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Revalorization of degraded maguey pulquero substrate for *Lycopersicon esculentum* germination

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ABSTRACT

The bagasse of *Agave salmiana* (maguey pulquero) is a residue generated during the exploitation of the plant to obtain pulque, inulin, honey, etc. Due to its chemical composition, it can be used for the cultivation of fungi of the *Pleurotus* genus and the subsequent use of the degraded material "degraded substrate (DS)" as a support for the germination of vegetables. The objective of the study was to characterize the bagasse of maguey pulquero biodegraded by *Pleurotus djamor* as a new perspective in its value chain, and subsequent use for the germination of *Lycopersicon esculetum* (tomato). The DS was recovered at 60 d from the *P. djamor* culture, characterized physicochemically and the conformation of the plant tissue was observed by scanning electron microscopy. The DS showed a decrease in protein (4.8–3.3 %) content and fibrous fraction (54–36 %), but dry matter digestibility increased from 47 to 71 %; in addition, changes in mineral composition were observed, mainly in calcium concentration (6 %). Due to its composition, it is possible to revalue DS in the germination of L. *esculetum* to reduce the use of peat moss (commercial peat). The results show that up to 25 % of maguey DS mixed with 75 % peat moss can be used (25:75), reaching a germination percentage of 85 % and increasing the seedling emergence speed index from 0.96 – 1.25. Concluding that it is possible to implement a circular strategy in which agave bagasse is used for mushroom cultivation and the subsequent recovery of the spent substrate for tomato germination.

1. Introduction

The trend towards healthy eating has led to an increase in mushroom cultivation. This involves the use of agro-industrial waste that serves as a support for growth and development, and once the cycle concludes, the material (degraded substrate (DS) or "spent") presents changes in its composition that make it an organic waste worth reusing Velázquez-De Lucio et al. (2023). Recent statistics suggest that the global market value of mushrooms will reach 20.84 million tons in 2026, causing the accumulation of DS and estimating a production of 60 million tons per year Atallah et al. (2021). Unfortunately, most of it is accumulated in large piles that give off unpleasant odors and cause the generation of insects and rodents; under normal circumstances, it is discarded without considering the environmental impact, including soil and groundwater contamination Ahlawat et al. (2011); Shanmugavelu and Sevugaperumal (2021).

In some countries, the management of DS is a major problem, and the search for solutions has led to the advancement of numerous scientific studies that propose areas of utilization for DS. Highlighting its use as a fertilizer (López et al., 2008), as a substrate for the cultivation of other edible fungi (Ashrafi et al., 2014), for the production of bacteria (Wu et al., 2014), as a biosorbent in wastewater treatment (Tay et al., 2016), as a substrate for vermicomposting and pest management (Rinker, 2017), feed for ruminants (Foluke et al., 2011), poultry (Foluke et al., 2014), and larvae of the yellow meal worm *Tenebrio molitor* Li et al. (2020).

However, the composition of the DS will vary according to the location, the fungus used, and other factors (Leong et al., 2022), such as the substrate (generally straw and stubble). In the case of fungi of the *Pleurotus* spp., genus, the literature mentions the ability to degrade different lignocellulosic residues, but recently agave bagasse has been used (Heredia-Solís et al., 2014, 2017; Palomo-Briones et al., 2018; Velázquez-De Lucio et al., 2022, 2023), which is a solid waste generated in the maguey industrial chain that constitutes an environmental

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concern due to the volume it accumulates (about 400 thousand tons of bagasse and 300 000 tons of plant leaves).

Current biotechnological methodologies allow the revaluation of agro-industrial wastes to generate clean energy and a circular economy. Therefore, agave bagasse is a new candidate for its use in the production of fungi of the genus *Pleurotus*, and the subsequent recovery of the DS as a germination support. The objective of this study was to characterize *Agave salmiana* bagasse biodegraded by *P. djamor* (DS) to evaluate the feasibility of its use in the germination of *Lycopersicon esculetum* (tomato) as a new circular perspective in the value chain of the maguey pulquero, the mushroom industry, and the producers of tomato seedlings.

2. Material and methods

2.1. Recovery of degraded substrate

The degraded substrate (DS) was obtained at 60 d from a culture of *P. djamor* (Strain HC001 isolated from *A. salmiana* with GenBank accession number MW581271) on maguey pulquero bagasse supplemented with urea as a nitrogen source, a byproduct generated in the work of Velázquez-De Lucio et al. (2022), where the treatments analyzed correspond to the percentage of total nitrogen (TN): T1: 0.77 % TN (T1), T2: 0.95 % TN, T3: 1.14 % TN, T4: 1.32 % TN, T5: 1.5 % TN, TV: 1.36 % TN; in addition, two types of primary inocula were used, one made with wheat seed (IG) and the other in pellet (IP).

2.2. Characterization of the degraded substrate

The DS was labeled and dried at 50 °C for 72 h in an oven, recording its wet weight and dry weight. These were characterized with the methodologies proposed by the A.O.A.C. (2005), moisture (H) was determined with procedure 925.10, crude protein (CP) with method 960.52, ethereal extract (EE) with method 945.16, ash (A) with method 923.03, organic matter (O.M.) by ash difference, total organic carbon (TOC) by calculation (O.M./1.74) (Gouleke, 1977) and C/N ratio.

