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Effects of Selenium and/or Arbuscular Mycorrhizal Fungal Inoculation on Strawberry Grown in Hydroponic Trial

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Abstract: Strawberry is considered as a fruit of high nutritional value, with appreciated benefits on human health. Arbuscular mycorrhizal fungi (AMF) are commonly used plant symbionts that affect plant growth and its effectiveness is plant species specific. Additionally, selenium (Se) projects a special interest to humans for its antioxidant specialties, and to plants, because of the potential to make them grow faster when added to the nutrient solution. Nonetheless, the performance of arbuscular mycorrhizal fungi (AMF) in Se biofortification in strawberry is unexplored. The purpose of the present study experiment was to determine whether mycorrhizal inoculation of AMF can have a positive impact on growth and quality of strawberries, and whether Se contributes in this effort or will adversely affect the plants. Four Se concentrations (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution, with or without mycorrhizal inoculation of AMF to the root system, were evaluated. Results demonstrated that Se of 10 mg L⁻¹ negatively affected plant growth, photosynthetic rates, decreased fruit firmness and total soluble solids, induced oxidative stress in fruits and affected nutrient accumulation in different plant organs. Mycorrhizal inoculation of AMF mainly stimulated antioxidative mechanisms of the fruits and increased nutrient accumulation for plants grown at high Se levels. Based on our observations, mycorrhizal inoculation can enhance the nutritional value of strawberry fruits and strawberry plants seem to be a strong candidate for Se biofortification, allowing the rise of Se of the consumers' intake.

Keywords: *Fragaria ananassa*; selenium; mycorrhizae; health benefits; hydroponics; antioxidants; symbiosis



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1. Introduction

Selenium (Se) is a microelement that is needed for human and animal nutrition and is extensively studied due to its beneficial effects on living organisms [1]. Selenium protects human immune system, participates in conditioning the cardiovascular system, improves thyroid function, protects from Alzheimer's or Parkinson's disease and poses anticancer functions [2–4]. Selenium's effectiveness as a cancer chemopreventive agent is dependent on the dosage and formulation of Se. Selenium is also an apoptosis inducer and a cell proliferation inhibitor, which may explain its cancer preventive properties [5,6]. According to recent estimates, a Se intake of >900 µg day⁻¹ is potentially harmful to humans whereas a Se intake of less than 30 µg day⁻¹ is insufficient [7]. The recommended daily intake for Se is between 55 and 70 µg and can be up to 200 µg in a dietary Se supplement [8,9] to fortify resistance against infections of HIV and decrease prostate cancers incidence [4,10]. Selenium deficiency affects between 0.5 and 1 billion people worldwide, according to estimates [11].

Food is the main important source of Se supplementation in human diet with onion, garlic and Brazil nuts, among others to be recognized as natural Se sources [5]. Vegetables species from *Allium* and *Brassica* have the ability to accumulate Se in high amounts and can be used as Se supplements, as natural dietary [12].

Selenium plays an antioxidant role against free radicals by decreasing hydrogen peroxide and organic peroxides [2] and the effects on plants are Se-dose dependent [13]. The antioxidative role of Se is often related to the selenoproteins (i.e., glutathione peroxidase), thioredoxin reductase and selenoprotein P [3]. Application of Se in high levels though can be toxic to plants [14] or even affect the accumulation of other essential macro- and micronutrients inside a plant's tissue [15]. Indeed, the optimal Se levels could have positive impacts on plant yield and quality-related attributes, control water status of plants [16,17] and delay senescence [14], even though Se has not been shown to be required for the growth of vascular plants [5].

In the presence of high levels of inorganic Se, some plants may metabolize and accumulate Se as organic derivatives. This process is important for the plant, because it decreases the chalcogen's toxicity, while allowing the production of Se-enriched foods that could be used as a potential nutraceutical for humans and animals, when bioaccumulation occurs in plants' edible tissues [18]. Furthermore, Se biofortification can stimulate the production of secondary metabolites, which may be beneficial to human health when consumed in conjunction with a healthy diet [8,19]. To that degree, biofortification strategies used to produce Se-enriched foods may aid in overcoming Se deficiency and its consequences for human health, and may increase food's nutraceutical value. Despite several scientific studies on Se-biofortification strategies, producing Se-enriched foods suitable for animal and human consumption remains difficult. Selenium enrichment has been applied in lettuce [4], spinach [20], tomato [8], radish [21], cabbage and carrots [22] and strawberry [23], while high interest has been on the forced Se enrichment with arbuscular mycorrhizal fungi (AMF) in shallot bulbs [24] and lettuce [25]. Regarding shallots, Se biofortification combined with pretreatment with an arbuscular mycorrhizal fungi based formulation increased bulb Se content by 5.3-times, whereas Se biofortification with selenate increased this value by 21%, compared to the control. Moreover, AMF inoculation increased several bulb quality indicators, nutrient content, ascorbic acid and antioxidant activity [24]. Mycorrhizal-based products are more cost-effective than conventional fertilizers especially in regions where phosphorus depletion in soils is a serious plant nutrition problem, thus driving the demand for large scale production [26].

Since Se is scarce in most soils and plants are the primary dietary source of this element for humans and animals, several studies have been conducted in recent years to investigate various methods for increasing Se content in crops [8]. Since inorganic Se absorbed by plants is converted into organic forms with a higher bioavailability, agronomic Se biofortification offers a number of advantages over direct Se supplementation [3]. Se biofortification efficiency is depended on several variables, including cultivation techniques (from soil to soilless culture, Se levels, plant species, fertilizer form and plant growth stage, to name a few). Notably, Se biofortification must be strictly controlled, as an overabundance in plant edible parts could be toxic for human consumption [20]. The present study examined the effects of Se levels alone or in combination with AMF on plant growth, the potential accumulation of Se and other nutrients, the antioxidant status and plant stress indicators, in hydroponically grown strawberries, in perlite.

2. Materials and Methods

2.1. Experimental Conditions

The present study took place at Cyprus University of Technology, in a climate automation greenhouse during late winter—spring. During day and night, the air temperature ranged from 25.4 ± 2 to 18.3 ± 2 °C, respectively.

Two-week old strawberry plants (*Fragaria × ananassa* Festival) (160 in total) were produced with stolon's from mother plants and remained under nursery conditions for additional two weeks for rooting. Commercial cocosoil was purchased, watered for the recovery of its physicochemical properties and then mixed with perlite in a 4:1 ratio (v/v). Plants were transplanted in plastic pots (4.5 L capacity) containing the cocosoil:perlite

mixture, and placed on twin trough (channels) of 4 m length and 0.3 m apart, while plants were separated in pots by 0.2 m.

The soilless culture system was open, with the excess nutrient solution (NS) drained away by the 2% slope that the channels had. A stock solution (1:100 *v/v*) in water was prepared, containing the following concentration of nutrients: NO_3^- -N = 14.30, K = 8.95, PO_4 -P = 1.50, Ca = 3.75, Mg = 2.85, SO_4^{2-} -S = 1.55 and Na = 1.30 mmol L^{-1} , respectively; and B = 18.55, Fe = 71.55, Mn = 18.20, Cu = 4.71, Zn = 1.53 and Mo = 0.52 $\mu\text{mol L}^{-1}$, respectively, based on previous studies [27]. Fertigation was applied (4 times) during daytime using an automatic scheduled timer (1 min every 3 h at a flow rate of 75 mL min^{-1}) and a drip irrigation system (via emitters) by means of pressure pumps. The target pH and electrical conductivity (EC) of the NS were 5.8 and 1.8 mS cm^{-1} respectively. The pH of the nutrient solution was adjusted (due to the alkalinity of water) with nitric acid (HNO_3 5% *v/v*). The corresponding EC of the nutrient solution was achieved by the appropriate addition of the stock solution in water [28].

In order to determine the effects of the AMF *Funneliformis mosseae* (formerly *Glomus mosseae*) [26], on strawberry crop, 80 plants were used and root trapped with mycorrhizal inoculation of AMF. Mycorrhizal fungus inoculum of *G. mosseae* BEG95 provided/customized by Symbiom (Symbiom s.r.o., Lanškroun, Czech Republic), in granules-powder form, grey color and neutral pH. Moreover, the effect of selenium levels in the nutrient solution was studied, considering four levels of Se (0, 1, 5 and 10 mg L^{-1}) supplied as Na_2SeO_4 (Sigma-Aldrich, reagent grade $\geq 98\%$). Therefore, eight (4 Se \times 2 AMF) treatments were tested, and each treatment had 20 replicated plants. Plants were grown for 120 days in total, after transplanting.

Following one week of fertigation with the above stock solution, four nutrient solution recipes were prepared, based on the different levels of the Se, with target pH and EC of 5.8 and 1.8 mS cm^{-1} . Different NS composition was used during the vegetative and reproductive stage of strawberry plants (Table S1). Selected pots were placed on plastic trays, to collect the drainage solution and monitor the composition of the NS.

2.2. Mycorrhizal Colonization Measurement

After the end of the experiment, the whole plant was removed from the cocosoil-based substrate. The root was obtained by removing the substrate by means of vacuum air. Mycorrhizal colonization rates were measured according to Phillips and Hayman [29], following modifications. In details, root was cut into pieces, rinsed with tap water and boiled in 10% KOH (*w/v*) for three times (or until color change from redness to yellowness) at 250 °C. Solution of 5% blue ink (Pelikan) in 5% acetic acid was prepared and roots were boiled in it for 3 min to removal excess of ink. Additional boiling for 20 min took place with 2N HCl whenever the ink was present. Several rinses with water were followed and mycorrhizal colonization rate was determined using microscopical observations.

2.3. Plant Growth and Physiological Parameters

Plant growth was monitored throughout the experiment, as the number of leaves, number of flowers and number of fruits were recorded at 20, 49, 80 and 110 days after transplanting (DAT) for 8 plants per treatment.

Strawberry leaf tissue (eight replications/treatment; each replication consisted of a pool of two plants tissue; 0.1 g) was incubated in heat bath at 65 °C for 30 min, in the dark, with 10 mL dimethyl sulfoxide (DMSO) for chlorophyll extraction. Photosynthetic leaf pigments, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total chlorophyll (t-Chl) content were calculated [30]. Leaf photosynthetic rate (*pn*), stomatal conductance (*gs*) and internal leaf concentration of CO_2 (*Ci*) were measured using a portable infrared gas analyzer (model Li-6400, Biosciences, Lincoln, Washington, USA). Measurements of *pn*, *gs* and *Ci* were carried out between 9:00 and 11:20 a.m., when the leaf temperature within the chamber was 28 ± 2 °C, with photon flux density of 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at ambient CO_2 concentration [31]. The Li-6400 was equipped with a leaf chamber with constant area inserts

(6.0 cm²). All gas-exchange measurements started 3 h after the onset of the photoperiod and were replicated with four plants for each treatment and two fully expanded, healthy, sun-exposed leaves per plant. The above measurements took place at 49 and 80 DAT.

