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ORIGINAL RESEARCH ARTICLE

Reuse of post-culture mushroom substrate in horticultural seedbeds

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ABSTRACT

The agronomic use of mushroom post-harvest substrates (SPCHs) in horticultural seedbeds could be an interesting alternative for the reuse of these wastes in line with the European circular economy strategy. This work evaluates the potential use of four treatments with different SPCHs, mushroom (-Ch), mushroom (-St), mushroom compost (-CO), and a mixture (SPCH-Ch and SPCH-St) as substrates for lettuce and chili pepper seed germination. The trial was carried out in a germination chamber using commercial compost as a control treatment. The evaluation was based on its chemical (salinity, N and C content), physical (bulk and real density, porosity and water retention) and plant effect (germination and biomass) characteristics. Of the chemical properties studied, the high salinity in SPCH-Ch and SPCH-CO was a limiting factor for the development of the horticultural species evaluated (electrical conductivity 1:2.5; p/v; ~11 dS m^{-1}), and low germination percentages were observed. Regarding physical properties, porosity and water retention, the SPCH-CO, SPCH-St and mixture treatments presented some values outside the optimal range established for germination substrates. In the case of SPCH-St, its high C/N ratio could be a limitation for supplying N to the crop. In relation to biomass production (aerial and root) of lettuce and chili pepper, all the treatments evaluated obtained similar values to the control treatment. The mixed treatment presented the highest biomass values, significantly higher in the lettuce crop. In general, the mixed treatment proved to be the best alternative for use in the seedbed.

Keywords: Chili Pepper; Lettuce; Physical Properties; Horticultural Planter; Chemical Properties; Organic Substrates

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1. Introduction

The agri-food industries generate a significant volume of waste, the reuse of which involves efficient waste management and the recovery of new resources that can be used as a source of production in other agricultural activities. This use of secondary raw materials is one of the objectives driven by the European circular economy plan[1].

The edible mushroom production industry generates an amount of organic material after production of about five kilograms of fresh mushroom post-culture substrate for every kilogram of mushrooms produced[2]. The forecast over the next decade for this in-industry is for a notable increase[3], which would mean an increase in the production of associated waste. The disposal of these wastes generated after mushroom cultivation represents a problem in mushroom cultivation industries[4]. The mushroom species most produced in the world are champignon *(Agaricus* spp. 15%) and seta *(Pleurotus* spp. 19%)[5], being the production of edible mushrooms worldwide in 2017 around 10.2 million tons. Spain occupies the fifth position (160,000 tons per year)[6], with production being geographically concentrated in Castilla la Mancha and La Rioja. After mushroom cultivation (~3–4 fructifications), the so-called post mushroom cultivation substrate (SPCH) is generated.

SPCH can be used agronomically as fertilizer, soil amendment or seedling substrate (medium container or seedling bed). Through these alternatives, these residues are incorporated as a resource in another agrarian system according to the guidelines of the circular economy[7–10]. The application of SPCHs in agricultural systems besides contributing to reduce production costs implies a reduction of their environmental impact[11].

This substrate could be used directly or after a composting process, as a seedbed for vegetable production. In both cases, it would be an interesting alternative to the use of conventional commercial compost. Compost from mushroom cultivation substrate remains is included in the list of organic products as cultivation substrates or as a mixture of cultivation substrates[12,13].

In a recent review by Stewart-Wade[14] on the efficacy of organic wastes used in the production of container plants, it was concluded that the characteristics, drawbacks and suitability should be reviewed for each specific waste.

In this sense, its reuse implies the need to evaluate its potential both from the point of view of its chemical characteristics[15], and physical properties[16,17], as well as its effect on germination and seedling production in the seedbed (aerial and root biomass).

In this study, four treatments with residues of post-cultivation substrates of mushrooms were evaluated with respect to a commercial compost treatment for the germination of seeds and growth of lettuce and chilli seedlings in a chamber under controlled conditions.

2. Material and methods

2.1 Origin of mushroom post-culture materials used

The champignon (*Agaricus*) and seta (*Pleurotus* sp.) post-culture substrates come from the Technological Center for Mushroom Research (CTICH, La Rioja, Spain) and are the organic material resulting from the mushroom cultivation process, so their composition is conditioned by the raw materials used in their elaboration-composting and the mycelial remains of the mushrooms after their cultivation.

