

## Effects of Mycorrhizal (*Rhizophagus irregularis*) and *Trichoderma Harzianum* Fungus on Strawberry cv. Camarosa Quality under Different Selenium Levels

A. Lachinani<sup>1</sup>, S. J. Tabatabaei<sup>2\*</sup>, A. Bostani<sup>2</sup>, V. Abdossi<sup>1</sup>, and S. Rezaee<sup>3</sup>

### ABSTRACT

The present study aimed at assessment of the beneficial role of fungal inoculation [Arbuscular Mycorrhizal Fungi (AMF) and *Trichoderma harzianum*] and Selenium (Se) treatments (0.0, 0.5, 1.0, 2.0 and 4.0 mg kg<sup>-1</sup> soil) on quality of strawberry cv. Camarosa by an emphasis on physiochemical characteristics. Fungal inoculation and Se treatment improved the fruit fresh and dry weights and fruit length, which was related to their capacity to enhance photosynthetic pigments (chlorophylls and carotenoid). Leaves protein, N, and P content as well as fruit total phenolic content and anthocyanin concentration were significantly affected by AMF and *T. harzianum* inoculation. It was found that strawberry plants inoculated with *T. harzianum* under 1.0 mg kg<sup>-1</sup> soil of Se treatment had better leaves and fruit physicochemical characteristics as compared with other treatments. Overall, inoculation of *T. harzianum* along with 1.0 mg kg<sup>-1</sup> soil of Se treatment could be recommended as an environmentally sustainable approach for improvement of the quality of strawberry cv. Camarosa fruit.

**Keywords:** Anthocyanin, Fruit weight, Nutrient elements, Photosynthetic pigments, Total phenolic.

### INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is one of the major horticultural species with high economic value. The main purpose of strawberry farming is to produce fruit with an attractive appearance (fruit size, color, and shape). Various approaches have been used to increase fruit production along with improving their quality (Kalantari *et al.*, 2020).

Today, specific consideration is given to sustainable and environmentally friendly production. The approaches associated with sustainable and ecological production techniques guarantee high fruit productions of appropriate nutritional quality, while limiting the application of mineral fertilizers and

synthetic plant protection factors (Kurokura *et al.*, 2017; Mikiciuk *et al.*, 2019). Due to the impoverishment of nutrient elements availability or improvement of soil organic elements and occurrence of different diseases, there is a requirement to apply bioproducts and beneficial microorganisms (mycorrhizal fungi, and filamentous fungi). These may enhance soil biodiversity and induce plant growth and have antagonistic impacts on microorganisms that are detrimental to plant yield (Mikiciuk *et al.*, 2019).

Arbuscular Mycorrhizal Fungi (AMF) are one of the main distributed species of endotrophic mycorrhizal fungi. AMF is recognized as an environment-friendly biofertilizer since it promotes plant growth,

<sup>1</sup> Department of Horticultural Science, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.

<sup>2</sup> Faculty of Agricultural Sciences, Shahed University, Tehran, Islamic Republic of Iran.

<sup>3</sup> Department of Plant Protection, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.

\* Corresponding author; e-mail: j.tabatabaei@shahed.ac.ir



performance, and quality. The process of re-establishing the natural amount of AMF richness can be a credible option to traditional fertilization systems, regarding sustainable agriculture (Berruti *et al.*, 2016; Chen *et al.*, 2017). Bona *et al.* (2015) found that inoculation of the strawberry cultivar 'Selva' with AMF significantly enhanced fruit growth traits and improved yield, fruit number, and fruit size as compared with conventional fertilization.

Also, *Trichoderma* is a fungal genus generally identified as a biocontrol factor in agriculture and *Trichoderma*-stimulated growth increment in plants. It induces systemic resistance of the host, thereby, promoting its health, and enhancing plant growth, development, and photosynthetic efficiency (López-Bucio *et al.*, 2015; Fraceto *et al.*, 2018). The beneficial effects of *Trichoderma* inoculation on strawberry have been previously reported by Debode *et al.* (2018).

Selenium (Se) is not considered an essential element for higher plants, but at low concentrations, it has been considered a beneficial element. Se biofortification can improve plant mineral composition, growth, yield, and quality, activation of the antioxidative defense system (Pilon-Smits, 2015; Zhu *et al.*, 2018). It was revealed that Se had various positive influences on the growth and yield of strawberry in normal and abnormal conditions (Zahedi *et al.* 2019).

Although there is some information about the effect of AMF (Bona *et al.*, 2015), *Trichoderma* (Debode *et al.*, 2018) and Se (Zahedi *et al.* 2019) on strawberry production, most of them are about quantitative parameters. Therefore, there is still a gap of research interest in their interaction effects on strawberry, especially based on quality characteristics. Therefore, this study aimed to evaluate the impacts of AMF, *Trichoderma*, and Se on some fruit, and leaves characteristics of strawberry cv. Camarosa.

## MATERIALS AND METHODS

### Plant Materials

This experiment was done in 2018 using the strawberry cv. Camarosa under greenhouse conditions. The 15-day-old uniform well-grown seedlings (three-leaf stage) were individually cultivated into plastic pots (7-liter volume, one plant per pot) filled with a mixture of perlite, coco peat and sand (5:7:23, w:w:w, respectively). Before planting, all the dead leaves and runners were removed. Along with planting, Arbuscular Mycorrhizal Fungi (AMF, *Rhizophagus irregularis*) and *Trichoderma harzianum* were mixed separately into the soil of cultivation media. All pots were placed in a research greenhouse with the following conditions: day/night temperatures of 25/15°C ( $\pm 1^\circ\text{C}$ ) and 70% ( $\pm 5\%$ ) relative humidity with a 14 h photoperiod at a light intensity of approx. 500-1000  $\mu\text{mol s}^{-1} \text{m}^{-2}$  Photosynthetic Photon Flux Density (PPFD) provided by cool-white fluorescent lights, controlled automatically (Kalantari *et al.*, 2020). Plants were irrigated daily with Hoagland solution (EC 1.7 dS  $\text{m}^{-1}$ , pH 6.0-6.5, 750 mL  $\text{pot}^{-1} \text{d}^{-1}$ ). Thereafter, plants with 5-6 fully expanded leaves were subjected to Se treatment (0.0, 0.5, 1.0, 2.0 and 4.0 mg  $\text{kg}^{-1}$  soil). Se treatments were applied by irrigation water to the cultivation media.

