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The spent mushroom substrate as a growing medium for strawberry (*Fragaria* × *ananassa* Duch.)

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Contents

1. Introduction

Strawberries are the most consumed and economically important soft fruit in the world. For many years, Poland has been a leader in the production of strawberries in the European Union and a significant global producer. According to Food and Agriculture Organisation, Poland's total strawberry production in 2019 amounted to almost 185 400 tonnes, with average productivity of 4 tonnes/ha.

In Poland, strawberries have been usually grown in the field for processing. In recent years, a growing interest in the soilless production of dessert cultivars employing new technologies in protected cultivation has been noticed. One of the main factors determining the qualitative and quantitative parameters of strawberries in soilless culture is the substrate. As of today, peat is the most widely used substrate for such production. However, considering the high cost, extensive utilisation, non-renewable nature of peat and the environmental concerns associated with peat mining, there is a great demand for peat reduced or peat-free sustainable alternatives. The possibility of utilising organic materials from agro-wastes which can partly or completely replace peat in soilless cultivation will be of great importance. The ideal growing medium should be derived from renewable sources being biodegradable, relatively cheap, and easily available.

Mushrooms are becoming more important in our daily diet due to their delicious taste, nutritional value, high protein and low-fat content. In recent years, they are considered a functional food because of the associated nutritious and health benefits. The rising demand for vegetarian food, a growing concern among the population towards a healthy diet and lifestyle has significantly increased mushroom production around the globe. In 2019, the global mushroom production was nearly 11 million tons. In European Union, Poland is a leader in mushroom production with a production of nearly 362 400 tons and a significant global producer. However, mushroom production can be considered as a non-sustainable agriculture activity due to the generation and accumulation of an enormous amount of waste materials (approximately five times the volume of fresh mushroom produced), its limited re-use and associated environmental concerns due to improper handling and disposal.

Spent mushroom substrate (SMS) is the residual material left off after commercial mushroom cultivation and is often treated as agro-waste or farm waste. SMS's generated from mushroom enterprises in large quantities are often burnt, discarded or simply thrown away. Further, to date, the Polish legislation categorises this potential waste as "other unspecified waste" and inadequately addresses the issues of its management and do not properly specify SMS storage conditions in the field, which is neither economical nor environmentally safe.

The substrate generated immediately after mushroom production is treated as fresh-SMS or simply SMS whereas, the same material after further decomposition or weathering or composting is regarded as weathered-SMS or spent mushroom compost (SMC). Immediate utilisation of SMS is not recommended in agriculture and horticulture, mainly due to unfavourable pH, high salinity (EC) and associated phytotoxicity. On the other hand, SMC is recommended to be used in some farming activities, where the excess salts and nutrients are leached out during the weathering process. However, weathering can be considered a time consuming, laborious and non-environmentally friendly approach, because of the long composting time (up to 36 months) and environmental problems associated with leachates which can significantly contaminate ground water and soils. The increase in mushroom production in recent years and the expected increase in the future generates and accumulates a significant amount of SMS every year. Hence, proper handling and effective utilisation of fresh SMS after mushroom production is a considerable issue.

As the successful greenhouse and nursery production of container-grown plants are largely dependent on growing media. In recent years, research on utilising organic materials derived from agro-waste streams are of great significance. Therefore, understanding the need to study the potentiality of locally available materials instead of overexploiting peat resources, importing expensive mineral and organic substrates, the present investigation was designed to study the immediate, potential and sustainable utilisation of an agro-waste generated from mushroom production (SMS). Among many agro-wastes, studying the possible utilisation of SMS as a potential substitute to commercial substrates can be beneficial as this material is easily available, cost-effective and has a considerable amount of nutrients. Effective utilisation of SMS can also help to overcome limited re-use and associated disposal problems achieving sustainability at the same time creating transition towards a circular economy. However, to the best of my knowledge, the scientific evidence supporting the immediate use of fresh SMS as a soilless substitute is not well documented and still in its infancy.

The present investigation was designed to study the suitability of the spent mushroom substrates obtained after commercial production of white button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus ostreatus*) and shiitake (*Lentinus edodes*) as peat substitutes in soilless strawberry cultivation (*Fragaria* × *ananassa* Duch.) in an unheated plastic tunnel.

The main aim of this investigation was to study the influence of different SMS's on strawberry morphological, pomological and physiological parameters as well as yield performances.

The experiment was carried out with the following objectives:

- to study the possibility of utilising fresh SMS as a peat substitute
- to evaluate the performances of strawberry cv. 'Honeoye' and 'Elsanta' grown on SMS substituting peat in various combinations
- to determine which SMS and in which combination with peat will be most suitable for soilless strawberry production

2. Review of literature

2.1. Strawberry – origin and botany

Strawberry (*Fragaria* × *ananassa* Duch.) is a herbaceous, perennial plant and is a member of the Rosaceae family of the genus *Fragaria* (Hancock 1999). The present-day cultivated strawberry is a monoecious octoploid (2n=56) and is a hybrid of two dioecious octoploids namely *Fragaria chiloensis* and *Fragaria virginiana* (Bowling 2000). As reported by Liston et al. (2014), the strawberry is one of the youngest domesticated plants. Botanically, strawberry fruits are 'etaerio of achenes' and they are referred as aggregate fruits, having seeds on the surface of a red fleshy receptacle (Darnell 2003). Being a non-climacteric crop, strawberry fruits mature only on the plant (Cordenunsi et al. 2005). Strawberries, based on bearing season, are classified as June-bearing, everbearing and day-neutral (Durner et al. 1984).

2.2. Strawberry – current scenario and health benefits

Strawberry is the most economically important soft fruit of the world. In the last decade, the global production of strawberries has increased nearly 40%, making it the second-largest berry fruit after grape (*Vitis vinifera*) in terms of production. Poland is the second-largest producer of strawberries in the EU and a significant global producer. In the European Union, Poland occupies the highest area of 49 900 ha under strawberry cultivation with a total production of 185 400 tons, with average productivity of 4 tons per ha (FAO 2019).

Strawberries are well known for their attractive red fruits, characteristic aroma and distinct taste. Apart from their appearance and taste, fruits have numerous dietary and health benefits. Strawberries are a significant source of vitamin B, vitamin C, vitamin E, potassium, folic acid and carotenoids, they also contain significant amounts of ellagic acid, tannins, and phytosterols (Stoner et al. 2006, Aaby et al. 2007, Basu et al. 2010). When compared to other berry fruits, strawberries contain a higher percentage of vitamin C, phenolics, flavonoids and phytochemicals (Hakkinen and Törrönen 2000, Trevino-Garza et al. 2015). Strawberries were recently included as one of the 100 richest sources of dietary polyphenols, they further secured a place among 89 food and beverages for providing more than 1 mg total polyphenols per 100 g (Perez-Jimenez et al. 2010).

Strawberries are now considered a functional food due to the multiple health benefits associated with their consumption beyond basic nutrition. The presence of several nutrients, phytochemicals and fibres, substantiated by the accumulating evidence on its antioxidant, antiinflammatory, anti-hyperlipidemic, anti-hypertensive and anti-proliferative properties plays a synergistic role in its characterisation as a functional food (Basu et al. 2014). The antioxidative properties of strawberry fruits are reported to be beneficial for blood sugar and heart health (Halvorsen et al. 2006, Giampieri et al. 2012, Basu et al. 2014).

2.3. Soilless strawberry production

Among many fruit crops, strawberries can be cultivated under wide ecological conditions, i.e. from arctic to tropic regions (Hancock 1999, Guerrero-Chavez et al. 2015). Recently, there is a growing interest worldwide in greenhouse strawberry production, this is because the soil culture of strawberries in the open field still faces various challenges due to soil pathogens, herbicide injury and high labour costs (Mattner et al. 2014, Kruistum et al. 2014). The development of protected cultivation systems allows offseason and year-round production of strawberries (Kadir et al. 2006, Medina et al. 2011).

In recent years, soilless strawberry production is becoming popular, as substrates represent a good alternative to conventional field production of strawberries and offer many advantages to growers by eliminating the need for chemical fumigations, crop rotations, non-fumigant soil disinfestations, better crop management, efficient utilisation of inputs and often resulting in higher yields (Altieri et al. 2010, Ameri et al. 2012, Fennimore et al. 2013, Adak et al. 2018). In addition, the short growing period until harvest and suitability for small farms are the main reasons for the interest in soilless production (Agüero et al. 2015).

Among berries, strawberry responds quite well to soilless production systems. Major yield-affecting factors for strawberries in soilless systems may be listed as: type and quality of growing medium, source and type of plants, cultivar, growing season and efficiency of fertigation system (Jafarnia et al. 2010, Ameri et al. 2012, Tariq et al. 2013, Kuisma et al. 2014, Abul-Soud et al. 2015, Grunert et al. 2016, Adak et al. 2018, Diel et al. 2018).

2.4. Factors influencing soilless strawberry production

2.4.1. Substrates on vegetative, generative and quality parameters

It has been reported that substrates greatly influence the strawberry yield and performance (Latigue et al. 2011, Ameri et al. 2012, Cecatto et al. 2013, Kuisma et al. 2014, Tomasi et al. 2015, Palencia et al. 2016, Martínez et al. 2017, Massetani et al. 2017, Adak et al.

2018, Alsmairat et al. 2018, Diel et al. 2018). Reports on these areas demonstrated that substrates influence leaf-level physiology and yield (Alsmairat et al. 2018), as well as plant height, crown diameter, leaf area and plant dry mass (Yavari et al. 2008, Tariq et al. 2013). These findings are supported by Adak et al. (2018), where growing media largely influenced the number of leaves, root-shoot dry mass and length of roots, as well as yield. In this study, authors also reported a positive correlation among yield and morphological traits, where an increase in the number of leaves, root-shoot dry masses, number and length of roots resulted in higher yield. Grijalba et al. (2015) observed that increase in leaf area and the number of crowns resulted in yield improvement. The study conducted by Ameri et al. (2012) showed that cultivars responded differently to different substrates. Cecatto et al. (2013) observed no difference among the cultivars in terms of production and yield. In contrast, Alsmairat et al. (2018) suggested that the strawberry growth and yield parameters are greatly affected by substrate composition but not by cultivars.

Substrates can also influence strawberry fruit quality parameters. In the study by Tehranifar et al. (2007) the soluble solids of fruits varied among different cultivars and different substrates. Jafarnia et al. (2010), Ameri et al. (2012) and Cecatto et al. (2013) also reported that growing media greatly influence the fruit quality parameters, such as total soluble solids (TSS). As reported by Silva et al. (2015), the TSS content characterises the sweetness of fruits. Ideal substrate characteristics can help in achieving higher yield along with good quality fruits (fruit diameter and fruit length), as concluded by Caso et al. (2009). These findings are in line with Ameri et al. (2012) and Alsmairat et al. (2018) who also reported that a suitable choice of substrate is extremely important to achieve better quality fruits.

The strawberry fruit colour is an important characteristic feature for consumer product acceptance and/or preferences (Trevino-Garza et al. 2015). A case study on consumer preferences and perception for strawberries revealed that nearly 95% of consumers preferred riped and red coloured strawberries (Bhat et al. 2015). The strawberry fruit colour was observed to be dependent on cultivars and time of harvesting, as reported by Nunes et al. (2006). Whereas, Alsmairat et al. (2018) reported that substrates used in soilless strawberry cultivation had no influence on strawberry fruit colour.

Fruit colour is determined by three colour coordinates $L*$, $a*$, and $b*$. Where $L*$ represents the lightness level of the colour, $a*$ indicates the positive/negative correlation to the red/green component, and $b*$ indicates the yellow/blue component of colour (Nunes et al. 2006, Schulze and Contreras 2017).

2.4.2. Substrate salinity, pH and nutrient content

Substrate grown strawberries are highly sensitive to high salinity (EC) and excessive or deficient amount of macro- and micronutrients, as well as pH (Lieten 2006 a, 2006 b). According to numerous authors, strawberries are highly salt-sensitive crops (Grattan 2002, Saied et al. 2003, Hoffman and Shannon 2007, Bryla and Scagel 2014). The negative effects of salinity on strawberry growth parameters, yield and quality of fruits produced under soilless systems have been well documented (Eshghi et al. 2017, Zahedi et al. 2020, Haghshenas et al. 2020). The ions of Na^+ and Cl^- are most commonly reported to cause these effects (Khan et al. 2000, Mansour 2000, Saied et al. 2005, Turhan and Eris 2009).

Saied et al. (2005) reported that EC above 2.5 $mS \cdot cm^{-1}$ had a negative impact on plant growth, fruit quality and yield for strawberries grown in the soil. Ameri et al. (2012) also reported similar results, where the substrate EC of 2.73 negatively influenced the plant growth and yield, at the same time resulting in the highest number of malformed fruits. In the study by Sun et al. (2015) the EC range of 3.3 to 4.4 $mS \cdot cm^{-1}$ significantly reduced the shoot dry weight, as well as the fruit yield. In contrast, D'Anna et al. (2003) recorded higher fruit yield, better fruit quality and higher fruit weights at EC of 2.5 $mS \cdot cm^{-1}$ than at lower EC values under soilless conditions. Keutgen and Pawelzik (2007) noticed that the osmotic effect of 80 mM NaCl, i.e. EC 7.5 $mS \cdot cm^{-1}$ significantly reduced the fruit yield of cv. 'Elsanta' up to 46%. In another study by Keutgen and Pawelzik (2009) the leaf area and dry weights of strawberry plants were negatively affected when exposed to 40 and 80 mM NaCl, i.e. EC 3.9 and 7.5 $mS \cdot cm^{-1}$, respectively). Bryla and Scagel (2014) reported that to achieve optimum growth in strawberry cv. 'Honeoye' the EC of the growing media (or the nutrient solution) should be maintained at $\leq$1.3 $mS \cdot cm^{-1}$ during the plant establishment stage, and later at $\leq$3.4 $mS \cdot cm^{-1}$.

Strawberry is highly susceptible to osmotic stress. Salinity directly influences plant growth through osmotic stress, specific ion toxicity, and ionic imbalances, which result in increased production of free radicals (Mahajan and Tuteja 2005). This increase in free radicals can damage cell biomolecules resulting in a significant decrease in photosynthetic capacity (Jiang et al. 2017). The plant's response to stress appears with a range of morphological, physiological, biochemical, and molecular changes, which are controlled by a large number of stress-responsive genes (Liu et al. 2014).

The most favourable pH reported for strawberry production is between 4.6 and 6.5 (Niskanen and Dris 2002, Milosevic et al. 2009). In the study carried out by Cieśliński et al.

(1996) pH of 6.8 helped strawberries to achieve better growth and development, as well as higher yield.

The nutrient content in growing media plays a critical role in plant growth and development, this is partly due to nutrient availability in the substrate, which can significantly influence shoot and root growth (Leskovar and Othman 2016). The availability of nitrates (NO_3^-) and phosphorus (P) in the substrate plays a vital role in the root system architecture and development (Linkohr et al. 2002). Pokhrel et al. (2015) reported that organic nutrition alone increased plant dry mass but significantly decreased yield due to the high pH, EC and NH_3^+/NO_3^- ratio around the root zone, because of cation imbalance and nutrient deficiencies. These findings are supported by Leskovar and Othman (2016) who also reported that the high level of NO_3^- in growing media reduced root length and root volume.

2.4.3. Substrate pH and EC on physiological performances of strawberry

Substrates have a significant influence on pH and EC value in the rhizosphere (Martínez et al. 2013). The unfavourable pH and EC of the substrate may negatively affect overall plant development (Roosta 2014, Garriga et al. 2015) and can alter photosynthetic processes due to abiotic stress (Gerloff-Elias et al. 2005, Ghaderi et al. 2018, Yaghubi et al. 2019, Shamsabad et al. 2020). In recent years, various non-destructive techniques have been developed to study abiotic stress responses in plants. The most promising and reliable results have been reported in processes that analyse photosynthesis which is closely associated with yield and overall plant performances (Kalaji et al. 2018, Stirbet et al. 2018, Kupper et al. 2019). 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 (Kalaji et al. 2018, Rastogi et al. 2020). Alsmairat et al. (2018) reported that substrates significantly affect the leaf level physiology in strawberries.

To study the impact of different stress factors on photosynthesis, chlorophyll *a* fluorescence has become a popular approach (Dai et al. 2009, Kuckenberg et al. 2009, Kalaji et al. 2018), including salt stress (Rastogi et al. 2020) and nutrient deficiencies (Samborska et al. 2019). According to Auriga et al. (2020) and Shamsabad et al. (2020), chlorophyll *a* fluorescence parameters can be a useful indicator for diagnosing the occurrence of salt and alkaline stress in strawberries. The stress-induced changes on the PSII electron acceptor are well reflected in the values of integrative OJIP-test parameters, known as Performance Indices (PIs). OJIP-test has been successfully used to understand the influence of several stress factors in plants (Bayat et al. 2018, Kalaji et al. 2018, Rastogi et al. 2019).

The F_0 defines fluorescence intensity at 50µs and F_m stands for maximal fluorescence intensity (Strasser et al. 2000). The F_v/F_0 value defines the ratio of photochemical to non-photochemical quantum efficiencies, F_v/F_m ratio indicates the maximum quantum yield of PSII photochemistry and these parameters are the most common indicators of chlorophyll fluorescence transient on plant leaves under stress conditions (Strasser et al. 2000, Kalaji et al. 2017, 2018).

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 Normalized Difference Vegetation Index (NDVI), Photochemical Reflectance Index (PRI) are a quantitative measure of reflectance change at 531 nm, which indicates the changes in the state of xanthophyll cycles and is strongly related to the photosynthetic light-use efficiency (Gamon et al. 1992, Trotter et al. 2002). Modified Chlorophyll Absorption in Reflectance Index (MCARI) helps to analyse crop growth, vigour, and several other vegetation properties including biomass and chlorophyll content (Sishodia et al. 2020).

2.5. Peat in soilless strawberry production – challenges and needs for peat-free or peat reduced growing media

Peat (sphagnum peat) is the most popular and commercial used substrate in soilless cultivation. Its favourable physical-chemical properties, suitability for many species and cultivation systems constitutes peat as the most widely used substrate (Raviv 2013, Dhen 2018, Sinclair et al. 2020). However, its extensive utilisation causes serious environmental issues and peat mining is considered ecologically unsustainable (Raviv 2013, Gruda 2019). As, peat mining exacerbates climate change due to the release of stable and sequestered carbon into the active carbon cycle (Dunn and Freeman 2011, Barrett et al. 2016), leading to loss of soil organic carbon (Carlile and Coules 2013) and its associated negative impacts on wetland ecosystems (Ceglie et al. 2015). It has been reported that due to over-exploitation, the global peat resources are at the edge of depletion (Basirat 2011, Sendi et al. 2013, Ünal 2015, Kitir et al. 2018). Hence, considering its non-renewability, relatively high cost, future availability, and environmental sustainability, growers around the globe need a high-quality, renewable and sustainable substitute that can substantially replace peat either in whole or in combination (Shober et al. 2010, Kitir et al. 2018).

As of today, cocopeat (coconut coir, coir dust or coir pith) is identified as a potential peat substitute because of its favourable physical-chemical properties (Recamales et al. 2007, Ayesha et al. 2011, Meena et al. 2017) and renewable nature (Noguera et al. 2000, Lieten et al. 2004). Several reports recommend cocopeat as one of the most promising substrates in soilless strawberry production (Miranda et al. 2014, Wang et al. 2016, Martínez et al. 2017, Massetani et al. 2017, Alsmairat et al. 2018). However, a mixed degree of success has been documented with cocopeat when compared to other substrates (Recamales et al. 2007, Ayesha et al. 2011, Kuisma et al. 2014, Adak et al. 2018). On the other hand, cocopeat is obtained only from coconut plantations and its cultivation is geographically limited to some parts of the USA, tropical regions of Africa and Asia (Barrett et al. 2016). It is estimated that annually many countries spend a considerable amount of money on cocopeat import (Shirani 2013). Henceforth, researchers and growers are still in need, and in search of sustainable, cost-effective and locally available growing media for container-grown plants (Drake et al. 2016, Gong et al. 2018, Gruda 2019).

2.6. Soilless strawberry production in agro-waste based peat substitutes

The growing media such as peat, gravel, sand, perlite, rock wool, coconut fibre, and vermiculite are popularly used in soilless strawberry production (Recamales et al. 2007, Ghazvini et al. 2007). In recent years, considering the easy availability, negligible cost and environmental hazards associated with improper handling or disposal of various designated agro-wastes and re-using such materials as growing media substitute appear to be a viable alternative in soilless crop production (Rostami et al. 2014, Abdelrahman et al. 2016, Dhen 2018). In this context, efforts have been made proposing wastes from agro-industrial streams to be used as commercial substrate additives in soilless strawberry production (Recamales et al. 2007, Dhen 2018).

Martínez-Nicolás et al. (2020) studied the possibility of utilising marine sediments as a peat substitute where the growing media for soilless strawberry were formulated using 100% peat as a control substrate, 100% dredged remediated sediment and 50% of each. The results from two years of strawberry cultivation using these substrate mixes demonstrated the possibility of using treated sediments as a viable substrate, confirming the suitability of strawberry fruits for fresh and/or processed consumption with no risk of toxicity.

Depardieu et al. (2016) evaluated three organic substrates to determine the productivity potential of strawberry, where peat-sawdust mixture in comparison with two commercial substrates, i.e. aged bark and coconut fibre was studies. The results suggested that a peat-sawdust mixture of 30% of white spruce (*Picea glauca*) sawdust and 70% of brown sphagnum peat and a mixture of aged bark, and sphagnum peat moss appeared suitable for soilless strawberry production. They concluded that the sawdust and bark-based materials can be used as substitutes to traditional coconut coir for the successful undercover cultivation of strawberries.

Abul-Soud et al. (2015) investigated the influence of vermicompost as an alternative organic substrate mixed with different mineral substrates (perlite, vermiculite and sand) on the growth and yield of strawberries. The results demonstrated that adding vermicompost positively influenced the growth, yield, quality and chemical composition of strawberries and also improved the physical and chemical properties of substrate mixes. Further, they concluded that utilising such organic matters will be a sustainable solution for the management of accumulating organic wastes, mitigate greenhouse gases and avoid indiscriminate use of nutrients.

Kuisma et al. (2014) aimed to evaluate the possibilities of utilising reed canary grass (*Phalaris arundinacea*) straw as a peat substitute in soilless strawberry cv. 'Elsanta' production. In this study mixture of reed canary grass straw and peat (50:50%), as well as coir were considered as growing media. The results indicated that the total yield, berry size and sugar to acid ratio were similar in all the tested substrates and demonstrated that ground reed canary grass may be used to replace traditional peat or coir in a soilless culture of strawberry.

Ameri et al (2012) investigated the effects of substrate and cultivar on biochemical characteristics of strawberry in soilless culture. The experiment consisted of three strawberry cultivars: 'Camarosa', 'Mrak', and 'Selva' and six substrate combinations: rice hull, sycamore pruning waste, cocopeat+perlite (50:50), vermicomposts+perlite+cocopeat in (5:45:50), (15:40:45) and (25:35:40), respectively. The results indicated that the substrates used in the study had different chemical and physical characteristics so, caused different biochemical characteristics of fruits obtained from each substrate. The response of each cultivar was different in each substrate. Therefore, the authors concluded that the substrates largely influence the quality of fruits and recommended that a suitable choice of substrate is important for the production of desirable fruit.

Latigui et al. (2011) used four substrates: 100% olive oil cake, 90% olive oil cake+10% vermiculite, 80% olive oil cake+20% vermiculite, 70% olive oil cake+30% vermiculite and reported that 100% olive oil cake and 90% olive oil cake gave better conditions for the growth of the strawberry plant.

Altieri et al. (2010) evaluated the suitability of olive oil mill waste mixture as a peat substitute in 0, 25, 50, 75% (v/v) for soilless strawberry cultivation. The results from yield and plant tissues analyses showed that 25 and 50% olive mill waste mixture performed adequately as a substitute for peat and showed great compatibility in soilless strawberry cultivation being an effective and cheap alternative to peat.

As reported by Papafotiou et al. (2001) and Maher et al. (2008), rice hulls both fresh and decomposed can be considered as a peat amendment in soilless growing conditions. These findings were supported by Caso et al. (2009) where four substrates including rice husk (100%), pumice (100%), rice husk:sand (75:25%) and rice husk:pumice (50:50%) were used in strawberry production. The authors reported that strawberry plants grown in rice husks (100%) achieved the best yield and fruit quality parameters.

The influence of soilless production on quality and yield aspects of strawberries has been studied extensively (Latigue et al. 2011, Ameri et al. 2012, Neocleous 2012, Cecatto et al. 2013, Marinou et al. 2013, Akhatou and Recamales 2014, Martínez et al. 2015, Tomasi et al. 2015, Adak et al. 2018). On the other hand, only a few studies reported possibilities of utilising designated agro-wastes as a peat substitute in strawberry cultivation (Kuisma et al. 2014, Abul-Soud et al. 2015, Depardieu et al. 2016). At the same time, many reports urged that there is a need for more studies on identifying the best alternative to peat from agro-industrial streams, which can address the effective use of resources, need for recycling organic wastes, necessary environmental impact and strengthen economic activity (Abul-Soud et al. 2015, Martínez-Nicolás et al. 2020). Hence, to come up with a potential eco-friendly, peat-reduced/peat-free growing media will be of great significance (Drake et al. 2016, Gong et al. 2018, Gruda 2019).

2.7. Spent mushroom substrate (SMS)

2.7.1. The process of mushroom production and associated problems

In the last decade, a significant increase in demand and production of mushrooms has been noticed, while *Agaricus bisporus* (white button mushroom), *Lentinus edodes* (shiitake) and *Pleurotus ostreatus* (oyster mushroom) are the most popular and commercially cultivated mushroom species around the world (Valverde et al. 2015, Royse et al. 2017). The global mushroom production has passed 11 million tons (FAO 2019). Poland is one of the leading producers of mushrooms in the world with a production of 362 400 tons and is the biggest producer of white button mushrooms in the European Union (EU STAT 2020).

The effective utilisation and disposal of a large amount of agro-industrial wastes generated annually is a great challenge. These wastes mainly consist of cellulose, hemicellulose and lignin, all of which are collectively termed lignocellulosic materials (Kumla et al. 2020). Mushroom cultivation is a lucrative agriculture business (Sendi et al. 2013) and can be also considered as a prominent biotechnological process of utilising such lignocellulosic agro-waste materials to produce mushroom fruiting bodies (Philippoussis 2009). On the other hand, mushroom production can be considered as a non-sustainable agriculture activity due to the accumulation of an enormous amount of spent mushroom substrate (Finney et al. 2009), its limited re-use and associated environmental issues (Cebula et al. 2013, González-Marcos et al. 2015, Magalhães et al. 2018).

2.7.2. Challenges and opportunities

The residual material left off after commercial mushroom production is termed as spent mushroom substrate (SMS). It is also considered as an organic solid waste that remains after mushroom cultivation (Gao et al. 2015). The global mushroom production has passed 11 million tons (FAO 2019). Poland is one of the leading producers of mushrooms in the world with a production of 362 400 tons and is the biggest producer of white button mushrooms in the European Union (EU STAT 2020). Approximately, five kilograms of SMS is left off after one kilogram of fresh mushrooms produced (Semple et al. 2001, Williams et al. 2001, Lau et al. 2003, Finney et al. 2009, Zisopoulos et al. 2016). On this account, the annual SMS generated from the global mushroom industry is estimated to be nearly 60 million tons, whereas Poland alone generates approximately 1.70 million tonnes of SMS annually from mushroom enterprises (mushroom production value multiplied by five times).

The spent mushroom substrate is no longer considered as an appropriate substrate for successive mushroom production, and also mushroom cultivation on the new substrate is much cheaper than processing SMS for the second cycle of mushroom production (Ashrafi et al. 2014, Rashid et al. 2016). Hence, the SMS is often regarded as agro-waste or farm waste (Hanafi et al. 2018). SMS's generated from mushroom enterprises in large quantities are often composted, burnt, discarded, incorporated into the soil or simply thrown away, which is neither economical nor environmentally safe (Phan and Sabaratnam 2012, Zhu et al. 2012).

