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ORIGINAL PAPER

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THE EFFECT OF SPENT MUSHROOM SUBSTRATE ENRICHED WITH SELENIUM AND ZINC ON THE YIELD AND PHOTOSYNTHETIC PARAMETERS OF LETTUCE (*Lactuca sativa* L.)

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ABSTRACT

The aim of the study was to investigate the influence of *Agaricus bisporus* spent mushroom substrate (A-SMS) enriched with selenium (Se) and zinc (Zn) on the yield and photosynthetic parameters of lettuce (*Lactuca sativa* L. var. *capitata*) of the 'Skindel' cultivar. The growing medium for the cultivation of lettuce consisted of A-SMS (10%) and commercial peat (90%). It was further enriched with Se and Zn concentrated at five levels, i.e. 0.1, 0.2, 0.4, 0.6, and 0.8 $mmol \cdot L^{-1}$ to obtain six growing medias. Se was added to the growing medium in the form of sodium selenite and sodium selenate at a 1 : 1 ratio, whereas Zn was added in the form of zinc nitrate hexahydrate. Lettuce was grown under controlled conditions in growth chambers. The experiment was conducted in a randomised complete block design in three replicates. The results indicated that the A-SMS added to the growing medium increased both the yield of lettuce and its biological value by increasing the content of Se and Zn. Consumable percent recommended daily allowance and safe hazard quotient for lettuce biofortified with Se and Zn were achieved. The experiment also showed that the addition of Se + Zn did not negatively affect photosynthesis and chlorophyll fluorescence parameters, which proved that these elements did not have toxic effect on lettuce in agronomic perspective.

Key words: *Lactuca sativa*, spent mushroom substrate, selenium, zinc, photosynthetic performances, chlorophyll fluorescence

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most popular and favourite leafy vegetable around the world. It can be considered a ready-to-eat minimally processed vegetable (MPV), which is most often consumed raw, usually as a salad. In recent years the production of such MPVs has been increasing to satisfy the growing number of consumers [Soliva-Fortuny and Martin-Belloso 2003]. Lettuce is popularly known for its health benefits because it is rich in vitamin C, polyphenols, and fibre [Materska et al. 2019]. It also has anti-inflammatory, cholesterol-lowering and anti-diabetic properties [Kim et al. 2016]. China is the largest producer and consumer of lettuce in the world [FAO 2018], whereas Spain is the leading producer (9.35 M tonnes) and exporter (7.95 M tonnes) of lettuce in the European Union [European statistics handbook 2020].

Nutritional deficiency is a widespread problem concerning both humans and animals around the world [Feng et al. 2013, Smoleń et al. 2016] and it is a challenge to feed the growing population with nutritious food. Selenium (Se) and zinc (Zn) play a vital role in human nutrition. These elements are important for all

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age groups, including pregnant and lactating women, infants, adults, and elderly people. The deficiency of either one or both elements may have negative influence on human health. Se and Zn deficiencies are reported to affect 30% and 15% of the global population, respectively [Smoleń et al. 2016].

Se is considered one of the well-established trace minerals essential for human health [Papp et al. 2007]. It plays an essential role in the antioxidative defence system and the modulation of growth and development [Finley et al. 2005, Alsiņa et al. 2012]. The Recommended Dietary Allowance (RDA) of Se is 55 $\mu g \cdot day^{-1}$ [Institute of Medicine 2001]. Se deficiency is the cause of hypothyroidism, cardiovascular disease, lower immunity, male infertility, cognitive decline, and increased incidence of various cancers [Fairweather-Tait et al. 2011, Rayman 2012, Fordyce 2013]. Zinc is identified as an essential component for more than 300 enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It plays a central role in the immune system, affecting several aspects of cellular and humoral immunity [Shankar and Prasad 1998]. According to the recommendations of the National Research Council, the daily RDA of Zn for an adult is about 11 mg. However, it is reported that the daily intake of a fifth of the world's population is deficient in this essential micronutrient [Beal et al. 2017].

In recent years agronomic biofortification with essential mineral elements has gained importance. It is considered the cheapest way to enrich plants with necessary elements, which can benefit the human diet [Zhao and McGrath 2009, White and Broadley 2009, Bouis and Saltzman 2017]. Agronomic biofortification of crops with Se is intended to increase its content and secure its adequate supply to people [Schiavon et al. 2020]. Studies have shown the positive influence of selenate and selenite biofortification on the yield, physiological, and biochemical properties of lettuce [Ríos et al. 2010, Esringu et al. 2015, Smoleń et al. 2019]. Se also influences the photosynthetic system F_v, F_m, F_v/F_m, F_0 [Zhang et al. 2014] and enhances the antioxidative property along with yield [Xue et al. 2001, do Nascimento da Silva and Cadore 2019]. Due to the dietary importance of Zn, the biofortification of lettuce with this element has been evaluated in different growing systems [Roosta et al. 2018, de Almeida et al. 2020, Sahin 2021]. Zn deficiency reduced the chlorophyll and fluorescence parameters, including the maximum quantum yield of PSII photochemistry and performance index, and resulted in lesser production of dry matter [Hawrylak-Nowak et al. 2018]. However, the combined effect of Se and Zn on lettuce has not been well documented. To the best of our knowledge, this is the first study reporting the combined effect of Se and Zn on peat substituted with designated waste from mushroom enterprises, i.e. spent mushroom substrate (SMS).