Fruits were harvested at the same ripening stage ( $> 75\%$  red surface color), and transferred immediately to the laboratory. To analyze fruits, 30 strawberries from each replication were used. The samples were rapidly cut, pooled, frozen in liquid nitrogen, and placed at  $-80^\circ\text{C}$  until use for future analysis.

### Characteristics Measurement

Fruit Fresh Weight (FFW), Fruit Dry Weight (FDW), and fruit length were determined immediately after harvest. The

pH and Total Soluble Solids (TSS) were assessed with juice achieved using the indelicate Super-Automatic extractor (model A2-104, China). The pH was determined by pH meter (model 691, Metrohm, AG, Herisau, Switzerland). TSS was determined with a digital refractometer (ATAGO, Tokyo, Japan). TPC was estimated using the Folin–Ciocalteu method as described by Singleton *et al.* (1999). Fruit anthocyanin concentration was assayed based on the Wagner *et al.* (1979) method.

The Chlorophyll a (Chl-a), Chlorophyll b (Chl-b), Total Chlorophyll (T-Chl), and carotenoid content were determined as described by Hiscox and Israelstam (1979). The protein amount was measured spectrophotometry based on the Bradford (1976) at 595 nm. The Nitrogen (N), Phosphorus (P), and potassium (K) concentration of leaves were assayed according to Waling *et al.* (1989) and Shiri *et al.* (2016).

### Statistical Analysis

The data were analyzed as a two-factor linear model by the PROC MIXED procedure by the SAS software (Ver. 9.1 2002–2003, SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

### Leaves Pigments Content

The results showed that the main and interaction effects of fungal inoculation and Se treatment significantly ( $P \leq 0.01$ ) affected the chlorophylls content (Table 1). As compared with the control, fungal inoculation significantly enhanced Chlorophyll a (Chl-a), Chlorophyll b (Chl-b), and Total Chlorophyll (T-Chl) content, and strawberry plants inoculated with *T. harzianum* had the highest chlorophylls content (Tables 1 and 2). As shown in Table 2, the highest Chl-a, Chl-b, and T-Chl content were found in inoculated plants with

*T. harzianum* under 1.0 and 0.5 mg kg<sup>-1</sup> soil of Se treatment (30.46 and 28.76 mg g FW<sup>-1</sup>, respectively), whereas, control plants had the lowest chlorophylls content.

Carotenoid content was significantly ( $P \leq 0.01$ ) affected by the main and interaction effects of fungal inoculation and Se treatment (Table 1). Similar to chlorophylls content, carotenoid content significantly increased in response to both AMF and *T. harzianum* inoculation (Tables 1 and 2). Among fungi-inoculated plants, the highest carotenoid content was obtained in strawberry plants inoculated with *T. harzianum* under 1.0 and 0.5 mg kg<sup>-1</sup> soil of Se treatment (Table 2).

Our findings are in accordance with Chauhan *et al.* (2010) who mentioned that inoculation of the strawberry plant (*Fragaria ananassa* Duch.) with AMF and *Trichoderma viride* significantly affected pigment content. It was reported that fungal inoculations may have down-regulated the activity of chlorophyllase and also up-regulated the expression of chlorophyll biosynthetic genes, resulting in higher pigment synthesis (Al-Arjani *et al.*, 2020). Similarly, it is well known that fungal inoculations can stimulate pigments metabolic pathways in plants (Baslam *et al.*, 2013). Furthermore, De Andrade *et al.* (2015) revealed that fungal inoculations can enhance N content in plants and results in higher pigment contents.

On the other hand, Zahedi *et al.* (2019) demonstrated that foliar application of Se significantly affected the chlorophylls and carotenoid content of strawberry plants. Previously, Germ *et al.* (2005) proposed that Se might stimulate photosynthetic pigments biosynthesis by promoting respiration and electron transport in the respiratory system. The change in photosynthetic pigments may be connected to Se impact on the protection of certain chloroplast enzymes required in the biosynthesis of photosynthetic pigments, especially, selenium plays as the catalytic center of selenoproteins, like glutathione peroxidase. Hence, it is essential to detoxify free radicals and thus protect the

**Table 1.** Effects of fungal inoculation (AMF and *T. harzianum*) and Se treatment on some leaves characteristics of strawberry cv. Camarosa at harvest.<sup>a</sup>

Treatments	Chl-a (mg FW <sup>-1</sup> )	g	Chl-b (mg FW <sup>-1</sup> )	g	TChl (mg FW <sup>-1</sup> )	g	Carotenoid (mg g FW <sup>-1</sup> )	Leaf protein (mg g <sup>-1</sup> )	N (%)	P (%)	K (%)
Fungal	**		**		**		**	**	*	**	NS
Se	**		**		**		**	**	NS	*	*
Fungal × Se	**		**		**		**	NS	NS	*	*
Fungal inoculation											
Control	5.30c		3.08c		8.38c		2.61c	0.657b	1.80b	0.183b	1.63a
AMF	7.11b		5.76b		12.87b		3.22b	0.667b	2.06a	0.210a	1.59a
<i>T. harzianum</i>	13.66a		10.52a		24.18a		4.54a	0.847a	2.04a	0.195b	1.53a
Se treatment (mg kg <sup>-1</sup> soil)											
0.0	8.54c		4.92c		13.46e		3.46d	0.76bc	1.97a	0.19b	2.07a
0.5	10.68b		8.09b		18.77c		3.64c	0.85ab	2.02a	0.22a	1.78b
1.0	12.13a		10.16a		22.29a		4.58a	0.95a	2.01a	0.19b	1.50c
2.0	12.13a		8.84b		17.29d		4.12b	0.57d	2.08a	0.20ab	1.21d
4.0	8.45c		8.69b		20.82b		3.58cd	0.66cd	2.18a	0.21ab	1.25d

<sup>a</sup> AMF: Arbuscular Mycorrhizal Fungi, Chl-a: Chlorophyll a, Chl-b: Chlorophyll b, T-Chl: Total Chlorophyll. (a-d) Means within each column followed by the same letter are not significantly different according to the Duncan's Multiple Range test. Data are the mean (n= 3). NS, \*, and \*\*: Non-Significant, Significant at P≤ 0.05, and P≤ 0.01, respectively.

