EFFECTS OF *TRICHODERMA HARZIANUM* AND BORON ON SPRING BROCCOLI

ROSA, R.* – FRANCZUK, J. – ZANIEWICZ-BAJKOWSKA, A. – HAJKO, L.

*Department of Vegetable Crops, Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities, Prusa 14 Street, 08-110 Siedlce, Poland
(phone: +48-25-643-12-76)*

**Corresponding author
e-mail: robert.rosa@uph.edu.pl*

(Received 9th Jan 2019; accepted 26th Feb 2019)

Abstract. This paper deals with the effects of *Trichoderma harzianum* application and boron (Nitrabor) topdressing on the growth, yield, and quality of spring broccoli. A field experiment was carried out in central-eastern Poland, in 2014-2015.The experiment was established as a split-block design with three replicates. There were the following combinations of *Trichoderma harzianum* treatment: added to the nursery substrate; added to the nursery substrate and as topdressing in a form of spray after seedlings were planted out; as pre-planting treatment just before seedlings were planted out, followed by topdressing in a form of spray; control without *Trichoderma harzianum*. Two types of late topdressing for broccoli were used: nitrogen topdressing or Nitrabor to the soil. The treatment significantly increased the marketable yield of broccoli curds. This increase relative to control ranged from 17 to 49%, depending on the *Trichoderma harzianum* treatment. The best results were obtained when boron topdressing was combined with *Trichoderma harzianum* added to the nursery substrate and then the fungus was applied in a form of spray after seedlings were planted out. Broccoli inoculation with *Trichoderma harzianum* also increased L-Ascorbic acid content in the plants. Topdressing with boron significantly increased the marketable yield of curds, their weight, the diameter of their circumference, the diameter of the stem, the leaf greenness index value (SPAD), as well as the content of dry matter, total sugars, and L-Ascorbic acid.
Keywords: *bio-stimulators, Brassica oleracea L. var. italica, mineral fertilization, nutritional value, yield*

Introduction

Broccoli (*Brassica oleracea* var. *italica*) is an important vegetable grown worldwide. The plant originates from the Mediterranean region and belongs to the *Brassicaceae* family. It is tasty and more nutritious than any other vegetables of the same kind, considered a valuable source of vitamins, antioxidants, glucosinolates, and other compounds of proven anticancer activity (Parente et al., 2013). Broccoli is an important component of human healthy diet, and demand for this vegetable on Polish and international markets is constantly growing. Its producers use new growing methods enhancing its growth and improving its quality. Hence, there is a need for research to improve the yield of broccoli and to increase its production; some biological agents should be implemented either on their own, or in combination with mineral fertilisers to increase its cultivation and production.

Among various biological methods currently used, microbial inoculants applied at the seedling stage could prove promising. Several symbionts like arbuscular mycorrhizal fungi and *Trichoderma* ssp. can be implemented for broccoli cultivation.

The efficacy of *Trichoderma* as a biostimulator agent has been proven when fungal species have been applied to soil, seeds, or plants. Their benefits are related to increased

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

nutrient solubility, improved nutrient uptake by roots, and to the mode of action of plant growth promoting bacteria (Molla et al., 2012; Martínez et al., 2015). *Trichoderma harzianum* releases volatile organic antibiotic compounds against pathogenic fungi, and it also stimulates plant growth (Vinale et al., 2008). *Trichoderma* species improve the growth and development of plants grown in vitro, in greenhouses, and in the field. Some studies have demonstrated beneficial effects of *Trichoderma* spp. on vegetable plants, like cabbage, cucumber, tomato, and lettuce, stimulating seed germination, or vegetative and generative growth (Bal and Altintas, 2006; Studholme et al., 2013).

Boron represents one of the essential micronutrients necessary for proper plant growth, the latter becoming limited both when it is deficient or at elevated level (Davies et al., 2011). It increases the growth and yield of plants because it stimulates division and elongation of the cell and development of its walls. Boron plays an important role in the metabolism of carbohydrates and proteins (Goldbach and Wimmer, 2007; Miwa et al., 2007). It is also crucial for the development of nitrogen-fixing cyanobacteria (Bolanos et al., 1996; Bonilla et al., 1997). Boron deficiency causes many anatomical, physiological, and biological disorders (Brown et al., 2002; Xu et al., 2007). Vegetable plants with the highest demand for boron include, among others, broccoli.

The natural content of boron in the soil depends mainly on the type of material from which it has developed. Clay soils are generally rich in boron, in contrast to sandy ones, in which this chemical element may be present in small amounts. Boron concentration in soils varies from 2 to 200 mg B kg^{-1}, but generally less than 5-10% of its content is in a form available to plants (Diana, 2006). In Poland sandy soils dominate, and with the content of this chemical element being too small it is necessary to apply boron with mineral fertilizers to the soil or as a foliar spray (Szulc and Rutkowska, 2013).

