agronomy

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Influence of Different Spent Mushroom Substrates on Yield, Morphological and Photosynthetic Parameters of Strawberry (*Fragaria* × *ananassa* Duch.)

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Abstract: The present study aimed to evaluate fresh spent mushroom substrate (SMS) as a growing medium in soilless strawberry cv. 'Honeoye' production. Fresh SMS after commercial production of *Agaricus bisporus*, *Lentinus edodes*, and *Pleurotus ostreatus* was used as a peat substitute in 15 and 25% (*v*/*v*), for strawberry cultivation in an unheated plastic tunnel. In the experiment, seven different substrates were studied, including peat (100%) as control and six substrate combinations (prepared by mixing SMSs with peat). The study was carried out in a randomized complete block design in five replicates. The results indicated that the electrical conductivity (EC), pH, and nutrient content varied among the studied substrates. The experiment also demonstrated that the substrates significantly influenced strawberry yield, leaf area, and fresh and dry plant weights. However, no significant differences were observed for selected photosynthetic parameters (F_v/F_m, F_v/F_0, and PI_{abs}) and Normalized Difference Vegetation Index (NDVI) values among the evaluated substrates. Differences were recorded for the Photochemical Reflectance Index (PRI) and Modified Chlorophyll Absorption in Reflectance Index (MCARI) values. The present investigation revealed that fresh SMSs can be an effective and inexpensive peat substitute in 15 and 25% (*v*/*v*). Therefore, such easy and immediate utilisation of SMSs could overcome associated disposal problems.

Keywords: abiotic stress; agro-waste; photosynthetic parameters; soilless cultivation; soilless substrate; spent mushroom substrate; strawberry

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Citation: Prasad, R.; Lisiecka, J.; Antala, M.; Rastogi, A. Influence of Different Spent Mushroom Substrates on Yield, Morphological and Photosynthetic Parameters of Strawberry (*Fragaria* × *ananassa* Duch.). *Agronomy* **2021**, *11*, 2086. https://doi.org/10.3390/agronomy11102086

Received: 3 September 2021
Accepted: 16 October 2021
Published: 19 October 2021

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is the most economically important soft fruit in the world. The global production of strawberries has risen almost 30% between 2009 and 2019, making it the second-largest berry fruit, after grape (*Vitis vinifera* L.), in terms of production [1]. Strawberries are known for their characteristic aroma and taste. They are also an excellent source of vitamin C and have considerable amounts of vitamin B9. The antioxidative properties of strawberry fruits are reported to be beneficial for blood sugar and heart health [2–4].

In recent years, greenhouse strawberry production around the globe has gained importance over traditional soil culture. The advantages of greenhouse production over traditional cultivation include better cultivation practices, improved pest and disease management, and more efficient nutrient and irrigation use resulting in higher yield. In addition to these advantages, the short cultivation period and suitability for small family farms are the key reasons for the interest in soilless strawberry production [5]. Peat predominates as a commercial substrate for greenhouse strawberry production. However, due to extensive utilization, peat resources are at the edge of depletion [6–8]. Considering its non-renewability, increasing cost, future availability, and environmental sustainability, growers around the globe need a high-quality, renewable, and sustainable substitute [8]. At the same time, many reports also stress the need for finding potential peat-free growing media [9–11].

Increasing environmental concerns and need for effective utilisation and disposal of agro-waste to achieve sustainability has encouraged investigations into utilizing accumulating potential agro-industrial residues in horticulture. Some studies have reported the influence of agro-waste-based substrates as a substitute for peat in strawberry production [12–16]. Among many agro-wastes, the possibility of utilizing spent mushroom substrate (SMS) as a potential substitute can be beneficial. This agro-waste has a high organic composition, is readily available, and the cost is negligible [17–20]. SMS is the residual material left over after commercial mushroom production. The substrate obtained immediately after mushroom production is fresh-SMS (F-SMS) or SMS, and the same material after further decomposition or weathering from 3–24 months is called weathered-SMS (W-SMS) or spent mushroom compost (SMC) [21]. Mushroom production can be considered as a non-sustainable agriculture activity due to the accumulation of an enormous amount of SMS and its limited re-use. Global mushroom production has passed ten million tons [1]. Approximately five kilograms of SMS is generated for each kilogram of mushrooms produced [18,22,23]. On this account, the annual SMS generated from the global mushroom industry is estimated to be more than 50 million tons. SMSs generated from mushroom enterprises in large quantities are often burnt, discarded, or simply thrown away, which is neither economical nor environmentally safe [24]. Increasing mushroom production in recent years and the expected increase in the future will lead to a significant accumulation of SMS. The generated amount of SMS can pose potential environmental threats if not properly disposed of or effectively utilised.

Researchers over decades reported that SMC can be utilised for agricultural and horticultural purposes [25], as a soil conditioner [26–28], as nursery media [7,29,30], and as a soilless growing medium [31,32]. On the contrary, fresh SMS is recommended only for use after further decomposition/weathering process ranging from 3–24 months [21,29,33]. It has been reported that even after 24 months of passive weathering, SMS can still release a significant amount of soluble solids [21], and the leachates from SMS would significantly increase the salt content of underlying soil and groundwater [21,34]. Fresh SMS has high EC due to excess accumulation of salts during mushroom cultivation and unfavourable pH, which are the major limiting factors for its immediate use and hence using fresh SMS is not recommended [21,35,36]. The unfavourable pH and EC of growing media may negatively influence overall plant development [37,38] and alter photosynthetic processes due to stress [39–42].

In recent years, various techniques have been developed to indicate stress processes in plants. The most promising and reliable results have been obtained in processes that analyse photosynthesis, which is closely associated with plant performance and yield [43–45]. The decline in photosynthetic activity, either directly or indirectly due to various abiotic stress factors may largely influence the overall performance and yield of the plant [44,46]. To study the impact of different stress factors on photosynthesis, chlorophyll *a* fluorescence has become a popular approach [44,47,48], including salt stress [46] and nutrient deficiencies [49]. The stress-induced changes on the Photosystem II (PSII) electron acceptor are well reflected in the values of integrative OJIP-test parameters, known as Performance Indices (PIs). The OJIP-test has been successfully used to understand the influence of several stress factors in plants [44,50,51].