At the end of the experiment, fresh plant biomass (upper part), roots and fruits weight were determined. The corresponding dry weight (Dw) was also measured by drying samples at 65 °C to constant weight. The number of harvested strawberries and their fresh weight was measured throughout the harvesting period (2 harvests per week; 17 harvests in total), and total yield per plant was computed.

2.4. Plant Nutrient Content

Dried leaf, fruit and root tissue (four replicates/treatment) were grounded to pass a 40 mesh screens as described in Chrysargyris et al. [32]. Subsamples (0.2 g) were acid (2N HCl) digested for mineral extraction. Nitrogen (N) content was determined by the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Switzerland) [33]. Potassium (K) and sodium (Na) were determined photometrically (Flame photometer, Lasany Model 1832, Lasany International, Haryana, India), phosphorus (P) was determined spectrophotometrically (Multiskan GO, Thermo Fisher Scientific, Boston, USA) and Se by an atomic absorption spectrophotometer (PG Instruments AA500FG, Leicestershire, UK) following Chrysargyris et al. [33]. Data were expressed in g kg⁻¹ of dry weight.

The Se accumulation rate (AR), bioaccumulation coefficient (BAC), and translocation factor (TF) of strawberry were calculated by equations described by Benimeli et al. [34], Amin et al. [35] and Azooz et al. [36] as follows:

The accumulation rate (AR) was calculated as the sum up of Se concentration in each plant tissue × plant Dw divided by the number of days under Se levels by the total plant Dw.

$$\text{Accumulation rate mg kg}^{-1} \text{ Dw day}^{-1} = \frac{([\text{Se}]_{\text{leaf}} \times \text{Dw leaf} + ([\text{Se}]_{\text{fruit}} \times \text{Dw fruit} + ([\text{Se}]_{\text{root}} \times \text{Dw root}))}{\text{Days} \times (\text{Dw leaf} + \text{Dw fruit} + \text{Dw root})}$$

The bioaccumulation coefficient (BAC) was calculated as the ratio of Se concentration in plant tissue to that of Se concentration in nutrient solution:

$$\text{Bioaccumulation coefficient} = \frac{\text{Se concentration in plant tissue (mg per kg Dw)}}{\text{Se concentration in nutrient solution (mg per L)}}$$

The translocation factor (TF) was calculated as the ratio of Se concentration in plant tissue to that of Se concentration in plant roots:

$$\text{Translocation factor} = \frac{\text{Se concentration in plant tissue (mg kg Dw)}}{\text{Se concentration in plant root (mg per kg Dw)}}$$

2.5. Fruit Quality

Strawberry quality attributes were assessed after they were freshly collected. Each treatment consisted of at least eight biological replicates (pool of 2–3 fruit subsamples or submeasurements).

Fruit firmness was assessed at one point on each fruit's shoulder using a texture-meter FT 011 (TR Scientific Instruments, Forli, Italy) with a 3 mm plunger. The amount of force (in Newtons; N) needed to break through fruit's radial pericarp (i.e., surface) in eight replicates was measured at room temperature [37].

Color was measured using the Hunter Lab System and a Minolta colorimeter model CR400 (Konica Minolta, Osaka, Japan) for each fruit for the individual *L** (lightness), *a** (green to red) and *b** (blue to yellow) values, chroma value (C), hue (h), whiteness index (WI) and color index (CI) were calculated as described previously [37].

Strawberry juice was obtained from 2–3 pooled fruits for each replication (with eight replicates per treatment), and total soluble solids (TSS, expressed in percentage) measured

with a temperature-compensated digital refractometer (model Atago PR-101, Atago Co. Ltd., Tokyo, Japan) at 20 °C. Titratable acidity (TA) was measured via potentiometric titration (Mettler Toledo DL22, Columbus, OH, USA) and results were expressed in citric acid percentage [27]. The fruit sweetness/ripening index was calculated using TSS/TA ratio.

Ascorbic acid (AA) was determined by the 2,6-dichloroindophenol titrimetric method as described previously [37]. Data were expressed as mg of AA per gram of fresh weight.

Fruit polyphenols were extracted from eight replicates (three pooled tissue/replication) for each treatment as reported previously [38]. The obtained supernatants were used for the analysis of total phenolic content and antioxidant capacity. The total phenolic content was determined using the Folin–Ciocalteu method at 755 nm, according to Chondraki et al. [38] and the results were expressed as equivalents of gallic acid (Scharlau, Spain) per g of fresh weight (mg of GAE g⁻¹ fresh weight). Total antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays, according to Wojdylo et al. [39] with some modifications [37]. The results were expressed as equivalents of trolox per g of fresh weight. The total flavonoid content was determined according to the aluminum chloride colorimetric method [40] and results were expressed as rutin equivalents (mg rutin per gram of fresh tissue).

Damage index was determined by the content of hydrogen peroxide (H₂O₂) using the method of Loreto and Velikova [41] and in terms of lipid peroxidation according to Azevedo-Neto et al. [42]. The results were expressed as μmol H₂O₂ per g of fresh weight, while lipid peroxidation was calculated through the malondialdehyde (MDA) content (nmol of MDA per g of fresh weight).

Fresh produce marketability, size and appearance were marked by using a 1–5 scale with 0.5 intermediate scores; 1: not marketable quality (i.e., malformation, wounds and small size); 3: marketable with few defects i.e., decolorization (medium quality); 5: marketable with no defects (extra quality) and results were expressed in a percentage [37].

2.6. Statistical Methods

A two-factor (mycorrhizal presence and selenium levels) factorial experiment was conducted. Results were statistically analyzed with a two-way analysis of variance (ANOVA) with the IBM SPSS v.22 software for Windows. Following one-way ANOVA, the Duncan's multiple range test (DMRT) was used to compare means in case of the effect of factors and their interaction, at $p \leq 0.05$. Mean values \pm standard error (SE) of eight biological replications ($n = 8$) for plant growth-physiology and of four biological replications ($n = 4$) for nutrient content analysis were used.

3. Results

3.1. Overall Effects of Se and Mycorrhizal Inoculation

Table 1 presents the effects of Se levels, mycorrhizal inoculation and their interaction on plant-related parameters. Selenium levels in the NS affected significantly plant biomass Fw, fruit H₂O₂ concentration and MDA levels, leaf P and Se, fruit Na and Se, Root K, P and Se at $p < 0.001$; fruit Hue, phenolics, flavonoids, FRAP and P at $p < 0.01$; fruit DM, color b^* and K, root Na at $p < 0.05$. Mycorrhizal colonization affected significantly fruit flavonoids and Na, root K at $p < 0.001$; leaf Se and root Na at $p < 0.01$; root N and chlorophyll a content at $p < 0.05$. The interaction of Se \times mycorrhizal affected significantly fruit H₂O₂ and MDA, Root N, K, Na, Se and P at $p < 0.001$; leaf Se, fruit DPPH and ABTS at $p < 0.01$; fruit TSS, color b^* and Hue, leaf K and Na at $p < 0.05$.

Table 1. Effects of selenium (Se) levels, arbuscular mycorrhizal fungi (AMF) inoculation and their interaction on strawberry plant growth, physiology and nutrient content.

	Selenium (Se)	Arbuscular Mycorrhizal Fungi (AMF) Inoculation	Se × AMF
Plant growth, physiology			
Plant biomass Fw (g)	***	ns	ns
Plant biomass DM (%)	ns	ns	ns
Yield (g plant ⁻¹)	ns	ns	ns
Fruit No	ns	ns	ns
Fruit Fw (g)	ns	ns	ns
Fruit DM (%)	*	ns	ns
Photosynthetic rate (μmol m ⁻¹ s ⁻¹)	ns	ns	ns
Stomatal conductance (μmol m ⁻¹ s ⁻¹)	ns	ns	ns
Internal CO ₂ concentration (μmol mol ⁻¹)	ns	ns	ns
Chlorophyll <i>a</i> (mg g ⁻¹ Fw)	ns	*	ns
Chlorophyll <i>b</i> (mg g ⁻¹ Fw)	ns	ns	ns
total Chlorophylls (mg g ⁻¹ Fw)	ns	ns	ns
Fruit quality			
Firmness (N)	ns	ns	ns
Total soluble solids (TSS in °Brix)	ns	ns	*
Titrateable acidity (TA in %)	ns	ns	ns
Sweetness	ns	ns	ns
Color <i>L</i> *	ns	ns	ns
Color <i>a</i> *	ns	ns	ns
Color <i>b</i> *	*	ns	*
Chroma	ns	ns	ns
Hue	**	ns	*
Whitening index (WI)	ns	ns	ns
Colour index (CI)	*	ns	ns
Total phenolics (mg GAE g ⁻¹ Fw)	**	ns	ns
Total flavonoids (mg rutin g ⁻¹ Fw)	**	***	ns
Ascorbic acid (mg AA g ⁻¹ Fw)	ns	ns	ns
FRAP (mg trolox g ⁻¹ Fw)	**	ns	ns
DPPH (mg trolox g ⁻¹ Fw)	ns	ns	**
ABTS (mg trolox g ⁻¹ Fw)	ns	ns	**
H ₂ O ₂ (nmol g ⁻¹ Fw)	***	ns	***
MDA (nmol g ⁻¹ Fw)	***	ns	***
Nutrient content			
Leaf N (g kg ⁻¹)	ns	ns	ns
Leaf K (g kg ⁻¹)	ns	ns	*
Leaf P (g kg ⁻¹)	***	ns	ns
Leaf Na (g kg ⁻¹)	ns	ns	*
Leaf Se (g kg ⁻¹)	***	**	**
Fruit N (g kg ⁻¹)	ns	ns	ns
Fruit K (g kg ⁻¹)	*	ns	ns
Fruit P (g kg ⁻¹)	**	ns	ns
Fruit Na (g kg ⁻¹)	***	***	ns
Fruit Se (g kg ⁻¹)	***	ns	ns
Root N (g kg ⁻¹)	***	*	***
Root K (g kg ⁻¹)	***	***	***
Root P (g kg ⁻¹)	***	ns	***
Root Na (g kg ⁻¹)	*	**	***
Root Se (g kg ⁻¹)	***	ns	***

*, **, *** Significant difference at $p \leq 5\%$, 1% , and 0.1% following two-way ANOVA. ns: non-significant. Fresh weight (Fw); dry matter (DM); hydrogen peroxide (H₂O₂); malondialdehyde (MDA); 2,2-diphenyl-1-picrylhydrazyl (DPPH); ferric reducing antioxidant power (FRAP); 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS).