The purpose of this research was to determine the effect of *Trichoderma harzianum*, applied as a biostimulator to nursery substrate and after seedlings were planted out, and of boron topdressing on the size and quality of the broccoli yield.

Material and methods

Experimental site

The experiment was carried out between 2014 and 2015 at the Experimental Station of the Siedlce University of Natural Sciences and Humanities, located in central-eastern Poland (52°03'N, 22°33'E). The soil was classified as luvisol (IUSS, 2015) with the average organic carbon content of 0.97%, the humus layer reaching the depth of 30-40 cm, and pH_{KCl} of 6.0. The content of available forms of nutrients ($mg \cdot kg^{-1}$) was as follows: 4.7 NO_3-N, 2.5 NH_4-N, 12 P, 27 K, 10.8 Mg, 87.5 Ca, 0.11 B.

Experimental design

The experiment was established as a split-block design with three replicates, and it included two factors: factor I – mineral fertiliser, factor II – different methods of using *Trichoderma harzianum* (*Table 1*). The studies used Trianum-G and Trianum-P produced by the Koppert B.V. company (Netherlands). Trianum-G is microbiological granular containing spores of *Trichoderma harzianum (T. harzianum)* Strain T-22 Rifai (1.5×10^8 spores per 1 g). It is insoluble in water and used as pre-planting treatment, mixing granules with the soil. Trianum-P is a microbiological product in a form of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

granules to prepare aqueous suspension. It contains 1.5×10^9 spores of *T. harzianum* Strain T-22 Rifai per 1 g. The combinations of Trianum-G and Trianum-P application are presented in *Table 1*. In the experiment both Trianum-G and Trianum-P were used in doses recommended by the manufacturer. Nitrabor (Yara Poland) contains calcium nitrate with 15.4% N (including 14.1% NO_3-N and 1.3% NH_4-N), 25.6% CaO, and 0.3% B. It is recommended for use on vegetable plants, especially on species that require increased doses of boron.

Table 1. *Factors of the experiment*

	Two mineral fertiliser combinations	
Factor I	NPK	Basic pre-planting treatment NPK (206 kg N, 146 kg P_2O_5, 273 kg K_2O per 1 ha – in the form of ammonium nitrate, granular superphosphate and 60% potassium chloride, respectively) + nitrogen topdressing to the soil (62 kg·ha^{-1} – in the form of ammonium nitrate)
	NPK+B	Basic pre-planting treatment NPK (206 kg N, 146 kg P_2O_5, 273 kg K_2O per 1 ha – in the form of ammonium nitrate, granular superphosphate and 60% potassium chloride, respectively) + Nitrabor topdressing to the soil (400 kg·ha^{-1})
	Five combinations with *Trichoderma harzianum*	
Factor II	Th_0	control – without *Trichoderma harzianum*
	Th_1	*T. harzianum* added to the substrate before planting seeds (Trianum-G 750 g per 1 m^3 of the substrate)
	Th_2	*T. harzianum* added to the substrate + spraying plants in the field with *Trichoderma harzianum* (Trianum-G 750 g per 1 m^3 of the substrate + Trianum-P 40 g·100 m^{-2} dissolved in 15 dm^3 H_2O, plants sprayed 21 days after transplanting seedlings to the field)
	Th_3	*T. harzianum* added to the soil before transplanting seedlings (Trianum-G 1 g per 1 plant)
	Th_4	*T. harzianum* added to the soil before transplanting seedlings + spraying plants in the field with *Trichoderma harzianum* (Trianum-G 1 g per 1 plant + Trianum-P 40 g·100 m^{-2} dissolved in 15 dm^3 H_2O, plants sprayed 21 days after transplanting seedlings to the field)

Seedling preparation

Broccoli seedlings ‘Wiarus’ (PNOS, Poland) were grown in a non-heated greenhouse. Seeds were sown in the successive growing seasons on 17 and 20 March to multi-trays with the size of 400 × 600 mm and 54 cells with the diameter of 54 mm. 60% of the seedlings were produced on substrate without *Trichoderma harzianum*, and 40% on substrate with *Trichoderma harzianum* (Trianum-G). The Aura substrate produced by Hollas - Greenyard Horticulture Poland Ltd. was used for the production of seedlings. It was made of de-acidified ‘highmoor’ peat with 5.5-6.5 pH and salinity not greater than 2 g NaCl per litre. The substrate was enriched with mineral fertiliser containing NPK (14-16-18%) and Mg (5%). On average nutrient content in the substrate was as follows (in mg·dm^{-3}): 238 NO_3-N, 18 NH_4-N, 70 P, 207 K, 1016 Ca, and 158 Mg.