The raw materials for the preparation of the mushroom growing substrate were wheat straw, chicken manure, gypsum, urea and water. This mixture starts the biodegradation process when it reaches a humidity of 76%. Under aerobic conditions, it reaches temperatures up to 80 °C, with successive turning; this phase lasts 17–20 days and is followed by pasteurization and thermophilic conditioning. The substrate obtained is mixed with the mycelium and transferred to the culture room, where the fungus colonizes the substrate, after which a covering layer based on peat (corrected with $CaCO_3$) is applied, on which fruiting takes place. After the end of mushroom production, the post-culture mushroom fungus substrate (SPCH-Ch) is obtained, which includes the degrated compost and the cover layer.

In the case of mushroom substrate, it is produced from wheat straw that is moistened and turned under aerobic conditions until obtaining a humidity of around 65–70%. Subsequently, the substrate is pasteurized and the mycelium is added. After the mushroom production is finished, the post-culture mushroom substrate (SPCH-St) is obtained.

For the composting of SPCH-Ch, an open system of plateaus is used, which are turned for a minimum period of 8 weeks under conditions of temperature and humidity controlled for maturation. Under these conditions, a product called composted post-culture mushroom compost substrate (SPCH-CO) is generated. In this process, biodegradation causes the mycelium to disappear, homogenizes and reduces the humidity of the resulting substrate, modifying the physico-chemical parameters and organic matter of the original product. A commercial vegetable seedbed compost (a mixture of black peat, blond peat and vegetable compost) was used as a control treatment.

The procedure for preparing the mushroom substrates, the origin and the mixing composition of the materials were always the same. The composition of SPCH is stable between batches over time, which makes the product obtained relatively homogeneous in composition.

2.2 Description of the test

The post-culture mushroom substrates SPCH-Ch, SPCH-St, SPCH-CO and the mixture of SPCH-Ch and SPCH-St (50% by weight of each substrate) were used in comparison with a commercial compost as a control treatment (**Table 1**). To evaluate the chemical and physical characteristics of the SPCH treatments as semi-soil substrates, the chemical parameters were determined: pH and electrical conductivity (EC) in solution 1:2.5 (p/v) on fresh sample, dry matter (DM) determination at 105 °C[18], organic carbon by oxidation method with potassium dichromate[19] considering a recovery factor (1.29), total N by Kjeldahl[18] and physical parameters determined on fresh sample (smf): bulk density (Da) and real density (Dr) on unaltered samples of substrate according to Hao *et al.*[20]. The Da was determined with a test tube, establishing the relationship between the weight and the volume it occupies, and the Dr was determined based on the pycnometer methodology, determining the volume occupied by a given weight of substrate. The relationship between both densities allows the calculation of the total porosity (ε) of the sample.

Table 1. Description of treatments and initial moisture conditions for germination of lettuce and chili pepper cultivars

Treatment	Substrate weight per fresh alveolus (g)	Initial humidity (%)	Initial water content per alveolus (g)
SPCH-Ch	25	72	18
SPCH-St	17	82	14
SPCH-CO†	24	44	13
Mixture†	21	41	12
Compost	17	73	12

SPCH-Ch: champignon post-culture substrate; SPCH-St: seta post-culture substrate; SPCH-CO: composted SPCH-Ch; Mixture: SPCH-Ch and SPCH-St; Compost: commercial compost. †At the initial moment of the SPCH-CO and mixture treatments, 2.5 mL of distilled water were added.

Table 2. Characterization of the main chemical parameters of the materials used as substrates

Material	pH (1:2.5) Ud. pH	EC (1:2.5) dS m^{-1}	Dry matter % smf	Organic carbon % sms	Total N (N mineral) % sms	C/N
SPCH-Ch	7.36 ± 0.01^{c}	10.87 ± 0.06^{b}	29.4 ± 0.9^{b}	29.9 ± 0.5^{c}	2.7 ± 0.07^{a} (0.5)	11
SPCH-St	8.14 ± 0.04^{a}	2,79 ± 0.02^{d}	22.7 ± 0.7^{c}	48.1 ± 0.8^{a}	1.1 ± 0.03^{d} (0.2)	43
SPCH-CO	7.44 ± 0.20^{c}	11.20 ± 0.20^{a}	54.7 ± 0.4^{a}	24.4 ± 0.3^{d}	1.8 ± 0.04^{c} (0.3)	13
Mixture	7.93 ± 0.05^{b}	7.62 ± 0.15^{c}	23.3 ± 1.9^{c}	34.0 ± 1.1^{b}	2.1 ± 0.02^{b} (NA)	17
Compost	7.14 ± 0.05^{d}	1.14 ± 0.08^{e}	55.7 ± 1.1^{a}	18.6 ± 0.5^{e}	0.7 ± 0.01^{e} (NA)	27
Significance	***	***	***	***	***	-

