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Abstract: The aim of this study was to determine the effect of a mycorrhizal inoculation (AMF) and irrigation regime on certain yield morphological parameters and the biological value of fruits of open field-grown sweet pepper under temperate climate conditions. A study on the Polish hybrid cultivar 'Roberta F_1 ' was conducted over the period 2016–2018 in a private certified organic farm. Sweet pepper was harvested at physiological maturity from the second 10 days of August to the first 10 days of October. AMF and irrigation were shown to significantly modify the selected morphological parameters of the peppers. Fruits with the highest weight, length, and width were harvested from AMF-inoculated plants, both irrigated and non-irrigated ones. The chemical composition and antioxidant activity (AA) of pepper fruit extracts were significantly affected by AMF and irrigation. AMF application contributed to a decrease in the percentage of dry matter, vitamin C, reducing sugars, extract, carotenoids, and AA. Irrigation, on the other hand, had a beneficial effect on enhancing the biological value of pepper fruits (except for vitamin C), also increasing their AA. The highest levels of carotenoids (4.64 mg 100 g⁻¹ of fresh matter (FM) were found in the fruits of irrigated plants without AMF, whereas the highest levels of vitamin C (134.10 mg 100 g⁻¹ FM) were accumulated by the fruits of plants grown without AMF and without irrigation.

Keywords: *Capsicum annuum* L.; arbuscular mycorrhizal fungi; organic farming; vitamin C; carotenoids; DPPH

1. Introduction

Capsicum annuum L. (sweet pepper) is one of the five domesticated pepper species, which was probably domesticated in one or two areas of Mexico, notably the north-eastern and central-eastern regions [1–3]. Sweet pepper is now grown across the world as a vegetable, spice, and medicinal plant in order to obtain fresh and dried peppers as well as processed products. Pepper cultivars differ in many morphological characters and the content of biologically active substances [4], but also in their resistance to environmental stress [5,6] and to diseases caused by fungi, bacteria, and viruses [7]. Sweet peppers are characterized by a high content of vitamin C [165.4 mg 100 g⁻¹] as well as of sugars, carotenoids, tocopherols, polyphenols, and minerals [2].

The cultivation method and certain agronomic practices can significantly increase the quality of pepper fruits and their biological value [8–12]. Water availability is the most limiting factor in plant production in dry areas. Pepper is very sensitive to water stress and water deficit reduces fresh fruit yield [13,14]. The irrigation level and frequency have an impact on the yield quality parameters of peppers grown under field conditions and an appropriate irrigation system is recommended in order to obtain higher yields of better quality [15–17]. Irrigation management techniques based on soil sensors are helpful in achieving a high pepper yield [18]. Irrigation in mulched soil ensures higher productivity of peppers with lower water consumption, thus increasing water use efficiency [19].



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The importance of microbiological fertilizers and their beneficial effects on crop plants have been stressed recently. Such fertilizers are considered to be an ecological alternative to overused and environmentally harmful chemical fertilizers and agrochemicals [20]. Biostimulants, among others arbuscular mycorrhizal fungi (AMF), arouse great interest among the scientific and production community because they not only modify the response of plants to environmental changes but also modulate their metabolism, thus influencing the biosynthesis of organic substances. AMF use is becoming common practice in sustainable horticultural production [21–24]. Mycorrhization improves the biological properties of the rhizosphere, particularly under water stress and elevated salinity conditions [25,26]. Mycorrhizal inoculation has a protective effect on young pepper plants infected with *Fusarium oxysporum* and also increases their photosynthetic activity [27]. Application of microorganism-enriched fertilization in pepper cultivation contributes to an increase in fruit yield as well as in polyphenol content and antioxidant activity [28–30]. Buczkowska and Sałata [31] found the highest yield and number of pepper fruits when simultaneous AMF inoculation and irrigation throughout the entire growing season were applied, but AMF colonization was of greater importance than irrigation.