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to Van Soest et al. (1991). Hemicellulose (HEM) was calculated by the difference between ADF and NDF. Dry matter digestibility (% DMS) was calculated with the results obtained from ADF using Eq. (1) Linn and Martin (1991); Boga et al. (2014). From treatments T0, T1, T2, T3, T4, and T5, the concentration of calcium, phosphorus, and copper in the digested biomass was determined, for calcium and copper, the A.O.A.C. 975.03 methodologies was used with ICP-OES (inductively coupled plasma optical emission spectrometry); for phosphorus, the spectrophotometry method was used with the methodology proposed by A.O.A.C. 965.17; and for T0 and TV, a chemical elemental mapping was performed. Microscopic analysis of *A. salmiana* bagasse fibers from T0 and TV treatments was performed by scanning electron microscopy (MEB) and elemental chemical analysis with a high-energy X-ray fluorescence spectrometer, through an external service with the national center for innovation and technology transfer of Hidalgo.

$$\%DMS = 88.9 - 0.779 * ADF \quad (1)$$

2.3. Germination

Tomato seed T48109, corresponding to L. *esculetum*, Syngenta ROGERS brand, lot PTF11336, sublot 10,888,717, with a purity percentage of 99.98 %, was used. A completely randomized experimental design of 5 treatments with two controls, 10 replicates per treatment, and 4 replicates was used.

The DS was mixed with commercial peat moss as follows (w/w): T1: 15 % DS of *P. djamor* + 85 % peat; T2: 25 % DS of *P. djamor* + 75 % peat; T3: 50 % DS of *P. djamor* + 50 % peat; T4: 75 % DS of *P. djamor* + 25 % peat; T5: 100 % DS of *P. djamor* + 0 % peat.

The controls were 100 % peat (Control +), 100 % undegraded agave bagasse (Control −).

Germination was carried out in 200-well polystyrene seedbeds, watered until percolation, and placed in black bags for 5 d The parameters recorded were, time to hypocotyl emergence (days), hypocotyl length when reaching verticality, number of plants with two fully unfolded cotyledon leaves, germination percentage (Eq. (2)), germination rate, seedling height at 12 and 17 d, emergence speed index (Eq. (3)) (Maguire, 1962), peak day, and survival percentage at 30 d At 35 d, 10 % of the seedlings were sacrificed to determine root length, stem length, and dry weight.

$$Germination\ percentage\ (\%PG) = \frac{Number\ of\ germinated\ seeds}{Number\ of\ seeds\ sown} 100 \quad (2)$$

$$Germination\ speed\ (M) = \sum \left(\frac{ni}{t}\right) \quad (3)$$

2.4. Statistical analysis

For the characterization of the degraded substrate, a one-way ANOVA analysis and a Tukey's multiple comparisons test with a control with a confidence level of 95 % were carried out in the Minitab 19 statistical program. For the characterization of the mixtures used for germination, the determination of the effect of substrates on germination speed, seedling height, the determination of the effect of substrate on dry weight, stem height and root length of L. *esculentum* seedlings, a one-way ANOVA analysis and a Tukey's multiple comparisons test with a control at 95 % confidence level were performed to verify if there were significant differences among all the trials and a comparison of means by Dunnet at 95 % confidence level to verify if there was a significant difference between the control (CNT+ "commercial peat") and the treatments (mixtures at different proportions of degraded substrate with peat) and thus verify if there is a change regarding traditional germination. Data analysis was performed with the Minitab 19 statistical program.

3. Results

3.1. Characterization of the DS

The results of the physicochemical characterization of DS and non-biodegraded of agave bagasse by *P. djamor* are shown in Tables 1 and 2. The effect on plant tissues of agave bagasse was obtained using a Lelca DMS 1000 Modular Digital Microscope. Fig. 1a shows the bagasse of *A. salmiana* intact and shiny, without traces of fungal degradation, while Fig. 1b shows the bagasse tissue opaque and invaded by mycelium of *P. djamor*, and fungal degradation is evident by presenting a spongy appearance with small fibrils protruding from the damaged tissue and weak to the touch.

The analysis of the fibers by scanning electron microscopy is detailed in Fig. 2, in section a., the bagasse tissue of *A. salmiana* T0 is shown, where the fibrous bundles of bagasse can be appreciated, constituted mainly by a set of cellulose microfibrils, which can be aggregated in more elongated forms adopting a heliocoidal arrangement within the lignin and hemicellulose matrix. In section b., we observe the bagasse fibrils invaded by the mycelium of *P. djamor*, responsible for degrading the lignocellulosic polymers present in the fibers.

The results of the elemental chemical mapping performed on the T0 and TV tissues are shown in Figs. 3a and 3b, of which only carbon, oxygen, phosphorus, potassium, and calcium were considered; the first column shows a microscopy of the tissues that allows identifying the presence of the studied elements on the substrate, or mycelium. In section, a. the fibers of the bagasse of *A. salmiana* are observed, in which the presence of C and O distributed homogeneously over the surface of the whole material can be appreciated. While phosphorus and potassium

Table 1
Composition of substrate degraded by *Pleurotus djamor* inoculated with IG in dry weight.