Spent mushroom substrate is a designated waste material left after the commercial cultivation of mushrooms. The global mushroom production has exceeded ten million tonnes [FAO 2019]. It is estimated that on average five kilograms of the substrate is spent to produce one kilogram of fresh mushrooms [Semple et al. 2001, Williams et al. 2001], on this account the annual SMS (spent mushroom substrate) generated from global mushroom industries can be approximately 50–55 million tonnes. It is extremely important to utilise or dispose SMS effectively, as improper handling of this potential agro-waste may lead to various environmental hazards [Cebula et al. 2013, Atila 2016, Magalhães et al. 2018]. Numerous reports are made available for utilising composted SMS i.e. spent mushroom compost (SMC), where the fresh SMS is passively weathered/composted for 6–24 months [Ahlawat et al. 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]. However, the immediate and effective utilization of SMS is still in its infancy [Medina et al. 2009, Eudoxie and Alexander 2011, Collela et al. 2019]. According to few reports, SMS can be effectively utilised for agricultural and horticultural purposes [Suess and Curtis 2006, Medina et al. 2009, Demir 2017, Prasad et al. 2021]. It can be used as a nursery medium [Chong 2005, Medina et al. 2009, Ribas et al. 2009, Eudoxie and Alexander 2011, Zhang et al. 2012, Gao et al. 2015, Unal 2015] and as a soilless growing medium [Raviv 2011, Prasad et al. 2021]. There were positive results in lettuce seedling production when SMS was used along with commercial peat at lower concentrations of <50% [Liu et al. 2016, Marques et al. 2014]. As the global peat resources are running out [Sendi et al. 2013], non-peat substrates such as SMS can be a potential and sustainable peat substitute in the near future.

In our study a designated agro-waste (SMS) after the commercial production of *Agaricus bisporus* was used as a peat substitute. The *A. bisporus* mushroom substrate was further enriched with well-established mineral trace elements Se and Zn to assess their influence on the yield as well as the photosynthetic performance and chlorophyll fluorescence of lettuce. Further percent recommended daily allowance and hazard quotient for both added elements were calculated. In view of the decreasing area of peatlands and the global popularity of lettuce, studying the possible utilisation of A-SMS as peat substitute with the benefits of Se and Zn for human health can be expected. To the best of our knowledge, the present investigation is the first of its kind reporting the bio fortification of Se and Zn in *A. bisporus* SMS substituted peat substrate. We hypothesise that, the lettuce produced on a SMS based substrate enriched with Se and Zn may have high nutritional value by providing the necessary mineral intake in the human diet. Furthermore, effective utilisation of A-SMS as a peat substitute can contribute to a sustainable and peat-reduced horticulture production technology.

MATERIALS AND METHODS

The experiment was conducted in a phytotron belonging to the Department of Vegetable Crops, Faculty of Agronomy, Horticulture and Bioengineering, Poznań University of Life Sciences, Poland. Lettuce cv. 'Skindel' (*Lactuca sativa* L. var. *capitata*) was grown on substrate mixes in growing chambers in 2020. The growing media (GM) for the cultivation of lettuce were prepared on the basis of the volume concentration (v/v), with 10% of *Agaricus bisporus* SMS (A-SMS) and 90% of commercial peat (Klasmann-Deilmann TS1, pH 6.0, EC 1 $mS \cdot cm^{-1}$, 1 kg PG Mix (NPK 14–16–18 + micro per 1 m^3)]. The A-SMS: peat concentration used in the present investigation was based on the findings of Prasad et al. [2021] where SMS from *Agaricus bisporus*, *Lentinus edodes* and *Pleurotus ostreatus* were evaluated as commercial peat substitute for greenhouse strawberry cultivation. Where, A-SMS was recommended to be used in lower concentration (10–25%) along with peat for soilless culture. The A-SMS used in the study was obtained after commercial cultivation of *A. bisporus* (pH 7.1, EC 6.73 $mS \cdot cm^{-1}$, NPK 2.56–0.54–1.97 $g \cdot 100g^{-1}$ d.m.) obtained from HAJDUK Pvt. Ltd. Poland (HAJDUK Grupa Producentów Pieczarek sp. z o.o.)

The GM (10% A-SMS: 90% peat, pH 6.54, EC 1.89 $mS \cdot cm^{-1}$) was further enriched with selenium and zinc at five levels, i.e. 0.1, 0.2, 0.4, 0.6, and 0.8 $mmol \cdot L^{-1}$ (Tab. 1). These five enriched GM were compared with the control sample of GM without added Se + Zn. Se was applied to the GM in the form of sodium selenite and sodium selenate (Acros Organics, New Jersey, NJ, USA) at a 1 : 1 ratio. Zn was added to the GM in the form of zinc nitrate hexahydrate (Sigma-Aldrich, Saint Louis, MO, USA), dissolved in sterile distilled water to reach the five desired concentrations. The Se and Zn concentrations were the same as in a previous study conducted at the Department

Table 1. The amount of Se and Zn ($mg \cdot kg^{-1}$ D.W.) added to the growing media

Treatments	Concentrations of elements added ($mmol \cdot L^{-1}$)	Selenium	Zinc
GM0	control	5.86 ±0.33	27.49 ±0.59
GM1	0.1	9.51 ±0.16	31.35 ±0.36
GM2	0.2	12.52 ±0.87	36.33 ±0.92
GM3	0.4	16.63 ±0.30	41.92 ±0.83
GM4	0.6	19.26 ±0.63	51.36 ±0.74
GM5	0.8	22.64 ±0.34	60.42 ±0.73

of Vegetable Crops, in which *Agaricus bisporus* was cultivated on a substrate enriched with Se, Zn, and Cu [Rzymski et al. 2017].

Lettuce seeds were initially sown in pro trays with a substrate capacity of 54 cm^3/cell, with one seed per cell. The seedlings were transplanted after about 30 days into pots with a capacity of 3 dm^3 of prepared GM. The lettuce was harvested about 45 days after transplanting (DAT). The yield and photosynthetic performance of the lettuce cultivated on the GM were recorded. Nine plants were maintained in each treatment (GM) with three plants in each replication.

