

Department of Plant Protection and Quarantine, University of Life Sciences in Lublin  
S. Leszczyńskiego 7, 20-068 Lublin  
e-mail: aguto@wp.pl

AGNIESZKA JAMIOŁKOWSKA

### **Effect of some biotechnical preparations on the growth of sweet pepper plants in the field production**

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Wpływ niektórych preparatów biotechnicznych na wzrost papryki  
uprawianej w polu

**Summary.** The aim of the study was to estimate the influence of Bioczos Płynny, Biosept 33 SL and Bio-algeen S90 Plus on the growth of sweet pepper plants cultivated in the field. Field experiments were carried out during the period 2010–2012 in the farm in Zezulín, near Lublin. Plants of sweet pepper ('Roberta F<sub>1</sub>') were the objects of research. The biotechnical preparations used for protection of pepper against diseases were: Bioczos Płynny, Biosept 33SL and Bio-algeen S90 Plus. Leaves, stems and roots of pepper were measured in the fourth week after treatment of plants. The biometric measurements of plants showed that the size of plants was different and depended on the type of preparation and weather conditions. The biotechnical preparations do not have the positive influence on the length of stems and roots of pepper but they increase the size of the leaf blade. The biggest leaves were observed on the plants sprayed with Bioczos Płynny and Bio-algeen S90 Plus. The microscopic analysis shows that Bioczos Płynny, Bio-algeen S90 Plus and Biosept 33 SL increase the thickness of the stem secondary cortex of pepper plants.

**Key words:** Bioczos Płynny, Biosept 33SL, Bio-algeen S90 Plus, biotechnical preparations, *Cap-sicum annuum*, plants growth, size of leaf blade, secondary cortex of stem, SEM

#### INTRODUCTION

One of the most popular method of plant protection against pathogenic fungi is the chemical control with use of different fungicides [Smith *et al.* 1999, Yaqub and Shahzad 2006]. Chemical control not only induces the resistance of fungi to fungicides and environment pollution but above all affects directly the human health [Zhang *et al.* 2007, Damalas and Eleftherohorinos 2011]. Recently, the use of chemicals in plant production has been reduced. The methods of plant control against pests are the use of natural preparations [Saniewska 2000, Orlikowski and Skrzypczak 2003, Pięta and Pastucha 2004, Maleshko 2005, Gorczyca 2007, Patkowska 2006a, b, Jamiołkowska 2009, 2013,

Jamiołkowska and Wagner 2007, 2011]. More and more often preparations based on natural substances are applied for protecting plants against diseases. Recently, plant extracts and essential oils are more often used by producers to protect plants against pathogenic fungi both in ecological and integrated production [Wagner *et al.* 2003, Jamiołkowska and Wagner 2007, Jamiołkowska 2009]. Biological preparations protect the plants against pathogens and induce the various resistance mechanisms in the plants. Many of the commercially used formulations of this type are known inducers of plant resistance and are named biostimulators [Jamiołkowska 2013].

Among natural preparations, this effect show Bioczos Płynny, Biosept 33 SL and Bio-algeen S90 Plus [Orlikowski and Skrzypczak 2003, Dobromilska and Gubarewicz 2008, Portz *et al.* 2008, Jamiołkowska and Wagner 2011, Jamiołkowska 2013].

Bioczos Płynny is produced on the base of garlic pulp. The literature provides more information about possibility of using the garlic extract in the plant's protection against diseases. Garlic extract has antibacterial and antifungal properties [Saniewska 2000, Jamiołkowska and Wagner 2011, Hadian 2012, Horoszkiewicz-Janka *et al.* 2012, Jamiołkowska 2013]. The antimicrobial activity of garlic juice had long been known and this was due to allicin [Miron *et al.* 2000]. This natural biological substance is a volatile phytoanticipin produced in garlic upon wounding, is active against a broad range of phytopathogenic organisms *in vitro* and *in vivo* [Curtis *et al.* 2004, Jamiołkowska and Wagner 2011]. In addition the garlic extract might contain substances which are able to induce systemic acquired resistance (SAR) in the host [Uknes *et al.* 1992].

