ^{B}	7.85 ^{B}	5.34 ^{B}	6.21 ^{B}
	CV	32.72	Average	9.96
Biological Efficiency (%)				
Control	34.03 ^{A}	41.28 ^{A}	42.54 ^{A}	36.60 ^{A}
Added water	17.79 ^{B}	22.43 ^{B}	15.25 ^{B}	17.74 ^{B}
	CV	32.72	Average	28.46
Number of Mushroom (un)				
Control	41.7 ^{A}	32.2 ^{A}	24.2	34.7 ^{A}
Added water	15.5 ^{B}	19.0 ^{B}	12.5	12.2 ^{B}
	CV	36.09	Average	24.0

Table 3. *Cont.*

2nd Flush				
Irrigation/Strain	**ABI 18/02**	**ABI 18/04**	**ABI 19/03**	**ABI 11/19**
Weight of Mushrooms (g)				
Control	7.29 ^{b}	11.42 ^{b}	15.18 ^{Aa}	9.49 ^{b}
Added water	10.64	10.22	7.95 ^{B}	12.64
	CV	22.21	Average	10.60

Different capital letters in the same column within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. Different lowercase letters in the same row within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. The absence of letters indicates that there was no statistical difference.

During the third flush, we observed the lowest yields and BE, which were consistent with the added water method, except for the ABI 19/03 strain. The ABI 19/03 strain achieved the highest yield and BE using the control method (Table 4). It is worth noting that the reduced yield of the ABI 18/02 strain can be attributed to its strong performance in the first two flushes, indicating precocity and infeasibility to conducting the third flush. The ABI 11/19 strain showed a high weight of mushrooms when water was added to the compost. Subjected to the same method, the ABI 18/04 and ABI 19/03 strains showed mushrooms with a low weight.

Table 4. Yield (%), biological efficiency (%), number (un), and weight of mushrooms (g) in the third flush.

3rd Flush				
Irrigation/Strain	**ABI 18/02**	**ABI 18/04**	**ABI 19/03**	**ABI 11/19**
Yield (%)				
Control	3.18 ^{b}	5.65 ^{b}	7.93 ^{Aa}	4.61 ^{b}
Added water	4.40	5.24	4.87 ^{B}	5.27
	CV	39.94	Average	5.14
Biological Efficiency (%)				
Control	9.07 ^{b}	16.14 ^{b}	22.65 ^{Aa}	13.17 ^{b}
Added water	12.57	14.97	13.91 ^{B}	15.05
	CV	39.94	Average	14.69
Number of Mushrooms (un)				
Control	6.0	13.2	16.2	10.7
Added water	6.7	11.2	11.5	7.7
	CV	44.83	Average	10.4
Weight of Mushrooms (g)				
Control	15.24	10.55	13.66	11.55 ^{B}
Added water	16.52 ^{a}	11.48 ^{b}	9.95 ^{b}	19.48 ^{Aa}
	CV	39.07	Average	13.55

Different capital letters in the same column within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. Different lowercase letters in the same row within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. The absence of letters indicates that there was no statistical difference.

Analyzing the total production data, a similar yield and BE can be observed for the two water management methods, with a significant difference only for the ABI 18/04 strain (Table 5). However, considering the strains in the method of adding water to the colonized compost, the ABI 18/02 and ABI 11/019 strains were more efficient.

Table 5. Total of number (un), biological efficiency (%), weight (g), and yield of mushrooms (%).

		Total		
Irrigation/Strain	**ABI 18/02**	**ABI 18/04**	**ABI 19/03**	**ABI 11/19**
		Yield (%)		
Control	32.24	32.25 ^{A}	30.32	32.04
Added water	30.53 ^{a}	24.64 ^{Bb}	25.30 ^{b}	31.48 ^{a}
	CV	13.67	Average	29.85
		Biological Efficiency (%)		
Control	92.11	92.14 ^{A}	86.62	91.54
Added water	87.22 ^{a}	70.40 ^{Bb}	72.28 ^{b}	89.94 ^{a}
	CV	13.67	Average	85.28
		Number of Mushrooms (un)		
Control	83.2 ^{Aa}	59.7 ^{Ac}	47.0 ^{c}	69.2 ^{b}
Added water	52.0 ^{B}	43.7 ^{B}	49.0	61.2
	CV	15.33	Average	58.1
		Weight of Mushrooms (g)		
Control	9.72 ^{Bc}	13.52 ^{b}	16.04 ^{Aa}	11.52 ^{c}
Added water	14.84 ^{A}	14.16	12.88 ^{B}	13.24
	CV	12.07	Average	13.25

Different capital letters in the same column within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. Different lowercase letters in the same row within the same variable indicate a significant difference according to the Scott–Knott test at a 5% probability. The absence of letters indicates that there was no statistical difference.