The F_v/F_0 value defines the maximum quantum efficiency of PSII photochemistry and is the most common indicator of chlorophyll fluorescence transient on plant leaves under stress conditions [52]. F_v/F_m indicates the maximum quantum yield of PSII photochemistry, whereas $PI_{(abs)}$ is a photosynthetic parameter that takes several different phenomena related to PSII photochemistry into consideration [53]. Vegetation indices (VIs) are radiative transfer-based methods, with some mathematical combination or transformation of spectral bands that accentuate the spectral properties of plants. The expressed values of different VIs, including the Normalized Difference Vegetation Index (NDVI), Photochemical Reflectance Index (PRI), and Modified Chlorophyll Absorption in Reflectance Index (MCARI) help to analyse crop growth, vigour, and several other vegetation properties including biomass and chlorophyll content [54]. Vegetation indices are also reported to be a reliable indicator of plant health [55,56] and abiotic stress in plants [57,58].

The scientific information supporting the utilisation of SMC (weathered SMS) is evident. However, the process of further decomposition can be considered laborious, time-consuming and the leachates released during this process can alter underlying soil and water chemistry, which may lead to various environmental hazards. Hence, this study was aimed to come up with an easy, effective, immediate, and cheap utilisation of fresh SMS, which may be environmentally and economically beneficial.

Strawberry greenhouse production is progressively gaining importance around the globe [13,59,60]. However, the yield and overall performance of strawberries largely depends on the substrate [12–16,59,60]. Considering the growing popularity of soilless strawberry cultivation, which relies mainly on peat, and the increasing demand for peat-reduced media, the development of a peat alternative is of great importance.

The present investigation was designed to study the possible utilization of fresh SMS as a renewable, easily available, comparatively cheap and sustainable alternative to non-renewable, relatively expensive, and non-sustainable peat in soilless strawberry production. In the present study, fresh SMS was obtained after the cultivation of white button mushroom—*Agaricus bisporus* (J.E. Lange), shiitake—*Lentinus edodes* (Berk.) and oyster mushroom—*Pleurotus ostreatus* (Jacq.). The obtained SMSs were used as a peat substitute in 15 and 25% (*v*/*v*) for soilless strawberry cv. 'Honeoye' cultivation in an unheated plastic tunnel. The investigation was carried out with three objectives: to evaluate the suitability of fresh SMS as a peat substitute, to study the strawberry growth and yield performances on SMS based substrates, and to determine selected abiotic stress responses and vegetation indices.

2. Materials and Methods

2.1. Substrate Composition

2.1.1. *Agaricus bisporus*

Substrate for the cultivation of *Agaricus bisporus* was prepared using 1000 kg wheat straw, 750 kg poultry manure, 80 kg gypsum, and 3000 kg of water. Fermentation was carried out at 75–85 °C and pasteurization at 54–60 °C. Black peat was used as a casing soil along with 20 kg chalk per 1 m^2 of peat. A layer of 5 cm peat was embedded on top of the compost block at the rate of 80 kg m^2. The total duration of *A. bisporus* cultivation was approximately 11 weeks (from spawning to final harvest). The mushroom substrate after commercial production of *A. bisporus* (A-SMS) without post-crop heat treatment/sterilization was used in the study.

2.1.2. *Lentinus edodes*

For *Lentinus edodes* cultivation, the substrate was prepared using a sawdust mixture of beech and oak in a ratio of 1:1 (*v*/*v*). It was mixed along with 10% bran and 10% flour (dry matter of sawdust). The moisture of 65% was maintained in the substrate; later, this substrate was pasteurization for 10 h at 90–95 °C. The substrate was spawned at 5% wet weight and incubated for 90 days prior to commencement of the harvest. The total duration of *L. edodes* cultivation was approximately 12 weeks. Fresh SMS (without post-crop heat treatment/sterilization) after commercial production of *L. edodes* (L-SMS) was used in the study.

2.1.3. *Pleurotus ostreatus*

Pleurotus ostreatus was cultivated on the substrate prepared from wheat straw and wheat bran 20% (dry matter of straw) with an optimum moisture content of 70%. Pasteurization was carried out for 48 h at 60 °C. After cooling to room temperature, the substrate was spawned at 3% wet weight. Inoculated substrates were incubated for 18 days at 25 °C. The total duration of *P. ostreatus* cultivation was approximately 10 weeks. Fresh SMS (without post-crop heat treatment/sterilization) after commercial production of *P. ostreatus* (P-SMS) was used in the study.

2.1.4. Peat

Superior quality professional peat (peat clear-class H2 according to Van Post classes) with enhanced hydrophilic capacity obtained from Hartmann Polska Sp. z o.o. (Poznań 60-307, Poland) was used as a control substrate in the experiment.

2.2. *Growing Conditions, Cultivar and Substrate Preparation*

The experiment was conducted during spring 2019 (April to June) in an unheated plastic tunnel 30 × 7 m (length × width) at The Experimental Station Marcelin (52°24′20″ N and 16°51′35″ E) belonging to the Faculty of Agronomy, Horticulture and Bioengineering, Poznan University of Life Sciences, Poland. The mean temperature and relative humidity (RH) inside the tunnel during the growing period were 21.7 °C and 55.5%, respectively.

The studied cultivar in the present investigation 'Honeoye' is a June bearing cultivar (bred in New York and released in 1979) [61]. The tray plants (A+ grade) were planted on the 1 April 2019 and the strawberry fruits were harvested from the last week of May to the end of June 2019.

Three SMSs after commercial cultivation of *Agaricus bisporus* (A-SMS), *Pleurotus ostreatus* (P-SMS), and *Lentinus edodes* (L-SMS) were evaluated as a substitute for peat in the study. Seven different substrates were studied in the experiment, which included: Peat 100% as control, A-15 A-SMS:Peat (15:85%), A-25 A-SMS:Peat (25:75%), L-15 L-SMS:Peat (15:85%), L-25 L-SMS:Peat (25:75%), P-15 P-SMS:Peat (15:85%), and P-25 P-SMS:Peat (25:75%) prepared based on (*v*/*v*). The nutrient content of all substrates used in the study is presented in Table 1.

Table 1. Macro- and micronutrient concentrations ($mg \cdot L^{-1}$) of different substrates used in the study.