Other preparation using in plant protection is Biosept 33 SL (33% grapefruit extract). This product contains widely spectrum of active substances (endogenous flavonoids) that have strong antibiotic properties, and at the same time, it does not invoke any side-effects. Inhibition of many disease forming bacteria and fungi has been reported in studies upon grapefruit extracts [Saniewska 2002, 2004, Patkowska 2006a, b, Sadowski *et al.* 2009, Jamiołkowska 2009, 2013]. Biosept 33 SL not only inhibits the development of harmful microorganisms, but also improves plant's immune system [Orlikowski and Skrzypczak 2001, Jamiołkowska 2013]. The literature provides with information on the effectiveness of Biosept 33 SL in the control vegetables and ornamental plants [Dłużniewska 2004, 2006, Patkowska 2006a, Lenc 2007, Saniewska 2002].

Bio-algeen S90 Plus preparation contains sea algae *Ascophyllum nodosum*. This product is using for stimulation the growth and development the plants. Sea algae containing brown pigment, which have been used as a fertilizer since long time, are characterized by special nutritive value. The contain a lot of vitamins, carbohydrates and amino acids [Dobromilska and Gubarewicz 2008, Sultana *et al.* 2011].

The aim of the study was to estimate the influence of Bioczos Płynny, Biosept 33 SL and Bio-algeen S90 Plus on the growth of sweet pepper plants cultivated in the field.

## MATERIAL AND METHODS

### Field experiment

The experiment was carried out in 2010–2012 in the horticultural farm in Zezulin in Lublin province (N51°20', E22°49'). The objects of research were the plants of sweet pepper (*Capsicum annuum* L.) 'Roberta F<sub>1</sub>'. The experiment was set up in the soil with

a pH of 6.5. Wheat was the forecrop for pepper in three years. In the year preceding pepper cropping, organic fertilization was applied at the rate of  $40 \text{ t} \cdot \text{ha}^{-1}$ . Spring mineral nutrition was done according to the soil analysis ( $\text{kg} \cdot \text{ha}^{-1}$ ): N-100 (nitrogen nitrate), P-60 (superphosphate), K-140 (potassium sulphate). Foliar nutrition was applied as post-crop: once with Florovit (0.5%), twice with calcium nitrate (1.0%).

Four-week pepper seedlings were planted in the field in the second decade of May in the spacing of  $0,67 \times 0,35 \text{ m}$ . Twenty seedlings were planted in each plot of  $4.69 \text{ m}^2$ . The experiment was set up in randomized blocks in 4 replications. The investigated factors were the biotechnical preparations which types and concentrations are described in table 1. There were two control combinations: absolute (with plants without any treatment) and relative (with plants treated with the fungicide Amistar 250 SC). Around experiment plots one row of pepper plants was planted. Those plants were not used in the experiment. Plants were weeded manually during the growing period. The application of preparations started 1 month after planting of seedlings. Preparations were applied every 7–10 days, by spraying and watering of plant with 100–200 ml liquid for each plant (tab. 1).

Table 1. Combinations of field experiment in 2010–2012  
Tabela 1. Kombinacje doświadczenia polowego w latach 2010–2012

Experimental combination Kombinacja doświadczalna	Concentration of preparation Stężenie preparatu (%)	Number of treatments Liczba zabiegów
Control absolute, plants without treatments Kontrola absolutna, rośliny niechronione	–	–
Amistar 250 SC (azoxystrobin) – relative control Amistar 250 SC (azoksystrobina) – kontrola względna	0.1	2
Bioczos Płynny (garlic extract/ wyciąg z czosnku)	2.0	6
Biosept 33SL (grapefruit extract/ wyciąg z grejpfruta)	0.2	6
Bio-algeen S90 Plus ( <i>Ascophyllum nodosum</i> sea algae/ algi morskie)	0.3	6

### Biometric features of plants

The plants analysis were conducted in the 2010–2012. Stems, roots and leaves of pepper were collected and measured in the fourth week after spraying (third decade of August). For each combinations twelve uniform plants were selected. The length (in cm) of roots and stems were measured for each plants. Leaves for analysis were collected from the middle part of the plant. Leaf length was measured including leaf blade and petiole, while the width was measured at the widest point of the leaf blade.

