

Growth, Nutrient Uptake, and Foliar Gas Exchange in Pepper Cultured with Un-composted Fresh Spent Mushroom Residue

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Abstract

Spent mushroom substrate (SMS) can be used as the component of growing medium for the culture of crop plants. Fresh SMS may have the potential as an alternative to peat to raise horticultural plants. In this study, five container media characterized by the proportions of SMS to commercial peat in 0% (control), 25%, 50%, 75%, and 100% were used to raise pepper (*Capsicum annuum* L.) plants. Initial SMS was found to have low available nitrogen (N) content (<20 mg kg⁻¹) but moderate extractable phosphorus (P) content (900 mg kg⁻¹). In the second month photosynthetic rate was found to decline in the 75% treatment. At harvest in the third month, plants in the 100% treatment nearly died out. The 25% treatment resulted in the highest height (19 cm) and diameter growth (0.3 cm), shoot (0.6 g) and root biomass accumulation (0.13 g), fruit weight (3 g), and shoot carbohydrate content (98 mg g⁻¹), but lowest foliar acid phosphatase activity (30 μg NPP g⁻¹ FW min⁻¹). With the increase of SMS proportion in the substrate, the medium pH and electrical conductance (EC) increased with the decrease of foliar size. The available N and P contents in the substrates showed contrasting relationship with N and P contents in pepper plants. Therefore, fresh SMS cannot be directly used as the substrate for the culture of pepper plants. According to our findings fresh SMS was recommended to be mixed in the proportion of 25% with commercial peat for the culture of horticultural plants.

Keywords: nutrient utilization; solid waste; spent mushroom residue; un-compost; weathered material

Introduction

Currently, mushroom production is one of the largest solid-state fermentation industries all over the world (Soccol and Vandenberghe, 2008). After mushroom is harvested, the spent residue is considered “spent out” and abandoned as weathered fresh substrate, generating a by-product of mushroom cultivation. Every production of 1 kg of mushroom would generate 2.5-5 kg of spent mushroom substrate (SMS) (Semple *et al.*, 2001; Ünal, 2015). A huge amount of SMS is produced every year in the world: 0.2 million tons in Turkey, 0.99 million tons in Spain, and 38.93 million tons in China (FAO, 2016). To properly dispose SMS can not only increase the income of by-product of mushroom industry but also eliminate the potential environmental contamination by disposable manner.

SMS is usually utilized as the spent mushroom compost (SMC). SMC can function as an amendment to improve soil physical properties, which benefits from decreased bulk

density, reduced clod, mitigatory crust-formation, promoted infiltration, enhanced water content (Stewart *et al.*, 1998), and increased aggregate stability (Stewart *et al.*, 1998; Curtin and Mullen, 2007). SMC contains abundant content of nutrients that is generally harmless to plants (Tam and Wang, 2015; Paredes *et al.*, 2016). SMC is also rich in humus content, which can provide nutrients for plant growth in a long term as slow-release source (Kadiri and Mustapha, 2010). SMC application to soils can also improve nutrient availability for vegetable growth (Rhoads and Olson, 1995; Wuest *et al.*, 1995). In addition, the porosity of SMC would probably favour the root develop to increase the efficiency of nutrient and water uptake. These properties all contribute to the suggestion of SMC as one component in the growing media for crop plant culture (Chong *et al.*, 1994; Tam and Wang, 2015; Nguyen and Wang, 2016).

Currently, peat is the main component in the commercial substrates for the cultivation of containerized plants globally (Ribeiro *et al.*, 2007). The heavy extraction of peat source for the usage of commercial substrate for

horticultural plants has resulted in the wetland depletion. This also causes the problems of non-renewable source loss from degraded wetland ecosystem and CO₂ permission through peat decomposition (Bustamante *et al.*, 2008). Therefore, SMC has been aroused as an alternative to the commercial peat substrate (Kandemir *et al.*, 2009; Ünal, 2015; Zhang *et al.*, 2012, 2013). The use of SMC in the container substrate can efficiently inhibit the pathogen damage on horticultural crops (Segarra *et al.*, 2007; Chen *et al.*, 2015), promote nutrient uptake (Paredes *et al.*, 2016; Sönmez *et al.*, 2016), and promote crop growth (Ünal, 2015). However, the high pH value and electrical conductance (EC) prevent SMS to be used as the main component in the substrate (Szmidski and Chong, 1995; Gonani *et al.*, 2011). Therefore, SMC was usually applied to replace some peat in the substrate where horticultural plant tended to respond in better germination and growth in the lower SMS proportion of 20~30 % (Chong *et al.*, 1994; Khan *et al.*, 2006; Medina *et al.*, 2009; Gonani *et al.*, 2011; Kwack *et al.*, 2012).

Fresh SMSs are those being weathered but have not been composted. The application of fresh SMS requires less time consumption, site occupation, labour forces, and additional input compared to the compost. Like SMC, the fresh SMS can also amend soils thorough decreasing compactness (Peregrina *et al.*, 2012; Kato *et al.*, 2013) and increasing porosity and fractal dimension (Nakatsuka *et al.*, 2016). However, fresh SMS is usually characterized by high salt content therefore tends to be unsuitable as the main component of growing media (Chong *et al.*, 1994). However, little is known about the effect of mixed fresh SMS and peat on containerized crops.