Field work

The crop preceding broccoli was triticale. In the autumn the field was ploughed, and in the spring, two weeks before seedlings were planted, disc harrowing was used. After that, mineral fertilizers were applied up to the optimal level for broccoli: 206 kg N, 146 kg P_2O_5, 273 kg K_2O per 1 ha. Seedlings were planted on 18 and 22 April, at a spacing of 50 × 50 cm. The area of a plot (unit) was 9 m^2 (3 m × 3 m), with 36 plants in

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

each of them. The area of the whole field together with paths between experimental combinations and replicates was 650 m^2. Then *Trichoderma harzianum* was added to adequate plots, in accordance with the combinations set out in *Table 1*. For three weeks the plants were covered with Pegas Agro 17UV polypropylene fibre (Rybnik, Poland). After removing the fibre the plants were topdressed with mineral fertilizers. To plants with the NPK treatment 62 $kg \cdot ha^{-1}$ of nitrogen was applied (the same amount of nitrogen as the NPK applied, together with B, in a form of Nitrabor. On the NPK+B combination a Nitrabor dose of 400 $kg \cdot ha^{-1}$ was used, which made it 1.2 $kg \cdot ha^{-1}$ of Boron. The Nitabor dose to be applied in the experiment had been determined by taking into account boron content in the soil and in the fertilizer, as well as broccoli requirements for this chemical element. 21 days after planting seedlings *Trichoderma harzianum* was applied as a spray to Th_2 and Th_4 combinations (*Table 1*).

Sample collection and laboratory analysis

Broccoli was harvested by hand on 12 June in 2014, and on 11 June in 2015. The area of each plot to be harvested was 3 m^2. Marketable yield, weight of marketable curd, length of curd circumference, and stalk diameter were determined during the harvest. From each plot a broccoli sample was also taken (four randomly selected curds) for chemical analysis to determine: dry matter content by drying to the constant weight at 105°C; L-ascorbic acid content with the Tillmans method (PN-A-04019, 1998); monosaccharide content with the Luff-Schoorl method (EU, 2009); protein content with the Kjeldahl method (using a factor of 6.25). Seven days before the plants were harvested the leaf greenness index (SPAD) of broccoli curds was measured with SPAD-502 Plus Chlorophyll Meter (Konica Minolta Inc., Tokyo, Japan).

Statistical analysis

The results were statistically processed with ANOVA for the split-block design. The significance of differences was determined with Tukey’s test at the significance level of $P \leq 0.05$. All the calculations were performed with Statistica software (version 10, Statsoft, USA).

Weather conditions

The basic weather conditions of the experimental area in individual experimental years are presented in *Table 2*. To assess pluvio-thermal conditions during the growing season Selyaninov’s hydrothermal coefficient was calculated (Eq. 1):

$$K = \frac{Mo \times 10}{Dt \times days} \qquad \text{(Eq.1)}$$

where: K – hydrothermal coefficient for individual months; M_o – total monthly precipitation; D_t – mean daily temperatures in a particular month (Szymańska et al., 2017).

During the broccoli growing season from April to June 2014 the air temperatures and rainfall were higher than in 2015. However, the second year, 2015, turned out to be more favourable for broccoli growth. High air temperatures during the final ten days of April and in May 2014 negatively affected survival and growth of seedlings despite the large amount of rainfall. This translated into the smaller yield of curds.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

Table 2. *Weather condition in the experiment area in 2014 and 2015 (Zawady Meteorological Station, Poland)*

Month	2014			2015		
	T (°C)	P (mm)	*K*	T (°C)	P (mm)	*K*
April	9.8	44.7	1.53 o	8.2	30.0	1.22 qd
May	13.5	92.7	2.29 h	12.3	100.4	2.71 vh
June	15.3	55.4	1.20 qd	16.5	43.3	0.87 d
Mean	12.9	-	-	12.3	-	-
Total	-	192.8	-	-	173.7	-

T – temperature; P – precipitation; K – Sielyaninov's hydrothermal index: d – dry, qd – quite dry, o – optimum, h – humid, vh – very humid

Results and discussion

For two years of the research the marketable yield of broccoli curds was on average 13.1 t·ha^{-1}, and the mass of the marketable curd was 352.5 g (*Table 3*). In 2015, when weather conditions for broccoli growth were more favourable, the yield was 20.2% higher than in 2014.