	T0	T1	T2	T3	T4	T5	TV
DM (%)	89.4 ± 0.1^{d}	90.8 ± 0.3^{c}	91.8 ± 0.3^{b}	91.9 ± 0.2^{b}	91.9 ± 0.2^{b}	92.5 ± 0.3^{b}	95.0 ± 0.3^{a}
Ash (%)	10.6 ± 0.1^{e}	22.5 ± 0.8^{bc}	21.0 ± 0.1^{cd}	22.3 ± 0.8^{bc}	23.8 ± 0.2^{b}	25.4 ± 0.6^{a}	20.2 ± 0.3^{d}
O.M. (%)	89.3 ± 0.1^{a}	77.5 ± 0.8^{bc}	78.9 ± 0.1^{b}	77.7 ± 0.8^{bc}	76.2 ± 0.2^{cd}	74.6 ± 0.6^{d}	74.6 ± 0.7^{b}
CP (%)	4.8 ± 0^{a}	3.8 ± 0 ^{c}	4.3 ± 0^{b}	4.3 ± 0^{b}	3.5 ± 0.3^{cd}	3.3 ± 0^{d}	4.4 ± 0.3^{ab}
EE (%)	4.35 ±0.3^{a}	3.26 ± 0.05 ^{b}	4.22 ± 0.5 ^{a}	4.07 ± 0.04^{ab}	4.08 ± 0.4 ^{ab}	4.07 ± 0^{ab}	2.42 ± 0.1^{c}
N (%)	0.7 ± 0^{a}	0.6 ± 0^{c}	0.7 ± 0^{b}	0.7 ± 0^{b}	0.6 ± 0.04^{cd}	0.5 ± 0^{d}	0.7 ± 0^{ab}
NDF (%)	54.1 ± 0^{a}	40.6 ± 0.9^{c}	36.9 ± 0.7^{d}	39.8 ± 0.1^{c}	39.5 ± 0.7^{c}	36.9 ± 0.2^{d}	44.0 ± 0.2^{b}
ADF (%)	58.7 ± 0^{a}	26.2 ± 1^{c}	21.7 ± 0.8^{d}	24.5 ± 0.6^{cd}	35.6 ± 0.7^{b}	26.7 ± 1^{c}	24.9 ± 1^{c}
TOC (%)	51.4 ± 0.1^{a}	43.0 ± 0.5^{cd}	43.9 ± 0.1^{bc}	43.2 ± 0.4^{cd}	42.3 ± 0.1^{d}	41.4 ± 0.4^{e}	44.3 ± 0.4^{b}
HEM (%)	4.7 ± 0.1^{c}	14.4 ± 1^{ab}	15.1 ± 0.9^{ab}	15.3 ± 0.5^{ab}	3.8 ± 0.1^{c}	12.6 ± 6^{b}	20.2 ± 0.9^{a}
DMS	47.0 ± 0.4^{e}	68.5 ± 0.8^{bc}	71.9 ± 0.6^{a}	69.7 ± 0.5^{abc}	61.1 ± 0.6^{d}	68.1 ± 1^{c}	70.5 ± 0.7^{ab}
Relation C/N	63.2 ± 0.7^{c}	70.0 ± 0.7^{b}	64.0 ± 0.1^{c}	63.0 ± 0.6^{c}	79.8 ± 0.1^{a}	78.4 ± 0.9^{a}	60.5 ± 0.4 ^{d}
Ca (g/100 g)	4.02	6.46	6.26	5.67	6.06	5.63	NA
P (g/100 g)	0.21	0.15	0.17	0.16	0.19	0.15	NA
Cu ppm	11.98	29.03	19.33	15.36	23.06	17.16	NA

TOC: Total organic carbon; HEM: Hemicellulose; DMS: Digestibility of dry matter ± Standard deviation.
Means not sharing a letter are significantly different according to Tukey Method, α=0.05.

Table 2
Composition of substrate degraded by *Pleurotus djamor* inoculated IP in dry weight.

	T0	T1	T2	T3	T4	T5
DM (%)	89.4 ± 0.1^{c}	90.7 ± 0.3^{b}	91.8 ± 0.3^{a}	91.9 ± 0.2^{a}	91.9 ± 0.2^{a}	92.5 ± 0.3^{a}
Ash (%)	10.6 ± 0.1^{d}	24.9 ± 0.8^{a}	24.8 ± 0.4^{a}	19.8 ± 0.5^{bc}	20.3 ± 1^{b}	18.4 ± 0.3^{c}
O.M.(%)	89.3 ± 0.1^{a}	75.1 ± 0.8^{d}	75.2 ± 0.4^{d}	80.2 ± 0.5^{bc}	79.7 ± 1^{c}	81.6 ± 0.3^{b}
CP (%)	4.8 ± 0^{a}	3.2 ± 0.3^{c}	3.0 ± 0.3^{c}	3.2 ± 0.3^{c}	3.8 ± 0^{b}	3.8 ± 0^{b}
EE (%)	4.3 ± 0^{a}	3.7 ± 0.7^{ab}	3.5 ± 0.7^{b}	4.7 ± 0.2^{a}	4.3 ± 0.2^{ab}	4.5 ± 0.3^{a}
N (%)	0.7 ± 0^{a}	0.5 ± 0^{c}	0.5 ± 0^{c}	0.5 ± 0^{c}	0.6 ± 0^{b}	0.6 ± 0^{b}
NDF (%)	54.1 ± 0^{a}	48.9 ± 0.9^{b}	42.0 ± 0.7^{c}	35.9 ± 0.5^{d}	30.6 ± 0.9^{e}	37.2 ± 0.8^{d}
ADF (%)	58.7 ± 0^{a}	41.1 ± 0.7^{b}	39.9 ± 0.3^{b}	33.8 ± 0.2^{c}	20.7 ± 0.7^{d}	14.4 ± 0.9^{e}
TOC (%)	51.4 ± 0.1^{a}	41.7 ± 0.4^{d}	41.8 ± 0.2^{d}	44.5 ± 0.3^{bc}	44.3 ± 0.6^{c}	45.3 ± 0.1^{b}
HEM (%)	4.7 ± 0.1^{c}	7.7 ± 0.8^{b}	2.1 ± 0.8^{c}	2.1 ± 0.4^{c}	9.8 ± 1^{b}	22.8 ± 1^{a}
DMS	47.0 ± 0.4^{e}	56.9 ± 0.5^{d}	57.8 ± 0.2^{d}	62.5 ± 0.2^{c}	72.7 ± 0.5^{b}	77.7 ± 0.5^{a}
Relation C/N	63.2 ± 0.7^{b}	82.3 ± 8^{a}	86.8 ± 7^{a}	88.0 ± 8^{a}	72.5 ± 1^{ab}	73.8 ± 0.2^{ab}
Ca (g/100 g)	4.02	2.74	2.64	2.99	2.02	2.21
P (g/100 g)	0.21	0.30	0.07	0.08	0.04	0.06
Cu ppm	11.98	13.28	7.96	9.36	8.52	8.27

TOC: Total organic carbon; HEM: Hemicellulose; DMS: Digestibility of dry matter ± Standard deviation.
Means not sharing a letter are significantly different according to Tukey Method, α=0.05.

are scarce, calcium, on the other hand, is concentrated in the elongated rectangular fibers of the bagasse, rather than in the rest of the cell wall. In section b., the first micrograph shows the microscopy of the DS with mycelium marked with an arrow. The elemental chemical change of the substrate can be appreciated once it has been degraded by the fungus, mainly carbon, oxygen, and phosphorus are found in greater abundance in the section of the substrate invaded by the mycelium, unlike calcium, which has a marked presence on the surface of the cell wall. On the other hand, potassium is distributed along the entire surface of the organic material.