Nutrients	**Peat**	**A-15**	**A-25**	**L-15**	**L-25**	**P-15**	**P-25**
Ammonia-Nitrogen ($N\text{-}NH_4$)	4	189	476	7	14	7	7
Nitrate-Nitrogen ($N\text{-}NO_3$)	28	77	63	21	14	7	7
Phosphorus (P)	49	334	843	28	23	126	77
Potassium (K)	94	1144	1216	120	160	67	114
Calcium (Ca)	1209	2123	1606	181	278	1765	955
Magnesium (Mg)	119	529	610	19	42	164	80
Sulphur ($S\text{-}SO_4$)	145	407	317	1	5	3	3
Sodium (Na)	21	135	198	24	20	14	17
Chlorine (Cl)	10	25	38	27	9	5	11
Iron (Fe)	13.0	101.4	74.6	90.8	47.0	102.6	110.2
Manganese (Mn)	1.7	45.6	55.3	24.9	33.3	4.3	5.9
Zinc (Zn)	0.8	24.6	27.9	5.5	5.8	10.3	6.9
Copper (Cu)	0.3	4.9	11.8	0.8	0.8	1.0	1.0

A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

2.3. Substrate Filling, Planting, and Experimental Setup

The growing containers 90 × 13.5 × 12 cm (L × B × D) were filled with 12 L of prepared substrates. Four strawberry plants were planted in each container (3 L of substrates per plant), maintaining a spacing of 20 cm between each plant. The experiment was laid out in a Randomized Complete Block Design (RCBD), with seven substrates in five replications. Then, 40 plants (10 growing containers) were maintained in individual studied substrates. The growing containers were organised into 5 rows (each row as a replicate) with a spacing of 95 cm between rows and 35 cm between each growing container.

Automatic micro drip irrigation (three bent arrow emitters per container with a flow rate of 2 $L \cdot h^{-1}$) was provided. During the vegetative phase (April), plants were irrigated two times a day for 120 s (60 s in each interval), and during the flowering and fruiting phase (from the first week of May till the end of June) plants were irrigated three times a day for 360 s (120 s in each interval). The irrigation duration and interval were controlled by Galcon GAE2S0002U1 8006 AC station zone irrigation controller. The average pH and EC of irrigation water were 7.11 and 0.72 $mS \cdot cm^{-1}$, respectively.

Plants were fertilised with a water-soluble nutrient solution based on Kristalon Blue (N:P:K 19:06:20 + microelements) and Calcinit (15.5% N + 26.3% CaO) from YARA (Yara Poland Sp. z o. o, Szczecin, Poland). The nutrient solution prepared from a 10% stock solution of each fertiliser was furthered diluted to obtain a working concentration of 0.25%. Two doses of each nutrient solution (500 mL per container) were manually applied to each growing container (to ensure the same amount of nutrients added to each growing container) during the crop cycle at the 15th, 30th, 45th and 60th days after planting (Table 2). The pH and EC of 10% Kristalon Blue and Calcinit ranged between 5.9–6.2 and 1.3–1.5 $mS \cdot cm^{-1}$ and 6.0–6.2 and 1.0–1.2 $mS \cdot cm^{-1}$, respectively.

Table 2. A standard stock solution of nutrients at 0.25% (*v*/*v*) dilution rate, used in different strawberry growing stages.

Nutrients	Dose-1	Dose-2	Dose-3	Dose-4
	Vegetative Stage		Generative Stage	
Macronutrients				
Nitrogen (N)				
N-NO_3	7.9%	14.4%	7.9%	14.4%
N-NH_4	12.1%	1.1%	12.1%	1.1%
Phosphorus (P)	5%		5%	
Potassium (K_2O)	10%		10%	
Magnesium (MgO)	2%		2%	
Sulfur (S)	10%		10%	
Calcium (CaO)		26.3%		26.3%
Micronutrients				
Iron (Fe) EDTA	0.07		0.07	
Manganese (Mn) EDTA	0.04%		0.04%	
Zinc (Zn) EDTA	0.025%		0.025%	
Copper (Cu) EDTA	0.01%		0.01%	
Boron (B)	0.025%		0.025%	
Molybdenum (Mo)	0.004%		0.004%	

ethylenediaminetetraacetic acid (EDTA).

2.4. Yield and Morphological Parameters

The strawberry fruits were harvested from the last week of May to the end of June. The fruits from each plant in the individual substrate were harvested and cumulative yield (g·plant^{-1}) per harvest was recorded. At the end of the growing season, the cumulative yields (g·plant^{-1}) in each substrate were added to obtain the total yield (g·plant^{-1}). The total and marketable yield from each substrate was determined on fruits harvested through May–June. The diseased, damaged, misshaped fruits and fruits with less than 18 mm of diameter were categorised as unmarketable fruits. Harvested fruits free from any infections and fruits with a diameter between 18–25 mm, and >25 mm were considered as marketable fruits [62]. At the end of the growing season (June), strawberry plants were separated from the growing media (carefully washed in running water till no trace of substrates was attached to the roots) and plant morphological parameters including shoot, root, and total plant fresh as well as dry weights (dried at 105 °C for 24 h) were recorded. The shoot to root ratio was calculated based on the obtained dry weight. In individual substrates five fully developed (2nd leaf from the node) strawberry leaves were scanned using the WINDIAZ leaf measurement system and the leaf area was calculated using WINDIAZ software (Delta-T Devices Ltd., Cambridge, UK). The obtained leaf area was expressed in cm^2.

2.5. Chlorophyll a Fluorescence

Chlorophyll a fluorescence transients were measured using a handheld PAM fluorometer FluorPen FP 110 (Photon Systems Instruments Ltd., Drásov, Czech Republic). The measurements were taken during the strawberry generative phase (15–20 June 2019). The leaf samples were dark-adapted for a minimum of 30 min (using FP 110 leaf clip) and the measurements were made on a fully mature leaf (2nd leaf from the node). The measurements were carried out on 3 plants (one fully mature leaf from each plant), accounting for 15 measurements in individual studied substrates. The recorded data were later exported using FluorPen software and subsequent OJIP (rapid fluorescence transient) analysis was undertaken. This OJIP rise measured at saturating light has been widely used to calculate F_v/F_m, a fluorescence parameter that serves as a proxy for the maximum quantum yield of PSII photochemistry [63]. These Performance Indices (PIs) are proposed to combine information on the performance of PSII and efficiencies of specific electron transport reactions in the thylakoid membrane during the OJIP rise to provide a sensitive tool for photosynthetic stress tolerance [43]. Selected PIs measured in the present study are given in Table 3.

Table 3. Selected Performance Indices (PIs).

Parameter	Formula	Authors	Sensitive to Identify	Intended in the Present Study
F_v/F_0	$=(F_m - F_0)/F_0$	[52,64]	Identify abiotic stress responses in plants [51,52]	To study abiotic stress responses in strawberry plants due to varying chemical properties (pH, EC and nutrient content) of studied substrates
F_v/F_m	$=(F_m - F_0)/F_m$	[52,64]		
PI_{abs}	$=(RC/ABS) \times [\varphi P0/(1 - \varphi P0)] \times [\psi E0/(1 - \psi E0)]$	[43,52]		

F_v/F_0 (the maximum primary yield of the photochemistry of photosystem II), F_v/F_m (maximal photochemical efficiency of PSII), and PI_{abs} (performance index).