3.2. Plant Growth and Physiology

Plant growth related parameters as affected by mycorrhizal inoculation of AMF and/or Se levels are presented in Table 2. Plant fresh weight biomass was significantly increased at Se concentration of 1 mg L⁻¹, compared to control or ≥ 5 mg Se L⁻¹, while mycorrhizal inoculation of AMF did not affect the biomass production. Plant yield, fruit number and fresh fruit weight were decreased at 10 mg L⁻¹, compared to the control

and/or lower Se levels, while mycorrhizal inoculation of AMF seemed to further stimulate those parameters. Dry matter content in plant biomass remained at similar levels (averaged in 22.7%) after all treatments of Se and/or mycorrhizal inoculation of AMF presence. In the case of fruit dry matter content, Se at 10 mg L⁻¹ with mycorrhizal inoculation of AMF decreased the fruit DM compared to the lower Se levels.

Table 2. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on plant biomass (g plant⁻¹), dry matter content (%), yield (g plant⁻¹), fruit number and fruit weight (g plant⁻¹) in hydroponically grown strawberries in cocosoil.

Treatments		Plant Biomass Fw	Plant Biomass DM	Yield	Fruit No	Fruit Fw	Fruit DM
– AMF	Se-0 mg L ⁻¹	45.5 ± 7.81 ^c	21.5 ± 1.90 ^a	97.1 ± 11.27 ^a	11.0 ± 1.30 ^a	9.1 ± 0.59 ^{ab}	9.41 ± 0.81 ^a
	Se-1 mg L ⁻¹	111.1 ± 7.96 ^a	22.1 ± 1.27 ^a	98.5 ± 18.75 ^a	9.5 ± 1.09 ^a	10.5 ± 0.33 ^a	9.83 ± 0.95 ^a
	Se-5 mg L ⁻¹	77.6 ± 6.81 ^b	23.3 ± 1.44 ^a	82.8 ± 26.05 ^a	7.9 ± 1.86 ^{ab}	17.9 ± 8.04 ^a	8.69 ± 0.99 ^a
	Se-10 mg L ⁻¹	54.3 ± 10.85 ^{bc}	22.5 ± 0.60 ^a	38.7 ± 4.85 ^b	5.5 ± 0.75 ^b	7.5 ± 0.74 ^b	7.79 ± 1.19 ^a
+ AMF	Se-0 mg L ⁻¹	72.3 ± 8.10 ^b	23.6 ± 1.38 ^a	102.7 ± 23.13 ^a	11.5 ± 2.12 ^a	8.6 ± 0.48 ^a	9.79 ± 0.80 ^a
	Se-1 mg L ⁻¹	141.3 ± 23.98 ^a	23.4 ± 0.66 ^a	126.8 ± 18.35 ^a	12.8 ± 1.69 ^a	9.6 ± 0.33 ^a	9.88 ± 0.49 ^a
	Se-5 mg L ⁻¹	62.5 ± 9.50 ^b	22.9 ± 0.72 ^a	103.6 ± 10.09 ^a	10.7 ± 0.87 ^a	9.6 ± 0.26 ^a	8.87 ± 0.62 ^a
	Se-10 mg L ⁻¹	37.9 ± 5.80 ^c	22.3 ± 0.52 ^a	42.3 ± 6.82 ^b	7.1 ± 1.14 ^b	6.3 ± 0.50 ^b	7.07 ± 0.16 ^b

Values (n = 8) in each column followed by the same letter are not significantly different, $p < 0.05$.

The number of leaves, flowers and fruits produced on a monthly basis, are presented in Figure S1. Leaf number was decreased at Se of 10 mg L⁻¹ for both mycorrhizal inoculation of AMF presence or absence after 110 DAT (Figure S1A,B). Flower number was increased at 80 DAT with Se at 10 mg L⁻¹ compared to control, however, a corresponding increase in fruit number was not observed for the same period (Figure S1D,F). In general, a consistent trend was not observed after the application of the examined treatments regarding the flowers and the fruit number produced, on a monthly basis, rather than a decrease in fruit number only at high Se levels (Table 2).

Leaf photosynthetic rates were decreased at Se of 10 mg L⁻¹ compared to the control or lower Se levels, at 80 DAT for both the presence or absence of mycorrhizal inoculation of AMF (Figure 1A,B). Leaf stomatal conductance was decreased with the presence of Se and mycorrhizal inoculation of AMF (Figure 1D), with a stronger decrease at the high (10 mg L⁻¹) Se levels, but no effects were observed in the case of mycorrhizal inoculation of AMF absence at 80 DAT (Figure 1C). Neither mycorrhizal inoculation of AMF nor Se levels affected the rates of leaf photosynthesis, stomatal conductance and internal CO₂ concentration at 49 DAT, and averaged in 15.64 μmol m⁻² s⁻¹, 0.29 μmol m⁻² s⁻¹ and 252.24 μmol mol⁻¹, respectively. In general, chlorophyll *a*, chlorophyll *b* and total chlorophylls were not changed in different Se levels and/or mycorrhizal inoculation of AMF at 49 and 80 DAT (Figure S2), with the exception of a slight increase in plants of Se at 5 mg L⁻¹ compared to control at 80 DAT, without mycorrhizal inoculation of AMF (Figure S2A).

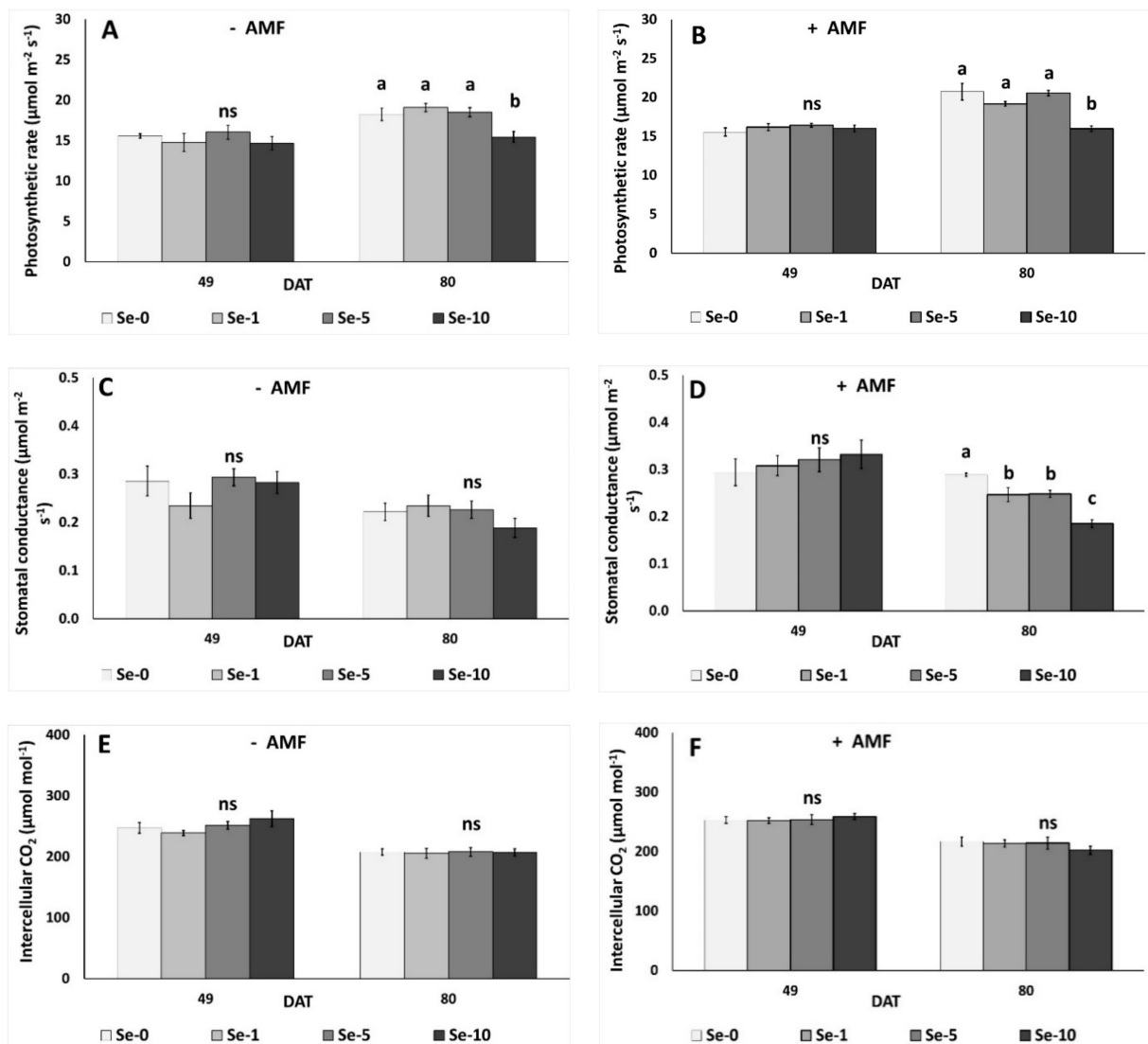


Figure 1. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L^{-1}) in the nutrient solution plant physiology in hydroponically grown strawberries in cocosoil. (A,B) photosynthetic rate, (C,D) stomatal conductance, and (E,F) internal CO_2 concentration. Significant differences ($p < 0.05$) among selenium treatments are indicated by different letters. Error bars show SE ($n = 8$). Days after transplanting: (DAT).

3.3. Fruit Quality-Related Attributes

The effect of Se levels and the mycorrhizal inoculation of AMF presence on strawberry fruit quality are presented in Table 3 and Table S1 and Figures 2 and 3. Fruit firmness was decreased up to 42.4% and 39.4% at Se of 10 mg L^{-1} in comparison to control, without and with mycorrhizal inoculation of AMF, respectively (Table 3). Similarly, without mycorrhizal inoculation of AMF, TSS were decreased at the Se of 5 and 10 mg L^{-1} in comparison to control, while TSS were unaffected by the Se levels when mycorrhizal inoculation of AMF was present and averaged in 7.16 °Brix. Titratable acidity and sweetness remained unaffected after the tested treatments and averaged at 0.76% citric acid and 9.21, respectively. The higher fruit marketability was found at 5 mg L^{-1} , scored with 4.21/5.0 and the lower was found at 10 mg L^{-1} , scored with 2.37/5.0 (data not presented).

Table 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on fruit firmness (N), total soluble solids (°Brix), titratable acidity (citric acid %) and sweetness (TSS/TA) in hydroponically grown strawberries in cocosoil.