### Microscopic analysis

Material for microscopic analysis was collected in 2012, during the pepper fruiting period (third decade of August). The fragments of the stems were collected from the half of analyzed part of the plants. The plant material was treated 24 hours in the 3% glutaralde-

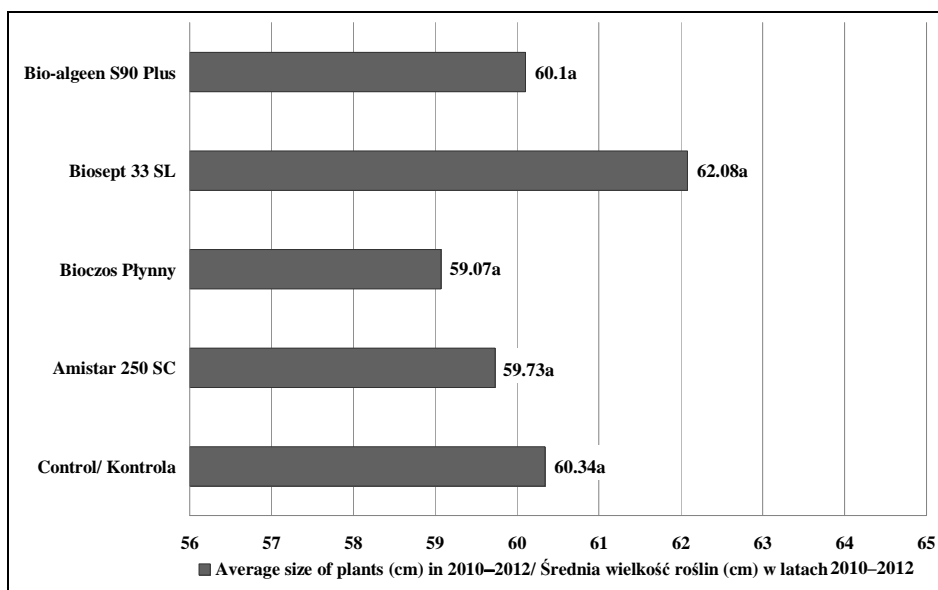
hyde, washed in 0.1 M phosphate buffer ( $3 \times 10$  min), dehydrated in ethanol, dried in critical point dried and sprayed with precious metal. The cross-sections were observed in scanning electron microscopy (SEM) at the magnification  $\times 110$ .

### Statistical analysis

The data were analyzed by the analysis of variance (Tukey's test) at 5% significance level using the SAS statistical system (SAS Version 9.1, SAS Inst., Cary, N.C., USA).

## RESULTS

The biometric measurements of plants conducted in the vegetation period showed that the size of plants depend on the type of preparation and weather conditions (fig. 1, tab. 2). The growth of plants varied on the year cultivation and weather (fig. 1, tab. 2). The highest plants were in 2010 and the lowest in 2012. It depends on the amount of rainfall that were the highest in 2010 (tab. 2). The size of plants also depend on the type of formulation. The highest stems were observed in the plants sprayed with Biosept (mean 47.12 cm) (except 2010) and the biggest roots were noted in the plants coming from a combination with Bio-algeen (mean 15.59 cm). The noted differences however, were not statistically significant to the control (tab. 3).