Coupled to the scanning electron microscope, a high-energy X-ray fluorescence spectrometer (EDS) was used to determine the elemental composition of the tissues only at T0 and TV (Table 3). The calcium content was from 4.67 % in T0 determined by EDS (4.02 % determined by ICP) to 15.42 % in TV, oxygen increased from 11.71 % in T0 to 14.79 % in TV, while according to this technique the phosphorus content in T0 was 0.11 % and in TV 0.12 %, copper also increased as observed in the previous analyses. However, the content calculated by EDS in T0 was 67.70 ppm and 78.10 ppm in TV, the inconsistencies in the observed values can be attributed to the sensitivity of the techniques used, in spite of this, it is proved that the biologically DS suffer changes in the mineral composition, which differs from the initial one.

3.2. Germination

A. salmiana bagasse degraded by 60-day-old *P. djamor* was evaluated as a substrate for *Lycopersicum esculetum* germination. The pH and electrical conductivity (EC) are important chemical parameters in the substrate intended for seed germination, since their value determines the availability of micro- and macronutrients. The pH values of the mixtures in this study are within the optimum range for this purpose (Table 4). On the other hand, it has been reported that EC increases as the addition of spent substrate in the mixture also increases; salinity is closely related to this parameter, so it is a factor to be considered when germinating seeds and verifying the tolerance of the crop to salts.

When using the biodegraded substrate in the germination process, percentages between 70 and 85 % were obtained, being the T2 treatment (25 % DS +75 % Pm) the one with the highest germination percentage reaching 85 % (Fig. 4). However, the results did not show statistically significant differences regarding the positive control (Peat moss, commercial substrate), which means that the inclusion of DS up to 75 % in the mixture does not influence seed germination, and it is even possible to use up to 100 % of spent substrate for this purpose.

In relation to germination speed, where the number of germinated seeds is related to time, the highest germination speed was reached in treatment 2, which presented the highest germination percentage (Table 5).

Fig. 5a shows the daily germination graph (plants with visible hypocotyl) where the number of germinated seeds per day is indicated, it also describes the distribution of germination through time and allows visualizing the peak day of germination, being treatment 2 with the highest number of germinated seeds on days 6 and 7, for CNT + and T1 the peak day was the sixth day, with a decrease in the number of seed emergence through time, on the other hand, T3 had its maximum on day 7. Fig. 5b corresponds to the cumulative germination by time interval,

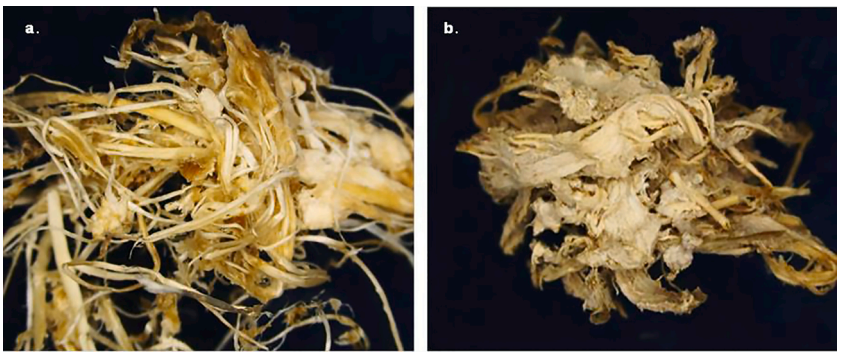

Fig. 1. Plant tissue of *A. salmiana* a. uninoculated bagasse without urea addition (T0); b. degraded substrate TV 60 days post-culture; obtained in Lelca DMS 1000 Modular Digital Microscope.

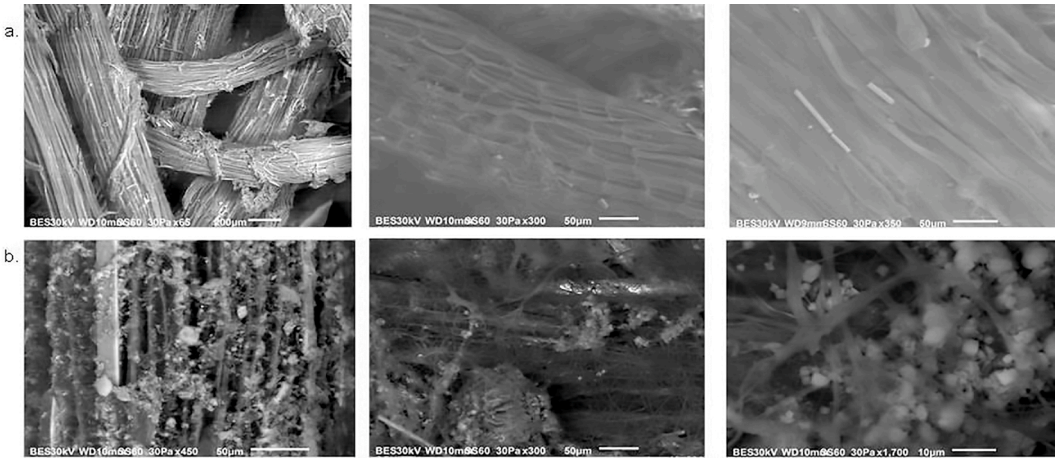

Fig. 2. a. *A. salmiana* bagasse tissue (T0), b. 60-day post-culture degraded substrate tissue with 1.36 % NT, both images obtained by MEBA.

