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ORIGINAL RESEARCH

Application of spent *Agaricus subrufescens* compost in integrated production of seedlings and plants of tomato

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Abstract

Purpose Spent mushroom compost (SMC) is a major solid waste product of the mushroom industry and is the material which remains at the end of a mushroom crop.

Methods Different proportions of the SMC from *Agaricus subrufescens* cultivation were tested to produce seedlings and plants of tomato. A commercial substrate was used, both as a control and in combination with the spent compost in different proportions. So two experiments were carried out, the first was the production and evaluation of tomato seedlings and the second was tomato cultivation from seedlings produced in the first experiment.

Results The use of different proportions of spent *A. subrufescens* compost resulted in a decreasing trend of all the parameters in the production of the tomato seedlings. However, in tomato cultivation for all periods of harvest, a positive effect was observed in fruiting when the seedlings were produced with spent *A. subrufescens* compost in comparison to the commercial control.

Conclusion It was concluded that the use of spent *A. subrufescens* compost for seedling production led to a higher total tomato production compared to previously reported production levels in organic cultivation systems with green, organic and other types of fertilization. These results demonstrated the great potential of spent *A. subrufescens* compost for use in organic tomato production because of the better quality of harvested fruit.

Keywords Almond mushroom · *Agaricus blazei* · Reuse of compost · Waste

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Introduction

Agaricus subrufescens, which is synonymous with *Agaricus blazei* ss. Heinemann and *Agaricus brasiliensis*, is a basidiomycete fungus that has aroused great interest in various parts of the world because of its medicinal properties, which is used in the prevention or treatment of diabetes, atherosclerosis, hepatitis, hypercholesterolemia, and heart disease (Dias et al. 2013; Zied et al. 2013).

Cultivation of mushrooms generates a large amount of spent mushroom compost (SMC) that must be rapidly discarded by the grower to avoid the contamination of new cultivation cycles and infestation by flies or other insects (Marques et al. 2014). Furthermore, after cultivation, uncovered SMC should not be stockpiled due to the risk of contaminating groundwater by leaching after rain events (Ribas et al. 2009).

Depending on the type of mushroom and substrate materials, the SMC can be directly used as animal feed because it is nutritionally rich in proteins, easy to digest and a stimulator of the immune system of animals (Sánchez 2004; Machado et al. 2007; Kwak et al. 2008; Azevedo et al. 2009).

Apart from use in animal feeds, compost produced by mushroom cultivation could be used directly as an organic fertilizer (Rinker 2002). However, under these conditions, the compost is not yet fully stable and can undergo changes if it is stored wet over long periods of time and may affect plant growth because of the presence of heavy metals, poor physical properties and excessive salts, that result in high electrical conductivity (Abad et al. 2001; Bustamante et al. 2008; Medina et al. 2009).

In addition to fertility and plant nutrition, SMC that is produced using the appropriate technology also has the advantage of containing a rich microbiota that can ensure the necessary balance to guarantee the phytosanitary requirements of plant cultures (Elo et al. 2000; Viji et al. 2003). It is also possible that some species of microorganisms have the potential to induce resistance in seedlings pre-grown on this substrate.

The use of microorganisms to induce plant resistance to pathogens of root and leaves is now well documented, showing the great potential of this strategy (Ishida et al. 2008; Fontenelle et al. 2011). The microbial community that is present in organic compost and in the substrate after cultivation of mushrooms might play an important role in controlling the microbiota in the rhizosphere region.

Therefore, this type of SMC could be an important source of microorganisms of different species with the potential to induce resistance against diseases. In addition, the mushroom species itself produce biotic resistance inducers, substances that elicit plant defense responses (Silva et al. 2013). Considering all this, the use of SMC can have a much greater potential than simply an organic fertilizer or soil conditioner.

In the present manuscript, tomato plants were chosen to evaluate the potential use of spent mushroom compost after cultivation of the mushroom *A. subrufescens* in different proportions in a mixture with a commercial substrate as growing media for tomato production in an organic system.