Values designated with the same letter do not significantly differ at 5% error (Tukey's test)  
Wartości oznaczone tą samą literą nie różnią się istotnie przy poziomie błędów 5% (test Tukeya)

Fig. 1. The effect of biotechnical preparations treatment on the average size of sweet pepper plants in 2010–2012

Rys. 1. Wpływ preparatów biotechnicznych na średnią wielkość roślin papryki słodkiej w latach 2010–2012

Table 2. The average temperature (°C) and monthly rainfall (mm) of Agrometeorological Observatory in Felin in 2010–2012  
Tabela 2. Średnie miesięczne temperatury (°C) i średnie miesięczne sumy opadów (mm) z Obserwatorium Agrometeorologicznego w Felinie w latach 2010–2012

Month Miesiąc	Average temperature Średnia temperatura (°C)				Monthly rainfall Średnia miesięczna suma opadów (mm)			
	2010	2011	2012	1951–2010	2010	2011	2012	1951–2010
V	14.5	14.3	15.0	13.0	156.7	42.2	56.3	60.7
VI	18.0	18.6	17.3	16.3	65.6	67.8	62.8	65.9
VII	21.6	18.4	21.4	18.0	101.0	189.0	52.3	82.0
VIII	20.2	18.8	19.2	17.2	132.8	65.3	37.6	70.7
IX	12.5	15.2	15.0	12.6	119.0	5.4	35.5	53.7
X	5.6	7.9	8.0	7.6	11.2	28.5	88.8	40.1

Table 3. The effect of biotechnical preparations treatment on the vegetative growth of sweet pepper plants (2010–2012)

Tabela 3. Wpływ preparatów biotechnicznych na wzrost wegetatywny roślin papryki słodkiej (2010–2012)

Experimental combination Kombinacja doświadczalna	Length of stem Długość łodygi (cm)				Length of root Długość korzenia (cm)			
	2010	2011	2012	mean średnia	2010	2011	2012	mean średnia
Control/ Kontrola	54.33a	46.5a	35.0b	45.27a	13.72b	18.5a	13.0 ab	15.07a
Amistar 250 SC	48.5ab	46.0a	40.0ab	44.83a	15.46ab	15.5ab	13.75a	14.9a
Bioczos Płynny	47.75b	48.75a	37.5b	44.67a	16.21ab	14.75b	12.25ab	14.4a
Biosept 33SL	46.35b	49.0a	46.0a	47.12a	19.64a	15.0b	10.25b	14.96a
Bio-algeen S90 Plus	52.33ab	45.5a	35.7b	44.51a	17.28ab	17.25ab	12.25ab	15.59a
LSD <sub>0.05</sub> /NIR <sub>0.05</sub>	6.3759	7.4140	7.2565	4.3406	4.5939	3.1126	3.3920	2.2924

Values designated with the same letter in columns do not significantly differ at 5% error (Tukey's test)  
Wartości oznaczone tą samą literą w kolumnach nie różnią się istotnie przy poziomie błędów 5% (test Tukeya)

Table 4. The effect of biotechnical preparations treatment on the size of leaf blade of pepper plants (2010–2012)

Tabela 4. Wpływ preparatów biotechnicznych na wielkość blaszki liściowej papryki (2010–2012)

Experimental combination Kombinacja doświadczalna	Length of leaf Długość liścia (cm)				Width of leaf Szerokość liścia (cm)			
	2010	2011	2012	mean średnia	2010	2011	2012	mean średnia
Control/ Kontrola	12.4	11.1	10.5	11.33b	3.8	4.0	3.2	3.66b
Amistar 250 SC	12.7	12.9	11.6	12.4ab	5.2	4.2	3.4	4.26ab
Bioczos Płynny	14.9	13.5	15.1	14.5a	5.0	5.8	5.2	5.33a
Biosept 33SL	12.2	12.9	12.3	12.46ab	4.1	4.2	4.0	4.10ab
Bio-algeen S90 Plus	12.5	13.7	11.0	12.4ab	4.8	5.5	4.5	4.93a
LSD <sub>0.05</sub> /NIR <sub>0.05</sub>	3.0123				1.0954			

