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THE INFLUENCE OF EFFECTIVE MICROORGANISMS ON THE OCCURRENCE OF FUNGAL DISEASES, GROWTH AND THE QUALITY OF THE STRAWBERRY FRUITS

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Abstract

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The aim of this study was to establish the possibility of using different forms of the Effective Microorganisms preparation to limit the occurrence of the fungal diseases of the strawberry leaves and fruits and estimation of their influence on the growth, crop and quality parameters of the fruits. The EM preparation effectively limited the occurrence of strawberry red-leaf spot (*Diplocarpon earliana*) and white-leaf spot (*Mycosphaerella fragariae*) and to a lesser extent – the powdery mildew on the fruits (*Botrytis cinerea*). From the studied forms of the EM preparation, the best effects were achieved when plants were sprayed with the mixture of two forms EM-A and EM-5 and the EM-5 preparation enriched with the onion extract. In combination with their application the plants had a high diameter increment of the collar root, the highest weight of the root system and a high average mass of the fruits. No significant influence of different forms of Effective Microorganisms on the quality parameters of the fruits was shown.

Key words: EM, strawberry diseases, mass of the fruits, extraction content

Introduction

The deterioration of the productive properties of the soil in the modern commodity crops and the improvement of diseases and pests become increasingly important in economic terms. The basic way of supplementing the insufficiency of the nutrients in the soil is the use of mineral fertilizers, and to fight the pathogens are used the chemical plant protection products. Notwithstanding the unquestionable benefits, such solutions may lead to resistance of some pests and pathogens to chemical preparations, and consequently to the creation of environmental risks (Chowdhury et al., 2008; Torriani et al., 2008). An alternative to the chemical means of production are the preparations of natural origin, containing one or more biologically active substances. Such preparations do not have a phytotoxic effect and do not require any withdrawal periods so that they may constitute a valuable addition for the chemical protection.

In this research a preparation named Effective Microorganisms (EM) was used which is a mixture of different forms of microorganisms. It consists of lactobacilli (*Lactobacillus casei, Streptococcus lactis*), phosphate-synthesizing bacteria (*Rhodopseudomonas palustrus, Rhodobacterspae*), yeast (*Saccharomyces albus, Candida utilis*), actinomyces (*Streptomyces albus*) and moulds (*Aspergillusotyzae, Mucorhiemalis*) (Daly and Steward, 1999). The mechanism of operation of the Effective Microorganisms is to displace the "harmful" organisms (Kaczmarek et al., 2008; Mrugalska, 2008; Górski and Góra, 2009; Wolna-Maruwka et al., 2010), support the useful soil microflora (Sahain et al., 2007; Klama et al., 2010), improve the growth parameters of plants, including the root system and to enrich the diversity of biological life in soil (Valarini et al., 2002; Kaczmarek et al., 2008).

The aim of this study was to establish the possibility of using different forms of the Effective Microorganisms preparation to limit the occurrence of the fungal diseases of the

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strawberry leaves and fruits and to estimate their influence on the growth, crop and quality parameters of the fruits.

Materials and Methods

The study was conducted at the area of the experimental station of the Faculty of Horticulture of the Poznan University of Life Sciences in the years of 2011-2013. Strawberry cuttings of the Senga-Sengana cultivar were planted in 6-liter containers. The base was the lessive soil, proper, generated from the loamy sands lying on boulder clay. The slightly acidic soil contained the optimal quantity of nutrients. The care treatments in the vegetation period were limited to regular weeding and watering of the plants.

In the experience three forms of the Effective Microorganisms were used: EM-A (the active form), EM-5 and EM-5 enriched with the onion extract. Five combinations represented by six replications were provided (one container constituted one replication). The list of combinations is as follows: combination 1 (control) - with no preparations used, combination 2 – use of a chemical preparation (according to the plant protection program, combination 3 – use of the EM-5 preparation, combination 4 – use of the EM-5 preparation enriched with a plant extract, combination 5 – use of a mixture of two forms of preparations EM-A and EM-5.

For the preparation of the EM-A composition 50 ml cane molasses dissolved in warm water were used to which 50 ml of EM concentrate was added and the mixture was supplemented with water to the capacity of 1 litre. This preparation was stored in a warm, shaded spot for the period of 7 days. During this time the proliferation of microorganisms took place. For the preparation of the EM-5 composition 450 ml EM-A was used to which 50 ml of alcohol and 50 ml of vinegar was added. The preparation was ready to use after 7 days. The third composition was prepared with the use of 500 ml of EM-A and 100 gr of finely chopped onion. The preparation was ready to use after 7 days.