**Table 2.** The interaction effects of fungal inoculation (AMF and *T. harzianum*) and Se treatment on some leaves characteristics of strawberry cv. Camarosa at harvest.<sup>a</sup>

Fungal inoculation	Se (mg kg <sup>-1</sup> soil)	Chl-a (mg g FW <sup>-1</sup> )	Chl-b (mg g FW <sup>-1</sup> )	TChl (mg FW <sup>-1</sup> )	g	Carotenoid (mg g FW <sup>-1</sup> )	P (%)	K (%)
Control	0.0	5.30de	3.08d	8.38d		2.61e	0.180b	1.63cd
AMF	0.0	4.56e	2.55d	7.11d		2.69de	0.200ab	1.84bc
AMF	0.5	13.04b	7.93c	20.97b		4.11b	0.237a	2.12ab
AMF	1.0	5.20de	3.59d	8.79d		2.93cde	0.203ab	1.65cd
AMF	2.0	6.75d	7.37c	14.12c		3.09cd	0.200ab	1.17e
AMF	4.0	6.00de	7.36c	13.36c		3.24c	0.210ab	1.20de
<i>T. harzianum</i>	0.0	12.51bc	7.28c	19.79b		4.24b	0.193ab	1.35de
<i>T. harzianum</i>	0.5	16.16a	12.60a	28.76a		4.98a	0.193ab	1.45cde
<i>T. harzianum</i>	1.0	17.51a	12.95a	30.46a		5.06a	0.183ab	2.30a
<i>T. harzianum</i>	2.0	10.89c	10.33b	21.22b		4.16b	0.200ab	1.25de
<i>T. harzianum</i>	4.0	11.22bc	9.46b	20.68b		4.23b	0.203ab	1.30de

<sup>a</sup> AMF: Arbuscular Mycorrhizal Fungi, Chl-a: Chlorophyll a, Chl-b: Chlorophyll b, T-Chl: Total Chlorophyll. (a-e) Means within each column followed by the same letter are not significantly different according to the Duncan's Multiple Range test. Data are the mean (n= 3).

photosynthetic system (Hussein *et al.*, 2019). Therefore, the changes in the concentration of photosynthetic pigments in Se treated plants may be due to the restoration of the photosynthetic ability and consequent growth attributes.

### Leaves Protein Content

It was revealed that the main effects of fungal inoculation and Se treatment significantly (P≤ 0.01) affected the leaves' protein content of strawberry cv. Camarosa, while their interaction effects were not significant (Table 1). While the control

plants ( $0.657 \text{ mg g}^{-1}$ ) and AMF inoculated plants ( $0.667 \text{ mg g}^{-1}$ ) showed no significant difference, strawberry plants inoculated with *T. harzianum* had the highest leaves protein content ( $0.847 \text{ mg g}^{-1}$ ) as compared with others. Furthermore, under Se treatment, the highest leaves protein content was observed at 1.0 and 0.5  $\text{mg kg}^{-1}$  soil of Se ( $0.95$  and  $0.85 \text{ mg g}^{-1}$ , respectively) (Table 1).

It is reported that *T. harzianum* application increases availability of N for metabolic reactions, which can provide higher levels of amino acids and proteins. Moreover, foliar spray of *Trichoderma* can stimulate the enzyme activity associated with protein metabolism (Fraceto *et al.*, 2018). Increases in protein content in response to *Trichoderma* inoculation may be attributed to higher absorption of nitrates and other ions. *Trichoderma sp.* action enhances biological nitrogen fixation in soil and nitrogen absorption by plants. *T. harzianum* could provide nitrogen oxide that is coding for an enzyme associated with L-arginine, which is essential for protein biosynthesis (Akladiou and Abbas, 2014). Also, Ryant *et al.* (2020) indicate the impact of Se on plant protein content.

### Leaves N, P and K Content

As shown in Table 1, leaves N content was significantly ( $P \leq 0.05$ ) affected by the main effect of fungal inoculation. According to Table 2, both AMF and *T. harzianum* inoculation significantly promoted the leaves N content of strawberry cv. Camarosa (2.06% and 2.04%, respectively) as compared with the control plants (1.80%). Under Se treatments, the leaves N content ranged from 1.97 to 2.18% but no significant differences were observed between different concentrations of Se treatments (Table 1).

The main and interaction effects of fungal inoculation and Se treatment significantly affected leaves P content of strawberry cv. Camarosa (Table 1). The results indicated that the highest leaves P content was obtained in strawberry plants inoculated

with AMF under 0.5  $\text{mg kg}^{-1}$  soil of Se treatment (0.237%), while the control plants showed the lowest leaves P content (0.180%) (Table 2). Meantime, other treatments had no significant difference with each other and with these treatments.

While the main effect of fungal inoculation was not significant on leaves K content, the main effect of Se treatment as well as the interaction effects of fungal inoculation and Se treatment significantly ( $P \leq 0.05$ ) affected leaves K content (Table 1). As shown in Table 2, strawberry plants inoculated with *T. harzianum* under 1.0  $\text{mg kg}^{-1}$  soil of Se treatment and also AMF inoculated plants under 0.5  $\text{mg kg}^{-1}$  soil of Se treatment had the highest leaves K content (2.30 and 2.12%, respectively).