Accumulation of this potential waste in large quantities over time has a negative impact on the environment (González-Marcos et al. 2015), in terms of environmental concerns such as soil, water, and air pollutions (Atila 2016, Magalhães et al 2018). Annually, a large amount of SMS is generated from mushroom farms and its effective disposal and/or utilization is one of the most serious challenges. According to the Polish law and Regulation of the Minister of Environment from 27 September 2001 to the most recent Regulation of the Minister of Climate from 2 January 2020, in the management of the waste catalogue, the spent mushroom substrate is classified in the group of wastes from agriculture, horticulture, aquaculture, fisheries, forestry and food processing, under catalogue number 02 01 99 specified as "other unspecified waste" (Rozporzadzenie Ministra Klimatu z 2020 w sprawie katalogu odpadow. Dz.U. 2020 poz.10). However, to date, the Polish legislation inadequately addresses the issues of its management and do not properly specify SMS storage conditions in the field (Cebula et al. 2013), which is neither economical nor environmentally safe.

Many studies have reported that the SMS still holds considerable levels of organic matter and nutrients, which could be of potential use in agriculture, horticulture, or plant disease management (Lau et al. 2003, Medina et al. 2009, Adedokun and Orluchukwu 2013, Atikmen et al. 2014, Kang et al. 2017). However, the immediate use of fresh SMS is restricted (Ahlawat and Sagar 2007, Zhang et al. 2012, Cebula et al. 2013, Atikmen et al. 2014). It has been documented that fresh SMS has high EC due to excess accumulation of salts during mushroom cultivation and has unfavourable pH, which are the major limiting factors for its immediate use (Medina et al. 2009, Eudoxie and Alexander 2011, Cebula et al. 2013). The unfavourable pH and EC of SMS may negatively influence overall plant development (Roosta 2014, Garriga et al. 2015) and alter photosynthetic processes due to abiotic stress (Gerloff-Elias et al. 2005, Ghaderi et al. 2018, Yaghubi et al. 2019, Shamsabad et al. 2020) and hence fresh SMS is not recommended for use in agriculture or horticulture activity.

SMS requires stabilisation before considering to be utilised in agriculture and/or horticulture (García-Delgado et al. 2013, Paula et al. 2017). The stabilisation of SMS is carried out through a weathering or composting process (Cebula et al. 2013, González et al. 2015, Medina et al. 2012), where the composting process involves the succession of microorganisms load, moisture content, C/N ratio, pH and EC level (Khater 2015, Pascual et al. 2018).

2.8. Spent mushroom compost (SMC)

The substrate generated immediately after mushroom production is called fresh-SMS (F-SMS) or simply SMS whereas, the same material after further decomposition or weathering for several months is regarded as weathered-SMS (W-SMS) or spent mushroom compost-SMC (Cebula et al. 2013). Passive weathering of SMS in open fields is one of the popular methods of disposal, where SMS is dumped in piles for further decomposition. Generally, the weathering process can range from 3-24 months (sometimes up to 36 months), during this process, rain and snowmelt water percolate through SMS piles and large amounts of salts are leached (Chong and Rinker 1994, Kaplan et al. 1995, Chefetz et al. 2000, Guo et al. 2001 a, b, Cebula et al. 2013). However, it has been reported that even after 24 months of passive weathering SMS can still release a significant amount of soluble solids (Cebula et al. 2013) and the leachates from SMS can significantly increase the salt content of underlying soils and groundwater (Guo et al. 2001 a, b, Cebula et al. 2013). Few reports suggest that the weathering (6-24 months) of SMS is not enough to reduce salinity to a satisfactory level, and proposed leaching as one of the possible options to reduce the salinity of SMC which include washing it for a short period (Riahi and Arab 2004, Riahi and Azizi 2006, Gonani et al. 2011). According to Riahi and Arab (2004) and Riahi and Azizi (2006), the leached SMC has a lower salinity level than the weathered compost and most of the essential elements, as well as the microbial properties, remain the same as spent mushroom compost.

Numerous studies reported that SMC can be utilised for agricultural and horticultural purposes (Suess and Curtis 2006) such as: soil conditioner (Kadiri and Mustapha 2011, Jonathan et al. 2013, Roy et al. 2015), nursery medium (Chong 2005, Medina et al. 2009, Ribas et al. 2009, Eudoxie and Alexander 2011, Zhang et al. 2012, Gao 2015, Ünal 2015), soilless growing medium (Chong 2005, Raviv 2011) in vegetable cultivation (Ahlawat and Sagar 2007, Polat et al. 2009, Ribas et al. 2009, Aktas et al. 2013, Idowu and Kadiri 2013, Sendi et al. 2013, Roy et al. 2015, Rahman et al. 2016) and fruits production (Danai et al. 2011, Adedokun and Orluchukwu 2013, Cabilovski et al. 2014). However, considering the composting time (6-36 months) and environmental issues associated with improper disposal of the spent mushroom substrate, as well as the negative influence of the leachates on soil, water and environment during the weathering process (Cebula et al. 2013), it is extremely important to come up with a simple, effective, efficient and immediate way of utilising such potential agro-wastes (Duque-Acevedo et al. 2020, Adejumo and Adebiyi 2021). This can help to overcome the limited re-use, disposal problems and environmental concerns associated with spent mushroom substrates at the same time achieving sustainability and creating a transition towards a circular economy (Danai et al. 2011, Adedokun and Orluchukwu 2013, Grimm and Wösten 2018).

2.9. Applications of SMS and SMC in horticulture

Most of the work to date has focused on the possible utilisation of SMC but not SMS, mainly due to its elevated salinity, unfavourable pH, and associated phytotoxicity (Maher et al. 2000, Sanchez-Monedero et al. 2004, Jordan et al. 2008, Demir 2017). Hence, SMS is recommended to use only after further decomposition or composting, or after the stabilisation process (Cebula et al. 2013, Roy et al. 2015, Paula et al. 2017). To date, only a few studies have been carried out evaluating its potential utilisation (Medina et al. 2009, Atikmen et al. 2014). The scientific evidence focusing on immediate and effective utilisation of fresh SMS in horticulture is not well documented and still in its infancy (Rinker 2017).

2.10. Spent mushroom substrate as a peat substitute

Medina et al. (2009) studied possibilities of utilising spent mushroom substrate after cultivation of *Agaricus bisporus* and *Pleurotus ostreatus* as growing media components replacing peat in seedling production of three vegetable species with different salt sensitivity. In the study SMS substituting peat in 25, 50 and 75% (v/v) was compared with 100% peat on the performance of tomato (*Lycopersicon esculentum*), courgette (*Cucurbita pepo*) and pepper (*Capsicum annuum*). The results indicated that both SMS's tested in the study can be potentially used as a peat substitute in seedling production, preferably in lower concentrations (25-50%).

These findings were supported by Eudoxie and Alexander (2011), where two fractions of *A. bisporus* SMS (fine >2 mm and course <6.25 mm) mixed with commercial peat moss in 50:50% (v/v) for seedling production of tomato. The seedlings produced in SMS were superior to those in peat and the fine fraction of SMS with peat (50:50%) was recommended to be the best substrate combination, further, the high level of EC in SMS had no negative influence on tomato seed germination and the total dry matter.

Collela et al. (2019) studied *A. bisporus* SMS (composted for 15 days) as nursery substrate in seedling production and as an organic fertiliser in crop production for tomato in comparison with two commercial peat-based substrates and three fertilisers (SMS of *A.*

bisporus, mineral fertiliser and animal manure). The seedlings produced on SMS presented higher rates of germination time and also 20% higher field productivity when compared to commercial substrates and fertilisers tested. The researchers concluded that SMS performed as good as commercial substrate and further recommended using SMS as organic fertiliser in seedling production.

Gao et al. (2015) replaced peat in traditional Swedish biobeds (consist of soil:peat:wheat straw in 1:1:2) with different SMS (*Pleurotus eryngii*, *Flammulina velutipes* and *Lentinus edodes*). Among the SMS bio-mixtures tested in the study, *L. edodes* SMS based bio-mixture was most biologically active and the authors concluded that SMS can replace peat in biodeds.

Paula et al. (2017) substituted commercial peat in Italian grass (*Lolium multiflorum*) production with *A. bisporus* SMS. The authors observed improved biomass up to 300% when compared to control and also reported no phytotoxicity.

2.11. The spent mushroom substrate as an organic fertiliser

Danai et al. (2011) studied the possibility of utilising fresh SMS as an organic soil amendment in comparison with popular and widely used cattle manure compost in avocado orchards. The soils amended with SMS showed better soil porosity, water holding capacity and also higher yield when compared with animal manure treated soils. The authors further concluded that SMS is relatively cheap and easily available when compared to animal manure, and the commercial application of SMS in avocado orchards should be done carefully to avoid unnecessary damage due to high EC.

These findings were supported by Adedokun and Orluchukwu (2013), where soil incorporation of *P. osteratus* SMS (composted for 2 months) showed a positive effect on the overall performance of pineapple (*Ananas comosus*). These authors concluded that SMS can be considered as a potential organic fertiliser which promotes organic farming and further recommended mushroom growers to sell SMS rather than disposing of it in an open field. This can help to overcome disposal problems associated with SMS and to generate additional income for mushroom growers.

Gobbi et al. (2015) evaluated SMS as organic fertiliser in comparison with mineral fertilisers for lettuce (*Lactuca sativa*) and leek (*Allium porrum*) production. The results indicated that soil amended with SMS had a positive effect on both lettuce and leek performance comparable with mineral fertilisation.

In the study conducted by Nakatsuka et al. (2016) the soil incorporation with *P. ostreatus* SMS improved the soil structure and the porosity in both topsoil and subsoil, at the same time improved soil aggregates and micromorphology.

These findings are in line with Orluchukwu et al. (2016), who reported that the soil incorporated with *P. ostreatus* SMS had higher values of N, P, K and further improved morphological performance of fluted pumpkin (*Telfairia occidentalis*).

Paredes et al. (2016) used *A. bisporus* and *P. osteratus* SMS as organic soil amendments to a calcareous clayey-loam soil in comparison with mineral fertiliser. The addition of both SMS's, in particular *A. bisporus* SMS, improved soil physiochemical properties and fertility. Further, the authors concluded that SMS's had no negative influence on the yield and nutritional composition of lettuce.

2.12. Comparing utilisation of SMS and SMC

Atikmen et al. (2014) conducted a greenhouse study comparing fresh SMS and SMC (weathered for 24 months) as organic substrates along with commercial peat and perlite in chrysanthemum (*Chrysanthemum morifolium*) cultivation. They reported that the fresh SMS can be used preliminary at 12.5% and 25% being the highest dose in crops that are not sensitive to salinity like chrysanthemum. On the other hand, the authors recommended to use SMC at 25% and 50%.

These findings were supported by Roy et al. (2015) who studied, fresh SMS, SMC and leachates obtained from *P. osteratus* and *A. bisporus* mixed with soil in a pot experiment. It was evident that the use of the different forms of SMS of both mushroom species positively influenced the overall growth of pepper (*Capsicum annuum*) when compared to un-amended soil. In contrast, Demir et al. (2017) reported that fresh SMS had a negative influence on the overall performance of pepper seedlings (*C. annuum*) when used as a substrate and further concluded that only aged SMC (weathered for 6 months) along with 30% perlite can be recommended as an alternative to the peat-based substrate.

2.13. Spent mushroom substrate – other applications and needs for further studies

As reviewed by Rinker (2017) and Hanafi et al. (2018), major applications of the spent mushroom substrate are bioremediation, animal feedstock, fertiliser and energy production.

The reviews also discussed the possible utilisation of fresh SMS in successive mushroom production and the need for further research exploring immediate, cheap utilisation and safe disposal. Considering the limited scientific information on immediate utilisation of fresh SMS in agriculture and horticulture (Rinker et al. 2017, Hanafi et al. 2018), mixed degree of success of fresh SMS when compared to SMC (Demir et al. 2017), environmental issues associated with improper disposal and weathering of SMS (Cebula et al. 2013, Roy et al. 2015) and a need of stabilisation before direct use in horticulture and/or agriculture demonstrates the importance of further research in this regard (Paula et al. 2017). As the nature of spent mushroom substrate can largely vary depending on materials used in substrate preparation, composting process and the type of mushroom cultivated, comparative study in this regard will be of great importance (Peksen and Yamac 2016, Catal and Peksen 2020).

Among many agro-wastes studying the possibilities of utilising waste after commercial mushroom production can stand out, as its easy availability (Finney et al. 2009), relatively low cost (Danai et al. 2011, Adedokun and Orluchukwu 2013), the residual amount of nutrients (Catal and Peksen 2020) and the environmental problems associated with its improper disposal or handling (Atila 2016, Magalhães et al 2018).

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3. Materials and methods

3.1. Geographical location of the experimental site

The experiment was conducted in an unheated plastic tunnel at the Experimental Station 'Marcelin' (lat. 52^{o}24'25.4" N, long. 16^{o}51'38.0" E and at 282 m elevation) belonging to the Faculty of Agronomy, Horticulture and Bioengineering, Poznan University of Life Sciences, Poland.

3.2. Substrates and their characteristics

3.2.1. Spent Mushroom Substrates

3.2.1.1 *Agaricus bisporus*

The white button mushroom (*A. bisporus*) was cultivated on the substrate prepared following the standard method, i.e. using 1000 kg wheat straw, 750 kg poultry manure, 80 kg gypsum and 3000 kg of water. Fermentation was carried out at 75-85^{o}C and pasteurisation at 54-60^{o}C. Black peat was used as a casing material along with 20 kg chalk per 1 sq. meter of peat. The layer of 5 cm peat was embedded on top of the compost block at the rate of 80 kg compost per 1 sq. meter. The time taken from fructification to the end of cultivation was three weeks. The fresh SMS after commercial production of *A. bisporus* (A-SMS) was obtained from "Hajduk Grupa Producentow Pieczarek Sp. z o.o."

3.2.1.2. *Lentinus edodes*

Shiitake (*L. edodes*) was cultivated on the sawdust based substrate. The beech and oak sawdust in the ratio 1:1 based on volume (v/v) were mixed with additives such as corn flour, wheat bran and millet grain (additives constituted 20% of dry matter of sawdust). This mixture at 65% moisture was used as the substrate for *L. edodes* cultivation. Pasteurisation was carried out for 10 hours at 90-95^{o}C. Five per cent mushroom spawn of the wet substrate was incubated for 90 days. The time taken from fructification to the end of cultivation was three weeks. The fresh SMS after commercial production of *L. edodes* (L-SMS) was obtained from "Uprawa Grzybow shiitake Alicja Hamrol".

3.2.1.3. *Pleurotus ostreatus*

The oyster mushroom (*P. 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%. Pasteurisation was carried out for 48 hours at 60^{o}C. The substrate was inoculated with 3% of *P. ostreatus* spawn. The inoculated substrate was incubated for 18 days at 25^{o}C.

The duration from fructification to the end of cultivation was four weeks. The fresh SMS after commercial production of *P. ostreatus* (P-SMS) was obtained for the experiment from "TYRMYCEL Wytwornia grzybow uprawowych i kostki boczniaka".

3.2.2. Peat

A superior quality professional peat (Peat clear-class H2 according to Van Post classes) with enhanced hydrophilic capacity was purchased from "Hartmann Polska Sp. z o.o."

3.3. Experimental details

3.3.1. Experimental design and layout

The investigation was divided into three studies: experiment 1, 2 and 3 for the better implementation of methodology and interpretation of results. Experiments 1 and 2 were carried out during spring 2018 and 2019 from April to June, respectively. Experiment 3 was conducted from November 2019 to June 2020. The experimental timeline is given in Tab. 1.

Table 1. The experimental timeline from 2018 to 2020

Particulars	**Experiment 1**	**Experiment 2**		**Experiment 3**
Year	**2018**	**2019**		**2020**
Cultivars	'Honeoye'	'Honeoye'	'Elsanta'	'Elsanta'
Planting season	Spring-2018	Spring-2019		Autumn-2019
Planting time	10^{th} April	10^{th} April		10^{th} November
Planting material	Tray plants (A+ grade)	Tray plants (A+ grade)		Tray plants (A+ grade)
Growing season	Spring	Spring		Winter-Spring
Fruit harvesting	May-June	May-June		May-June

The experiments were laid out in a randomised completely block design (RCBD) with seven substrate combinations (S1-S7) and five replications (R1-R5). In each experiment, 40 plants were maintained in an individual substrate facilitating 10 plants in each replication. Additionally, eight plants were placed at the beginning and end of each row as border plants. The substrate combinations were allotted randomly according to definite laws of probability. The plan and layout of experiments 1, 2 and 3 are given in Fig. 1

N

R_1	**R_2**	**R_3**	**R_4**	**R_5**
border plants	border plants	border plants	border plants	border plants
S_7	S_2	S_4	S_1	S_3
S_6	S_3	S_1	S_4	S_7
S_5	S_7	S_6	S_3	S_4
S_4	S_1	S_2	S_6	S_5
S_3	S_6	S_5	S_2	S_6
S_2	S_4	S_7	S_5	S_1
S_1	S_5	S_3	S_7	S_2
border plants	border plants	border plants	border plants	border plants

Figure 1. Plan and layout of experiments (2018-2020)

R_1-R_5 (Replication 1-Replication 5), S_1-S_7 (Substrate 1-Substrate 7)

3.3.2. Substrate combinations

The different SMS's from three mushroom species *Agaricus bisporus* (A-SMS), *Lentinus edodes* (L-SMS) and *Pleurotus ostreatus* (P-SMS) were selected as growing media substitutes for the study. Peat was substituted with varying concentrations of SMS's and used as a soilless growing media (Substrate 2-7) in the present study. The 100% commercial peat (Substrate 1-S1) was considered as a control. Whereas, Substrate 2 (S2) and Substrate 3 (S3) was formulated using A-SMS:Peat, Substrate 4 (S4) and Substrate 5 (S5) using L-SMS:Peat, Substrate 6 (S6) and Substrate 7 (S7) using P-SMS:Peat in varying concentrations. The substrate combinations were prepared based on volume (v/v). The substrate combinations are as described in Tab. 2, constituting seven different substrates (S1-S7) as a growing media.

Table 2. Substrate combinations used in the study (2018-2020)

Substrate combinations	**Experiment 1**	**Experiment 2**	**Experiment 3**
S1	Peat-100% (control)	Peat-100% (control)	Peat-100% (control)
S2	A-SMS:Peat (10:90%)	A-SMS:Peat (15:85%)	A-SMS:Peat (15:85%)
S3	A-SMS:Peat (20:80%)	A-SMS:Peat (25:75%)	A-SMS:Peat (25:75%)
S4	L-SMS:Peat (25:75%)	L-SMS:Peat (15:85%)	L-SMS:Peat (15:85%)
S5	L-SMS:Peat (50:50%)	L-SMS:Peat (25:75%)	L-SMS:Peat (25:75%)
S6	P-SMS:Peat (25:75%)	P-SMS:Peat (15:85%)	P-SMS:Peat (15:85%)
S7	P-SMS:Peat (50:50%)	P-SMS:Peat (25:75%)	P-SMS:Peat (25:75%)

Since the scientific information recommending standard substitution rates of different SMS along with peat in soilless strawberry production is not well documented, different substitution rates were investigated in the present study. Experiment 1 was considered as a preliminary study, where A-SMS was substituted with peat at 10% and 20%, while L-SMS and P-SMS were substituted at 25% and 50%. Based on the results of experiment 1, A-SMS, L-SMS and P-SMS, were supplemented to peat in 15% and 25% in experiment 2. The results of experiment 2 indicated that the supplementation rates tested in experiment 2 were suitable for soilless strawberry production, so the same substitution rates were tested in experiment 3. The fresh SMS's, peat (S1) and substrate combinations based on SMS (S2-S7) used in the study (2018-2020) are shown in photos 1-4.

Photo 1. The fresh spent mushroom substrate of *Agaricus bisporus* (A) *Lentinus edodes* (B) *Pleurotus ostreatus* (C) obtained immediately after commercial mushroom cultivation (Photo: R. Prasad)

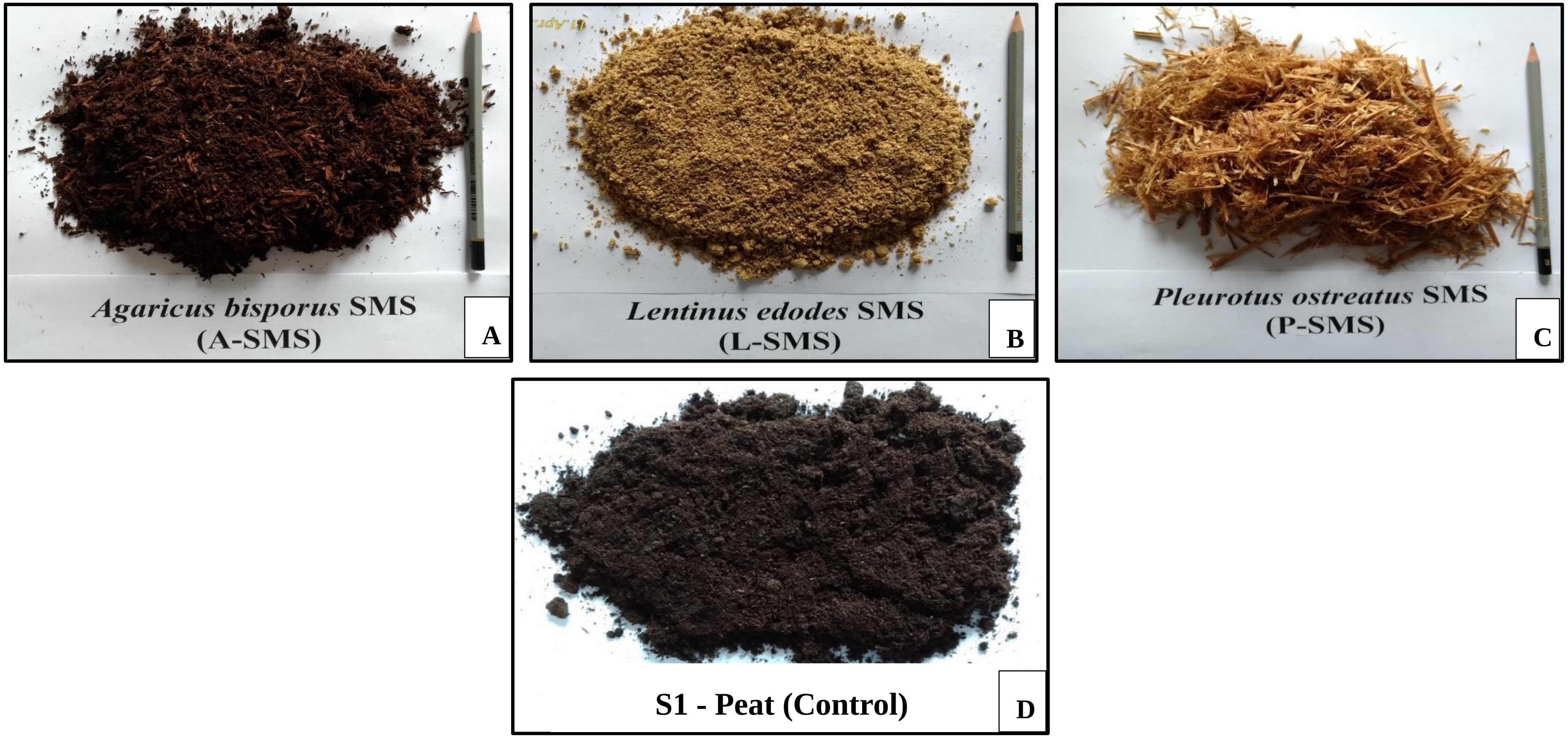

Photo 2. The 100% fresh spent mushroom substrate of *Agaricus bisporus* (A), *Lentinus edodes* (B) and *Pleurotus ostreatus* (C) after manual pre-processing in comparison with peat (D) (Photo: R. Prasad)

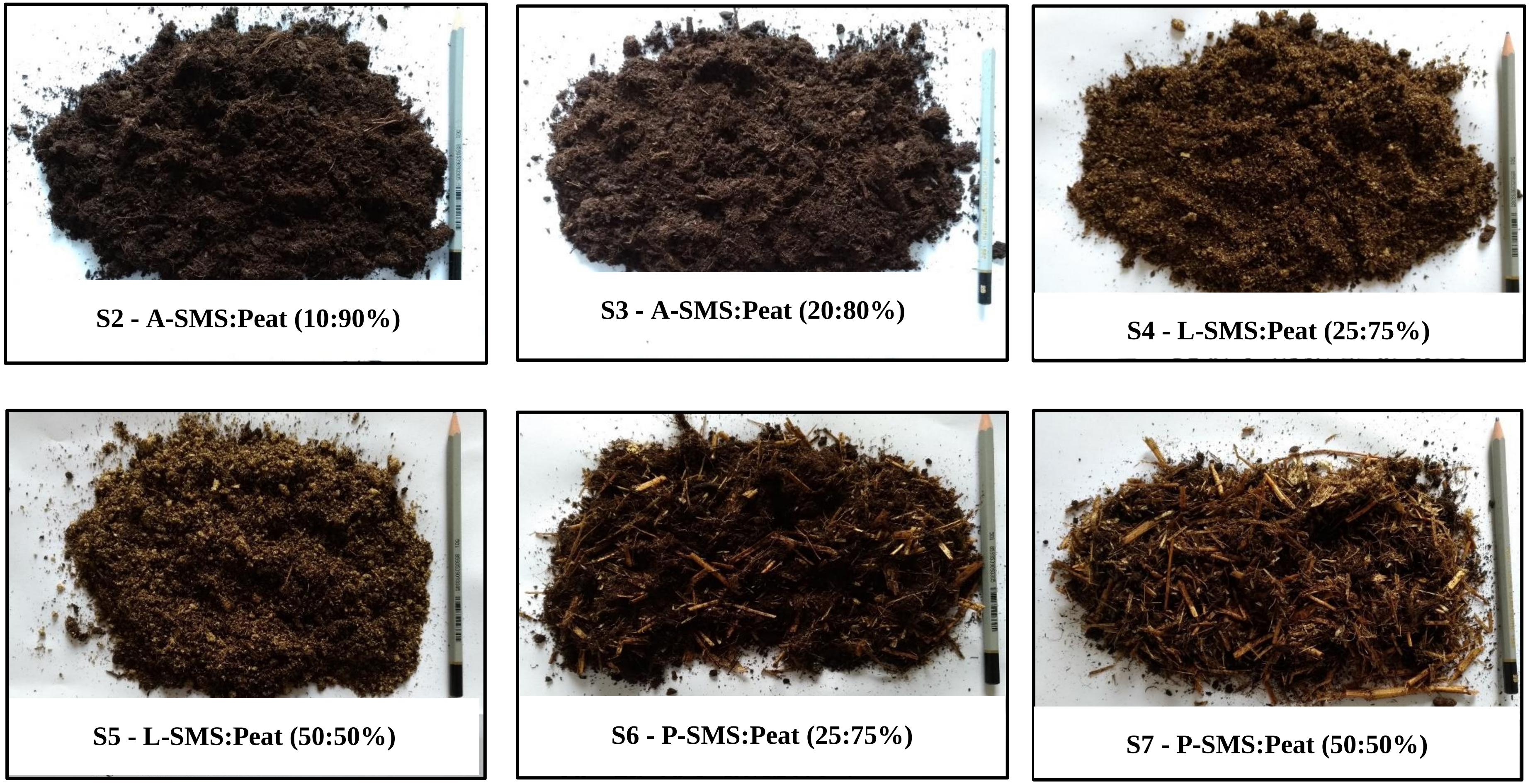

Photo 3. The substrate combinations based on SMS (S2-S7) used for strawberry cultivation in 2018 for experiment 1 (Photo: R. Prasad)

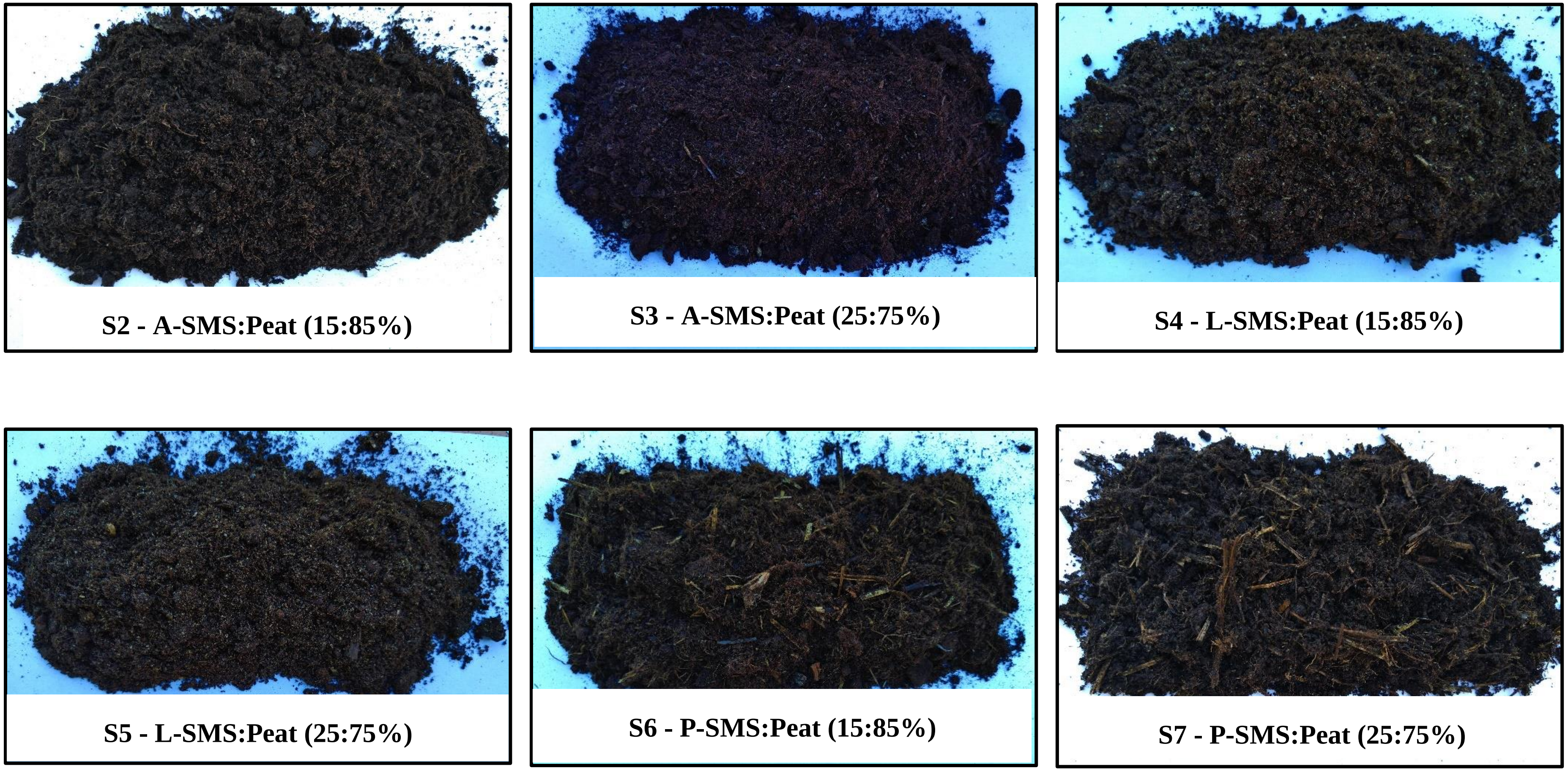

Photo 4. The substrate combinations based on SMS (S2-S7) used for strawberry cultivation in 2019 and 2020 for experiment 2 and 3 (Photo: R. Prasad)

3.4. Horticultural operations

The details of various horticultural operations carried out during investigation are as mentioned below:

3.4.1. Tunnel preparation

A low cost unheated plastic tunnel of 30×7 m (L×B) was preliminarily prepared by removal of weeds, alignment of surface and covering the surface with black geotextile mulch. Chemical sprays before 30 days of the scheduled experiment were carried out to make the tunnel free from any residual pests and diseases. The plastic tunnel at the beginning of the experiment is shown in Photo 5.