Pepper (*Capsicum annuum* L.) is an important horticultural vegetable in the world (Marín *et al.*, 2014). Globally the cultivation of pepper plants occupied about 0.53 million ha with the total production of 0.55 ton (FAO, 2016). By 2016, Asia has the largest area for pepper crop cultivation of 0.45 million ha, which accounted for 85% of the whole world area (FAO, 2016). During the culture of pepper plants, the involvement of SMC in the medium can effectively suppress the fungal disease (Marín *et al.*, 2014), enhance the vegetative growth (Ahlawat *et al.*, 2007), and promote the fruit quality (Eudoxie *et al.*, 2014). It was also indicated that un-composted solid-waste was more efficient to promote the pepper growth than aerated composted one (Marín *et al.*, 2014). The effect of fresh SMS substrate on pepper may contain more uncertainty than expected because pepper is salt-sensitive (Medina *et al.*, 2009).

In this study, pepper plants were cultured in a controlled environment with the media of commercial peat and fresh SMS in different proportions. The main objective of this work was to quantify the chemical properties of the substrate and the plant quality and fruiting performance. We also determined physiological parameters to detect the potential mechanism for plant response. It was hypothesized that: (i) both growth and fruiting performance were best in the treatment with low proportion of fresh SMS, which resulted in (ii) higher photosynthesis and gas exchange and (iii) better nutrient availability.

Materials and Methods

Study site and condition

The present study was conducted in a growth chamber at the platform of combined manipulations of illumination and fertility on plant growth regulation (43°59'46" N, 125°23'34" E) (Zhilunpudao Agric. S&T Co., Ltd., Changchun, China). Throughout the experiment all environmental factors of light, temperature and moisture were manually controlled to the best condition for plant growth. Light was supplied for 16 h per day by artificial illumination of high-pressure sodium (HPS) lamps to the intensity of 4000-5000 lx with the spectrum of 43.9% red (600-700 nm), 54.7% green (500-600 nm), and 1.4% blue (400-500 nm). This illumination condition has been proven to favor plant growth even for slowly growing species (Wei *et al.*, 2013; Zhu *et al.*, 2016). Under this illumination condition, the photosynthetic photon flux density (PPFD) was measured to be 72-73 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the tip of pepper shoot. Temperature was measured to be 16.8/31.7 °C (night/day). The relative humidity (RH) ranged between 49% and 94%.

Experiment design

The experiment was conducted as a completely randomized design with different media substrate proportions of fresh SMS and commercial peat. SMS was added to peat in proportions of 100%, 75%, 50%, and 25% (v/v). The peat substrate with 0% proportion (v/v) of SMS added was tested as the control. Each treatment was replicated for three times. SMS was obtained from a local mushroom factory in Changchun after the production of *Pleurotus eryngii*. The raw material of SMS included 20% cottonseed hull, 20% wood bits, 24% corncobs, 20% brans, 5% corn flour, 2% lime carbonate, 1% gypsum powder, and 8% bean pulp. Before the experiment, SMS were prepared to be oven-dried at 45°C for 2 d. Dried SMS were ground to pass 5-mm sieve to generate homogeneous granules. The commercial peat was supplied by a local peat-manufacturer (Mushro-Dust, Chuangfeike S&T Co., Ltd., Jingyu, Baishan, China). This peat production was mainly manufactured by peat (80%), perlite (15%), and vermiculite (5%). Annual sale number of this commercial peat was around 0.55 million, 20% of which were sold for the culture of pepper plants. Both chemical nutrients and nutrient-release controller were added to the peat substrate to guarantee plants can be fed by nutrients for at least three months. Chemical properties of the substrate in each treatment are shown in Table 1.

Pepper plant culture

On 23 April 2017, pepper seeds were sown in a seeding tray filled with pure peat. On 14 May 2017, germinant seedlings were transplanted to planting trays. There were 32 plug holes (4×8 spacing) in the tray with each hole of 13 cm in height and 7 cm in diameter. Before planting, seedlings were firstly screened for uniform size to eliminate the possible difference among plant units. Seedlings in one tray were assigned as a treatment unit and three trays were arranged as replicates. Trays were placed in tanks (35 cm × 55 cm, width × length) to enable the sub-irrigation, which

has been fully proven to ensure the root moisture with uniform water supply in the controlled environment (Wang *et al.*, 2017; Zhao *et al.*, 2017). During the experimental stage, seedlings were watered every two days by maintaining the water table in the tank at the height of about 4 cm. All Seedling trays were rearranged after each irrigation manipulation to eliminate the possible edge effect.

Measurement of photosynthesis and gas exchange

On 17 July 2017 when leaves at the top of the stem had fully expanded, four seedlings in one tray were randomly chosen and measured for photosynthesis and gas exchange using Li-Cor 6400 portable photosynthetic detector (Li-Cor Co., Lincoln, U.S.A.). Fully expanded leaves at the top of the stem were chosen for measurement. During the measurement, the PPFD was controlled at $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, while the carbon dioxide (CO_2) concentration was controlled at $400 \mu\text{mol mol}^{-1}$. Because seedlings were cultured under uniformly artificial lighting environment, the measuring time was not stipulated to any specific period of the day. However, all measured were finished in four hours from 12:30 to 16:30. Water use efficiency (WUE) was calculated as the ratio of photosynthetic rate to transpiration rate (Guo *et al.*, 2016).