Materials and methods

Location of the experiment

The experiment was conducted under greenhouse conditions for the production of tomato seedlings and under field conditions for the production of tomatoes (*Solanum lycopersicum*) in the area located at 21°14′S and 45°00′W and at 920 m of altitude. The average annual temperature is 19.3 °C, and the average annual rainfall is 1411 mm, with a concentration of rainfall during summer. The average relative humidity is 78 %. The soil was an oxisol, and the soil showed good depth, a clayey texture, good water retention capacity, good aeration and a flat topography. The analysis of the soil is described in Table 1.

Table 1 Physicochemical characteristics of the soil at the experimental location

Characteristics	Values
pH in water (v:v; 1:2.5)	5.9
P (mg dm^{-3})	9.3
K (mg dm^{-3})	116
Ca^{2+} ($cmol_c$ dm^{-3})	3.4
Mg^{2+} ($cmol_c$ dm^{-3})	1.4
Al^{2+} ($cmol_c$ dm^{-3})	0.1
SB ($cmol_c$ dm^{-3})	5.1
CEC ($cmol_c$ dm^{-3})	8.0
Clay (g kg^{-1})	600
Silt (g kg^{-1})	220
Sand (g kg^{-1})	180
H + Al ($cmol_c$ dm^{-3})	2.9

SB sum of bases, *CEC* cation exchanged capacity

Procurement of substrates for the production of seedlings

Bioplant© a commercial product[1] was used as a substrate for the production of tomato seedlings, both as a control and in combination with the spent *A. subrufescens* compost (SASC) in different proportions, depending on each treatment. The SASC was obtained from the study carried out by Figueiredo et al. (2013), who used the CS10 strains (University of Lavras, Brazil), compost based on sugarcane bagasse, and coastcross hay with a casing layer of soil. In total, there were six treatments: Treatment 1–100 % Bioplant; Treatment 2–80 % Bioplant +20 % SASC; Treatment 3–60 % Bioplant +40 % SASC; Treatment 4–40 % Bioplant +60 % SASC, Treatment 5–20 % Bioplant +80 % SASC; Treatment 6–100 % SASC. The moisture contents of both substrates were determined after drying the samples in an oven at 60 °C for 48 h. The dry weight was determined by the weight difference between dry and wet weight of each sample (Bioplant and SASC) used in each treatment. The analyses of the SASC and chemical properties of the mixtures were done with colorimetric determination methods and are described in Tables 2 and 3 (Ansorena 1994; Pardo-Gimenez et al. 2010).

Production and evaluation of tomato seedlings

The seedlings were grown in a greenhouse covered with a plastic sheet and sided with white screens. The seeds were

[1] Composition: *Pinus* bark, aggregating agents, vermiculite, NPK and micronutrients with pH of 8.2 and e Electrical conductivity 0.96 mS cm^{-1}.

Table 2 Chemical composition of the spent *A. subrufescens* compost (SASC)

pH	P (mg dm^{-3})	K (mg dm^{-3})	Ca^{+2} (mg dm^{-3})	Mg^{+2} (mg dm^{-3})	Al^{+3} (mg dm^{-3})	H + Al^{+3} (mg dm^{-3})	CEC (mg dm^{-3})	*M* (mg dm^{-3})	*V* (%)	OM (%)	*P* (mg L^{-1})
5.0	0.9	624	2.0	0.7	0.0	2.1	6.4	0.0	67.2	63.0	1.5

CEC cation exchanged capacity, *M* percentage of Al saturation, *V* percentage of base saturation, *OM* Organic matter

Table 3 Chemical properties of the growing medium SASC based on tomato seedlings production

Treatment	pH	Electrical conductivity (mS cm^{-1})
T1: 0 % SASC	8.2	0.96
T2: 20 % SASC	7.6	1.5
T3: 40 % SASC	6.6	3.1
T4: 60 % SASC	6.5	4.13
T5: 80 % SASC	6.1	5.7
T6: 100 % SASC	6.0	5.51

sown in plastic trays with 200 cells, each containing the substrate of each of the six treatments, totaling 1200 seedlings. The seedlings were irrigated daily and were transferred to the field after 17 days, when they showed four true leaves.