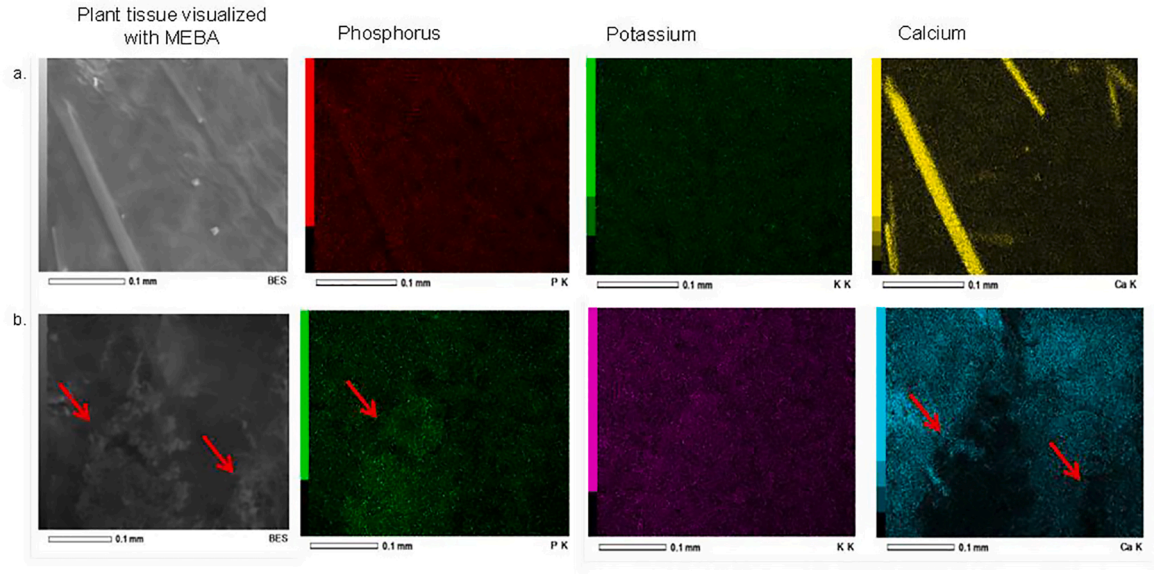

Fig. 3. Elemental mapping by Energy-dispersive X-ray spectroscopy (EDS) a. *A. salmiana* bagasse fibers; b. substrate degraded with 1.36 % NT.

which indicates the maximum germination capacity and the time in days in which it is reached, the way germination increases and the time of onset.

Plants with visible hypocotyl are an indication of the speed and uniformity of seed germination, for this purpose, those in stage 09 of the BBCH scale were considered, and the time to reach stage 10 of the BBCH scale was recorded, that is, the time in which the seedlings presented two fully unfolded cotyledons; the seedlings that reached stage 10 in less time were T1 to T3, the T4 and T5 treatments reached stage 10 from day 11. The average height of the seedlings at 12 and 17 days in mm

Table 3
Elemental composition of T0 and TV tissues obtained by X-ray fluorescence spectrometer.

Element	TO (%)	TV (%)
Nitrogen	0.20	0.16
Carbon	79.94	64.84
Oxygen	11.71	14.79
Magnesium	0.22	1.80
Aluminum	0.23	0.15
Silicon	0.56	0.37
Phosphorus	0.11	0.12
Sulfur	0.09	0.06
Chlorine	0.015	0.022
Potassium	1.31	1.09
Calcium	4.67	15.42
Titanium	0.026	0.026
Sodium	0.040	0.073
Iron	0.52	0.47
Zinc	0.074	0.051
Copper (ppm)	67.70	78.10
Rubidium (ppm)	58.60	67.00

Table 4
pH and electrical conductivity (CE) of the mixtures used for *Lycopersicum esculetum* germination.

Treatment	Composition	pH	EC (µS/cm)
Control (+)	100 % Peat moss	6.36 ± 0.1 ^{bA}	72.40± 2 ^{fA}
Control (-)	100 % *Agave salmiana* bagasse	5.5 ± 0 ^{d}	240 ± 5 ^{d}
T1	15 % Degraded substrate *P. djamor* - 85 % Peat moss	7.36 ± 0.1 ^{a}	175 ± 6 ^{e}
T2	25 % Degraded substrate *P. djamor* - 75 % Peat moss	6.46 ± 0.1 ^{bA}	250.33± 5 ^{d}
T3	50 % Degraded substrate *P. djamor* - 50 % Peat moss	5.73 ± 0.05 ^{cd}	309.33 ± 4 ^{c}
T4	75 % Degraded substrate *P. djamor* - 25 % Peat moss	5.88 ± 0.02 ^{c}	545 ± 4 ^{b}
T5	100 % Degraded substrate *P. djamor* - 0 % Peat moss	5.53 ± 0.05 ^{d}	654± 5 ^{a}

Means that do not share a letter are significantly different Tukey (α=0.05).
Means not labeled A are significantly different from the control level mean (-) Dunnett (α=0.05).

respectively are presented in Tables 6 and 7.

Regarding seedling survival at 30 days (Fig. 6), 100 % was achieved in the control +, T1 and T2, and from T3 onwards, the survival rate began to decrease, with T5 showing the lowest number of live seedlings at 30 days.

After 30 days, 10 % of the seedlings were sacrificed to determine stem height, root length and seedling dry weight (Table 8). Differences between treatments may be due to the chemical composition and salt content of the spent substrate. Seedling dry weight was statistically different regarding the control + (Dunnet α=*0.05*) in all treatments except for T2.

