

# Spent Mushroom Compost as a Substrate for the Production of Lettuce Seedlings

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## Abstract

The successful production of marketable lettuce heads with full exploitation of the genetic potential of a particular cultivar depends on the availability of seedlings of the highest quality. The aims of the present study were (i) to evaluate the effects of substrates containing different proportions of spent mushroom compost on the growth of lettuce seedlings, and (ii) to determine the enduring effects of substrates containing spent mushroom substrate on the vigor of mature lettuce plants derived from such seedlings. The substrates employed in the germination and development of lettuce seedlings were obtained by mixing spent compost from the cultivation of *Agaricus subrufescens* and commercial vegetable substrate in different proportions. Seedlings were evaluated with respect to the number of leaves and the height, fresh mass and dry mass of the aerial parts, while mature plants were appraised according to stem height and circumference, fresh mass and dry mass of the lettuce head. Substrate containing between 42 to 48% spent mushroom substrate provided the most adequate conditions for the growth and development of crisphead lettuce seedlings and, consequently, of vigorous marketable plants. Under such conditions, the fresh mass of the aerial parts of seedlings attained a level of 0.89 g plant<sup>-1</sup>, while the fresh mass of the marketable heads achieved a maximum of 233.45 g plant<sup>-1</sup>. The data presented herein verify that top quality lettuce seedlings yield high quality marketable heads, and that quality improvement can be brought about by the addition of spent mushroom substrate.

**Keywords:** spent *Agaricus subrufescens* substrate, growing media, lettuce seedling production

## 1. Introduction

Lettuce (*Lactuca sativa* L.) is the most common of the salad leaf crops and is currently grown in nearly all countries of the world. According to the Food and Agriculture Organization (2012), the global production of lettuce was around 24,946,142.00 tons in 2012 with a total cropping area of 1,116,220.40 ha. In Brazil, 576,338 tons of lettuce were produced in 2006 (Instituto Brasileiro de Geografia e Estatística, 2006), corresponding to a value of US\$ 220 million, with 69.7% of the cultivation located in the southeast region of the Country. Since lettuce production can be conducted successfully in the form of a small-scale agricultural operation, it is particularly appropriate for family farming and can generate a substantial income (Steffen, Antonioli, Steffen, & Machado, 2010).

The successful production of lettuce with full exploitation of the genetic potential of a particular cultivar depends on the availability of high quality seedlings since the use of inferior planting material results in a significant diminution in yield. Lettuce crops are generally initiated from seeds that have been germinated on good quality substrate contained in expanded polystyrene trays (employed to save space and minimize substrate employed), conditions that typically produce seedlings of premium standard and with high survival index (Rodrigues, Leal, Costa, Paula, & Gomes, 2010). Additionally, selection of uniform seedlings, preservation of root integrity by appropriate handling during transplantation, and an adequate supply of a suitable nutrient are essential prerequisites for the production of vigorous lettuce crops that will require minimal levels of pest control and generate superior products of high commercial value (Leal, Guerra, Peixoto, & Almeida, 2007).

The utilization of spent mushroom compost as an organic manure and soil conditioner in horticulture, or as a dietary supplement in poultry farming, has received some consideration (Rinker, 2002; Santos et al., 2005; Machado, Souza Dias, Santos, & Freitas, 2007; Azevedo, Ávila, Souza Dias, Bertechini, & Schwan, 2009; Ribas, Mendonça, Camelini, & Soares, 2009). Typically, mushroom farms are generally eager to dispose of compost that is no longer capable of producing viable yields of mushrooms since the residue attracts flies and other insects that can carry diseases, and constitutes a potential source of water and air pollution (obnoxious odors) (Zied, Pardo-Gonzalez, Minhoni, & Pardo-Gimenez, 2011). On the other hand, spent compost from the cultivation of *Agaricus* spp. represents a possible alternative substrate for the production of lettuce since the material exhibits some characteristics that are appropriate for the growth of seedlings (Uzun, 2004; Medina, Paredes, Pérez-Murcia, Bustamante, & Moral, 2009; Ribas, Mendonça, Camelini, & Soares 2009; Fidanza, Sanford, Beyer, & Aurentz, 2010; Zhang, Duan, & Li, 2012).