Application of biostimulants changes the metabolic profile of peppers and results in an accumulation of carotenoids, saponins, and phenolic compounds in plants [32]. Beneficial microbes and combined microbial inoculation have different potentials to modulate the defensive enzymes and positively influence pepper fruit yield under field conditions [33]. Recently, with changing climatic conditions, including increasing humidity deficiencies, the use of plant-friendly and environment-friendly biostimulation seems to be the key task of modern agrotechnics. In addition, in case of insufficient humidity, it becomes necessary to irrigate crops, which especially applies to plants with high water requirements. The aim of the present study was to determine the effect of mycorrhizal inoculation and irrigation on some morphological parameters and the biological value of fruits of open field-grown sweet pepper under temperate climate conditions.

2. Materials and Methods

2.1. Materials and Growing Conditions

The study was carried out over the period 2016–2018 (the weather conditions are listed in Table S1) in a private certified organic farm (Agrobiotest 04557), located in south-eastern Poland (51.36° N, 22.83° E). A Polish hybrid cultivar of sweet pepper (C. annuum L.), 'Roberta F_1 ' (breeder-Department of Plant Genetics, Breeding and Biotechnology SGGW Warsaw), was selected for the study. The selection of this cultivar was guided by its great practical importance for producers and high yield reliability, regardless of weather conditions during cultivation [34]. A two-factor experiment was conducted in a randomized block design in 4 replicates, with 20 plants per plot (plot area 4.7 m²). The experimental factors were as follows: 1-Mycorrhiza; Arbuscular Mycorrhizal Fungi (AMF): plants with AMF (AMF); plants without AMF (non-AMF); 2-Irrigation: plants with irrigation (irrigation); plants without irrigation (non-irrigation), and Control: plants without AMF and without irrigation. The experiment used a commercial mycorrhizal inoculant (Mycoflor, Końskowola, Poland) containing spores and dormant mycelium of Mycorrhizal Fungi (Rhizophagus aggregatus, R. intraradices, Claroideoglomus etunicatum, Endogone mosseae, *Funneliformis caledonium,* and *Gigaspora margarita*) mixed with peat [27]. Pepper was grown after runner bean with application of organic fertilization: in the autumn, manure (the composition of the fertilizer, expressed in kg t⁻¹: 4.7 N, 2.8 P_2O_5 , 6.5 K_2O , 4.3 CaO, 1.5 Mg, and 1.0 Na) was applied at a rate of 30 t ha^{-1} . Plant seedlings were fertilized with Fertikal organic fertilizer (10 kg 100 m $^{-2}$) (FERTIKAL, Beveren, Belgium) two weeks before planting. For 2, 4, and 6 weeks after planting seedlings, the plants were foliar fed with the organic fertilizer Bio-Algeen S90 (Schulze & Hermsen GmbH, Dahlenburg, Germany) at a concentration of 0.5%. The fertilization scheme was designed based on a soil analysis performed in the spring (Table 1).

Years	Soil Humidity (%)	Soil Density (g cm ⁻³)	Total Porosity (%)	Mineral Components (mg dcm ⁻³)					- U	Salinity
				N-NO ₃	Р	К	Ca	Mg	рп	(mgKCl dcm ⁻³)
2016	18.2	1.47	43.2	40	90	160	1540	110	6.7	0.30
2017	16.3	1.48	42.9	25	68	140	1280	95	6.4	0.25
2018	18.0	1.47	43.4	37	75	128	1480	105	6.5	0.17

Table 1. Physical and chemical characteristics of a soil with bell pepper grown with or without mycorrhizal inoculation and irrigation.

Peppers were grown from potted seedlings prepared in a greenhouse of the Experimental Station of the University of Life Sciences in Lublin. Seeds were sown in the third 10 days of March, while seedlings were planted in the field at the turn of the second and third 10 days of May at a spacing of 0.67×0.35 m, while at the same time applying the mycorrhizal inoculant to the soil at an amount of 3 mL per plant. Mycorrhizal inoculation was applied under the plant to the dug hole before planting the plants in a permanent location. Drip tape irrigation was provided using a T-Tape irrigation system (Milex, Dobrzyków, Poland) with emitters spaced every 30 cm. The irrigation started when the soil water potential value at a depth of 25 cm was equal to or lower than 30 kPa, applying a single dose of 15–20 mm. The soil water potential value was measured with a tensiometer (TENSJOMETR MMM, STNDARD, Agrosimex, Goliany, Poland). During the research period, depending on the humidity, the following frequency of irrigation was used: 6 (2016), 5 (2017) and 6 (2018) water doses. The total water dosage that was used in the irrigation treatments over the period 2016–2018 was 1200, 1000, and 900 m⁻³ per hectare, respectively.