The Se and Zn concentrations were measured both in the GM before the start of cultivation and in the lettuce leaves after harvesting. The climatic conditions in the growth chambers were as follows: temperature – 18/16°C (day/night), photoperiod – 16 h, relative humidity – 60%, and photosynthetic photon flux density – 150 $\mu mol \cdot m^{-2} s^{-1}$ and CO_2 – 350 ppm.

The GM moisture content was monitored with soil moisture probes. The plants were initially watered to 100% field capacity and irrigated when the GM moisture/water capacity was 60% (v/v). During the growing period the plants were fertigated with water-soluble fertilisers based on 0.5% calcium nitrate (15.5% N + 26% CaO) and 0.5% Kristalon blue (NPK 19-6-20 + micro). The plants were fertilised two times at 10–12 day intervals.

Analyses of Se and Zn content

The chemical analysis of Se and Zn in growing media and lettuce heads after harvesting were carried out according to Rzymski et al. [2017]. The growing media before lettuce cultivation and lettuce after harvesting were dried with an SLW 53 STD electric dryer (Pol-Eko, Wodzisław Śląski, Poland) at 50 ±2°C for 48 h, to the constant weight, and then ground in a Cutting Boll Mill 200 (Retsch GmbH, Haan, Germany) for 1 min. The powdered samples were sieved through a 0.02 mm sieve, and samples weighing 1.000 ±0.001 g were extracted with 1 mol/L phosphoric acid in an ultrasonic bath at an ambient temperature for 30 min. The samples were then passed through filter paper (washed with 200 mL of water and 20 mL of phosphate buffer). The pH of the samples was adjusted to 6.0–6.5 by adding 10 mol/L of a sodium hydroxide solution. Finally, the samples were diluted to 20 mL with phosphate buffer.

The Se content was measured by means of electrothermal atomic absorption spectrometry (ETAAS) with a SpectrAA 280Z (Agilent, Santa Clara, CA, USA) with Zeeman background correction. A selenium hollow cathode lamp (wavelength – 196.0 nm, slit – 1.0 nm, current – 10 mA) was used and a temperature programme was optimised as follows: drying 85–120°C for 55 s, ashing 1,000°C for 8 s, atomisation 2,600°C. Pyrolytic graphite tubes and a palladium solution was used as a chemical modifier (10 μL of 500 mgL^{-1} per 20 μL of the sample). The Zn content was measured by means of flame atomic absorption spectrometry (FAAS) with a SpectrAA 22FS (Varian) with acetylene (2.0 $L \cdot min^{-1}$) and air (13.5 $L \cdot min^{-1}$) stoichiometric flame. Hollow cathode lamps (Varian) with the following parameters were used: wavelength – 213.9 nm, slit – 1.0, current – 5 mA, with background correction with a deuterium lamp.

Physiological measurements

The net photosynthetic rate (A), transpiration (E), stomatal conductance (gs), and internal CO_2 concentration (Ci) were measured. The measurements were made on the second pair of leaves (counting from the apex) collected from individual lettuce plants 40–41 days after the transplantation with the LCpro + system (ADC BioScientific Ltd., UK), which automatically set levels of CO_2 (360 ppm), PPFD (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$), RH (50%) and air temperature (20°C) depending on the programme selected. It enabled automatic change of the parameters while taking measurements. The gas exchange was determined in a leaf chamber with the LCpro + system (an area of 6.25 cm^2). The net photosynthetic rate was automatically calculated as the difference between CO_2 concentrations in the air coming in and out of the measurement chamber ($\mu mol \cdot m^{-2} \cdot s^{-1}$). The rate of the airflow through the LCpro + chamber was approximately 200 $ml \cdot min^{-1}$. The measurements were made when all the parameters were stabilised.

Chlorophyll fluorescence parameters were measured with an OS5p fluorometer (OptiSciences Inc., USA) on the same lettuce leaves from individual plants 41–42 days after the transplantation. The fluorescence parameters: F_0 (dark-adapted initial fluorescence), F_m (dark-adapted maximum fluorescence), and ETR (apparent photosynthetic electron transport rate) were determined after 8 h of dark adaptation, by means of photosynthetic photon flux density (PPFD) <0.15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. F_m was measured after 0.8 s of saturating white light pulse (>15.000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD). F_v/F_m (maximum photochemical efficiency of PSII) was calculated from the obtained values. Quantum yield of electron transport (Y) was calculated according to the method described by Genty et al. [1989]. Whereas the qP (photochemical fluorescence quenching) and qN (non-photochemical fluorescence quenching) parameters were calculated according to the method described by Schreiber et al. [1986]. The chlorophyll content index (CCI) was measured with an OSI CCM-200 Plus leaf chlorophyll meter (ADC BioScientific Ltd., UK) on the same leaves which were used for the chlorophyll fluorescence measurements.

Biofortification target, consumer safety of Se and Zn enriched lettuce

In the present investigation, the daily intake of Se (D-Se), Zn (D-Zn) and the recommended daily allowance of Se (% RDA-Se) and Zn (% RDA-Zn) from 100 g of fresh lettuce heads were calculated. The considered values of RDA-Se for adults of age 19+ (male and female) was 55 µg $Se \cdot day^{-1}$ as suggested by National Academies of Sciences, Engineering, and Medicine /NASEM/, USA [Institute of Medicine 2000] and Andersson et al. [2007]. The RDA-Zn values taken was 11 $mg \cdot day^{-1}$, based on the recommendation of National Research Council [2001] for adults of age 19+ (male).