Treatments		Firmness	TSS	TA	Sweetness
- AMF	Se-0 mg L ⁻¹	1.18 ± 0.13 ^a	7.92 ± 0.55 ^a	0.81 ± 0.03 ^a	9.95 ± 1.04 ^a
	Se-1 mg L ⁻¹	1.14 ± 0.11 ^{ab}	6.87 ± 0.41 ^{ab}	0.81 ± 0.02 ^a	8.42 ± 0.23 ^a
	Se-5 mg L ⁻¹	0.99 ± 0.13 ^{ab}	6.45 ± 0.38 ^b	0.80 ± 0.05 ^a	8.10 ± 0.21 ^a
	Se-10 mg L ⁻¹	0.68 ± 0.20 ^b	6.12 ± 0.34 ^b	0.70 ± 0.01 ^a	9.10 ± 1.14 ^a
+ AMF	Se-0 mg L ⁻¹	0.99 ± 0.08 ^a	6.77 ± 0.29 ^a	0.72 ± 0.02 ^a	9.35 ± 0.16 ^a
	Se-1 mg L ⁻¹	1.06 ± 0.11 ^a	7.17 ± 0.43 ^a	0.79 ± 0.05 ^a	9.27 ± 1.12 ^a
	Se-5 mg L ⁻¹	1.05 ± 0.09 ^a	7.80 ± 0.45 ^a	0.74 ± 0.06 ^a	10.77 ± 1.16 ^a
	Se-10 mg L ⁻¹	0.60 ± 0.18 ^b	6.90 ± 0.33 ^a	0.75 ± 0.07 ^a	9.50 ± 1.03 ^a

Values (n = 10) in each column followed by the same letter are not significantly different, $p < 0.05$.

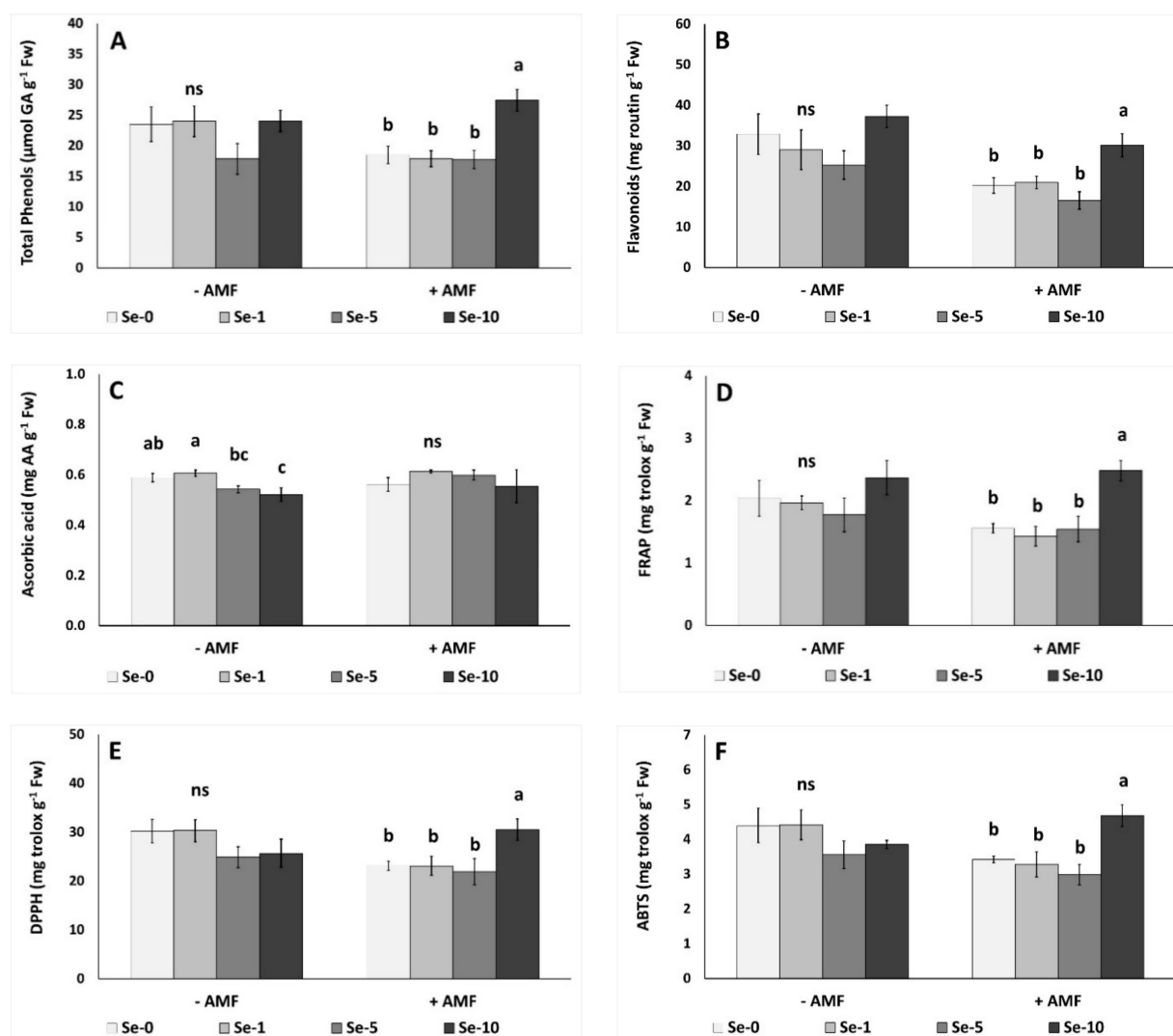


Figure 2. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on fruit total phenols, flavonoids, ascorbic acid and antioxidant activity in hydroponically grown strawberries in cocosoil. (A) Total phenols, (B) flavonoids, (C) ascorbic acid-AA, (D) FRAP, (E) DPPH and (F) ABTS. Significant differences ($p < 0.05$) among selenium treatments are indicated by different letters. Error bars show SE (n = 8).

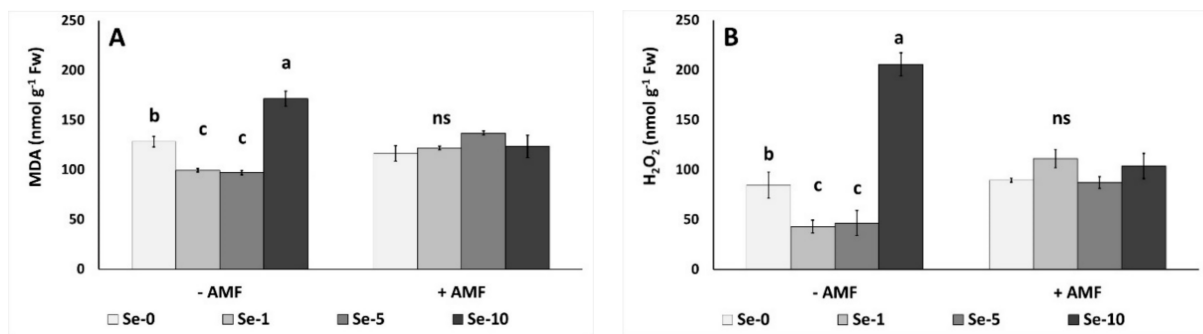


Figure 3. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on fruit lipid peroxidation and hydrogen peroxide in hydroponically grown strawberries in cocosoil. (A) Lipid peroxidation-MDA and (B) hydrogen peroxide-H₂O₂. Significant differences ($p < 0.05$) among selenium treatments are indicated by different letters. Error bars show SE ($n = 8$).

Fruit color was not affected from the Se levels in the NS in the absence of mycorrhizal inoculation of AMF (Table S1). With the presence of mycorrhizal inoculation of AMF, color b^* was decreased at Se of 10 mg L⁻¹ compared to the control or lower Se levels. Chroma was decreased at Se of 10 mg L⁻¹ in comparison to Se of 5 mg L⁻¹, while the greatest hue and color index values were found in Se of 1 mg L⁻¹ and Se of 10 mg L⁻¹, respectively (Table S1). Color L^* , a^* and whitening index did not differ among the examined treatments.

The effects on total phenols, flavonoids, ascorbic acid and antioxidant capacity of the strawberry fruits harvested from plants that were subjected to Se levels and/or mycorrhizal inoculation of AMF are presented in Figure 2. Indeed, ascorbic acid levels were decreased at Se of 10 mg L⁻¹ compared to control and ≤ 1 mg Se L⁻¹ (Figure 2C), while total phenols, flavonoids and antioxidants (FRAP, DPPH and ABTS) were remained unaffected in different Se levels without mycorrhizal inoculation of AMF (Figure 2A,B,D–F). The presence of mycorrhizal inoculation of AMF reversed the effects, as Se of 10 mg L⁻¹ increased total phenols (up to 48.4%), flavonoids (up to 48.9%), FRAP (up to 59.6%), DPPH (up to 32.0%) and ABTS (up to 36.7%) compared to the control or ≤ 5 mg Se L⁻¹, while ascorbic acid remained unaffected.

Both lipid peroxidation and hydrogen peroxide production were affected only in the case of the mycorrhizal inoculation of AMF absence (Figure 3). Therefore, low Se levels (1 and 5 Se mg L⁻¹) revealed decreased MDA and H₂O₂ levels compared to the control, while Se of 10 mg L⁻¹ increased MDA up to 33.6% (Figure 3A) and H₂O₂ up to 143.5% (Figure 3B), respectively, compared to the control.

3.4. Nutrient Accumulation and Mycorrhizal Colonization

Nutrient content in leaves, fruits and roots as affected by the Se levels and mycorrhizal colonization is presented in Figure 4. Plants grown with mycorrhizal colonization revealed increased N content in fruits but decreased N in roots at Se of 10 mg L⁻¹ (Figure 4A2,A3), increased K content in leaves at ≥ 5 mg Se L⁻¹, in fruits at Se of 5 mg L⁻¹, in roots at Se of 1 and 10 mg L⁻¹, compared to the control treatment (Figure 4B1–B3). Sodium content was increased at Se of 10 mg L⁻¹ in leaves and fruits and at ≥ 1 mg Se L⁻¹ in roots for the mycorrhizal-colonized plants (Figure 4D1–D3). In contrast, P content found decreased at ≥ 5 mg Se L⁻¹ in leaves, decreased at 5 mg Se L⁻¹ in fruits but increased at ≥ 1 mg Se L⁻¹ in roots (Figure 4C1–C3). Selenium content was increased in leaves at ≥ 5 mg Se L⁻¹, accumulated in fruits at 10 mg Se L⁻¹, but found decreased in roots at ≥ 1 mg Se L⁻¹, (Figure 4E1–E3).