2.2. Raw Material Collection and Post-Harvest Treatments

Pepper fruits were harvested at full physiological maturity, every 7–10 days. The harvest lasted from the second 10 days of August until the first 10 days of October. Peppers were collected separately for each treatment combination. Evaluation of the quality of pepper fruits was performed using morphological (fruit weight, length, and width, fruit shape ratio, pericarp thickness) and biochemical criteria (dry matter, L-ascorbic acid, reducing sugars, extract, carotenoids, and antioxidant activity). From each replicate and treatment, 20 fruits were randomly selected for evaluation.

2.3. Sample Preparation and Analyses

From the peppers harvested at the turn of the first and second 10 days of September, randomly mixed samples were prepared for each treatment for analytical analysis of the selected parameters. The chemical analysis was carried out in 3 replicates.

2.3.1. Dry Matter

Aliquots of about 1 g (0.0001 g accuracy) of raw and ground fruits were weighed. Samples were placed in a drier and dried at 105 °C for 6 h. The drying process was repeated until a constant weight of samples (the difference between two subsequent weighings should not be greater than 0.5 g). The difference of weights before and after drying was water loss; the result was then recalculated into the percentage of dry matter.

2.3.2. Vitamin C

The fruits (10 g) were extracted twice for 30 min with 2.5 mL 4.0% (m/V) L-cysteine and 10.0 mL water by sonification. All aqueous extracts were combined and diluted with water to 25 mL. Vitamin C (ascorbic acid plus dehydroascorbic acid) in the fruit of the peppers was quantified by high-performance liquid chromatography (HPLC) analysis, with a reverse phase C18-silica analytical column (LiChrospher 100RP dp = 5 μ M 4 mm \times 250 mm dimensions). The mobile phase, standard solutions, and samples were prepared as explained by using the method described by Najda et al. [35]. The results were expressed in mg of vitamin C per 100 g of fresh fruit matter.

2.3.3. Reducing Sugars

Reducing sugars (% FM) were determined by the titration method following Schoorl–Luff [36]. The extracts were prepared using water by grinding 10 g of fruit and 50 mL of distilled water in a mortar. A quantity of 10 mL of hydrolyzed sample was diluted up to 20 mL with distilled water and inserted into a 250 mL flask, to which was added 25 mL of Luff–Schoorl reactant and the mix was heated in a Bunsen burner and kept boiling for 2 min. It was quickly cooled in an ice bath to room temperature; 10 mL of potassium iodide 30% (w/v) solution, 10 mL of 10 N sulfuric acid and 3 drops of 1% starch were added. The iodine produced was titrated with 0.1 N sodium thiosulfate until total disappearance of the blue–black color.

The blank test was performed using distilled water instead of the extract.

2.3.4. Extract (Total Extract)

The total content of water-soluble non-volatile substances up to a temperature of 100 °C, was determined using a refractometer (RE 50, Mettler Toledo, Greifensee, Switzerland) according to the PN-90 A-75101/02 standard [37].

2.3.5. Carotenoid

Carotenoid content was determined through a spectrophotometric method proposed by Hornero and Mínguez [38]. A quantity of 2 g of fruit was extracted in a volumetric flask containing 100 mL of acetone, then filtered, and absorbance measurements were made in a diode array spectrophotometer (Spectrophotometer UV-Vis Hitachi U-2900, Tokyo, Japan) at 472 and 508 nm. In order to obtain both isochromic carotenoid and total carotenoid fractions, the absorbance values obtained were introduced in the following equations:

$$C^{\rm R} = \frac{A_{508} \times 2144 - A_{472} \times 403.3}{270.9} \tag{1}$$

$$C^{Y} = \frac{A_{472} \times 1724.3 - A_{508} \times 403.3}{270.9}$$
(2)

$$C^{T} = C^{R} + C^{Y}$$
(3)

where C^R represents the red isochromatic fraction content, C^Y represents the yellow isochromatic fraction content, and C^T represents total carotenoid content. All carotenoid determinations were carried out in triplicate.