For the chemical protection of the strawberry a preparation named Topsin M500 SC was used. The fungicide was used twice during the vegetation period – during blossom and after harvesting at a dose of 2.5 l/ha. The frequency of application and doses resulted from the fruit plant protection programs which were valid during the period of the research (Program 2011, 2012, 2013). Spraying with the microbiological preparations at a dose of 70 ml/l of water was conducted three times during the vegetation period, in May, June and July. The exertions were conducted with a hand sprinkler until the leaf blades were totally covered with the liquid.

In this research the effectiveness of the used preparations in limiting the occurrence of such fungal diseases of strawberry leaves as: white-leaf spot (*Mycospaerella fragariae*) and red-leaf spot (*Diplocarpon earliana*) was estimated. On the fruits the occurrence of the powdery mildew (*Botrytis cinerea*) was evaluated. The observation of the occurrence of fungal diseases was conducted three times at the interval of seven days from the spraying of the plants.

The recommendations established by the European-Mediterranean Plant Protection Organization (EPPO) were used to evaluate the preparations in limiting the occurrence of fungal diseases of strawberries. These recommendations provide fixing such parameters as: percentage of leaf infection, disease severity index and the percentage of effectiveness of preparation.

The percentage of leaf infection was assessed visually. The disease severity index was counted on the basis of the following formula:

$$DSIn = \frac{(a \times 0) + (b \times 10) + (c \times 30) + (d \times 60) + (e \times 100)}{T},$$

where: a – no leaf infection, b – infection up to 10% of the leaf area, c – infection from 10 to 25% of the leaf area, d – infection of 25 – 50% of the leaf area, e – infection of > 50% of the area, T – total number of the leaves.

The percentage of the effectiveness of the preparation was calculated on the basis of the following formula:

$$PEP = \frac{A - B}{A} \times 100,$$

where: A – percentage of leaf infection in the control combination, B – percentage of leaf infection in the studied contamination.

■ The strength of the vegetative growth of the strawberry bush was evaluated on the basis of the results of measurements of the collar root diameter. The measurements were conducted at the end of the vegetative period twice – before and after the study. Additionally, after removing the plants form the containers were measured the average mass of the root system.

■ The evaluation of the quality of the fruits consisted of measuring their average mass, firmness and the extract capacity. From each combination 15 fruit were taken which were then weighed with an accuracy of 1 g. The measurements of the firmness (g/cm^{-2}) were conducted at two sides of the fruits with the use of penetrometer Fruit Pressure Tester (so-called Magness-Taylor test). The extract capacity was measured with the use of refractometer.

■ All measurements and observations during the study were conducted on the same plants. The results of the measurements and observations were statistically established with the use of the multivariate analysis of variance sepa-

rately for each studied characteristic with the use of the Duncan test at the significance level of α = 0,05.

The climatic data was acquired from the local meteorological station. The year 2011 was warm. It is proved by the high average annual air temperature of +10.7ºC, exceeding the average temperature of the multi-year (Table 1). In terms of the moisture conditions the year of 2011 was unfavourable for plant growth. It results both from the low amount of precipitation during vegetation period (368.2 mm) and its very uneven distribution in particular months. The meteorological conditions in the following years of the research – 2012 and 2013 can be regarded as more favourable for the plant growth and development, particularly in terms of precipitation. Its amount in the vegetation period was noticeably higher in comparison to the average value of the multi-year (Table 1).

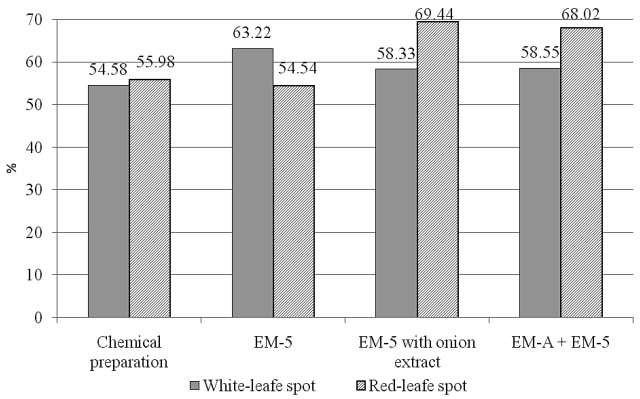

Fig. 1. Percentage of effectiveness of the preparations in limitation the occurrence of strawberry leaf disease in the years 2012–2013

Results and Discussion

In the years of 2011–2013 the strawberry leaves were mostly infected by the red-leaf spot and white-leaf spot in the control combination. Both the highest percentage of leaf infection by fungal diseases and the highest disease severity index were noticed here (Table 2). The effectiveness of three forms of the Effective Microorganisms preparation in limiting the occurrence of red-leaf spot and white-leaf spot was comparable to the effectiveness of the chemical preparation. Both in 2012 and 2013 the percentage of the strawberry leaves infection with the red-leaf spot was noticeably higher than with the white-leaf spot.