Figure 3 illustrates the quantity of water utilized in the cultivation of *A. bisporus* and the duration of irrigation per square meter. In the treatment involving the addition of water to the colonized compost, less water was applied overall, with the exception of the day before the casing layer was introduced, when 25 L m^{-2} was added. Future research could reduce the amount of water to adapt the methodology and construct a larger experiment, at a commercial level.

In this method, sprinkler irrigation became necessary only on the 14th day following the addition of the casing layer. In contrast, for the control treatment, irrigation commenced on the second day, which means that adding water to the compost at the start of cultivation reduced the need for frequent irrigation throughout the crop cycle, as the casing layer remained adequately moist because of the water provided by the compost for at least 11 days after casing.

In terms of the total water consumption for crop irrigation, the control method utilized approximately 26% less water compared to the treatment involving the addition of water to the colonized compost (23.79 and 32.40 L m^{-2}, respectively). Despite this difference of approximately 8.61 L m^{-2}, the approach of adding water to the compost can be seen as a viable alternative, aiming to reduce both the time required for application (257 s m^{-2}) and the labor involved (6 days) when compared to the control irrigation method (with a total time of 446.6 s m^{-2} and a frequency of irrigation every 10 days). It is worth noting that in both methods, the last irrigation was carried out on the 31st day, two days prior to the third flush harvest and seven days before the end of the cultivation cycle.

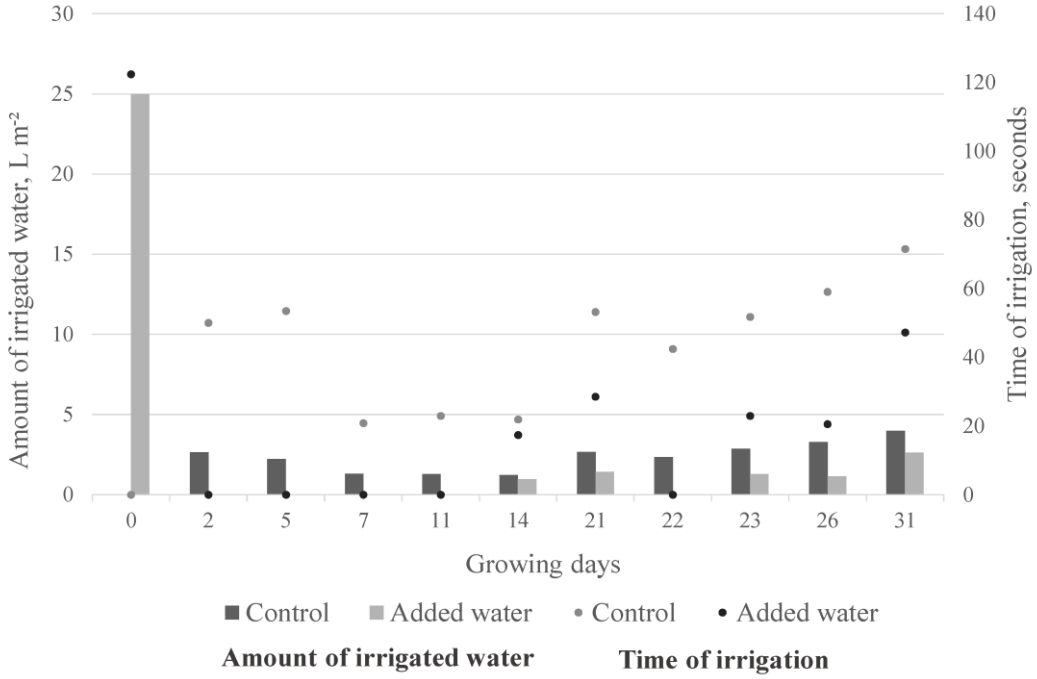

Figure 3. Amount of water and irrigation time per m^2 in the cultivation of *Agaricus bisporus* mushrooms.