The consumer safety of Se and Zn bio fortified lettuce was evaluated on the basis of the HQ (hazard quotient) values that describe the risk to human health resulting from the intake of Se and/or Zn by consumption of fresh lettuce heads. The calculations of HQ-Se and HQ-Zn values were performed based on United States Environmental Protection Agency (USEPA) protocol and as described by Pannico et al. [2019], Smoleń et al. [2019] and Sularz et al. [2020], following the equation: HQ = ADD / RfD. Where ADD is the average daily dose of Se or Zn (mg Se or Zn per kg body weight per day) and RfD represents the recommended dietary tolerable upper intake level of Se or Zn [Smoleń et al. 2016, Smoleń et al. 2019, Sularz et al. 2020]. The average daily dose (ADD) was determined according to Smoleń et al. [2019] and Sularz et al. [2020], where ADD = (MI·CF·DI) / BW, where MI is the Se or Zn concentration in lettuce heads ($mg \cdot kg^{-1}$ d.w.), CF is the fresh to dry weight conversion factor for plant samples (dry weight to fresh weight ratio; 0.162 on average), DI is the daily intake of lettuce (0.1 kg) and BW is the considered body weight (70 kg).

The taken RfD values of Se and Zn for the sake of calculations were: 400 µg $Se \cdot day^{-1}$ or 5.71 µg $Se \cdot kg^{-1} \cdot day^{-1}$ for a 70 kg adult [Institute of Medicine 2000] and 40 mg $Zn \cdot day^{-1}$ [Institute of Medicine 2001], respectively.

Statistical analysis

The experiment was conducted in a Randomised Complete Block Design (RCBD) with one factor. It included six treatments (GM) with three replications (6 × 3). Three plants were maintained in each replication with nine plants in each treatment (GM). The yield parameters are means of three replicates, whereas the mean values of the photosynthetic performance and chlorophyll fluorescence represent three replicates. Individual lettuce plants were harvested about 45 DAT and analysed to determine their Se and Zn content. The results were analysed with the STATISTICA 10.0 software (Stat-Soft, Tulsa, OK, USA). The significance of differences between the GM in the yield, Se + Zn content, photosynthetic parameters, percent recommended daily allowance, daily intake and hazard quotient were assessed with analysis of variance (ANOVA) with the Newman-Keuls post-hoc test at a significance level of $P \leq 0.05$.

RESULTS

Lettuce yield. In comparison with the control combination, the addition of Se + Zn significantly increased the yield of lettuce (Fig. 1). The GM (GM1-GM5) biofortified with Se + Zn resulted in a higher yield, which ranged from 956.48 to 1,042.48 g. The lowest yield (858.20 g) was recorded in GM0 with no Se + Zn added.

Se and Zn content in lettuce leaves. The Se and Zn concentrations in the lettuce leaves differed depending on the concentrations of these elements added to the growing media (Tab. 2). The highest Se content was observed in GM5 (14.87 $mg \cdot kg^{-1}$ D.W.), whereas the lowest was in GM0 (2.99 $mg \cdot kg^{-1}$ D.W.). The highest Zn content was found in GM4 (23.21 $mg \cdot kg^{-1}$ D.W.), whereas the lowest was in GM0 (7.85 $mg \cdot kg^{-1}$ D.W).

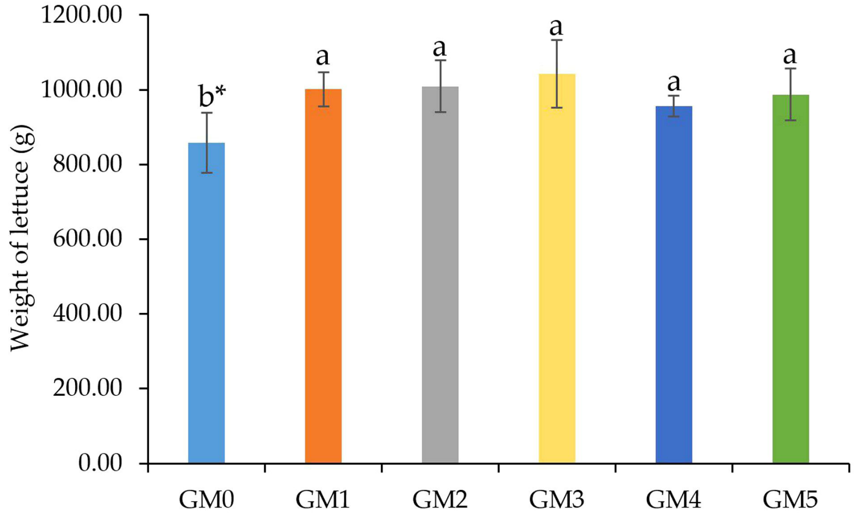

Fig. 1. The weight of harvested single lettuce heads cultivated on growing media enriched with Se + Zn. The data represent mean ±SD (n = 3)

* Different letters indicate significant differences between the substrates according to the Newman-Keuls test at $P \leq 0.05$.

Table 2. The Se and Zn content ($mg \cdot kg^{-1}$ D.W.) in the leaves harvested from lettuce cultivated on growing media enriched with Se + Zn (mean ±SD, n = 3)

Treatments	Selenium	Zinc
GM0	2.99 ±0.33f*	7.85 ±0.59f
GM1	4.36 ±0.16e	12.82 ±0.36e
GM2	5.99 ±0.87d	15.98 ±0.92d
GM3	8.38 ±0.30c	18.75 ±0.83c
GM4	12.06 ±0.63b	23.21 ±0.74a
GM5	14.87 ±0.34a	20.87 ±0.73b

* Means in each column followed by different letters are significantly different at $P \leq 0.05$.