2.3.6. Antioxidant Activity (AA)

The radical scavenging activity of the plant extracts against the 2,2-diphenyl-1-picryl hydrazyl (DPPH•) radical was determined using method [39] with slight modifications. Aliquots of the extracts of various concentrations (10–100 μ g/mL) were prepared in methanol. One milliliter of these extract concentrations was placed in test tubes and methanol (3 mL) was added followed by 1 mM methanol solution of DPPH• (0.5 mL). A blank solution containing the same amount of methanol and DPPH• was also prepared. After 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH• as a percentage (%) was calculated using the following formula:

% inhibition of DPPH• =
$$\{(Ab - Aa)/Ab\} \times 100$$
 (4)

where Ab is the absorption of the blank sample and Aa is the absorption of the extract.

2.3.7. Minerals

Determination of potassium, calcium, iron, and sodium was made by Flame Atomic Absorption Spectrometry (AAS). The concentration of K, Na, Ca, Mg, and Fe ions was determined by the atomic absorption spectroscopy (AAS) method using a spectrometer Spectr AA 280 FS with an autosampler SPS 3 (Varian, Belrose, Australia), which was equipped with a deuterium lamp, a hollow cathode lamp for each element, and an airacetylene burner. The Instrumental parameters related to each element are summarized in Table S2. In order to avoid sample ionization during potassium analysis, a Schinkel buffer solution (mixture contents 10 g/L cesium chloride and 100 g/L lanthanum chloride) was used.

2.3.8. Preparation of Peppers Fruits Sample

Approximately 0.5 g of pepper fruit samples were placed in a Teflon vessel, 10 mL of 65% $\rm HNO_3$ (suprapur grade[®], Merck, Darmstadt, Germany) was added into the vessel and the sealed vessel was put into a microwave mineralizer MARS Express (CEM, Stallings Rd, Charlotte, CO, USA). The microwave mineralization was performed stepwise at 400 W and 363 K, at 800 W and 393 K, and at 1600 W and 483 K. The cooled digestion solution was then diluted to 50 mL using high purity de-ionized water. All sample solutions were clear before determination of the mineral content in the pepper fruit sample.

Phosphorus was determined by colorimetry using ammonium vanadium molybdate.

2.3.9. Chemicals

All reagents and solvents were analytical grade chemicals from Merck (Darmstadt, Germany) or Sigma Chemical Co. (St. Louis, MO, USA) and POCH (Gliwice, Poland).

2.4. Statistical Analysis

The results were statistically analyzed using STATISTICA 13.2 software (TIBCO Software Inc., Palo Alto, CA, USA). Significant differences were evaluated on the basis of Tukey's multiple test at a significance level of $\alpha = 0.05$.

3. Results

3.1. Fruit Morphological Parameters

Application of the four AMF/irrigation treatments in pepper cultivation significantly modified the selected fruit morphological parameters (Table 2). The shape ratio of the investigated fruits ranged from 1.54–1.55, regardless of the treatment used, which indicates that fruit shape is a stable characteristic of the species studied. The fruits of irrigated plants subjected to mycorrhizal inoculation were distinguished by the greatest thickness of the pericarp (on average 5.3 mm). Similar to AMF and non-irrigation, AMF + irrigation contributed to the production of peppers with the highest weight, length, and width compared to the other treatments. Irrigated plants produced longer fruits with a larger diameter compared to non-irrigated ones.

Table 2. Quality parameters of bell pepper fruits grown with or without mycorrhizal inoculation and irrigation (mean for 2016–2018).