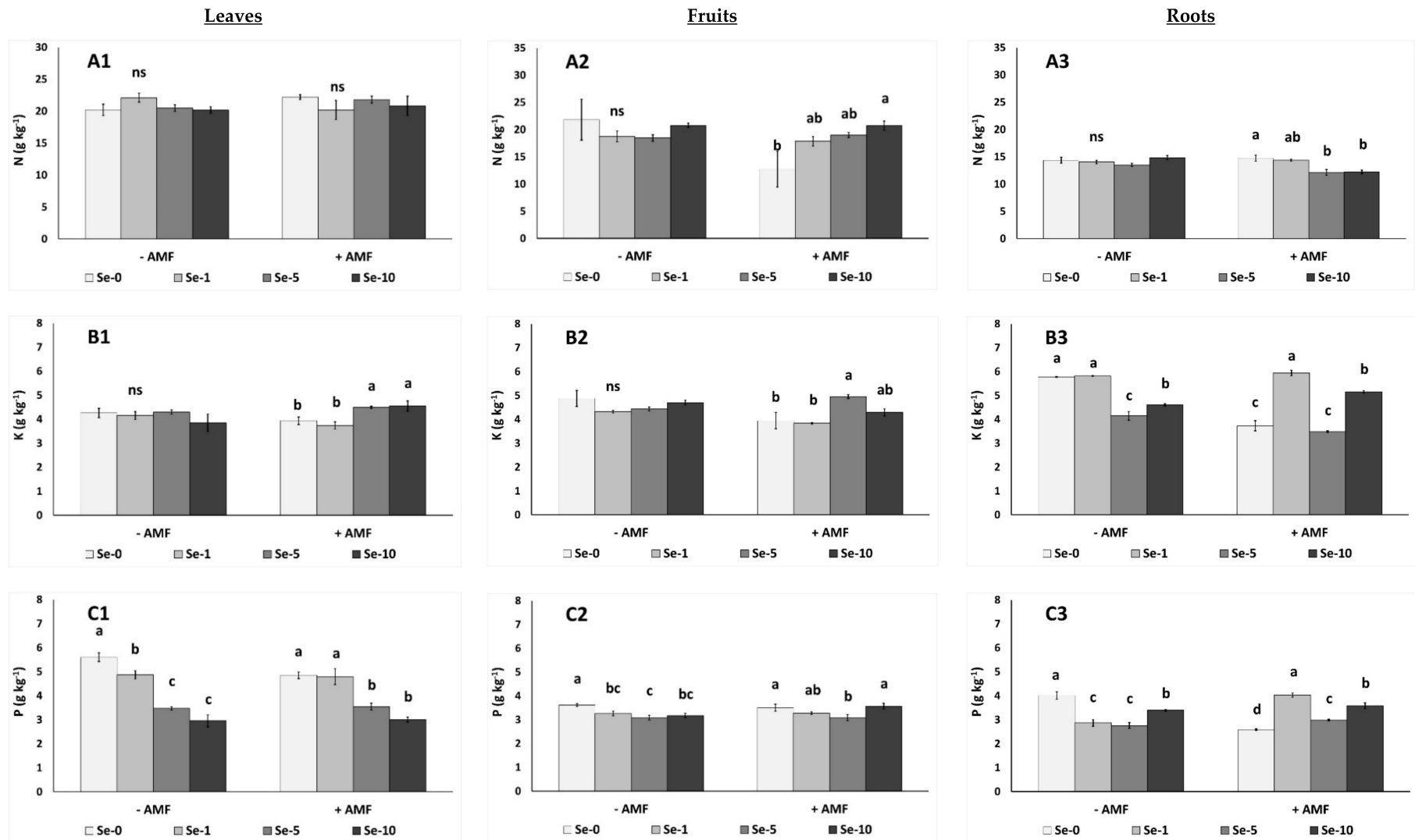


Figure 4. Cont.

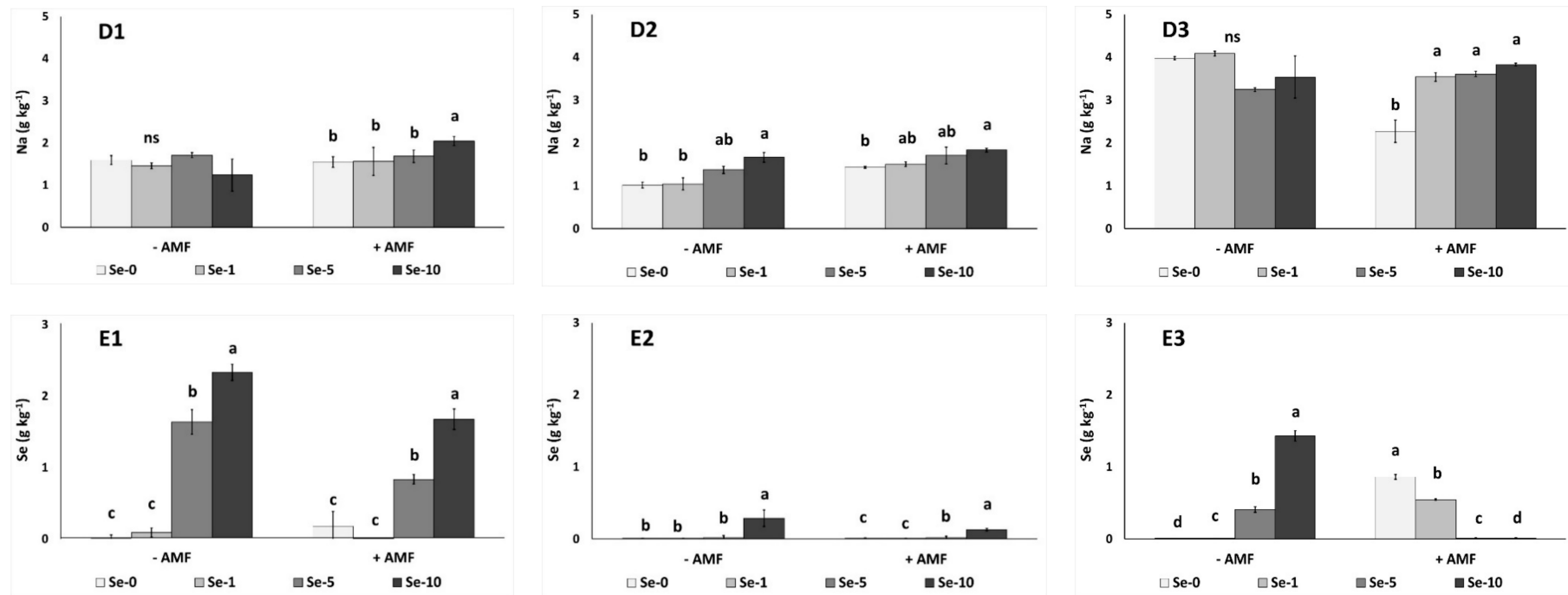


Figure 4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on nutrient content in hydroponically grown strawberries in cocosoil. (A1–E1) leaves, (A2–E2) fruits and (A3–E3) roots. Significant differences ($p < 0.05$) among selenium treatments are indicated by different letters. Error bars show SE ($n = 4$).

With the absence of mycorrhizal inoculation of AMF, K content was decreased at ≥ 5 mg Se L⁻¹ in roots (Figure 4B3), P was decreased at ≥ 1 mg Se L⁻¹ in all plant organs (leaves, fruits and roots) (Figure 4C1–C3), while Na content found increased at 10 mg Se L⁻¹ in fruits (Figure 4D2). Selenium content was increased in leaves and roots at ≥ 5 mg Se L⁻¹, and accumulated in fruits at 10 mg Se L⁻¹ (Figure 4E1–E3). There was no variation in N content between the treatments for leaves, fruits and roots (Figure 4A1–A3).

Selenium accumulation rate was significantly increased at ≥ 5 mg Se L⁻¹ with the absence of mycorrhizal inoculation of AMF, while the mycorrhizal inoculation of AMF and Se in the nutrient solution did affect the AR (Table 4). In non-mycorrhizal inoculation of AMF treated plants, BAC-leaves/stems and BAC-roots were increased at ≥ 5 mg Se L⁻¹, and BAC-fruits were increased at 10 mg Se L⁻¹. The greatest TF values in leaves/stems and fruits were observed at 5 and 1 mg Se L⁻¹, respectively. Strawberry plants inoculated with mycorrhizal inoculation of AMF, revealed increased BAC-leaves/stems at ≥ 5 mg Se L⁻¹, increased BAC-fruits at 10 mg Se L⁻¹ and increased BAC-roots at roots at 1 mg Se L⁻¹. Interestingly, mycorrhizal inoculation of AMF decreased BAC-roots at ≥ 5 mg Se L⁻¹ compared with the control treatment. Translocation factor was decreased in leaves/stems at ≥ 5 mg Se L⁻¹ while the greatest TF in fruits was found at 5 mg Se L⁻¹ (Table 4).

Table 4. Accumulation rate—AR (mg kg⁻¹ Dw day⁻¹), bioaccumulation coefficient—BAC and translocation factor—TF for Se in strawberry plants grown hydroponically in cocosoil:perlite (4/1 v/v) with arbuscular mycorrhizal fungi (AMF) inoculation.

Treatments	Accumulation Rate—AR (mg kg ⁻¹ Dw day ⁻¹)	Bioaccumulation Coefficient—BAC			Translocation Factor—TF		
		Leaves/Stems	Fruits	Roots	Leaves/Stems	Fruits	
– AMF	Se-0 mg L ⁻¹	−0.09 ± 0.03 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^{ab}	0.00 ± 0.00 ^c	0.03 ± 0.14 ^c	1.12 ± 0.01 ^b
	Se-1 mg L ⁻¹	0.12 ± 0.03 ^b	70.30 ± 62.56 ^b	−332.79 ± 0.27 ^c	−169.27 ± 1.32 ^d	−0.42 ± 0.37 ^c	1.96 ± 0.06 ^a
	Se-5 mg L ⁻¹	5.04 ± 0.77 ^a	324.43 ± 34.11 ^a	−33.02 ± 18.84 ^b	82.16 ± 7.91 ^b	4.05 ± 0.71 ^a	−0.41 ± 0.22 ^d
	Se-10 mg L ⁻¹	3.43 ± 1.10 ^a	231.54 ± 11.20 ^a	28.91 ± 11.44 ^a	143.03 ± 7.21 ^a	1.62 ± 0.08 ^b	0.19 ± 0.06 ^c
+ AMF	Se-0 mg L ⁻¹	0.69 ± 0.17 ^a	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.17 ± 0.22 ^a	−0.42 ± 0.22 ^b
	Se-1 mg L ⁻¹	−0.01 ± 0.01 ^a	−11.07 ± 0.22 ^b	−345.22 ± 4.01 ^c	547.80 ± 7.73 ^a	−0.02 ± 0.00 ^a	−0.63 ± 0.01 ^c
	Se-5 mg L ⁻¹	1.92 ± 0.91 ^a	163.37 ± 13.64 ^a	−7.39 ± 3.72 ^b	−42.45 ± 1.23 ^c	−3.84 ± 0.26 ^b	0.17 ± 0.08 ^a
	Se-10 mg L ⁻¹	1.26 ± 0.21 ^a	165.87 ± 14.24 ^a	12.79 ± 1.81 ^a	−33.12 ± 0.79 ^c	−4.99 ± 0.31 ^c	−0.38 ± 0.05 ^b

Values (n = 4) in each column followed by the same letter are not significantly different, $p < 0.05$.