Seedling harvest and determination

On 17 August 2017, after three months' culture two trays of seedlings in the 100% SMS treatment died out leaving quite few seedlings alive in the last tray (Fig. 1). Thereafter, the experiment was terminated and all seedlings were sampled in the rest of treatments. Ten seedlings were randomly sampled from one tray and measured for height, diameter, boll number, fruit number, and fruit weight. The results of the ten seedlings were averaged as the value for one tray as one replicate of the treatment. Thereafter, the ten seedlings were grouped into two halves.

The first half of five seedlings was divided into shoot and root parts and measured for biomass after drying in the ventilated oven at 70°C for 3 d. Dried samples were ground to pass 2-mm sieve and determined for sugar, starch, N, and P concentrations. Soluble sugars (glucose, fructose and sucrose) and starch concentrations were determined by the colorimetric method (Wei *et al.*, 2014) using a spectrophotometer at 490 nm (UV-Visible 8453, Agilent Technologies Inc., Santa Clara, CA, USA). Briefly, 0.5-g sample was added to 50 mL of distilled water, steamed by the high pressure for two hours and determined for the

concentration of soluble sugars; thereafter the residual was washed by distilled water, oven-dried, added to hydrochloric acid, extracted in boiling water bath for eight hours, added with sodium hydroxide solution and determined for starch concentration. Total N and P concentrations were determined with the modification of the method described by Li *et al.* (2017). The 0.2 g sample was digested in 5 mL mixture of hydrogen peroxide and sulfuric acid. The digestion solution was diluted to 50 mL with deionized water. Total N concentration was determined using the Kjeldahl method. Total P concentration was determined by the Molybdenum-anticolorimetry method.

The other half of five seedlings was used for the measures on foliar size and green degree and the determination of enzyme activities of acid phosphatase (ACP) and glutamine synthetase (GS). Four leaves were randomly collected from one seedling and scanned for the projected image at the dpi of $118.11 \text{ pixels cm}^{-1}$ (HP Deskjet 1510 scanner, HP Inc., Palo Alto, CA, USA). The leaf image was transformed to the .gif format by Photoshop CS V 8.0 (Adobe, San Jose, CA, USA) and removed for all background colours. The leaf-pattern zone was targeted by one marquee and opened as an intact histogram. The leaf area was calculated by the total pixels of the histogram divided by the square of dpi. The foliar green degree was given by the averaged value of the green channel from the RGB panel in the histogram.

Statistical analysis

The chemical properties in substrates, photosynthesis, and gas exchange were analyzed for all five treatments. Because seedlings in the 100%-SMS treatment died out a harvest, data about growth, biomass, physiology, carbohydrate, and nutrient content were analyzed for the four treatments in SMS proportions of 0% (control), 25%, 50%, and 75%. All data were tested for the normality and the homogeneous variances and no data needed to be transformed. Data were analyzed using SAS software (SAS Institute Inc., NC, USA). The analysis of variance (ANOVA) was conducted to detect the effect of treatments on parameters using the GLM procedure. When the significant effect was indicated by ANOVA at the 0.05 level, values were compared and ranged by Tukey test. The relationship between the chemical properties of the substrates and N and P contents and concentrations in shoot and root parts were analyzed using the Pearson correlation with the CORR procedure.

Table 1. Chemical properties of the substrates with fresh spent mushroom substrate mixed with commercial peat in different proportions (v/v)

Treatment	$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	$\text{PO}_4^{3-}\text{-P}$ (mg kg^{-1})	pH	EC (dS m^{-1})
0%	124.74±4.92a	152.51±8.63a	232.29±18.26c	4.29±0.05d	0.31±0.20c
25%	121.75±2.02a	137.63±3.56a	364.88±3.45d	4.48±0.06cd	0.99±0.12b
50%	113.33±1.66a	49.11±4.08b	510.52±8.78c	4.66±0.05c	1.54±0.23b
75%	74.35±10.79b	36.96±5.70b	637.86±10.43b	5.10±0.19b	2.35±0.29a
100%	19.52±8.18c	11.07±4.13c	910.01±13.67a	5.66±0.08a	2.74±0.09a
M.S.	5976.84	12078.55	20423.17	0.94	2.94
F value	124.79	353.14	1274.12	79.51	66.17
$P_r > F$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Results

Chemical properties in substrates

The treatments of different proportions of SMS in the substrate resulted in significantly different changes of chemical properties of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$, pH, and EC (Table 1). Generally, with the increase of SMS proportion in the substrate, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations tended to decline but the $\text{PO}_4^{3-}\text{-P}$ concentration, pH, and EC showed increasing trends. The $\text{NH}_4^+\text{-N}$ concentration did not change with the SMS proportion increasing from 0% to 50% but significantly declined since the 75% proportion to the lowest value in the 100% SMS proportion treatment. The $\text{NO}_3^-\text{-N}$

concentration declined by about 70% in the 50% and 75% treatments than that in the 0% and 25% treatments and also showed lowest value in the 100% treatment. The $\text{PO}_4^{3-}\text{-P}$ concentration was lowest in the control and highest in the 100% treatment, while the latter increased by 290% than the earlier. Compared to pH and EC values in the control, those in the 100% treatment were increased by 32% and 820%, respectively.