Overall, as the amount of Se + Zn added to the GM increased, i.e. from 0.1 to 0.8 $mmol \cdot L^{-1}$, so did the selenium content in the lettuce leaves, whereas the zinc content tended to increase from 0.1 to 0.6 $mmol \cdot L^{-1}$, but it decreased significantly at 0.8 $mmol \cdot L^{-1}$.

Influence of GM on photosynthetic and chlorophyll parameters. The GM biofortified with Se + Zn at various concentrations did not influence the values of the A, Ci, gs, E, and CCI parameters (Tab. 3), which ranged within 1.28–1.98, 325.83–343.22, 0.09–0.11, 0.80–0.89, and 37.81–41.47, respectively. Only the A parameter exhibited a certain dependency, because its value tended to decrease as the Se + Zn content in the GM increased. However, these differences were not statistically significant.

Like the photosynthetic parameters, the chlorophyll fluorescence parameters were also not influenced by the GM with varying Se + Zn concentrations (Tab. 4 and Fig. 2). The values of the chlorophyll fluorescence parameters F_v/F_m, Y, qP, qn, ETR, F_0, and F_m ranged within 0.81–0.83, 0.53–0.55, 0.77–0.84, 0.53–0.61, 80.13–84.50, 227.33–251.33, and 1326.00–1546.67, respectively.

Table 3. The photosynthetic performances of lettuce cultivated on growing media enriched with Se + Zn (mean ±SD, n = 3)

Treatments	A	C_i	gs	E	CCI
GM0	1.85 ±0.97a*	325.83 ±15.31a	0.11 ±0.02 a	0.89 ±0.08 a	41.15 ±0.37 a
GM1	1.98 ±0.56a	326.61 ±10.40a	0.09 ±0.03 a	0.88 ±0.15 a	40.61 ±2.79 a
GM2	1.63 ±0.74a	329.61 ±7.52a	0.11 ±0.04 a	0.81 ±0.18 a	39.24 ±4.49 a
GM3	1.74 ±0.93a	343.92 ±8.09a	0.11 ±0.02a	0.85 ±0.12 a	41.47 ±2.81 a
GM4	1.48 ±0.61a	333.22 ±8.62a	0.09 ±0.01a	0.81 ±0.06 a	41.42 ±1.75 a
GM5	1.28 ±0.28a	337.89 ±15.45a	0.10 ±0.01 a	0.80 ±0.03 a	37.81 ±2.27 a

A – the net photosynthetic rate, Ci – internal CO_2 concentration, gs – stomatal conductance, E – transpiration, CCI – chlorophyll content index.
* Means in each column followed by the same letters are not significantly different at $P \leq 0.05$.

Table 4. The chlorophyll fluorescence parameters of lettuce cultivated on growing media (mean ±SD, n = 3)

Treatments	F_v/F_m	Y	qP	qN	ETR
GM0	0.81 ±0.01a*	0.53 ±0.03a*	0.77 ±0.13a*	0.61 ±0.04a*	80.13 ±4.54a*
GM1	0.82 ±0.01a	0.53 ±0.02a	0.82 ±0.03a	0.59 ±0.05a	80.97 ±3.81a
GM2	0.82 ±0.01a	0.53 ±0.03a	0.82 ±0.02a	0.56 ±0.03a	83.50 ±2.69a
GM3	0.83 ±0.02a	0.55 ±0.02a	0.80 ±0.02a	0.53 ±0.01a	84.50 ±1.35a
GM4	0.82 ±0.02a	0.55 ±0.01a	0.84 ±0.02a	0.56 v 0.02a	84.27 v 0.76a
GM5	0.82 ±0.02a	0.54 ±0.01a	0.83 ±0.03a	0.60 v 0.03a	81.93 v 0.29a

F_v/F_m – maximum photochemical efficiency of PSII, Y – quantum yield of electron transport, qP – photochemical fluorescence quenching, qN – non-photochemical fluorescence quenching, ETR – apparent photosynthetic electron transport rate.
* Means in each column followed by the same letters are not significantly different at $P \leq 0.05$.

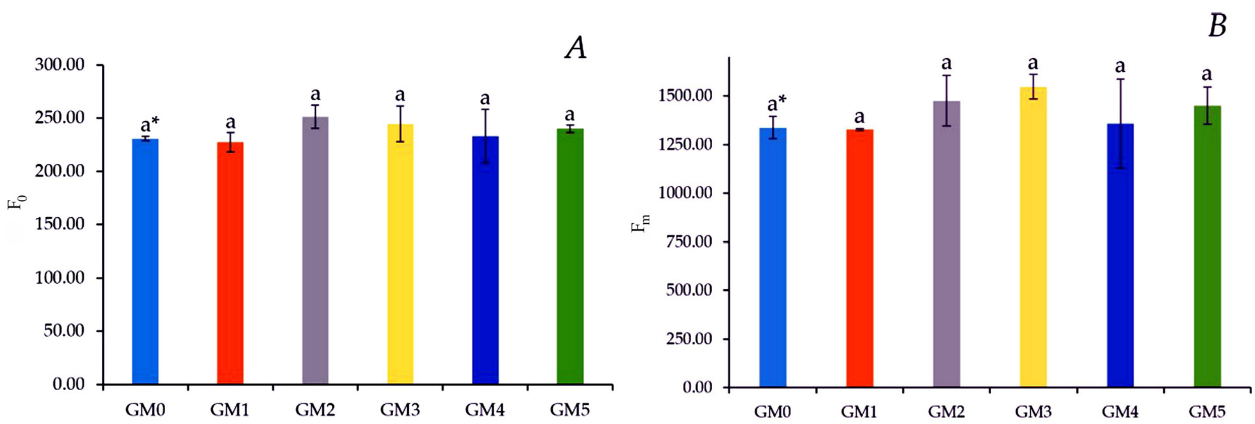

Fig. 2. F_0 – dark-adapted initial fluorescence (A) and F_m–dark-adapted maximum fluorescence (B) of lettuce cultivated on growing media enriched with Se and Zn. The data represent mean ±SD (n = 3)
* Means in each column followed by the same letters are not significantly different at $P \leq 0.05$.