Photo 5. Pre-preparation of the unheated plastic tunnel for the experiment by covering the surface with black geotextile mulch (Photo: R. Prasad)

3.4.2. Substrates preparation

The fresh SMS's obtained from commercial mushroom farms were pre-processed manually before mixing with commercial peat. A-SMS was passed through a 2 mm sieve and mixed well to maintain homogeneity. The L-SMS blocks were finely powdered and passed through a 2 mm sieve. P-SMS blocks of wheat straw were manually separated and mixed well to make sure that no wheat straws were in aggregates, which may alter the homogeneity of prepared substrates. The manually processed 100% SMS's were later manually mixed with commercial peat based on volume (v/v) in varying concentrations as previously mentioned in Tab. 2. The manual preparation of substrate combinations served as growing media for soilless strawberry cultivation are shown in Photo 6.

Photo 6. Preparation of substrate combinations (Photo: R. Prasad, J. Lisiecka)

3.4.3. Growing containers

In the study, white plastic boxes 90×13.5×12 cm (L×B×D) were used as growing containers. The volume of the individual growing container was calculated (approximately 13 litres) and provided with three drainage holes at the bottom to facilitate adequate drainage.

3.4.4. Media filling

The substrate combinations were filled into individual growing containers covering 90% (12 litres) of total volume. Understanding the expandable nature of growing media, approximately 10% of total volume was left unfilled facilitating free pore space for better exchange of gases, moisture and nutrients to provide optimum growing conditions for plants. The growing containers filled with prepared substrate combinations are shown in Photo 7.

Photo 7. Growing containers filled with prepared substrate combinations (Photo: R. Prasad)

3.4.5. Planting material and planting

The strawberry (*Fragaria* × *ananassa* Duch.) tray plants (A+ grade) were used as planting material during the present study and were obtained from the Experimental Station ‘Marcelin’. The tray plants of cv. ‘Honeoye’ was used as planting material for experiment 1. The tray plants of strawberry cv. ‘Honeoye’ and cv. ‘Elsanta’ were used as planting material for experiment 2 and the tray plants of cv. ‘Elsanta’ was used as planting material for experiment 3.

The strawberry plants were planted in growing containers filled with substrates. Four plants per one container (3 dm^3 substrate/plant) were planted maintaining 20 cm between each plant leaving 15 cm from both ends of the growing container. The strawberry planting operation and growing containers with plants are as shown in Photo 8 and Photo 9.

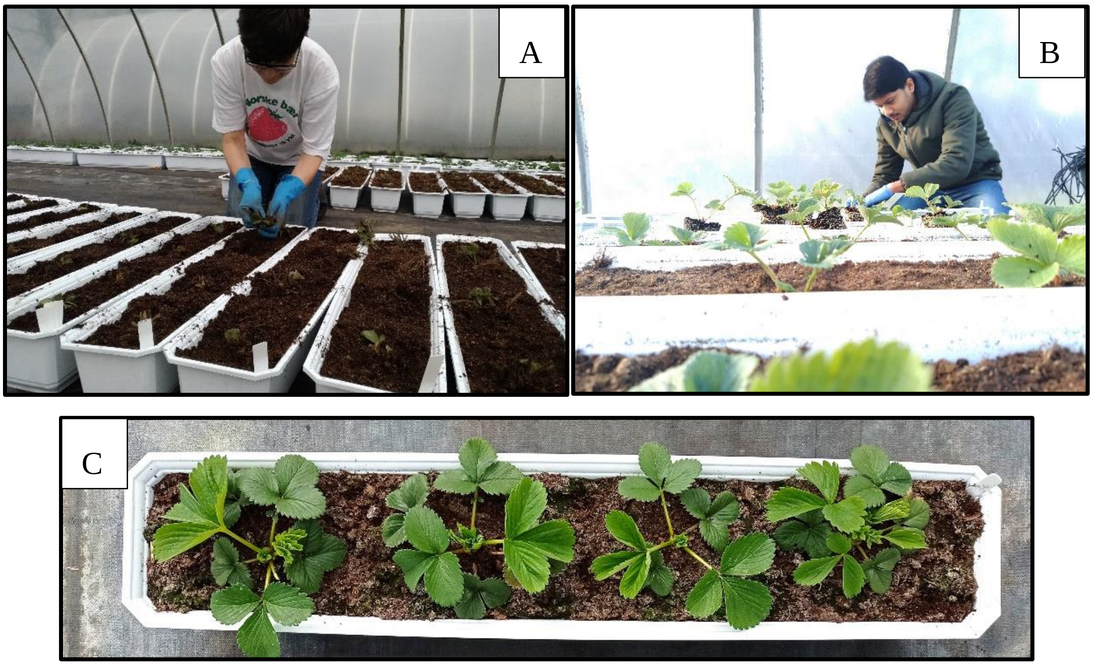

Photo 8. Planting strawberry plants in growing containers (A and B), growing containers with strawberry plants (C) (Photo: R. Prasad)

Photo 9. Strawberry plants after one week of transplanting (Photo: R. Prasad)

3.4.6. Establishment of drip irrigation system

The facility of an automatic drip irrigation system was established for efficient water management. The system was furnished with micro-tubes attached to drippers for effective and uniform distribution and utilisation of water. The laterals were provided for individual substrates in each replication and the system was established in such a way that individual substrates receive a sufficient amount of water depending on the moisture content of the substrate. The growing containers were provided with three bent arrow emitters with a flow rate of 2 litres/ hour (Photo 10).

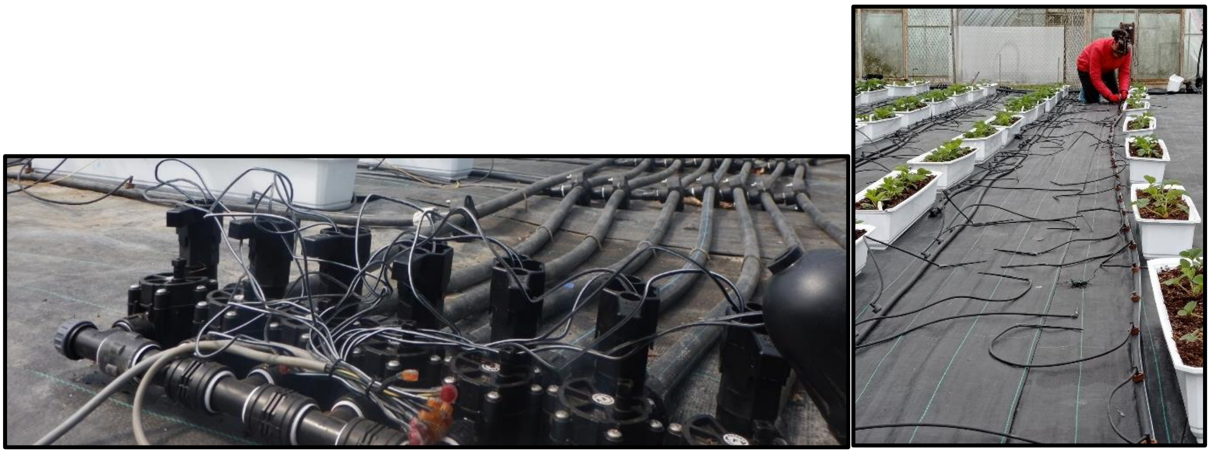

Photo 10. Installation of drip irrigation system (Photo: R. Prasad)

3.4.7. Organising growing containers according to experimental plan and layout

The growing containers with plants were organised in rows as previously given in Fig. 1 in an unheated plastic tunnel. The spacing of 60×25 cm (between rows and between containers) was maintained. Immediately after planting plants were irrigated to ensure sufficient substrate moisture. The general view of the study (2018-2020) is given in Photo 11.

Photo 11. General view of the experiment during experiment 1 (A), experiment 2 (B) and experiment 3 (C) (Photo: R. Prasad)

3.4.8. Irrigation and application of fertilisers

During the vegetative phase, plants were irrigated two times a day for 120 s (60 s in each interval) and during the flowering and fruiting stage (from the first week of May) 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 electrical conductivity (EC) of irrigation water during the study were between 7.09-7.11 and 0.70-0.72 $mS \cdot cm^{-1}$, respectively.

The strawberry plants were fertilised with water-soluble nutrient solution based on Kristalon Blue 19:6:20 ($N:P_2O_5:K_2O$) + microelements (MgO 3%, SO_3 7.5%, B 0.025%, Cu 0.01%, Fe 0.07%, Mn 0.04%, Mo 0.004% and Zn 0.025% by Yara Polska Sp. z o.o.) and Calcinit comprising 15.5% total N (14.4% $N\text{-}NO_3$ and 1.1% $N\text{-}NH_4$) and calcium 26.5% CaO and 19.0% Ca by "Yara Polska Sp. z o.o.". 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 growing container) were alternatively applied during the crop cycle with an interval of 10-12 days (Tab. 3).

Table 3. The nutrient solution used for fertilisation

Nutrient	**Vegetative phase**		**Generative phase**	
	dose-1	**dose-2**	**dose-3**	**dose-4**
Ammonia-Nitrogen ($N\text{-}NH_4$)	12.1%	1.1%	12.1%	1.1%
Nitrate-Nitrogen ($N\text{-}NO_3$)	7.9%	14.4%	7.9%	14.4%
Phosphorus (P)	5%		5%	
Potassium (K_2O)	10%		10%	
Magnesium (MgO)	2%		2%	
Sulfur (S)	10%		10%	
Calcium (CaO)		26.3%		26.3%
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%	

The pH and EC of 10% Kristalon Blue and 10% 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.

3.4.9. After care and plant protection

The runners and old dry leaves were frequently removed from strawberry plants, to maintain good field sanitation. The experimental plot was maintained weed-free during the period of investigation, manual weeding operation was carried out to make the tunnel free from any possible weeds.

The plant protection activity was carried out immediately after identifying the visible symptoms. The use of chemicals was kept minimum and only when necessary. All chemical plant protection operations were carried out during the vegetative stage and the use of chemical sprays were restricted once the plants achieve the generative stage. The chemicals were prepared based on standard chemicals in recommended concentrations for spraying (Tab. 4).

Table 4. Plant protection chemicals used during the studies (2018-2020)

Experiment	**Insect/pest**	**Chemical and concentration**	**Interval**	**Number of sprays**
Experiment 1	Spider mites *Tetranychus urticae*	Vertigo 1.8% EC (0.1%) (based on Abamectin)	10-12 days	2
	Aphids *Chaetosiphon fragaefolii*	Fastac® 100 EC (0.05%) (based on Alphacypermethrin)	10-12 days	2
Experiment 2	Aphids *Chaetosiphon fragaefolii*	Fastac® 100 EC (0.05%) (based on Alphacypermethrin)	10-12 days	2
Experiment 3				

3.5. Observations recorded

3.5.1 Meteorological data

The temperature, relative humidity (RH) and dew point during the experiment inside the plastic tunnel was recorded using HOBO Onset data loggers and the mean values during the growing season are given in Fig. 2.

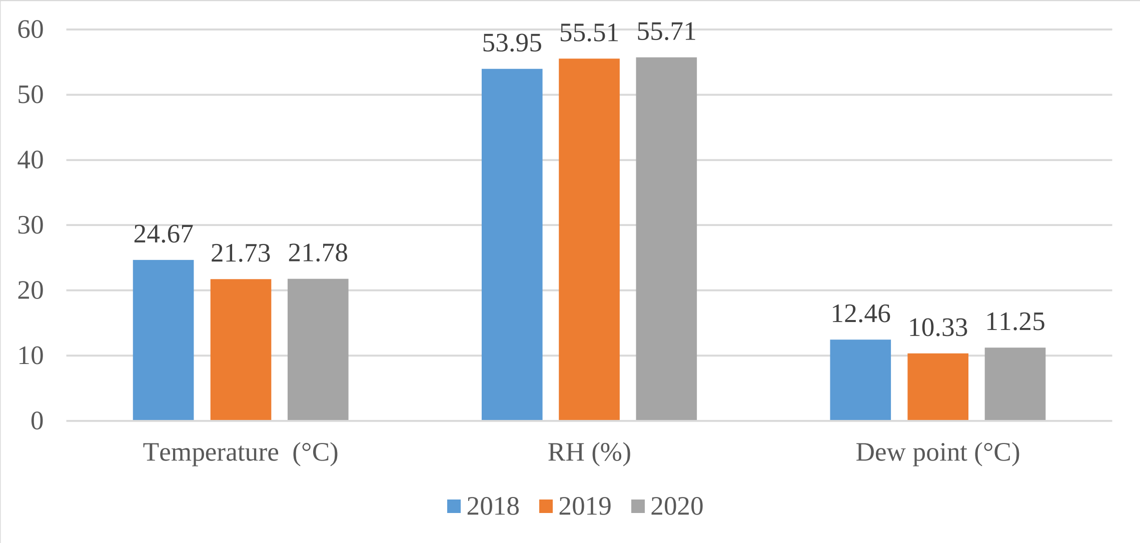

Figure 2. The average temperatures, RH and dew point inside the plastic tunnel during the studies (2018-2020)

3.5.2. Substrate analysis

All substrates (S1-S7) were collected both before and after each experiment for chemical analysis. The substrate sample of two litres from individual studies substrate before the experiment were collected during substrate preparation and filling. The substrates at the end of the experiment were collected from growing containers after the plants were separated from growing media. The materials such as roots and dry leaves were carefully removed from the collected samples and the samples were mixed well before analysis. The chemical analysis of substrates was carried out at the Department of Plant Nutrition, Faculty of Agronomy, Horticulture and Bioengineering, Poznan University of Life Sciences as described by Schroeter-Zakrzewska et al. (2021).

For chemical analyses, 20 cm^3 of the substrate with actual moisture were taken using the Drews' instrument which permits obtaining the same sample density (Bres et al. 2008). The volumetric sample was later transferred to a round bottom flask and then, to extract macroelements, 200 cm^3 of extraction solution (acetic acid - 0.03 MCH_3COOH) were added in 1:10 proportion of substrate to extraction solution. The suspension was shaken for 30 minutes in a rotational agitator, and then it was filtered. To determine microelements, a successive substrate sample was taken and it was flooded with Lindsay's solution containing 10 dm^3: 50 g of EDTA (ethylenediamine-tetraacetic acid), 90 cm^3 of 25 % NH_4OH solution, 40 g of citric acid, 20 g of Ca $(CH_3COO)_2 \times 2H_2O$ (IUGN 1983, Bres et al. 2008) and then the same procedure was carried out as in case of the extraction with acetic acid.

The pH, salinity (EC), macro- (N, P, K, Ca, Mg, S, Na, Cl) and micro- (Fe, Cu, Mn, Zn) nutrient analysis of collected substrate samples were carried out. Nitrate nitrogen ($N\text{-}NO_3$) and ammonia nitrogen ($N\text{-}NH_4$) were determined using micro distillation (according to Bremner in Starck's modification), phosphorus (P) using the colourimetric method with ammonium vanadomolybdate, potassium (K), calcium (Ca) and sodium (Na) by flame photometry, chlorides (Cl) by nephelometrically with AgNO3, sulphate sulphur ($S\text{-}SO_4$) using nephelometrically with $BaCl_2$, boron (B) using the colourimetric method with curcumin, magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) by atomic absorption spectrometry (AAS) on Carl Zeiss Jena AAS apparatus. Furthermore, the salinity (EC) using a conductometric method, with the substrate: water ratio of 1:2 (v/v) and pH by a potentiometric method with the substrate: water ratio of 1:2 (v/v) was also determined.

3.5.3. Morphological parameters

Five plants were selected randomly in individual substrate combinations (S1-S7) and labelled for recording observations on morphological, pomological, yield and physiological parameters. The mean value of recorded data was selected to represent each parameter. The observations of morphological parameters like plant height and number of leaves were taken at the monthly interval after planting. At the end of the growing season, strawberry plants were carefully separated from media and morphological parameters including shoot length, root length, number of leaves, crown diameter, number of crowns, fresh and dry masses of plant samples were recorded. The strawberry plants during different growing stages were as given in Photo 12.

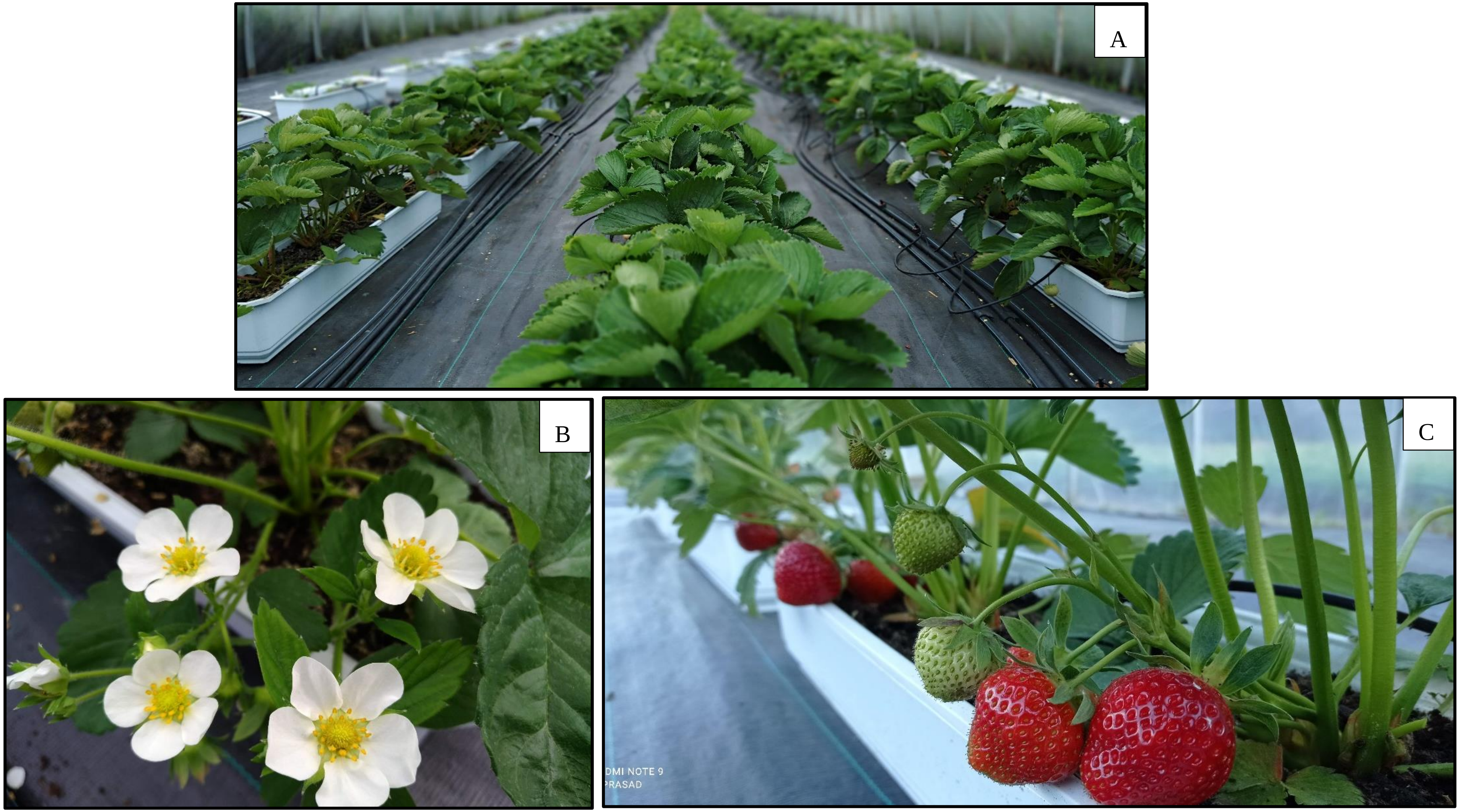

Photo 12. Strawberry plants during vegetative stage (A), generative stage (B) and reproductive stage (C) (Photo: R. Prasad)

- Plant height

The plant height was measured from the media level, i.e. first node of the plant to the tip of the plant at 45 and 60 days after transplanting and after final plant harvest. The average was expressed in centimetres.

- Number of leaves per plant

The total number of fully opened trifoliate leaves produced by the individual plant at 45 and 60 days after transplanting and at final plant harvest was counted manually and expressed in numbers.

- Leaf area

During each experiment, five fully matured leaves from individual substrate combinations (S1-S7) were collected and scanned using the WinDIAS leaf area scanner. Then leaf area was computed using WinDIAS leaf area measurement system (Photo 13 a, b) WD-E3 manufactured by Delta-T Devices and expressed in cm^2.

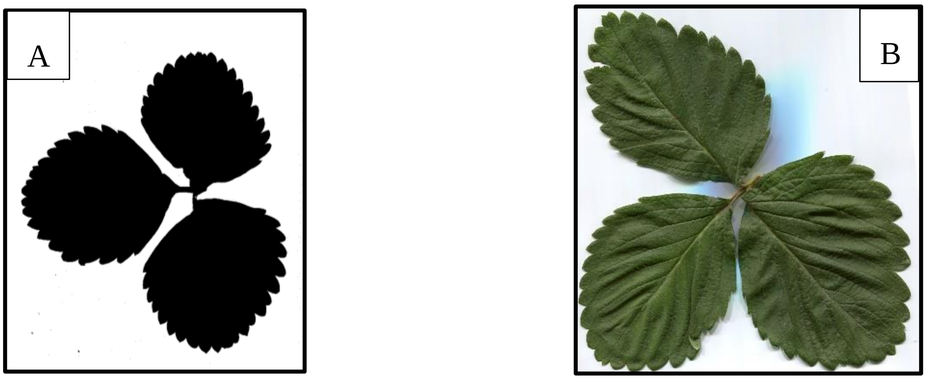

Photo 13. Scanned strawberry leaf (A-grayscale and B-colour) using WinDIAS for leaf area measurement

- Plant fresh weight at final harvest

At the end of each experiment, five plants from each substrate combination (S1-S7) were carefully uprooted and thoroughly washed in running water until there were no substrate traces attached to roots. The washed plant samples were placed in a paper bag and labelled. The fresh weight was recorded for an individual plant concerning the whole plant, shoots and root weights.

- Crown diameter

The plants uprooted for recording fresh weight were used to measure the crown diameter (measured at the widest section) using a digital vernier calliper and it was expressed in millimetres (mm).

- Plant dry weight

After recording fresh weight, the plant's samples were dried at 70ºC (till they reach a constant weight) in a hot air oven. Final dry masses of individual plant samples including the whole plant, shoots and root were determined and expressed in grams.

- Shoot to root ratio

The shoot/root ratio was calculated based on obtained shoot and root dry weight.

3.5.4. Pomological parameters

The matured berries were picked along with stalks from tagged plants. In an interval of 3-4 days, fresh fruits were harvested at their proper maturity stage. The harvesting operation was done in the early morning or late evening hours to reduce the transpiration losses. After harvesting the following fruit quality and yield-related measurements were made. The strawberry fruits at proper maturity and harvested strawberries from individual substrates are given in Photos 14 and 15.

