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Spent oyster mushroom substrate in a mix with organic soil for plant pot cultivation

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ABSTRACT

Disposal of spent mushroom substrates can pose a problem to the environment. The re-use in crop production by its recycling as an ingredient of growing mixes would provide an environmentally safe disposition. We show the use of *Pleurotus* sp. spent substrate, obtained after cultivation on a sunflower seed hulls based substrate and mixed with organic soil from local nurseries. *Salvia officinalis* was used to study plant growth in pot cultivation under greenhouse conditions. Treatments were: C, control (soil used in a local nursery); T1, soil : *Pleurotus* spent substrate (2:1 v/v); and T2, equivalent to T1, but using washed *Pleurotus* spent substrate in order to reduce its salt content. T1 substrate had 3.3 times higher electrical conductivity (7 mS cm^{-1}) than that of the control, which is high for the growth of most plants. Air porosity was greater in T1 (7.4%) and T2 (10.2%) than the control (2.8%). The content of certain nutrients also increased with regard to the control, such as phosphorus and sulphur (T1 and T2 substrates), as well as potassium (T1 substrate). After growing 29 days on T1 substrate, plants showed a marked increase in biomass (*ca.* 21%, $p < 0.05$) and some minerals compared to the control. T2 plants grew poorly, possibly because of nitrogen deficit. T1 substrate was adequate to sustain the growth of *S. officinalis* plants in pots, by improving air porosity and mineral content.

Key words: Oyster mushrooms, plant pot cultivation, *Salvia officinalis*, spent mushroom substrate, waste recycling.

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INTRODUCTION

At present, the commercial production of edible and medicinal mushrooms is carried out on substrates formulated with lignocellulosic materials of different sources (*i.e*., sawdust, straw, corncobs, coatings of oleaginous seeds), alone or mixed with supplements to overcome nutritional limitations and to provide suitable substrate structure and pH. After mushroom cultivation, a considerable amount of spent substrate remains as residual material. Several studies have shown the potential use of the spent substrate of different mushroom species (mainly *Agaricus bisporus* and *Pleurotus* spp.) in purification of water and soils, cultivation of other mushroom species, cultivation of vegetables, biological control of pests, vermiculture, as well as its use as animal feed, alternative fuel, and source of degradative enzymes[18].

Sunflower seed hulls are a residue of the edible oil industry in many countries, including Argentina (processing plants in Bahía Blanca city may produce 200 ton of sunflower seed hulls per day[5]). It accounts for 18-20% of the processed seed[12], and it is scarcely utilized, although research is being carried out in Argentina on its possible applications as soil amendment[19] and as a component of a substrate for the cultivation of ornamental plants in nurseries[9]. The use of sunflower seed hulls as a basal substrate for the cultivation of several edible mushrooms, such as *Pleurotus* spp.[8], *Ganoderma* spp.[11], *Lentinula* spp.[6], *Hericium erinaceus*[10], and *Agaricus* spp. (unpublished data), has also been investigated.

There is paucity of information about the use of the spent substrate from *Pleurotus* spp. (PSS) as soil co-substrate for the cultivation of plants[18], particularly the PSS derived from the cultivation of oyster mushrooms on substrates based on sunflower seed hulls. This spent substrate has mineral nutrients, high content of organic matter, and low apparent density. The feasibility of using the PSS in soil mixtures for plant (*Salvia officinalis*, common sage) culture in pots was investigated. Physical, chemical, and nutritional properties of soil mixed with PSS and washed PSS (WPSS) were evaluated, as well as their physiological effects on plant growth and nutritional status.

MATERIALS AND METHODS

Plant material and substrates. Seeds of *S. officinalis* L. (pretreated with 0.2% captan fungicide, Feltrin, Farroupilha, Brazil) were sown in multicell trays containing a substrate consisting of soil (argiudoll, 10.8% organic matter content, previously disinfected for one hour by autoclave at 1 atm, 121 C) used in local nurseries, sphagnum peat (Simonetta, Berazategui, Argentina), and perlite in a mixture 1:2:1 by volume, respectively. The soil used was from Sierra de la Ventana region, in Buenos Aires Province, Argentina. The soil and perlite were passed through a 2 mm mesh sieve.