Treatment	Average Fruit Weight (g)	Average Pericarp Thickness (mm)	Average Fruit Length (cm)	Average Fruit Width (cm)	Shape Ratio
AMF and irrigation	136.7 ± 1.8 a	5.3 ± 0.6 a	10.7 ± 1.6 a	6.9 ± 0.9 a	1.55
AMF and non-irrigation	$30.9\pm2.5~\mathrm{a}$	$5.0\pm0.6\mathrm{b}$	$10.9\pm1.7~\mathrm{a}$	7.1 ± 1.4 a	1.54
non-AMF and irrigation	$121.1\pm2.8\mathrm{b}$	$5.0\pm0.2\mathrm{b}$	$10.0\pm1.3\mathrm{b}$	$6.5\pm1.0~\mathrm{b}$	1.54
non-AMF and non irrigation	$124.2\pm3.0b$	$5.0\pm0.3\mathrm{b}$	$10.2\pm0.9\mathrm{b}$	$6.6\pm1.0~\mathrm{b}$	1.55
Mean for AMF	$133.8\pm2.3~\mathrm{A}$	$5.1\pm0.6~\mathrm{A}$	$10.8\pm1.6~\mathrm{A}$	$7.0\pm1.1~\mathrm{A}$	1.54
Mean for non-AMF	$122.7\pm2.9~\mathrm{B}$	$5.0\pm0.2~\mathrm{A}$	$10.1\pm1.1~\mathrm{B}$	$6.5\pm1.0~\mathrm{B}$	1.55
Mean for irrigation	$128.9\pm2.5~\mathrm{A}$	$5.1\pm0.5~\mathrm{A}$	$10.8\pm1.5~\mathrm{A}$	$6.7\pm0.9~\mathrm{A}$	1.55
Mean for non-irrigation	$127.5\pm2.7~\mathrm{A}$	$5.0\pm0.4~\mathrm{A}$	$10.1\pm1.4~\mathrm{B}$	$6.9\pm1.2~\mathrm{B}$	1.54

Arbuscular Mycorrhizal Fungi (AMF). The same letters indicate no statistically significant differences; lowercase letters refer to the significance of interactions, uppercase letters refer to differences in the mean values for the studied factors.

3.2. Biological Value of Fruits

The chemical composition and antioxidant activity of the pepper fruits were significantly affected by AMF and irrigation, but it is difficult to show a clear trend in the effects (Tables 3 and 4). The highest dry matter was accumulated in irrigated plants without AMF and in non-irrigated plants without AMF (statistically insignificant differences), while the lowest amount was accumulated by the fruits of irrigated plants with AMF (6.65%). In turn, the vitamin C level was highest in non-irrigated plants (without AMF and with AMF), respectively 134.0–135.31 mg per 100 g FM.

Table 3. Selected fruit quality parameters of bell pepper grown with or without mycorrhizal inoculation and irrigation (mean for 2016–2018).