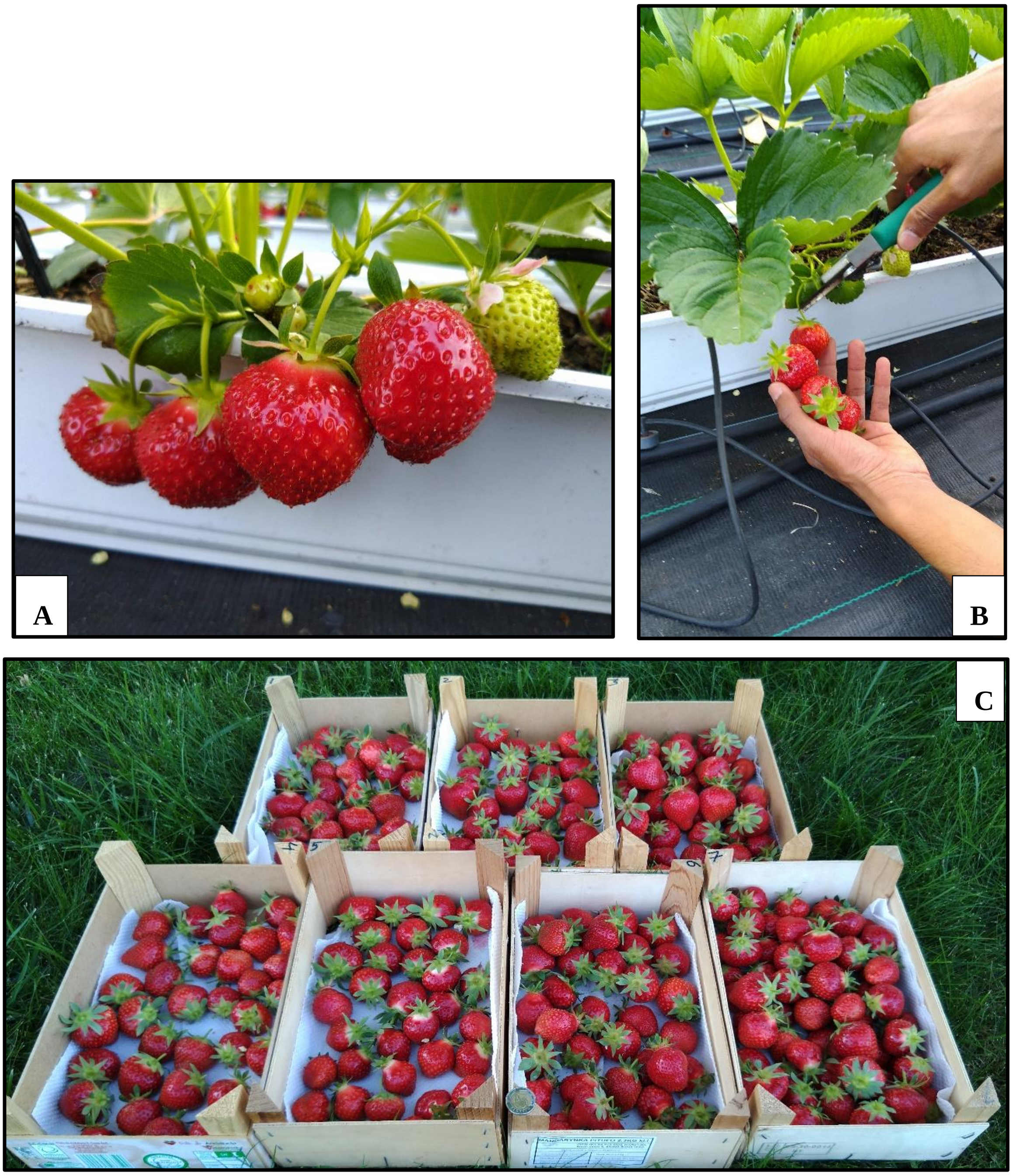

Photo 14. Strawberry fruits ready for harvesting (A), harvesting strawberries at proper maturity (B), harvested fruits from individual substrate combinations (C) (Photo: R. Prasad)

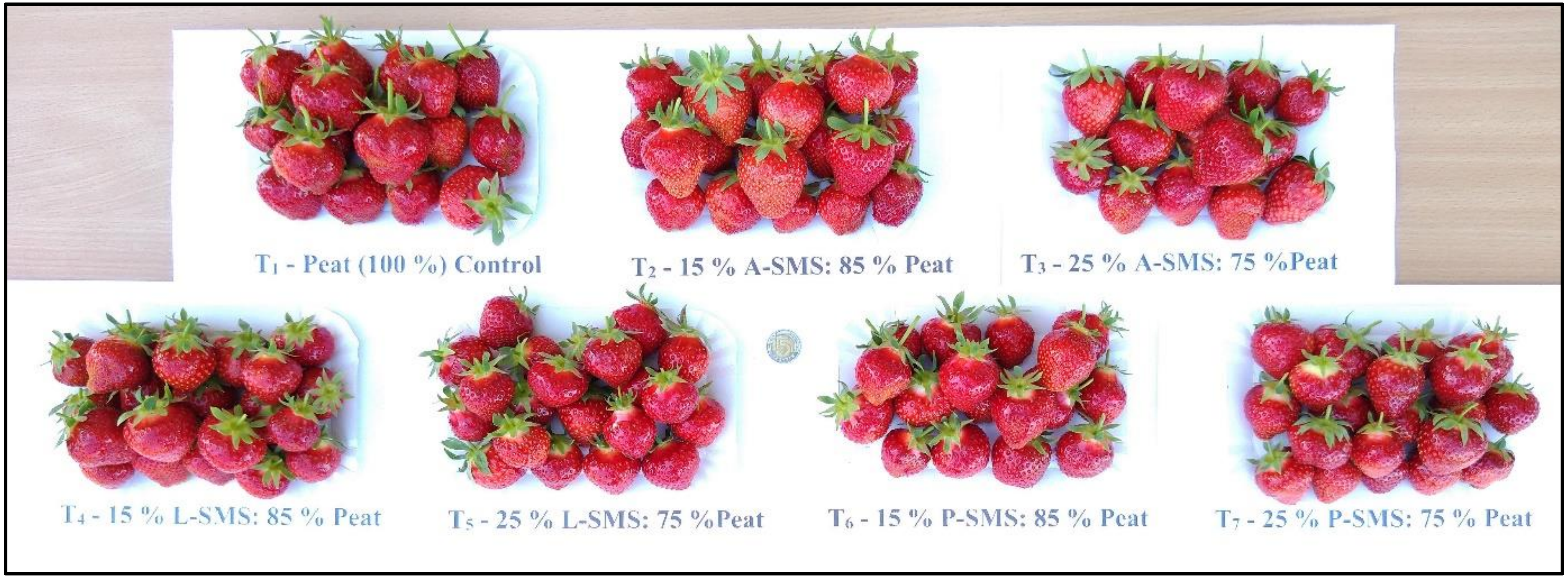

Photo 15. Harvested strawberry fruits from individual substrate combinations (Photo: R. Prasad)

3.5.4.1. Fruit quality parameters

- Fruit diameter

The fruit diameter was measured at the maximum width of fruits with the help of a digital vernier calliper and it was expressed in mm.

- Individual fruit weight

The weight of fifteen fruits in each harvest were recorded using a digital balance, then the average value for individual fruit in individual substrate combinations (S1-S7) was calculated and expressed in grams.

- Total Soluble Solids

Fifteen marketable fruits from individual substrates in each harvest were selected to determine total soluble solids (TSS). The TSS was recorded with the extracted fruit juice using a digital refractometer at room temperature and expressed as degree Brix (ºB) or %.

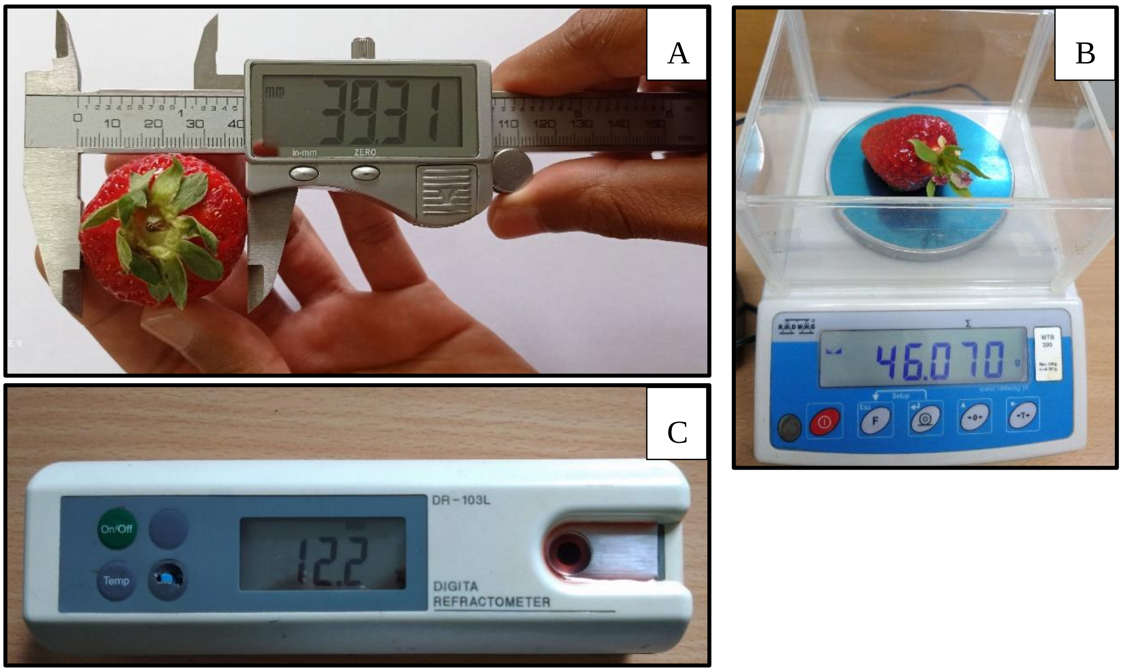

Photo 16. Measuring fruit diameter – a digital vernier caliper (A), individual fruit weight – a digital balance (B) TSS – a digital refractometer (B) (Photo: J. Lisiecka and R. Prasad)

- Fruit colour

During experiment 3 (2020), fifteen marketable grade strawberry fruits previously used to measure fruit diameter and individual fruit weights from individual substrate combinations were selected for colour measurement. Strawberry fruit colour was measured employing a non-destructive method by using a top-port colour measuring instrument (Konica Minolta CM-5 Spectrophotometer, USA) and expressed as colour coordinates L*, a* and b*. Where L* represented the lightness level of the colour, a* and b* indicated the positive/negative correlation to the red/green component, and the yellow/blue component of colour, respectively.

3.5.4.2 Yield parameters

During each experiment, the observations on fruit yield per plant were recorded after every harvest from each substrate (S1-S7). The total yield per plant was calculated by adding the values obtained in different picking and expressed as total yield per plant (grams). All harvested fruits were further classified into marketable yield per plant and unmarketable yield per plant.

- Grading: marketable and un-marketable

The extra class (minimum fruit diameter of 25 mm), class-I and class-II (minimum fruit diameter of 18 mm) fruits were regarded as commercial or marketable yields. Whereas the misshaped, damaged, diseased and fruits with diameter less than 18 mm were considered as un-marketable yield.

The harvested strawberries were classified as an extra class, class-I and class-II (Commission delegated regulation (EU) 2019/428 of 12 July 2018, amending Implementing Regulation (EU) No 543/2011 as regards marketing standards in the fruit and vegetable sector).

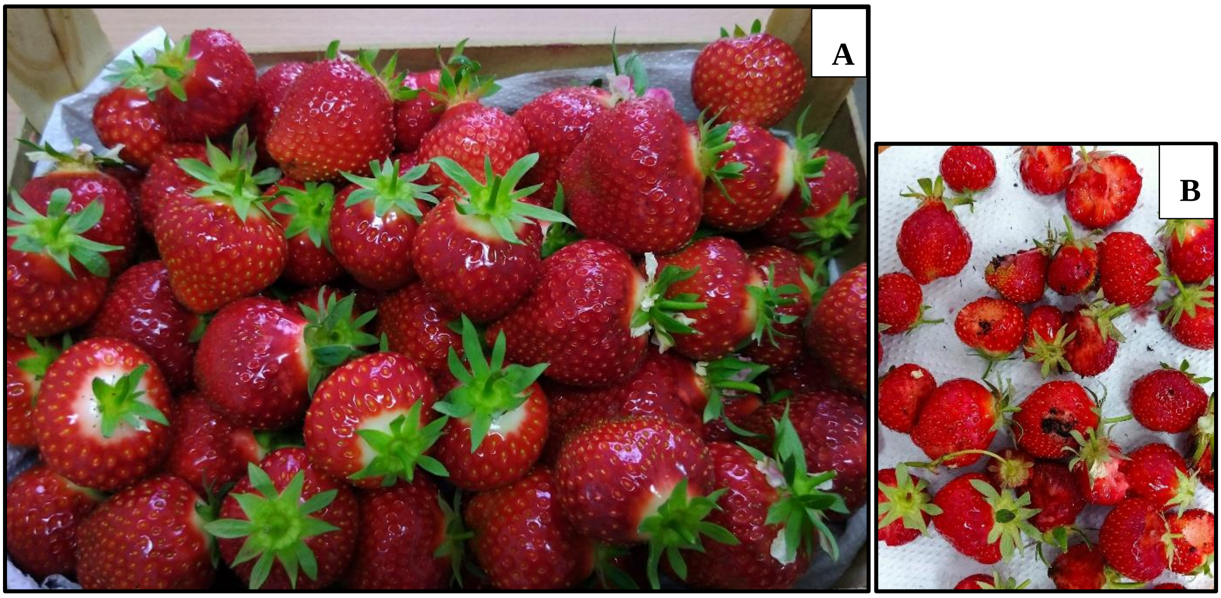

Photo 17. Marketable grade (A) and unmarketable grade (B) strawberry fruits (Photo: R.Prasad)

3.6. Physiological parameters

During experiment 2 and 3, selected physiological measurements were recorded, to study the influence of prepared substrate combinations on photosynthetic performances of plants and also to study the abiotic stress responses in plants influenced by substrate characteristics, i.e. pH, EC and nutrient content.

3.6.1. Selected Performance Indices (PIs)

Chlorophyll *a* fluorescence is a rapid, non-destructive technique that is used successfully in the evaluation of plant photosynthetic activity. This measurement will help to understand the influence of any stress induced by the growing medium on the performance of strawberries. Chl *a* fluorescence transients were measured using a handheld PAM fluorometer FluorPen FP 110, Photon Systems Instruments, Ltd. Drásov, Czech Republic (Tab. 5). The measurements were taken after leaf samples were dark-adapted for a minimum of 30 minutes (using leaf clip). The recorded data was later exported using FluorPen software and then subsequent OJIP (rapid fluorescence transient) analysis was done. The handheld device used for PIs measurement is given in Photo 18a.

Table 5. Evaluated Performance Indices (PIs)

Sl. No.	Parameter	Explanation	Formula	Authors
1	F_0	minimal fluorescence intensity (at 50 μs),	–	Strasser et al. 2000, Kalaji et al. 2017
2	F_m	maximum fluorescence intensity	–	
3	F_v/F_0	the maximum quantum yield of PSII photochemistry	$= (F_m – F_o)/F_0$	
4	F_v/F_m	the maximum quantum efficiency of PSII photochemistry under dark adaptation	$= (F_m – F_o)/F_m$	

3.6.2. Spectral Vegetation Indices (VIs)

Spectral VIs were measured using the handheld device PolyPen RP 410, Photon Systems Instruments, Ltd. Drásov, Czech Republic (Tab. 6). The measurements were recorded during daylight in fully matured strawberry leaves. Leaves (hold in place with a mechanical leaf holder) were exposed to a light source (Xenon incandescent lamp with a spectral range of 380-1050 nm) with a UVIS sensor (380-970 nm). The recorded data was later downloaded and exported using integrated software from the provider. VIs measurements were recorded between 11 a.m. and 1 p.m. from leaves fully exposed to the sun. Five plants were measured from each replicate in an individual substrate combination (S1-S7). The handheld device used for VIs measurement is given in Photo 18b.

Table 6. Measured spectral Vegetation Indices (VIs)

Sl. No.	Parameter	Explanation	Formula	Authors
1	NDVI	Normalised Difference Vegetation Index	$= (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED})$	Rouse et al. (1974)
2	PRI	Photochemical Reflectance Index	$= (R_{531} - R_{570}) / (R_{531} + R_{570})$	Gamon et al. (1992)
3	MCARI	Modified Chlorophyll Absorption in Reflectance Index	$= [(R_{700} - R_{670}) - 0.2 \times (R_{700} - R_{550})] \times (R_{700}/R_{670})$	Daughtry et al. (2000)

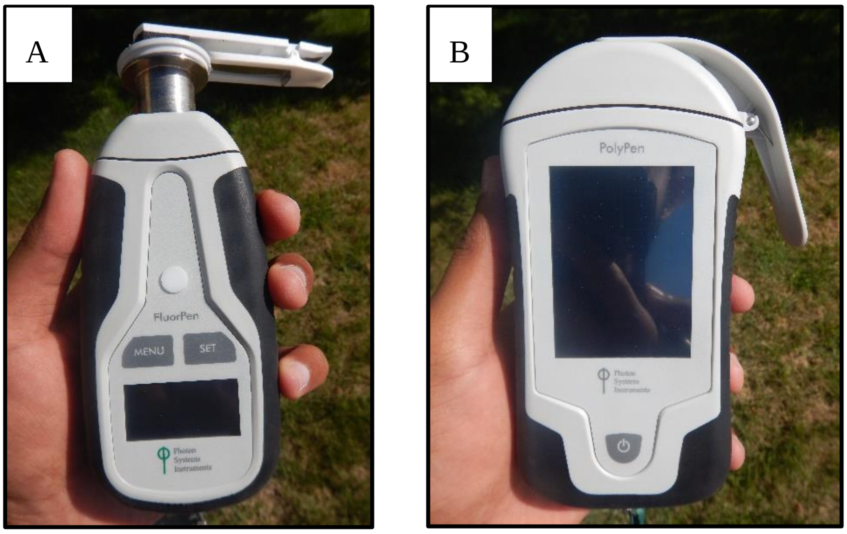

Photo 18. Handheld devices used to measure Performance Indices (A) and Vegetation Indices (B)

3.7. Statistical analysis of the experimental data

The experiments were laid in a Randomised Complete Block Design (RCBD) with seven substrates (S1-S7) in five replications facilitating 40 plants in individual substrates. The results of chemical characteristics concerning pH, EC and nutrient concentrations of A-SMS, L-SMS, P-SMS and peat in whole (100%) and the prepared substrate combinations were not subjected to statistical analysis rather compared to commercial peat values studied in the investigation. The data of all the morphological, pomological and physiological parameters were tabulated and subjected for statistical analysis. The represented morphological and yield parameters are means of five replicates, whereas, fruit quality parameters and measured selected performance indices and vegetation indices are the means of 15 plants. All recorded data during experiment 1 was evaluated by one-way analysis of variance (ANOVA). The data obtained from experiment 2 was subjected to two-way ANOVA to study the main and interaction between substrate combinations (S1-S7) and cv. 'Honeoye' and cv. 'Elsanta'. The experimental data obtained during experiment 3 was subjected to one way ANOVA. The mean differences were compared by post hoc test at a $P<0.05$ according to Tukey's HSD. Pearson's correlation analysis was performed between marketable yield and tested morphological parameters. Statistical analysis was performed using STATISTICA 10.0 (Stat-Soft, Tulsa, Oklahoma, USA). Correlograms were prepared using collerplot application in Origin 2020 (Origin Lab Corporation, USA).

4. Results

4.1 Selected chemical parameters of 100% SMS and 100% peat substrates (2018-2020)

4.1.1. The pH and EC values of 100% peat and 100% fresh SMS's

The pH and EC values differed among 100% peat and 100% fresh SMS's used in the study (Tab. 7). The average pH values among the fresh SMS's varied from 4.53 to 8.15, whereas the EC was from 2.17 to 7.34 mS·cm^{-1}.

During the study, the pH values of peat, A-SMS, L-SMS and P-SMS was in the range 6.30-6.45, 8.15-7.93, 4.09-4.84 and 4.63-5.96, respectively while the EC was 0.51-0.63, 7.10-7.53, 1.67-2.76 and 1.25-2.69 mS·cm^{-1}, respectively. The highest average pH values was recorded in 100% A-SMS (8.15) when compared to peat (6.30). Similarly, the average highest EC values was also recorded in 100% A-SMS (7.34 mS·cm^{-1}) in comparison with peat (0.56 mS·cm^{-1}).

Overall, the results of chemical analysis concerning pH and EC revealed that both the pH and EC values of 100% fresh A-SMS (*Agaricus bisporus*), L-SMS (*Lentinus edodes*) and P-SMS (*Pleurotus ostreatus*) were at the levels that limit their immediate use as a substrate in strawberry soilless cultivation.

Table 7. The pH and EC values of 100% peat and 100% fresh SMS's used in the study (2018-2020)

Substrate	Average pH	Range		Average EC (mS·cm^{-1})	Range	
		Min.	Max.		Min.	Max.
Peat (100%)	6.30	6.30	6.45	0.56	0.51	0.63
A-SMS (100%)	8.15	7.93	8.46	7.34	7.10	7.53
L-SMS (100%)	4.53	4.09	4.84	2.18	1.67	2.76
P-SMS (100%)	5.19	4.62	5.96	2.17	1.25	2.69

Abbreviation: A-SMS (*Agaricus bisporus*- spent mushroom substrate), L-SMS (*Lentinus edodes*- spent mushroom substrate), P-SMS (*Pleurotus ostreatus*- spent mushroom substrate)

4.1.2. The macro- and micronutrient concentrations of 100% peat and 100% fresh SMS's

The concentration of nutrients varied among100% peat and 100% fresh SMS's obtained for the study (Tab. 8). Most of the macro- and microelement concentrations were observed to be higher in 100% A-SMS when compared to commercial peat, L-SMS and P-SMS. The A-SMS (100%) had the highest concentrations of $N\text{-}NH_4$, $N\text{-}NO_3$, K, Ca, Mg, Na and Zn (399.0, 129.7, 3901.2, 3838.4, 587.6, 542.0 and 16.7 mg·dm^{-3}, respectively). The obtained values of SMS's concerning nutrient concentrations were higher and were not comparable to the values of commercial peat.

Table 8. The average nutrient concentrations of 100% peat and 100% fresh SMS's obtained for the study (2018-2020)

Nutrients (mg·dm^{-3})	**Peat (100%)**	**A-SMS (100%)**	**L-SMS (100%)**	**P-SMS (100%)**
Ammonia-Nitrogen ($N-NH_4$)	21.2	399.0	217.0	25.7
Nitrate-Nitrogen ($N-NO_3$)	32.7	129.7	3.7	2.3
Phosphorus (P)	68.7	ND*	ND	ND
Potassium (K)	118.7	3901.2	532.9	1322.8
Calcium (Ca)	1497.1	3838.4	1015.4	384.0
Magnesium (Mg)	152.0	587.6	459.4	117.0
Sulphur ($S-SO_4$)	218.3	ND	ND	ND
Sodium (Na)	19.8	542.0	52.7	51.1
Chlorine (Cl)	13.7	ND	ND	ND
Iron (Fe)	35.1	48.0	49.7	10.5
Manganese (Mn)	4.0	22.2	69.8	8.5
Zinc (Zn)	1.2	16.7	15.3	4.3
Copper (Cu)	0.8	1.7	1.4	0.4

*ND- value not detected

4.2 Selected chemical parameters of studied substrate combinations (2018-2020)

4.2.1 The pH and EC values of different substrate combinations (S1-S7)

The substrate combinations were prepared for experiment 1, 2 and 3 as previously described in Tab. 2. The pH values varied among the prepared substrate combinations before and after the experiments (Tab. 9a). In experiment 1, 2 and 3 the initial value of pH in substrate combinations ranged from 5.28-6.52, 5.49-7.75 and 5.96-6.68, respectively when compared to peat values of 6.46, 6.37 and 6.38. Whereas the pH values after experiments 1, 2 and 3 were in the range of 6.10-6.91, 6.15-6.74 and 6.02-6.70, respectively compared to the peat values of 6.33, 6.57 and 6.7.

The chemical analysis results concerning initial pH revealed that after mixing SMS's with peat in various combinations the pH value was almost neutralised and nearly comparable to the commercial peat values. The pH values after the experiment in studied substrate combinations revealed that acidic and alkaline pH of some substrate combinations were nearly neutralised.

Table 9a. The pH values of substrate combinations before and after strawberry cultivation

Substrate combinations	pH					
	Experiment 1 (2018)		Experiment 2 (2019)		Experiment 3 (2020)	
	before	After	before	after	Before	After
S1	6.46	6.33	6.37	6.57	6.38	6.71
S2	6.35	6.10	7.18	6.34	6.58	6.62
S3	6.34	6.63	7.75	6.29	6.68	6.70
S4	5.54	6.76	5.62	6.17	6.07	6.05
S5	5.28	6.80	5.49	6.15	5.96	6.02
S6	6.32	6.80	7.57	6.74	6.24	6.50
S7	6.52	6.91	7.58	6.63	6.10	6.34

The EC values among substrate combinations varied before and after the experiments (Tab. 9b). In the experiment 1, 2 and 3 the initial EC value of substrate combinations ranged from 0.64-2.39, 0.08-1.26, 0.56-2.58, respectively when compared to peat values of 0.63, 0.51 and 0.55. Whereas, the EC values of substrates after the experiments were in the range of 0.88-2.92, 1.14-4.05 and 1.29-4.09 when compared to peat values of 2.60, 2.03 and 2.54.

Generally, during the study, the highest EC values both before and after the experiments were noticed in S2 and S3 substrates, which were mixtures of A-SMS and peat in varying substitution rates.

Table 9b. The EC values of substrate combinations before and after strawberry cultivation

Substrate combinations	EC ($mS \cdot cm^{-1}$)					
	Experiment 1 (2018)		Experiment 2 (2019)		Experiment 3 (2020)	
	before	After	before	after	before	After
S1	0.63	2.60	0.51	2.03	0.55	2.54
S2	2.05	2.28	1.15	3.68	2.18	3.70
S3	2.39	2.92	1.26	4.05	2.58	4.09
S4	0.64	1.26	0.12	1.65	0.79	1.53
S5	0.78	1.45	0.10	2.28	0.80	1.94
S6	0.86	1.07	0.11	1.14	0.56	1.51
S7	1.52	0.88	0.08	1.35	0.60	1.29

4.2.2. The macro- and micronutrient concentrations in substrate combinations (S1-S7)

4.2.2.1. The nutrient concentrations in substrate combinations during experiment 1 (2018)

The substrate combinations (S1-S7) exhibited varying concentrations of macro- and micronutrients during experiment 1 (Table 10a). Among the studied substrate combinations

S1-S7, the higher N-NH_4 P, K, Ca, Mg, S-SO_4 and Na were observed in S3 (173.5, 152.0, 798.0, 2299.0, 269.0, 1313.0 and 86.0 mg·dm^{-3}). While N-NO_3 was higher in S1 (66.5 mg·dm^{-3}), Cl was higher in S6 (28.0 mg·dm^{-3}), Fe was higher in S2 (66.9 mg·dm^{-3}), Mn and Zn were higher in S5 (42.0 and 7.3 mg·dm^{-3}).

Table 10a. Nutrient concentrations of substrate combinations at the beginning of experiment 1 (2018)

Nutrients (mg·dm^{-3})	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
N-NH_4	52.5	143.5	173.5	21.0	35.0	28.0	14.0
N-NO_3	66.5	28.0	21.0	ND*	ND	ND	ND
P	66.0	122.0	152.0	95.0	ND	80.0	ND
K	109.0	508.0	798.0	183.0	249.0	374.0	929.0
Ca	1851.0	1909.0	2299.0	1219.0	1322.0	1622.0	982.0
Mg	146.0	229.0	269.0	195.0	243.0	154.0	128.0
S-SO_4	211.0	813.0	1313.0	286.0	ND	266.0	ND
Na	24.0	66.0	86.0	38.0	42.0	37.0	40.0
Cl	19.0	11.0	14.0	22.0	ND	28.0	ND
Fe	57.9	66.9	46.0	64.2	36.9	44.8	37.4
Mn	4.5	8.8	27.9	11.9	42.0	5.5	7.8
Zn	1.1	4.9	4.5	7.1	7.3	2.3	2.9
Cu	1.4	1.6	1.4	1.6	1.0	1.1	0.9

*ND- value not detected

4.2.2.2. The nutrient concentrations in substrate combinations during experiment 2 (2019)

The macro- and micronutrients concentrations among the substrate combinations (S1-S7) differed during experiment 2 (Table 10b). In comparison with peat (S1), the higher N-NH_4 P, K, Ca, Mg, Na, Cl, Mn, Zn and Cu were observed in S3 (276.0, 843.0, 1216.0, 2123.0, 610.0, 198.0, 38.0, 55.3, 27.9 and 11.8 mg·dm^{-3}). While the N-NO_3 content was higher in S2 (77.0 mg·dm^{-3}), S-SO_4 was higher in S2 (407.0 mg·dm^{-3}) and Fe was higher in S7 (11.02 mg·dm^{-3}).

Table 10b. Nutrient concentrations of substrate combinations at the beginning of experiment 2 (2019)

Nutrients (mg·dm^{-3})	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
N-NH_4	4.0	189.0	276.0	7.0	14.0	7.0	7.0
N-NO_3	28.0	77.0	63.0	21.0	14.0	7.0	7.0
P	49.0	334.0	843.0	28.0	23.0	126.0	77.0
K	94.0	1144.0	1216.0	120.0	160.0	67.0	114.0
Ca	1209.0	1606.0	2123.0	181.0	278.0	1765.0	955.0

Mg	119.0	529.0	610.0	19.0	42.0	164.0	80.0
$S-SO_4$	145.0	407.0	317.0	1.0	5.0	3.0	3.0
Na	21.0	135.0	198.0	24.0	20.0	14.0	17.0
Cl	10.0	25.0	38.0	27.0	9.0	5.0	11.0
Fe	13.0	101.4	74.6	90.8	47.0	102.6	110.2
Mn	1.7	45.6	55.3	24.9	33.3	4.3	5.9
Zn	0.8	24.6	27.9	5.5	5.8	10.3	6.9
Cu	0.3	4.9	11.8	0.8	0.8	1.0	1.0

4.2.2.3. The nutrient concentrations in substrate combinations during experiment 3 (2020)

The substrate combinations (S1-S7) exhibited varying concentrations of macro- and micronutrients during experiment 3 (Table 10c). In comparison with peat (S1), the higher $N-NH_4$ P, K, Ca, Mg, $S-SO_4$ and Na were observed in S3 (219.0, 234.0, 866.0, 3476.0, 387.0, 1037.0 and 85.6 $mg \cdot dm^{-3}$). While $N-NO_3$ was higher in S2 (74.9 $mg \cdot dm^{-3}$), Cl was higher in S6 (28.0 $mg \cdot dm^{-3}$), Fe was higher in S7 (36.9 $mg \cdot dm^{-3}$), Mn, Zn and Cu were higher in S5 (31.1, 4.6 and 1.2 $mg \cdot dm^{-3}$).