Table 3. *The effect of different treatment combinations on the yield of broccoli and the weight of curds*

Treatment	Marketable yield (t·ha^{-1})					Weight of marketable curd (g)				
	Year		Mineral fertilization		Mean	Year		Mineral fertilization		Mean
	2014	2015	NPK	NPK+B		2014	2015	NPK	NPK+B	
Th_0	10.46^{a}	9.89^{a}	9.20^{aA}	11.15^{aB}	10.18^{a}	320.7	336.5^{a}	314.8	342.3	328.6
Th_1	10.41^{a}	13.51^{b}	11.27^{bA}	12.64^{bB}	11.96^{b}	278.0	390.8^{ab}	350.7	318.2	334.4
Th_2	13.23^{b}	17.12^{d}	14.12^{cA}	16.23^{dB}	15.17^{d}	318.2	420.2^{b}	353.2	385.2	369.2
Th_3	11.65^{ab}	15.14^{cd}	12.01^{bA}	14.78^{cB}	13.39^{bc}	292.0	423.3^{b}	344.8	370.5	357.7
Th_4	13.73^{b}	15.87^{d}	14.01^{cA}	15.60^{dB}	14.81^{cd}	304.2	441.3^{b}	359.8	385.7	372.8
Mean	11.90^{A}	14.30^{B}	12.12^{A}	14.08^{B}	13.10	302.6^{A}	402.4^{B}	344.7	360.4	352.5

Source of variation	F-value	*P*	$HSD_{0.05}$	F-value	*P*	$HSD_{0.05}$
Th	35.01	<0.001	1.469	2.38	>0.05	NS
MF	299.59	<0.001	0.278	2.93	>0.05	NS
Y	59.11	<0.001	0.650	73.57	<0.001	24.21
Th × MF	3.03	0.03	0.750	1.23	>0.05	NS
MF × Th	3.03	0.03	0.650	1.23	>0.05	NS
Y × Th	6.51	0.003	2.088	3.55	0.02	77.56
Y × MF	5.91	>0.05	NS	1.35	>0.05	NS

Means followed by different lowercase letters in columns and different uppercase letters in rows differ significantly at $p \leq 0.05$; NS – not significant; Th – *Trichoderma harzianum*; MF – mineral fertilization; Y – years; Th_0, Th_1, …, NPK, NPK+B – combinations with *Trichoderma harzianum* and mineral fertilization, see *Table 1*

Similarly, the weight of the marketable curd was significantly higher. Inoculation of broccoli with *Trichoderma harzianum* contributed to the growth of the marketable yield of curds relative to uninoculated control. The effect was especially visible in 2015, when a significant increase in the yield compared to control was observed on all combinations of *T. harzianum*, regardless of the manner of application. The average increase in the curd yield for both years ranged from 17.5 to 49%, with the highest for

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

the Th_2 combination (*T. harzianum* added to the nursery substrate and as topdressing in a form of spray after seedlings were planted out). The beneficial effect of broccoli inoculation with *T. harzianum* on the curd marketable yield was recorded only in 2015. Tanwar et al. (2013) also noted a significant increase in the weight and size of broccoli curds as a response to inoculation with *Trichoderma viride*. Abd Alla and El-Shoraky (2017) found a significant increase in the yields of white cabbage and cauliflower after *Trichoderma harzianum* inoculation. Altintas and Bal (2008) noted an upward trend in the yield of onion after applying *T. harzianum*, but the increase, when compared with control, was not statistically significant.

Regardless of the manner of *Trichoderma harzianum* application there was a beneficial effects of boron treatment on broccoli yields. The marketable curd yield on plots with Nitrabor (NPK+B) increased on average by 2.68 $t \cdot ha^{-1}$ in relation to the combinations without boron (NPK). The largest marketable yield was reported after combined application of *Trichoderma harzianum* (Th_2) and NPK+B topdressing. An increase in the yield of broccoli curd as a response to boron application was also noted by Moniruzzaman et al. (2007), Hussain et al. (2012), Singh et al. (2015), Islam et al. (2015) and Farooq et al. (2018). Additionally, Moniruzzaman et al. (2007), Hussain et al. (2012) and Singh et al. (2015) observed a statistically significant increase in the mass of curds. Optimum levels of NPK and boron applied to broccoli positively affect photosynthetic efficiency and improve other processes like enzyme activation, protein and carbohydrate accumulation, and translocation of sugar and starch; because of all that the yield also increases (Shahah et al., 2010; Singh et al., 2015).

There was no effect of *Trichoderma harzianum* on the curd circumference length and stem diameter. It was noted, however, that the SPAD value evolved under the influence of the inoculation, depending on how it was applied (*Table 4*).