At the transfer to the field, ten seedling samples from each of the six treatments were randomly selected to evaluate and quantify the level of growth of the tomato seedlings based on measurements and fresh weight of the seedlings. The following characteristics were analyzed: mass of the aerial portion, height of aerial portion, mass of root, length of root and total fresh weight.

Tomato cultivation from seedlings produced on different substrates

The tomato plants were cultivated with a spacing of 45 × 60 cm in three rows with staking. The experiment was a randomized block design with six repetitions, in which each plant is considered as a repetition. The buds were thinned when they were 3–5 cm long, and the tip was pruned when the last bunch had sprouted, reaching the desired amount of 4 bunches per plant.

To analyze the production of tomatoes, all the fruits from each plant were harvested in each of the four production flushes. The fruits were harvested when the "breaker" stage was attained, that is, when each tomato showed a reddish color on the apex in the weekly harvest. Only the commercially acceptable production was evaluated, and all damaged fruits were discarded.

The fruits were then evaluated with regard to the number of fruits per plant, the size according to the classification of the Ministry of Agriculture of Brazil (Table 4) and the total weight (tomato production, g plant^{-1}).

Regarding culture management, insecticides and fungicides were not used to control pests and diseases. Nutrients were also not supplemented through the use of fertilizers. Likewise, the weed growth was controlled manually rather than through the use of herbicides. Irrigation was performed daily from the moment of transplanting until the harvest period.

Table 4 Classification of tomato fruits with regard to size, according to the norms of the Ministry of Agriculture of Brazil

Classification	Fruit diameter (mm)
Extra-AA (coarse)	>52
Extra-A (medium)	47 < 52
Extra (small)	40 < 47
Especial (extra small)	33 < 40

Statistical analysis

All the parameters were subjected to regression analysis using the program SISVAR.

Results and discussion

Production of the tomato seedlings

In the production of the tomato seedlings, the use of different proportions of SASC resulted in a decreasing tendency of all the parameters (Figs. 1, 2, 3).

Considering the treatments with SASC, the best results were generally obtained with the treatments using 20 and 40 % SASC. The fresh mass of roots and size of the root were the parameters most negatively affected by the use of SASC, sharply decreasing with the addition of SASC, which may indicate a deficiency in the physical characteristics of the SASC.

Costa et al. (2012) used different substrates for the production of tomato seedlings. The results reported for all the tested parameters (plant height, stem diameter, total weight, mass of the shoots and mass of the root) were worse than those shown here. Steffen et al. (2010) tested substrates of rice husk and cow manure and also obtained worse results for plant height and fresh weight of the aerial portion than those obtained in this work with the use of SASC.

Medina et al. (2009) evaluated the use of SMC of *Agaricus bisporus* and *Pleurotus ostreatus* for the seedlings' production of different vegetables species. None of the treatments containing SMC matched the peat (control) as growing medium for tomato seedlings production, but SMC from *A. bisporus* was better than *P. ostreatus* as component of the growing media. In the present study, although the best results were obtained with the control substrate (100 % Bioplant), all the treatments with different ratios of SASC achieved results as good as or even better than those obtained with other substrates in other studies.

Cultivation of tomatoes from seedlings grown on substrates based on SASC

In all flushes of tomato production, a positive effect was observed when the seedlings were produced with SASC in comparison to the control (Fig. 4). For each flush, variations among the treatments regarding the best production were observed. However, the treatment with 100 % SASC generally led to the best production in two flushes and the second-best production in the remaining two flushes. Considering the total production, the seedlings produced in the substrate with 100 % SASC had the greatest tomato production (Fig. 5). It is worth noting that, in addition to the treatment with 100 % of SASC, all the other treatments