Pepper growth, biomass and fruiting performance

Because pepper plants in the 100% treatment died out at harvest (Fig. 1), results were not analyzed for this treatment. Height and diameter growth both tended to increase from the control to the 25% treatment thereafter declined in the

Table 2. Growth and fruiting performance of pepper plants in fresh spent mushroom substrate mixed with commercial peat in different proportions (v/v)

Treatment	Height (cm)	Diameter (cm)	Boll number	Fruit number	Fruit weight (g FW)
0%	14.73±0.40b	0.27±0.01ab	4.97±1.09a	1.00±0.00a	1.28±1.39ab
25%	19.03±1.20a	0.32±0.04a	3.32±1.25ab	1.08±0.14a	3.00±0.93a
50%	16.09±0.94b	0.23±0.02bc	3.74±0.92ab	0.67±0.55ab	0.66±0.80ab
75%	8.81±0.42c	0.18±0.00c	2.05±0.17b	0.00±0.00b	0.00±0.00b
M.S.	55.38	0.01	4.34	0.73	4.96
F value	74.78	15.19	4.29	8.22	5.17
Pr > F	<0.0001	0.0011	0.0442	0.0080	0.0282

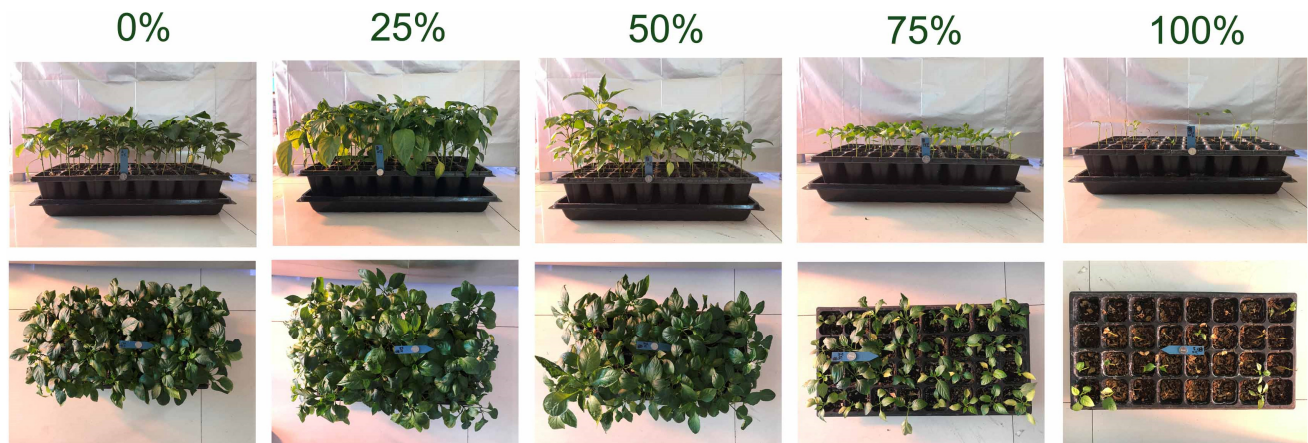


Fig. 1. The appearance of pepper (*Capsicum annuum* L.) plants cultured in the media mixed with commercial peat and fresh spent mushroom substrate (SMS) in proportions of 0% (control), 25%, 50%, 75%, and 100%. Seedlings in the 100% SMS substrate treatment nearly died out

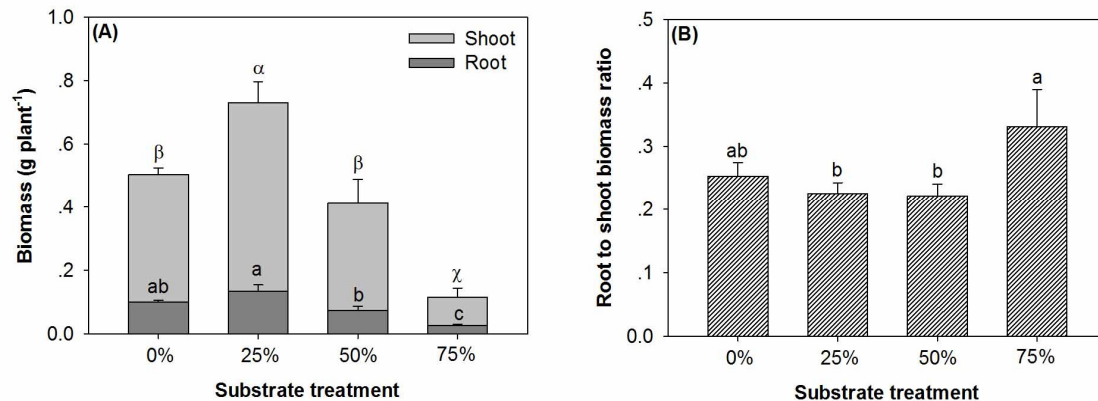


Fig. 2. Biomass accumulation in shoot and root parts (A) and root to shoot biomass ratio (B) in pepper (*Capsicum annuum* L.) plants cultured in the media mixed with commercial peat and fresh spent mushroom substrate (SMS) in proportions of 0% (control), 25%, 50%, and 75%. Different letters indicate significant difference in Tukey test at $\alpha=0.05$. In the cell A, letters of α , β , and χ indicate difference for shoot part; letters of a, b, and c indicate difference for root part

50% and 75% treatments (Table 2). Boll number decreased in the 75% treatment by 59% than in the control. Fruit number and weight in the 75% treatment both decreased by 100% compared to those in the 25% treatment.