Treatment	Dry Matter (%)	Vitamin C (mg·100 g ^{−1} FM)	Reducing Sugars (g∙100 g ⁻¹ FM)	Extract (%)	Carotenoids (mg·100 g ^{−1} FM)	DPPH• (%)
AMF and irrigation	$7.11\pm0.57\mathrm{b}$	$110.96\pm9.44~\mathrm{c}$	$4.84\pm0.07~\mathrm{a}$	7.52 ± 0.14 a	$3.32\pm0.11~\mathrm{c}$	$63.4\pm6.2~\mathrm{d}$
AMF and non-irrigation	$6.65\pm0.34~\mathrm{c}$	$135.31\pm4.23~\mathrm{a}$	$4.17\pm0.16~\mathrm{d}$	$7.19\pm0.81~\mathrm{b}$	$2.37\pm0.09~\mathrm{d}$	$64.6\pm3.4b$
non-AMF and irrigation	$7.40\pm0.29~\mathrm{a}$	$118.82\pm6.89\mathrm{b}$	$4.33\pm0.24~\mathrm{c}$	$7.53\pm0.31~\mathrm{a}$	4.64 ± 0.15 a	69.7 ± 1.2 a
non-AMF and non irrigation	$7.37\pm0.31~\mathrm{a}$	$134.10\pm4.23~\mathrm{a}$	$4.78\pm0.10\mathrm{b}$	$7.60\pm0.71~\mathrm{a}$	$3.50\pm0.10~\mathrm{b}$	$64.2\pm1.4~\mathrm{c}$
Mean for AMF	$6.88\pm0.52~\mathrm{B}$	$123.13 \pm 15.29 \text{ B}$	$4.51\pm0.37~\mathrm{B}$	$7.35\pm0.59~\mathrm{B}$	$2.85\pm0.11~\mathrm{B}$	$64.0\pm4.9~\mathrm{B}$
Mean for non-AMF	$7.36\pm0.29~\mathrm{A}$	$126.46\pm9.60~\mathrm{A}$	$4.56\pm0.29~\mathrm{A}$	$7.56\pm0.54~\mathrm{A}$	$4.07\pm0.16~\mathrm{A}$	$67.0\pm3.1~\mathrm{A}$
Mean for irrigation	$7.26\pm0.47~\mathrm{A}$	$114.89\pm9.03~\mathrm{B}$	$4.59\pm0.31~\mathrm{A}$	$7.52\pm0.24~\mathrm{A}$	$3.98\pm0.15~\mathrm{A}$	$66.5\pm5.4~\mathrm{A}$
Mean for non-irrigation	$7.01\pm0.49~\mathrm{B}$	$134.71\pm6.69~\mathrm{A}$	$4.48\pm0.34~\text{B}$	$7.40\pm0.77~\mathrm{B}$	$2.94\pm0.11~\mathrm{B}$	$64.4\pm2.5~B$

% Inhibition of DPPH•; Arbuscular Mycorrhizal Fungi (AMF). Means followed by the letters (a–d and A–B) do not significantly differ at $\alpha = 0.05$.

Table 4. Mineral	l composition of swe	et pepper growr	n with or with	out mycorrhizal	inoculation and	d irrigation	(mean for
2017–2018).							

T ()	Macro and Micronutrients (mg \cdot 100 g $^{-1}$ FM)								
Ireatment	Р	K	Ca	Mg	Fe	Na			
AMF and irrigation	$32.10\pm2.96~a$	$187.49\pm3.76~\mathrm{b}$	$14.96\pm0.50~\mathrm{a}$	10.96 ± 0.18 a	$0.29\pm0.04~b$	$1.22\pm0.17b$			
AMF and non-irrigation	$30.64\pm1.60~b$	$190.86\pm4.00~\mathrm{a}$	$12.56\pm0.44~\mathrm{c}$	$10.52\pm0.23~\mathrm{c}$	$0.25\pm0.06~\mathrm{c}$	$1.14\pm0.05~{\rm c}$			
non-AMF and irrigation	$31.01\pm1.60~b$	$190.61\pm4.95~\mathrm{a}$	$14.14\pm0.45b$	$10.84\pm0.38~b$	$0.33\pm0.06~\text{a}$	$1.44\pm0.20~\mathrm{a}$			
non-AMF and non irrigation	$27.10\pm2.08~c$	$148.50\pm2.69~c$	$12.92\pm0.82~c$	$9.51\pm0.20~d$	$0.26\pm0.02~c$	$0.84\pm0.31~d$			
Mean for AMF	$31.37\pm2.95~\text{A}$	$189.18\pm4.09~\mathrm{A}$	$13.80\pm1.29~\text{A}$	$10.74\pm0.31~\mathrm{A}$	$0.27\pm0.05~B$	$1.18\pm0.13~\mathrm{A}$			
Mean for non-AMF	$29.05\pm2.79~B$	$169.55\pm2.93~\text{B}$	$13.53\pm0.90~\text{A}$	$10.17\pm0.75~\mathrm{B}$	$0.29\pm0.06\;A$	$1.14\pm0.40~\mathrm{A}$			
Mean for irrigation	$31.56\pm2.34~\text{A}$	$189.05\pm9.10~\mathrm{A}$	$14.55\pm0.692~\text{A}$	$10.90\pm0.29~\text{A}$	$0.31\pm0.05~\text{A}$	$1.33\pm0.21~\mathrm{A}$			
Mean for non-irrigation	$28.87\pm3.08~\text{B}$	$169.68\pm2.65\text{ B}$	$12.79\pm0.64~B$	$10.02\pm0.57~B$	$0.25\pm0.04~\text{B}$	$0.99\pm0.26~\text{B}$			

Means followed by the letters (a–d and A–B) do not significantly differ at α = 0.05.