Table 10c. Nutrient concentrations of substrate combinations at the beginning of experiment 3 (2020)

Nutrients ($mg \cdot dm^{-3}$)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
$N-NH_4$	7.0	173.0	219.0	49.0	7.0	ND	4.0
$N-NO_3$	4.0	74.9	69.4	ND*	ND	ND	ND
P	91.0	229.0	234.0	ND	ND	93.0	120.0
K	153.0	672.0	866.0	239.0	268.0	238.0	339.0
Ca	1431.0	3077.0	3476.0	1145.0	1495.0	1271.0	913.0
Mg	191.0	381.0	387.0	268.0	263.0	149.0	200.0
$S-SO_4$	299.0	761.0	1037.0	ND	ND	359.0	297.0
Na	14.5	60.6	85.6	50.5	23.9	13.8	18.5
Fe	34.5	33.3	30.1	35.9	44.7	31.4	36.9
Mn	5.6	10.4	11.0	16.5	31.1	5.2	5.1
Zn	1.7	4.2	4.3	2.8	4.6	1.2	1.5
Cu	0.8	0.6	0.5	0.6	1.2	0.6	0.7

*ND- value not detected

Overall, the results of the substrate analysis (Tab. 10a, b and c) demonstrated that during experiment 1, 2 and 3 the macro- and micronutrient concentrations in A-SMS based substrates, i.e. S2 and S3 were comparatively greater than in other substrate combinations (S4-S7), as well as commercial peat values (S1).

4.2.3 Suitability of SMS's and peat substrate combinations as soilless growing media

The values of pH, EC and nutrient concentrations of all substrate combinations (S2-S7) prepared for strawberry cultivation were nearly comparable to commercial peat values tested in the study (S1). During experiment 1, A-SMS was substituted to peat in 10 and 20% whereas, L-SMS and P-SMS were added in 25 and 50%, respectively. While, during the experiment 2 and 3, each SMS (A-SMS, L-SMS and P-SMS) was substituted to peat in 15 and 25%, respectively. The results concerning pH, EC and nutrient concentrations demonstrated that the prepared substrate combinations (S2-S7) can be considered as a suitable substitute for peat-reduced growing media. Considering that, SMS's can be a potential and sustainable substitute to commercial peat in varying supplementation rates. In particular, for soilless strawberry production.

Experiment 1 (2018)

4.3. Influence of substrate combinations on morphological parameters

4.3.1. Plant height and number of leaves

In the present study, the substrate combinations had a significant influence on the strawberry plant height and the number of leaves (Tab. 11) at 45 and 60 days after planting (DAP). The plants grown in S1 (commercial peat) achieved the highest plant height (24.2 cm) while, the lowest plant height was recorded in S5, S4, S6 and S7 (12.0, 12.4, 14.0 and 14.2 cm), respectively at 45 DAP. Whereas, at 60 DAP the highest plant height was recorded in S1 (26.6 cm), S2 (27.8 cm) and S3 (27.6 cm). The plants grown in S4 and S5 achieved the lowest plant height (16.2 and 16.4 cm) at 60 DAP.

The highest number of leaves at 45 and 60 DAP was recorded in S2 (7.8 and 10.0) and S3 (7.6 and 9.8), respectively. Whereas, the lowest number of leaves was noticed in S4 (5.6 and 7.6) at 45 and 60 DAP, respectively.

Table 11. The plant height and the number of leaves of strawberry cv. 'Honeoye' at 45 and 60 DAP (mean ± SD)

Substrate combination		**Plant height (cm)**		**Number of leaves (no)**	
		45 DAP	**60 DAP**	**45 DAP**	**60 DAP**
S1	Peat-100% (control)	24.2 ± 3.77a*	26.6 ± 3.44a	7.4 ± 0.55ab	9.4 ± 1.14ab
S2	A-SMS: Peat (10:90%)	21.0 ± 1.58ab	27.8 ± 1.10a	7.8 ± 0.84a	10.0 ± 1.58a
S3	A-SMS: Peat (20:80%)	19.8 ± 0.84b	27.6 ± 1.14a	7.6 ± 0.54a	9.8 ± 0.84a
S4	L-SMS: Peat (25:75%)	12.4 ± 0.89c	16.2 ± 1.30c	5.6 ± 0.55c	7.6 ± 0.55b
S5	L-SMS: Peat (50:50%)	12.0 ± 0.71c	16.4 ± 0.89c	6.2 ± 0.45bc	8.0 ± 1.00ab
S6	P-SMS: Peat (25:75%)	14.0 ± 0.70c	20.6 ± 2.51b	6.6 ± 0.55bc	8.8 ± 1.10ab
S7	P-SMS: Peat (50:50%)	14.2 ± 0.84c	20.4 ± 1.14b	6.0 ± 0.84c	8.2 ± 0.84ab

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=15)
Abbreviations: A-SMS (*Agaricus bisporus*- spent mushroom substrate), L-SMS (*Lentinus edodes*- spent mushroom substrate), P-SMS (*Pleurotus ostreatus*- spent mushroom substrate)

4.3.2. Total plant fresh weight

The substrate combinations significantly influenced the total plant fresh weight of strawberry plants (Fig. 3). The highest total plant fresh weight was recorded in S1 (68.77 g), S2 (66.63 g), S3 (70.46 g) and S6 (66.41 g) whereas, the lowest value was recorded in S5 (23.83 g) and S4 (34.24 g).

The value for total plant fresh weight was observed to slightly increasing in S3 (20% A-SMS) when compared to S2 (10% A-SMS). Whereas, in S4 (25% L-SMS), S5 (50% L-SMS), S6 (25% P-SMS) and S7 (50% P-SMS) as the concentration of added SMS increased from 25 to 50% the value for total plant fresh weight decreased.

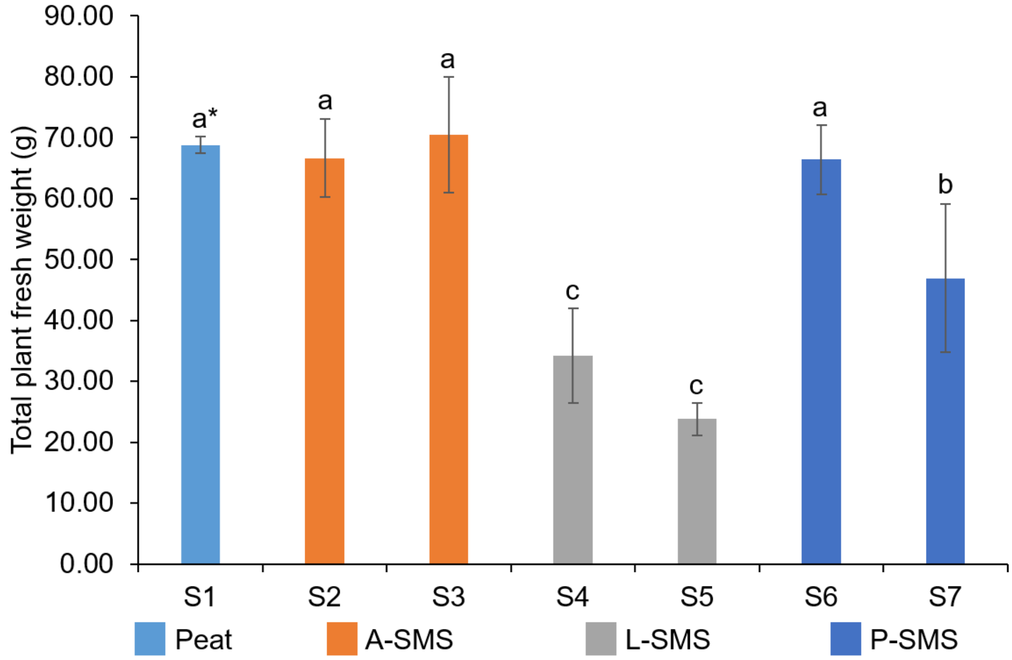

Figure 3. Total plant fresh weight (g) of strawberry cv. 'Honeoye' grown in different substrate combinations (mean ± SD). *means in each column followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=5).

4.3.3. Crown diameter and leaf area

The substrate combinations significantly influenced the crown diameter and leaf area (Tab. 12). The plants cultivated in S1 achieved the highest crown diameter (27.86 mm) followed by S6 and S3 (25.64 and 24.86 mm) and the lowest crown diameter was recorded in S5 (13.58 mm). The highest leaf area was observed in S1 (194.10 cm^2) and S2 (166.11 cm^2) followed by S3 (131.02 cm^2) and the lowest value was recorded in S5 (29.80 cm^2).

Table 12. Crown diameter and leaf area of strawberry cv. 'Honeoye' in different substrate combinations (mean ± SD)

Substrate combinations	**Crown diameter (mm)**	**Leaf area (cm^2)**
S1	27.86 ± 1.05a*	194.10 ± 26.53a
S2	21.24 ± 3.06b	166.11 ± 42.60a
S3	24.86 ± 1.08ab	131.02 ± 23.27b
S4	19.14 ± 4.57b	77.34 ± 13.45c
S5	13.58 ± 1.57c	29.80 ± 4.99d
S6	25.64 ± 1.78ab	68.38 ± 14.39c
S7	20.91 ± 2.47b	57.47 ± 14.06c

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=5).

4.3.4. Shoot, root and total plant dry weight

The substrates significantly influenced strawberry plant dry matter (Tab. 13). The highest shoot dry weight and total plant weight were recorded in S1 (19.70 and 24.91 g) followed by S2 (15.92 and 21.28 g) and S3 (15.54 and 21.29 g). Whereas, the lowest values were recorded in S5 (3.47 and 8.40 g).

The plants grown in S6 recorded the highest root dry weight (6.24 g) followed by other substrate combinations except for S4 which had the lowest value (3.80 g).

The highest shoot to root ratio was recorded in S1 (3.80) followed by S2, S3 and S6 (2.97, 2.77 and 2.02) while the lowest value was observed in S5 (0.72).

Table 13. Dry matter distribution of strawberry cv. 'Honeoye' cultivated in different substrate combinations (mean ± SD)

Substrate combinations	Shoot dry weight (g)	Root dry weight (g)	Total plant dry weight (g)	Shoot: root
S1	19.70 ± 2.05a*	5.21 ± 0.34ab	24.91 ± 2.07a	3.80 ± 0.51a
S2	15.92 ± 2.71b	5.37 ± 0.92ab	21.28 ± 3.63ab	2.97 ± 0.10b
S3	15.54 ± 1.17b	5.75 ± 0.87ab	21.29 ± 0.90ab	2.77 ± 0.60b
S4	7.01 ± 0.99d	3.80 ± 0.96b	10.81 ± 1.72c	1.92 ± 0.43c
S5	3.47 ± 0.49 e	4.92 ± 0.73ab	8.40 ± 0.91c	0.72 ± 0.14d
S6	12.03 ± 0.91c	6.24 ± 1.42a	18.26 ± 1.64b	2.02 ± 0.53b
S7	7.33 ± 1.71d	5.70 ± 1.64ab	13.03 ± 3.27c	1.31 ± 0.19c

*means followed by the same letters are not significantly different at P<0.05 according to Tukey's HSD (n=5).

Overall, the strawberry plant morphological performances determined by shoot dry weight, total plant dry weight, crown diameter and leaf area was observed to be increased in S2 and S3 (10 and 20% of added A-SMS). Whereas, in S4 (25% L-SMS), S5 (50% L-SMS), S6 (25% P-SMS) and S7 (50% P-SMS), as the concentration of added SMS's increased from 25 to 50% these morphological performances were noticed to be in a decreased.

4.4. Influence of substrate combinations fruit quality parameters

4.4.1. Fruit diameter, individual fruit weight and total soluble solids (TSS)

The fruit quality parameters of strawberry cv. 'Honeoye' varied among the studied substrate combinations (Tab. 14). The fruits produced on S2 had the highest fruit diameter of 42.21 mm followed by S3 (40.79 mm) and S6 (38.35mm) and the lowest fruit diameter was observed in S5 (17.83 mm).

The fruits obtained in S1 and S2 had the highest individual fruit weight (24.41 g and 24.06 g) whereas, S5 had the lowest value (12.86 g).

The fruits produced on S5 showed the highest value for TSS (13.50°Brix) followed by all other substrate combinations except for S7 (8.34°Brix) and S4 (8.30°Brix) where the lowest TSS values were recorded.

Table 14. Fruit quality parameters of strawberry cv. 'Honeoye' in different substrate combination (mean ± SD)

Substrate combinations	Fruit diameter (mm)	Individual fruit weight (g)	TSS (°Brix)
S1	38.25 ± 1.93b	24.41 ± 2.50a	9.56 ± 0.90bc
S2	42.21 ± 0.91a	24.06 ± 1.25a	9.68 ± 0.94bc
S3	40.79 ± 1.55ab	21.98 ± 1.44ab	10.48 ± 0.82bc
S4	34.04 ± 1.62c	15.22 ± 0.92c	8.30 ± 0.55c
S5	17.83 ± 0.91e	12.86 ± 0.16d	13.50 ± 0.74a
S6	38.35 ± 3.82ab	20.44 ± 2.09b	9.26 ± 0.82bc
S7	29.67 ± 1.11d	14.97 ± 1.01c	8.34 ± 0.79c

*means followed by the same letters are not significantly different at $P<0.05$ according to Tukey's HSD (n=15).

4.5. Influence of substrate combinations yield performances

4.5.1. Total yield, marketable and unmarketable yield

The results of the study revealed that the studied substrate combinations significantly influenced total, marketable and unmarketable yield per plant (Fig. 4 and Tab. 15). The highest total and marketable yield per plant were recorded in S1 (270.53 g and 257.74 g) and the lowest values were observed in S5 (58.72 g and 34.11 g). The highest unmarketable yield was recorded in S6 (29.97 g) and the lowest unmarketable yield was observed in S1 (12.80 g).

Overall, the total and marketable yields were observed to be increasing in S3 (20% A-SMS) compared to S2 (10% A-SMS). Whereas, in S4 (25% L-SMS), S5 (50% L-SMS), S6 (25% P-SMS) and S7 (50% P-SMS) as the concentration of added SMS's increased from 25 to 50% the total and marketable yield per plant decreased.

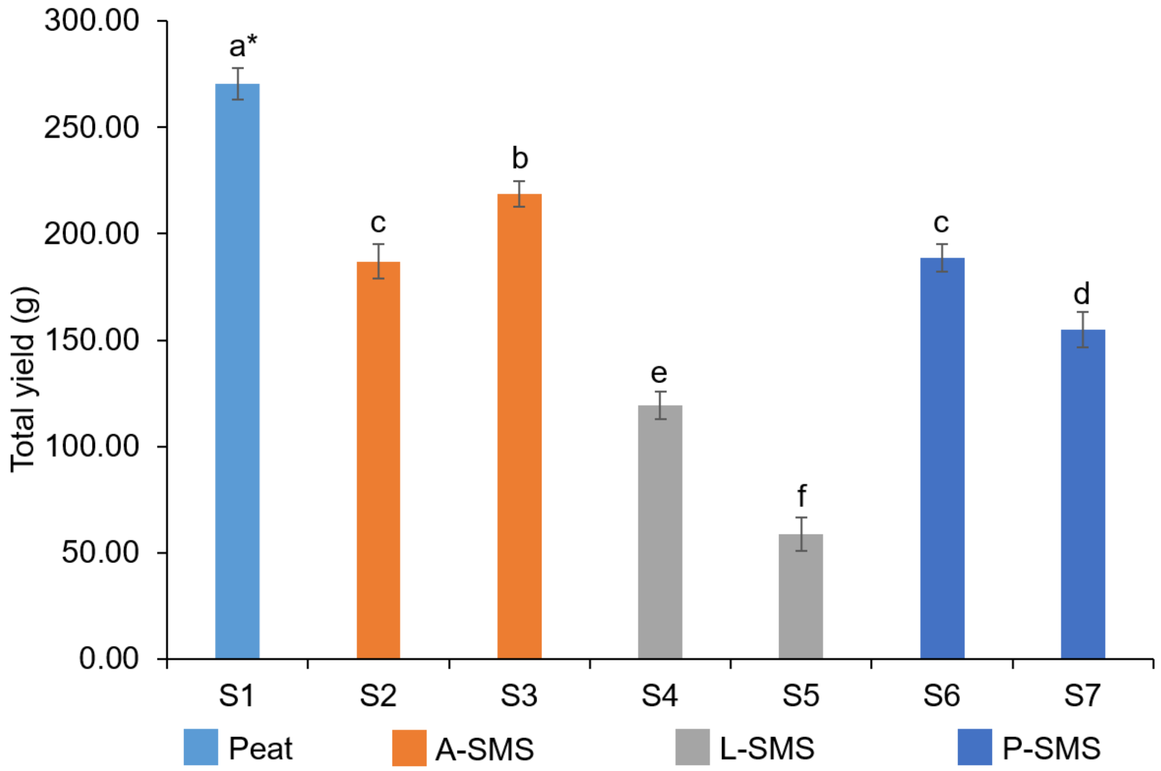

Figure 4. Total yield per plant (g) of strawberry cv. 'Honeoye' grown in different substrate combinations (mean ± SD)

*means followed by the same letter are not significantly different at P < 0.05 according to Tukey's HSD (n=5).

Table 15. Marketable and unmarketable yield per plant of strawberry cv. 'Honeoye' grown in different substrate combinations (mean ± SD)

Substrate combinations	**Marketable yield (g)**	**Unmarketable yield (g)**
S1	257.74 ± 9.73a*	12.80 ± 4.02c
S2	164.82 ± 5.97c	22.19 ± 3.92b
S3	194.19 ± 5.06b	24.52 ± 4.58ab
S4	94.79 ± 9.19e	24.57 ± 4.97ab
S5	34.11 ± 3.75f	24.61 ± 4.79ab
S6	158.67 ± 3.69c	29.97 ± 5.06a
S7	126.58 ± 5.93d	28.31 ± 4.90ab

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=5).

The obtained marketable and unmarketable yields in grams were converted into percentages to present the yield trends among substrate combinations (Fig. 5). The 95.27% of fruits obtained from S1 were considered as marketable yield when compared to 88.14%, 88.79%, 84.11% and 81.72% in S2, S3, S6 and S7, respectively. While the highest percentage of unmarketable yield was recorded in S5 (41.91%).

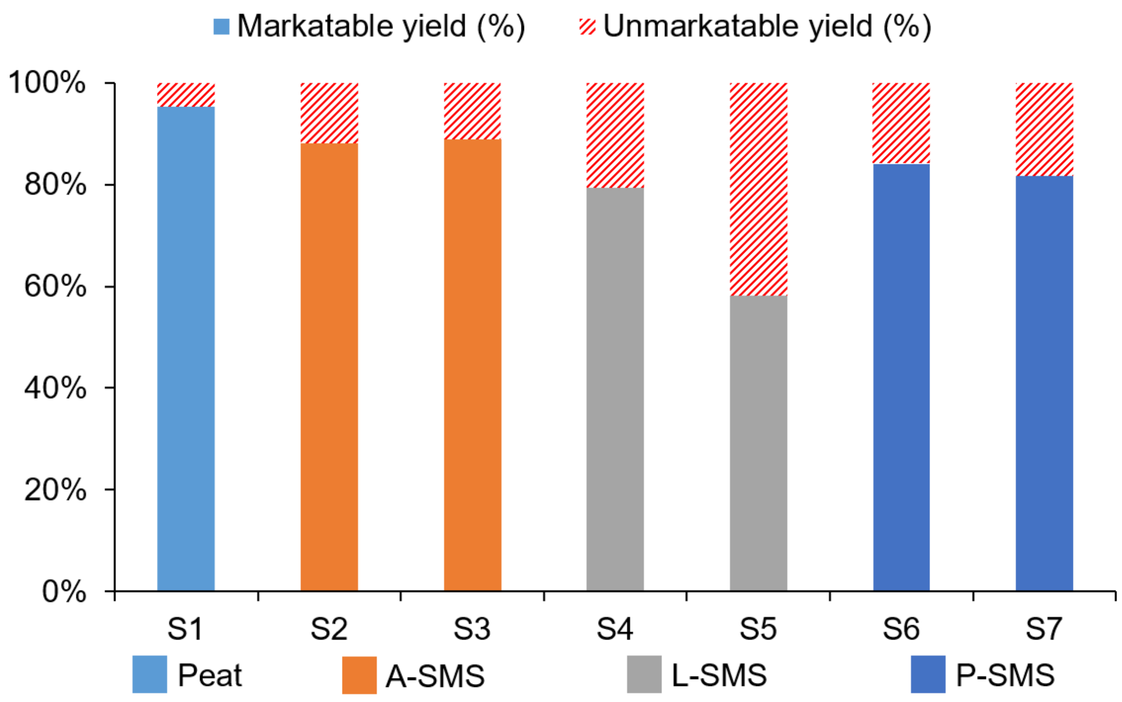

Figure 5. Per cent marketable and unmarketable yields among the studied substrate combinations

4.6. Correlation analysis among marketable yield and studied morphological parameters

Pearson's correlation analysis suggested positive correlations among the studied yield and morphological parameters (Fig. 6). According to the correlation coefficient values observed among the studied parameters, it can be inferred that the greater values obtained for plant height (PHt), number of leaves (NOL), crown diameter (CD), leaf area (LA), shoot dry weight (SDW) and total plant dry weight (TPDW) resulted in higher marketable yield (MY).

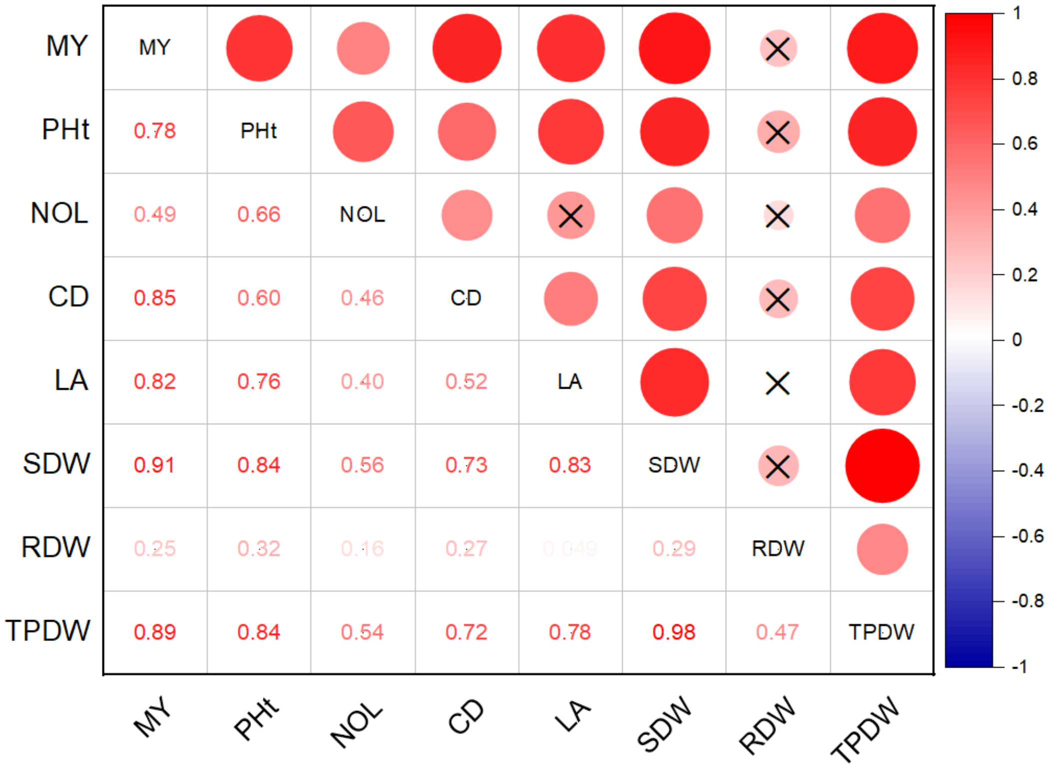

Figure 6. Correlation matrix among studied yield and morphological parameters.

Positive correlations are displayed in red and negative correlations are in blue. The size and colour intensity of circles are proportional to Pearson's correlation coefficient at P<0.01, circles marked with × indicates correlation values that are not significant at P<0.01. Numbers range from -1 to +1 are correlation coefficients for variables on the vertical and horizontal axis.

Experiment 2 (2019)

4.7. Influence of substrate combinations and cultivars on strawberry morphological parameters

4.7.1. Shoot dry weight, root dry weight and total plant dry weight

In the present study, the dry matter of strawberry plants determined by shoot, root and total plant dry weight were influenced significantly by substrate combinations and also differed between cultivars (Tab. 16). The plants cultivated in S3 achieved the highest shoot dry weight (14.98 g) followed by S4 and S5 (14.65 and 14.55 g) and the lowest value was recorded in S6 and S7 (11.52 and 11.67 g). The cv. 'Elsanta' had a higher shoot dry weight (15.99 g) in comparison to cv. 'Honeoye' (10.73 g).

The shoot dry weight among the studied substrate combinations and cultivars ranged from 6.49-18.09 g. The highest shoot dry weight was obtained for cv. 'Elsanta' in S2, S3, S4 and S7 (16.63, 18.09, 17.67 and 16.86). The lowest value concerning shoot dry weight was noticed for cv. 'Honeoye' in S7 (6.49 g).

The mean root dry weight was not significantly different among the studied substrates (6.90-9.47 g). Whereas, the cv. 'Elsanta' had a higher root dry weight (9.23 g) which was significantly superior to that of cv. 'Honeoye' (6.32 g). Among the substrate combinations and cultivars studied, cv. 'Elsanta' had the highest root dry weight in S6 (11.49 g) and S7 (11.37 g). The lowest value was noticed for cv. 'Honeoye' in S7 (3.96 g).

The total plant dry weight differed among the studied substrate combinations and both studied cultivars (Tab. 16). The highest total plant dry weight was recorded in S3 (35.65 g) followed by S5 (32.87 g), S4 (31.02 g) and S2 (30.31 g). The lowest total plant dry weight was in S7 (24.57 g). The performance of cv. 'Elsanta' concerning total plant dry weight was significantly superior (42.27 g) when compared to cv. 'Honeoye' (17.05 g).

The interaction among substrate combinations and cultivars concerning the total plant dry weight revealed that, the total plant dry weight of strawberry cv. 'Elsanta' among studied substrate combinations was not significantly different. While the lowest value was observed in cv. 'Honeoye' in S7 (10.45 g).

Table 16. Shoot, root and total plant dry weight of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate Combinations		**Shoot dry weight (g)**			**Root dry weight (g)**			**Total plant dry weight (g)**		
		Cultivar		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**
		'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'	
S1	Peat-100% (control)	9.73cd*	14.11bc	11.92BC*	4.19b	9.79b	6.99A	13.91bc*	37.82a	25.87BC
S2	A-SMS:Peat (15:85)	11.84c	16.63a	14.24BC	6.44b	7.44b	6.94A	18.28bc	42.35a	30.31AB
S3	A-SMS:Peat (25:75)	11.86c	18.09a	14.98A	10.51b	8.40b	9.47A	22.41b	48.89a	35.65A
S4	L-SMS:Peat (15:85)	11.63c	17.67a	14.65AB	5.51b	10.10b	7.80A	17.14bc	44.91a	31.02AB
S5	L-SMS:Peat (25:75)	15.04ab	14.05bc	14.55AB	7.79b	6.02b	6.90A	22.83b	42.90a	32.87AB
S6	P-SMS:Peat (15:85)	8.51cd	14.52ab	11.52C	5.82b	11.49a	8.65A	14.34bc	40.35a	27.34BC
S7	P-SMS:Peat (25:75)	6.49d	16.86a	11.67C	3.96c	11.37a	7.67A	10.45c	38.68a	24.57C
Mean (Cultivar)		10.73B	15.99A		6.32B	9.23A		17.05B	42.27A	

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=5), * small letters indicate the significant difference of interaction at $P<0.05$ and capital letters indicate the significant difference of each factor at $P<0.05$

Abbreviation: A-SMS (*Agaricus bisporus*), L-SMS (*Lentinus edodes*), P-SMS (*Pleurotus ostreatus*)

4.7.2. Shoot to root ratio and leaf area

There were no significant differences concerning shoot to root ratio among the studied substrate combinations as well as cultivars (Tab. 17a). The shoot to root ratio for substrates ranged from 1.57-2.14 and among the cultivars from 1.91-1.94.