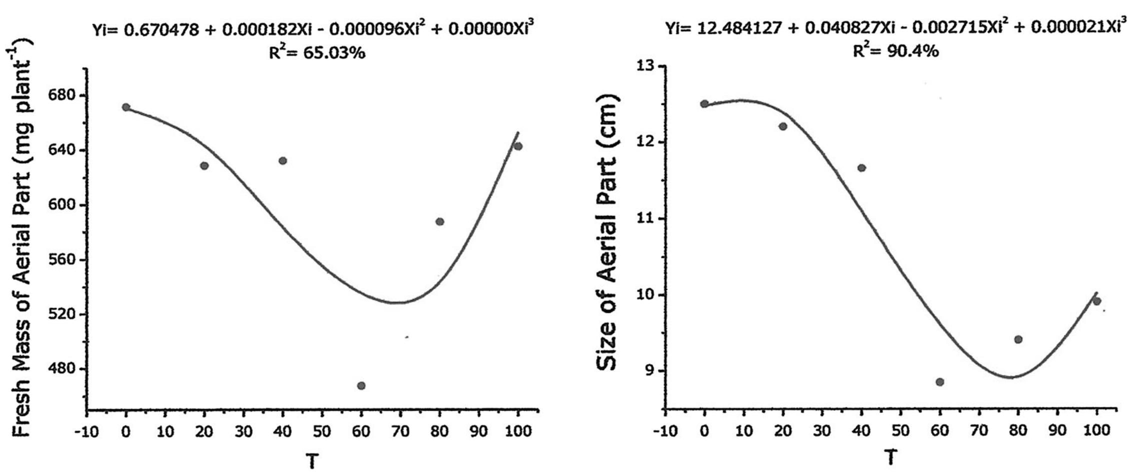

Fig. 1 Influence of spent *A. subrufescens* compost on the fresh mass and size of aerial part of tomato seedlings

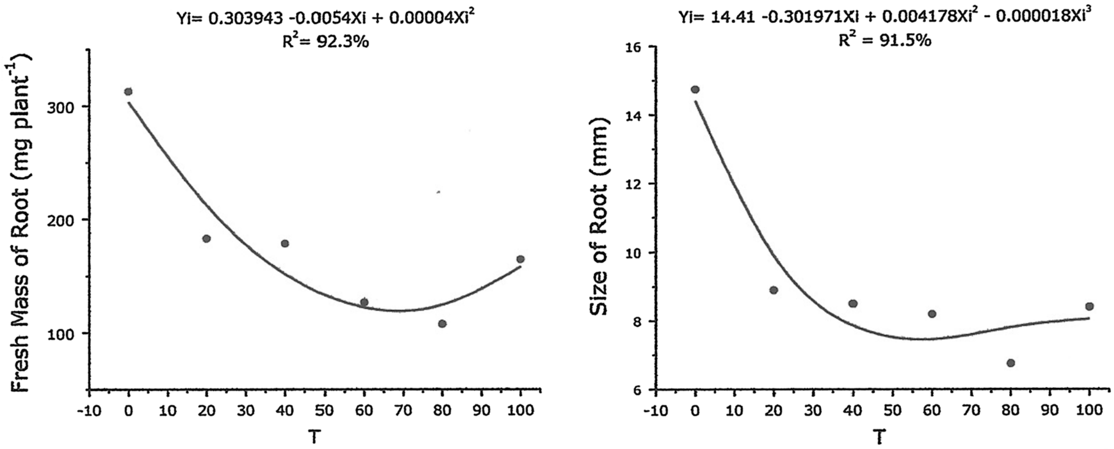

Fig. 2 Influence of spent *A. subrufescens* compost on the fresh mass and length of roots of tomato seedlings

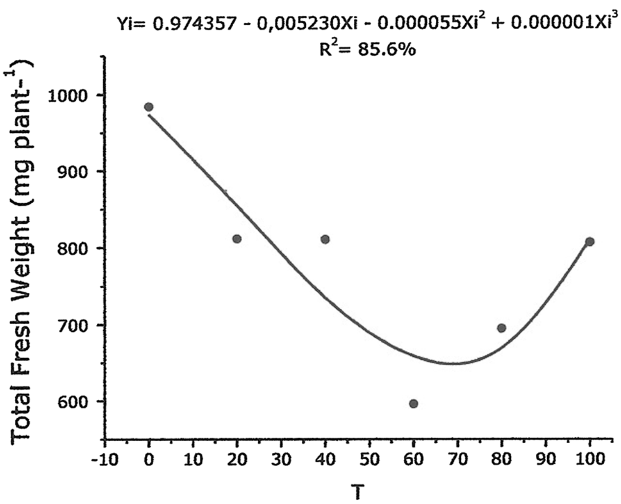

Fig. 3 Influence of spent *A. subrufescens* compost on the total fresh weight of tomato seedlings

led to a larger production than the control, showing the positive effect of this substrate.