Table 4. *The effect of different treatment combinations on broccoli curd biological parameters and SPAD*

Treatment	Curd circumference length (mm)	Stalk diameter (mm)	SPAD
Trichoderma harzianum			
Th_0	226	31.6	80.53^{a}
Th_1	225	28.8	81.83^{ab}
Th_2	221	32.3	86.94^{c}
Th_3	232	29.3	80.54^{a}
Th_4	224	32.1	84.46^{bc}
Mineral fertilization			
NPK	220^{a}	29.8^{a}	82.05^{a}
NPK+B	232^{b}	31.8^{b}	83.67^{b}
Years			
2014	239^{b}	31.9^{b}	82.99
2015	212^{a}	29.8^{a}	82.05
Mean	226	30.8	82.86
Source of variation	$HSD_{0.05}$		
T. harzianum	NS	NS	2.565
Mineral fertilization	5.2	1.71	0.710
Years	7.8	1.85	NS
Th × MF	NS	NS	NS
MF × Th	NS	NS	NS
Y × Th	NS	NS	NS
Y × MF	NS	NS	NS

Means followed by different letters in columns differ significantly at $p \leq 0.05$; NS – not significant; Th – *Trichoderma harzianum*; MF – mineral fertilization; Y – years; Th_0, Th_1, …, NPK, NPK+B – combinations with *Trichoderma harzianum* and mineral fertilization, see *Table 1*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

Compared to control a significant SPAD increase was reported on Th_2 and Th_4 combinations, where *T. harzianum* was applied additionally as a spray. The results indicated that this kind of treatment increased the amount of nitrogen taken up by plants. A significant increase in chlorophyll content in broccoli leaves after vaccination of the substrate with *Trichoderma viride* was noted, among others, by Tanwar et al. (2013). Harman et al. (2012) and Hermosa et al. (2012) report that *Trichoderma* strains colonise plant roots, establishing chemical communication and systemically altering the expression of numerous plant genes that alter plant physiology and may result in the improvement of abiotic stress resistance, nitrogen fertiliser uptake, and photosynthetic efficiency.

Both NPK+B topdressing significantly increased curd circumference length, the diameter of the stem, and the SPAD value compared with the basic fertilization (*Table 4*). Similar results were obtained by Husain et al. (2012) and Singh et al. (2015). Regardless of the dose size, they recorded a significant increase in the diameter of broccoli curds and stems after boron treatment. Similarly, Chatterjee and Bandyopadhyay (2017) recorded a significant SPAD increase in cowpea after boron application. The increase was directly proportional to the applied doses. In the present experiment, there was no significant effect of the interaction of *T. harzianum* and boron on the circumference length, the diameter of the stem, and the SPAD value.

The average content of dry matter in broccoli curds was 11.61%, with protein constituting 4.04% in fresh matter (FM), total sugars 2.66 $g \cdot 100g^{-1}$ FM, and monosaccharides 1.68 $g \cdot 100g^{-1}$ FM (*Table 5*).

Table 5. *The effect of different treatment combinations on dry matter, protein, and sugar content in broccoli*

Treatment	Dry matter (%)	Protein (% FM)	Total sugars ($g \cdot 100g^{-1}$ FM)	Monosaccharides ($g \cdot 100g^{-1}$ FM)
Trichoderma harzianum				
Th_0	11.51	4.05	2.68	1.64
Th_1	11.67	4.03	2.61	1.65
Th_2	11.52	4.04	2.66	1.62
Th_3	11.62	4.04	2.71	1.63
Th_4	11.74	4.06	2.66	1.86
Mineral fertilization				
NPK	11.17^a	4.04	2.71^b	1.68
NPK+B	12.05^b	4.06	2.61^a	1.68
Years				
2014	11.40^a	4.02	2.64	1.76^b
2015	11.82^b	4.07	2.69	1.60^a
Mean	11.61	4.04	2.66	1.68
Source of variation	$HSD_{0.05}$			
T. harzianum	NS	NS	NS	NS
Mineral fertilization	0.435	NS	0.039	NS
Years	0.231	NS	NS	0.136
Th × MF	NS	NS	NS	NS
MF × Th	NS	NS	NS	NS
Y × Th	NS	NS	NS	NS
Y × MF	NS	NS	NS	NS

Means followed by different letters in columns differ significantly at $p \leq 0.05$; NS – not significant; Th – *Trichoderma harzianum*; MF – mineral fertilization; Y – years; Th_0, Th_1, …, NPK, NPK+B – combinations with *Trichoderma harzianum* and mineral fertilization, see *Table 1*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

There was no significant effect of *Trichoderma harzianum* inoculation on the above content. However, the amount of dry matter and total sugars in plants was dependent on fertilizer treatment. Broccoli plants treated with boron contained significantly more dry matter and less total sugars than those grown without the Nitrabor fertilizer. A significant increase in dry matter content in broccoli curds as a response to boron was also noted by Islam et al. (2015). Ningawale et al. (2016) recorded an increase in dry matter content in cauliflower heads treated with Borax, but the increase was statistically insignificant. In the studies of Patel et al. (2017) broccoli treated with boron and without it had similar content of sugars, but Meena et al. (2015) found a significant increase in the content of total sugars and monosachcarides in the fruits of tomato treated with this chemical element. In the present experiment there was no significant effect of the interaction between *T. harzianum* and boron on the content of dry matter, protein, and sugars in broccoli. It was found, however, that weather conditions throughout the research differentiated the content of dry matter and monosaccharides in broccoli curds. In 2014 dry matter content was lower and monosaccharide content was greater than in 2015.