Table 17a. Shoot to root ratio of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combinations	**Shoot: root**		
	Cultivar		**Mean (Substrate)**
	'Honeoye'	'Elsanta'	
S1	2.36a*	1.60a	1.98A*
S2	1.90a	2.38a	2.14A
S3	1.35a	2.20a	1.77A
S4	2.32a	1.80a	2.06A
S5	2.10a	2.37a	2.23A
S6	1.71a	1.43a	1.57A
S7	1.86a	1.58a	1.72A
Mean (Cultivar)	1.94A	1.91A	

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=5).

The leaf area varied among the studied substrate combinations as well as between two cultivars (Tab. 17b). The highest leaf area was recorded in S5 (219.44 cm^2) and the lowest value was observed in S7 (158.78 cm^2).

The cv. 'Elsanta' was found to be superior concerning leaf area (221.99 cm^2) when compared to cv. 'Honeoye' (166.25 cm^2).

Among the substrate combinations and cultivars studied in the experiment, the highest leaf area was observed in S2, S3, S4 and S6 (225.38, 226.24, 224.57 and 226.32 cm^2) for cv. 'Elsanta' whereas, the lowest for cv. 'Honeoye' in S7 (101.59 cm^2).

Overall, in the present study the morphological performances of the cv. 'Elsanta' was observed to be superior to the cv. 'Honeoye'. Among the seven substrate combinations studied, the substrates S2-S6 performed better and/or equally with commercial peat (S1) except for S7.

Table 17b. Leaf area of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate Combinations	Leaf area (cm^2)		
	Cultivar		Mean (Substrate)
	'Honeoye'	'Elsanta'	
S1	169.52b*	217.84ab	193.68AB*
S2	160.01b	225.38a	192.70AB
S3	189.97ab	226.24a	208.10AB
S4	184.36ab	224.57a	204.46AB
S5	221.28ab	217.60ab	219.44A
S6	137.03c	226.32a	181.67B
S7	101.59d	215.97ab	158.78C
Mean (Cultivar)	166.25B	221.99A	

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=5).

4.8. Influence of substrate combinations and cultivars on fruit quality parameters

4.8.1. Fruit diameter, individual fruit weight and total soluble solids (TSS)

The fruit quality parameters, as determined by fruit diameter, individual fruit weight and TSS differed significantly among substrate combinations and cultivars (Tab. 18). The plants cultivated in S2 and S3 produced fruits with the highest fruit diameter (35.88 and 35.38 mm). Whereas, the fruits obtained from S7 had the lowest fruit diameter (26.01 mm). Plants from cv. 'Elsanta' had a greater fruit diameter (36.02 mm) when compared to cv. 'Honeoye' (31.12 mm).

Among the substrate combinations and cultivars, the fruits obtained in S2 had the greatest fruit diameter (33.79 mm) followed by S3 and S5 (37.63 and 35.84 mm) for cv. 'Elsanta'. The lowest fruit diameter was noticed in S7 for cv. 'Honeoye' (19.95 mm).

The individual fruit weight was observed to be the highest in S2 (21.24 g) followed by S3 and S6 (20.50 and 20.31g). The lowest individual fruit weight was in S7 (17.14 g).

The performance of cv. 'Elsanta' with respect to individual fruit weight was superior (22.03 g) when compared to the cv. 'Honeoye' (15.22 g).

The interaction among the substrate combinations and cultivars revealed that the individual fruit weight was the highest in S2 (24.87 g) for cv. ‘Elsanta’ whereas, the lowest value was reported in S7 for cv. ‘Honeoye’ (11.71 g).

The fruits obtained from S3 had the highest TSS value (11.30°Brix) whereas, the lowest TSS was observed in S5 (9.08°Brix). The fruits of cv. ‘Elsanta’ had greater TSS (10.41 °Brix) when compared to cv. ‘Honeoye’ (9.44 °Brix). Among the studied substrate combinations and cultivars, the greatest TSS was recorded in S3 (11.96 °Brix) for cv. ‘Elsanta’. The lowest TSS was noticed in S6 for cv. ‘Honeoye’.

Table 18. Fruit quality parameters of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combinations	**Fruit diameter (mm)**			**Individual fruit weight (g)**			**TSS (°Brix)**		
	Cultivar		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**
	'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'	
S1	32.04cd*	34.51bc	33.28B*	14.34c	18.56c	16.45C	9.53bc	9.67bc	9.60B
S2	33.79bc	37.97a	35.88A	17.60c	24.87a	21.24A	9.73bc	10.60ab	10.17AB
S3	33.13bc	37.63ab	35.38A	16.55c	24.44ab	20.50AB	10.63ab	11.96a	11.30A
S4	27.13d	33.83bc	30.48C	12.61c	20.01bc	16.31C	8.90bc	10.20ab	9.55B
S5	29.67d	35.84ab	32.75B	16.21c	20.65bc	18.43BC	8.73bc	9.43bc	9.08C
S6	29.07d	33.30bc	31.19C	17.51c	23.11ab	20.31AB	8.50c	10.07ab	9.28B
S7	19.95e	32.06cd	26.01D	11.71d	22.57ab	17.14C	10.03ab	10.93ab	10.48AB
Mean (Cultivar)	31.12B	36.02A		15.22B	22.03A		9.44B	10.41A	

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=15).

4.9. Influence of substrate combinations and cultivars on yield performances

4.9.1 Total yield, marketable and unmarketable yield

The substrate combinations and cultivars significantly influenced total, marketable and unmarketable yield (Tab. 19). The total and marketable yield was observed to be the highest in S3 (241.92 and 222.95 g) and S2 (230.37 and 212.36 g). Whereas, the lowest total and marketable yield were recorded in S7 166.47 and 142.99 g, respectively.

The performance of cv. 'Elsanta' with respect to the total and marketable yield was superior (267.09 and 243.76 g) when compared to the cv. 'Honeoye' (147.31 and 123.07 g).

The interaction between substrate combinations and cultivars revealed that the highest total yield was in S3 for cv. 'Elsanta' (295.39 g) while the lowest was in S7 for cv. 'Honeoye' (76.46 g). For marketable yield, the highest values were noticed in S3 and S2 for cv. 'Elsanta' (280.00 and 274.80 g) and the lowest was in S7 for cv. 'Honeoye' (60.98 g).

The unmarketable yield was observed to be the highest in S5 and S6 (33.13 and 32.30 g). Among the two cultivars, no significant difference was recorded for unmarketable yield (24.24 and 23.33 g). The interaction between the substrate combinations and cultivars showed that the highest unmarketable yield was in S6 for cv. 'Elsanta' (35.76 g) and in S5 for cv. 'Honeoye' (34.20 g). The lowest unmarketable yield was in S4 for cv. 'Elsanta' (12.28 g).

Overall, the yield performances among the substrate combinations studied in the experiment were observed to be superior in S2 (15% A-SMS) and S3 (25% A-SMS) followed by S5 (25% L-SMS) and S6 (15% P-SMS) when compared to S1 (commercial peat). Whereas, the S7 (25% P-SMS) showed the lowest yield performances among all the studied substrate combinations. The results also clearly demonstrated that among the two cultivars studied in the experiment, the cv. 'Elsanta' performed better when compared to the cv. 'Honeoye'.

Table 19. Yield performances of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combinations	Total yield (g)			Marketable yield (g)			Unmarketable yield (g)		
	Cultivar		Mean (Substrate)	Cultivar		Mean (Substrate)	Cultivar		Mean (Substrate)
	'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'	
S1	150.04e*	211.45c	180.75C*	129.77fg	190.33d	160.05C	20.27cd	21.13cd	20.70B
S2	170.73d	290.01ab	230.37A	149.91ef	274.80a	212.36A	20.82cd	15.21cd	18.02B
S3	188.46d	295.39a	241.92A	165.90e	280.00a	222.95A	22.56bc	15.39cd	18.97B
S4	126.76f	258.49b	192.62C	99.28h	246.20bc	172.74C	27.48ab	12.28d	19.88B
S5	173.81d	272.06b	222.94B	139.61f	240.10bc	189.81B	34.20a	32.06ab	33.13A
S6	144.89ef	285.76ab	215.32B	116.04gh	250.10b	183.02BC	28.84ab	35.76a	32.30A
S7	76.46g	256.49b	166.47D	60.98i	225.10c	142.99D	15.48cd	31.49ab	23.49B
Mean (Cultivar)	147.31B	267.09A		123.07B	243.76A		24.24A	23.33A	

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n = 5)

4.10. Correlation analysis among marketable yield and studied morphological parameters

Pearson's correlation analysis suggested positive correlations among the studied yield and morphological parameters (Fig. 7). According to the correlation coefficient values observed among the studied parameters, it can be inferred that the greater values obtained for leaf area (LA), shoot dry weight (SDW), root dry weight (RDW) and total plant dry weight (TPDW) resulted in higher marketable yield (MY).

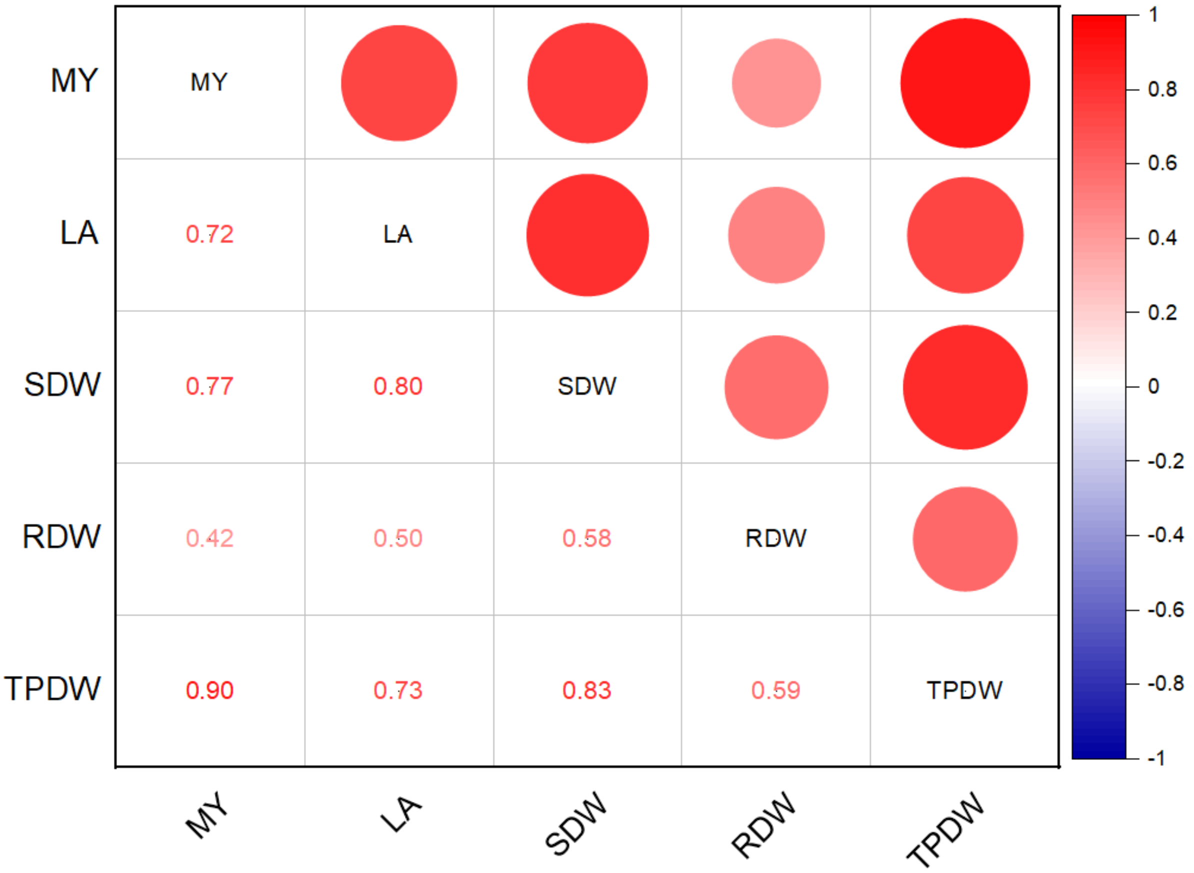

Figure 7. Correlation matrix among studied yield and morphological parameters.

Positive correlations are displayed in red and negative correlations are in blue. The size and colour intensity of circles are proportional to Pearson's correlation coefficient at P<0.01. Numbers range from -1 to +1 are correlation coefficients for variables on the vertical and horizontal axis.

4.11. Influence of substrate combinations and cultivars on physiological parameters

4.11.1 Selected Performance Indices (PIs)

There were no significant differences among the substrate combinations and cultivars concerning selected PIs values (Fig. 8). The F_v/F_m and F_v/F_0 values were observed to be in the range of 0.82-0.84 and 4.22-4.96 respectively.

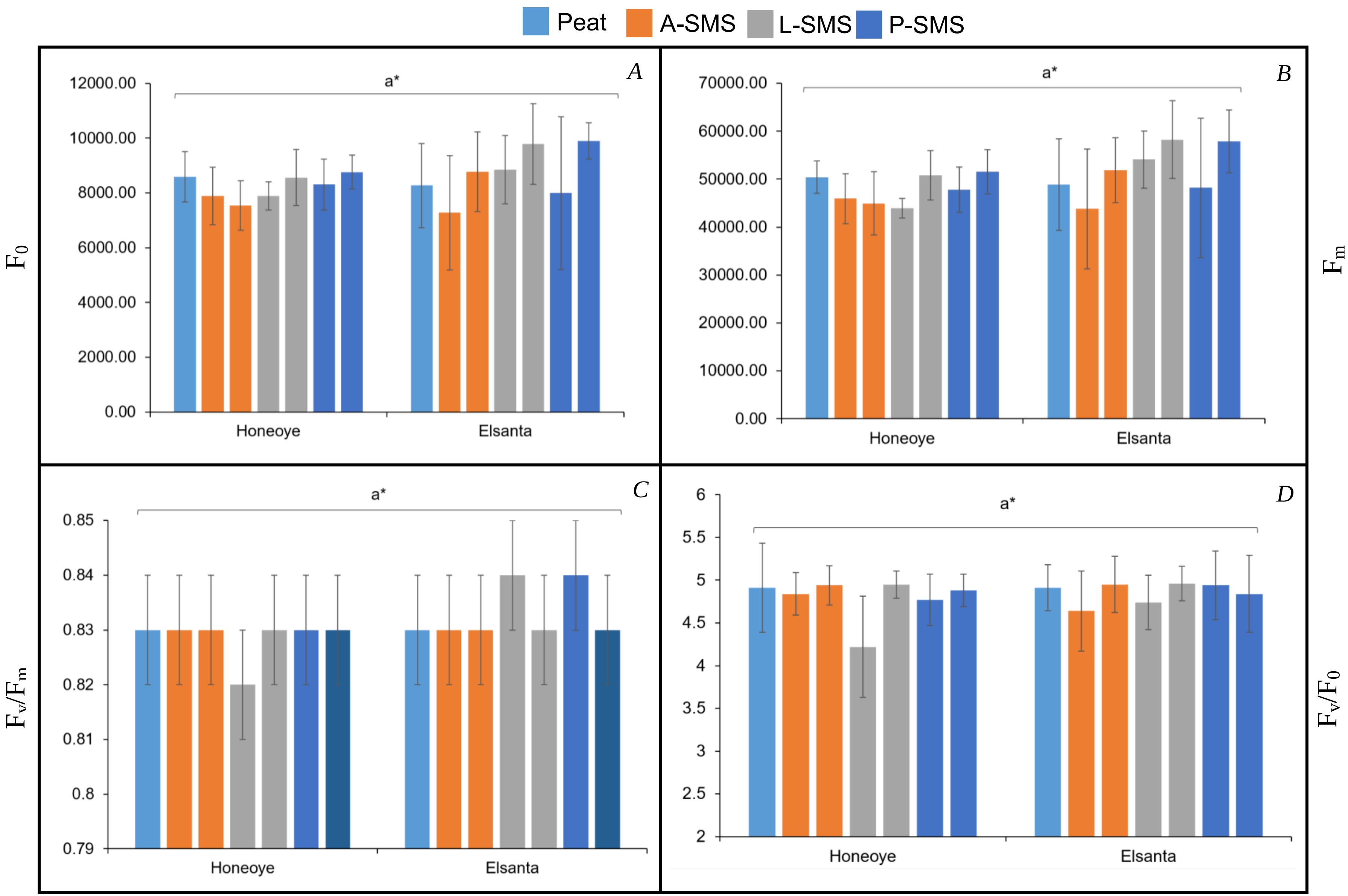

Figure. 8. F_0- fluorescence intensity at time 0 (*A*), F_m- maximum fluorescence intensity (*B*), F_v/F_m - (*C*) and F_v/F_o- (*D*) of strawberry cv. 'Honeoye' and cv. 'Elsanta' grown in different substrate combinations (mean ± SD)

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=15)

4.11.2. Selected Vegetation Indices (VIs)

The selected VIs (NDVI, MCARI and PRI) values varied among the studied substrate combinations and cultivars (Tab. 20). The highest and lowest NDVI values were recorded in S6 and S7 (0.78 and 0.75), respectively. No significant differences were observed among the two cultivars for NDVI values. The MCARI1 value was observed to be the highest and lowest in S5 and S6 (0.90 and 0.79), respectively. Among the cultivars, cv. 'Elsanta' has the highest MCARI (0.90) and cv. 'Honeoye' had a lower value (0.79). The PRI values were noticed to be the highest in S6 (0.039) and the lowest in S4 (0.025). Among the cultivars, the cv. 'Honeoye' had the highest PRI (0.051) when compared to cv. 'Elsanta' (0.017).

Table 20. NDVI, MCARI1 and PRI values of strawberry cv. 'Honeoye' and cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combinations	**NDVI**			**MCARI**			**PRI**		
	Cultivar		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**	**Cultivar**		**Mean (Substrate)**
	'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'		'Honeoye'	'Elsanta'	
S1	0.77b*	0.75b	0.76AB*	0.73b	0.91a	0.82B	0.050a	0.014c	0.032B
S2	0.78b	0.77b	0.77AB	0.81ab	0.84ab	0.82B	0.060a	0.013c	0.036B
S3	0.78b	0.77b	0.77AB	0.78ab	0.89ab	0.83B	0.048a	0.020b	0.034B
S4	0.77b	0.77b	0.77AB	0.83ab	0.92a	0.88A	0.038ab	0.011c	0.025C
S5	0.77b	0.76b	0.76AB	0.87ab	0.93a	0.90A	0.054a	0.025b	0.038B
S6	0.78b	0.79a	0.78A	0.71b	0.89ab	0.79C	0.058a	0.017b	0.039A
S7	0.74c	0.76b	0.75B	0.83ab	0.89a	0.86A	0.046a	0.021b	0.033B
Mean (Cultivar)	0.77A	0.77A		0.79B	0.90A		0.051A	0.017B	

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=15)

Experiment 3 (2020)

4.12. Influence of substrate combinations on morphological parameters

4.12.1. Shoot, root and total plant length

The results of experiment 3 revealed that the substrate combinations significantly influenced the morphological parameters of strawberry cv. 'Elsanta' as determined by shoot length, root length and total plant length (Tab. 21a). The shoot length among the studied substrates varied from 28.20-35.80 cm. The substrates S1-S6 performed equally (33.20-35.80 cm) concerning shoot length except for S7 where the lowest value was observed (28.20 cm).

The highest root length and total plant length (shoot + root length) were recorded in S5 (27.00 and 61.00 cm) and S3 (26.20 and 59.40 cm), respectively. Whereas, the lowest root length was recorded in S1, S4 and S2 (20.60, 22.20 and 22.60 cm, respectively). The lowest value for total plant length was recorded in S7 (51.80 cm).

Table 21a. Shoot, root and total plant length of strawberry cv. 'Elsanta' cultivated in different substrate combinations (mean ± SD)

Substrate combination	Substrates	Shoot length (cm)	Root length (cm)	Total plant length (cm)
S1	Peat-100% (control)	33.80 ± 0.76a*	20.60 ± 1.29c	54.40 ± 1.14b
S2	A-SMS:Peat (15:85)	35.60 ± 1.56a	22.60 ± 1.85c	58.20 ± 2.59ab
S3	A-SMS:Peat (25:75)	33.20 ± 1.48a	26.20 ± 2.39a	59.40 ± 2.41a
S4	L-SMS:Peat (15:85)	35.80 ± 1.79a	22.20 ± 1.30c	58.00 ± 2.35ab
S5	L-SMS:Peat (25:75)	34.00 ± 1.22a	27.00 ± 1.22a	61.00 ± 1.87a
S6	P-SMS:Peat (15:85)	33.80 ± 2.39a	23.20 ± 1.92b	57.00 ± 2.92ab
S7	P-SMS:Peat (25:75)	28.20 ± 1.92b	23.60 ± 1.14b	51.80 ± 2.86c

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=15)
Abbreviations: A-SMS (*Agaricus bisporus*), L-SMS (*Lentinus edodes*), P-SMS (*Pleurotus ostreatus*)

4.12.2. Number of leaves, leaf area, number of crowns and crown diameter

The strawberry leaf and crown morphological parameters as determined by the number of leaves, leaf area, number of crowns and crown diameter were significantly influenced by substrate combinations (Tab. 21b). The plants grown in S5 and S2 achieved the highest number of leaves (18.60 and 17.60) and the lowest number of leaves was recorded in S6 (13.20) and S7 (12.00). No significant differences were observed among the studied substrates concerning leaf area, where the leaf area was in the range of 254.01-322.29 cm^2.

The plants cultivated on S5 had the highest number of crowns (3.40) followed by S1 (2.60) and S2 (2.60). The lowest number of crowns were recorded in S6 (2.00) and S7 (2.00).

The highest crown diameter was observed in S5 (40.66 mm) and S4 (39.47 mm) while the lowest values were reported in S7 (26.97 mm) and S1 (32.95 mm).

Table 21b. Leaf and crown morphological parameters of strawberry cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combination	**Number of leaves**	**Leaf area (cm^2)**	**Number of crowns**	**Crown diameter (mm)**
S1	15.60 ± 1.52b	260.51 ± 67.53a	2.60 ± 0.53ab	32.95 ± 0.87c
S2	17.60 ± 3.51a	298.63 ± 53.41a	2.60 ± 0.55ab	38.17 ± 6.28b
S3	15.20 ± 1.64b	306.63 ± 26.10a	2.20 ± 0.45b	37.59 ± 1.67b
S4	15.60 ± 1.14b	320.07 ± 11.17a	2.40 ± 0.55b	39.47 ± 2.00a
S5	18.60 ± 2.30a	254.01 ± 41.66a	3.40 ± 0.55a	40.66 ± 4.57a
S6	13.20 ± 1.92c	322.29 ± 27.36a	2.00 ± 0.10c	34.21 ± 1.94b
S7	12.00 ± 1.58c	303.50 ± 33.62a	2.00 ± 0.11c	26.97 ± 1.10c

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=5)

4.13. Influence of substrate combinations on plant dry matter

4.13.1. Shoot, root and total plant dry weight

The substrate combinations significantly influenced the plant dry matter, as determined by shoot, root as well as total plant dry weights (Tab. 22). The shoot dry weight ranged from 34.00-49.51 g, where all the studied substrate combinations S2-S6 performed equally to S1 (commercial peat) except for S7 which had the lowest value (34.00 g).

The plants cultivated in S2 had the highest root dry weight (25.51 g) when compared to the other substrates, while the lowest value of 12.09 g was recorded in S5. The total plant dry weight was reported to be the highest in S2 (73.17 g) followed by all other substrates except for S7 with the lowest total plant dry weight (48.22 g). The shoot to root ratio varied among the studied substrates and was observed to be in the range of 1.95-4.14. The highest and lowest values were recorded in S5 and S2 (4.14 and 1.95, respectively).

Table 22. Plant dry matter of strawberry cv. Elsanta in different substrate combinations (mean ± SD)

Substrate combination	Shoot dry weight (g)	Root dry weight (g)	Total plant dry weight (g)	Shoot: root
S1	47.42 ± 9.06a*	20.69 ± 2.55ab	68.11 ± 9.84ab	2.31 ± 0.52b
S2	47.66 ± 12.47a	25.51 ± 8.51a	73.17 ± 20.10a	1.95 ± 0.41c
S3	45.88 ± 1.88a	21.26 ± 3.01ab	67.14 ± 4.18ab	2.19 ± 0.33b
S4	46.75 ± 4.06a	15.23 ± 3.41b	61.98 ± 3.39ab	3.20 ± 0.77ab
S5	49.51 ± 8.29a	12.09 ± 2.23c	61.60 ± 9.95ab	4.14 ± 0.61a
S6	44.77 ± 8.30a	17.20 ± 3.88b	61.97 ± 11.37ab	2.65 ± 0.43b
S7	34.00 ± 6.40b	14.23 ± 3.27b	48.22 ± 7.66b	2.47 ± 0.59b

*means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=5)

Overall, in the present study the morphological performances of strawberry cv. 'Elsanta' concerning shoot length, root length and total plant heights were observed to be superior in S3 and S5. The number of leaves, number of crowns were higher in S2 and S5. Whereas, S4 and S5 had higher crown diameter. Plants cultivated in S2 had the highest root dry weight. Whereas, the total plant dry weight was superior in S2. Among the seven substrate combinations studied, the substrates S2-S6 performed better and/or equally with commercial peat (S1) except for S7.

4.14. Influence of substrate combinations on fruit quality parameters

4.14.1. Fruit diameter, individual fruit weight and total soluble solids (TSS)

The substrate combinations significantly influenced the fruit quality parameters of strawberry cv. 'Elsanta' (Tab. 23). The fruits diameter among the studied substrate combinations were observed to be in the range of 34.29-39.43 mm. The fruit diameter was similar in all substrates except for S3 and S7 where the lowest fruit diameter was recorded (34.30 and 34.29 mm).

The individual fruit weight varied among the substrate combinations from 16.04 g to 25.06, where fruits obtained in S1-S4 had higher individual fruit weight (23.95, 25.06, 24.41 and 22.92 g, respectively) when compared to the fruits obtained in S7 (16.04 g) with the lowest value. The TSS content of fruits was not significantly different among the studied substrates and was in the range 9.80-11.92°Brix.

Table 23. Fruit quality parameters of strawberry cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combination	Fruit diameter (mm)	Individual fruit weight (g)	TSS (°Brix)
S1	37.20 ± 4.83a*	23.95 ± 7.52a	10.17 ± 1.24a
S2	39.43 ± 3.68a	25.06 ± 8.34a	11.23 ± 2.45a
S3	34.30 ± 4.17b	24.41 ± 9.26a	11.92 ± 1.17a
S4	36.88 ± 4.70a	22.92 ± 7.66a	9.80 ± 1.02a
S5	37.13 ± 2.47a	21.12 ± 6.77ab	11.24 ± 1.75a
S6	36.83 ± 5.16a	20.14 ± 9.69ab	10.87 ± 1.44a
S7	34.29 ± 4.54b	16.04 ± 4.94b	10.64 ± 1.02a

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=15)

4.14.2. Strawberry fruit colour coordinates

The fruit colour coordinates *L**, *a** and *b**were observed to be significantly influenced by different substrate combinations (Tab. X). No significant differences were reported for fruits harvested from S2-S6 concerning lightness (*L**) while, *L**was observed to be lower in fruits harvested from S7 (35.27) and S1 (35.29). While redness (*a**) was observed to be highest in fruits from S2 (38.42) and S4 (37.91) followed by other substrate combinations, except for fruits obtained from S1, which had the lowest *a** value (33.60). The obtained values concerning redness suggest that the fruits harvested in SMS based substrates (S2-S7) were more red when compared to fruits obtained from peat (S1). The highest yellowness was observed in fruits harvested in S2 (22.83) while the fruits harvested in S1 had the lowest *b** value (16.51).