In comparison to the results obtained with conventional crops, the tomato production was lower in this study because pesticides and fertilizers were not used. The maximum production (53 t ha^{-1}) was obtained when treating with 100 % SASC resulting in a yield of 1.43 kg of tomatoes per plant. When comparing organic and conventional cultivation, Bettiol et al. (2004) concluded that organic farming production corresponds to only 36.5 % of conventional production. Wamser et al. (2012) studied the productivity of tomato hybrids in super-high-density cultivation fields (44,444 plants ha^{-1}), reporting a maximum yield of 170.7 t ha^{-1}, whereas in traditional cultivation (22,222 plants ha^{-1}), the maximum yield was 128.5 t ha^{-1}. Assuming that the proportion of 36.5 % proposed by Bettiol et al. (2004) is correct, yields of 62.2 and 46.9 t ha^{-1} should be expected for super-dense and traditional cultivation, respectively, under an organic cultivation system. Therefore, the maximum yield obtained in the present work, 53 t ha^{-1}, falls in the expected range for organic system.

In hydroponic cultivation, Albuquerque Neto and Peil (2012) reported a maximum total production of 54.6 t ha^{-1}. The highest total yield reported by these authors is therefore practically the same as the maximum yield obtained in the present work. In addition to the numbers that were presented above, it is also important to emphasize that no fertilization procedures of any kind were used in the present work, either before or during the crop, because we aimed to evaluate the behavior of seedlings in the simplest crop management conditions possible. This information is important because despite the need to avoid chemical fertilizers in organic farming, the use of organic compost, green manure, bone meal and so forth is recommended.

Costa et al. (2011) evaluated the productivity of tomato cultivars tolerant to high temperature in an organic farming system using organic manure, bone meal and biofertilizer, with a maximum total production of 36.3 t ha^{-1}. Marouelli et al. (2011) evaluated the effect of this type of irrigation system (dripping vs. sprinkler) on organic tomato cultivation using green fertilization with Crotalaria (*Crotalaria pumila*), applying organic compost and thermophosphate as well as applying Bordeaux mixture and a biopesticide (*Bacillus thuringiensis*). Under these conditions, these authors obtained a total production of 58.8 and 64.8 t ha^{-1} for the dripping and the sprinkler systems, respectively. These results were better than those obtained in this study (53 t ha^{-1}). Nonetheless, it is important to emphasize that the authors used green fertilization, organic fertilizer and thermophosphate.