In the light of the above, the aim of the present study was to examine the hypothesis that the quality and yield of lettuce heads may be improved by incorporating spent mushroom compost from *A. subrufescens* into the substrate employed to raise lettuce seedlings. In order to test this hypothesis, experiments were carried out with the following objectives: (i) to evaluate the effects of substrates containing different proportions of spent mushroom compost on the growth of lettuce seedlings, and (ii) to determine the enduring effects of substrates containing spent mushroom compost on the vigor of mature lettuce plants derived from such seedlings.

## 2. Materials and Methods

### 2.1 Preparation of Substrates for the Production of Lettuce Seedlings

Spawn of *Agaricus subrufescens* (strain CS10) was inoculated into substrate obtained by composting 45 kg of sugarcane (*Saccharum officinarum* L.) bagasse, 45 kg of coast-cross (*Cynodon dactylon* L. Pers.) hay, 10 kg of wheat (*Triticum aestivum* L.) bran, 1 kg of superphosphate fertilizer, 2 kg of limestone, 2 kg of gypsum, and 1 kg of ammonium sulfate (Siqueira, Souza Dias, Silva, Martos, & Rinker, 2009). The fully colonized substrate was transferred to a mushroom house and maintained under the conditions described by Siqueira, Martos, Silva, & Souza Dias (2011) until the end of the cultivation cycle. Spent mushroom substrate (SMS), which still contained mycelia, was dehydrated and roughly triturated for subsequent blending. Substrates S1 to S6 employed in the production of lettuce seedlings were prepared by mixing SMS and Bioplant Ouro® (Bioplant Agricola Ltda, Nova Ponte, MG, Brazil) vegetable substrate (the characteristics of which are shown in Table 1) in the proportions indicated in Table 2.

### 2.2 Chemical Analyses

Samples of soil and SMS were sent to the Soil Fertility Laboratory/University of Lavras for chemical analyses according to EMBRAPA (1997).

### 2.3 Production of Lettuce Seedlings and Evaluation of Vigor

The lettuce cultivar Hortência (crisp group) was employed in the study since it presents a culture cycle of 60 to 65 days, is adapted for planting all year round under conventional or hydroponic cultivation conditions, and is resistant to premature bolting (Hortec Tecnologia de Sementes Ltd., 2006). Seeds were sown in substrates S1 to S6 contained in polystyrene trays comprising 200 cells (cell height 47 mm; cell volume 16 cm<sup>3</sup>) arranged in 20 x 10 cell arrays (120 seeds for each treatment). The percentage of germination was determined by counting the number of germinated seeds after 6 days from seeding. Cultures were maintained in the greenhouse under a natural light regime, and irrigation was provided by micro-aspersion. Fertirrigation was not used during the greenhouse experiment. Thinning was performed 17 days after sowing, and plants were transplanted to the field 27 days after sowing. Seedlings were evaluated at transplantation with regard to the number of fully developed leaves, and the height, fresh mass and dry mass of the aerial parts. Height (cm) was estimated using a graduated ruler, fresh mass (g plant<sup>-1</sup>) was determined using analytical scales, and dry mass (g plant<sup>-1</sup>) was measured after drying the aerial parts to constant weight in a forced-air oven at 65 °C.

### 2.4 Transplantation of Seedlings to the Field and Evaluation of Plant Vigor

The randomized experimental plots design comprised four repetitions per treatment. Each experimental unit consisted of 10 plants, spaced 0.30 m apart with 0.35 m between rows (4 rows), with the two central rows being considered usable. Lettuce seedlings were transplanted during the morning period into dystrophic red latosol soil (the characteristics of which are shown in Table 1) that had been treated five days earlier with NPK fertilizer (4:14:8) at a level of 722 kg ha<sup>-1</sup>. Additional treatments with ammonium sulfate at levels of 150, 225 and 225 kg ha<sup>-1</sup> were applied, respectively, at 15, 25 and 35 days after transplantation. Plantlets were irrigated by aspersion on a daily basis during the first two weeks, following which irrigation was reduced to two or three times a week in