Ascorbic acid (AA) content in broccoli curds was on average 67.20 mg per 100 g FM (*Table 6*). With 2.95 $mg \cdot 100g^{-1}$ FM in 2014 it was higher than in 2015, and this difference was statistically significant. There was a significant effect of broccoli inoculation with *Trichoderma harzianum* on AA content. On average, for both growing seasons, broccoli grown on plots with *T. harzianum* contained more AA than on the control plot. In the case of Th_2 and Th_3 combinations these differences were statistically significant. The beneficial effect of *T. harzianum* on AA content in broccoli was especially visible in 2015. Similarly, in the studies of Hale et al. (2012) it was found that *T. harzianum,* added to compost and applied in a form of solution in the cultivation of tomato, significantly influenced the growth of the content of AA, protein, and total sugars in fruits.

Table 6. *The effect of different treatment combinations on ascorbic acid content in broccoli (mg $100 \cdot g^{-1}$ FM)*

Treatment	**Year**		**Mineral fertilization**		**Mean**
	2014	**2015**	**NPK**	**NPK+B**	
Th_0	69.01	62.84^{a}	65.87	65.99^{a}	65.93^{a}
Th_1	69.78	64.13^{ab}	67.63	66.27^{ab}	66.95^{ab}
Th_2	70.06	66.43^{bc}	66.98^{A}	69.52^{cB}	68.25^{b}
Th_3	68.27	67.29^{c}	67.39	68.17^{bc}	67.78^{b}
Th_4	68.76	65.48^{bc}	65.86^{A}	68.38^{cB}	67.12^{ab}
Mean	68.18^{B}	65.23^{A}	66.75^{A}	67.67^{B}	67.20

Source of variation	*F-value*	*P*	$HSD_{0.05}$
T. harzianum	5.04	0.005	1.659
Mineral fertilization	6.64	0.04	0.888
Years	125.21	<0.001	0.733
Th × MF	3.79	0.02	1.778
MF × Th	3.79	0.02	2.051
Y × Th	6.94	0.003	2.347
Y × MF	5.84	>0.05	NS

Means followed by different lowercase letters in columns and different uppercase letters in rows differ significantly at $p \leq 0.05$; NS – not significant; Th – *Trichoderma harzianum*; MF – mineral fertilization; Y – years; Th_0, Th_1, …, NPK, NPK+B – combinations with *Trichoderma harzianum* and mineral fertilization, see *Table 1*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

A significant increase in AA content (on average by 1.35%) in broccoli was also noted after application of Nitrabor, a fertilizer containing boron. Beneficial effects of boron on the content of ascorbic acid in broccoli was also observed by Islam et al. (2015), and in the fruit of tomato by Lester (2006). However, in the studies of Patel et al. (2017) and Meena et al. (2015) there were no significant changes in the content of vitamin C in broccoli heads and tomato fruits after boron treatment, compared to plants treated with basic fertilizer.

Combined application of *T. harzianum* and boron had a significant impact on the amount of ascorbic acid. The highest amount of AA was recorded in plants topdressed with boron and with *T. harzianum* added to the substrate during seedling production and then sprayed on plants in the field (Th_2), and when *T. harzianum* were applied in the field first as pre-planting treatment and then as a spray (Th_4).

Conclusions

1. Trichoderma harzianum, irrespective of the application combination, had a significant impact on the growth of the marketable broccoli curd yield.
2. The highest yield increase was recorded after applying Trichoderma harzianum to seedling substrate, supplemented with spraying plants with Trichoderma harzianum during their growth. Favourable yield parameters resulted from greener leaves, with more efficient photosynthetic production.
3. Boron nutrition favourably affected yield parameters studied in the experiment. As a chemical element affecting synthesis and transport of carbohydrates boron contributed to a substantial increase in the curd marketable yield (on average by 16.2%), curd weight (on average by 4.6%) and an increase in the content of dry matter, total sugars, and L-Ascorbic acid in broccoli tissues in comparison to that after mineral fertilization without the addition of boron.
4. In the production of broccoli, the use of Trichoderma harzianum can be recommended, especially as a combined application, first to the seedling substrate, and then in a form of spray in the field, supplemented with boron.

Acknowledgements. The research carried out under the theme No 226/06/S was financed by the science grant of the Ministry of Science and Higher Education.

REFERENCES

[1] Abd Alla, M. A., El-Shoraky, F. S. (2017): Impact of biological agents and plant essential oils on growth, quality and productivity of cabbage and cauliflower plants correlated to some diseases control. – Journal of Sustainable Agricultural Sciences 43(1): 27-38. DOI: 10.21608/jsas.2017.3493.