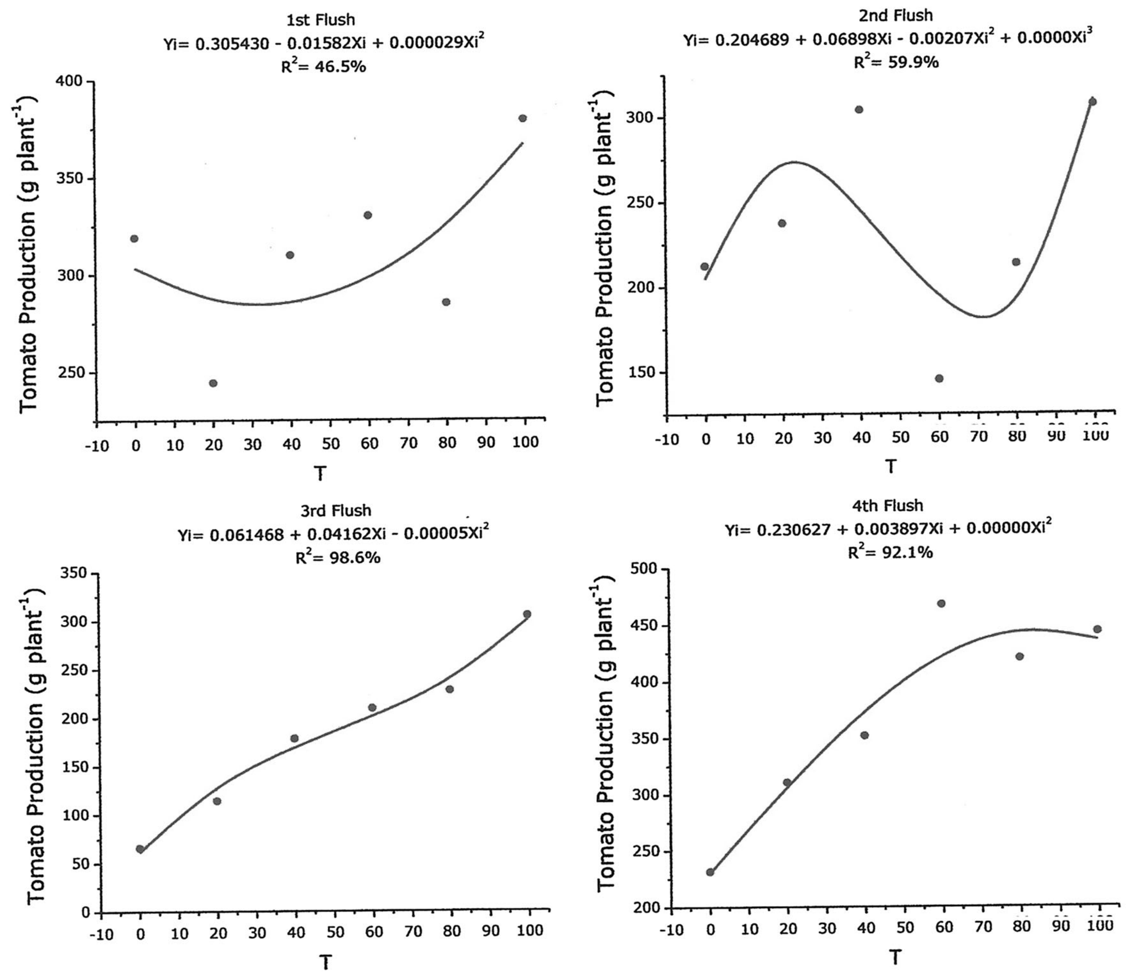

Fig. 4 Flushes of tomato production from the seedlings grown using different proportions of spent *A. subrufescens* compost

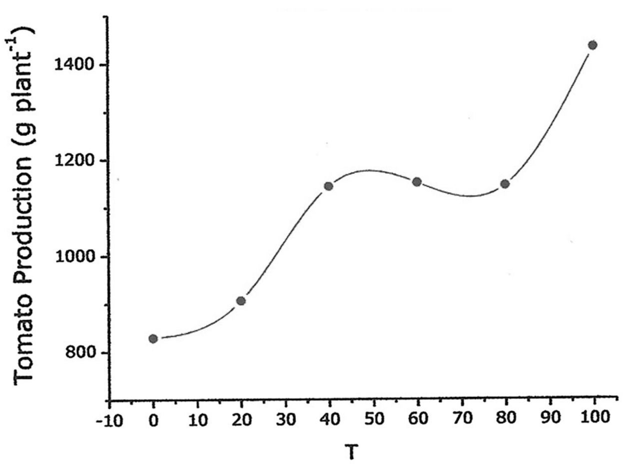

Fig. 5 Total tomato production from seedlings grown using different proportions of spent *A. subrufescens* compost

Spent mushroom compost and *Trichoderma harzianum* were effective when used against *Meloidogyne javanica* in tomato under field conditions in Dargai in Malakand Agency, Pakistan (Irfan-ud-Din et al. 2005). The author conclude that SMC stimulated flowering, plant height, fresh and dry shoot weight and increased yield by 101.9, 61.5, 27.9, 38.3 and 102.5 %, respectively; moreover, it reduced the number of galls/plant by 73.5 %. Modolon et al. (2012) used homeopathic preparations for pest control during the organic cultivation of tomatoes, also employing green fertilization procedures and applying compost. Under these conditions, the largest total production obtained was 15.5 t ha^{-1} in field cultivation and 29.8 t ha^{-1} in greenhouse cultivation.