Approximately 5% of the compost moisture was lost in the first days of cultivation, during the mycelium run, decreasing the compost moisture from 65% to 60% (Figure 4), showing the possibility of adding water to the colonized compost, as well as in the casing layer, depending on the water consumption/use of labor, and the cultivation method adopted in each production system. At seven days after the addition of the casing layer, the difference in the moisture found in the compost between treatments was high (71.7% for the treatment with water added to the colonized compost and 62.2% for the control).

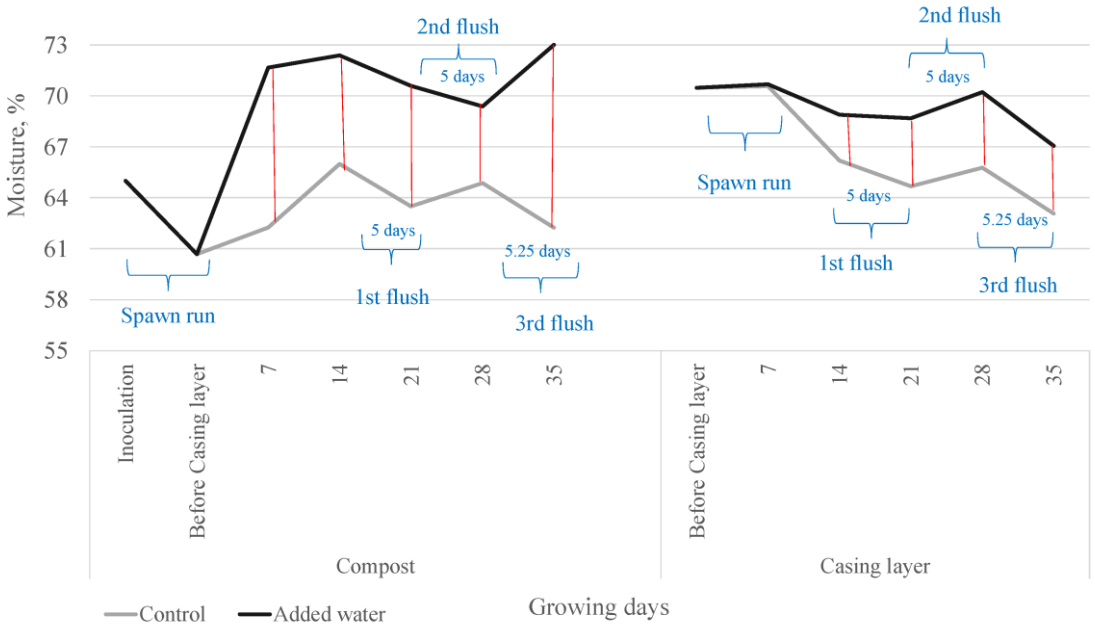

Figure 4. Moisture contents of the compost and casing layer during the growing cycle of *Agaricus bisporus*.

After the beginning of the first flush, the compost moisture varied according to the method of adding water, which justifies the reduction in yield in the second flush, when water was added to the colonized compost. Note that after the 14th day until the 28th day, the humidity of the compost decreased, while in the control method, on the 28th day the moisture increased, reaching 65% humidity. It is clear that the method of adding water to the compost always had the highest moisture in the compost and in the casing layer; however, frequent irrigation in the control method was essential to maintaining greater evaporation on the surface of the casing layer, causing greater stress in the formation of primordia, resulting in a greater yield and number of mushrooms harvested.

In the third flush, which was the end of the crop cycle, there was a greater drop in the moisture in the casing layer due to the absence of irrigation over the last 7 days of cultivation. After the application of water on the 31st day, the moisture in the treatment with water in the compost increased again, which shows similarity to the control treatment in the agronomic results of the 3rd flow. The absence of irrigation was due to a reduction of the presence of diseases in the mushroom, such as *Lecanicillium fungicola*, *Pseudomonas tolaasii*, and *Trichoderma* spp.

4. Discussion

The results obtained in the present work demonstrate the importance of both studied factors (method of water addition vs. genetic strain). The main objective was to evaluate, on an experimental pilot model, the method that used less water and resulted in better agronomic traits of *A. bisporus*, which was obtained by micro sprinkling with the ABI 18/04 strain. It should also be noted that in this method the variability in the yield and BE depending on the strains used was low, unlike the addition of water to the colonized compost, which recommends the use of strains ABI 18/02 and ABI 11/19. The ABI 18/02 strain, on the other hand, had a slightly lower yield and BE; however, fewer mushrooms of larger weight were harvested, which reduces the number of employees involved in this process.