Both shoot and root biomass tended to show highest value in the 25% treatment and be lowest in the 75% treatment (Fig. 2A). Compared to the biomass in the 25% treatment, biomass in the shoot and root parts in the 75% decreased by 85% and 79%, respectively. In contrast, the root to shoot biomass ratio (R/S) tended to increase from the proportion of SMS in the substrate (Fig. 2B). Compared to the R/S in the 25% and 50% treatments, that in the 75% treatment was increased by 32%.

Photosynthesis and gas exchange

With the increase of SMS proportion in substrate, the photosynthetic rate did not change from the 0% to the 50% treatments and decreased by 5% in the 75% treatment (Table 3). However, the conductance and transpiration rate

were not affected by SMS proportion treatments. The WUE was highest in the 75% treatment, which was higher by 354% than that in the 0%, 25%, and 50% treatments.

N and P concentrations and contents

With the increase of SMS proportion in the substrate, N and P concentrations showed contrasting responses (Fig. 3). Shoot and root N concentrations generally showed the decreasing trends but P concentration showed the increasing trend. Compared to shoot N concentration in the control, that in the 25% treatment declined by 24% ($P=0.0003$) (Fig. 3A). In the 75% treatment, shoot N concentration declined to be 68% of that in the control. Root N concentration did not change from the 0% to the 50% treatments, but declined by 33% in the 75% treatment than in the 50% treatment ($P=0.0015$) (Fig. 3A). Shoot P concentration decreased in the 25% treatment compared to that in the control by 18% than increased in the 50% and 75% treatments ($P<0.0001$) (Fig. 3B). Root P

Table 3. Photosynthesis and gas exchange of pepper plants in fresh spent mushroom substrate mixed with commercial peat in different proportions (v/v)

Treatment	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Conductance ($\mu\text{l H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Water-use efficiency ($\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ H}_2\text{O}$)
0%	11.44±0.04a	21.36±10.93	0.59±0.28	22.53±8.15c
25%	11.29±0.16a	17.71±3.07	0.46±0.08	25.40±4.40c
50%	11.37±0.08a	25.07±13.75	0.76±0.38	18.01±6.34c
75%	10.83±0.18b	4.49±1.34	0.14±0.04	99.70±4.97a
100%	11.10±0.05ab	13.88±5.39	0.40±0.15	62.40±5.98b
M.S.	0.18	0.00	0.16	3682.90
F value	11.31	2.40	2.84	88.50
Pr > F	0.0010	0.1188	0.0825	<0.0001

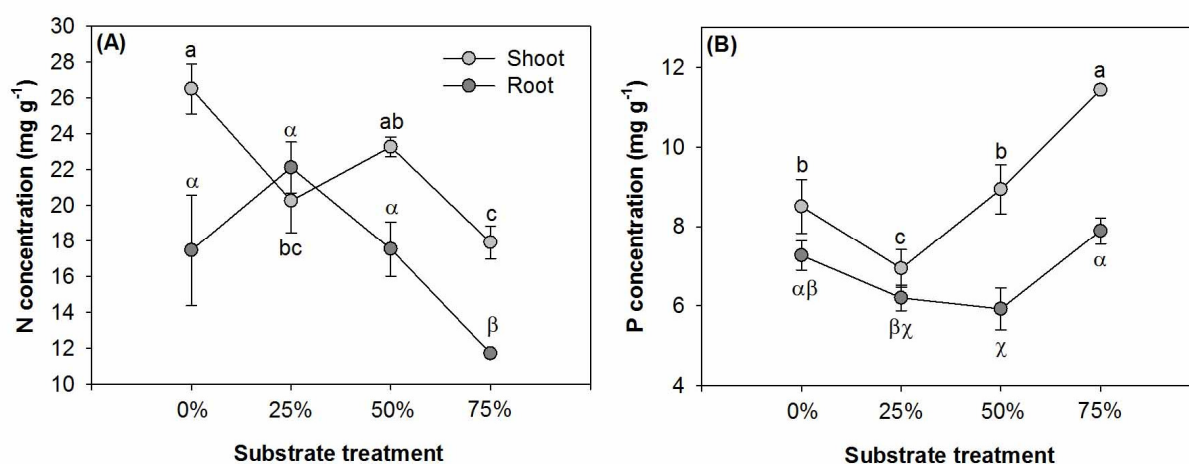


Fig. 3. N (A) and P (B) concentrations in shoot and root parts of pepper (*Capsicum annuum* L.) plants cultured in the media mixed with commercial peat and fresh spent mushroom substrate (SMS) in proportions of 0% (control), 25%, 50%, and 75%. Different letters indicate significant difference in Tukey test at $\alpha=0.05$. Letters of a, b, and c indicate difference for shoot part; letters of α , β , and χ indicate difference for root part