Table 24. Fruit colour (*L**, *a**, *b**) for strawberry cv. 'Elsanta' in different substrate combinations (mean ± SD)

Substrate combinations	Fruit colour		
	L* (lightness)	***a**** (redness)	***b**** (yellowness)
S1	35.29 ± 1.47ab*	33.60 ± 2.98b	16.51 ± 1.19b
S2	38.82 ± 2.60a	38.42 ± 0.92a	22.83 ± 3.89a
S3	36.49 ± 0.82a	35.03 ± 0.72ab	19.95 ± 2.01ab
S4	37.68 ± 1.45a	37.91 ± 2.16a	20.81 ± 0.67ab
S5	36.51 ± 1.36a	37.25 ± 0.71ab	19.96 ± 1.88ab
S6	37.13 ± 1.61a	37.20 ± 1.95ab	20.91 ± 2.56ab
S7	35.27 ± 1.29ab	36.30 ± 2.51ab	18.34 ± 2.25ab

*means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=15)

4.15. Influence of substrate combinations on yield performances

4.15.1. Total yield, marketable and unmarketable yield

The substrate combinations significantly influenced the total yield, marketable and unmarketable yields of strawberry cv. ‘Elsanta’ (Fig 8 and Tab. 24). The highest total and marketable yield per plant were recorded in S2 (581.09 g and 550.59 g) followed by S3 (575.05 g and 545.07 g). These values were significantly superior to S1 (commercial peat) and other substrate combinations studied in the experiment. The lowest total and marketable yields were observed in S7 (420.55 and 369.08 g). The highest unmarketable yield was recorded in S6 (69.84 g) and the lowest was in S2, S3 and S4 (22.34, 29.98 and 30.50 g), respectively.

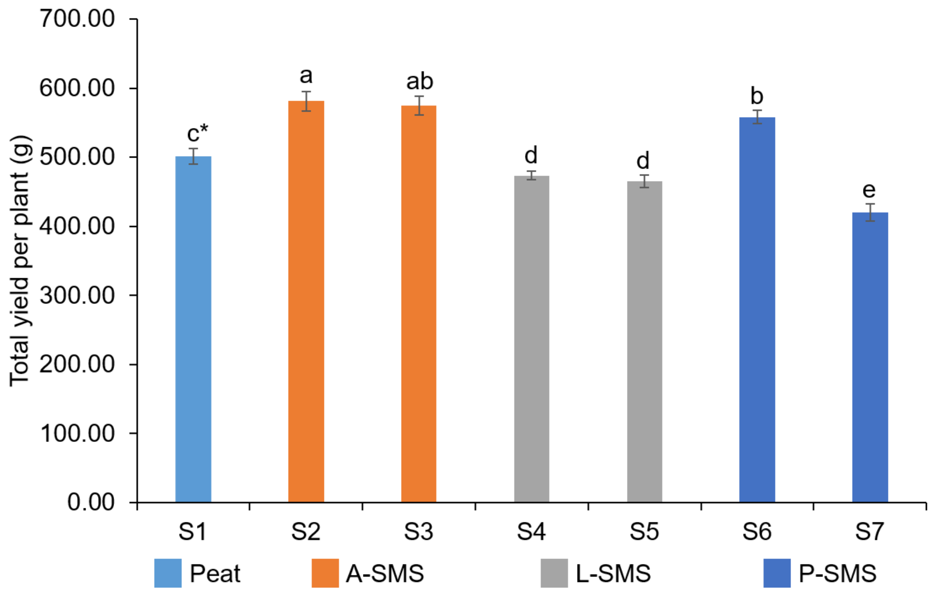

Figure 8. Total yield per plant (g) of strawberry cv. ‘Elsanta’ grown in different substrate combinations (mean ± SD) *means followed by the same letter are not significantly different at P<0.05 according to Tukey’s HSD (n=5).

Table 24. The marketable and unmarketable yield per plant of strawberry cv. ‘Honeoye’ grown in different substrate combinations (mean ± SD)

Substrate combination	**Marketable yield (g)**	**Unmarketable yield (g)**
S1	451.26 ± 12.93c*	50.03 ± 4.05b
S2	550.59 ± 15.15a	30.50 ± 4.32c
S3	545.07 ± 16.40a	29.98 ± 4.74c
S4	451.27 ± 7.94c	22.34 ± 8.67c
S5	410.27 ± 6.91d	54.70 ± 5.94b
S6	488.50 ± 13.32b	69.84 ± 6.94a
S7	369.08 ± 14.31e	51.47 ± 8.98b

*means followed by the same letters are not significantly different at P < 0.05 according to Tukey’s HSD (n=5)

The obtained marketable and unmarketable yields in grams were converted to percentages to present the yield trends among substrate combinations and are shown in Fig. 9. The percentage of marketable yields obtained in S2 (94.75%), S3 (34.79%) and S4 (95.28%) were greater than the marketable yield in commercial peat S1 (90.02%). While, the S5, S6 and S7 had a greater percentage of unmarketable yields (11.76%, 12.51% and 12.24%, respectively) when compared to commercial peat S1 (9.98%).

Overall, among all studied substrate combinations, the total and marketable yield was observed to be superior in S2 (15% A-SMS) and S3 (25% A-SMS). The total yield in S4 and S5 was similar at 15 and 25% of L-SMS supplementation whereas, the marketable yield in S4 (15% L-SMS) was superior when compared to S5 (25% L-SMS). In contrast, both the total and marketable yields were observed to be in decreasing trend in S6 and S7, where the substrate S7 with 25% P-SMS substitution resulted in lower yields when compared to S6 with 15% P-SMS substitution.

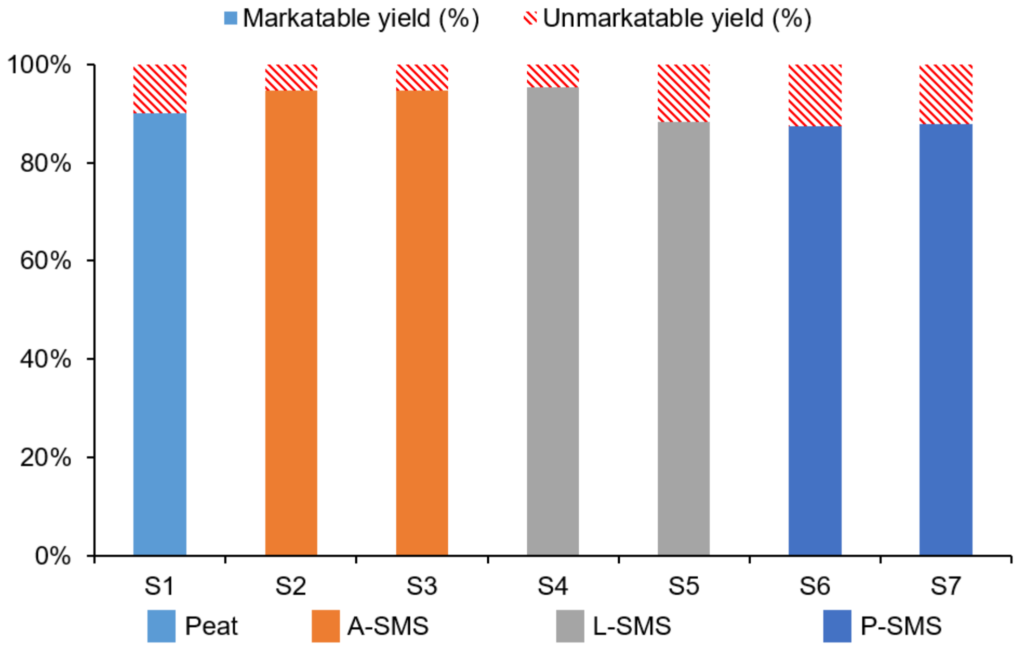

Figure 9. The per cent marketable and unmarketable yield among the studied substrate combinations

4.16. Correlation analysis among marketable and studied morphological parameters

Pearson's correlation analysis suggested positive correlations among the studied yield and morphological parameters (Fig 10). According to the correlation coefficient values observed among the studied parameters, it can be inferred that the greater values obtained for the number of leaves (NOL), plant height (PHt), crown diameter (CD), leaf area (LA), shoot dry weight (SDW), root dry weight (RDW) and total plant dry weight (TPDW) resulted in higher marketable yield (MY).

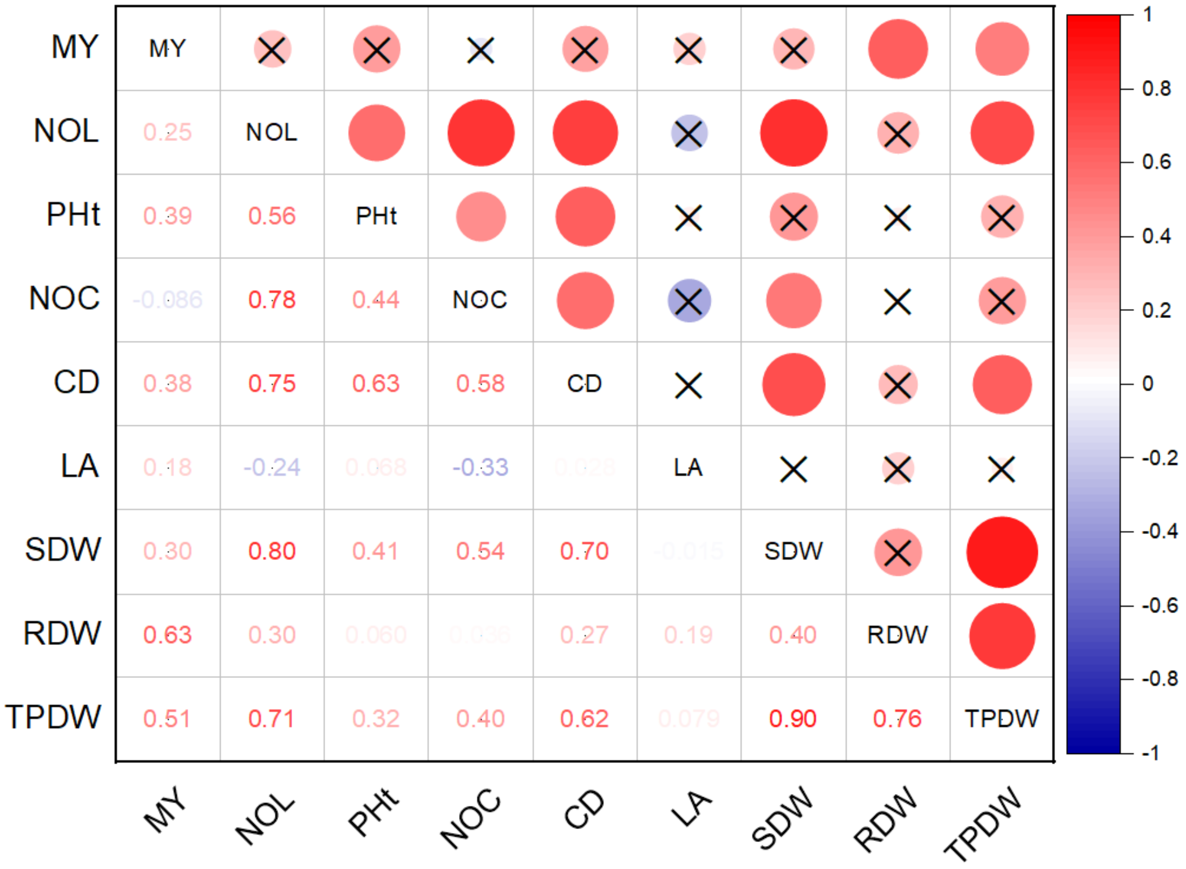

Figure. 10. Correlation matrix among studied yield and morphological parameters.

Positive correlations are displayed in red and negative correlations are in blue. The size and colour intensity of circles are proportional to Pearson's correlation coefficient at P<0.01, circles marked with × indicates correlation values that are not significant at P<0.01. Numbers range from -1 to +1 are correlation coefficients for variables on the vertical and horizontal axis.

4.17. Influence of substrate combinations on physiological parameters

4.17.1. Selected Performance Indices (PIs) and Vegetation Indices (VIs)

There were no significant differences among the plants grown in different substrate combinations for selected PIs values (Fig 11). The F_v/F_m, F_v/F_0, F_0 and F_m values ranged between 0.79-0.81, 4.00-4.50, 300.20-323.20 and 1608.20-1740.60, respectively.

Likewise PIs values among the measured VIs, the NDVI values were also found to be not significantly different among the studied substrate combinations (Fig. 12). The PRI and MCARI values significantly varied among the substrate combinations (Fig. 12). The highest

PRI value was observed in S5 and S7 (0.025) and the lowest was in S2 (0.015). The highest value for MCARI was in S2, S3 and S6 (0.94, 0.95 and 0.93, respectively) and the lowest values were observed in S5 and S7 (0.88 and 0.87).

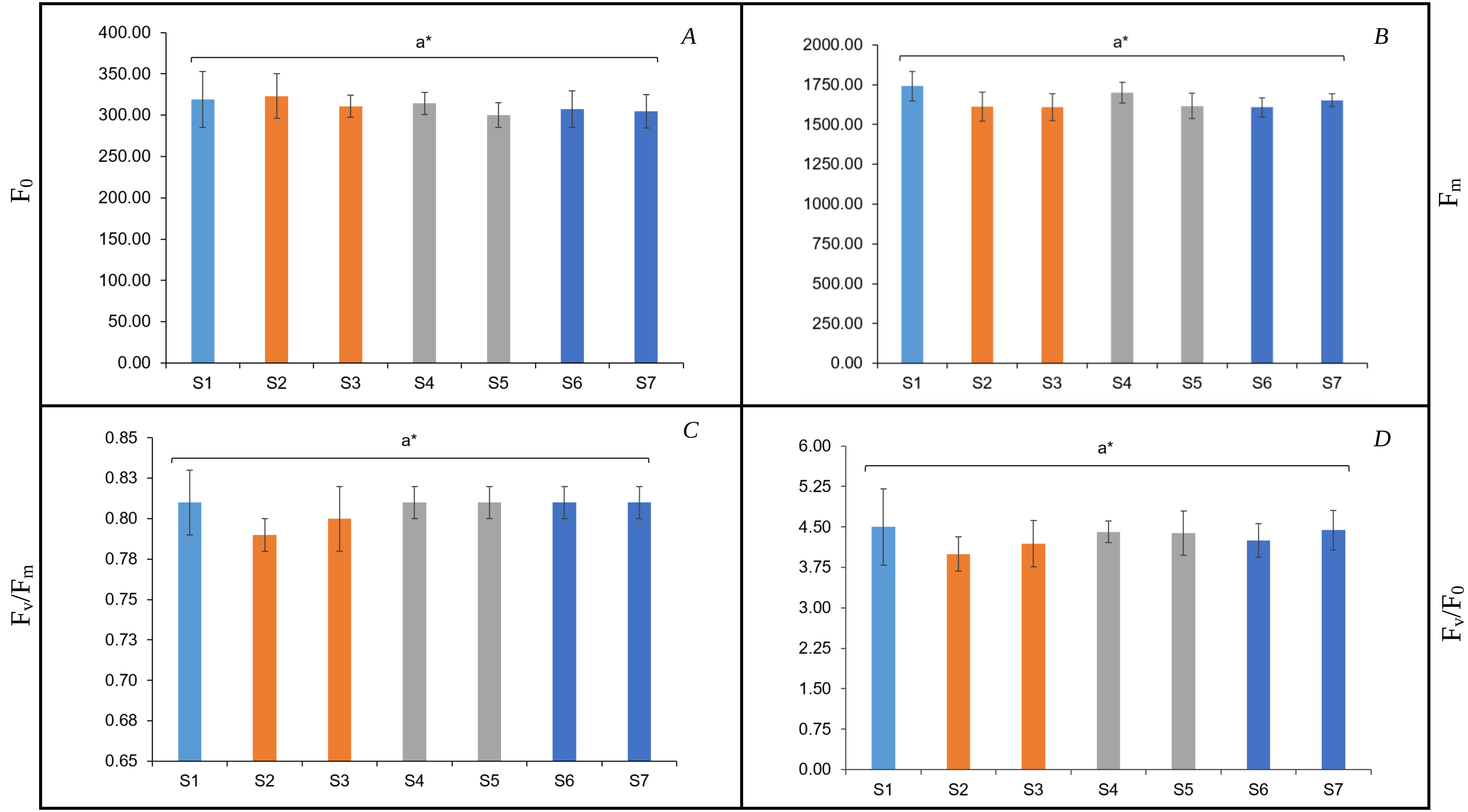

Figure 11. F_0- fluorescence intensity at time 0 (*A*), F_m- maximum fluorescence intensity (*B*), F_v/F_m - (*C*) and F_v/F_0- (*D*) of strawberry cv. 'Elsanta' grown in different substrate combinations (mean ± SD), *means followed by the same letter are not significantly different at P<0.05 according to Tukey's HSD (n=15)

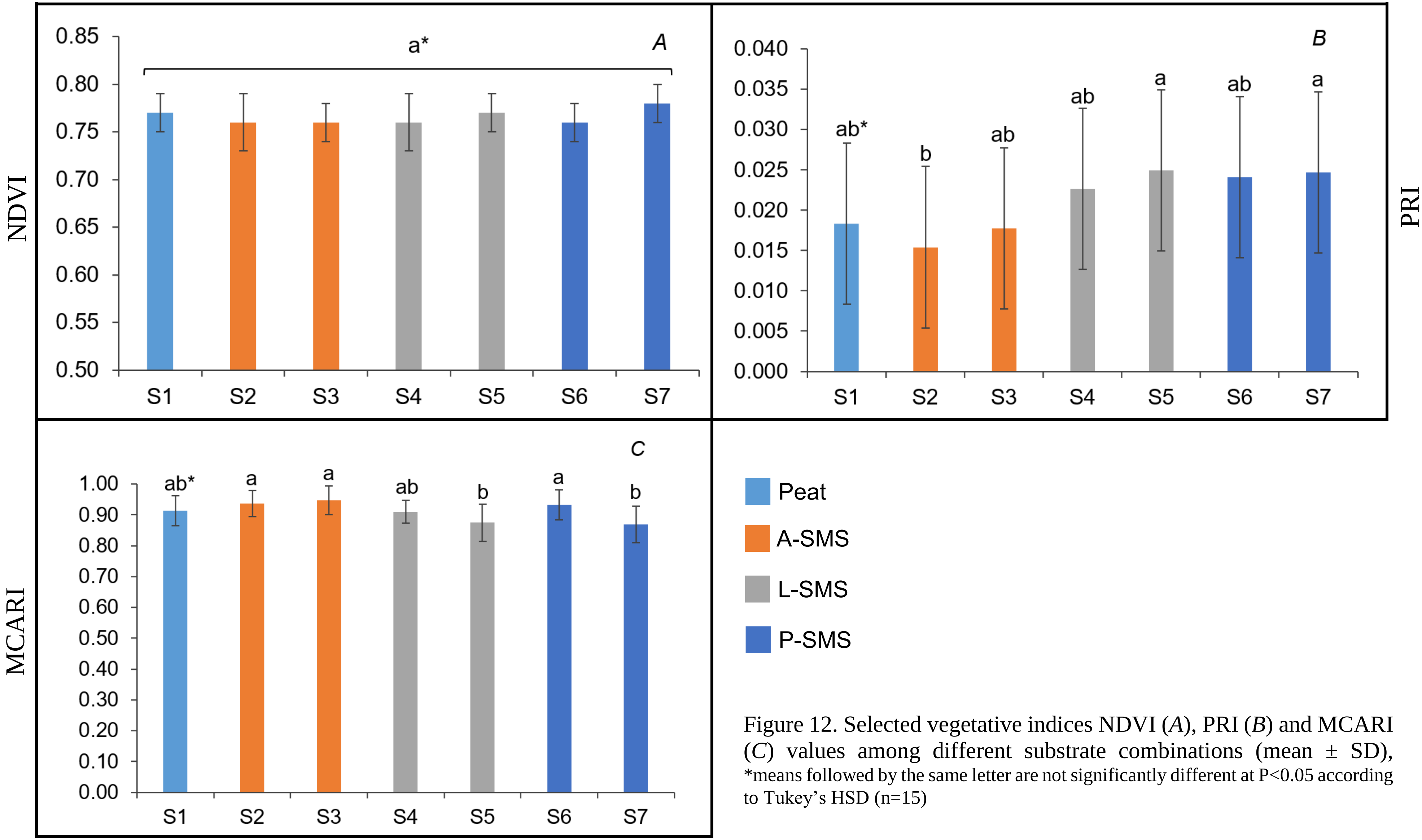

Figure 12. Selected vegetative indices NDVI (*A*), PRI (*B*) and MCARI (*C*) values among different substrate combinations (mean ± SD), *means followed by the same letter are not significantly different at $P<0.05$ according to Tukey's HSD (n=15)

5. Discussion

Peat remains as most popular and commercial substrate in soilless production (Dhen 2018, Sinclair et al. 2020). The environmental concerns associated with peat extraction (Barrett et al. 2016), its associated negative impact on wetland ecosystem (Ceglie et al. 2015) and the need for peat-free and/or peat-reduced growing media (Kitir et al. 2018) have led researchers to investigate more environmentally friendly substrates (Gruda 2019). Among many organic and/or agro-waste based substitutes, studying the possible utilisation of spent mushroom substrate (SMS) can stand out, considering the easy availability (Finney et al. 2009), relatively low cost (Danai et al. 2011) and considerable nutrient load in SMS (Adedokun and Orluchukwu 2013). The use of SMC (spent mushroom compost) is evident (Rinker 2017) while the scientific information on the effective use of fresh SMS in horticulture is still in its infancy (Cebula et al. 2013, Demir et al. 2017). Hence, to come up with an easy and effective utilisation of SMS can therefore help to reduce environmental problems related to improper disposal of SMS (Cebula et al. 2013) and fill the research gap supporting immediate use of a potential waste from mushroom enterprises.

Poland being the leader in mushroom production in the EU with the production of 362 400 tons (FAO 2019), accumulates nearly 1.8 million tons of SMS annually (mushroom production value multiplied by five). Despite the negative environmental impacts of SMS due to improper storage, handling and leaching of excess salts as well as nutrients during its field storage, Polish legislation still categorises SMS as ‘other unspecified waste’ and inadequately addresses the issues on its proper handling, processing and field storage (Cebula et al. 2013).

Considering the limited reuse of SMS in horticulture and the dependence on peat in greenhouse strawberry production, as well as the environmental problems associated with the extensive use of peat and improper handling of SMS, this investigation was carried out. The present study aimed to evaluate the potential utilisation of fresh SMS left after commercial production of three commercial mushroom species *Agaricus bisporus* (A-SMS), *Lentinus edodes* (L-SMS) and *Pleurotus ostreatus* (P-SMS) as peat substitutes in soilless strawberry production. Based on the experimental results, this chapter presents the potential and immediate use of fresh SMS in strawberry soilless production.

5.1. The pH, EC and nutrient concentration of 100% SMS and 100% peat

The choice of the substrate should be based on its characteristics, availability and cost (Lieten et al. 2004). Moreover, the selection of suitable substrate is crucial for plant nutrition, growth and eco-sustainability of horticultural systems (Grunert et al. 2016). Abad et al. (2001) reported that, an ideal substrate for soilless cultivation should exhibit following chemical properties: pH 5.2-6.3, EC 0.75-3.49 $dS\cdot m^{-1}$, OM >80%, $N\text{-}NO^{-3}$ 100-199 $mg\cdot ml^{-1}$, K^{+} 150-249 $mg\cdot ml^{-1}$, Na^{+}<115 $mg\cdot ml^{-1}$, Cl<180 $mg\cdot ml^{-1}$ and $S\text{-}SO_4^{2}$<960 $mg\cdot ml^{-1}$.

The fresh SMS has high salinity (EC) due to excess accumulation of salts during mushroom cultivation and exhibits unfavourable pH, which are the major limiting factors for its immediate use and hence using fresh SMS is restricted and/or not recommended (Medina et al. 2009, Eudoxie and Alexander 2011, Cebula et al. 2013). One of the most important factors that restrict the use of fresh SMS as a growing medium is EC (Guo et al. 2001, Jordan et al. 2008, Catal and Peksen 2020) and associated phytotoxicity due to high salinity (Sanchez-Monedero et al. 2004). In the present study, EC values of 100% fresh SMS, i.e. A-SMS (7.10-7.53 $mS\cdot cm^{-1}$), L-SMS (1.67-2.76 $mS\cdot cm^{-1}$) and P-SMS (1.25-2.69 $mS\cdot cm^{-1}$) were comparable to the values reported by Maher et al. (2000), Jordan et al. (2008) for A-SMS (0.58-10.70 $mS\cdot cm^{-1}$) and Catal and Peksen (2020) for L-SMS (1.96 $mS\cdot cm^{-1}$) and P-SMS (0.89-4.01 $mS\cdot cm^{-1}$). The range of pH and EC observed in the present investigation and previously reported by other researchers varied, as the nature of SMS largely depends on the materials used in the preparation of substrates for mushroom cultivation, composting process and the mushroom species cultivated (Peksen and Yamac 2016, Catal and Peksen 2020).

The results of the chemical analysis revealed that the pH and EC values of 100% fresh A-SMS, L-SMS and P-SMS in the present study were at the levels that limit their immediate use as a soilless substrate (Medina et al. 2009, Catal and Peksen 2020). The values of 100% SMS's concerning pH, EC and nutrient concentrations were not comparable to commercial peat values in the study. Moreover, the chemical parameters of SMS's were not in the optimal range for ideal substrate as reported by Abad et al. (2001). Therefore, 100% fresh SMS should not be considered as an ideal substrate and its immediate use as a whole (100%) should be restricted.

5.2. The pH, EC and nutrient concentration of studied substrate combinations (S1-S7)

Strawberries are highly sensitive to salinity and to excessive or deficient amounts of macro- and, micronutrients, as well as pH (Lieten, 2006a, b). The chemical analysis of substrate combinations used in the present study revealed that most of the chemical parameters concerning pH, EC and nutrient concentrations among the studied combinations (S2-S7) during experiment 1, 2 and 3 were within the acceptable and optimal values for ideal substrate as recommended by Abad et al. (2001). Moreover, when compared to 100% fresh

SMS's the values concerning pH, EC and nutrient content of prepared substrate combinations (S2-S7) were greatly comparable to the commercial peat values (S1).

The optimum EC for strawberry soilless production is reported to be in the range of 1.4-2.5 $mS \cdot cm^{-1}$ (Depardieu et al. 2016) whereas, the EC 2.5 $mS \cdot cm^{-1}$ is considered to be an upper limit (D'Anna et al. 2003, Saied 2005). While Caso et al. (2009) reported that higher EC values (3.49 $mS \cdot cm^{-1}$) of the substrates had no negative influence on the overall performance of strawberries in soilless cultivation. These findings are supported by Bryla and Scagel (2014) where EC $\leq$3.4 $mS \cdot cm^{-1}$ was found to be optimum. In the present investigation, the initial EC values of all prepared substrate combinations (S2-S7) during the experiment 1 (0.64-2.39 $mS \cdot cm^{-1}$), 2 (0.08- 1.26 $mS \cdot cm^{-1}$) and 3 (0.56-2.58 $mS \cdot cm^{-1}$) were within optimum values recommended for strawberry soilless production by Depardieu et al. (2016). Maher et al. (2000), Jordan et al. (2008) and Demir et al. (2017) reported that the substrate mixes based on SMS were observed to be highly saline. Whereas, in the present study all substrate combinations (S2-S7) based on SMS (A-SMS, L-SMS and P-SMS) were not observed to be saline.

The pH values of all prepared substrate combinations (S2-S7) in experiment 1 (5.28-6.52), 2 (5.49-7.75) and 3 (5.96-6.68) were comparable to the values (4.5-6.5) recommended for strawberry cultivation by Niskanen and Dris (2002) and Milosevic et al. (2009).

During experiment 1 A-SMS was substituted to peat in 10 and 20%, whereas L-SMS and P-SMS were added in 25 and 50%. While, during the experiment 2 and 3 all SMS's, i.e. A-SMS, L-SMS and P-SMS were substituted to peat in 15 and 25%. Based on the obtained results from the current study, concerning the chemical properties of substrate combinations (S2-S7), it can be concluded that mixing fresh SMS's with peat in lower concentrations (10-50%) can nearly neutralise the limiting nature of fresh SMS's. These findings are in line with Eudoxie and Alexander (2011) and Holozlu (2013). Hence, the prepared substrate combinations (S2-S7) with A-SMS, P-SMS and L-SMS (<50%) can be considered as a suitable peat substitute and recommended to be used as a peat-reduced growing media (Medina et al. 2009, Atikmen et al. 2014).

5.3. Influence of substrate combinations on strawberry morphological parameters

In the current study, the substrate combinations greatly influenced the morphological performances of strawberries. These findings are in line with Yavari et al. (2009), Tariq et al. (2013) and Adak et al. (2018) who reported that plant height, crown diameter, leaf area and plant dry masses were influenced by the growing media.