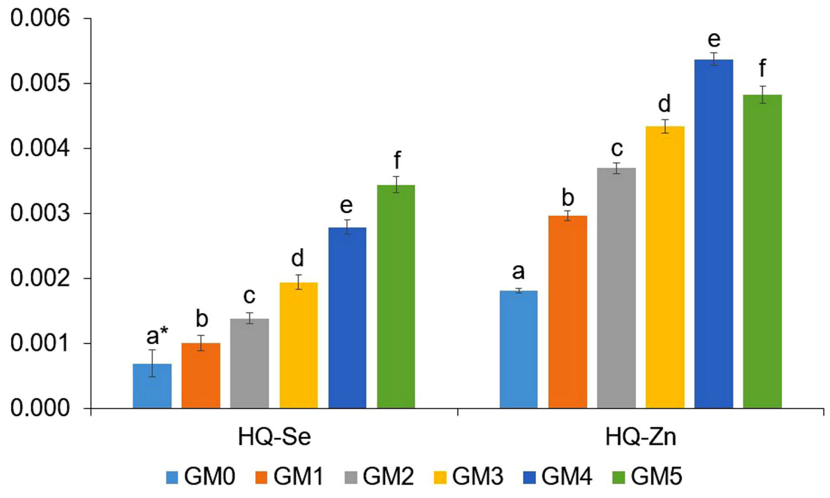

Fig. 3. Hazard quotient (HQ) for intake of Se (HQ-Se) and Zn (HQ-Zn)

* Means in each column followed by the same letters are not significantly different at $P \leq 0.05$.

Table 5. Percentage of recommended daily allowance (% RDA) for Se (% RDA-Se) and Zn (% RDA-Zn) for intake of Se and Zn through consumption of 100 g portions of fresh lettuce leaves by adults 70 kg body weight

Treatment	Daily intake of Se with 100 g of lettuce (mg Se·day^{-1})	Daily intake of Zn with 100 g of lettuce (mg Zn·day^{-1})	% RDA-Se	% RDA-Zn
GM0	0.03 ±0.01d	0.09 ±0.01d	48.48 ±10.50d	78.79 ±5.25d
GM1	0.04 ±0.01cd	0.13 ±0.01c	72.73 ±18.18c	118.18 ±9.09c
GM2	0.06 ±0.01c	0.16 ±0.01c	109.09 ±17.99c	142.42 ±5.25c
GM3	0.08 ±0.01c	0.17 ±0.01c	145.45 ±18.11c	157.58 ±10.50c
GM4	0.12 ±0.02b	0.24 ±0.02a	212.12 ±27.77b	215.15 ±13.89a
GM5	0.16 ±0.01a	0.20 ±0.01b	290.91 ±18.18a	184.85 ±10.50b

Percentage of recommended daily allowance (% RDA) for Se and Zn and hazard quotient (HQ). The Se and Zn daily intake and percentage of RDA-Se and RDA-Zn through consumption of 100 g fresh lettuce significantly differed among the studied GM (Tab. 5). The highest value of Se was recorded in GM5 (0.16 mg Se·day^{-1}) whereas, the highest content of Zn was noticed in GM4 (0.24 mg Zn·day^{-1}). The Se and Zn daily intake as well as % RDA-Se and % RDA-Zn linearly increased among the studied GM (GM0-GM5) with varying concentrations of added Se and Zn (0.1–0.8 mmol·L^{-1}, respectively). Consequently, Se reached the peak value of daily intake and percent RDA (0.16 mg Se·day^{-1} and % RDA-Se 290.91) in GM5 with 0.8 mmol·L^{-1} of added Se. While, Zn achieved its peak value of daily intake and percent RDA (0.24 Zn·day^{-1} and 215.15% RDA-Zn) in GM4 with 0.6 mmol·L^{-1} added Zn, respectively. When compared to the GM4 (0.6 mmol·L^{-1} Zn), the daily intake and percent RDA significantly decreased at the highest dose of added Zn (0.8 mmol·L^{-1}) in GM5 achieving daily intake of 0.20 Zn·day^{-1} with 184.85% RDA-Zn.

The HQ for Se and Zn ranged between 0.001–0.003 and 0.002–0.005, respectively among the studied substrates enriched with these essential elements (Fig. 3).

The highest HQ (0.003) for Se was recorded in GM5 and the lowest (0.001) was in GM0. While, for Zn the highest (0.005) HQ value was observed in GM4 and the lowest (0.002) in GM0.