The ABI 19/03 strain is closely related to ABI 11/19 and ABI 18/02 to ABI 18/04 strain; however, the agronomic parameters showed marked differences, such as, for example, a significant difference in the yield and BE using the micro sprinkling method in the first and third harvest flushes between ABI 19/03 and ABI 11/19. Another difference verified between these strains was related to the greater weight of the mushrooms in ABI 19/03 compared to ABI 11/19 in the first and third flushes.

Dong et al. [38] highlighted that most of the genes present in the genome of *A. bisporus* still do not have a characterization of their function in the development of the fungus. Other authors also mentioned that different responses in cultivation occur due to the enzymatic activity of laccase and peroxidase, as well as suitability to the environment and region [24,39,40]. In this sense, the agronomic traits were more influenced by a phenotypic question than a genotypic one, since the strains that presented the lowest genetic variability presented great differences in the yield, mushroom weight, and method of water application.

Concerning the water application method, there was no clear evidence to suggest that increased moisture levels in the compost and casing layer result in higher yields and BE. Consequently, the micro sprinkler irrigation system demonstrated its efficiency by delivering substantial water conservation. In a comprehensive assessment, it is essential to consider both factors. Yield represents the direct measurement of mushroom quantity, while BE gauges how efficiently resources are used in cultivation, discarding the casing layer and substrate moisture of the evaluation [13].

In this context, the micro sprinkler irrigation system proved efficient as it provided significant water savings. On the other hand, the method of adopting water in the compost reduces the irrigation time and frequency. It should be noted that in the present study, a casing layer was added on the compost in both situations with approximately 70% moisture,

and the harvest period finished with 63% for micro sprinkling and 67% moisture for adding water to the compost.

The use of casing layers based on materials with high water-holding capacity and porosity, such as peat, makes it easier for growers to manage the crop and reduces the variability associated with the casing layers in terms of the yield, quality, and uniformity, facilitating several functions. Among them, peat-based casing adequately contributes to the maintenance of a moist microclimate to help feed the mycelium, supplies sufficient water for the growth and development of mycelium and fruit bodies by providing a reservoir for maturing mushrooms and supplementing the water provided by de compost, facilitates the transport of dissolved nutrients to the carpophores, provides the mycelium with a suitably aerated environment by facilitating the diffusion of oxygen, carbon dioxide and other volatile gases, and finally, protects the compost surface from excessive drying out [12,41].

In addition to the method of water application in the crop being related to the use of appropriate strains, the production system and the casing layer can be crucial factors for choosing the best management to be used, considering the reduction of water and operational demand involved. Dhar [42] presented the existing cropping systems for *A. bisporus* production. It should be noted that the application of water to the colonized compost would generate one more operational step if the compost of phase II was inoculated and incubated in bags. Therefore, this method would only be recommended for compost colonized in pasteurization tunnels (phases II and III, together) which would be replaced in shelves or trays.

The concept of supplying water directly to the compost can also be applied with drip irrigation, as described by Navarro et al. [11]. The authors obtained productivities around 30%, as well as in this work. In addition, the mushrooms showed better quality (bigger and cleaner), since the water was supplied directly to the compost, thus reducing the contact surface for the development of pathogens. However, drip irrigation involves a higher installation cost and the need to collect spent compost when disposing of it. Depending on the quality of the plastic used to manufacture the drip hoses, the practice of cook out can reduce the time they are used, increasing the cost of production in the growing system.

5. Conclusions

Traditionally, irrigation in mushroom cultivation has been viewed as an art closely tied to the grower's expertise. However, the evolution of cultivation methods towards sophisticated, air-conditioned facilities with advanced technology has made it possible to enhance production in terms of both yield and quality. The experimental pilot model presented in this study advances this endeavor through the integration of three interconnected factors:

(a) The selection of appropriate strains based on their agronomic characteristics, market demands, and climatic prerequisites;
(b) The optional application of water to the phase III compost before the casing layer, primarily influenced by the initial moisture content of the substrate;
(c) The assessment of economic expenditure on employees and working time combined with the moderate use of water aiming for better sustainability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13102626/s1, Table S1: Precocity from the strains of *Agaricus bisporus*.

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