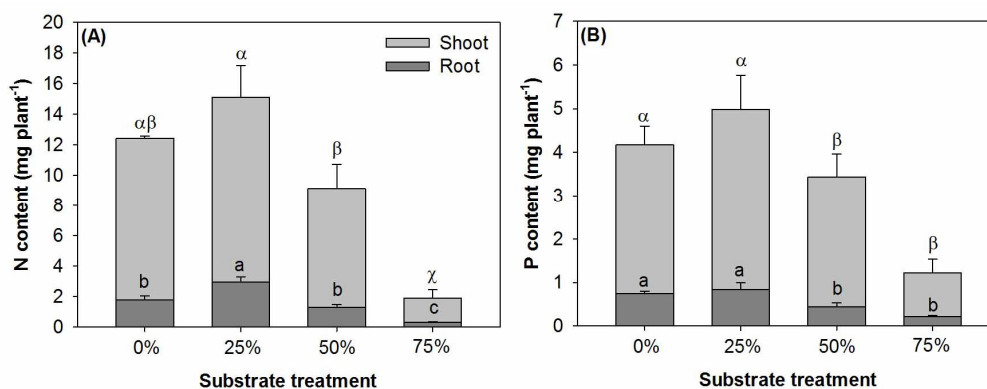


Fig. 4. N (A) and P (B) contents in shoot and root parts of pepper (*Capsicum annuum* L.) plants cultured in the media mixed with commercial peat and fresh spent mushroom substrate (SMS) in proportions of 0% (control), 25%, 50%, and 75%. Different letters indicate significant difference in Tukey test at $\alpha=0.05$. Letters of α , β , and γ indicate difference for shoot part; letters of a, b, and c indicate difference for root part

concentration showed a decreasing trend from 0% to the 50% treatment then increased by 33% in the 75% treatment ($P=0.0013$) (Fig. 3B).

Both N and P contents tended to be highest in the 25% treatment among all treatments with different proportions of SMS (Fig. 4). Compared to shoot N content in the 25% treatment, that in the 50% and 75% treatments declined by 35% and 87%, respectively ($P<0.0001$) (Fig. 4A). Root N content increased by 68% in the 25% treatment than in the control ($P<0.0001$) (Fig. 4A). Compared to N content in the 25% treatment, that in the 50% and 75% treatments declined by 56% and 89%, respectively. Compared to shoot P content in the 25% treatment, that in the 50% and 75% treatments declined by 28% and 76%, respectively ($P=0.0003$) (Fig. 4B). Compared to root P content in the 25% treatment, that in the 50% and 75% treatments declined by 47% and 74%, respectively ($P=0.0003$).

Foliar area and green degree

Foliar area did not change among the treatments of 0%, 25%, and 50% treatments ($P<0.0001$) (Fig. 5A). Foliar area in the 75% treatment declined by 68% than that in the 25% treatment. Foliar green degree was not statistically different among the treatments ($F=2.55$; $P=0.1291$). Foliar green degree ranged from 89.06 ± 14.30 to 104.62 ± 5.55 and had a negative relationship with shoot N concentration (Fig. 5B).

Carbohydrate content and foliar enzyme activity

Soluble sugar content was not statistically different among treatments in either shoot ($F=0.68$; $P=0.5873$) or root ($F=1.38$; $P=0.3161$) parts. Soluble sugar content in shoot ranged from 208.79 mg g⁻¹ to 255.89 ± 35.45 mg g⁻¹, while that in root ranged from 88.66 mg g⁻¹ to 158.43 ± 55.81 mg g⁻¹. In contrast, starch content had a significant response to SMS substrate proportion

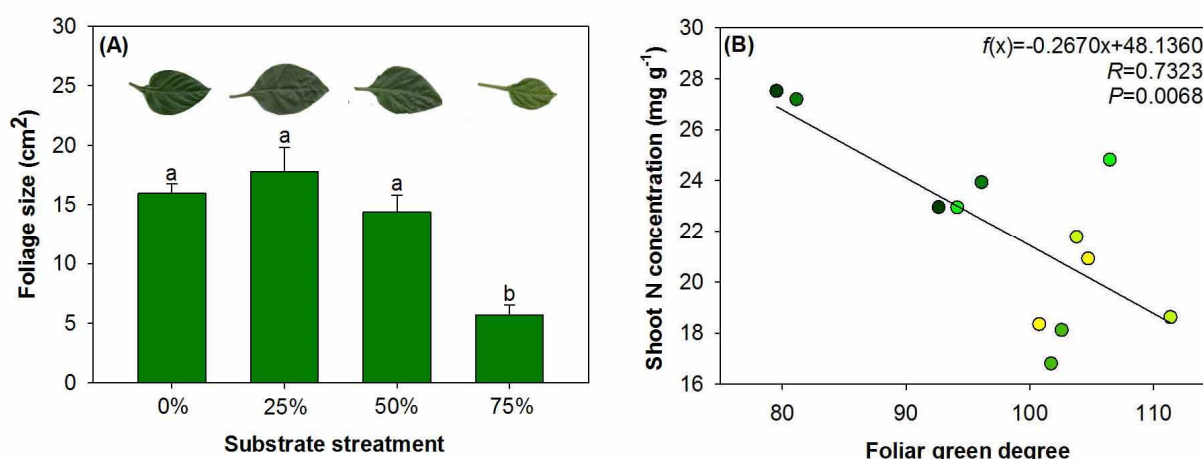


Fig. 5. A: the area of leaves in pepper (*Capsicum annuum* L.) plants cultured in the media mixed with commercial peat and fresh spent mushroom substrate (SMS) in proportions of 0% (control), 25%, 50%, and 75%. Different letters indicate significant difference in Tukey test at $\alpha=0.05$. B: the linear correlation between foliar green degree and shoot N concentration

Table 4. Starch content and foliar enzyme activity in pepper plants in fresh spent mushroom substrate mixed with commercial peat in different proportions (v/v)