Thirty six seedlings of *S. officinalis* (25 days old, 1 cm height) were transplanted to individual 12 cm high (*ca*. 710 cm^3) pots containing the substrates studied (12 plantlets per treatment). Tested substrates were made with soil (same as the one described previously, but passed through a 1 cm mesh sieve and without disinfection) and either fresh PSS or WPSS. The PSS used derived from a sunflower seed hulls based substrate prepared according to Curvetto[7]. It was obtained after 2 mushroom flushes, as almost 85% of the maximum potential yield has normally been harvested

at this stage. The PSS was passed through a chopper (1 cm mesh sieve) in order to disintegrate the compact PSS. In the case of WPSS, disintegrated PSS was submerged in tap water overnight (1:3 v/v ratio). Then it was drained, and dried for 72 h at 60 C. Experimental treatments were: soil as control (C); soil and PSS, 2:1 v/v (T1); and soil and WPSS, 2:1 v/v (T2).

Pots were placed in a greenhouse with minimum and maximum temperatures of 16 C and 27 C, respectively, and a photoperiod corresponding to spring (September-October). They were randomly distributed according to a 9x4 block design, and rotated once a week until the end of the experiment, approximately 65 days after plantlet transplanting. Water was provided as needed, every 2-3 days, avoiding excess watering.

Plant growth. Plant height and biomass were determined. Height, from the base of the shoot to the apical bud, was measured in all plants (12 per treatment) every 2-3 days during 13 days (starting from the end of second week after plant transplant to substrates). For biomass determination, the dry weight of shoots and leaves of the plants (8 per treatment) was determined 29 days after transplanting. Drying was carried out at 70 C until constant weight.

Relative water content (RWC). Thirty seven days after plant transplanting, totally expanded leaves (n= 4), from plants watered a day before, were sampled to determine their fresh weight (FW); turgid weight (TW, obtained after storing the leaves submerged in distilled water for 24 h at 4 C, in darkness); and dry weight (DW, obtained after drying at 70 C until constant weight). Relative water content (%) was then calculated as follows: RWC (%) = [(FW-DW)/(TW-DW)] x100.

Chemical Analysis

Chlorophyll leaf content. The chlorophyll extraction was basically done according to the method described by Holden[13]. Fresh leaf material from three different plants per treatment (n=3) was homogenized with 80% aqueous solution of acetone (Ac80), saturated with $MgSO_4$ crystals to avoid phaeophytin formation. Chlorophyll absorbance was measured at 645 nm (A_{645}) and at 663 nm (A_{663}) using a Metrolab 1600 Plus spectrophotometer (UV-Vis Metrolab, Bernal, Argentina). Chlorophyll content was determined using the formula corrected by Porra[17]: Total Chlorophyll (µg/ml)= 0.895 [20.2 (A_{645}) + 8.02 (A_{663})]. Results were expressed in g of chlorophyll per g of leaf dry matter.

Analysis of tissue and substrate mineral content. The plant leaves and shoots, previously used in biomass determination, were milled with a Willey mill (1,430 rpm) using a #40 mesh sieve. Samples corresponding to each treatment were then pooled to obtain material for mineral tissue analysis. Each of these samples (100 mg) were then subjected to a humid digestion, according to the procedure described by Campbell and Planck[3].

Dried substrate samples (4 g) were milled in mortar, sieved through 2 mm mesh, and mixed with Mehlich 3 solution[14] in 1:10 w/v ratio to yield the extracted solution.

The content of P, K, Mg, Ca, S, Mn, Fe, Cu and Zn was measured in both substrates and plants, and Na was only measured in substrates. For mineral analysis, an Inductively Coupled Plasma Optical Emission Spectrometer, Shimadzu 1000 III (Shimadzu, Tokyo, Japan) was used.

Nitrogen content. Kjeldahl's method was used to determine the total nitrogen content in plant and substrate samples (50 mg and 250 mg aliquots, respectively). An

automated Büchi (Postfach, Switzerland) Distillation Unit B-324 was used for ammonia distillation.

Organic matter content. After dry matter (DM) determination of substrate samples (n= 3) by drying at 105 C for 72 h, they were calcined at 550 C for 4 h to determine ash content. The organic matter content (OM), as percentage of DM, was determined after subtracting the ashes from DM. This method usually results in OM values higher than those obtained by other methods[4].