The treatment without SASC induced a higher proportion of small and very small fruit in the tomato production, whereas the treatment with 100 % SASC led to a higher proportion of large and medium-sized fruit (Table 5).

Therefore, the use of this substrate in the production stage of the seedlings resulted in more vigorous plants, leading not only to greater yields but also to fruits with greater size compared to the commercial control. Interestingly, the treatment with 80 % SASC led to the highest

Table 5 Influence of spent *A. subrufescens* compost on the classification of the total tomato production regarding the fruit size

Treatment	Classification of tomatoes								Total number of fruits
	Extra small		Small		Medium		Coarse		
	N[a]	%[b]	*N*	%	*N*	%	*N*	%	
Control	12	12.0	49	49.0	35	35.0	4	4.0	100
20 % SMS	2	2.1	51	53.7	37	38.9	5	5.3	95
40 % SMS	0	0	55	43.3	72	56.7	0	0	127
60 % SMS	0	0	55	44.7	68	55.3	0	0	123
80 % SMS	3	2.1	73	51.4	66	46.5	0	0	142
100 % SMS	0	1.5	40	31.0	73	56.6	14	10.8	129

The values are presented as a percentage of the total number of fruits harvested

[a] Number of fruits

[b] Percentage of fruits with size classification

number of fruits among all the treatments. However, compared to the treatment with 100 % SASC, the production consisted almost exclusively of small and very small fruit, with not a single large fruit harvested.

Conclusions

We can conclude that the use of SASC for seedling production led to a higher, or at least equal, total tomato production compared to previously reported production levels in organic cultivation systems with green, organic and other types of fertilization. These results demonstrate the great potential of SASC for use in organic tomato production because of the better quality of harvested fruit.

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References

Abad M, Noguera P, Bures S (2001) National inventory of organic wastes for use as growing media for ornamental potted plant production: case study in Spain. Bioresour Technol 77:197–200

Albuquerque Neto AAR, Peil RMN (2012) Biological productivity of tomato plant genotypes in hydroponic systems during fall/winter. Hortic Bras 30(4):613–619

Ansorena J (1994) Sustratos. Propiedades y caracterización. Mundi-prensa S.A., Madrid, p 172

Azevedo RS, Ávila CLS, Bertechini G, Schwan R (2009) Utilization of the spent substrate of *Pleurotus sajor caju* mushroom in broiler chicks ration and the effect on broiler chicken performance. Acta Scient Anim Sci 31(2):139–144

Bettiol W, Ghini R, Galvão JAH, Siloto RC (2004) Organic and conventional tomato cropping systems. Sci Agric 61(3):253–259

Bustamante MA, Paredes C, Moral R, Agulló E, Pérez-Murcia MD, Abad M (2008) Composts from distillery wastes as peat substitutes for transplant production. Resour Conserv Recy 52:792–799

Costa CA, Silva AC, Sampaio RA, Martins ER (2011) Productivity of determinate growth tomato lines tolerant to heat under the organic system. Hortic Bras 29(4):590–593

Costa E, Leal PAM, Benett CGS, Benett KS, Salamene LCP (2012) Production of tomato seedlings using different substrates and trays in three protected environments. Eng Agric 32(5):822–830

Dias ES, Zied DC, Rinker DL (2013) Physiologic response of *Agaricus subrufescens* using different casing materials and practices applied in the cultivation of *Agaricus bisporus*. Fungal Biol 117(7):569–575

Elo S, Maunuksela L, Salkinoja-Salonen M, Smolander A, Haahtela K (2000) Humus bacteria of Norway spruce stands: plant growth promoting properties and birch, red fescue and alder colonizing capacity. FEMS Microbiol Ecol 31:143–152

Figueiredo VR, Martos ET, Siqueira FG, Maciel WP, Silva R, Rinker DL, Dias ES (2013) Microbial inoculation during composting improves productivity of sun mushroom (*Agaricus subrufescens* Peck). Afr J Microbiol Res. doi:10.5897/AJMR2013.5944