2.6. Spectral Vegetation Indices (VIs)

Spectral vegetation indices such as NDVI, PRI, and MCARI measured in the study are presented in Table 4. They were measured using the handheld device PolyPen RP 410 (Photon Systems Instruments Ltd., Drásov, Czech Republic). The measurements were recorded in a greenhouse during daylight from a fully mature leaf (2nd leaf from the node). The same leaves previously used to measure PIs were used for VI measurements. The leaf (held in place with a RP 410 mechanical leaf holder) was exposed to an internal light source of RP 410 (Xenon incandescent lamp with a spectral range of 380–1050 nm) with a UVIS sensor (380–790 nm). The recorded data were later downloaded and exported using integrated software from the provider.

Table 4. Evaluated spectral vegetation indices (VIs).

Parameter	Formula	References	Sensitive to Identify	Intended in the Present Study
NDVI	$=(R_{780} - R_{630})/(R_{780} + R_{630})$	[65,66]	Plant health [55], abiotic stress [56]	To study plant health status and abiotic stress responses induced by different substrate characteristics (pH, EC and nutrient content)
PRI	$=(R_{531} - R_{570})/(R_{531} + R_{570})$	[66,67]	Abiotic stress [57]	
MCARI	$=[(R_{700} - R_{670}) - 0.2 \times (R_{700} - R_{550})] \times (R_{700}/R_{670})$	[66,68]	Abiotic stress [58]	

Normalized Difference Vegetation Index (NDVI), Photochemical Reflectance Index (PRI) and Modified Chlorophyll Absorption in Reflectance Index (MCARI).

2.7. Substrate Analysis

The substrate samples were collected to analyse the pH, EC, and macro-and micronutrients before and after strawberry cultivation. The substrate samples (500 mL) before the study were collected (in a plastic zip-lock bag) during substrate preparation, and at the end of the growing season the substrate samples were collected from growing containers (100 mL from 5 growing containers, 500 mL total of substrate per sample).

The substrate analysis was carried out at the Department of Plant Nutrition, the Faculty of Agronomy, Horticulture and Bioengineering, Poznań University of Life Sciences. The pH, EC, micro- and macronutrients were analysed as described by Schroeter-Zakrzewska et al. [69]. Collected substrate samples were chemically analysed by the universal method. Extraction of macronutrients (N-NH_4, N-NO_3, P, K, Ca, Mg, S-SO_4), Cl and Na were carried out in 0.03 M acetic acid with a quantitative 1:10 proportion of substrate to extraction solution. After extraction, the following determinations were made: N-NH_4, N-NO_3-by micro distillation according to Bremer in Starck's modification; P-colourimetrically with ammonium vanadomolybdate; K, Ca, Na-photometrically; Mg-by atomic absorption spectrometry; S-SO_4-nephelometrically with $BaCl_2$; Cl-nephelometrically with $AgNO_3$. Micronutrients (Fe, Mn, Zn and Cu) were extracted from the soil with Lindsay's Solution containing 1 dm^3: 5 g EDTA (ethylenediaminetetraacetic acid); 9 cm^3 of 25% NH4 solution, 4 g citric acid; 2 g Ca $(CH_3COO)_2 \cdot 2H_2O$. Micronutrients were determined by the ASA method. Salinity was identified conductometrically as an electrolytic conductivity (EC in $mS \cdot cm^{-1}$) (substrate:water = 1:2), and pH was determined by the potentiometric method (substrate:water = 1:2).

2.8. Statistical Analysis

The experiment was laid out in a Randomized Complete Block Design (RCBD) with seven substrates in five replications, facilitating 40 plants in individual substrates studied. The yields and morphological parameters are means of 5 replicates (3 plants were measured in each replicate (row), the mean value from each replicate constituting 5 observations from each substrate was statically analysed). However, OJIP and VIs are the means of 15 plants (3 plants from each replication). All recorded data were evaluated by one-way analysis of variance (ANOVA), and the mean differences were compared by post hoc test at a p level of <0.05 according to Tukey's HSD. Statistical and correlation analyses were performed using SPSS 13.5 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Selected Chemical Parameters of Substrates

All fresh SMSs (100% A-SMS, L-SMS, and P-SMS) and peat (100%) showed differences in pH and EC values (Table 5). The highest pH and EC values were recorded in 100% fresh A-SMS when compared to peat and two other fresh SMSs. The pH and EC values of fresh SMSs were at levels that limit the use of pure (100%) SMS as a soilless substrate.

Table 5. The pH and EC value of 100% SMSs and 100% peat used in the study (mean ± SD).

Substrate	pH	EC ($mS \cdot cm^{-1}$)
Peat	6.46 ± 0.59 b	0.56 ± 0.33 c
A-SMS	8.05 ± 0.20 a	7.77 ± 0.17 a
L-SMS	4.98 ± 0.11 c	2.88 ± 0.04 b
P-SMS	4.55 ± 0.13 c	1.28 ± 0.06 c

Means in each column followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD ($n = 3$). Electrical conductivity (EC), *A. bisporus* spent mushroom substrate (A-SMS), *L. edodes* spent mushroom substrate (L-SMS) and *P. ostreatus* spent mushroom substrate (P-SMS).

The pH and EC values varied among studied substrates before and after the experiment (Table 6). The pH range before the experiment was 5.51–7.56 and after the experiment between 6.15–6.72. The higher pH values before the experiment in A-25, P-15, P-25, and A-15 substrates were observed to be lower after strawberry production.

Table 6. The pH and EC values of substrates before and after cultivation of strawberry cv. 'Honeoye'.

Substrates	pH		EC ($mS \cdot cm^{-1}$)	
	Before	After	Before	After
Peat	6.46 ± 0.59 c	6.57 ± 0.01 b	0.56 ± 0.33 b	2.08 ± 0.06 d
A-15	7.16 ± 0.01 b	6.32 ± 0.01 c	1.28 ± 0.03 a	3.20 ± 0.07 b
A-25	7.72 ± 0.03 a	6.29 ± 0.01 c	1.20 ± 0.06 a	3.69 ± 0.01 a
L-15	5.70 ± 0.13 d	6.18 ± 0.02 d	0.15 ± 0.04 c	1.67 ± 0.03 e
L-25	5.51 ± 0.03 d	6.15 ± 0.01 d	0.13 ± 0.04 c	2.29 ± 0.01 c
P-15	7.54 ± 0.03 a	6.72 ± 0.02 b	0.15 ± 0.07 c	1.17 ± 0.03 g
P-25	7.56 ± 0.01 a	6.61 ± 0.02 a	0.11 ± 0.05 c	1.38 ± 0.03 f

Means in each column followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD ($n = 3$). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

The EC values before and after the experiment were between 0.11–1.28 $mS \cdot cm^{-1}$ and 1.17–3.69 $mS \cdot cm^{-1}$, respectively. The EC values were observed to be higher in A-SMS substrates (A-15 and A-25) both before and after the experiment. While the lowest EC before the study was observed in both L-SMS (L-15 and L-25) and P-SMS substrates (P-15 and P-25). Whereas the substrate P-15 had the lowest EC among all studied substrates.