SPCH-Ch: post champignon cultivation substrate; SPCH-St: post seta cultivation substrate; SPCH-CO: composted SPCH-Ch; Mixture: SPCH-Ch and SPCH-St; Compost: commercial compost. EC: electrical conductivity; smf: expressed on fresh material; sms: expressed on dry matter, the determination of organic carbon by oxidation considers 58% of the organic matter as organic carbon (n = 3 ± standard deviation). NA: not analyzed. Mineral N: $N\text{-}NH_4^+ + N\text{-}NO_3^-$.
Different letters between substrates in each column indicate significant differences $p < 0.05$; Duncan's test.

Water retention was determined according to UNE 77332:2003[21], which consists of saturating the fresh substrate with distilled water, occupying all the pores with water, and then allowing the water to drain freely by gravity until its cessation (24–48 h). The difference between the weight of the substrate before saturation and after drainage is the water retained, expressed in fresh weight of the substrate.

The water of constitution of the substrate (substrate moisture) is obtained by drying the fresh sample at 105 °C.

To evaluate the effect of different treatments on germination and seedling development, 40-day germination chamber trials were conducted with lettuce seeds (*Lactuca sativa* L., variety 'Venegia Seminis') coated with fungicide treatment (thiram) (16 July-26 August 2019) and Ibarra chili pepper seeds *(Capsicum annuum* L., variety 'Ibarroria') (5 November-17 December 2019).

Germination was evaluated after 10 days, but because a delay in germination was observed in certain treatments, the percentage was calculated from the count data of viable seedlings before seeding. After the end of the trial, the plants were harvested and the aerial part and root were separated and the biomass was determined.

Both trials were carried out under controlled conditions: 16 h of light, 25 °C in day and 18 °C in night. The environmental humidity was kept high by placing trays with distilled water in the lower part of the chamber.

At the beginning of each trial, the treatments were moistened to obtain 12–18 g of water per alveolus to guarantee germination (**Table 2**). Irrigation was about 3 times per week, controlling that no leaching occurred.

Each trial consisted of 3 blocks (3 replicates), one per tray of alveoli, and in each block 3 seeds per treatment (total 9 seeds per treatment).

2.3 Statistical analysis

Statistical analysis was performed using the SAS v8 statistical package[22], based on the analysis of variance (complements 1 to 4), considering the significance levels (p): * ($0.05 \leq p < 0.01$); ** ($0.01 \leq p < 0.001$); *** ($0.001 \leq p < 0.0001$). Values of $p > 0.05$ were considered non-significant (NS). Separation of means was performed using the DUNCAN test.

3. Results and discussion

3.1 Chemical characterization of the materials used as substrates for seedbeds

The chemical properties of the materials used (**Table 2**) show a pH between neutral and basic (7.1–8.1), which does not limit the availability of nutrients as a horticultural substrate. The EC shows important differences between the substrates used, varying between 1.1 dS m^{-1} and 11.2 dS m^{-1}. The SPCH-Ch and SPCH-CO treatments show the highest values, which conditions the germination of the seeds of both crops (**Figure 1**). These data are in agreement with those found by Postemsky and López-Castro[15], indicating that high EC values are related to the effects of osmotic drought and toxicity due to salt accumulation. The effect of salinity on SPCH-Ch had no effect when it was applied as a soil amendment for lettuce production[23], presumably due to a dilution effect in the soil.

In relation to DM content, SPCH-CO and compost substrates presented about twice as much (~55%) with respect to the rest (29–23%). In general, the weight loss of the substrates depends on the carbon source[24]. These differences are going to have implications from the point of view of handling and transport of these materials. The results obtained in relation to organic carbon and total N content show differences among all treatments, highlighting SPCH-St with the highest carbon content and a low total N content, leading to a high C/N ratio above 40. The C/N ratio is an appropriate chemical indicator of the stability of a plant substrate. The value of this ratio should not be higher than 30, since at higher values microorganisms can immobilize N, and compete for this nutrient with the plant[25]. In these cases, the addition of mineral N to the substrate would prevent its immobilization[15]. The commercial compost had the lowest organic carbon and total N content, with a C/N ratio slightly below 30. The rest of the treatments had C/N ratios between 11 and 17, which favor N availability for the crop.