One of the indicators for the evaluation of the effectiveness of the use of preparations for fighting fungal diseases is the so-called percentage of effectiveness. A value exceeding 40% shows the ability of the preparation to limit the development of a fungal disease. Both the fungicide in the control

Table 1
Meteorological conditions in the years 2011-2013

Years	Average annual air temperature (ºC)	Average air temperature in the vegetation period IV–IX (ºC)	Amount annual of precipitation (mm)	Amount of precipitation in the vegetation period (mm)
2011	10.7	16.5	466.3	368.2
2012	9.0	14.4	576.8	444.4
2013	8.8	14.6	516.8	411.2
Average for years 1955–1998	8.1	13.4	529.1	372.5
Average for years 1997–2007	9.5	16.6	471.7	271.8

Table 2
Occurrence of the strawberry leaf diseases in the years 2011-2013

Lp.	Combinations	Strawberry white-leaf spot		Strawberry red-leaf spot	
		Percentage of leaf infection	Disease severity index	Percentage of leaf infection	Percentage of leaf infection
1	Control	19.19 b[1]	22.29 c	4.01 b	4.34 c
2	Chemical preparation	7.32 a	8.87 b	1.13 a	1.57 ab
3	EM-5	5.94 a	9.53 b	1.02 a	1.71 b
4	EM-5 with onion extract	7.23 a	5.18 a	1.28 a	1.05 a
5	EM-A + EM-5	6.22 a	5.79 a	1.27 a	1.18 ab

[1] means marked with the same letter do not differ at the significance level of α = 0,05 according to the Duncan's test. Statistical analyses were made separately for each disease

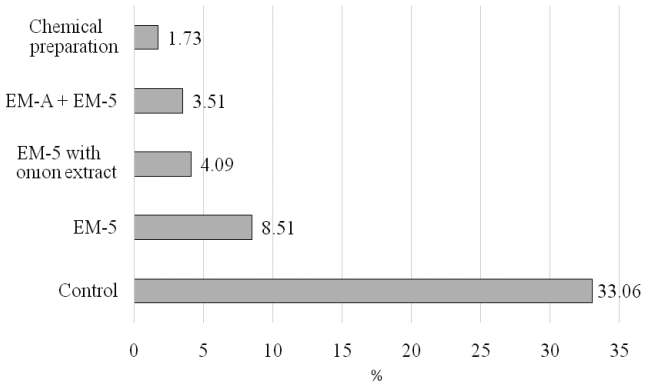

Fig. 2. Average percentage share of fruits infected with powdery mildew in the yeras 2012-2013

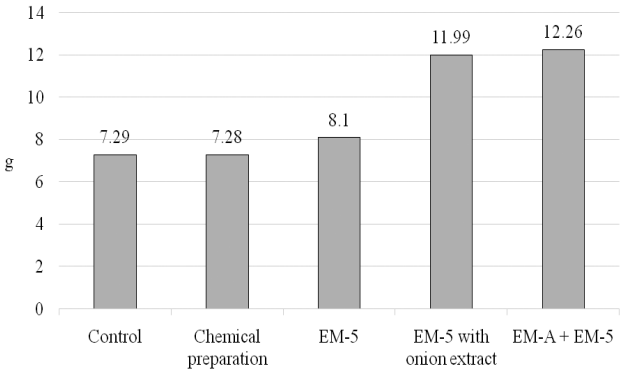

Fig. 3. Average of the root system mass (g) of strawberry in 2013 years

combination and the other forms of EM preparation limited the occurrence of the strawberry red-leaf spot and white-leaf spot (Figure 1). The best effect was achieved with mixing the two forms of EM-A and EM-5 preparation and with enriching the EM-5 preparation with onion extract.