4. Discussion

The cultivation of fungi, such as *Pleurotus* spp., continues to expand, and their cultivation is an efficient technique to generate value-added products Velázquez-De Lucio et al. (2022, 2023). During its cultivation, solid-state fermentation is involved, which offers a relatively inexpensive and environmentally friendly technology to reduce the lignin mass and thus give them a potential use for already enriched lignocellulosic residues Van Kuijk et al. (2015). Spent or degraded substrate is the residue left after fungal production, which presents changes in its composition derived from fungal degradation and its increasing accumulation has prompted governments and researchers to address the potential use of this material Rinker (2017); Velázquez-De Lucio et al. (2022). Recent studies point to the use of agave bagasse as a new alternative substrate for mushroom cultivation (*A. salmiana* or maguey bagasse), which is a residue from the maguey production chain; despite the potential of the material to generate other value-added products, its use is still limited and little explored Valle-Pérez et al. (2021); Velázquez-De Lucio et al. (2022). Because of its structure, the fibrils are assembled in parallel layers bonded by cementitious materials

Table 5
Effect of substrates on germination speed.

	M
CTN +	0.96 ± 0.3 ^{ab}
CNT -	0.66 ± 0.2 ^{b}
T1	1.04 ± 0.3 ^{ab}
T2	1.25 ± 0.1 ^{aA}
T3	1.07 ± 0.1 ^{ab}
T4	0.94 ± 0.3 ^{ab}
T5	0.79 ± 0.1 ^{ab}

CNT (+): Peat moss (Pm), CNT (-):Agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS.
Means that do not share a letter are significantly different Tukey (α=0.05).
Means not labeled A are significantly different from the control level mean (-) Dunnett (α=0.05).

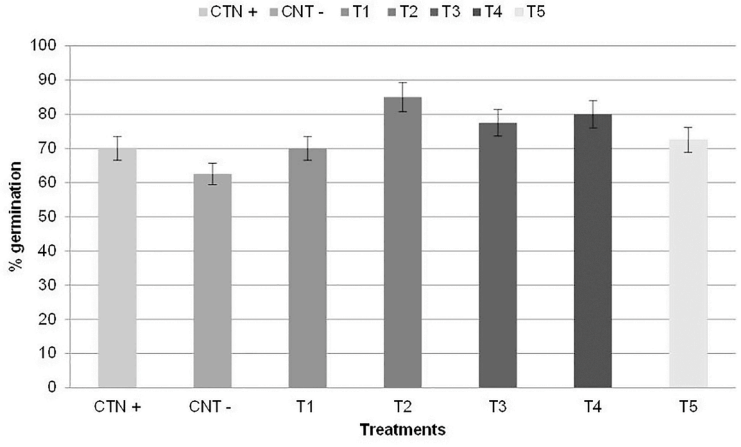

Fig. 4. Effect of substrates on germination percentage. CNT (+): Peat moss (Pm), CNT (-): agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS. Means not sharing a letter are significantly different Tukey (α=0.05).

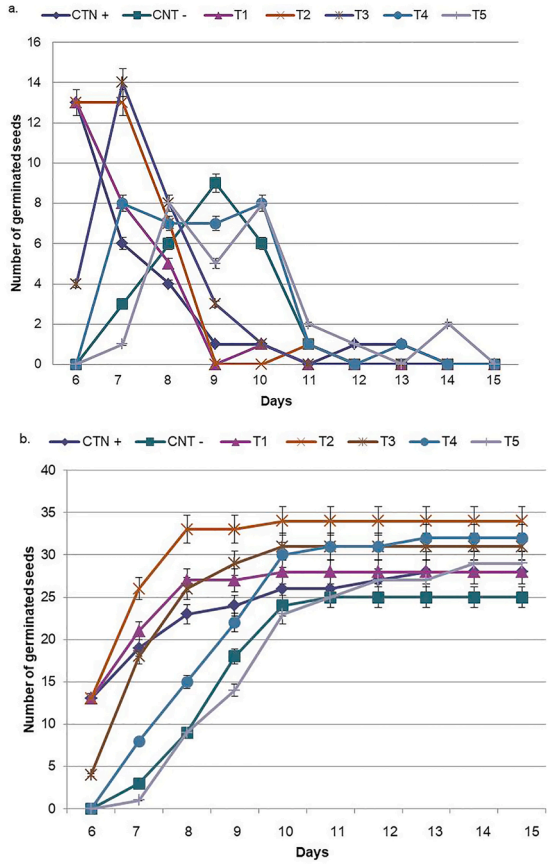

Fig. 5. a) Daily germination graph, b) Cumulative germination graph by time interval.

such as pectins, lignin, and hemicellulose giving rise to the fiber structure Bessadok et al. (2009). In addition to these substances, the fiber surface is also covered by waxes due to fatty acids present in the plant Madhu et al. (2020). De Jesús-Rivera et al. (2009) point out that *Pleurotus* species can simultaneously degrade cellulose, hemicellulose and lignin, with lignin being the first to degrade completely, leading to the formation of inter-fibrillar spaces, leaving the cellulose clean.

The agave bagasse recovered in this study was supplemented with different concentrations of urea and type of inoculum (Velázquez-De Lucio et al., 2022), shows changes in its composition at 60 days, possibly because of enzymatic degradation by the fungus Van Kuijk et al. (2015); Velázquez-De Lucio et al. (2022). A statistically significant decrease in CP and nitrogen was observed in the DS, possibly due to the incorporation of nitrogen from the fruiting body, stems or mycelium, given that this element is necessary for the production of protein, nucleic acid, purine synthesis, pyrimidine, and polysaccharides constituents of the fungal cell wall Ruiloba et al. (2014); Bellettini et al. (2019). Regarding the behavior of ashes, this has also been mentioned by Ruiloba et al. (2014), Velázquez-De Lucio et al. (2020), and Amido et al. (2021) among others, this may be a consequence of the degradation of organic matter and the decomposition of silicates in the lignified structures releasing silica and consequently increasing the ash content Assi and King (2008); Phutela et al. (2012).