Electrical conductivity (EC) and pH. These variables were measured following the saturated paste extract method described by Warncke[20]. Readings of electrical conductivity were performed on the saturated extracts using an electronic conductimeter (Lutron WA-300).

Physical properties of substrates. Cylinders of 440 cm^3 capacity (7.6 cm in diameter, 9.8 cm in height), with a central drainage hole in their base, were used. Each cylinder was provided with an upper end removable supplementary ring, which increased the cylinder height approximately 4 cm. All determinations were done using 3 replications per treatment.

Apparent density (AD), air porosity (AP), and container capacity (CC) determinations were performed adapting techniques described by Ansorena-Miner[1] to cylinders previously described. Effective porosity (EP) was also determined. These variables were calculated as follows: AD= DW of the substrate/cylinder volume; AP (%)= (drained volume/cylinder volume) x 100; and CC (%)= 100 x [(Weight of moist sample-Weight of dry sample)/(cylinder volume) (specific weight of pure water)]; and iv) EP (%)= AP + CC.

Statistical analysis. All designs were balanced, and a 5% significance level was chosen for all analysis. Depending on the case, variables were analyzed using the t test (assuming either equal or unequal variances, depending on the F test results), or analysis of variance (ANOVA). When ANOVA was significant, multiple comparisons of the means were done using the Tukey's test. Previous to t tests, when n>5, the homoscedasticity was tested using the F test (data not shown).

RESULTS AND DISCUSSION

The PSS used was rich in mineral nutrients (data not shown), and as expected with a very high EC (> 15 mS cm^{-1}) and a high content of organic matter (**Table 1**). Washing produced a marked diminution of its salt content, as shown by EC values of WPSS. PSS would not be suitable as sole component of plant growing media, because its high AP and low CC (**Table 1**). A low CC leads to very frequent watering in most pots (CC depends on substrate column height) in order to fulfill plant demands. **Table 2** shows physical characteristics of the substrates used in this experiment, as well as optimum ranges. The addition of either PSS or WPSS to the soil reduced AD in both T1 and T2 substrates compared to the control. This reduction can be explained by the low AD values of PSS and WPSS (**Table 1**), which is a desirable effect, as labor required for transporting and handling of pots is reduced.

According to Ansorena-Miner[1], AP is probably the most important physical characteristic of substrates. Media must have an adequate proportion of air filled pores to adequately support plant growth. AP did not differ significantly between T1 and T2 ($p = 0.1215$) but, in both cases, it was significantly different and higher than the control. Possibly, there was an

Table 1. Chemical and physical characteristics of *Pleurotus* spent substrate (PSS) and washed *Pleurotus* spent substrate (WPSS).

Characteristic	PSS	WPSS
MC (%)	46.9 ± 0.3	43.5 ± 0.1
pH	6.9-7	7.5-7.6
EC (mS cm^{-1})	15.42 ± 0.34	2.85 ± 0.29
OM (%)	87.5 ± 0.1	92.0 ± 0.0
AD (g cm^{-3})	0.109 ± 0.001	0.108 ± 0.001
AP (%)	60.3 ± 1.8	59.2 ± 1.9
CC (%)	26.2 ± 1.8	27.4 ± 1.5
EP (%)	86.5 ± 0.3	86.7 ± 0.5

MC= Moisture content. EC= Electric conductivity. OM= Organic matter. AD= Apparent density. AP= Air porosity. CC= Container capacity. EP= Effective porosity. ± = Standard error.
n= 3, except in pH and EC, in which case n= 2.
pH determined in saturated paste; EC determined in saturated paste extract.

increase in the proportion of larger pores due to the addition of either PSS or WPSS. The increase in AP was accompanied by a reduction in CC (**Table 2**), although no significant differences were found among treatments (p = 0.1398). Decreases in CC values of T1 (1.1%) and T2 (3.5%), with regard to the control, were smaller than increases in corresponding AP values (T1: 4.6%; T2: 7.4%). This resulted in EP values for T1 and T2 larger than that of the control (**Table 2**), although these differences were not significant (but not far from significance level, since p = 0.0885). These results may be explained by the presence of material having larger mean particle size with respect to the control, and a sufficiently large proportion of smaller internal pores capable of retaining water at a tension higher than that determined by substrate column height[2]. PSS and WPSS had these characteristics, they were, at first sight, a coarser material than the soil used in this assay. Besides, "dead volume" of lignified cells, and the space or volume between cells present in sunflower seed hulls might become open pore space due to hyphal growth.