Mycorrhizal inoculation of AMF structures were found in strawberry roots inoculated with AMF, independently if they were fertilized with Se or not (Figure 5). Plants that had not been inoculated with AMF did not have fungal structures in their roots, as the AMF presence was indicated with a blue color.

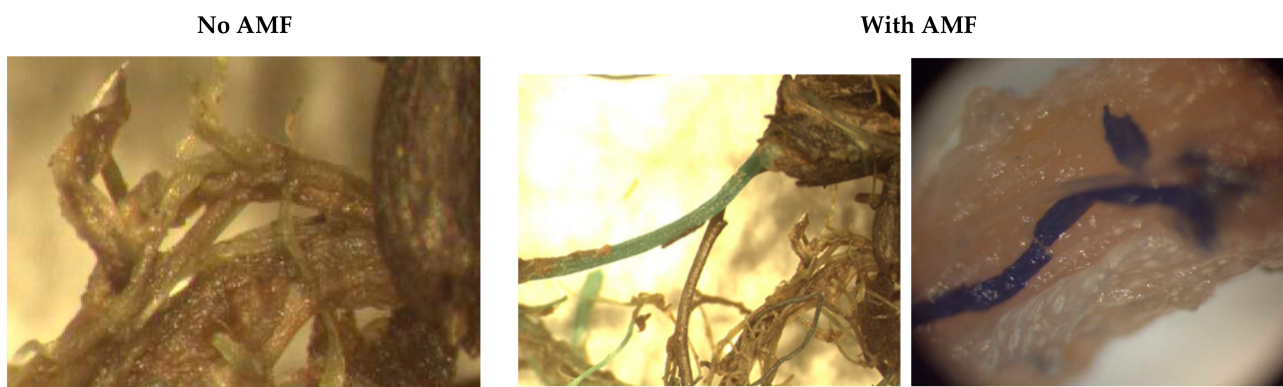


Figure 5. Cont.

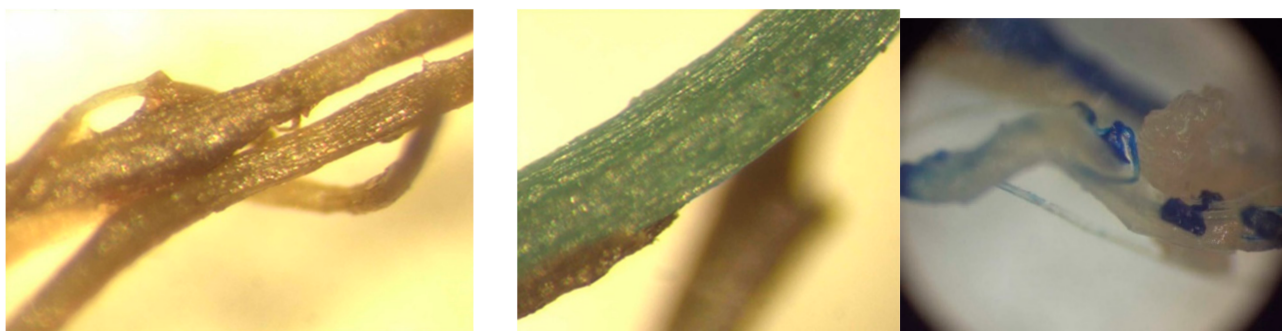


Figure 5. Arbuscular mycorrhizal fungi (AMF) inoculation measurement. Blue colored roots indicate the colonization of AMF.

4. Discussion

The aim of the present research was to investigate the feasibility of Se biofortification and the role of arbuscular mycorrhizal fungi on berry crops using hydroponically grown strawberry plants. The application of Se in low levels (e.g., $10 \mu\text{M}$ or 0.79 mg L^{-1}) has been proved to reveal growth-promoting effects [23], while higher Se levels on the other hand, resulted in toxic effects in plants, as it has been reported for tomato [8] and cucumber [43]. Therefore, in the present work, Se levels of $0\text{--}1\text{--}5\text{--}10 \text{ mg L}^{-1}$ were addressed for potential induced plant growth/metabolism and/or toxic-stress related effects.

Low and medium Se levels (i.e., $1\text{--}5 \text{ mg L}^{-1}$) induced a significant accumulation of plant biomass (up to + 144%) with no effects on fruit setting, yield and plant growth-related attributes as compared to the controls. In contrast, high Se levels (i.e., 10 mg L^{-1}) negatively influenced leaf and fruit number, mean fruit weight, which resulted to the decreased plant yield. Malorgio et al. [44] reported increased chicory and lettuce yield in plants grown hydroponically at low Se levels ($0.5\text{--}1 \text{ mg L}^{-1}$ Se). However, in other leafy vegetables as spinach, Ferrarese et al. [20], reported similar yield on hydroponically grown spinach when used low Se levels ($\leq 0.41 \text{ mg L}^{-1}$ Se). Mycorrhizal inoculation of AMF seems to support better the plant yield at control and/or low Se levels (i.e., $1\text{--}5 \text{ mg L}^{-1}$), however, mycorrhizal presence did not alleviate the negative effects that the 10 mg L^{-1} of Se caused on yield, fruit number and fruit fresh weight.

Chlorophylls content were not highly affected by Se levels, which is consistent with previous research on chicory and lettuce, when plants exposed to low Se levels of $0.5\text{--}1 \text{ mg L}^{-1}$ [44]. Plant photosynthetic rate was decreased in the case of the Se of 10 mg L^{-1} while leaf stomatal conductance was further decreased in mycorrhizal-treated plants when subjected to Se. The decreased photosynthetic rates might be correlated with the decreased P levels at the Se-exposed plants, as P plays an important role in the optimization of photosynthetic metabolism and as a prominent component of nucleic acids and phospholipids [45]. Interestingly, Sanmartín et al. [25] reported that Se addition to lettuce plants may induce the expression of genes encoding proteins implied in the photosynthesis, and finally reducing the levels of total soluble proteins in leaves, photosynthetic rates and the production of photoassimilates. This is the case with the high Se levels in our study, while mycorrhizal inoculation of AMF presence stimulates that photosynthetic rates reduction.

Fruit firmness was maintained in $\leq 5 \text{ mg L}^{-1}$ of Se in the nutrient solution, while softer fruits (decreased firmness) were observed in the case of Se of 10 mg L^{-1} . Lower fruit firmness was reflecting a shorter duration of postharvest storage for the fruits, which is of great concern, especially for strawberry fruits that have short postharvest and shelf life period and are up to decay in a great percentage. Decreased fruit firmness is a feature often derived from lower calcium accumulation in fruits [23] that makes them more sensitive to several physiological and pathological disorders [46]. Furthermore, TSS were decreased in $\geq 5 \text{ mg L}^{-1}$ of Se but this decrease was alleviated and maintained to similar levels among the examined Se concentrations in the nutrient solution, when mycorrhizal inoculation of

AMF was present, suggesting that mycorrhizal presence might have a positive effect on fruit taste at high Se levels. Titratable acidity was remained unaffected in different Se levels as described previously in strawberries, when 0.79 and 7.9 mg L⁻¹ of Se were used [23]. Strawberry fruits exhibiting sweetness index equal to 6 are considered to be of acid flavors, while when it increases to 7, it indicates a sweet fruit [47], being in accordance with the present findings and with previous reports [23].

Fruits subjected to Se biofortification accumulated Se only at 10 mg L⁻¹ of Se, while small amounts were found at ≤5 mg L⁻¹ of Se. Considering the recommended Se intake of 55–200 µg for humans [9] and the average weight per fruit (9.1 g) found in the present study, consumers could safely consume 110 g of strawberries, exposed to ≤5 mg L⁻¹ of Se, in order to reach the recommended intake. For fruits subjected to 10 mg L⁻¹ of Se, 7.45 g should be consumed (practically one fruit per day) while for fruits subjected to 10 mg L⁻¹ of Se and treated with mycorrhizal inoculation of AMF, the daily consumption could be 19.67 g of strawberries (practically two fruits per day). The above variations and recommendations are of great importance, since Se levels, cultivation practices, plant species and variety and environmental conditions can influence the accumulation rates of nutrients, and in this case, the accumulation of Se. Mimmo et al. [23] reported a safe consumption of 150 g strawberries (intake of 60 µg Se day⁻¹) when subjected to 0.8 mg L⁻¹ of Se but same portion of 150 g strawberries when subjected to 8 mg L⁻¹ would supply approximately 600 µg Se day⁻¹, which exceeds the toxic limits for humans (400 µg day⁻¹) [48]. In our study, a portion of 150 g strawberries would supply a range of 1525–4023 µg day⁻¹, which would be toxic for humans. Ferrarese et al. [20], reported the amount of spinach suggested for consumption (ranging from 25.7 to 14.2 g) for the RDA for Se, when plants were subjected to 2.6–5.2 µM Se, respectively. It is noteworthy that in the human diet, food containing less than 0.1 mg Se kg⁻¹ causes deficiency, while food containing more than 1 g Se kg⁻¹ causes toxicity [49].

Low Se levels did not affect fruit's content in total phenolics, total flavonoids, ascorbic acid and antioxidant activity, while restrained lipid peroxidation and the overproduction of hydrogen peroxide. However, Se of 10 mg L⁻¹ revealed oxidative stress as evidenced by increased MDA and H₂O₂ levels and this was reflected to the decreased ascorbic acid levels, probably due to the detoxification of the reactive oxygen species (ROS) produced by the high Se stress. Similarly, increased MDA content was found in rape and wheat plants when subjected to Se levels [45]. Interestingly, this ROS produced stress was absent in the case of mycorrhizal-treated plants, as both MDA and H₂O₂ levels were remained to similar levels as the ones of the control treatment. This effect could be explained with the increase of the non-enzymatic antioxidant mechanisms, as total phenolics, total flavonoids, ascorbic acid and antioxidant activity were induced at Se of 10 mg L⁻¹. The antioxidative effect of Se, according to Hartikainen et al. [50], was linked to α-tocopherol biosynthesis (a non-enzymatic antioxidative mechanism), but not with the increase of superoxide dismutase activity (enzymatic antioxidants). Previous reports indicating no changes on total phenolics, flavonoids and carotenoids in tomato fruits from plants grown in soil and sprayed with Se [8]. However, in the same study, it was reported that several phenolics compounds were produced in low Se levels and within short time (i.e., 24 h at 10 µM Se or 5 d at 5 Mm Se) in tomato leaves [8]. Mimmo et al. [23] also reported no changes on the content of total phenolics in Se-treated strawberry plants, however, other studies indicate an increased content of phenolics in shoots of spinach plants exposed under Se levels [51].