Treatment	Shoot starch (mg g ⁻¹)	Root starch (mg g ⁻¹)	Foliar ACP (μg NPP g ⁻¹ FW min ⁻¹)	Foliar GS (A μg ⁻¹ protein h ⁻¹)
0%	69.17±0.71ab	138.77±38.42a	45.21±2.05a	6.71±1.06a
25%	98.04±23.31a	80.95±6.38b	30.36±0.51b	10.82±3.65a
50%	48.52±5.95b	72.93±6.32b	35.61±2.61ab	8.34±2.15a
75%	71.45±5.04ab	88.75±7.86ab	41.47±9.24ab	12.59±2.17a
M.S.	1237.62	2638.53	128.10	0.00
F value	7.33	5.84	4.75	3.07
Pr > F	0.0110	0.0205	0.0348	0.0911

Table 5. The Pearson correlation between the chemical properties and nutrient concentrations and contents in pepper plants (n=12)

Substrate		N concentration		P concentration		N content		P content	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
NH ₄ ⁺ -N	R	0.6667	0.7342	-0.8638	-0.5300	0.8964	0.7616	0.8489	0.8201
	P	0.0178	0.0056	0.0003	0.0763	<0.0001	0.0040	0.0005	0.0011
NO ₃ ⁻ -N	R	0.5128	0.6565	-0.7391	-0.1505	0.8137	0.7824	0.7401	0.8765
	P	0.0882	0.0204	0.0060	0.6407	0.0013	0.0026	0.0059	0.0002
PO ₄ ³⁻ -P	R	-0.7252	-0.5981	0.7125	0.1852	-0.8456	-0.6923	-0.7751	-0.8541
	P	0.0076	0.0399	0.0093	0.5644	0.0005	0.0126	0.0031	0.0004
pH	R	-0.7326	-0.6881	0.7841	0.3710	-0.8538	-0.7212	-0.7820	-0.8240
	P	0.0067	0.0134	0.0025	0.2351	0.0004	0.0081	0.0027	0.0010
EC	R	-0.7535	-0.6212	0.7070	0.2486	-0.8458	-0.6801	-0.7791	-0.8236
	P	0.0047	0.0311	0.0101	0.4359	0.0005	0.0149	0.0028	0.0010

Note: The light gray color of the cell indicates positive correlation; the dark gray color of the cell indicates negative correlation.

treatments (Table 4). Shoot starch content tended to be highest in the 25% treatment which was higher than that in the 50% treatment by 102%. However, root starch content tended to be highest in the control which was higher than that in the other treatments by about 70%. Foliar ACP activity also tended to be highest in the control, which was higher by 49% compared to that in the 25% treatment (Table 4). Foliar GS activity did not change among treatments.

Correlation analysis

Generally, both substrate NH₄⁺-N and NO₃⁻-N concentrations were positively correlated with N and P parameters except for P concentrations (Table 5). In contrast, substrate PO₄³⁻-P concentration, pH, and EC were positively correlated with shoot P concentration but negatively correlated with other nutrient parameters.

Discussion

Growth and biomass accumulation

Using SMC as the component of the mixture in substrate, a proportion of 20~30 % could result in better responses of growth and biomass for shrubs (Chong *et al.*, 1994), rough lemon (*Citrus jambhiri* L.) (Khan *et al.*, 2006), and horticultural plants (Medina *et al.*, 2009; Gonani *et al.*, 2011; Kwack *et al.*, 2012). Although we used fresh SMS as one component in the substrate, we also found that the SMS proportion of 25% can result in better height and diameter growth (Table 2) and greater shoot and root biomass accumulation (Fig. 2A). Former results, as

well as those found in our study, together suggest that SMS, no matter composted or fresh, is not suitable to be used as the main component in the substrate for horticultural plant culture. Furthermore, using pepper as the material Marín *et al.* (2014) reported that the highest stem length (seedling height) and diameter were measured to be 15.13 cm and 3.06 mm, respectively, in response to SMC. These values were both lower than those in the 25% treatment in our study (Table 2). In addition, the total dry weight in our study (0.73±0.07 g) was also greater than that (0.50 g) in Marín *et al.* (2014). The promotion of SMS substrate on shoot parameters was also supported by R/S, which was not statistically different among the control and the 25% treatment but higher ratio in the 75% treatment (Fig. 2B). This suggests that the substrate will not drive dry mass allocated to roots unless the SMS proportion was over 50%. Therefore, the 25% proportion of fresh SMS in the substrate in our study can be recommended for the practical use to improve growth and biomass accumulation in pepper plants.

Fruiting performance

In line with growth and biomass results, our results also showed that both fruit number and fruit weight showed best performance in the 25% treatment (Table 2). (Szmidt and Chong, 1995; Gonani *et al.*, 2011). In agreement with our results, Ahlawat *et al.* (2007) found that the involvement of SMC composting for 12 months in the substrate can also enhance the yield of pepper plants. Gonani *et al.* (2011) found more fruits out in the cucumber (*Cucumis sativus*) plants receiving the substrate of 25% (v/v) SMC than that of 25% SMC. The addition of SMS

to the substrate can promote the fruit quality of pepper plants except for vitamin C content (Eudoxie *et al.*, 2014). Other studies revealed that the soils amended by SMS can be effective in promoting yield and quality of melon (Nguyen and Wang, 2016) and maize (Wuest *et al.*, 1995). Therefore, the promotion of fruit yield in crop plants appeared to be a general effect by SMS addition to substrate. However, when the SMS proportion in the substrate increased to excess 50% the boll number significantly declined compared to the control (Table 2). Therefore, the SMS proportion in the substrate cannot affect boll generation unless it occupied the half volume. The 25% proportion of SMS in the substrate did not show any superiority in boll numbers; hence this proportion cannot be recommended for the potential of flowering and yield in pepper plants.