Our findings are linked with Baslam *et al.* (2013) who concluded that fungal inoculation can change the mineral nutrients of plants. Furthermore, Castellanos-Morales *et al.* (2010) and Bona *et al.* (2015) indicated that mineral nutrients content of strawberry were significantly affected by fungal inoculation, which is an agreement with our results. It was found that fungal inoculation can change acidification of the soil rhizosphere and increases mobilization and availability of different mineral nutrients elements and hence increases micronutrient uptake (Arcand and Schneider, 2006).

On the other hand, the antagonistic or synergistic impacts between Se and other nutrient elements reported earlier can affect plant nutrient levels (Zhu *et al.*, 2017). Similar to our finding, Narváez-Ortiz *et al.* (2018) concluded that the Se application significantly affected macro- and micronutrients in strawberries.

### Fruit Fresh Weight (FFW), Fruit Dry Weight (FDW) and Fruit Length

FFW and FDW were significantly ( $P \leq 0.01$ ) affected by the main and interaction effects of fungal inoculation and Se treatments (Table 3). Generally, under

**Table 3.** Effects of fungal inoculation (AMF and *T. harzianum*) and Se treatment on some fruit physiochemical characteristics of strawberry cv. Camarosa at harvest.<sup>a</sup>

Treatments	Fruit FW (g)	Fruit DW (g)	Fruit length (mm)	TSS (°Brix)	pH	TPC (mg GAE g <sup>-1</sup> FW)	Anthocyanin (mg g <sup>-1</sup> )
Fungal	**	**	**	NS	NS	**	**
Se	**	**	**	**	NS	NS	**
Fungal × Se	**	**	**	**	NS	**	**
Fungal inoculation							
Control	5.54c	0.40c	25.58b	4.96a	3.60a	6.28a	0.23b
AMF	6.83b	0.45b	25.26b	5.50a	3.73a	5.41b	0.21b
<i>T. harzianum</i>	7.55a	0.61a	27.74a	5.59a	3.50a	6.39a	0.33a
Se treatment (mg kg <sup>-1</sup> soil)							
0.0	5.66c	0.43c	23.47c	5.15c	3.58a	6.10a	0.36a
0.5	6.64b	0.52b	25.87b	5.83a	3.70a	5.85a	0.22b
1.0	7.68a	0.53b	27.62a	5.62b	3.72a	5.78a	0.25b
2.0	7.93a	0.58a	27.33ab	5.53b	3.63a	6.12a	0.24b
4.0	8.04a	0.59a	28.21a	5.58b	3.45a	5.65a	0.26b

<sup>a</sup> AMF: Arbuscular Mycorrhizal Fungi, FFW: Fruit Fresh Weight, FDW: Fruit Dry Weight, TSS: Total Soluble Solids, TPC: Total Phenolic Content. (a-c) Means within each column followed by the same letter are not significantly different according to the Duncan's Multiple Range test. Data are the mean (n= 3).

NS, \*, and \*\*: Non-Significant, Significant at P≤ 0.05, and P≤ 0.01, respectively.

**Table 4.** The interaction effects of fungal inoculation (AMF and *T. harzianum*) and Se treatment on some fruit physiochemical characteristics of strawberry cv. Camarosa at the time of harvest.<sup>a</sup>

Fungal inoculation	Se (mg kg <sup>-1</sup> soil)	FFW (g)	FDW (g)	Fruit length (mm)	TSS (°Brix)
Control	0.0	5.53d	0.403fg	25.58c	4.97c
AMF	0.0	5.59d	0.365g	26.41bc	5.40b
AMF	0.5	7.27bc	0.464de	27.32bc	5.60b
AMF	1.0	6.97c	0.437ef	20.78d	5.63b
AMF	2.0	7.17bc	0.486d	25.39c	5.63b
AMF	4.0	7.15bc	0.488d	26.38bc	5.67b
<i>T. harzianum</i>	0.0	5.72d	0.493d	27.92bc	4.90c
<i>T. harzianum</i>	0.5	8.82a	0.573c	25.33c	5.60b
<i>T. harzianum</i>	1.0	8.69a	0.683a	30.04a	6.07a
<i>T. harzianum</i>	2.0	8.39ab	0.680a	29.27ab	5.43b
<i>T. harzianum</i>	4.0	6.14cd	0.623b	26.15c	5.50b

<sup>a</sup> AMF: Arbuscular Mycorrhizal Fungi, FFW: Fruit Fresh Weight, FDW: Fruit Dry Weight. (a-f) Means within each column followed by the same letter are not significantly different according to the Duncan's Multiple Range test. Data are the mean (n= 3).

different Se treatments, strawberry cv. Camarosa plants inoculated with AMF and *T. harzianum* had higher FFW and FDW compared with the control plants (Table 4). The *T. harzianum*-inoculated plants under 1.0 mg kg<sup>-1</sup> soil of Se treatment showed the highest FFW and FDW (8.82 and 0.683 g, respectively).

According to Table 3, the main and interaction effects of fungal inoculation and Se treatment significantly (P≤ 0.01) affected the fruit length of strawberry cv. Camarosa. While the change of fruit length depended on AMF and *T. harzianum* inoculation and Se concentration (Table 4), strawberry plants inoculated with *T. harzianum* under 1.0 and 2.0 mg kg<sup>-1</sup> soil of Se treatment had

the longest fruits (30.04 and 29.27 mm, respectively).