The mineral N content in SPCH-Ch was presented in ammoniacal form, representing 19% of total N. This value may be partly due to the presence of poultry manure in its initial composition. In the case of SPCH-CO, the composting process involved a reduction of mineral N attributed to losses occurring during the process and to the mineralization of part of the ammoniacal N to nitrate[26].

3.2 Physical characterization of materials used as substrates for seedbeds

Physical parameters such as density (real and apparent), water retention, air space and total porosity are shown in **Table 3** and **Figure 2**.

Although there are no accepted standards regarding the physical properties of substrates, some authors have observed the optimal ranges most commonly used for horticulture[16,17]. These ranges expressed in volume are: total porosity between 50% and 85%, air space between 10% and 30%, available water between 25% and 35%, unavailable water (make-up water) between 20% and 35% and bulk density expressed on dry matter (sms) between 150 kg sms m^{-3} and 700 kg sms m^{-3}. In relation to the values obtained in this test, the total porosity in all substrates is in the proposed range, except SPCH-CO (44%) which presents a slightly lower value. In small volumes of containment, the substrate must have a maximum water retention without losing aeration capacity[27]. Therefore, both physical properties, air porosity and water retention of substrates are considered important properties when substrates are used in small containers, due to their impact on the conditions for plants to ensure adequate oxygen and water supply. In relation to air space, SPCH-St and the mixture exceed the limit of the optimum range (59% and 42% respectively vs. optimum 10–30%), and present values lower than the optimum range, both in relation to available water (18% and 19% respectively vs. optimum 25–35%, equivalent expressed on fresh matter (smf) to 820 kg water t^{-1} smf and 486 kg water t^{-1} smf) and Da (50 kg sms m^{-3} and 90 kg sms m^{-3}). These differences could possibly be associated with the presence of macropores. In the SPCH-St, the presence of straw (little evolved) is visually identifiable.

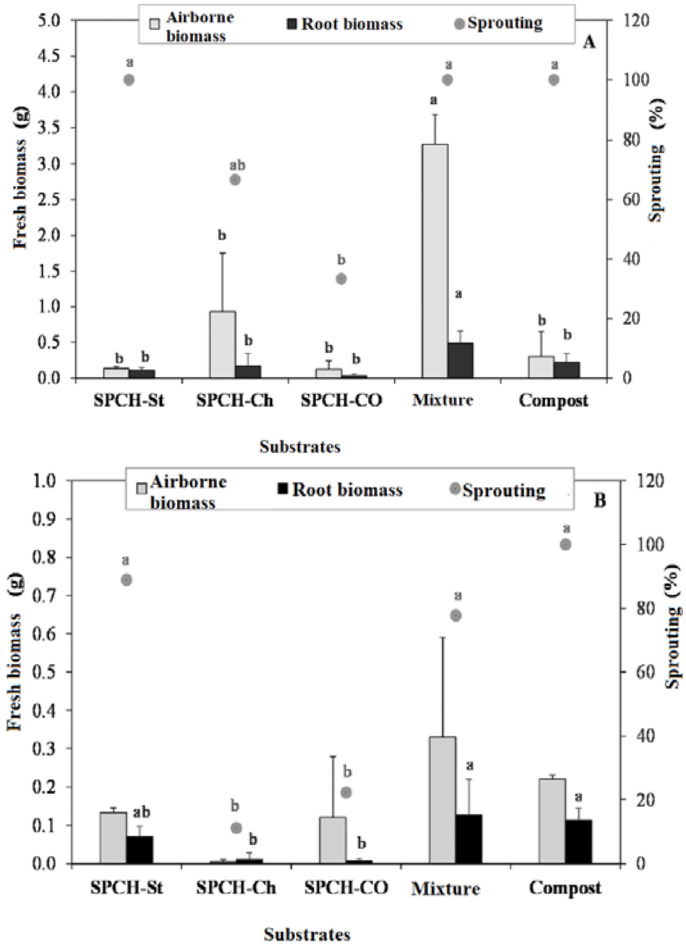

Figure 1. Mean values (n = 3) of the evaluated parameters of fresh biomass in lettuce (A) and chili pepper (B) at the end of the trial. Bars represent the standard deviation.
SPCH-St: seta post-culture substrate; SPCH-Ch: champignon post-culture substrate; SPCH-CO: composted SPCH-Ch; Mixture: SPCH-Ch and SPCH-St; Compost: commercial compost. Different letters between substrates for each parameter indicate significant differences (*p* < 0.05; Duncan's test).