Selenium at ≥5 mg L⁻¹ in the nutrient solution increased Se AR, BAC-leaves, BAC-roots and BAC-fruits (only at the 10 mg L⁻¹ of Se for the latter). The highest TF-leaves was observed at 5 mg L⁻¹ of Se while the highest TF-fruits was observed at 1 mg L⁻¹ of Se. Noticeably, the high Se levels (10 mg L⁻¹) did not reflect the greatest TF-leaves and TF-fruits, probably due to the increased stress (MDA and H₂O₂ levels) and the decreased photosynthetic performance observed at 10 mg L⁻¹ of Se. Indeed, mycorrhizal presence increased BAC-leaves at ≥5 mg L⁻¹ of Se and increased BAC-fruits at 10 mg L⁻¹, but this was not evident for BAC-roots at ≥5 mg L⁻¹ of Se, indicating a decreased TF-leaves. In

addition, mycorrhizal inoculation of AMF alleviated the increased AR found at $\geq 5 \text{ mg L}^{-1}$ of Se, as AR was remained unaffected among the treatments. Sanmartín et al. [25] and Yu et al. [52], respectively, reported that mycorrhizal inoculation decreased Se accumulation in lettuce leaves and maize plants, being in agreement with the present findings. Moreover, Yu et al. [52] reported that mycorrhizal inoculation might enhance binding of Se on hyphae and on the root surface, thus inhibiting further the translocation of Se into roots and, consequently, to shoots, as this was evident in our study, with Se-treated plants and inoculated with mycorrhizal inoculation of AMF had lower TF-leaves/stems than the relevant TF in non-mycorrhized plants.

Most of the studies on the Se accumulation in the plant tissue, are focusing mainly on the accumulation in shoots [43,45,51,53], without considering the interaction of Se with other basic elements in other plant organs, fruits or roots. Besides Se, the accumulation of nutrients in plant tissues was also affected by the Se levels and/or the mycorrhizal presence. Different absorption mechanisms and Se metabolism in plants have been reported; selenate is very mobile in the xylem of plants, while selenite is quickly converted to organic forms with low mobility and are slowly translocated from roots to the shoots [4]. These differences seem to be less pronounced when working in an hydroponic environment compared to soil [12]. Selenium at the highest level (i.e., 10 mg L^{-1} of Se) in the nutrient solution resulted in Se content of 0.23% on a dried weight basis, a feature that indicates a hyperaccumulating plant [54]. Se presence, especially at high levels, decreased P in leaves, fruits and roots, decreased K in roots but increased Na in fruits. In contrast, Mimmo et al. [23] reported that P levels were not influenced by the Se concentrations in the nutrient solution, and this can be due to the different variety tested, the plant's growth stage and the experimental conditions. Broyer et al. [55] found that the alleviation of P toxicity caused a positive growth response to Se fortification, and that the antagonistic relationship between selenite and P was much less pronounced at low P levels. Phosphorus content was mainly affected by the Se levels, rather than the mycorrhizal inoculation of AMF itself, being in accordance with previous findings on lettuce [25]. Interestingly, mycorrhizal inoculation of AMF presence interfered with the nutrient accumulation and the interaction of Se with other nutrients, as leaf K was accumulated $\geq 5 \text{ mg L}^{-1}$ of Se, leaf Na, fruit N and Na were accumulated at 10 mg L^{-1} of Se when compared to the control (0 mg L^{-1} of Se with mycorrhizal). In roots of mycorrhizal-treated plants, K, P and Na were accumulated but N and Se were decreased, when compared to the control treatment. To that direction, the BAC-roots decreased at $\geq 5 \text{ mg L}^{-1}$ of Se. Sodium accumulation in plant tissue, as observed at high Se levels and combination with mycorrhizal inoculation of AMF is of importance to maintaining the membrane integrity of plant cells [4]. Based on their binding energy, selenate may have the greatest effect on the accumulation of tri- and tetravalent cations and have a lesser effect on mono- and bivalent cations [4]. Sanmartín et al. [25] investigated the role of arbuscular mycorrhizal fungi in Se biofortification in lettuce, finding that inoculated plants had higher mineral, protein and/or sugar content than the non-inoculated controls supplied with Se.

5. Conclusions

Strawberry plants appeared to be a potential target for Se biofortification to increase human intake of this important micronutrient without affecting growth and yield parameters, based on the current findings, when Se was used in $\leq 5 \text{ mg L}^{-1}$. However, Se at 10 mg L^{-1} negatively affected plant growth, yield and nutritive value of the fruits, resulting in lower firmness and TSS. Consumption of fruits harvested from strawberry plants subjected to high Se levels require high attention, as it was proved in the present study that Se was accumulated on fruits and resulted in edible parts that may be harmful to human health and only few berries could be consumed daily. Mycorrhizal inoculation of AMF can enhance the nutritional value of strawberry fruits and alleviate the induced stress that Se may cause on the plants. Biofortified strawberries can be considered a fresh produce of high nutritive value and consumers could increase the Se intake through this process. Foliar application of Se can also be examined in strawberry cultivation, while possible

nutrient solution composition amendments might be required in order to eliminate any nutrient antagonism and deficiencies, during plant growth in hydroponics.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11040721/s1>, Figure S1: Effects of mycorrhizal inoculation of AMF and selenium (Se) levels on plant development in hydroponically grown strawberries in cocosoil for 20, 49, 80 and 110 days after transplant-DAT. Figure S2: Effects of mycorrhizal inoculation of AMF and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution leaf chlorophylls content in hydroponically grown strawberries in cocosoil for 49 and 80 days after transplant-DAT. Table S1: Effects of mycorrhizal inoculation of AMF and selenium (Se) levels (0, 1, 5 and 10 mg L⁻¹) in the nutrient solution on fruit color values (*L**, *a**, *b**, chroma, hue, whitening index—WI, color index—CI) in hydroponically grown strawberries in cocosoil.

Author Contributions: Conceptualization, A.C. and N.T.; methodology, O.A., P.X., A.C. and N.T.; software, A.C.; validation, P.X. and A.C.; formal analysis, A.C.; investigation, O.A., P.X., and A.C.; resources, O.A. and N.T.; data curation, A.C.; writing—original draft preparation, A.C. and N.T.; writing—review and editing, N.T.; visualization, A.C.; supervision, A.C. and N.T.; project administration, N.T.; funding acquisition, N.T. All authors have read and agreed to the published version of the manuscript.

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References

1. Pérez, M.B.; Lipinski, V.M.; Filippini, M.F.; Chacón-Madrid, K.; Arruda, M.A.Z.; Wuilloud, R.G. Selenium biofortification on garlic growth and other nutrients accumulation. *Hortic. Bras.* **2019**, *37*, 294–301. [[CrossRef](#)]
2. Bodnar, M.; Szczygłowska, M.; Konieczka, P.; Namiesnik, J. Methods of Selenium Supplementation: Bioavailability and Determination of Selenium Compounds. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 36–55. [[CrossRef](#)] [[PubMed](#)]
3. D'Amato, R.; Regni, L.; Falcinelli, B.; Mattioli, S.; Benincasa, P.; Dal Bosco, A.; Pacheco, P.; Proietti, P.; Troni, E.; Santi, C.; et al. Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. *J. Agric. Food Chem.* **2020**, *68*, 4075–4097. [[CrossRef](#)]
4. Da Silva, E.d.N.; Cidade, M.; Heerd, G.; Ribessi, R.L.; Morgon, N.H.; Cadore, S. Effect of selenite and selenate application on mineral composition of lettuce plants cultivated under hydroponic conditions: Nutritional balance overview using a multifaceted study. *J. Braz. Chem. Soc.* **2018**, *29*, 371–379.
5. Ellis, D.R.; Salt, D.E. Plants, selenium and human health. *Curr. Opin. Plant Biol.* **2003**, *6*, 273–279. [[CrossRef](#)]
6. Sinha, R.; El-Bayoumy, K. Apoptosis is a Critical Cellular Event in Cancer Chemoprevention and Chemotherapy by Selenium Compounds. *Curr. Cancer Drug Targets* **2005**, *4*, 13–28. [[CrossRef](#)] [[PubMed](#)]
7. Fairweather-Tait, S.J.; Bao, Y.; Broadley, M.R.; Collings, R.; Ford, D.; Hesketh, J.E.; Hurst, R. Selenium in human health and disease. *Antioxidants Redox Signal.* **2011**, *14*, 1337–1383. [[CrossRef](#)]
8. Schiavon, M.; Dall'Acqua, S.; Mietto, A.; Pilon-Smits, E.A.H.; Sambo, P.; Masi, A.; Malagoli, M. Selenium fertilization alters the chemical composition and antioxidant constituents of tomato (*Solanum lycopersicon* L.). *J. Agric. Food Chem.* **2013**, *61*, 10542–10554. [[CrossRef](#)]
9. USDA. *USDA National Nutrient Database for Standard Reference*; U.S. Department of Agriculture, Agricultural Research Service: Washington, DC, USA, 2012.
10. Stone, C.A.; Kawai, K.; Kupka, R.; Fawzi, W.W. Role of selenium in HIV infection. *Nutr. Rev.* **2010**, *68*, 671–681. [[CrossRef](#)] [[PubMed](#)]
11. Combs, G.F. Selenium in global food systems. *Br. J. Nutr.* **2001**, *85*, 517–547. [[CrossRef](#)]
12. Lavu, R.V.S.; Du Laing, G.; Van De Wiele, T.; Pratti, V.L.; Willekens, K.; Vandecasteele, B.; Tack, F. Fertilizing soil with selenium fertilizers: Impact on concentration, speciation, and bioaccessibility of selenium in leek (*Allium ampeloprasum*). *J. Agric. Food Chem.* **2012**, *60*, 10930–10935. [[CrossRef](#)] [[PubMed](#)]
13. Zhao, Y.; Wu, P.; Wang, Y.; Feng, H. Different approaches for selenium biofortification of pear-jujube (*Zizyphus jujuba* M. cv. Lizao) and associated effects on fruit quality. *J. Food Agric. Environ.* **2013**, *11*, 529–534.
14. Xue, T.; Hartikainen, H.; Piironen, V. Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil* **2001**, *237*, 55–61. [[CrossRef](#)]