Improvement in strawberry growth characteristics by AMF and *Trichoderma viride* inoculation is previously reported by Chauhan *et al.* (2010), in agreement with our findings. The increased FFW, FDW, and fruit length in fungal inoculation plants may be due to higher absorption of mineral ions, such as N. Macro-elements control essential physiological and biochemical processes, like cell division, enzyme activity, and proteins synthesis (Elkelish *et al.*, 2020). Additionally, fungal inoculated strawberry plants had higher chlorophylls content, which presumably leads to an increase in photosynthetic rates of inoculated plants, thus benefiting fruit growth and development.

In agreement with our results, Narváez-Ortiz *et al.* (2018) mentioned that morphological characteristics of strawberry plants improved in response to Se application. It was proposed that the variations in the growth and morphological characteristics of groundnut plants due to Se treatment may be associated with the action of plant hormones, modification of the antioxidant systems, raising transpiration rate, net photosynthetic rate, and nitrogen metabolism (Hussein *et al.*, 2019).

### Fruit pH Content

The pH content of strawberry cv. Camarosa ranged from 3.50 to 3.73, but no significant differences were observed between different inoculated and control plants (Table 3). This result is in accordance with Cecatto *et al.* (2016), who noted that fungal inoculation had no significant effect on some phytochemical content in strawberry fruits, such as pH content.

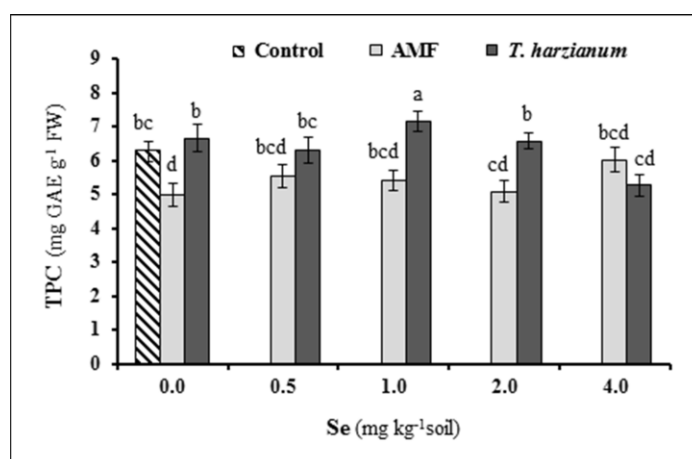
### Fruit TSS and TPC

The results indicated that TSS content was significantly ( $P \leq 0.01$ ) affected by the main

effect of Se treatment and the interaction effects of fungal inoculation and Se treatment, while the main effect of fungal inoculation was not significant (Table 3). As shown in Table 4, under different Se concentration, both AMF and *T. harzianum* inoculation significantly enhanced fruit TSS content as compared with control fruits, except *T. harzianum* inoculated plants under 0.0 mg kg<sup>-1</sup> soil of Se treatment. In the meantime, the highest fruit TSS content (6.07 °Brix) was found in strawberry plants inoculated with *T. harzianum* under 1.0 mg kg<sup>-1</sup> soil of Se treatment (Table 4).

It was revealed that the main effect of fungal inoculation and the interaction effects of fungal inoculation and Se treatment significantly ( $P \leq 0.01$ ) affected the fruit TPC, whereas the main effect of Se treatment was not significant (Table 3). Generally, AMF inoculation slightly reduced fruit TPC as compared with the control (Figure 1). The highest fruit TPC was found in *T. harzianum* inoculated plants under 1.0 mg kg<sup>-1</sup> soil of Se treatment (7.16 mg GAE g<sup>-1</sup> FW), while other *T. harzianum* inoculated plants showed no significant difference compared with the control.

Similar to our findings, Castellanos-Morales *et al.* (2010) and Ansari *et al.* (2018) reported that fungal inoculation altered some biochemical compounds of strawberry fruit such as soluble sugars, phenolic acids, and flavonols content. Furthermore, Hussein *et al.* (2019) mentioned that the Se application significantly affected TSS and TPC in groundnut. It seems that these results might be due to the higher chlorophylls and carotenoids content in treated plants, which could improve photosynthetic rate, the importance of carbohydrates to fruit, and increase metabolic processes in fruit. Additionally, it is hypothesized that fungal inoculation has a critical function in the activation of enzymes and, consequently, induce the biosynthesis of various biochemical compounds (Ansari *et al.*, 2018).



**Figure 1.** Effects of fungal inoculation [Arbuscular Mycorrhizal Fungi (AMF) and *T. harzianum*] and Se treatment on Total Phenolic Content (TPC) of strawberry cv. Camarosa at the harvest time. Vertical bars indicate standard error ( $n=3$ ). Means followed by the same letter are not significantly different according to the Duncan's Multiple Range test.

### Fruit Anthocyanin Content

Fruit anthocyanin content of strawberry cv. Camarosa was significantly ( $P \leq 0.01$ ) affected by the main and interaction effects of fungal inoculation and Se treatment (Table 3). AMF inoculated plants under 0.0 and 4.0 mg kg<sup>-1</sup> soil of Se significantly reduced fruit anthocyanin content as compared with the control, while no significant difference was observed between the control plants and AMF inoculated plants under 0.5, 1.0 and 2.0 mg kg<sup>-1</sup> soil of Se treatment (Figure 2). Additionally, *T. harzianum* inoculation significantly enhanced fruit anthocyanin content as compared with the control. The highest fruit anthocyanin content (0.363 mg g<sup>-1</sup>) was obtained in *T. harzianum* inoculated plants under 1.0 mg kg<sup>-1</sup> soil of Se.