The Senga-Sengana cultivar used in the experiment is sensitive to the *Botrytis cinerea* fungus. In the control combination the percentage share of the fruits with the powdery mildew was even several times higher than in the other combinations (Figure 2). The chemical preparation limited the occurrence of the powdery mildew the most (1.73% infected fruits). The EM-5 preparation form was far less effective in fighting the *Botrytis cinerea* fungus. In the combination with its use the number of fruits infected with the powdery mildew (8.51% of infected fruits) was even five times higher than in the combinations protected with fungicide (Figure 2). The improvement of the efficiency of Effective Microorganisms was obtained by enriching the EM-5 with the onion extract and with mixing the two forms of EM-A and EM-5 (3.51% and 4.09% infected fruits respectively). In the literature there's no lack of opinion about the positive influence of the Effective Microorganisms on the improvement of immunity of plants to the fungal diseases (Boligłowa and Gleń, 2008; Górski and Góra, 2009; Wolna-Maruwka, 2010). Sometimes the effect is apparent only in the ecological crops (Wachowska et al., 2015).

In 2011 no statistically proved differences in the strawberry collar root diameter were observed (Table 3). In the last year of the research, plants treated with the mixture of EM-A and EM-5 as well as EM-5 enriched with the onion extract had the greatest collar root diameter. After their use the plants also had the highest collar root diameter increment in the three-year period. In the combination with the mixture of two forms of preparations the collar root diameter in 2013 was over 96% larger in comparison to the measurements of 2011 (Table 3). For comparison, in the control combination the collar root diameter increment in the three-year period slightly exceeded 50%.

The measurements of the root system mass conducted in the last year of the experiment show the positive impact of the Effective Microorganisms to this parameter. The root mass of the plants treated with EM-5 with the onion extract and the mixture of EM-A and EM-5 was significantly higher in comparison to the root mass in the other combinations (Figure 3). In addition to improving strawberry growth the Effective Microorganisms may increase the length of apple

Table 3
The colar root diameter of strawberry in the years 2011–2013

Lp.	Combinations	Years		Root diameter increment in the three-year period (%)
		2011	2013	
1	Control	19.49 a	30.09 a	54.4
2	Chemical preparation	17.71 a	32.95 ab	86.1
3	EM-5	18.21 a	32.82 ab	80.3
4	EM-5 with onion extract	19.20 a	35.65 bc	85.67
5	EM-A + EM-5	20.14 a	39.52 c	96.22

[1] means marked with the same letter do not differ at the significance level of $\alpha = 0{,}05$ according to the Duncan`s test. Statistical analyses were made separately for each year.

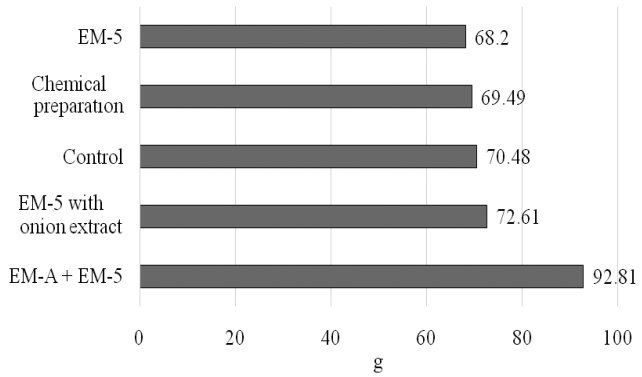

Fig. 4. The strawberry fruit mass (g) in years 2012-2013

shoots and leaf surface (Sahain et al., 2007), cause the growth of the fresh root weight and increase their length (Okorski et al., 2008; Klama et al., 2010), significantly raise the content of macro-nutrients in leaves (Kleiber et al., 2014).

Different forms of the EM preparation had a differentiated influence in the strawberry fruit mass. From the plants treated with the EM-5 fruits with a lower mass were harvested than from the plants in the control combination (Figure 4). A much better result was obtained by using the mixture of two forms of the EM-A and EM-5 preparations, the use of which allowed for the increase in the fruit mass up to 92.81 g with the average mass of 70.48 g in the control combination. A higher mass of the fruits may contribute to a higher yielding level. In the literature, there is contradictory information as to the impact of the Effective Microorganisms on the amount of crop. Piskier (2006) as well as Okorski and Majchrzak (2007) show no significant increase in the crop after application of the EM preparation or this growth occurs only when connecting EM with NPK fertilizers (Khaliq et al., 2006), while Kleiber et al. (2014) and Shah et al. (2001) are of a different opinion.

Apart from the preparations the average weight of the strawberry fruits was affected by the harvest time. Fruits of the lowest mass (52.05 g) were harvested during the first harvest – at the beginning of June, and of the highest (89.90 g) - during the second one at the end of June.