The substrate used for mushroom cultivation at the end of the process (spent substrate) turns out to be a material with high nutritional values that include mushroom mycelia, degraded cellulosic fibers, degraded lignin, proteins, and minerals that make it a valuable resource, to be reused in other processes Velázquez-De Lucio et al. (2022, 2023). In this sense, degraded substrates are rich in macronutrients such as phosphorus, potassium, calcium, magnesium, and some micronutrients such as iron and manganese Chang-Yun et al. (2009); Postemsky et al. (2016).

The distribution of elements on *A. salmiana* bagasse fibers coincides with that reported by Heredía-Solís et al. (2014). Who conducted an elemental chemical mapping of *A. salmiana* and *A. weberi* fibers and found abundant calcium on the elongated fibers of the residues, and carbon and oxygen distributed in all parts of the analyzed fibers. According to this technique, the increase in calcium concentration in TV would explain the decrease in its aluminum content to 0.15 %, this fact is relevant, since the presence of aluminum can decrease the acquisition of nutrients and energy in plants Yang et al. (2007); Postemsky etal. (2016); Chávez-Guerrero et al. (2020).

Of the variations in the mineral content, the Cu content was the one that attracted attention, so the possible reasons why it increased in the spent substrates were evaluated. The Cu content of the wheat seeds used to prepare the initial inoculum was determined, and it was found that the wheat seed contained 5.59 ppm of Cu. The inoculum with grain presented 9.66 ppm and the inoculum with mycelium or pellet contains 3.89 ppm, so that a possible cause of the increase in Cu in the degraded substrates may be the type of inoculum used, since the seed is rich in this mineral; however, the increase in Cu can also be attributed to other circumstances such as those referred by Chang-Yun et al. (2009) who suggest that during the incubation of the fungus and the maintenance of relative humidity above 85 %, it is possible that the high mineral content in the post-harvest substrates may have originated from the water supplied.

Researchers have reported on the nutritive value and increased

Table 7
Seedling height at 17 days in mm.

	Cnt (-)	Cnt (+)	T1	T2	T3	T4	T5
Average (mm)	24.0 cA	39.4 aA	37.2 aA	36.2 a	29.2 b	22.2 c	15.7 d
Standard deviation	5.9	9.4	5.3	7.1	6.4	4.5	5.6
Maximum	33.0	57.0	45.0	55.0	41.0	30.0	28.0
Minimum	12.0	18.0	25.0	23.0	15.0	10.0	7.0

Cnt (+): Peat moss (Pm), Cnt (-):Agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS. *Means not sharing a letter are significantly different Tukey (α=0.05). Means not labeled with A are significantly different from the control level mean (+) Dunnett (α=0.05).*

Table 6
Seedling height at 12 days in mm.

	Cnt (-)	Cnt (+)	T1	T2	T3	T4	T5
Average (mm)	18.82 cA	35.16 aA	31.50 aA	35.03 a	24.84 b	18.82 c	17.50 bc
Standard deviation	6.89	8.31	6.79	6.77	6.12	2.94	5.68
Maximum	25	51	43	50	40	25	25
Minimum	5	23	13	23	15	15	10

Cnt (+): Peat moss (Pm), Cnt (-):Agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS.
Means not sharing a letter are significantly different Tukey (α=0.05).
Means not labeled with A are significantly different from the control level mean (+) Dunnett (α=0.05).

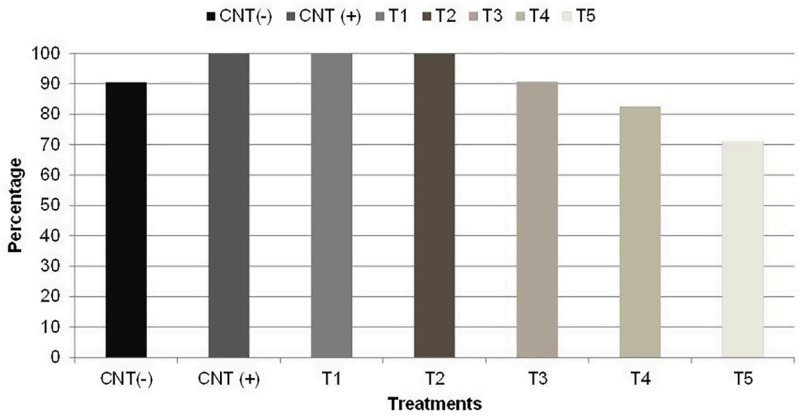

Fig. 6. Effect of substrates on seedling survival at 30 days. CNT (+): Peat moss (Pm), CNT (-): agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS. Means that do not share a letter are significantly different Tukey (α=0.05).

Table 8
Effect of substrate on dry weight, stem height and root length of *Lycopersicon esculentum* seedlings.

	Cnt (-)	Cnt (+)	T1	T2	T3	T4	T5
Dry weight (g)	0.005 ± 0^{de}	0.0132 ± 0^{aA}	0.01 ± 0^{bc}	0.01 ±0^{abA}	0.008 ±0^{cd}	0.007 ± 0^{d}	0.003 ±0^{e}
Stem height (cm)	2.9 ± 0.4^{cd}	4.8 ± 0.4^{a}	4.3 ± 0.6^{ab}	4.6 ± 0.6^{a}	3.7 ± 0.2^{bc}	3.1 ± 0.2^{c}	2.1 ± 0.2^{d}
Root length (cm)	7.1 ± 0.4^{abc}	5.5 ± 1^{abc}	7.9 ± 1^{a}	7.2 ± 3^{ab}	4.0 ± 2^{bcd}	3.0 ± 1^{cd}	0.8 ± 0.3^{d}

Cnt (+): Peat moss (Pm), Cnt (-):Agave bagasse, T1: 15 % DS+85 % Pm, T2: 25 % DS+75 % Pm; T3: 50 % DS+50 % Pm, T4: 75 % DS+25 % Pm, T5: 100 % DS. Means not sharing a letter are significantly different Tukey (α=0.05).
Means not labeled with A are significantly different from the control level mean (+) Dunnett (α=0.05).

digestibility of dry matter from agro-industrial residues inoculated by fungi of the genus *Pleurotus* (Fazaeli et al., 2004; Velázquez-De Lucio et al., 2020), the enzymatic degradation of substrate macromolecules results in increased digestibility and availability of the carbohydrates that compose it Fazaeli et al. (2014).