DISCUSSION

The GM with Se + Zn significantly increased the yield of lettuce in our study (Figure 1). When the Se + Zn content in the additive was 0.6 and 0.8 mmol·L^{-1}, there was a slight decrease in the yield, but it was not statistically significant. These results are in line with the observations made by Ramos et al. [2010], where the addition of selenium (in the form of sodium selenate and sodium selenite) at amounts ranging from 0.4 to 0.8 mmol·L^{-1} significantly increased the yield of lettuce, but higher Se doses (>16–64 mmol·L^{-1}) decreased the yield. According to Hawrylak-Nowak [2013], selenate applied at higher doses than 10 mmol·L^{-1} and selenite applied at doses exceeding 15 mmol·L^{-1} decreased the yield of lettuce. The decrease in the fresh weight of cucumber plants when the concentration of selenate in the solution was 80 mmol·L^{-1} was reported, whereas, the concentration of selenite was 20 mmol·L^{-1} [Hawrylak-Nowak et al. 2015]. Both selenite and selenate increased nitrogen metabolism in lettuce, but selenite had stronger influence on physiological processes, because it changed the enzymatic activity of nitrate reductase, glutamine synthetase, and glutamate synthase. Selenite is also characterised by greater phytotoxicity and it reduces the production of biomass, even though the assimilation of nitrogen increases [Ríos et al. 2008a, 2008b]. As reported by Smoleń et al. [2019], the combined application of iodine (I), Se and salicylic acid (SA) had negative influence on mass of lettuce heads and whole plant. Similarly, Pannico et al. [2019] reported that the application of sodium selenate solution to lettuce at 0, 8, 16 and 24 μM reduced green lettuce fresh yields. However, in our study such negative influenced on lettuce yields up on combined application of Se and Zn were not observed. In fact, the GM bio fortified with Se + Zn (GM1-GM5) resulted in superior yield when compared to lettuce cultivated in control GM (GM0), this positive effect can be mainly due to symbiotic relation among biofortified elements and A-SMS microorganism load. As, reported by Siqueira et al. [2011], the microbiota and nutrient load in SMS can be beneficial to achieve better growth.

As the amount of Se + Zn added to the GM increased, i.e. from 0.1 to 0.8 mmol·L^{-1}, so did the selenium content in the lettuce leaves, whereas the zinc content tended to increase at lower concentrations, i.e. from 0.1 to 0.6 mmol·L^{-1}, but it decreased significantly at 0.8 mmol·L^{-1} (Tab. 2). The decrease in the zinc content after the addition of Se + Zn at an amount of 0.8 mmol·L^{-1} may have been caused by the interaction of these two elements. This finding is consistent with the results of the research on *Synapis alba* conducted by Fargašová et al. [2006]. However, according to Silva et al. [2011], selenium had minimal effect on the accumulation of zinc in sunflowers. When wheat was grown at an optimal soil water content, the use of selenium did not increase the zinc content, but under drought stress, selenium decreased the zinc content [Nawaz et al. 2015]. Germ et al. [2013] found that when selenium was used in combination with zinc, the Se content in wheat was higher than when only a selenium compound was used. Selenate is a chemical analogue of sulphate. Both of these compounds compete with each other when taken up by plants. A higher concentration of zinc influences the expression of genes responsible for sulphur transport and thus increases the uptake of selenium. According to Ei et al. [2020], not only zinc had beneficial effect on the selenium uptake, but also vice versa, i.e. increased selenium content also resulted in higher zinc uptake.

The level and place of accumulation of selenium in the plant depend on the form in which this element was applied, i.e. selenate or selenite [Hawrylak-Nowak et al. 2015, Łukaszewicz et al. 2018]. In our experiment, Se was biofortified using selenate and selenite at a 1 : 1 ratio. This combined Se biofortification had no negative effect on lettuce yields. Further, negative influence of such combined Se enrichment were not reflected in measured photosynthetic parameters. Although both compounds differ in the manner of uptake and transport in the plant, both processes are interdependent. Selenate is actively taken up and rapidly transported through the xylem to the shoots. Selenite is rapidly transformed into organic form and only small amounts are transported to the shoots. The presence of selenite reduces the uptake of selenate and its transport through the xylem [Li et al. 2008].

Both Se and Zn significantly influence the efficiency of photosynthesis. Small doses of both elements have beneficial effect on photosynthesis, but the process is seriously disturbed when high doses are used [Wang et al. 2012, Sidhu 2016, Gupta and Gupta 2017]. In our study the application of increasing doses of Se + Zn to the GM did not affect the values of A, Ci, gs, E, and CCI (Tab. 3).

The measurement of chlorophyll fluorescence is a simple method of characterising the condition of the photosynthetic apparatus in plants, especially when they are exposed to stress [Björkman and Demmig 1987, Roháček 2002]. The chlorophyll fluorescence parameters measured in our study, i.e. F_0, F_m, F_v/F_m, Y, qP, qN, and ETR, remained at a constant level and were not significantly different (Tab. 4 and Fig. 2). This means that the commercial substrate substituted with 10% A-SMS and the amounts of Se + Zn added to the lettuce GM were safe and not detrimental for the plants. At the same time, these values showed that the lettuce plants were not under abiotic stress as influenced by the substrate composition (A-SMS : peat) and chemical nature of Se + Zn.

To date, there have been few studies on the combined effect of selenium and zinc on the photosynthesis and fluorescence parameters [Fargašová et al. 2006, Ramos et al. 2010]. However, there have been numerous studies analysing how these two elements independently affected the physiological parameters of different plants [Feng et al. 2015, Das et al. 2019, Jiang et al. 2015, Vassilev et al. 2011]. When selenite was applied in a cucumber plantation at doses of 2.4 and 6 $mg \cdot L^{-1}$, it did not affect the gs value, but it decreased the internal stomata CO_2 concentration and stomatal conductance [Haghigh et al. 2016]. When a selenium additive was applied in a wheat plantation, it increased the A, E, and gs [Hawrylak-Nowak et al. 2015]

According to Garousi et al. [2016], when selenate was applied in a sunflower plantation at doses of 0.1 and 0.3 $mg \cdot L^{-1}$, an increase in the values of the A and E parameters was observed. The activity of the photosynthetic apparatus also increased due to an increase in the F_v/F_m and F_v/F_o. However, higher doses of both selenite and selanate had the opposite effect. Similarly, Garousi et al. [2017] observed that when selenite was applied to peas at a dose exceeding 30 $mg \cdot kg^{-1}$, the maximum photochemical efficiency of PSII (F_v/F_m) decreased. Ghasemi et al. [2016] sprayed a broccoli plantation with sodium selenate at doses ranging from 10 to 100 μg Se ml^{-1} and found that selenium did not affect chlorophyll fluorescence parameters such as F_0, F_v/F_m, and Y.