Overall, the pH and EC values of all SMS-based substrates before strawberry production in the present investigation achieved nearly ideal values after substituting peat by 15 and 25% (v/v). This demonstrated the possible utilization of fresh SMS as a peat substitute in lower concentrations (<25%), which could help overcome the limiting nature of 100% fresh SMS.

3.2. Yield Performance

The total and marketable cumulative yield ($g \cdot plant^{-1}$) among the studied substrates were found to be significantly different (Figure 1 and Table 7). However, variations were observed among studied substrates between the total and marketable yields. The highest cumulative total yield was recorded in A-25 (186.46g) and the lowest values were observed in P-25 (74.46 g). Similar to cumulative total yield, the marketable yield was also observed to be the highest in A-25, while the lowest marketable yield was observed in P-25.

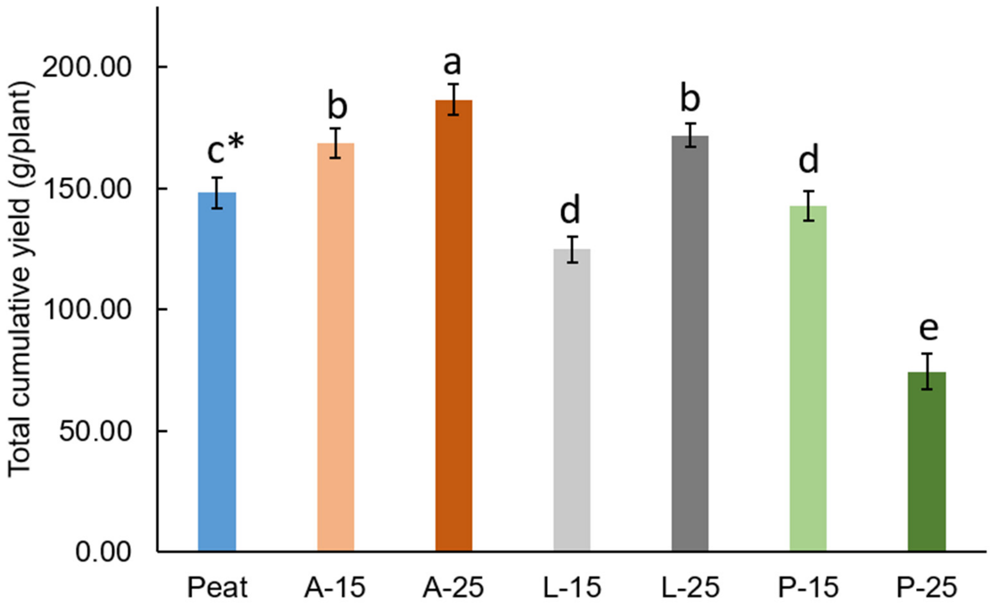

Figure 1. Total cumulative yield (g·plant^{-1}) of strawberry cv. 'Honeoye' in different substrates. * Means followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD ($n = 5$). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

Table 7. Cumulative marketable yield of strawberry cv. 'Honeoye' cultivated in different substrates (mean ± SD).

Substrates	Marketable Yield (g·plant^{-1})
Peat	136.78 ± 6.13 bc
A-15	148.58 ± 6.03 c
A-25	162.99 ± 6.40 a
L-15	96.54 ± 5.45 d
L-25	135.65 ± 4.86 bc
P-15	114.56 ± 6.11 c
P-25	59.89 ± 7.53 e

Means followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD ($n = 5$). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

Overall, the cumulative total and marketable yield trend were observed to be in increasing order in 15 and 25% of A-SMS (A-15 and A-25) and L-SMS (L-15 and L-25) substitution. Whereas in P-SMS (P-15 and P-25), as the concentration of added SMS increased from 15% to 25%, the strawberry yield performance significantly decreased.

3.3. Morphological Parameters

The results of recorded strawberry morphological parameters as determined by shoot, root, and total plant fresh weight, as well as leaf area, were significantly different in the studied substrates (Table 8). The plants cultivated in the L-25 substrate achieved the highest shoot fresh weight indicating superior shoot development, while the highest root fresh weight was observed in A-25 substrate. The total plant (shoot + root) weights were recorded to be the highest in A-25 substrate, followed by all studied substrates except for P-SMS-based substrate P-25 and the control peat substrate. The highest leaf area was recorded for plants grown in L-25, whereas the lowest value was recorded in P-25. The leaf area in A-25 and L-15 was similar to peat values.

Table 8. Morphological parameters of strawberry cv. 'Honeoye' cultivated in different substrates (mean ± SD).

Substrates	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Total Plant Fresh Weight (g)	Leaf Area (cm^2)
Peat	25.18 ± 1.85 bc	11.35 ± 1.57 b	36.53 ± 1.89 b	168.52 ± 13.77 ab
A-15	33.09 ± 5.64 ab	20.66 ± 3.86 ab	53.75 ± 6.32 ab	159.01 ± 39.18 b
A-25	33.87 ± 10.32 ab	35.48 ± 20.40 a	69.35 ± 28.56 a	188.97 ± 27.42 ab
L-15	35.89 ± 8.49 ab	15.25 ± 6.77 ab	51.15 ± 14.72 ab	183.36 ± 34.02 ab
L-25	41.83 ± 6.15 a	22.69 ± 7.31 ab	64.52 ± 12.95 ab	220.28 ± 26.12 a
P-15	26.01 ± 5.76 bc	22.49 ± 11.99 ab	48.51 ± 17.16 ab	136.03 ± 15.01 bc
P-25	17.74 ± 7.64 c	16.31 ± 9.27 ab	34.06 ± 16.69 b	100.59 ± 23.53 c

Means in each column followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD (n = 5). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

3.4. Dry Matter Distribution

The strawberry dry matter accumulation was significantly influenced by different substrates (Table 9). The plants cultivated in L-25 had the highest shoot dry weight while the lowest was in P-25. The highest root dry weight was recorded in A-25 and the lowest root dry weight was observed in the P-25 substrate. The highest total plant dry weights were recorded in A-25 and L-25 substrates, while the plants cultivated in P-25 had the lowest value. The shoot to root ratio among different substrates ranged from 1.23 to 2.12, and the differences were not significant.