The photosynthetic production and gas exchange

In this study, the shoot starch content tended to be highest in the 25% treatment, where root starch content was lower than the control (Table 4). These results suggest that seedlings in this treatment would allocate more photosynthetic productions in the shoot part to support the growth, biomass, and fruiting quality. Because we did not find any response of sugars, it cannot be put forward that SMS substrate had any effect on starch hydrolyzation into sugars. However, photosynthetic rate did not change in the substrates where the SMS proportion was lower than 75% (Table 3). Because SMS is of high risk of EC, the high proportion of SMS in the substrate (~75%) may have interrupted the photosynthesis through high ion concentration (Szmidski and Chong, 1995; Gonani *et al.*, 2011). Even the rhizosphere-condition in the 75% treatment had unlikely caused the salinity stress on pepper plants because no gas exchange responded (Guo *et al.*, 2016). Furthermore, we suspect the 100% SMS substrate has caused severe saline stress because nearly all seedlings died off (Fig. 1). The high WUE in the 75% treatment mainly resulted from the relatively lower transpiration rate in spite it was not statistically different from that in other treatments. As a photosynthetic organ, the area of leaves responded to SMS substrate with the same trend of photosynthetic rate. This was because the foliar area was related with the photosynthetic rate and the 25% proportion of SMS in the substrate failed to affect the foliar area. In contrast, Marín *et al.* (2014) reported that pepper plants in the treatment with best growth performance also had largest leaves. The foliar area in our study was about a quarter of that in Marín *et al.* (2014). This may be because seedlings in our study were cultured under artificial lighting and the PPFD of around 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ might be insufficient for the growth of large leaves.

Nutrient availability

The fresh SMS used in this study had less extractable N concentration but higher available P concentration than the commercial peat (Table 1). In other studies, both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations were much higher than those in ours. For example, the $\text{NH}_4^+\text{-N}$ was recorded to be 2,800 mg kg^{-1} (Szmidski and Chong, 1995), 186 mg kg^{-1} , 327 mg kg^{-1} (Paredes *et al.*, 2016), and 250 mg kg^{-1} (Paula *et al.*, 2017). The $\text{NO}_3^-\text{-N}$ was recorded to be 5,800 mg kg^{-1}

(Szmidski and Chong, 1995), 53 mg kg^{-1} , and 81 mg kg^{-1} (Paredes *et al.*, 2016). The composting process can increase the extractable N concentration by at least 100% (Paula *et al.*, 2017). Fresh mushroom had low concentration of available N because it had been composted. Available P concentration in the fresh SMS in our study was found to be about three-fold higher than that in the commercial peat (Table 1). Our results about available P concentration were higher than that reported by Unal (2015) (13.79~206.84 mg kg^{-1}) but much lower than that reported by Sönmez *et al.* (2016) (25,280~28,280 mg kg^{-1}) and that by Paredes *et al.* (2016) (3,740~6,800 mg kg^{-1}). Therefore, the fresh SMS in our study can be generally characterized by poor N availability and moderate P availability.

Compared to the control, shoot N concentration declined in the 25% treatment where root N concentration tended to increase but results were not statistically different (Fig. 3A). Regarding that the foliar GS activity was not different among treatments (Table 4) while biomass was greatest in the 25% treatment, it can be speculated that N was assimilated in root and utilized in shoot in the substrate with 25% SMS. The difference of N and P content among treatments was similar to that of biomass and starch content. Therefore, the positive relationship between substrate available N and N and P should be the result of promotion on carbohydrate production and thereafter biomass accumulation. In addition, the negative relationship between available N concentration in the substrate and P concentration was the result of P dilution by biomass increment. It was summarized that the high pH value and EC in SMS can impact its usage as the substrate for horticultural plants (Szmidski and Chong, 1995; Gonani *et al.*, 2011). In our study, P concentration increased with the increase of SMS proportion in the substrate (Fig. 3B). This trend concurs with that of available P concentration in the substrate with increasing proportion of SMS, which also caused increasing pH and EC (Table 1). Therefore, the negative relationship between available P concentration in the substrate and N concentration and N and P contents should be result of increasing pH and EC with the accumulating of SMS in the substrate.

Conclusions

With the increase of fresh SMS in the substrate mixing with the commercial peat, available N concentration declined but extractable P, pH and EC all increased. These gradient changes were mainly accompanied by the unimodal pattern of response of pepper plants in response to different proportions of SMS. As a result, the 25% proportion of SMS in the substrate was found to be the best treatment because it induced best height and diameter growth, biomass accumulation in shoot and root parts, fruit number and weight. These results can be attributed to by the accumulation of starch in shoot of seedlings under the 25% SMS condition. The SMS substrate did not affect photosynthetic rate and gas exchange in the proportion lower than 75%. High proportion of SMS caused the declines of N concentration and N and P contents. Seedlings in the 100% SMS substrate nearly died out. Therefore, fresh SMS cannot be directly used as the substrate for the culture of pepper plants. According to

findings in our study and others, fresh SMS was recommended to be mixed in the proportion of 25% with commercial peat for the culture of horticultural plants.

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