Chemical analyses of substrates tested are shown in **Table 3**. In all cases, the pH was slightly basic (7.2-7.6), which could be inadequate for some plants. T1 and T2 had higher OM content than the control, which reflects the high content of OM present in PSS and WPSS. There were remarkable relative increases in the content of P, Zn and S, in response to the addition of either PSS or WPSS to the soil. There was also a marked increase in the content of K (24%) in T1 with respect to the control, while a decrease in T2. Similar was the case of S content, which was also higher in T1. With respect to Na, both T1 and T2 had a higher content than the control (*ca*. 20%). This is an undesirable effect of PSS and WPSS addition, because Na is not an essential element for most plants, but it is capable of lowering the osmotic potential of the substrate solution and, therefore, reducing water availability. Tap water used to wash PSS did not have high Na concentration (60 mg L^{-1}), therefore lower Na levels were expected in T2 with respect to T1. It is also possible that most of the Na in PSS was adsorbed in the exchange complex of this material, and therefore it was not washed off by water. Low levels, with regard to the control, of Mn, Ca, and Cu present in PSS and WPSS, affected these nutrient levels in T1 and T2, respectively, whose values were 15-25% lower in comparison with the control.

EC values in the soil and T2 substrate were

Table 2. Physical characteristics of substrates: nursery soil used as control (C), T1 (soil and PSS, 2:1 v/v), and T2 (soil and WPSS, 2:1 v/v). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

Characteristic	Optimum values*	C	T1	T2
MC (%)		21.3 ± 0.1	25.9 ± 0.1	26.4 ± 0.1
AD (g cm^{-3})	< 0.4	0.795 ± 0.004^a	0.624 ± 0.002^b	0.588 ± 0.005^c
AP (%)	10-30	2.8 ± 0.5^b	7.4 ± 0.7^a	10.2 ± 1.1^a
CC (%)	55-70	59.4 ± 0.5^a	58.3 ± 0.8^a	55.9 ± 1.6^a
EP (%)		62.2 ± 0.9^a	65.7 ± 0.4^a	66.1 ± 1.6^a

* According to Ansorena-Miner[1].
MC= Moisture content. AD= Apparent density. AP= Air porosity. CC= Container capacity. EP= Effective porosity. ± = Standard error. Different superscript letters indicate significant differences ($p< 0.05$) among columns according to Tukey's multiple comparison test (n= 3).

similar and within the optimum range for plant growth (2.0-3.5 $mScm^{-1}$)[20]. By contrast, the addition of 1/3 on a volume basis of PSS to the soil (T1) resulted in an increase of EC up to a level considered excessively high for cultivation of most plants. This high level remained in T1 throughout the experiment, although EC decreased 35%, from an initial value of 7 mS cm^{-1} to 4 mS cm^{-1} at the end of the experiment, approximately 40 days later. Nevertheless, T1 plants had a mean biomass significantly higher than that of the control (**Table 4**). More leaves sprouting from axillary buds were observed in T1 compared to the control, whereas its occurrence in T2 was scarce as shown in **Fig. 1**. Moreover, the height of T1 plants was not significantly lower ($p = 0.2916$) than that in the control. The mean height of plants belonging to different treatments is shown in **Fig. 2**. Plants of T2 treatment presented a remarkably lower height and biomass being 49.5% and 74.5% smaller compared to the control, respectively (**Table 4**, **Fig. 1**).

The RWC of plants grown on soil used as the control was significantly higher than that of plants grown on substrates T1 and T2 (**Table 4**). Leaf wilting was not observed in any treatment. It should be mentioned that common sage is a species of known drought tolerance[16].