Table 9. Dry matter distribution in strawberry cv. 'Honeoye' cultivated in different substrates (mean ± SD).

Substrates	Shoot Dry Weight (g)	Root Dry Weight (g)	Total Plant Dry Weight (g)	Shoot to Root Ratio
Peat	8.73 ± 0.64 bc *	4.19 ± 0.55 b	12.91 ± 0.67 ab	1.23 ± 0.38 **
A-15	10.84 ± 1.85 ab	6.44 ± 1.25 ab	17.28 ± 2.03 ab	1.50 ± 0.52
A-25	10.86 ± 3.31 ab	10.54 ± 6.21 a	21.41 ± 8.81 a	1.53 ± 0.54
L-15	10.63 ± 2.51 ab	5.51 ± 2.28 ab	16.14 ± 4.65 ab	1.74 ± 0.53
L-25	14.04 ± 2.07 a	7.79 ± 2.49 ab	21.83 ± 4.38 a	1.95 ± 0.59
P-15	7.51 ± 1.67 bc	5.82 ± 3.23 b	13.34 ± 4.72 ab	2.10 ± 0.49
P-25	5.49 ± 2.3 6c	3.96 ± 2.34 c	9.45 ± 4.63 b	2.12 ± 0.43

* Means in each column followed by the same letters are not significantly different at $p < 0.05$ according to Tukey's HSD (n = 5), ** not significant. A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

3.5. Correlations Matrix among Yield and Morphological Parameters

There were positive correlations between marketable yield (Y) and leaf area (LA), shoot dry weight (SDW), root dry weight (RDW), as well as total plant dry weight (TPDW) as shown in Figure 2. Negative and non-significant differences were observed among tested yield and morphological parameters and shoot to root ratio (S:R).

The correlations agreed with the results presented in Table 7 and Figure 1 for a given substrate. The higher the marketable yield value is, the greater the values obtained for shoot, root, and total plant dry weights as well as for leaf area. For instance, the greatest root dry weight and total plant dry weight were obtained in A-25 in which the highest marketable yield was obtained.

Figure 2. Correlation matrix analysis among marketable yield and studied morphological parameters. The size and colour intensity of circles are proportional to Pearson's correlation coefficient at $p < 0.01$. Red circles indicate positive correlations, while blue are negative correlations. In the correlogram scale from −1 to +1, Pearson's correlation coefficient for variables is on the vertical and horizontal axis. × indicates values that are not statistically different at $p < 0.01$. Marketable yield (Y), shoot dry weight (SDW), root dry weight (RDW), total plant dry weight (TPDW), shoot to root ratio (S:R) and leaf area (LA).

3.6. Photosynthetic Parameters

There were no significant differences observed among plants grown in different substrates for F_v/F_0, F_v/F_m, and $PI_{(abs)}$ (Table 10). The F_v/F_0 values ranged between 4.66–4.93, F_v/F_m and $PI_{(abs)}$ were between 0.82–0.83 and 2.89–3.60, respectively. Likewise, the NDVI values were also not significantly different among substrates and were observed to be in a range of 0.77–0.79 (Figure 3A). Significant differences were observed in PRI and MCARI values (Figure 3B,C). The highest PRI was observed in L-25 (0.054), followed by P (0.053) and A-15 (0.053), and the lowest was in P-25 (0.043). The highest value for MCARI was in L-15 (0.27) and L-25 (0.26) and the lowest values were observed in peat (0.20) and P-15 (0.20).

Table 10. Selected chlorophyll fluorescence parameters of strawberry cv. 'Honeoye' cultivated in different substrates (mean ± SD).

Substrates	F_v/F_0	F_v/F_m	$PI_{(abs)}$
Peat	4.66 ± 0.55	0.82 ± 0.01	3.11 ± 1.19
A-15	4.93 ± 0.26	0.83 ± 0.01	3.60 ± 0.77
A-25	4.69 ± 0.33	0.82 ± 0.01	3.13 ± 0.59
L-15	4.54 ± 0.43	0.83 ± 0.01	2.89 ± 0.63
L-25	4.52 ± 1.28	0.82 ± 0.01	3.20 ± 1.02
P-15	4.75 ± 0.45	0.83 ± 0.01	3.21 ± 1.10
P-25	4.72 ± 0.30	0.82 ± 0.01	2.91 ± 0.59
Significance	Ns	ns	ns

ns = not significant at $p < 0.05$ according to Tukey's HSD (n = 15). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