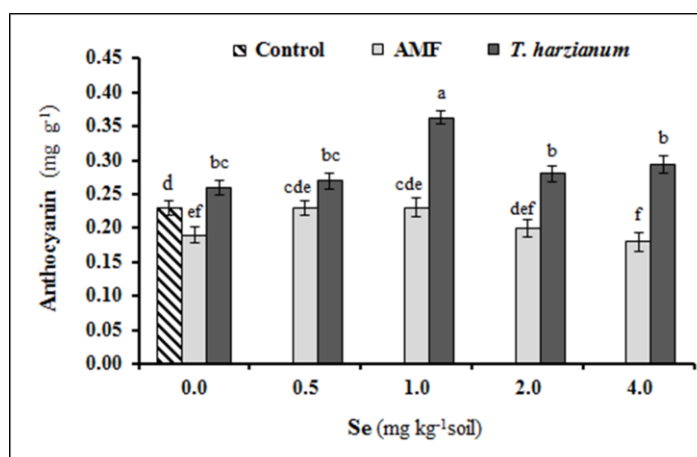
Increase in anthocyanin content in response to fungal inoculation is previously mentioned by Castellanos-Morales *et al.* (2010), Lingua *et al.* (2013) and Cecatto *et al.* (2016) in strawberry fruit, associated with increase in the activity of the enzyme in anthocyanin production pathway. Furthermore, Mechora *et al.* (2017) reported that anthocyanin content significantly increased under Se application in broccoli

plants. These might be related to the effect of Se on the molecular regulation of anthocyanin synthesis and the expression levels of some genes such as F3H and UFGT (Liu *et al.*, 2017).

It should be noted that in some cases, we found that Se treatment in higher concentration (especially at 4 mg kg<sup>-1</sup> soil) had inhibitory effect on the evaluated characteristics. These results are in agreement with Narváez-Ortiz *et al.* (2018) in the strawberry plant. It is known that the impact of Se on plants depends on its concentration (Hamilton, 2004): a low Se content will promote growth, but high levels can inhibit it (Boldren *et al.*, 2016). Ahmed (2010) observed a decrease in the fresh weight of tomato plants after applying Se in the forms of sodium selenite and organic Se in doses of 2 and 30 mg kg<sup>-1</sup> of soil, respectively. Ramos *et al.* (2010) indicated a reduction in the DW of lettuce plants when Se was applied in concentrations more than 0.6 mg L<sup>-1</sup>; both the selenite and sodium selenate forms were used, with selenite having a more significant effect.

It has been suggested that the negative effects of Se on growth is partly due to the substitution of sulfur by Se in proteins, which could modify the functionality of those proteins and sulfur metabolism in the plant (Boldren *et al.*, 2016). Perhaps this





**Figure 2.** Effects of fungal inoculation [Arbuscular Mycorrhizal Fungi (AMF) and *T. harzianum*] and Se treatment on anthocyanin content of strawberry cv. Camarosa at the harvest time. Vertical bars indicate standard error (n= 3). Means followed by the same letter are not significantly different according to the Duncan's Multiple Range test.

effect is different from one plant organ to another, which could explain the positive results in some organs and the absence of response, or undesirable effects, in others.

## CONCLUSIONS

The present results showed that the chlorophylls, carotenoids, proteins, N, and P content of strawberry cv. Camarosa leaves could increase in fungal inoculated plants under different Se treatments. Moreover, FFW, FDW, fruit length, TSS, TPC, and anthocyanin content were significantly affected by AMF and *T. harzianum* inoculation as well as Se treatments. Since *T. harzianum* inoculated plants under 1.0 mg kg<sup>-1</sup> soil of Se treatment showed better leaves and fruit physicochemical characteristics, this combined treatment would be a highly recommended practice in the production of strawberry cv. Camarosa fruit.

## REFERENCES

- Ahmed, H. K. 2010. Differences between Some Plants in Selenium Accumulation from Supplementation Soils with Selenium. *Agric. Biol. J. N. Am.*, **1**: 1050-1056.
- Akladios, S. A. and Abbas, S. M. 2014. Application of *Trichoderma harzianum* T22 as a Biofertilizer Potential Maize Growth. *J. Plant Nutr.*, **37**(1): 30-49.
- Al-Arjani, A. B. F., Hashem, A. and Abd\_Allah, E. F. 2020. Arbuscular mycorrhizal Fungi Modulates Dynamics Tolerance Expression to Mitigate Drought Stress in *Ephedra foliata* Boiss. *Saudi J. Biol. Sci.*, **27**(1): 380-394.
- Ansari, M. H., Hashemabadi, D., Mahdavi, M. and Kaviani, B. 2018. The Role of Pseudomonas Strains and Arbuscular Mycorrhiza Fungi as Organic Phosphate-Solubilizing in the Yield and Quality Improvement of Strawberry (*Fragaria×ananassa* Duch., cv. Selva) Fruit. *Acta Sci. Pol. Hortorum Cultus*, **17**(4): 93-107.
- Arcand, M. M. and Schneider, K. D. 2006. Plant- and Microbial-Based Mechanisms to Improve the Agronomic Effectiveness of Phosphate Rock: A Review. *An. Acad. Bras. Ciênc.*, **78**(4): 791-807.
- Baslam, M., Garmendia, I. and Goicoechea, N. 2013. Enhanced Accumulation of Vitamins, Nutraceuticals and Minerals in Lettuces Associated with Arbuscular Mycorrhizal Fungi (AMF): A Question of Interest for Both Vegetables and Humans. *Agric.*, **3**: 188-209.
- Berruti, A., Lumini, E., Balestrini, R. and Bianciotto, V. 2016. Arbuscular Mycorrhizal Fungi as Natural Biofertilizers:



- Let's Benefit from Past Successes. *Front. Microbiol.*, **6**: 1559.
8. Boldren, P. F., De Figueiredo, M. A., Yang, Y., Luo, H., Giri, S., Hart, J. J., Faquin, V., Guilherme, L. R. G., Thanhauser, T. W. and Li, L. 2016. Selenium Promotes Sulfur Accumulation and Plant Growth in Wheat (*Triticum aestivum*). *Physiol. Plant.*, **158**: 80-91.
  9. Bona, E., Lingua, G., Manassero, P., Cantamessa, S., Marsano, F., Todeschini, V., Copetta, A., D'Agostino, G., Massa, N., Avidano, L., Gamalero, E. and Berta, G. 2015. AM Fungi and PGP Pseudomonads Increase Flowering, Fruit Production, and Vitamin Content in Strawberry Grown at Low Nitrogen and Phosphorus Levels. *Mycorrhiza*, **25**: 181-193
  10. Bradford, M. M. 1976. A Rapid Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein Dye Binding. *Anal. Biochem.*, **72**: 248-254.
  11. Castellanos-Morales, V., Villegas, J., Wendelin, S., Vierheilig, H., Eder, R. and Cárdenas-Navarro, R. 2010. Root Colonisation by the Arbuscular Mycorrhizal Fungus *Glomus intraradices* Alters the Quality of Atrawberry Fruits (*Fragaria×ananassa* Duch.) at Different Nitrogen Levels. *J. Sci. Food Agric.*, **90**: 1774-1782.
  12. Cecatto, A. P., Ruiz, F. M., Calvete, E. O., Martínez, J. and Palencia, P. 2016. Mycorrhizal Inoculation Affects the. *Acta Sci. Agron.*, **38(2)**: 227-237.
  13. Chauhan, S., Kumar, A., Mangla, C. and Aggarwal, A. 2010. Response of Strawberry Plant (*Fragaria ananassa* Duch.) to Inoculation with Arbuscular Mycorrhizal Fungi and *Trichoderma viride*. *J. Appl. Nat. Sci.*, **2(2)**: 213-218.
  14. Chen, S., Zhao, H., Zou, C., Li, Y., Chen, Y., Wang, Z., Jiang, Y., Liu, A., Zhao, P., Wang, M. and Ahammed, G. J. 2017. Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings. *Front. Microbiol.*, **8**: 2516.
  15. De Andrade, S. A. L., Domingues, A. P. and Mazzafera, P. 2015. Photosynthesis Is Induced in Rice Plants that Associate with Arbuscular Mycorrhizal Fungi and Are Grown under Arsenate and Arsenite Stress. *Chemosphere*, **134**: 141-149.
  16. Debode, J., De Tender, C., Cremelie, P., Lee, A. S., Kyndt, T., Muylle, H., De Swaef, T. and Vandecasteele, B. 2018. *Trichoderma*-Inoculated Miscanthus Straw Can Replace Peat in Strawberry Cultivation, with Beneficial Effects on Disease Control. *Front. Plant Sci.*, **9**: 213.
  17. Elkelish, A. A., Alhaithloul, H. A. S., Qari, S. H., Soliman, M. H. and Hasanuzzaman, M. 2020. Pretreatment with *Trichoderma harzianum* Alleviates Waterlogging-Induced Growth Alterations in tomato Seedlings by Modulating Physiological, Biochemical, and Molecular Mechanisms. *Environ. Exp. Bot.*, **171**: 103946.
  18. Fraceto, L. F., Maruyama, C. R., Guilger, M., Mishra, S., Keswani, C., Singh, H. B. and de Lima, R. 2018. *Trichoderma harzianum* Based Novel Formulations: Potential Applications for Management of Next-Gen Agricultural Challenges. *J. Chem. Technol. Biotechnol.*, **93(8)**: 2056-2063.
  19. Germ, M., Kreft, I. and Osvald, J. 2005. Influence of UV-B Exclusion and Selenium Treatment on Photochemical Efficiency of Photosystem. II. Yield and Respiratory Potential in Pumpkins (*Cucurbita pepo* L.). *Plant Physiol. Bioch.*, **43**: 445-448.
  20. Hamilton, S. J. 2004. Review of Selenium Toxicity in the Aquatic Food Chain. *Sci. Total Environ.*, **326**: 1-31.
  21. Hiscox, J. D. and Israelstam, G. F. 1979. A Method for the Extraction of Chlorophyll from Leaf Tissue without Maceration. *Can. J. Bot.*, **57(12)**: 1332-1334.
  22. Hussein, H. A. A., Darwesh, O. M. and Mekki, B. B. 2019. Environmentally Friendly Nano-Selenium to Improve Antioxidant System and Growth of Groundnut Cultivars under Sandy Soil Conditions. *Biocatal. Agric. Biotechnol.*, **18**: 101080.
  23. Kalantari, M. R., Abdossi, V., Mortazaeinezhad, F., Golparvar, A. R., and Shahshahan, Z. 2020. Foliar Application of Ethinyl Estradiol and Progesterone Affects Morphological and Fruit Quality Characteristics of Strawberry cv. Camarosa. *Hortic. Sci. Technol.*, **38(2)**: 146-157.
  24. Kurokura, T., Hiraide, S., Shimamura, Y. and Yamane, K. 2017. PGPR Improves Yield of Strawberry Species under Less-