Fig. 1. Appearance of 54-day-old *Salvia officinalis* plantlets after growing for the last 29 days in pots containing (from left to right) nursery soil (rows 1-2), soil and WPSS (2:1 v/v; rows 3-4), and soil and PSS (2:1 v/v; rows 5-6). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

Table 3. Chemical analysis of substrates: nursery soil used as control (C), T1 (soil and PSS, 2:1 v/v) and T2 (soil and WPSS, 2:1 v/v). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

Characteristic	C	T1	T2
pH [a]	7.5-7.6	7.2	7.5
EC (mS cm^{-1}) [b]	2.1 ± 0.14	7.00 ± 0.04	2.25 ± 0.12
OM (%) [c]	10.8 ± 0.0	15.4 ± 0.1	15.2 ± 0.1
Elements (mg dm^{-3}) [d]			
N	3,896 ± n/a	3,469 ± n/a	3,034 ± n/a
Na	60.0 ± 3.2	70.5 ± 5.1	71.5 ± 1.7
Mg	382 ± 1	437 ± 11	385 ± 6
P	52.6 ± 3.2	71.2 ± 0.7	73.0 ± 1.1
S	73 ± 2.1	433 ± 10	122 ± 0.9
K	906 ± 34	1,127 ± 21	837 ± 13
Ca	7,346 ± 100	5,902 ± 0	6,259 ± 92
Mn	89.2 ± 1.3	65.1 ± 2.9	65.1 ± 0.6
Fe	64.1 ± 0.3	64.0 ± 2.6	63.6 ± 0.3
Cu	3.2 ± 0.2	2.6 ± 0.0	2.7 ± 0.1
Zn	3.6 ± 0.3	4.6 ± 0.3	5.7 ± 0.2

EC= Electrical conductivity. OM= Organic matter. ± = Standard error.
[a] n= 2; determined in saturated paste.
[b] n= 2; determined in saturated paste extract.
[c] n= 3.
[d] n= 2; the unit mass/volume is used instead of mass/mass because of differences in the apparent density among substrates.

During the experiment, no chlorosis symptoms were seen in plants belonging to all treatments. However, in total chlorophyll content analysis of leaves (**Table 4**), T2 plants had a significantly lower value than those in the control and T1. In terms of the chlorophyll content, there was a tendency to decrease from the control to T1 and T2 plants. This tendency was also registered for RWC. The relationship between chlorophyll content and RWC was also observed by Munné-Bosch and Alegre[15] in *S. officinalis* plants exposed to water deficit. They suggested that it could be a protective mechanism against activated oxygen species in water stress conditions.

Chemical analysis of leaves and shoots showed that T2 plants had a markedly lower N content (40%) with regard to that of the control and T1 plants (**Table 5**). Nitrogen deficiency, which is consistent with a lower N content (22%) of T2 substrate with regard to the soil, may also explain the lower chlorophyll levels and the impaired growth observed in these plants. T2 plants showed, for P and S content, values much higher than those present in plants grown

Table 4. Height, biomass, relative water content (RWC), and leaf chlorophyll content of *Salvia officinalis* plants grown on different substrates during 27-37 days. C, nursery soil used as control; T1, soil and PSS (2:1 v/v); and T2, soil and WPSS (2:1 v/v). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

Characteristic	C	T1	T2
Height (cm) [1]	9.3 ± 0.3 [ns 5]	8.5 ± 0.7	4.7 ± 0.4
Biomass (mg) [2]	423 ± 1.7	514 ± 2.5 * [6]	108 ± 0.9
RWC (%) [3]	89 ± 1.1 [a]	84 ± 0.9 [b]	83 ± 0.7 [b]
Chl (mg g^{-1}) [4]	8.51 ± 0.59 [a]	8.47 ± 1.71 [a]	5.50 ±0.83 [b]

Chl= Chlorophyll.
[1] 27 days after transplanting, n = 12.
[2] 29 days after transplanting, n = 8.
[3] 37 after transplanting, n = 4.
[4] 37 days after transplanting, n = 3.
[5] *t* test assuming unequal variances (C vs. T1). ns= Non significant differences.
[6] *t* test assuming equal variances (C vs. T1), the asterisk indicates a highly significant difference ($p < 0.01$).
Different superscript letters indicate significant differences ($p < 0.05$) among columns, according to Tukey's multiple comparison test.