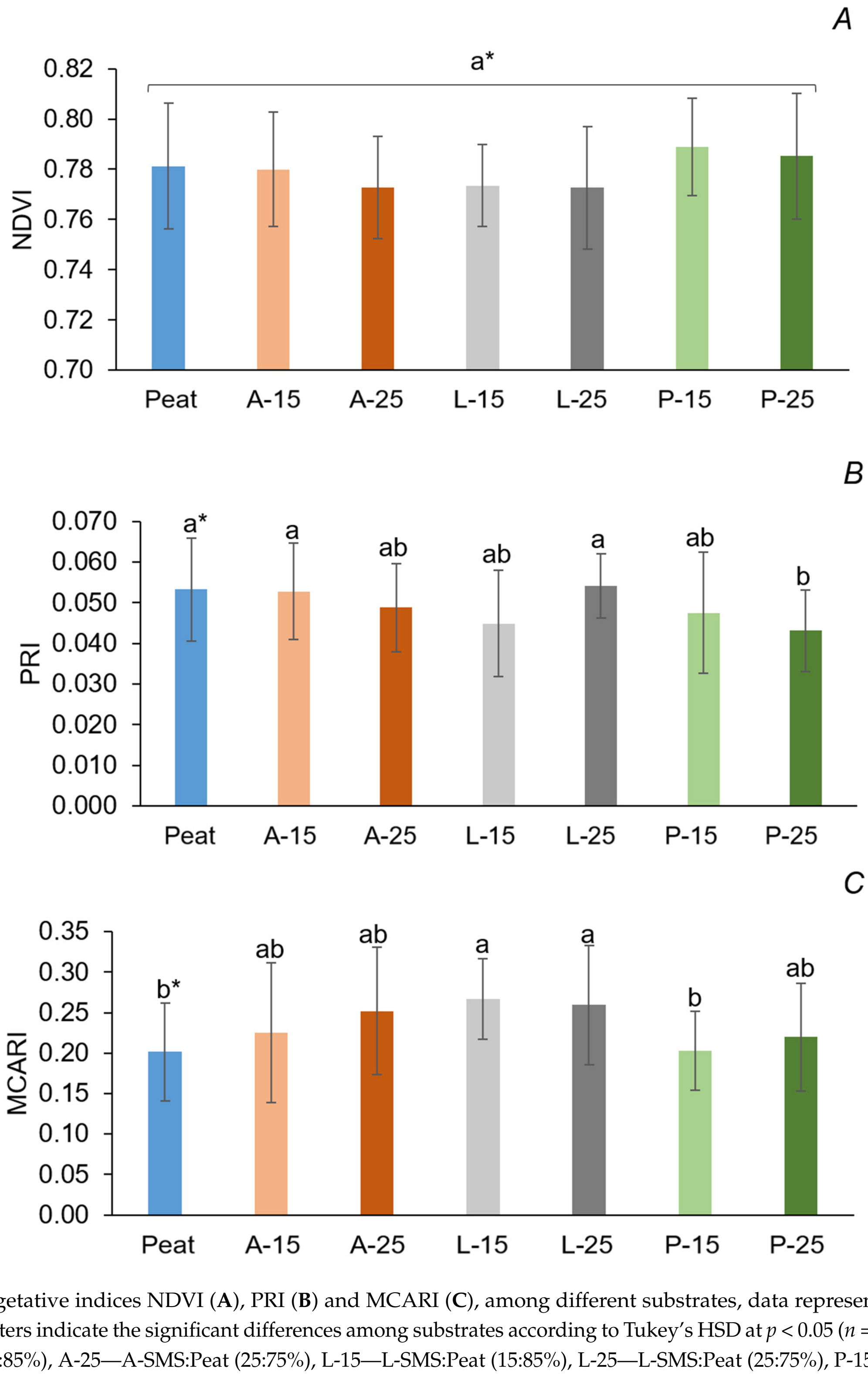

Figure 3. Vegetative indices NDVI (**A**), PRI (**B**) and MCARI (**C**), among different substrates, data represent mean ± SD. * Different letters indicate the significant differences among substrates according to Tukey's HSD at $p < 0.05$ ($n = 15$). A-15—A-SMS:Peat (15:85%), A-25—A-SMS:Peat (25:75%), L-15—L-SMS:Peat (15:85%), L-25—L-SMS:Peat (25:75%), P-15—P-SMS:Peat (15:85%) and P-25—P-SMS:Peat (25:75%).

4. Discussion

The high value of EC is one of the most important factors limiting the use of SMS as a growing medium [34,70,71]. Many researchers have reported that EC values of *Agaricus*-SMS, *Lentinus*-SMS, and *Pleurotus*-SMS range between 0.58–10.70 mS·cm^{-1} [71–73], 1.96 mS·cm^{-1}, and 0.89–4.01 mS·cm^{-1} [74], respectively. However, these values may vary as the composition of SMS greatly depends on the mushroom species cultivated [75]. The optimum EC for soilless strawberry production is reported to be 1.4–2.5 [76,77], whereas the EC of 2.5 mS·cm^{-1} is considered to be an upper limit [77,78]. Strawberries are a highly salt-sensitive crop [79,80]. A high salt concentration of the substrate can negatively affect overall crop performance [40,81–83]. Salinity stress in strawberries both directly or indirectly affects chlorophylls and carotenoids, which may lead to low productivity [84].

A high level of EC restricts the use of fresh SMS. In the present study, none of the substrate EC values before the experiment were reported to be saline, as the values ranged between 0.11–1.28 mS·cm^{-1} and nearly optimum EC for strawberry soilless culture was achieved in A-15 and A-25 with A-SMS (Table 6). The chemical analysis of substrates after the experiment revealed that the EC values increased among all tested substrates (Table 6). Substrates supplemented with A-SMS in 15 and 25% showed higher EC values of 3.20 and 3.69 mS·cm^{-1}, respectively. Bryla and Scagel [80] reported that to achieve optimum growth of strawberry cv. 'Honeoye', the EC of the growing media should be maintained at $\leq$1.3 mS·cm^{-1} during the early stage, and at $\leq$3.4 mS·cm^{-1} once the plants have matured.

The unfavourable value of pH is another factor that limits the usage of SMS as a growing medium. It has been reported that strawberries can perform well in acidic soil pHs of 4.6–6.5 [85,86]. In the present study, the pH values of 100% A-SMS, L-SMS, and P-SMS were outside of this optimal pH range (Table 5). Optimum pH levels were found in L-15 and L-25 with L-SMS when compared to other substrates.

The EC and pH values of all substrates studied in the present investigation were lowered to nearly peat values when fresh A-SMS, L-SMS, and P-SMS were mixed with peat in 15 and 25% (v/v) (Table 6). These results are in agreement with Eudoxie and Alexander [36], who concluded that mixing SMS with peat (50:50%) nearly neutralised the limiting nature of the SMS.

Results of the study showed that strawberry plants grown in A-25 and L-25 had the highest shoot dry weight, root dry weight, total plant dry weight, and leaf area when compared to peat. In previous studies, promising results with agro-waste-based substrates for strawberry cultivation were reported. In the study by Altieri et al. [12], olive mill waste mixture (OMWM) was found to be an effective and cheap alternative to peat. Kuisma et al. [14] reported using ground reed canary grass (*Phalaris arundinacea* L.) as a peat or coir substitute (50%). Depardieu et al. [16] revealed that a peat–sawdust mixture and aged bark were found to be promising alternative substrates to coconut coir dust for strawberry cultivation. However, others reported peat- and coconut-coir-based substrates were found to be the best substrates for strawberries [13,87–90].

The plants grown in A-SMS (A-15 and A-25) had significantly higher total cumulative and marketable yield than plants grown in peat (Figure 1 and Table 7). The initial nutrient concentration of A-SMS, especially macronutrients including N, P, K, as well as secondary elements like Ca and Mg, was observed to be higher when compared to other substrates, which may have contributed to higher yield and better morphological parameters. As reported by Medina et al. [35] and Benito et al. [91], SMS has been proven to increase the nutrient status of growing media. The amount of N-NH_4 in the growing media, which is easily available for plant uptake, can also benefit plant growth and yield [92].

Based on Cohen [93], the correlation coefficient values obtained in the present study suggested a strong relationship. The observed 'r' values among marketable yield and tested morphological parameters except for root to shoot ratio are positively correlated (0.445–0.552). Correlation values greater than 0.50 indicates that the relationship is strong. The observed correlation results are in agreement with Adak et al. [94], who also reported a positive correlation between marketable yield to root dry weight and marketable yield to shoot dry weights.