order to maintain adequate moisture for plant growth. Weeding was performed every eight days or so throughout the culture cycle. The 10 plants comprising the middle two rows of each of the treatment plots were harvested 41 days after transplantation of seedlings when the heads were considered suitable for commercialization. Plants were cut close to the ground and leaves external to the head were removed. Head circumference (cm) was determined using a measuring tape, while the height of the stem (cm) was estimated with a ruler after cutting the head longitudinally in order to expose the stem. The fresh mass of the head ( $\text{g plant}^{-1}$ ) was determined using analytical scales, and the dry mass ( $\text{g plant}^{-1}$ ) was measured after drying the head to constant weight in a forced-air oven at  $65^\circ\text{C}$ .

### 2.5 Statistical Analysis

Data were submitted to analysis of variance (ANOVA) followed by logistic regression analysis in order to determine which of the substrate combinations exerted the greatest positive effect on the cultivation of lettuce.

## 3. Results and Discussion

### 3.1 Characteristics of Lettuce Seedlings

Germination of lettuce seeds was observed four days after sowing, and emergence was estimated at 92% after six days. While none of the six substrates studied exerted significant effects on the seedlings in respect of number of leaves or height of the aerial parts, statistically significant differences were observed with regard to the fresh and dry masses of the aerial parts (Table 3).

Logistic regression analysis revealed that the fresh mass of the aerial parts of seedlings initially increased according to the proportion of spent mushroom compost in the substrate and, according to the quadratic equation function shown in Figure 1A, attained a maximum value of  $0.89 \text{ g plant}^{-1}$  when the substrate contained 47% of spent compost. Further increases in the proportion of mushroom compost gave rise to incremental decreases in the fresh mass of seedlings. Similar results were obtained with respect to the dry mass of seedlings (Figure 1B), in which case the quadratic function showed a maximum value of  $75.1 \text{ mg plant}^{-1}$  when the substrate contained 42% of spent mushroom compost. These results indicate that substrate with 45% of spent mushroom compost provided the most appropriate conditions for the germination and development of the lettuce seedlings as demonstrated by the superior biomass and vigor of the 27-day old plantlets (Figure 1).

Trani, Novo, Cavallaro-Júnior, and Telles (2004) evaluated the effects of various commercial substrates on the growth of lettuce seedlings and observed significant differences between treatments, with the best results being provided by a substrate based on a mixture of composted *Pinus* bark, peat, charcoal and vermiculite. Smiderle, Salibe, Hayashi, and Minami (2001) tested the addition of soil and sand to a similar type of substrate, but the results were analogous or inferior to those obtained with the substrate alone. Lopes, Boaro, Peres, and Guimarães (2007) reported that the performance of a substrate based on coconut fiber was inferior to those obtained with Plantmax or Golden Mix substrates, indicating that a substrate containing only one component was not suitable for the production of vegetable seedlings. Plantmax is a substrate similar to Bioplant while Golden Mix substrate is constituted basically of coconut fiber and both are commercial substrates commonly used for seedlings production.

Commercial substrates Bioplant and Plantmax are reported to have appropriate physicochemical characteristics for the production of vegetable seedlings (Castoldi, Freiburger, Pivetta, Pivetta, & Echer, 2014) and do not require the inclusion of further components (Rinker, 2002). In contrast, others studies have reported that composts can also show limiting factors for horticultural use, such as the presence of heavy metals, poor physical properties and excess in salts or nutrients that result in high electrical conductivity (Bustamante, Paredes, Moral, Agulló, Pérez-Murcia, & Abad, 2008). In the present study, we have demonstrated that the addition of spent mushroom compost from *A. subrufescens* elicits a significant and increasing improvement in the quality of the commercial substrate until the amount of 45%. Above 45%, the quality of the substrate starts to decrease, probably in function of some limiting factor present in SMS. However, it is important to emphasize that even for the higher amount of SMS (75%) we observed higher seedlings quality compared to the control (0% SMS).