The content of reducing sugars and extract was highest with inoculation and irrigation (4.84 g per 100 g FM), while the extract content was highest in the treatments with AMF and irrigation, in irrigated plants without inoculation as well as in non-irrigated and non-inoculated plants. The highest proportion of carotenoids and the highest AA were found in the fruits of irrigated plants without AMF (respectively 4.64 mg per 100 g FM and 69.7%).

Irrigation of pepper plants positively affected the biological value of harvested fruits and AA. An exception was vitamin C whose level was higher in the fruits of non-irrigated plants (134.7 mg per 100 g FM) than in irrigated ones (114.89 mg per 100 g FM) (Table 3).

Application of biostimulation and irrigation contributed to a higher content of phosphorus, calcium, and magnesium in the pepper fruits (Table 4). Most potassium was found in the fruits of AMF-inoculated plants without irrigation and in irrigated plants without AMF, whereas the fruits of irrigated plants without AMF accumulated the highest amount of ion and sodium. The analysis of the effect of each of the investigated factors on the mineral composition indicates that AMF contributes to higher levels of phosphorus, calcium, and magnesium, and also to a decrease in iron content. Irrigation, in turn, contributed to an increase in the content of all the studied minerals in the peppers (Table 4).

4. Discussion

In the previous paper [31], the highest yield of pepper fruits was obtained with simultaneous AMF inoculation and irrigation. The present data demonstrate that similar relationships also apply to pepper fruit quality. The fruits of inoculated and irrigated plants were of the highest quality, as well as the fruits of inoculated and irrigated ones. The effects of AMF on sweet pepper plants are different and dependent on several factors (environmental and biotic) [40]. The development of fungal colonization in the host plant includes changes in morphology and gene expression as well as introduces compounds similar to plant hormones that help in plant growth and development [22]. Ecophysiological research reveals that AM symbiosis is a key element that helps plants cope with water stress and increases their resistance to drought [41]. The present study did not find significant differences between irrigated and non-irrigated plants inoculated with AMF in terms of the selected fruit quality characters, which was probably associated with the fact that pepper plants inoculated with AM fungi did not feel water stress. It could also be related to a onetime application of mycorrhiza. In the previous paper describing the biological properties of sweet pepper rhizosphere [25], the significant impact of AMF inoculation on the reduction of the total number of bacteria in the plant rhizosphere was shown, while irrigation increased the total number of rhizosphere fungi. The highest biodiversity and metabolic activity was found in the rhizospheres from the mycorrhized and irrigated plants and mycorrhized plants, and a lower catabolic activity in the control and irrigated samples [25]. When considering the individual effects of the factors studied, AMF application in pepper cultivation resulted in an increase in fruit weight, length, and width. However, AMF was not found to have a significant impact on pericarp thickness. The results of other studies confirm that biostimulant-induced modifications improve the health of pepper plants from the vegetative phase, promoting a stable increase in fruit yield [32,33,42]. Pepper plants inoculated with mycorrhiza or treated with brassinosteroids exhibit better vegetative growth as well as fruit weight and yield than plants inoculated with Bacillus megaterium and control plants, at all salinity levels [42]. Mycorrhizal inoculation significantly increases the plant biomass parameters of pepper and also fruit yield [43]. Duc et al. [33] proved that inoculation with different microbes positively influenced pepper fruit yield, though the significant differences were dependent on specific microbe-cultivar treatments. In turn, inoculation with native fungi reduced stress associated with transplant of chili pepper plants, thus accelerating ripening and resulting in higher and better yield quality. Furthermore, no differences in fruit yield quality were found between non-inoculated plants and those treated with a commercial inoculant [44]. The effects of mycorrhizal inoculation are probably best seen when the inoculant composition is selected appropriately for the genotype of the pepper under specific growing conditions (open field/greenhouse, soil type, and climate).