The superior vegetative performances among substrate combinations (S2-S7), when compared to peat (S1), was partly due to the nutrient status and nutrient availability in the substrates. In particularly, A-SMS substituted substrates S2 and S3 exhibited considerably higher amounts of macro- and micronutrients which eventually resulted in higher vegetative and yield performances of strawberries. Previous studies by other researchers also suggest that the vegetative performance of strawberries is related to the availability of an adequate amount of N, P, Mg, Mn (Roosta and Afsharipoor 2012) and Ca (Tagliavini et al. 2005). The form of N can also greatly influence the growth, yield and fruit quality (Tabatabaei et al. 2006). In the current study strawberry plants grown on A-SMS based substrates were found to have better shoot and root growth when compared to peat and other substrate combinations. These findings are supported by Leskovar and Othman (2016) where the substrate nutrients concentration significantly influenced shoot and root growth.

5.4. Influence of substrate combinations on strawberry fruit quality parameters

In the present investigation, the substrate combinations also affected the fruit quality parameters including fruit total soluble solids (TSS), individual fruit weight (IFW) and fruit diameter. These findings are supported by Jafarnia et al. (2010) and Ameri et al. (2012) where TSS was greatly influenced by substrates. Caso et al. (2009) and Martínez et al. (2017) reported that substrates influenced fruit quality parameters including individual fruit weight and fruit diameter.

The TSS value characterises the sweetness of strawberry fruits (Silva et al. 2015). Galletta et al. (1995) reported that the TSS in strawberry fruit generally ranges between 7-12%. According to Cordenunsi et al. (2003) and Pelayo et al. (2003), for strawberry fruits to have an acceptable taste and/or flavour a minimum TSS of 7% is recommended. In the present study, the TSS values among the studied substrate combinations were in this acceptable range (8.30-11.96%). In experiment 1, TSS in all substrate combinations ranged from 8.30 to 10.48% except for S5 (13.50%). Whereas, during experiments 2 and 3 in all substrate combinations TSS was between 8.50-11.96% and 9.80-11.92%, respectively. These values demonstrated that the fruits produced on substrates supplemented with different SMS's in varying concentrations achieved acceptable and recommended TSS values. During experiment 2, among the studied cultivars, cv. 'Elsanta' had higher TSS (10.41%) when compared to cv. 'Honeoye' (9.44%). The obtained results concerning fruit TSS are in line with Jafarnia et al. (2010) and Ameri et al. (2012) where the TSS value was reported to be influenced by both substrates and cultivars studied in their experiments.

During the present study, the highest IFW and fruit diameter were observed in 15% A-SMS based substrate combination in experiment 1 (24.06 g and 42.21 mm), 2 (24.87 g and 37.97 mm) and 3 (25.06 g and 39.43 mm). These obtained values concerning IFW and fruit diameter are greater than the values reported by Caso et al. (2009) in an agro-waste based substrate, i.e. 100% rice husk (11.85 g and 29.9 mm), as well as Martínez et al. (2017) in commercial coconut fibre (21.41g and 29.09 mm).

In the present investigation, the IFW in SMS based substrate combinations ranged during experiment 1 (12.86-24.06 g), 2 (11.71-24.87 g) and 3 (16.04-25.06 g). Similar values were reported by Altieri et al. (2010) where an agro-waste, i.e. olive mill waste mix was used as peat substitute which resulted in an IWF of 11.3-23.6 g and Alsmairat et al. (2018) in cocopeat + perlite mixture (22.5 g). In contrast, the IWF values obtained in the present study were higher than that in coir (8.9 g), peat (10.1 g), peat + reed canary grass straw (8.5 g) and reed canary grass straw (9.0 g) reported by Kuisma et al. (2014), as well as in tuff (14.3 g) by Alsmairat et al. (2018).

The strawberry colour is an important characteristic feature for consumer product acceptance/preference (Trevino-Garza et al. 2015). Fruit colour coordinate $L*$ represents the lightness level of the colour, $a*$ and $b*$ is the positive/negative correlation to the red/green component, and the yellow/blue component of colour, respectively (Nunes et al. 2006, Schulze and Contreras 2017).

The results concerning fruit colour coordinates obtained in the present investigation (experiment 3) were nearly comparable to the results reported in strawberries by Nunes et al. (2006) and Alsmairat et al. (2018). In the current study the initial lightness of strawberries ($L*$) ranged from 35.27-38.82, the positive/negative correlation to the red component ($a*$) ranged from 33.60-37.91, and the yellow component of colour ($b*$) ranged from 16.51-22.83. In the study conducted by Nunes et al. (2006) and Alsmairat et al. (2018) the $L*$, $a*$ and $b*$ were observed to be ranged from 39-40, 34 to 35, and 29 to 30, respectively.

In the study by Alsmairat et al. (2018) the different soilless substrates mixes (cocopeat+perlite, peat moss+perlite, tuff, tuff+cocopeat and tuff+peat moss) did not influence strawberry fruit colour coordinates, $L*$, $a*$, and $b*$. While the results of the present study demonstrated that the fruits harvested from SMS based substrates (S2-S7) had superior redness ($a*$) in comparison with fruits obtained in peat (S1). Based on these results it can be concluded that the type of substrate significantly influenced strawberry fruit colour coordinates, $L*$, $a*$, and $b*$.

5.5. Influence of substrate combinations on strawberry yield performances

Several studies demonstrated that the substrate and/or growing media used in strawberry soilless production influenced yield parameters (Wang et al. 2002, Cantliffe et al. 2007, Latigue et al. 2011, Ameri et al. 2012, Cecatto et al. 2013, Tariq et al. 2013, Kuisma et al. 2014, Adak et al. 2018). The results of the present study with respect to yield performances support these findings and suggest that substrates largely influence total yield, marketable and unmarketable yield per plant. The results of experiment 2 revealed that strawberry performance and yield was influenced both by substrates and cultivars. These findings are in agreement with Ameri et al. (2012) where cultivars responded differently to different substrates. In contrast, Alsmairat et al. (2018) and Cecatto et al. (2013) observed variations among studied substrates but not cultivars. Whereas, Palencia et al. (2016) reported differences among studied cultivars but not among substrates.

Total yield

During experiment 1, A-SMS was substituted to peat in 10 and 20% whereas, L-SMS and P-SMS were added in 25 and 50%. While, during the experiment 2 and 3, each SMS (A-SMS, L-SMS and P-SMS) was substituted to peat in 15 and 25%. During experiment 1, the yield performances (total and marketable yield) in peat substrate (S1) was observed to be superior compared to other studied substrate combinations (S2-S7). Similar results were reported by Kuisma et al. (2014) and Alsmairat et al. (2018) where total yield was greater in commercial peat when compared to other substrates and/or substrate mixes. Whereas, the total yield in SMS based substrates (S2-S6) in experiment 2 and 3 were observed to be superior and/or equal to the peat substrate (S1). Similarly, superior total yield was reported in olive mill waste mix when compared to peat by Altieri et al. (2010) and in aged bark substrate when compared to coconut fibre by Depardieu et al. (2016).

Marketable yield

The marketable yield among all substrate combinations (S1-S7) during experiments 2 and 3 was superior in S2 (274.80 g and 550.59 g) and S3 (280.00g and 545.07 g). The substrates S2 and S3 were substituted with A-SMS in 15 and 25%, respectively. This superior yield performance in A-SMS can be explained by the initial nutrient concentrations in the A-SMS based substrates. According to Benito et al. (2005), Medina et al. (2009), Demir et al. (2017) and Collela et al. (2019), the high amounts of nutrients present in SMS's serve as a source of nutrition. In the present investigation superior yields were observed when SMS were substituted in lower concentration <25%. Similarly, an increase in plant growth and yield parameters were usually observed when compost based organic additives constituted in small proportions (25-50%) along with commercial peat and/or growing media (Grigatti et al. 2007, Bustamante et al. 2008).

Concurrently, the substrates S2 and S3 with A-SMS which achieved the highest marketable yields had the highest initial and final EC values. It proves that the high EC had no negative impact on the overall performance and yield of strawberries. These findings are in agreement with Medina et al. (2009) where higher salinity of A-SMS had no negative impact on tomato seedling dry matter content. D'Anna et al. (2003) recorded higher strawberry fruit yield, better fruit quality and higher fruit weights at EC of 2.5 $mS \cdot cm^{-1}$ than at lower EC values under soilless conditions. Whereas, as reported by Saied et al. (2005), Ameri et al. (2012) and Sun et al. (2015), EC value (2.5 to 4.4 $mS \cdot cm^{-1}$) had a negative effect on yield. Keutgen and Pawelzik (2007) noticed that the EC of 7.5 $mS \cdot cm^{-1}$ significantly reduced the fruit yield of cv. 'Elsanta' up to 46%. Sun et al. (2015) and Sandhu et al. (2019) concluded that the plant tolerance to the substrate salinity depends on their genotype/cultivar. In the present investigation, both studied cultivars 'Elsanta' and 'Honeoye' are categorised as salt-sensitive (Keutgen and Pawelzik 2009, Bryla and Scagel 2014). Hence, based on the results it can be inferred that the yield performances among the studied cultivars were mainly influenced by substrate combinations.

The achieved marketable yields during experiments 2 and 3 among the studied substrate combinations (S2-S7) were comparable to the values reported by Caso et al. (2009), Altieri et al. (2010) and Depardieu et al. (2016). Caso et al. (2009) recorded the highest marketable yield (496.73 g) in 100% rice husk, Alteiri et al. (2010) in 75% olive mill waste mix (224.0 g) and Depardieu et al. (2016) in aged bark based substrate (342.88 g). Overall, the studied substrate combinations (S2-S7) exhibited favourable chemical parameters and also resulted in higher strawberry yield and better fruit quality in comparison with peat (S1), the results are in line with Caso et al. (2009), who reported that rice husk (75-100%) resulted in higher yields and better fruit quality when compared to commercial peat.

5.6. Correlation among the marketable yield and studied morphological parameters

In the present study, positive correlations were observed among selected morphological parameters and marketable yield. The correlation coefficient (r) values among the selected morphological and yield parameters were observed to be greater than 0.50. Based on Cohen (1988), the values greater than 0.50 among variables suggested a strong relationship, which indicates that yield performances in the present investigation was largely dependent on plant morphological parameters. The positive correlations among studied morphological (plant dry weights, leaf area, crown diameter, number of crowns) and marketable yield are in agreement with Grijabla et al. (2015) where an increase in crown number and leaf area contributed to higher yields. In the study of Adak et al. (2018) an increase in root and shoot dry masses increased the yield performances of strawberries under soilless cultivation.

Overall, the varying SMS substitution rates, as well as the chemical properties (pH, EC and nutrient concentrations) of prepared substrate combinations (S2-S7) used in the study, had no negative effect on the overall performances of strawberries. Even A-SMS based substrates, where higher substrate EC values were observed after the strawberry production, had no negative effect on morphological and yield performances of strawberries. The overall results from the present investigation demonstrated that SMS can be used as a potential peat substitute in lower concentrations (<25%) without negatively affecting plant performances. These findings are in line with Medina et al. (2009) and Atikmen et al. (2014). According to Siqueira et al. (2011), the microbiota and nutrient load in SMS can be beneficial to achieve better growth.

5.7. Selected physiological parameters

Salinity stress caused significant reductions in strawberry leaf area, shoot and root dry biomass, as reported by Saied et al. (2005) and Turhan and Eris (2009). According to Mahajan and Tuteja (2005), salinity directly influences plant growth through osmotic stress, specific ion toxicity, and ionic imbalances, which result in increased production of free radicals. The plant's response to stress appears with a range of morphological, physiological, biochemical, and molecular changes, which are administered by a large number of stress-responsive genes (Liu et al. 2014).

The negative effects of salinity on strawberry growth parameters, yield, and quality of strawberry fruits produced under soilless systems have been well documented (Karlidag et al. 2009, Eshghi et al. 2017, Haghshenas et al. 2020, Zahedi et al. 2020). The alkalinity stress due to high pH may destroy the photosynthetic activity of plants (Gerloff-Elias et al. 2005, Shamsabad et al. 2020). Similar results were also reported under salinity stress conditions (Yaghubi et al. 2019, Auriga et al. 2020). Collectively, the unfavourable pH and EC of growing media may negatively influence overall plant development (Roosta 2014, Garriga et al. 2015) and alter photosynthetic processes due to stress (Ghaderi et al. 2018, Yaghubi et al. 2019, Shamsabad et al. 2020).

In the present investigation, no substrate combinations based on SMS (S2-S7) were reported to be saline and despite high EC values (>2.5 $mS \cdot cm^{-1}$) in A-SMS based substrates after soilless strawberry production, these high EC values had no negative effect on the overall performances of strawberry. The substrate pH values before the experiment in all substrate combinations (S2-S7) were comparable to peat (S1) and the acidic and basic pH observed initially in some substrate combinations were nearly neutralised after strawberry production.

Fluorescence is a highly sensitive photosynthetic plant retraction analysis that can detect any changes in the overall bioenergy status of a plant (Schweiger et al. 1996). In the present study, the influence of varying pH, EC and nutrient composition of substrate combinations on the strawberry growth and overall performances can be possibly explained by Performance Indices (PIs). Among several photosynthetic parameters measured during the present study F_0, F_m, F_v/F_0 and F_v/F_m ratios were selected to be presented due to their proven sensitivity to identify the different abiotic stress responses in plants (Kalaji et al. 2016, Kalaji et al. 2017, Rastogi et al. 2019). The results of measured PIs suggested that the plants grown on SMS substituted substrate combinations (S2-S7) were not under abiotic stress influenced by varying chemical characters (pH, EC and nutrient concentration) of substrate combinations.

Performance Indices (PIs): F_v/F_m and F_v/F_0

Angelini et al. (2001) 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. Roosta (2014) and Shamsabad et al. (2020) noticed alkalinity stress lowered the maximal quantum yield of PSII photochemistry (F_v/F_m) in strawberry cv. ‘Camarosa’. Significant differences in F_v/F_m values (0.77-0.81) among three substrates studied in strawberry soilless production were reported by Ebrahimi et al. (2012). In wild strawberries (*Fragaria vesca* L.), the 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 (Auriga et al. 2020). Shamsabad et al. (2020) observed that the F_v/F_m (0.39-0.82) and F_v/F_0 (0.37-4.49) parameters declined among all six cultivars with increasing sodium bicarbonate (alkalinity).

In the present investigation, during experiment 2 and 3, no significant differences were reported concerning the F_0, Fm, Fv/F_0 and F_v/F_m values. During experiment 2, the F_v/F_m value ranged from 0.82 to 0.84 and F_v/F_0 from 4.22 to 4.96. While, in experiment 3, the Fv/F_0 and F_v/F_m values were 0.79-0.81 and 4.00-4.50, respectively. The F_v/F_m values recorded in the present study were found to be in the optimum range, as recommended by Maxwell and Johnson

(2000) and Angelini et al. (2001). In turn, the large range of observed values concerning F_v/F_m and F_v/F_0 and a decrease in these parameters, as reported by Ebrahimi et al. (2012) and Auriga et al. (2020), indicated that the plant exposed to abiotic stress factors can significantly alter PSII functions. The numerical difference for F_v/F_m and F_v/F_0 values among the studied substrate combinations in the present study indicates that the data points are spread out over a small range of values which demonstrates that the strawberry plants grown on SMS based substrates were not exposed to abiotic stresses. These values can further demonstrate that the strawberry plant performances in substrates with A-SMS, L-SMS, and P-SMS substitutes, when compared to standard peat, were not influenced by abiotic stress factors induced by different substrate combinations due to varying chemical properties, i.e. pH, EC and nutrients content.

Vegetation Indices (VIs): NDVI, MCARI and PRI

The PIs values achieved in the present study was supported by NDVI values among substrate combinations which were also not significantly different. The NDVI values recorded during experiment 2 (0.75-0.78) and 3 (0.76-0.78) were found to be in the optimum range, indicating normal vegetation, as reported by Li et al. (2010). NDVI is a spectral vegetation index widely used in the determination of plant N status and can further explain the difference in yield (Jackson 1982, Li et al. 2001 a, b). The strawberry nitrogen content of leaves and marketable yield were found to be positively correlated to the NDVI values, as reported by España-Boquera et al. (2006) and Li et al. (2010). Based on this, it can be concluded that in the present study the obtained NDVI values among substrate combinations represented sufficient N status in strawberry plants which later contributed to achieving respective marketable yields.

PRI is a quantitative measure of reflectance change at 531 nm, which indicates the changes in the state of carotenoids particularly xanthophyll cycles and is strongly related to the photosynthetic light-use efficiency (Gamon et al. 1992, Trotter et al. 2002). The value for PRI range from –1 to +1 and the values from –0.2 to +0.2 indicates normal, green and/or healthy vegetation (Gamon et al. 1997, ENVI 2009). The value of PRI during experiment 2 for cv. ‘Honeoye’ was 0.038-0.060 which was observed to be higher than that of the values specified for normal vegetation. While for cv. ‘Elsanta’ during experiment 2 the value was 0.011-0.025 and during experiment 3 was 0.015-0.025. The observed PRI values for cv. ‘Elsanta’ during experiment 2 and 3 were within the range of –0.2 to +0.2 indicating healthy vegetation (Gamon et al. 1997, ENVI 2009). The differences in PRI values among substrate combinations and cultivars in the present study were probably due to the involvement of multiple processes with separate time constants affecting reflectance and fluorescence to different degrees (Peñuelas et al. 1995). MCARI stands for Modified Chlorophyll Absorption in Reflectance Index and represents chlorophyll variations (Daughtry et al. 2000) and also exhibits great potentiality to predict the green leaf area index (Haboudane et al. 2004). Significant differences among studied substrate combinations concerning MCARI were recorded. The MCARI during experiment 2 was in the range of 0.79-0.90 and during experiment 3 from 0.87-0.95. According to Daughtry et al. (2000), this dependency in MCARI values among substrates was probably due to differences in the leaf chlorophyll concentrations. However, the changes in the content of photosynthetic pigments (chlorophyll and carotenoids) are also dependent on the tolerance of plants to the salinity of the substrate, i.e. their genotype (García-Sánchez et al. 2002, Noreen and Ashraf 2009).

5.8. The scientific impact of present investigation

The present investigation revealed the possibilities of immediate utilisation of fresh SMS's as a potential and sustainable peat substitute in strawberry soilless production, preferably in lower concentrations (<50%). These findings are greatly in line with the findings of Medina et al. (2009) and Atikmen et al. (2014). In the present study, the use of fresh SMS's as peat substitutes in varying concentrations resulted in higher vegetative, morphological and yield performances that were superior and/or equal to peat substrate, which supports the findings of Collela et al. (2019).

The fresh SMS's studied in the investigation can be regarded as an organic, eco-friendly and viable peat alternative. According to Rostami et al. (2014), Abdelrahman et al. (2016) and Dhen (2018), a rapidly renewable, locally/easily available material, which is relatively low cost and able to perform equally as commercial products can be considered as an eco-friendly and a viable alternative growing medium in the crop production system. On the other hand, organic substrates are greatly preferred because of their low costs, biodegradability and high productivity potential (Raviv et al. 2002, Caron et al. 2015).

The results of the present study revealed the possible immediate utilisation of fresh SMS as a peat substitute in soilless culture, which can contribute to reduce the use of non-renewable resources (Garcia-Delgado et al. 2013). Such immediate, easy and effective utilisation of fresh SMS, can overcome environmental and disposal problems associated with improper handling of fresh SMS, as reported by Cebula et al. (2013) and Magalhães et al. (2018), and can help to reduce partial dependency on peat-based growing media (Dhen 2018) achieve sustainability as well as create a transition towards a circular economy.

6. Conclusions

- All fresh SMS's studied in the present study, i.e. A-SMS (*Agaricus bisporus*), L-SMS (*Lentinus edodes*) and P-SMS (*Pleurotus ostreatus*) performed better and/or equally to peat. Hence, based on the chemical characteristics of prepared substrate combinations fresh SMS is recommended to be used as a potential peat substitute.
- The strawberry cv. 'Elsanta' performed better when compared to cv. 'Honeoye'. The morphological, pomological, yield parameters were superior in SMS substituted substrate when compared to peat. The selected photosynthetic performances further demonstrated that the plants cultivated on SMS's were not negatively influenced and were not under abiotic stress-induced by substrate characteristics (pH, EC and nutrient content).
- It can be recommended to use A-SMS and L-SMS as a potential peat substitute from 10-25% (v/v). A-SMS and L-SMS can be used primarily at 15% and 25% being the secondary and highest supplementation rate to achieve the best results. P-SMS is recommended to be used preferably in lower concentration 10-15%, and higher supplementation rates >25% are not recommended. Overall, all studied fresh SMS's in the present investigation should be restricted to be used as a peat substitute in higher supplementation rates of more than 50%.

General remarks and future recommendations

Based on the results of the present study it seems that peat can be replaced by fresh SMS in the horticultural sector, especially in soilless strawberry production. Considering the low cost, easy availability and large area of mushroom cultivation in Poland and worldwide, the simple, immediate and effective use of fresh SMS can help to overcome the disposal problems associated with fresh SMS. Such effective use of resources from agro-waste streams can achieve sustainability and create a transition towards the circular economy.

The scientific information supporting the immediate and effective use utilisation of fresh SMS's is still in its infancy. Despite many environmental problems associated with the weathering and improper disposal of fresh SMS, to date, Polish legislation inadequately addresses the issue of their storage and handling. Further research to support the effective use of fresh SMS, preferably in horticulture, and a reconsideration of legislation addressing proper handling, effective use and storage of fresh SMS need to be initiated.

7. References

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8. Summary

The present investigation was designed to study the possibility of utilising a designated agro-waste after commercial mushroom cultivation (spent mushroom substrate – SMS) as a potential peat substitute in soilless strawberry production in an unheated plastic tunnel. The experiment was carried out for three years (2018-2020), studying the feasibility of fresh SMS's, i.e. A-SMS (white button mushroom – *Agaricus bisporus*), L-SMS (shiitake – *Lentinus edodes*) and P-SMS (oyster mushroom – *Pleurotus ostreatus*) as growing media substitutes in various combinations with peat. The selected chemical parameters of SMS based substrate combinations, and strawberry morphological, pomological, yield as well as selected physiological parameters among different substrate combinations (S2-S7) in comparison with peat (S1) were examined. During experiment 1 (2018) A-SMS was substituted to peat in 10 and 20% (v/v), while L-SMS and P-SMS were substituted in 25 and 50%. During the experiment 2 (2019) and 3 (2020) all SMS's, i.e. A-SMS, P-SMS and L-SMS were substituted to peat in 15 and 25%.

The results of chemical analysis over the period of the study (2018-2020) revealed that SMS's in whole (100%) did not exhibit ideal substrate characteristics, particularly concerning pH, EC and nutrient concentration values which in turn restricts their immediate use as a soilless substrate. Whereas, the prepared substrate combinations (S2-S7) based on A-SMS:Peat, L-SMS:Peat and P-SMS:Peat in various combinations during experiment 1 (2018), 2 (2019) and 3 (2020) revealed favourable chemical properties, exhibiting ideal substrate characteristics concerning pH, EC and nutrient concentrations. These results strongly demonstrated the possibilities of potential utilisation of fresh SMS as peat substitutes in lower concentrations (10-25%) for strawberry soilless production.

The strawberry performances concerning morphological, pomological and yield parameters among the studied substrate combinations (S2-S7) were observed to be superior and/or equal to the commercial peat (S1). The cv. 'Elsanta' was found to perform better when compared to cv. 'Honeoye'. Further, the measured Performance Indices (F_0, F_m, F_v/F_0 and F_v/F_m) during the experiment 2 (2019) and 3 (2020) revealed that the strawberry plant photosynthetic performances were not altered and were not under abiotic stress influenced by varying chemical properties (pH, EC and nutrient concentrations) exhibited by prepared substrate combinations (S2-S7). These findings were also supported by Vegetation Indices (NDVI, PRI and MCARI) indicating normal and/or healthy vegetation of strawberry plants cultivated on SMS based substrate combinations (S2-S7).

Overall, the outcomes from the present investigation revealed potential possibilities of utilising fresh SMS as a sustainable and eco-friendly peat substitute in soilless culture, contributing towards peat/reduced and/or peat-free horticulture. Such easy, immediate and effective utilisation of a designated agro-waste (SMS) from mushroom enterprises can help to overcome disposal and environmental issues associated with improper handling of fresh SMS. At the same time, substituting peat with fresh SMS can partially reduce the dependency on an expensive and non-renewable natural resource such as peat and can decrease the production costs in soilless culture.

Potential use of agro-industrial and/or agro-wastes such as SMS can help to achieve a zero-waste crop cycle, to create sustainability and a transition to the circular economy.

9. Streszczenie

Badania miały na celu określenie możliwości wykorzystania podłoża po uprawie grzybów (SMS) jako potencjalnego substytutu torfu w bezglebowej produkcji truskawek. Badania prowadzono przez trzy lata (2018-2020) w nieogrzewanym tunelu foliowym, wykorzystując świeże podłoża po uprawie trzech gatunków grzybów jadalnych, tj. A-SMS (pieczarki dwuzarodnikowej – *Agaricus bisporus*), L-SMS (shiitake – *Lentinus edodes*) i P-SMS (boczniaka ostrygowatego – *Pleurotus ostreatus*) w różnych kombinacjach z torfem. Analizie poddano wybrane parametry chemiczne uzyskanych kombinacji substratów, parametry morfologiczne i pomologiczne truskawek oraz ich plonowanie, a także wybrane parametry fizjologiczne roślin truskawek wśród różnych kombinacji substratów (S2-S7) w porównaniu z torfem (S1). W doświadczeniu 1 (2018) A-SMS został zastąpiony torfem w 10 i 20% (obj.), natomiast L-SMS i P-SMS zostały zastąpione w 25 i 50%. W doświadczeniu 2 (2019) i 3 (2020) wszystkie podłoża po uprawie grzybów (SMS), tj. A-SMS, P-SMS i L-SMS zostały zastąpione torfem w 15 i 25%.

Wyniki analiz chemicznych przeprowadzonych w okresie badań (2018-2020) wykazały, że podłoża po uprawie grzybów (100%) nie miały optymalnych właściwości, szczególnie w zakresie wartości pH, EC i stężenia składników odżywczych, co ogranicza ich bezpośrednie zastosowanie w uprawie. Natomiast przygotowane kombinacje substratów (S2-S7) oparte na podłożach po uprawie grzybów (A-SMS:Torf, L-SMS:Torf i P-SMS:Torf) w różnych kombinacjach z torfem w eksperymencie 1 (2018), 2 (2019) i 3 (2020) wykazały korzystne właściwości chemiczne pod względem pH, EC i zawartości składników pokarmowych. Uzyskane wyniki dowodza możliwości potencjalnego wykorzystania świeżych podłoży po uprawie grzybów (SMS) jako substytutów torfu w niższych stężeniach (10-25%) do produkcji bezglebowej truskawek.

Stwierdzono, że właściwości truskawek w zakresie parametrów morfologicznych, pomologicznych i plonowania wśród badanych kombinacji substratów (S2-S7) były lepsze i/lub równe substratowi torfowemu (S1). Korzystniejsze wyniki uzyskano u odm. ‘Elsanta’ w porównaniu do odm. ‘Honeoye’. Ponadto, indeksy wydajności (F_0, F_m, F_v/F_0 i F_v/F_m) w doświadczeniu 2 (2019) i 3 (2020) wykazały, że wydajność fotosyntezy roślin truskawki nie uległa zmianie i nie podlegała stresowi abiotycznemu pod wpływem różnych właściwości chemicznych (pH, EC i zawartość składników pokarmowych) przygotowanych kombinacji substratów (S2-S7). Zostało to również poparte indeksami wegetatywnymi (NDVI, PRI

i MCARI), wskazującymi na normalną i/lub zdrową wegetację roślin truskawek uprawianych w kombinacjach substratów (S2-S7).

W podsumowaniu można stwierdzić, że wyniki badań wskazują na potencjalne możliwości wykorzystania świeżego podłoża po uprawie grzybów (SMS) jako zrównoważonego i przyjaznego dla środowiska substytutu torfu w uprawie bezglebowej. Bezpośrednie i efektywne wykorzystanie odpadów po uprawie grzybów (SMS) może pomóc w przezwyciężeniu problemów związanych z ich utylizacją i ochroną środowiska. Jednocześnie zastąpienie torfu świeżym podłożem po uprawie grzybów może częściowo zmniejszyć zależność od drogiego i nieodnawialnego zasobu naturalnego, takiego jak torf i może obniżyć koszty produkcji w uprawach bezglebowych. Potencjalne wykorzystanie rolno-przemysłowych i/lub rolniczych odpadów, takich jak podłoża po uprawie grzybów (SMS), może pomóc w osiągnięciu cyklu upraw o zerowej ilości odpadów, zapewnieniu zrównoważonego rozwoju i przejściu na gospodarkę o obiegu zamkniętym.