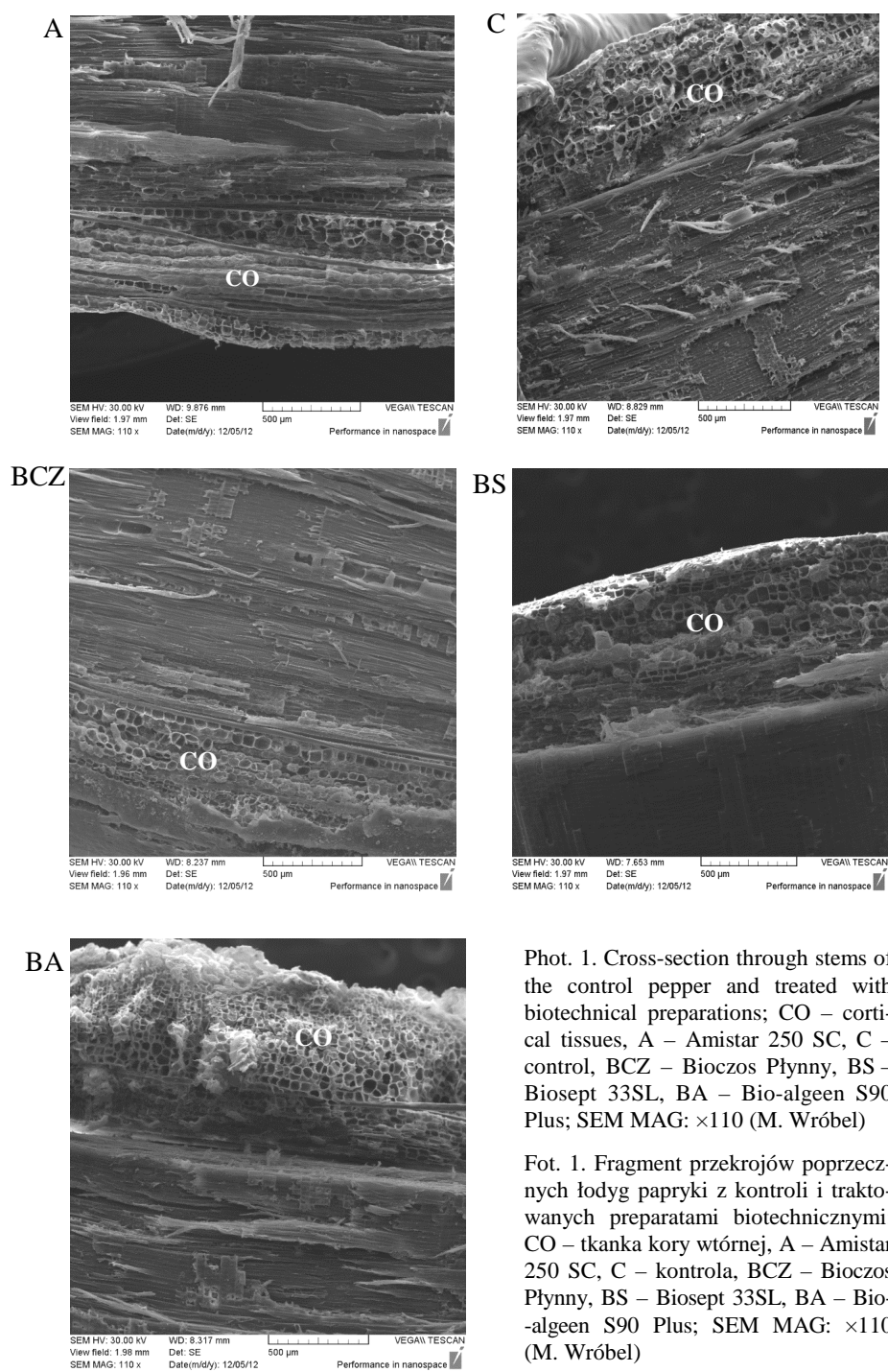
Values designated with the same letter in columns do not significantly differ at 5% error (Tukey's test)  
Wartości oznaczone tą samą literą w kolumnach nie różnią się istotnie przy poziomie błędów 5% (test Tukeya)