[2] Altintas, S., Bal, U. (2008): Effects of the commercial product based on *Trichoderma harzianum* on plant, bulb and yield characteristics of onion. – Scientia Horticulturae 116(2): 219-222. DOI: 10.1016/j.scienta.2007.11.012.

[3] Bal, U., Altintas, S. (2006): A positive side effect from *Trichoderma harzianum,* the biological control agent: Increased yield in vegetable crops. – Journal of Environmental Protection and Ecology 7: 383-387.

[4] Bolanos, L., Brewin, N. J., Bonilla, I. (1996): Effects of boron on *Rhizobium*-legume cell-surface interactions and nodule development. – Plant Physiology 110: 1249-1256.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

[5] Bonilla, I., Mergold-Villasefisor, C., Campos, M. E., Sanches, N., Perez, H., Lopez, L., Castrejon, L., Sanchez, F., Cassab, G. I. (1997): The aberrant cell walls of boron-deficient bean root nodules have no covalantly bound hydroxyproline-proline-rich proteins. – Plant Physiology 115: 1329-1340.

[6] Brown, P. H., Bellaloui, N., Wimmer, M. A., Bassil, E. S., Ruiz, J., Hu, H., Pfeffer, H., Dannel, F., Römheld, V. (2002): Boron in plant biology. – Plant Biology 4: 205-223.

[7] Chatterjee, R., Bandyopadhyay, S. (2017): Effect of boron, molybdenum and biofertilizers on growth and yield of cowpea (*Vigna unguiculata* L. Walp.) in acid soil of eastern Himalayan region. – Journal of the Saudi Society of Agricultural Sciences 16(4): 332-336. DOI: 10.1016/j.jssas.2015.11.001.

[8] Davies, M. J., Atkinson, C. J., Burns, C., Arroo, R., Woolley, J. (2011): Increases in leaf artemisinin concentration in Artemisia annua in response to the application of phosphorus and boron. – Industrial Crops and Products 34(3): 1465-1473. DOI: 10.1016/j.indcrop.2011.05.002.

[9] Diana, G. (2006): Boron in the soil, from deficit to toxicity. – Informatore Agrario 62: 54-58.

[10] EU (2009): Commission Regulation NO 152/2009. – Official Journal of the European Union L54: 1-130.

[11] Farooq, M., Bakhtiar, M., Ahmed, S., Ilyas, N., Khan, I., Saboor, A., Solangi, I. A., Khan, A. Y., Khan, S., Khan, I. (2018): Influence of sulfur and boron on the growth and yield of broccoli. – International Journal of Environmental & Agriculture Research 4(4): 9-16.

[12] Goldbach, H. E., Wimmer, M. A. (2007): Boron in plants and animals: is there a role beyond cell-wall structure? – Journal of Plant Nutrition and Soil Science 170(1): 39-48. DOI: 10.1002/jpln.200625161.

[13] Harman, G. E., Herrera-Estrella, A. H., Horwitz, B. A., Lorito, M. (2012): *Trichoderma* – from basic biology to biotechnology. – Microbiology 158(Special Issue): 1-2. DOI: 10.1099/mic.0.056424-0.

[14] Hermosa, R., Viterbo, A., Chet, I., Monte, E. (2012): Plant-beneficial effects of *Trichoderma* and of its genes. – Microbiology 158: 17-25. DOI: 10.1099/mic.0.052274-0.

[15] Hussain, M. J., Sirajul Karim, A. J. M., Solaiman, A. R. M., Haque, M. M. (2012): Effects of nitrogen and boron on the yield and hollow stem disorder of broccoli (*Brassica oleracea* var. *italica*). – The Agriculturists 10(2): 36-45. DOI: 10.3329/agric.v10i2.13140.

[16] Islam, M., Hoque, M. A., Reza, M. M., Chakma, S. P. (2015): Effect of boron on yield and quality of broccoli genotypes. – International Journal of Experimental Agriculture 5(1): 1-7.

[17] IUSS Working Group WRB (2015): World Reference Base for Soil Resources 2014 (update 2015). International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. – World Soil Resources Reports No. 106. Rome, FAO.

[18] Martínez, O. A., Crowley, D. E., Mora, M. L., Jorquera, M. A. (2015): Short-term study shows that phytate-mineralizing rhizobacteria inoculation affects the biomass, phosphorus (P) uptake and rhizosphere properties of cereal plants. – Journal of Soil Science and Plant Nutrition 15: 153-166. DOI: 10.4067/s0718-95162015005000013.