According to the preparation manufacture's declaration the positive effect of the Effective Microorganisms can be increased by combining its different forms or enriching the preparation with plant extracts. Results obtained in the experiment confirm this thesis. Plants treated with the EM-5 preparation enriched with the extract of onion, and in particular with a mixture of the two forms of preparations EM-A and EM-5 were attacked by the powdery mildew only slightly (Figure 2), had the highest increase in root collar diameter (Table 3) and a high root weight (Figure 3). One

Table 4
The extract capacity (% Brix) in the fruits of strawberry in the years 2012–2013

Lp.	Combinations	Years		Average for combination
		2012	2013	
1	Control	7.66 a[1]	7.55 a	7.61 a
2	Chemical preparation	7.68 a	7.96 ab	7.82 a
3	EM-5	7.83 a	8.15 abc	7.99 a
4	EM-5 with onion extract	8.77 bc	8.84 c	8.80 a
5	EM-A + EM-5	8.01 ab	8.07 abc	8.04 a
Average for years		7.99 a	8.11 a	-

[1] means marked with the same letter do not differ at the significance level of $\alpha = 0.05$ according to the Duncan's test

Table 5
Firmness (g/cm^{-2}) of the strawberry fruits in the years 2012–2013

Lp.	Combinations	Years		Average for combination
		2012	2013	
1	Control	136.94 abc[1]	136.11 ab	136.53 ab
2	Chemical preparation	152.78 d	149.11 cd	150.94 c
3	EM-5	147.22 bcd	146.67 bcd	146.94 c
4	EM-5 with onion extract	144.17 abcd	143.89 abcd	144.03 bc
5	EM-A + EM-5	135.56 ab	133.33 a	134.44 a
Average for years		143.33 a	141.82 a	-

[1] means marked with the same letter do not differ at the significance level of $\alpha = 0.05$ according to the Duncan's test

Table 6
Influence of harvest time on the fruit quality of strawberry in the years 2012-2013

Lp.	Fruit quality parameters	Terms		
		Beginning of June	End of June	Beginning of July
1	Fruit firmness	151.58 c[1]	143.08 b	133.07 a
2	Extract capacity	6.98 a	7.52 b	9.56 c

[1] means marked with the same letter do not differ at the significance level of α = 0,05 according to the Duncan's test. Statistical analyses were made separately for each parameters

of the reasons for achieving such good results, especially when it comes to the growth of plants, may be the presence in the combination with such a mixture of a double portion of cane molasses, which is used as a breeding ground for microorganisms occurring both in the form of EM-A and EM-5. Molasses, next to sucrose, contains amino acids, nitrogenous compounds, is rich in magnesium, potassium, calcium, sodium, copper, phosphorus, chromium, zinc and vitamins of the B group.

The influence of the Effective Microorganisms on the quality parameters of the strawberry fruits was indistinguishable. Statistical differences in the extract capacity of fruits in different combinations were not observed (Table 4). The fruit firmness to greater extent depended on their mass and harvest time. The least firm strawberry fruits (134.4 g/cm^{-2}) were harvested from the plants in the combination in which the mixtures of two forms of preparation was used (Table 5), where the fruits had the highest mass (Figure 4). Along with the end of the harvest period the fruit firmness significantly decreased. While at the first harvest it amounted to the average of 151,58 g/cm^{-2}, a month later it decreased to 133,07 g/cm^{-2} (Table 6). A reversed tendency was observed in studying the extract capacity in the fruits. At the end of the harvest (at the beginning of July) it was significantly higher than a month before.

In addition to the kinds of preparations used the intensity of strawberry infection with fungal diseases was also influenced by the course of the weather conditions. The occurrence of red-leaf spot – a disease developing best at high air humidity (Żurawicz, 2005), was intensified in the years of 2012 and 2013. It was a period of high rainfall (Table 1), especially in 2012, when the percentage of leaves of strawberry infected both with red-leaf spot and white-leaf spot was the highest. The powdery mildew develops rapidly in the conditions of high humidity, reducing the crop even by 70% (Żurawicz, 2005). In 2013, the number of fruit infected with the powdery mildew was significantly higher than in other years. In that year, at the time of the mass ripening of fruit (June, July) the average monthly precipitation exceeded 120 mm.

Conclusion

The presented results show the desirability of use of the Effective Microorganisms in crops of strawberries. It came out from the their positive influence on the growth of strawberry bushes and root system, low level of fruits damaged with the powdery mildew and reducing the occurrence of fungal diseases of leaves. Probably the EM preparations will not be able to completely replace chemical agents, particularly in the commodity crops, but they can be successfully used in conditions of limited possibilities of using chemical means of production (organic farming).

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