In this study also the leaf size was measured. The biggest leaves were observed on the plants sprayed with Bioczos Płynny (mean length 14.5 cm, width 5.33 cm) and the smallest in the control (mean length 11.33 cm, width 3.66 cm) (tab. 4). The treatments with Biosept and Bio-algeen had also positive effect on the size of leaves relative to control (respectively  $12.46 \times 4.1$  cm,  $12.4 \times 4.93$  cm). The results showed that the biotechnical preparations have a significant impact only on the size of leaf blade of sprayed plants (tab. 4).

The results of microscopic analysis were used to describe the influence of biotechnical preparations on stem cortex structure. There was the thickest of stem secondary cortex in plants treated with Bio-algeen (951.5  $\mu\text{m}$ ) and Biosept (625.0  $\mu\text{m}$ ). Thickness of secondary cortex of stems treated with Bioczos Płynny were similar to the control (respectively 579.8 and 548.6  $\mu\text{m}$ ) (tab. 5). Microscopic observation have shown that the use of Bio-algeen, Biosept and Bioczos Płynny caused an increase in the thickness of the pepper stems (phot. 1).

Table 5. Thickness of the stem secondary cortex of pepper in 2012 (SEM)  
Tabela 5. Grubość kory wtórnej łodyg papryki w roku 2012 (SEM)

Experimental combination Kombinacja doświadczalna	Thickness Grubość ( $\mu\text{m}$ )		
	No nr	measurement pomiar	mean średnia
Control/ Kontrola	1	555.6	548.6
	2	555.6	
	3	527.8	
	4	555.6	
Amistar 250 SC	1	902.7	885.4
	2	861.0	
	3	875.0	
	4	902.7	
Bioczos Płynny	1	625.0	579.8
	2	575.0	
	3	558.0	
	4	561.0	
Biosept 33SL	1	625.0	625.0
	2	611.1	
	3	638.8	
	4	625.0	
Bio-algeen S90 Plus	1	930.5	951.5
	2	972.2	
	3	972.2	
	4	931.0	



Phot. 1. Cross-section through stems of the control pepper and treated with biotechnical preparations; CO – cortical tissues, A – Amistar 250 SC, C – control, BCZ – Bioczos Płynny, BS – Biosept 33SL, BA – Bio-algeen S90 Plus; SEM MAG:  $\times 110$  (M. Wróbel)

Fot. 1. Fragment przekrojów poprzecznych łodyg papryki z kontroli i traktowanych preparatami biotechnicznymi; CO – tkanka kory wtórnej, A – Amistar 250 SC, C – kontrola, BCZ – Bioczos Płynny, BS – Biosept 33SL, BA – Bio-algeen S90 Plus; SEM MAG:  $\times 110$  (M. Wróbel)