Table 3. Mean values of bulk density (Da), real density (Dr), total porosity (ε) and water retention of the material used

Material	Da kg smf m^{-3}	Dr kg smf m^{-3}	ε % (v/v)	Water retained kg water t^{-1} smf
SPCH-Ch	472 ± 36 (140)^{b}	1,050 ± 137^{ab}	54.5 ± 6.9^{c}	649 ± 72^{c}
SPCH-St	224 ± 13 (50)^{d}	964 ± 21^{b}	76.9 ± 1.2^{a}	820 ± 108^{b}
SPCH-CO	640 ± 34 (350)^{a}	1,148 ± 64^{a}	44.2 ± 0.9^{d}	606 ± 89^{c}
Mix	387 ± 24 (90)^{c}	979 ± 56^{b}	60.5 ± 3.5^{b}	486 ± 18^{d}
Compost	472 ± 4 (263)^{b}	1,116 ± 58^{a}	57.7 ± 1.9^{bc}	952 ± 25^{a}
Significance	***	**	***	***

SPCH-Ch: mushroom post-culture substrate; SPCH-St: mushroom post-culture substrate; SPCH-CO: composted SPCH-Ch; Mixture: SPCH-Ch and SPCH-St; Compost: commercial compost. smf: on fresh matter; n = 6 ± standard deviation. Value in parentheses in the Da column is expressed kg sms m^{-3}.

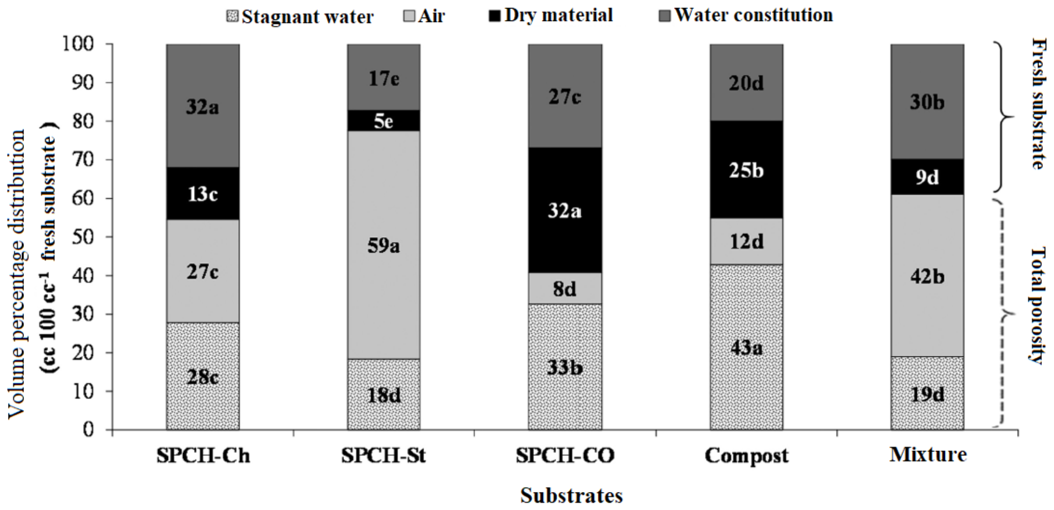

Figure 2. Percentage distribution in volume (n = 6) in total porosity: water and air volume retained after 24 h of dredging, and fresh substrate: dry matter and constituent water (substrate dried at 105 °C). SPCH-Ch: post champignon substrate; SPCH-St: post seta substrate; SPCH-CO: composted SPCH-Ch; Mixture: SPCH-Ch and SPCH-St; Compost: commercial compost. Different letters between substrates for each parameter indicate significant differences ($p < 0.05$; Duncan's test).

In contrast, SPCH-CO has a slightly lower air percentage (8% vs. optimum 10–30%), although the water retention percentage is slightly higher (43% vs. optimum 25–35%) than the optimum values. The water retention property of a substrate is related to the higher proportion of small-sized pores, due to their ability to adsorb water on their walls and the shape of the particles[15]. So the origin of organic materials affects the porosity and water-holding capacity of the substrate, presumably associated the shape and size of the particles[28]. A crushing or chopping process conditions the particle size, so that in these treatments, SPCH-St and mixing, in addition to uniformizing the product and reducing the macropores, would increase the Da and favor water retention in this process[15].