### 3.2 Characteristics of Marketable Lettuces

The effects of producing seedlings using substrates S1 to S6 on the characteristics of marketable lettuces were validated by the significant differences observed in the fresh and dry masses of the heads (Table 4). According to the quadratic function presented in Figure 2A, fresh head mass increased to a maximum value of  $233.45 \text{ g plant}^{-1}$  for plants derived from seedlings developed on substrate containing 48% of spent mushroom compost. Further increases in the proportion of mushroom compost employed in seedling production gave rise to incremental decreases in fresh head mass in the mature plants. Similar results were obtained for dry head mass (Figure 2B), where a maximum value of  $18.1 \text{ g plant}^{-1}$  was attained for plants derived from seedlings developed on substrate

containing 45% of spent mushroom compost. These results confirm that application of substrate S4 (45% of spent mushroom compost) for the germination and development of lettuce seedlings provided the most appropriate planting materials for the production of marketable plants with superior biomass and vigor.

It is important to emphasize that the spent mushroom compost was added exclusively to the substrate used in the production of seedlings and was not employed as fertilizer for plants grown on in the field. The results show that the addition of 45% mushroom compost exerted beneficial effects on seedling growth and development that were reflected in the quality of the mature lettuce plants. According to Uzun (2004), fresh SMS should not be used as fertilizer because it contains a lot of salt, mainly potassium and calcium. In function of this, SMS should be weathered for at least six months. For the present work, SMC was not weathered and, although it had shown a positive effect for any amount in the growing media, probably, excess of salts may be a limiting factor to use more than 45% of SMS in the substrate. Fidanza, Sanford, Beyer, and Aurentz (2010) reported the chemical and physical properties of the fresh mushroom compost produced in Pennsylvania. The content of macronutrients and trace elements we have found in the present work fit between the range reported by Fidanza, Sanford, Beyer, and Aurentz (2010). On the other hand, Medina, Paredes, Pérez-Murcia, Bustamante and Moral (2009) reported a high electrical conductivity for all *Agaricus* SMS-based growing media, which were higher than the reference level ( $\leq 0.5 \text{ dS m}^{-1}$ ) suggested by Abad, Noguera, and Bures (2001). Similar problem was observed for SMS from non *Agaricus* mushrooms but its electrical conductivity is not too high (Medina, Paredes, Pérez-Murcia, Bustamante, & Moral, 2009; Zhang, Duan, & Li, 2012). Considering that all *Agaricus* mushroom composts show a high electrical conductivity, due to their high concentrations of ions (nitrate, sulphate, chloride, etc) probably its addition will increase this property in the growing media. Besides other limiting factors, electrical conductivity limits the utilization of SMS to proportions between 25 and 50% (Medina, Paredes, Pérez-Murcia, Bustamante, & Moral, 2009). Bustamante, Paredes, Moral, Agulló, Pérez-Murcia, and Abad (2008) reported the utilization of traditional composts elaborated using wastes from the distillery industry and from livestock as growing media or growing media constituents for commercial vegetable transplant production. The authors concluded that the composts produced may be used as partial peat substitutes, especially at the rates of 25% and 50% of distillery compost. Similarly to SMS, the substrates showed electrical conductivity values higher than conventional substrates. Despite of this, as discussed before, even the higher proportion (SMS 75%) was better than the control (commercial substrate).

In general, previous studies have evaluated the effect of different substrates on the growth of seedlings on the assumption, without experimental validation, that higher quality seedlings would produce superior marketable plants. In the present study, this hypothesis was tested and confirmed through evaluation of the field production of plants derived from seedlings produced during the first experiment using different growing media. We observed seedlings with superior quality, resulting in higher lettuce production. These results suggest that SMS has other components like mushroom substances or microorganisms which have a positive effect during seedlings growing. These components probably compensate limiting factors such as poor physical properties and high electrical conductivity and should be subject of more detailed studies in the future.

#### 4. Conclusion

Substrate comprising a mixture of 45% spent mushroom compost from *A. subrufescens* provided the most adequate conditions for the growth and development of lettuce seedlings and, consequently, of vigorous marketable plants. The data confirmed that superior quality lettuce seedlings give rise to high quality marketable heads, and even the treatment with the highest amount of SMS (75%) resulted in seedlings of higher quality compared to commercial substrate. *Agaricus subrufescens* SMS is, therefore, an excellent substrate component to produce lettuce seedlings of high quality.

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### Supplementary File

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