Table 5. Elemental chemical composition of leaves and shoots (n= 2) of *Salvia officinalis* plants. Experimental treatments: C, nursery soil used as control; T1, soil and PSS (2:1 v/v); and T2, soil and WPSS (2:1 v/v). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

Elements	C	T1	T2
N (% DW)	3.14 ± 0.11	3.99 ± 0.04	1.88 ± 0.03
Mg (% DW)	0.60 ± 0.02	0.85 ± 0.03	0.62 ± 0.03
P (% DW)	0.33 ± 0.02	0.33 ± 0.02	0.56 ± 0.02
S (% DW)	0.22 ± 0.00	0.36 ± 0.00	0.65 ± 0.04
K (% DW)	2.70 ± 0.03	2.70 ± 0.10	2.90 ± 0.03
Ca (% DW)	1.46 ± 0.01	1.82 ± 0.04	1.52 ± 0.08
Mn (mg kg^{-1})	75 ± 0	68 ± 8	68 ± 3
Fe (mg kg^{-1})	100 ± 5	90 ± 5	78 ± 8
Cu (mg kg^{-1})	< 25	< 25	< 25
Zn (mg kg^{-1})	< 25	< 25	< 25

DW= Dry weight.

on soil and T1 substrate. With regard to element tissue content of T1 plants, it was either similar (P, K) or higher than that of the control plants. This was the case for S, Mg, N and Ca, which showed increases of 63%, 41%, 27%, and 25%, respectively, in comparison to control plants. Regarding micro-nutrients, in T1 and T2 plants, lower Mn and Fe levels, compared to the control plants, were observed. These lower Mn values reflect those in T1 and T2 substrates, which had been lower than those of the control soil. With regard to Fe, even though its levels had been similar in all substrates, both T1 and T2 plants showed, respectively, values 10% and 22% lower than that of plants grown on soil.

To account for the diminished growth response observed in T2 plants, aside from N deficit in the substrate caused by the addition of WPSS to soil, there was a 16.6% hydric saturation deficit in plants, contrasting with 15.7% deficit in T1 plants and 11.3% deficit in control plants. A significant reduction in chlorophyll content was also observed in T2 with its consequent negative impact on dry matter production by plants.

The addition of 33% PSS (v/v) to the soil, improved properties of the resulting substrate showing positive effects on AP and nutrient levels, which resulted not only in a plant biomass increase, but also in the improvement of the nutritional status of plants.

A marked decrease in EC values of T1 substrate at the end of the present assay (40 days) was observed. Watering was done as needed, every 2-3 days, in such a way that drainage was minimized. It is possible that more frequent and abundant watering could quickly decrease EC values to levels considered adequate for most plants. However, this could make additional fertilization necessary because of nutrient leaching from substrates. The

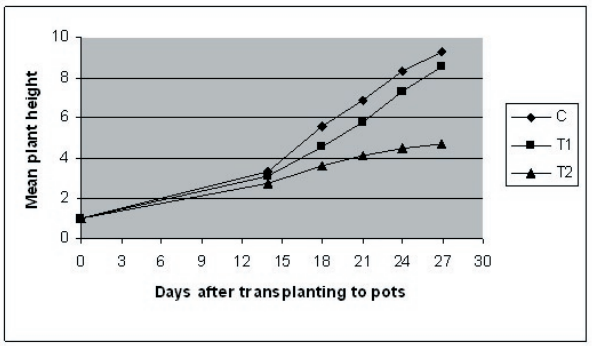

Fig. 2. Height (cm) of *Salvia officinalis* plantlets grown on different substrates during 27 days. C: nursery soil; T1: soil and PSS (2:1 v/v); and T2: soil and WPSS (2:1 v/v). PSS= *Pleurotus* spent substrate. WPSS= Washed *Pleurotus* spent substrate.

PSS could well be of use in the preparation of substrates for plants that, at least in some degree, are capable of tolerating/resisting salinity, *e.g*. geraniums, chrysanthemums, brooms, yuccas and hibiscus, among others[2]. With regard to WPSS, its potential use in substrate formulations is not discarded, because: i) Physical properties of T2 substrate were quite similar to those of T1, ii) Its EC was within optimum values, and iii) No fertilizers were added. In any case, chemical characteristics of a substrate may be modified by the grower during plant growth.

In conclusion, the use of a substrate composed of two parts soil with high organic matter content and one part oyster mushroom spent substrate (obtained after two crops of *Pleurotus* cultivation on a sunflower seed hulls based substrate) resulted in improvement of the growth and nutritional status of common sage plants cultivated in pots. This improvement can be due to the PSS contribution to higher air porosity and content of certain essential mineral nutrients to the substrate. It can also be suggested that washing out excess of salts present in the PSS, which demands time and labor, is not necessary for the cultivation of plants tolerant/resistant to drought or salinity.

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