In that sense, in relation to the physical properties of the substrates, the least adequate treatment would be SPCH-St for fresh use, because it is out of all the ranges in the parameters analyzed except for total porosity according to the standards for an optimal substrate according to Yeager *et al.*[16] and Bilderback *et al.*[17].

The physical properties of the substrates were evaluated before seedbedding, a fact to consider when using SPCH to grow plants in containers due to the low stability of MO, Da and porosity, parameters that can be affected throughout the plant culture and this is perceptible because the substrate "contracts" in the container[15]. This reduction in the volume of the substrates in the alveolus was detected viually in all treatments, including the commercial substrate as time elapsed in both trials.

3.3 Effect of the different treatments on germination and seedling development

In reference to lettuce germination at 10 days, the SPCH-St, mixture and compost treatments germinated all 9 seeds (100%); in the SPCH-Ch and SPCH-CO treatments only 1 of 9 seeds germinated per treatment. But at the end of this trial in these treatments germinated 6 of 9 seeds in the SPCH-Ch and 3 of 9 in SPCH-CO (67% and 33% respectively), which means that there was a delay in germination. In studies conducted with beans, the authors found maximum germination after 21 days regardless of the substrate used[29]. In the chili pepper trial, germination was evaluated at the end of the trial and showed that the compost treatment was the only one in which 100% of the seeds germinated; in SPCH-St and mixture, 8 and 7 seeds germinated out of 9 for each treatment respectively (89% and 78% respectively), and in SPCH-Ch and SPCH-CO only 1 and 2 out of 9 seeds germinated per treatment (11% and 22% respectively). In these last two treatments, in both trials the germination suppressing effect (**Figure 1**) is attributed to salinity due to its high EC values (**Table 1**) that would significantly affect seed germination.

The biomass (aerial and root) showed significantly higher values in the mixed treatment compared to the rest of the treatments in the lettuce trial. In the case of chilli, the highest biomass value was also obtained in the mixture, but no differences were detected, presumably due to the high variability between replicates.

Considering the low availability of mineral N in SPCH-St, associated with its high C/N ratio and the high salinity of SPCH-Ch and SPCH-CO, which could affect the development of the crops evaluated, the SPCH-St and SPCH-Ch mixture is a suitable option that, in addition to reducing salinity and C/N ratio, incorporates mineral N in ammoniacal form.

The sensitivity of the vegetable crop can be determinant for the response of the crop to the substrate used, Collela *et al.*[30] used SPCH-St as a seedling substrate and obtained vigorous and quality tomato seedlings as in the commercial substrates tested. Also the use of SPCH mixed with anaerobic digestion residues produced good results in vegetable seedlings for tomato and bell pepper[31]. These authors concluded that its use as a growth medium can replace peat for the production of these species.

When SPCH is mixed with peat in different proportions for lettuce seedlings, 50% SPCH mixture showed better values in relation to lettuce growth than when only peat was used, although without significant differences between them or in the different mixtures[32].

In our case, the mixed substrate treatment showed good germination and the best values in relation to plant development (aerial and radicular biomass) compared to the rest of the SPCH treatments evaluated, with values equal to or higher than the commercial substrate (**Figure 1**) in lettuce and chili pepper.

4. Conclusions

The biomass obtained in all treatments showed similar values to those obtained with the commercial substrate. The post-culture substrates of fresh and composted mushroom fungi (SPCH-Ch; SPCH-CO) had a negative effect on the nascence of lettuce and chili pepper, possibly due to their high salinity, so their direct use in seedbeds would not be advisable in salinity-sensitive crops. It would be advisable to carry out washes to reduce salinity or to use mixtures with other substrates. The use of SPCH-St, due to its high C/N ratio, would limit the availability of mineral N for crop development, making it necessary to provide N in the form of mineral fertilization.

The mixture of champignon and fresh seta substrates, combining the positive properties of both substrates, produced the highest biomass yields in lettuce seedlings. In the case of chili peppers, no differences were observed with respect to the commercial substrate associated with inter-run variability.

The results obtained confirm the possibility of reusing these materials for use as substrate in seedbeds. It would be advisable to carry out tests with different doses of each substrate mixture associated with the crop to be used, and to consider a pre-existing crushing to favor the uniformity of the mixture, reduce macroporosity and increase water retention.

Supplementary material

Supplementary material to this article can be found at URL https://doi.org/10.12706/itea.2021.004.

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