Fontenelle ADB, Guzzo SD, Lucon CMM, Harakawa R (2011) Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by *Trichoderma* spp. Crop Prot 30(11):1492–1500

Irfan-ud-Din Saifullah, Khan H, Khattak B (2005) Biological control of *Meloidogyne javanica* (Treub) Chitwood with *Trichoderma harzianum* Rifai and spent mushroom compost in tomato under field conditions. Pak J Phytopathol 17(2):144–145

Ishida AKN, Souza RM, Resende MLV, Cavalcanti FR, Oliveira DL, Pozza EA (2008) *Rhizobacterium* and acibenzolar-S-methyl (ASM) in resistance induction against bacterial blight and expression of defense responses in cotton. Trop Plant Pathol 33(1):27–34

Kwak WS, Jung SH, Kim YI (2008) Broiler litter supplementation improves storage and feed-nutritional value of sawdust-based spent mushroom substrate. Bioresour Technol 99(8):2947–2955

Machado AMB, Souza Dias E, Santos EC, Freitas RTF (2007) Spent mushroom substrate of *Agaricus blazei* in broiler chicks diet. R Bras Zootec 36(4):1113–1118

Marouelli WA, Medeiros MA, Souza RF, Resende FV (2011) Production of organic tomato irrigated by sprinkling or dripping in single cultivation or in consortium with coriander. Hortic Bras 29(3):429–434

Marques ELS, Martos ET, Souza RJ, Silva R, Zied DC, Dias ES (2014) Spent mushroom compost as a substrate for the production of lettuce seedlings. J Agric Sci 6(7):138–143

Medina E, Paredes C, Pérez-Murcia MD, Bustamante MA, Moral R (2009) Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. Bioresour Technol 100(18):4227–4232

Modolon TA, Boff P, Boff MIC, Miquelluti DJ (2012) Homeopathic and high dilution preparations for pest management to tomato crop under organic production system. Hortic Bras 30(1):51–57

Pardo-Gimenez A, Zied DC, Pardo-Gonzalez JE (2010) Utilización de compost agotado de champiñón como capa de coberturas en nuevos ciclos de producción. Pesq agropec bras 45(10):1164–1171

Ribas LCC, Mendonça MM, Camelini CM, Soares CHL (2009) Use of spent mushroom substrates from *Agaricus subrufescens* (syn. *A. blazei*, *A. brasiliensis*) and *Lentinula edodes* productions in the enrichment of a soil-based potting media for lettuce (*Lactuca sativa*) cultivation: growth promotion and soil bioremediation. Bioresour Technol 100(20):4750–4757

Rinker DL (2002) Handling and using "spent" mushroom substrate around the world. Mushroom biology and mushroom products. Universidad Autónoma del Estado de Morelos, Cuernavaca, pp 43–60

Sánchez C (2004) Modern aspects of mushroom culture technology. Appl Microbiol Biot 64(6):756–762

Silva RF, Pascholati SF, Bedendo IP (2013) Induced resistance in tomato plants to *Clavibacter michiganensis* subsp. *Michiganensis* by *Lentinula edodes* and *Agaricus subrufescens* (syn. *Agaricus brasiliensis*). J Plant Pathol 95(2):285–297

Steffen GPK, Antoniolli ZI, Steffen RB, Machado RG (2010) Rice husk and cow manure as substrates for worm multiplication and production of tomato and lettuce seedlings. Acta Zool Mex 26(2):333–343

Viji G, Uddin W, Romaine CP (2003) Suppression of gray leaf spot (blast) of perennial ryegrass turf by *Pseudomonas aeruginosa* from spent mushroom substrate. Biol Control 26:233–243

Wamser AF, Mueller S, Suzuki A, Becker WF, Santos JP (2012) Productivity of tomato hybrids subjected to super-dense cultivation. Hortic Bras 30:168–174

Zied DC, Pardo Giménez A, Pardo González JE, Souza Dias E, Carvalho MA, Minhoni MTA (2013) Effect of cultivation practices on the β-Glucan content of *Agaricus subrufescens* basidiocarps. J Agric Food Chem 62(1):41–49