Fluorescence is a highly sensitive photosynthetic plant retraction analysis that can detect any change in the overall bioenergy status of a plant [95]. Unfavourable pH and EC values can significantly influence strawberry growth and overall performance, which can be explained by measured PIs and VIs. Alkalinity stress due to high pH may destroy the photosynthetic activity of plants [34,42]. Similar results were reported under salinity stress conditions [41,96]. Alkalinity stress lowered the maximal quantum yield of PSII photochemistry (F_v/F_m) in strawberry cv. 'Camarosa' [37,42]. Chlorophyll *a* fluorescence parameters might be a useful indicator for diagnosing the occurrence of salt and alkaline stress in strawberries [42,96].

Among several photosynthetic parameters measured in the study, F_v/F_0, F_v/F_m, and $PI_{(abs)}$ were selected to be presented in this article due to their proven sensitivity in identifying different abiotic stress responses in plants [51,52,97–100]. Angelini et al. [98] reported that the maximum photochemical yield of PSII (F_v/F_m) is a reliable indicator of the photochemical activity of the photosynthetic apparatus. For the majority of plants at the stage of full development and under optimal growing conditions, the value of F_v/F_m was found to be around 0.83. Significant differences in F_v/F_m values (0.77–0.81) among three substrates studied in soilless strawberry production [88]. In wild strawberries (*Fragaria vesca* L.), substrate salinity caused a significant decrease in the F_v/F_m (0.71–0.74) and F_v/F_0 (2.61–2.90) values [93]. Shamsabad et al. [42] observed that the F_v/F_m (0.39–0.82), F_v/F_0 (0.37–4.49), and PI_{abs} (0.22–14.6) parameters declined among all six cultivars with increasing sodium bicarbonate (alkalinity).

In turn, the large range of observed values among these studies and a decrease in these parameters indicated that the plant exposed to stress factors can alter PSII functions. The structural damage of thylakoids and decrease in efficiency of water fission reaction may lead to slow relaxing quenching processes and a reduction in the efficiency of electron transport [101–103]. Changes in the content of photosynthetic pigments are also dependent on the tolerance of the plants to the salinity of the substrate, i.e., their genotype [104,105]. However, strawberry cv. 'Honeoye' used in the study is a salt-sensitive cultivar [80].

In the present investigation, the F_v/F_m values (0.82–0.83) were found to be in the optimum range [98,106]. The F_v/F_0 and $PI_{(abs)}$ values were observed to be in the range of 4.52–4.93 and 2.91–3.60, respectively. The observed values suggest that the plants were not under stress and the photosynthetic activity did not differ among plants grown in substrates with A-SMS, L-SMS, and P-SMS substitutes when compared to standard peat. This is also supported by NDVI values (0.77–0.79), which were found to be in the range indicating normal vegetation [107]. NDVI is a spectral vegetative index widely used in the determination of plant N status and can further explain the difference in yield [108–110]. The strawberry nitrogen content of leaves and marketable yield were found to be positively correlated to the NDVI values, as reported by Li et al. [107] and España-Boquera et al. [111].

PRI is a quantitative measure of reflectance change at 531 nm, which indicates changes in the state of xanthophyll cycles and is strongly related to photosynthetic light use efficiency [112]. The differences in PRI values among substrates (0.043–0.054) in the present study were probably due to the involvement of multiple processes with separate time constants affecting reflectance and fluorescence to different degrees [113]. Significant differences among substrates concerning MCARI (0.20–0.27) were probably due to differences in leaf chlorophyll concentrations [68]. The numerical difference among PIs and VIs in the present study indicates that the data points are spread out over a small range of values. These values further demonstrate that the plants were not influenced by stress due to varying pH and EC levels of substrates.

Several reports have demonstrated that the type of growing media used in soilless strawberry production influences yield parameters [12–14,94,114–117]. The obtained results in the present investigation also suggest that substrates largely influence marketable and total yield, as well as morphological parameters of strawberries.

The results of the present investigation are in line with Atikmen et al. [33], who reported that fresh mushroom substrate can be used as a substitute for peat primarily at 12.5% and 25% in greenhouse chrysanthemum production. In contrast, many reports recommend using SMS only after a weathering process in lower concentrations (5–50%) for better plant growth [29,36,118,119]. Some authors even claim that weathering alone is not enough to reduce salinity to a satisfactory level, recommending further leaching of weathered SMS for short times (up to 20 days) to remove excess salt (EC) before using it as a soilless substitute in crop production [120–122].

5. Conclusions

It can be concluded from the present study that fresh A-SMS and L-SMS substituting peat in 15 and 25% (*v*/*v*) in soilless strawberry production showed better compatibility and achieved better results when compared to commercial peat and P-SMS substrates. The superior yield and dry masses recorded in substrates A-15 and A-25 with A-SMS supplementation were mainly due to the initial nutrient concentrations in these substrates. Overall, our results support the fact that growing media largely influences overall strawberry performance. The measured photosynthetic parameters demonstrated that the photosynthetic processes were not altered at 15 and 25% of SMS supplementation, and the VI values were also observed to indicate normal vegetation. The results also demonstrated that chlorophyll *a* fluorescence parameters, including F_v/F_0, F_v/F_m, and $PI_{(abs)}$, are suitable indices to identify pH and saline (EC) induced stress, which may alter PS II activity.

An easy, immediate, and effective utilisation of agro-waste from the mushroom industry (SMS), preferably as a soilless substitute in lower concentrations of 15–25%, could provide an economically acceptable cultivation alternative, reducing the costs of production and overcoming environmental problems associated with improper handling of SMS. To the best of our knowledge, this is the first study reporting immediate utilization of different fresh SMSs as peat substitutes in soilless strawberry production, and hence further research in this regard is recommended to study varying concentrations of SMSs and other strawberry cultivars.

Author Contributions: Conceptualization, R.P. and J.L.; methodology, R.P. and J.L.; software, R.P.; validation, R.P., M.A. and A.R.; formal analysis, R.P.; investigation, R.P. and J.L.; resources, R.P., J.L., M.A. and A.R.; data curation, R.P., M.A. and A.R.; writing—original draft preparation, R.P. and J.L.; writing—review and editing, R.P., J.L., M.A. and A.R.; visualization, R.P., M.A. and A.R.; supervision, J.L. and A.R. All authors have read and agreed to the published version of the manuscript.

Funding: The research was financed by the Department of Vegetable Crops, Faculty of Agronomy, Horticulture and Bioengineering, Poznan University of Life Sciences, Poland. The publication was co-financed within the framework of the Polish Ministry of Science and Higher Education's program: "Regional Initiative Excellence" in the years 2019–2022 (No. 005/RID/2018/19), financing amount 12,000,000 PLN.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data presented in the study will be available upon request from the correspondence authors.

Conflicts of Interest: The authors declare no conflict of interest.

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