15. Longchamp, M.; Castrec-Rouelle, M.; Biron, P.; Bariac, T. Variations in the accumulation, localization and rate of metabolism of selenium in mature *Zea mays* plants supplied with selenite or selenate. *Food Chem.* **2015**, *182*, 128–135. [[CrossRef](#)] [[PubMed](#)]
16. Kuznetsov, V.V.; Kholodova, V.; Kuznetsov, V.; Yagodin, B. Selenium regulates the water status of plants exposed to drought. *Dokl. Biol. Sci.* **2003**, *390*, 266–268. [[CrossRef](#)] [[PubMed](#)]
17. Funes-Collado, V.; Morell-Garcia, A.; Rubio, R.; López-Sánchez, J.F. Study of selenocompounds from selenium-enriched culture of edible sprouts. *Food Chem.* **2013**, *141*, 3738–3743. [[CrossRef](#)]
18. Achibat, H.; AlOmari, N.A.; Messina, F.; Sancineto, L.; Khouili, M.; Santi, C. Organoselenium compounds as phytochemicals from the natural Kingdom. *Nat. Prod. Commun.* **2015**, *10*, 1885–1892. [[CrossRef](#)] [[PubMed](#)]
19. D’Amato, R.; De Feudis, M.; Guiducci, M.; Businelli, D. Zea mays L. Grain: Increase in Nutraceutical and Antioxidant Properties Due to Se Fortification in Low and High Water Regimes. *J. Agric. Food Chem.* **2019**, *67*, 7050–7059. [[CrossRef](#)]
20. Ferrarese, M.; Sourestani, M.; Quattrini, E.; Schiavi, M.; Ferrante, A. Biofortification of spinach plants applying selenium in the nutrient solution of floating system. *Veg. Crop. Res. Bull.* **2012**, *76*, 127–136. [[CrossRef](#)]
21. Schiavon, M.; Berto, C.; Malagoli, M.; Trentin, A.; Sambo, P.; Dall’Acqua, S.; Pilon-Smits, E.A.H. Selenium biofortification in radish enhances nutritional quality via accumulation of methyl-selenocysteine and promotion of transcripts and metabolites related to glucosinolates, phenolics amino acids. *Front. Plant Sci.* **2016**, *7*, 1371. [[CrossRef](#)]
22. Bañuelos, G.S.; Arroyo, I.; Pickering, I.J.; Yang, S.I.; Freeman, J.L. Selenium biofortification of broccoli and carrots grown in soil amended with Se-enriched hyperaccumulator *Stanleya pinnata*. *Food Chem.* **2015**, *166*, 603–608. [[CrossRef](#)]
23. Mimmo, T.; Tiziani, R.; Valentiniuzzi, F.; Lucini, L.; Nicoletto, C.; Sambo, P.; Scampicchio, M.; Pii, Y.; Cesco, S. Selenium biofortification in *Fragaria* × *ananassa*: Implications on strawberry fruits quality, content of bioactive health beneficial compounds and metabolomic profile. *Front. Plant Sci.* **2017**, *8*, 1–12. [[CrossRef](#)]
24. Golubkina, N.; Zamana, S.; Seregin, T.; Poluboyarinov, P.; Sokolov, S.; Baranova, H.; Krivenkov, L.; Pietrantonio, L.; Caruso, G. Effect of selenium biofortification and beneficial microorganism inoculation on yield, quality and antioxidant properties of shallot bulbs. *Plants* **2019**, *8*, 102. [[CrossRef](#)] [[PubMed](#)]
25. Sanmartín, C.; Garmendia, I.; Romano, B.; Díaz, M.; Palop, J.A.; Goicoechea, N. Mycorrhizal inoculation affected growth, mineral composition, proteins and sugars in lettuces biofortified with organic or inorganic selenocompounds. *Sci. Hortic.* **2014**, *180*, 40–51. [[CrossRef](#)]
26. Basiru, S.; Mwanza, H.P.; Hijri, M. Analysis of arbuscular mycorrhizal fungal inoculant benchmarks. *Microorganisms* **2021**, *9*, 1–18.
27. Marinou, E.; Chrysargyris, A.; Tzortzakis, N. Use of sawdust, coco soil and pumice in hydroponically grown strawberry. *Plant Soil Environ.* **2013**, *59*, 452–459. [[CrossRef](#)]
28. Tzortzakis, N. Influence of NaCl and calcium nitrate on lettuce and endive growth using nutrient film technique. *Int. J. Veg. Sci.* **2009**, *15*, 44–56. [[CrossRef](#)]
29. Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **1970**, *55*, 158–IN18. [[CrossRef](#)]
30. Richardson, A.D.; Duigan, S.P.; Berlyn, G.P.; Richardson, A.D. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol.* **2002**, *153*, 185–194. [[CrossRef](#)]
31. Tzortzakis, N. Potassium and calcium enrichment alleviate salinity-induced stress in hydroponically grown endives. *Hortic. Sci.* **2010**, *37*, 155–162. [[CrossRef](#)]
32. Chrysargyris, A.; Xylia, P.; Akinci, G.; Moustakas, K.; Tzortzakis, N. Printed Paper Waste as an Alternative Growing Medium Component to Produce Brassica Seedlings under Nursery Conditions. *Sustainability* **2020**, *12*, 5992. [[CrossRef](#)]
33. Chrysargyris, A.; Antoniou, O.; Tzionis, A.; Prasad, M.; Tzortzakis, N. Alternative soilless media using olive-mill and paper waste for growing ornamental plants. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35915–35927. [[CrossRef](#)] [[PubMed](#)]
34. Benimeli, C.S.; Medina, A.; Navarro, C.M.; Medina, R.B.; Amoroso, M.J.; Gómez, M.I. Bioaccumulation of copper by *Zea mays*: Impact on root, shoot and leaf growth. *Water Air Soil Pollut.* **2010**, *210*, 365–370. [[CrossRef](#)]
35. Amin, H.; Arain, B.A.; Jahangir, T.M.; Abbasi, A.R.; Mangi, J.; Abbasi, M.S.; Amin, F. Copper (Cu) tolerance and accumulation potential in four native plant species: A comparative study for effective phytoextraction technique. *Geol. Ecol. Landscapes* **2019**, *5*, 53–64. [[CrossRef](#)]
36. Azooz, M.M.; Abou-Elhamd, M.F.; Al-Fredan, M.A. Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat (*Triticum aestivum* cv. Hasaawi) at early growing stage. *Aust. J. Crop Sci.* **2012**, *6*, 688–694.
37. Xylia, P.; Clark, A.; Chrysargyris, A.; Romanazzi, G.; Tzortzakis, N. Quality and safety attributes on shredded carrots by using *Origanum majorana* and ascorbic acid. *Postharvest Biol. Technol.* **2019**, *155*, 120–129. [[CrossRef](#)]
38. Chondraki, S.; Tzerakis, C.; Tzortzakis, N. Influence of Sodium Chloride and Calcium Foliar Spray on Hydroponically Grown Parsley in Nutrient Film Technique System. *J. Plant Nutr.* **2012**, *35*, 1457–1467. [[CrossRef](#)]
39. Wojdyło, A.; Oszmiański, J.; Czemerys, R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* **2007**, *105*, 940–949. [[CrossRef](#)]
40. Meyers, K.J.; Watkins, C.B.; Pritts, M.P.; Liu, R.H. Antioxidant and Antiproliferative Activities of Strawberries. *J. Agric. Food Chem.* **2003**, *51*, 6887–6892. [[CrossRef](#)]
41. Loreto, F.; Velikova, V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.* **2001**, *127*, 1781–1787. [[CrossRef](#)]

42. De Azevedo Neto, A.D.; Prisco, J.T.; Enéas-Filho, J.; De Abreu, C.E.B.; Gomes-Filho, E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.* **2006**, *56*, 87–94. [[CrossRef](#)]
43. Hawrylak-Nowak, B. Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. *Biol. Trace Elem. Res.* **2009**, *132*, 259–269. [[CrossRef](#)]
44. Malorgio, F.; Diaz, K.E.; Ferrante, A.; Mensuali-Sodi, A.; Pezzarossa, B. Effects of selenium addition on minimally processed leafy vegetables grown in a floating system. *J. Sci. Food Agric.* **2009**, *89*, 2243–2251. [[CrossRef](#)]
45. Filek, M.; Zembala, M.; Kornaś, A.; Walas, S.; Mrowiec, H.; Hartikainen, H. The uptake and translocation of macro- and microelements in rape and wheat seedlings as affected by selenium supply level. *Plant Soil* **2010**, *336*, 303–312. [[CrossRef](#)]
46. Wójcik, P.; Lewandowski, M. Effect of calcium and boron sprays on yield and quality of “Elsanta” strawberry. *J. Plant Nutr.* **2003**, *26*, 671–682. [[CrossRef](#)]
47. Wozniak, W.; Radajewska, B.; Reszelska-Sieciechowicz, A.; Dejwor, I. Sugars and acid content influence organoleptic evaluation of fruits of six strawberry cultivars from controlled cultivation. *Acta Hortic.* **1997**, *439*, 333–336. [[CrossRef](#)]
48. Rayman, M.P. Selenium and human health. *Lancet* **2012**, *379*, 1256–1268. [[CrossRef](#)]
49. Whanger, P.D. Selenocompounds in plants and animals and their biological significance. *J. Am. Coll. Nutr.* **2002**, *21*, 223–232. [[CrossRef](#)]
50. Hartikainen, H.; Xue, T.; Piironen, V. Selenium as an anti-oxidant and pro-oxidant in ryegrass. *Plant Soil* **2000**, *225*, 193–200. [[CrossRef](#)]
51. Saffaryazdi, A.; Lahouti, M.; Ganjeali, A.; Bayat, H. Impact of Selenium Supplementation on Growth and Selenium Accumulation on Spinach (*Spinacia oleracea* L.) Plants. *Not. Sci. Biol.* **2012**, *4*, 95–100. [[CrossRef](#)]
52. Yu, Y.; Luo, L.; Yang, K.; Zhang, S. Influence of mycorrhizal inoculation on the accumulation and speciation of selenium in maize growing in selenite and selenate spiked soils. *Pedobiologia* **2011**, *54*, 267–272. [[CrossRef](#)]
53. Hawrylak-Nowak, B.; Matraszek, R.; Pogorzelec, M. The dual effects of two inorganic selenium forms on the growth, selected physiological parameters and macronutrients accumulation in cucumber plants. *Acta Physiol. Plant.* **2015**, *37*, 41. [[CrossRef](#)]
54. DeTar, R.A.; Alford, É.R.; Pilon-Smits, E.A.H. Molybdenum accumulation, tolerance and molybdenum-selenium-sulfur interactions in *Astragalus selenium* hyperaccumulator and nonaccumulator species. *J. Plant Physiol.* **2015**, *183*, 32–40. [[CrossRef](#)] [[PubMed](#)]
55. Broyer, T.C.; Johnson, C.M.; Huston, R.P. Selenium and nutrition of *Astragalus*—II. Ionic sorption interactions among selenium, phosphate, and the macro- and micronutrient cations. *Plant Soil* **1972**, *36*, 651–669. [[CrossRef](#)]