Irrigation of the studied pepper plants produced a positive effect only in the case of some of the fruit quality parameters. The studies of other authors reveal that irrigation frequency and level significantly affect fresh pepper yield and some quality parameters, while smaller amounts of irrigation at a higher frequency result in a significant decrease in yield [10,15,17,45]. Adeoye et al. [46] suggest that the amount and frequency of irrigation determine pepper fruit yield. Treatment with biostimulants induces secondary metabolism and results in accumulation of carotenoids, saponins, and phenolic compounds in plants [32]. The latest research has shown that the soil microbiome can be effective in controlling the harmful effects of abiotic stresses, but the mechanism of this phenomenon has not been fully explained. Mycorrhization can alleviate the effects of drought stress and the tolerance effect is achieved through osmotic adjustments mediated by the accumulation of compounds such as proline, chlorophyll, and carotenoids. AM fungi improve tolerance to drought by enhancing water use efficiency and stomatal conductance or by increasing

the amount of glycoproteins such as glomalin [22]. Pepper plants in the present experiment developed well in symbiosis with AMF, regardless of water moisture conditions. Mycorrhizal fungi stimulate the uptake of nutrients and water and their transport to the cells of the symbiotic host. As a consequence, plant growth improves, as well as the production of sugars, apocarotenoids, flavonoids, jasmonate, and triterpenoids [23,26,32].

The presence of microbes affects the quality of tomato fruits, increasing the level of lycopene and polyphenols as well as antioxidant activity. Nonetheless, the efficiency of AMF application depends on the cultivar and the amount of water used [47]. When analyzing the results of the experiment in terms of biostimulation, it can be noted that AMF inoculation contributes to a lower percentage of dry matter, vitamin C, reducing sugars, extract, and carotenoids as well as to a reduced AA. This could be due to the specific adaptability of microorganisms to local conditions. Sensoy et al. [48] found that in eight inoculated pepper genotypes, five cultivars had higher dry weight, while three had similar values to plants without inoculation. Castillo et al. [44] report that inoculation with Glomus intraradices does not improve chili pepper productivity or quality. Symbiosis of AM fungi in plants substantially contributes to the synthesis of secondary metabolites [22,32]. Peppers inoculated with mycorrhiza or treated with brassinosteroids are characterized by a higher percentage of chlorophyll a and b and antioxidants, expressed as the total concentration of soluble phenols and proline, compared to plants inoculated with Bacillus megaterium and control plants that exhibit severe growth delay, in particular at higher salinity [42]. The obtained results concerning changes in the biological value of pepper fruits under the influence of irrigation are confirmed in the literature [10,45]. The fruit of the peppers are characterized by the presence of secondary metabolites with substantial antioxidant properties [2]. The results presented in this paper show that the irrigation treatment contributed to increased antioxidant potential of the raw material by increasing the level of carotenoids. The mineral content in the fruit is modified by many factors that can act simultaneously [10]. In this study, the effects of AMF proved to be significant in the case of most of the minerals analyzed, whereas irrigation caused an increase in the content of all the compounds investigated.

5. Conclusions

- 1. AMF and irrigation affected fruit quality in field-grown sweet peppers beneficially. Biofertilization seems to have a slightly greater effect on the selected morphological parameters than irrigation.
- 2. AMF-inoculated plants produced fruits with a higher weight, length, and width compared to non-inoculated ones, whereas pericarp thickness proved to be a typically cultivar-specific trait that was not affected by the studied factor. Irrigation of peppers resulted in the production of longer and wider fruits.
- 3. Inoculation with AM fungi adversely influenced the biological value and antioxidant activity of the pepper fruits while at the same time increasing the fruit mineral content.
- 4. Irrigation contributed to an increase in the biological value of the peppers, also increasing their antioxidant potential. The level of vitamin C remained an exception and it was highest in the fruits of plants grown without AMF and without irrigation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/agronomy11112349/s1, Table S1. Weather conditions in the years 2016–2018 during vegetation of sweet pepper compared with means from 1951–2010. Table S2. Instrumental conditions for mineral element determination in pepper fruits.

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