## DISCUSSION

The influence of biotechnical preparations on the yield quality and plant health were studied by many authors [Pięta 2006, Dobromilska and Gubarewicz 2008, Kossak and Dyki 2008, Sadowski *et al.* 2009, Gajc-Wolska *et al.* 2009, Cwalina-Ambroziak and Amarowicz 2012, Jamiołkowska 2013]. In the literature do not have more information about the impact of biotechnical preparations on the plant growth. The results of our study, showed that there was no significant influence of the applied preparations on the size of stems and roots of pepper. The similar results presented Gajc-Wolska and co-authors [2009], who proved that the use of biotechnical preparations Goëmar Goteo and BM 86 (sea algae) do not have the impact on the physical traits of tomato fruits. Other results presented by Dobromilska and Gubarewicz [2008] shown that the preparation Bio-algeen have the significant influence on the height of tomato plants. They proved that the plants sprayed three times with Bio-algeen were statistically higher than the control. Tomatoes treated with Bioalgeen formed more leaves and flowers than plants untreated. The our research have shown interesting information on the impact of the biotechnical preparations on the size of the sprayed leaves. Bioczos Płynny and Bio-algeen increased significantly the length and width of leaves peppers compared to control combination. The leaves sprayed with Biosept and Amistar (azoxystrobin) were also biggest than the control but this difference was not significant. Jamiołkowska [2013] indicate that the using of Bioczos and Bio-algeen in protection of sweet pepper increased the thickness of the leaf blade. Pepper leaves sprayed with Bioczos Płynny, Bio-algeen and Boni Protect Forte were thicker than to control (respectively 365.19, 482.99, 341.38, 244.15  $\mu\text{m}$ ). The results of our study proved that biotechnical preparations increase not only the thickness of leaves but also the thickness of stems. Research presented by Kossak and Dyki [2008] shows that tomato plants treated with Goëmar Goteo and Bio Jodis biostimulators have more numerous and bigger cells of xylem and phloem vascular bundles in the tomato stems. It could contribute to more efficient transport of water with mineral substances in the plant and as a result to increase of fruits weight. It can suppose also that the biotechnical preparations increase in the thickness of the secondary cortex including epidermis who are a physical barrier to pathogens of plants. Based on our results and literature data Bioczos Płynny, Bio-algeen and Biosept can be regarded as biostimulators.

## CONCLUSIONS

1. Biotechnical preparations such as Bioczos Płynny, Bio-algeen S90 Plus and Biosept 33 SL do not increase the length of stems and roots of pepper plants.
2. Bioczos Płynny and Bio-algeen S90 Plus have a positive impact on the length and width of leaf blade of pepper.
3. Bioczos Płynny, Bio-algeen S90 Plus and Biosept 33 SL increase in the thickness of stem secondary cortex of pepper plants.

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**Acknowledgments.** Scientific research was supported by the Center of Sciences in Poland in 2010–2013 years (Personal Project No. N N N310 449538).

**Streszczenie.** Celem pracy była ocena wpływu preparatów Bioczos Płynny, Biosept 33 SL i Bio-algeen S90 Plus na wzrost roślin papryki słodkiej uprawianej w polu. Badania polowe przeprowadzono w latach 2010–2012 w gospodarstwie w miejscowości Zezulin koło Lublina. Obiektem badań były rośliny papryki słodkiej odmiany 'Roberta F<sub>1</sub>' uprawianej w polu. Do ochrony roślin stosowano preparaty biotechniczne: Bioczos Płynny, Biosept 33 SL i Bio-algeen S90 Plus. Liście, łodygi i korzenie papryki mierzono w czwartym tygodniu po zakończeniu oprysków. Pomiar biometryczny wykazały, że wielkość roślin była różna i zależała od rodzaju stosowanego preparatu oraz od warunków pogodowych. W wyniku przeprowadzonych badań stwierdzono, że stosowane preparaty biotechniczne nie wpływały istotnie na wielkość badanych roślin (długość łodygi i korzeni). Na podstawie uzyskanych wyników wykazano jednak pozytywny wpływ preparatów Bioczos Płynny i Bio-algeen S90 Plus na długość i szerokość opryskiwanych liści. Również badania z użyciem mikroskopu skaningowego wykazały, że preparaty Bioczos Płynny, Bio-algeen S90 Plus i Biosept 33SL wpływały na zwiększenie grubości kory wtórnej łodygi papryki.

**Słowa kluczowe:** Bioczos Płynny, Biosept 33SL, Bio-algeen S90 Plus, preparaty biotechniczne, *Capsicum annuum*, wzrost rośliny, wielkość blaszki liściowej, kora wtórna łodygi, SEM