[19] Meena, D. C., Maji, S., Meena, J. K., Govind Kumawat, R., Meena, K. R., Kumar, S., Sodh, K. (2015): Improvement of growth, yield and quality of tomato (*Solanum lycopersicum* L.) cv. Azad T-6 with foliar application of zinc and boron. – International Journal of Bio-resource and Stress Management 6(5): 598-601. DOI: 10.5958/0976-4038.2015.00091.3.

[20] Miwa, K., Takano, J., Omori, H., Seki, M., Shinozaki, K., Fujiwara, T. (2007): Plants tolerant of high boron levels. – Science 318(5855): 1417-1417. DOI: 10.1126/science.1146634.

[21] Molla, A. H., Manjurul Haque, M., Amdadul Haque, M., Ilias, G. N. M. (2012): *Trichoderma*-Enriched biofertilizer enhances production and nutritional quality of tomato

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/1702_43974407
© 2019, ALÖKI Kft., Budapest, Hungary

(*Lycopersicon esculentum* Mill.) and minimizes NPK fertilizer use. – Agricultural Research 1(3): 265-272. DOI: 10.1007/s40003-012-0025-7.
[22] Moniruzzaman, M., Rahman, S. M. L., Kibria, M. G., Rahman, M. A., Hossain, M. M. (2007): Effect of boron and nitrogen on yield and hollowstem of broccoli. – Journal of Soil and Nature 1(3): 24-29.
[23] Ningawale, D. K., Singh, R., Bose, U. S., Gurjar, P. S., Sharma, A., Gautam, U. S. (2016): Effect of boron and molybdenum on growth, yield and quality of cauliflower (*Brassica oleracea* var *botrytis*) cv. Snowball 16. – Indian Journal of Agricultural Sciences 86(6): 825-829.
[24] Parente, C. P., Reis Lima, M. J., Teixeira-Lemos, E., Moreira, M. M., Barros, A. A., Guido, L. F. (2013): Phenolic content and antioxidant activity determination in broccoli and lamb's lettuce. – International Journal of Agricultural and Biosystems Engineering 7: 70-73.
[25] Patel, A., Maji, S., Meena, K. R., Malviya, N. K., (2017): Use of boron and molybdenum to improve broccoli production. – Journal of Crop and Weed 13(2): 20-24.
[26] PN-A-04019 (1998): Food products – Determination of vitamin C content. – Produkty spożywcze – Oznaczanie zawartości witaminy C [in Polish].
[27] Sahah, D. A., Narayan, R., Ahmad, N., Narayan, S., Wani, K. P. (2010): Influence of boron and zinc on growth, yield and quality of Knol-khol cv. Early white Vienna. – Indian Journal of Horticulture 67(special issue): 323-328.
[28] Singh, M. K., Chand, T., Kumar, M., Singh, K. V., Lodhi, S. K., Singh, V. P., Sirohi, V. S. (2015): Response of different doses of NPK and boron on growth and yield of broccoli (*Brassica oleracea* L. var. *italica*). – International Journal of Bio-resource and Stress Management 6(1): 108-112. DOI: 10.5958/0976-4038.2015.00016.0.
[29] Studholme, D. J., Harris, B., Le Cocq, K., Winsbury, R., Perer, A. V., Ryder, L., Ward, J. L., Beale, M. H., Thornton, C. R., Grant, M. (2013): Investigating the beneficial traits of *Trichoderma hamatum* GD 12 for sustainable agriculture-insights from genomics. – Frontiers in Plant Science 4: 1-13.
[30] Szulc, W., Rutkowska, B. (2013): Diagnostics of boron deficiency for plants in reference to boron concentration in the soil solution. – Plant, Soil and Environment 59: 372-377.
[31] Szymańska, G., Faligowska, A., Panasiewicz, K., Szukała, J., Koziara, W. (2017): The productivity of two yellow lupine (*Lupinus luteus* L.) cultivars as an effect of different farming systems. – Plant, Soil and Environment 63(12): 552-557. DOI: 10.17221/639/2017-pse.
[32] Tanwar, A., Aggarwal, A., Kaushish, S., Chauhan, S. (2013): Interactive effect of AM fungi with *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield of broccoli. – Plant Protection Science 49(3): 137-45. DOI: 10.17221/54/2012-pps.
[33] Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Barbetti, M. J., Li, H., Woo, S. L., Lorito, M. (2008): A novel role for *Trichoderma* secondary metabolites in the interactions with plants. – Physiological and Molecular Plant Pathology 72: 80-86.
[34] Xu, F., Goldbac, H. E., Brown, P. H., Bell, R. W., Fujiwara, T., Hunt, C. D., Goldberg, S., Shi, L. (eds.) (2007): Advances in Plant and Animal Boron Nutrition. – Dordrecht: Springer, ISBN: 978-1-4020-5381-8.

© 2019, ALÖKI Kft., Budapest, Hungary