A zinc additive applied in a maize plantation with the optimal soil water content increased the values of A, E, gs, and F_v/F_m [Wang et al. 2012]. The increased zinc level disturbed the structure of chloroplasts and the synthesis of chlorophyll pigments. The transport of electrons was impeded, the stomata closed, and as a result, the CO_2 fixation capacity was reduced [Vassilev et al. 2011]. In our study the Se + Zn additives did not change the CCI value (Tab. 3). In contrast, Hawrylak-Nowak [2013] observed that when selenium was applied in the form of selenite, it decreased the content of chlorophyll a in lettuce. However, it had minimal influence on the content of other pigments. When selenate was applied, the content of photosynthetic pigments initially increased. However, a higher dose of selenate, i.e. 30 nM, decreased the values of these pigments. Ghasemi et al. [2016] found that as the concentration of sodium selenate applied to broccoli increased, so did the total chlorophyll content. The Se and Zn additive decreased the levels of photosynthetic pigments in *Synapis alba* L. [Fargašová et al. 2006]. When the elements were combined, the decrease in these values was lower than when they were applied separately.

The main aim of biofortification process is to increase the content of mineral components to such quantities as to effectively increase the possibility of covering the feeding allowance of consumers for particular elements in plants [White and Broadley 2009], and the results of our study demonstrate the possibilities of combined biofortification of two essential elements Se + Zn (Tab. 2). Further, in order to meet and confirm such requirements, the percentage of recommended daily allowance for Se (%RDA-Se) and Zn (%RDA-Zn) in one serving of 100 g fresh biofortified lettuce leaves were calculated using the results of Se and Zn content in fresh lettuce leaves as well as the recommended daily intake of these two essential elements for adults 55 μg Se and 11 mg Zn daily [Institute of Medicine 2000, 2001, Andersson et al. 2007]. The results indicated that, the influence of the applied Se and Zn in substrates had directly reflected on the level of Se and Zn accumulation in leaves (Tab. 2). These levels were also observed to be reflected in the calculated percentage coverage of the required amounts of RDA-Se and RDA-Zn. For a consumer point of view, as a result a theoretical consumption of 100 g of fresh leaves grown in GM1-GM5 can supply adequate amount of Se and Zn when compared to the lettuce leaves cultivated in GMO (Tab. 5). The obtained results are in line with findings of Smolen et al. [2019] and Pannico et al. [2019] where I + Se + SA and Se, respectively was biofortified successfully in lettuce, meeting the necessary daily intake and % RDA.

Further, in the present investigation in order to assess the risks to human health upon consumption of biofortified lettuce, the HQ was calculated according to the United States Environmental Protection Agency (USEPA) Protocol following the methodology described by Pannico et al. [2019]. The results concerning HQ-Se and HQ-Zn among the studies growing medium (GM0-GM5) were below 1.00 indicating the lettuce enriched with Se + Zn is safe for human consumption (Fig. 3). In our study, the HQ-Se increased with applied Se rate ranging from 0.001 to 0.003 and HQ-Zn from 0.002 to 0.005, therefore the 100g daily portion of biofortified lettuce can be considered as safe since the values of HQ-Se and HQ-Zn are less than 1.00 among varying Se and Zn enrichment rates (GM1-GM5). In particular, these lower HQ values indicates that even if the standard 100 g portion represented in this study is doubled, these lettuce would not be in any case detrimental and/or hazardous to human health. These results are in line with the findings of Pannico et al. [2019], where lettuce enriched with Se (sodium selenate) with nutrient solution at 0, 8, 16, 24, 32, or 40 μM achieved the safe HQ consumption levels (0.01–0.91) of Se biofortified lettuce. The results of these calculations in our study also demonstrated that the biofortification of Se and Zn in lettuce can effectively increase the possibility of fulfilling the necessary requirements of the RDA-Se and RDA-Zn, similar conclusions were reported by Smolen et al. [2019] demonstrating possibilities of covering the RDA (I and Se) requirements where, six different lettuce varieties were biofortified with combination of I, Se and SA.

Overall, the results of the present investigation demonstrated possible utilisation of A-SMS as peat substitute, where superior biofortified lettuce with Se + Zn were acthieved. Further, based on the results it can be inferred that, the lettuce constitutes as an ideal target for Se and Zn biofortification and such soilless cultivation can be an effective method for producing Se and Zn enriched lettuce heads of high nutraceutical values [Pannico et al. 2019].

CONCLUSIONS

In conclusion, the *A. bisporus* spent mushroom substrate and peat-based growing media enriched with Se and Zn not only increased the yield of lettuce but above all, improved its biological value. The highest dose of the Sn + Zn additive caused a fourfold increase in the selenium content in the lettuce leaves, whereas the zinc content was three times higher than in the control group. The combined use of Se and Zn did not have negative influence on the plants' performances, as the photosynthesis and chlorophyll fluorescence parameters remained at a constant level. Until now, to the best of our knowledge, no studies have been conducted aiming at the determination of the effectiveness of combined bio-fortification of Se and Zn in A-SMS based substrates. Hence, this study can be a reference for further research in this regard. Furthermore, commercial peat supplemented with A-SMS at an amount of 10% yielded bio fortified lettuce, which may have high dietary value. Such, immediate utilization of A-SMS as peat substitute can contribute towards peat-reduced horticulture.

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