- Fertilized Conditions. *Environ. Control Biol.*, **55**(3): 121-128.
25. Lingua, G., Bona, E., Manassero, P., Marsano, F., Todeschini, V., Cantamessa, S., Copetta, A., D'Agostino, G., Gamalero, E. and Berta, G. 2013. Arbuscular Mycorrhizal Fungi and Plant Growth-Promoting Pseudomonads Increases Anthocyanin Concentration in Strawberry Fruits (*Fragaria×ananassa* var. Selva) in Conditions of Reduced Fertilization. *Int. J. Mol. Sci.*, **14**: 16207-16225.
  26. Liu, D., Li, H., Wang, Y., Ying, Z., Bian, Z., Zhu, W., Liu, W., Yang, L. and Jiang, D. 2017. How Exogenous Selenium Affects Anthocyanin Accumulation and Biosynthesis-Related Gene Expression in Purple Lettuce. *Pol. J. Environ. Stud.*, **26**(2): 717-722.
  27. López-Bucio, J., Pelagio-Flores, R. and Herrera-Estrella, A. 2015. *Trichoderma* as Biostimulant: Exploiting the Multilevel Properties of a Plant Beneficial Fungus. *Sci. Hortic.*, **196**: 109-123.
  28. Mechora, Š., Torre, D. P., Bruns, R. E., Škof, M. and Ugrinović, K. 2017. Effect of Selenium Treated Broccoli on Herbivory and Oviposition Preferences of *Delia radicum* and *Phyllotreta* spp. *Sci. Hortic.*, **225**: 445-453.
  29. Mikiciuk, G., Sas-Paszt, L., Mikiciuk, M., Derkowska, E., Trzciński, P., Głuszek, S., Lisek, A., Wera-Bryl, S. and Rudnicka, J. 2019. Mycorrhizal Frequency, Physiological Parameters, and Yield of Strawberry Plants Inoculated with Endomycorrhizal Fungi and Rhizosphere Bacteria. *Mycorrhiza*, **29**: 489-501.
  30. Narváez-Ortiz, W. A., Martínez-Hernández, M., Fuentes-Lara, L. O., Benavides-Mendoza, A., Valenzuela-García, J. R. and González-Fuentes, J. A. 2018. Effect of Selenium Application on Mineral Macro- and Micronutrients and Antioxidant Status in Strawberries. *J. Appl. Bot. Food Qual.*, **91**: 321-331.
  31. Pilon-Smits, E. A. H. 2015. Selenium in Plants. In: "*Progress in Botany*", (Eds.): Luttge, U. and Beyschlag, W. Springer International Publishing, Switzerland, PP. 93–107.
  32. Ramos, S. J., Faquin, V., Guilherme, L. R. G., Castro, E., Ávila, F. W., Carvalho, G. S., Bastos, C. E. A. and Olivera, C. 2010: Selenium Bio-Fortification and Antioxidant Activity in Lettuce Plants Fed with Selenate and Selenite. *Plant Soil Env.* **56**: 584-588.
  33. Ryant, P., Antošovský, J., Adam, V., Ducsay, L., Škarpa, P. and Sapáková, E. 2020. The Importance of Selenium in Fruit Nutrition. In: "*Fruit Crops Diagnosis and Management of Nutrient Constraints*". Publisher: Elsevier, PP. 241-254.
  34. Shiri, M. A., Ghasemnezhad, M., Fatahi Moghadam, J. and Ebrahimi, R. 2016. Efficiency of CaCl<sub>2</sub> Spray at Different Fruit Development Stages on the Fruit Mineral Nutrient Accumulation in 'Hayward' Kiwifruit. *J. Elementol.*, **21**(1): 195-209.
  35. Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R.S. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin- Ciocalteau Reagent. *Meth. Enzymol.* **299**: 152-178.
  36. Wagner, G. J. 1979. Content and Vacuole/Extra Vacuole Distribution of Neutral Sugars, Free Amino Acids and Anthocyanin in Protoplast. *Plant Physiol.*, **68**: 88-93.
  37. Waling, I., Van Vark, W., Houba, V. J. G. and Van der Lee, J. J. 1989. Soil and Plant Analysis, a Series of Syllabi. Part 7. Plant Analysis Procedures. Wageningen Agriculture University.
  38. Zahedi, S. M., Abdelrahman, M., Sadat Hosseini, M., Fahadi Hoveizeh, N. and Tran, L. S. P. 2019. Alleviation of the Effect of Salinity on Growth and Yield of Strawberry by Foliar Spray of Selenium-Nanoparticles. *Environ. Pollut.*, **253**: 246-258.
  39. Zhu, Z., Zhang, Y., Liu, J., Chen, Y. and Zhang, X. 2018. Exploring the Effects of Selenium Treatment on the Nutritional Quality of Tomato Fruit. *Food Chem.*, **252**: 9-15.
  40. Zhu, L., Wang, P., Zhang, W., Hui, F. and Chen, X. 2017. Effects of Selenium Application on Nutrient Uptake and Nutritional Quality of *Codonopsis lanceolata*. *Sci. Hortic.*, **225**: 574-580.



## اثرات قارچ‌های میکوریز (*Rhizophagus irregularis*) و *Trichoderma Harzianum* بر کیفیت توت‌فرنگی رقم کاماروسا تحت سطوح مختلف سلنیوم

ع. لچینانی، س. ج. طباطبائی، ا. بستانی، و. عبدوسی، و س. رضائی

### چکیده

بررسی حاضر با هدف ارزیابی نقش سودمند تلقیح قارچی [قارچ‌های میکوریز آربوسکولار (AMF) و *Trichoderma harzianum*] و تیمار سلنیوم (۰/۰، ۰/۵، ۱/۰، ۲/۰ و ۴/۰ میلی‌گرم بر کیلوگرم در خاک) بر کیفیت گیاهان توت‌فرنگی رقم کاماروسا با تأکید بر ویژگی‌های فیزیکیوشیمیایی انجام شد. تلقیح قارچی و تیمار سلنیوم وزن تر و خشک و طول میوه را بهبود بخشید که با افزایش ظرفیت رنگدانه‌های فتوسنتزی (کلروفیل‌ها و کاروتنوئید) آن‌ها در ارتباط بود. میزان پروتئین برگ، نیتروژن و پتاسیم و همچنین محتوای فنل کل و غلظت آنتوسیانین به طور معنی‌داری تحت تأثیر تلقیح AMF و *Trichoderma harzianum* قرار گرفت. مشخص شد که گیاهان توت‌فرنگی که با *Trichoderma harzianum* تلقیح شده بودند، تحت سطوح ۱/۰ میلی‌گرم بر کیلوگرم در خاک تیمار سلنیوم در مقایسه با سایر تیمارها دارای ویژگی‌های فیزیکیوشیمیایی میوه و برگ بهتری بودند. در مجموع، تلقیح *Trichoderma harzianum* به همراه ۱/۰ میلی‌گرم بر کیلوگرم خاک سلنیوم می‌تواند به عنوان رویکرد پایدار زیست محیطی برای بهبود تولید میوه توت‌فرنگی رقم کاماروسا توصیه شود.