On the other hand, hemicellulose reduction is the likely result of the degradation of structural carbohydrates by xylanase enzymes that hydrolyze hemicellulose in the fungal metabolic process, since hemicellulose, cellulose, and lignin serve as energy sources for fungal growth because they contain carbon, hydrogen, and oxygen Mohammed et al. (2021); Herrera-Pérez et al. (2021).

Annually, the mushroom industry needs to dispose of >50 million tons of DS and its valorization is demanding, as the substrate composition varies locally, seasonally and during the harvest period Beckers et al. (2019). However, there is a wide range of possibilities within which are the removal of copper (Tay et al., 2010) and nickel contaminated water (Tay et al., 2011), degradation of polycyclic aromatic hydrocarbons (Lau et al., 2003), remediation of mining contaminated soils (Frutos et al., 2010), degradation of pesticides such as linuron, diazinon and myclobutanil (Marín-Benito et al., 2016), as soil conditioner, since due to its organic composition and mineral abundance the degraded substrate can favor soil fertility thus allowing healthy crops in the field, (Lou et al., 2017; Rajavat et al., 2022), as biofertilizer (Zhu et al., 2013), substrate for the new cultivation of fungi such as *Pleurotus* spp., (Pardo-Giménez et al., 2012) and *Agaricus blazei* (González-Matute et al., 2011) or as raw material for ruminant feed given the increased digestibility of dry matter (Fazaeli et al., 2014) and chickens Foluke et al. (2014).

In this sense, one way of using DS is through its incorporation into agricultural or forest soils, functioning as an organic fertilizer or as an improver of the physical and chemical characteristics, but its use for this purpose requires the reduction of particle size and the dilution of excess salts (Velázquez-De Lucio et al., 2023). It is estimated that 47 % of the substrates degraded by fungi are used for the cultivation of plants under greenhouse conditions; one of the plants used is *Lycopersicum esculetum* (tomato), *Cucurbita pepo* l. var Afrodite F1 (zucchini), *Capsicum annum* L. var Lamuyo F1 (pepper) Medina et al. (2009); Luna et al. (2013); Postemsky (2016). The DS from *Agaricus bisporus* cultivation has been used for the production of vegetables and fruits including asparagus, beets, cauliflower, cabbage, peppers, cucumber, tuce, green chickpea, mustard, onion, potato, radish, bean, tomato, zucchini, prunes, and apple seedlings; within large area crops the DS of *Agaricus bisporus* has been tested in the cultivation of corn, rye, wheat, and barley. The spent substrate of *Lentinula edodes* has been tested to produce tomatoes, that of *Flammulina velutipes* for melon seedling production; while the spent substrate of *Pleurotus spp*., has been evaluated for the cultivation of lettuce, tomato, zucchini, bell pepper and cucumber Rinker (2017). For their part, Luna et al. (2013) tested the germination of *Lycopersicum esculetum* on biodegraded sawdust + soil fertilized with urea (ASB+SF), biodegraded rice husk + soil fertilized with urea (CB+SF) and a mixture of rice husk + biodegraded sawdust + soil fertilized with urea (CB+ASB+SF) in addition to other treatments, where they reached lower germination percentages than those achieved in the present study, for ASB+SF the germination percentage was 77.33 %, for CB+SF of 20.66 % and for CB+ASB+SF of 32 %. A factor that influences the germination processes is the pH and EC of the substrates, the average pH value for seedling cultivation should be between 6.5 −7.5 since at higher values, root development is affected; biologically biodegraded materials normally present pH between 6.0 and 8.0 which is considered favorable for root development; values higher than 8.5 and lower than 5.5 can be limiting for some nutrients (Luna et al., 2013), the values found in this study are in the intermediate values, according to the literature.

Seedling development in the initial stage is related to the uniformity of germination, which in turn is related to the characteristics of the substrate (Fernández-Bravo et al., 2006), such as salinity and EC (described above), which have an effect on the germination percentage (as salinity increases, germination decreases). This work evidences that the initial vegetative organs suffer alterations as EC increases, the root system is not developed and consequently, there is not enough water and nutrient absorption, causing the size of stems, leaves and seedling weight to decrease. In addition, as EC increases, cotyledons suffer alterations in shape and size.

5. Conclusions

The degraded substrate showed a decrease in the fibrous fraction and variation in the mineral content, mainly Ca and Cu, because of fungal growth, enzymatic activity of the fungus, initial composition of the agave bagasse, as well as conditions and time of cultivation. Due to its composition, it can be used in animal feed, bioremediation and as a substrate to germinate plants or cultivate fungi. In this study, the DS was recovered for seed germination in tomato cultivation, and it was demonstrated that it can be mixed with Peat moss (25:75) and reach a germination percentage of 85 % and significantly increase the speed of seedling emergence.

CRediT authorship contribution statement

B.S. Velázquez-De Lucio: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **E.M. Hernández-Domínguez:** Formal analysis, Data curation. **M.P. Falcón-León:** Writing – review & editing. **A. Téllez-Jurado:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing – original draft, Writing – review & editing. **J Álvarez-Cervantes:** Conceptualization, Methodology, Formal analysis, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Does not apply

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