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ARTYKUŁ ORYGINALNY

Możliwość wykorzystania produktów naturalnych w zwalczaniu czarnej plamistości róży (*Diplocarpon rosae* Wolf.)

A possible use of natural products in black spot control on rose (*Diplocarpon rosae* Wolf.)

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Streszczenie

Przeprowadzono doświadczenia polowe w celu oceny wpływu nawozów dolistnych – Actifos, Solfan PK, stymulatorów wzrostu roślin – Atonik SL, Biosept Active, Huwa-San TR-50, PronTech, środka biotechnologicznego – płynny Bioczos, olejków – Atpolan 80 EC, Olejan 85 EC oraz mleka UHT stosowanych do opryskiwania róż cztery razy co 7 dni (kuracyjnie) lub dziewięć razy co 14 dni (profilaktycznie) w celu ograniczenia rozwoju objawów chorobowych wywoływanych przez *Diplocarpon rosae*. W zastosowaniu profilaktycznym najwyższą skuteczność wykazały: Olejan 85 EC, PronTech, Solfan PK oraz mleko UHT. W pierwszym doświadczeniu skuteczność produktów stosowanych kuracyjnie oceniana trzy dni po zakończeniu czterech zabiegów wyniosła ponad 62% z wyjątkiem mleka UHT, a w drugim doświadczeniu wyniosła ponad 89% dla wszystkich produktów. Testy kiełkowania zarodników wykazały, że jedynie Huwa-San TR-50, Olejan 85 EC i mleko UHT wykazały stosunkowo niską skuteczność. Żaden z testowanych produktów nie był fitotoksyczny dla roślin róż.

Słowa kluczowe: *Diplocarpon rosae*, róża, ochrona, produkty syntetyczne i naturalne

Abstract

Field experiments were conducted to evaluate the effects of foliar fertilizers – Actifos, Solfan PK, plant growth promoters – Atonik SL, Biosept Active, Huwa-San TR-50, PronTech, a biotechnological agent – liquid Bioczos, oils – Atpolan 80 EC, Olejan 85 EC and UHT Milk used for spraying roses four times every 7 days (curatively) or nine times every 14 days (preventively) to control the development of disease symptoms caused by *Diplocarpon rosae*. When used preventively, the highest efficacy was shown by: Olejan 85 EC, PronTech, Solfan PK and UHT Milk. In the first experiment, the efficacy of the products used curatively assessed three days after the completion of four treatments was above 62% except for UHT Milk, and in the second experiment it was above 89% for all the products. Spore germination tests showed that only Huwa-San TR-50, Olejan 85 EC and UHT Milk showed relatively low efficacy. None of the tested products was phytotoxic to rose plants.

Keywords: *Diplocarpon rosae*, rose, protection, synthetic and natural products

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Wstęp / Introduction

Black spot, caused by the fungus *Marssonina rosae* (Lib.) (perfect stage *Diplocarpon rosae* Wolf.), is the most common and most serious disease of roses cultivated in the open field, and in recent years, following the introduction of new cultivars, also under cover (Ljubojević and Božanić Biljana 2025). For many years, the main method of protecting roses against the occurrence of black spot symptoms has been the use of fungicides (Wojdyła and Łyś 2000; Wojdyła 2009). However, their frequent use carries the risk of danger to humans and animals, and causes environmental pollution, and may lead to the development by pathogens of resistance to the applied chemical agents. Searching for other effective means as alternatives to fungicides seems fully justified. One possibility is the introduction into the protection of roses against black spot of natural and synthetic products with a different mechanism of action against pathogens (Wojdyła 2016a). It should be emphasized that the European Commission in the Regulation of 22 June 2022 envisages a reduction in the use of pesticides throughout the European Union by 2030. Depending on the current consumption of the active substance per hectare, this reduction is to be 35% in the case of Poland, and in some countries where significantly more active substance is used per hectare, the reduction is to be 50% or even 65%. The withdrawal from the use of conventional active substances of plant protection products is associated with the need to search for other substances that are usually not plant protection products, which are completely safe for humans, animals and the environment. Unconventional products presented in the work may be particularly useful for this purpose, which in previous studies have shown high effectiveness in protecting plants against pathogens, the causes of leaf spot, powdery mildew, rust, gray mold (Wojdyła 2001a, 2001b, 2010, 2012a, 2016b; Crisp et al. 2006; Jamar et al. 2007; Slusarenko et al. 2008). Some of them, such as Actifos, liquid Bioczos, Biosept Active, Huwa-San TR-50 can also be recommended for the protection of plants against soil pathogens caused by species: *Pythium* spp., *Phytophthora* spp. and *Fusarium* spp. (Saniewska and Orlikowski 1994; Orlikowski 2001; Orlikowski et al. 2001).

The aim of the experiments was to determine the efficacy of foliar fertilizers, plant growth promoters, a biotechnological agent, oils and UHT Milk in reducing the development of *D. rosae* black spot.

Materiały i metody / Materials and methods

The experiments evaluated the efficacy of foliar fertilizers – Actifos (ammonium phosphite + microelements B, Cu, Fe, Mn, Mo, Zn) and Solfan PK (48% potassium carbonate, 48% monopotassium phosphate), plant growth promoters – Atonik SL (0.3% sodium para-nitrophenolate + 0.2% sodium ortho-nitrophenolate + 0.1% sodium 5-nitroguaiacolate), Biosept Active (33% extract from grapefruit seed and pulp), Huwa-San TR-50 (493 g of hydrogen peroxide + 0.32 g colloidal silver in 1 kg), PronTech (40% alkyl dimethyl benzyl ammonium chlorides from the group of quaternary ammonium compounds, and 60% urea), biotechnological agent – liquid Bioczos (liquid garlic extract), oils – Atpolan 80 EC (76% mineral oil SN) and Olejan 85 EC (85% rapeseed oil), and UHT Milk with a 3.2% fat content in the protection of roses against black spot. For comparison, the fungicide Domark 100 EC (100 g/l tetraconazole) was used. The preparation concentrations were in accordance with the manufacturer's recommendations. The experimental design, natural plant infection through spores formed on fallen leaves found under shrubs and observations were carried out according to the previously adopted methodology for the evaluation of the effectiveness of fungicides in the protection of rose against black spot (Wojdyła and Łyś 2000; Wojdyła 2013).

The experiments were conducted on roses cultivar Polastern and *Rosa canina* cultivar Schmid's Ideal. The rose bushes were planted in 2013 in sandy-loam soil at the experimental field of the National Institute of Horticultural Research in Skierniewice. The organic matter content of the soil was 1.5% and the pH was 6.7. The experiments were conducted on two-year-old bushes from 2014 and three-year-old bushes from 2015, cultivated conventionally. These bushes had suffered from black spot in previous seasons. In spring, after pruning, the bushes were fertilized with Azofoska at a rate of 2 kg per 100 m^2. Aphid control on the roses was carried out in the second half of June using Confidor 200 SL 0.04%. To prevent weed growth, the field was covered with a black mat. Roses were sprayed using a laboratory pneumatic sprayer with a tank capacity of 1.5 dm^3, a 0.8 mm diameter swirl nozzle, and a spray pressure of 0.2 MPa (manufactured by the Institute of Plant Protection – National Research Institute in Poznań). The roses were sprayed with the test products four times at 7-day intervals or nine times at 14-day intervals. The treatments were performed in the morning using 1 dm^3 of liquid per 10 m^2 of surface area. During spraying, the upper and lower surfaces of the leaf blade were thoroughly coated with the liquid. The efficacy of the test products was determined before the experiments and three days after the 2nd and 4th treatment (*R. canina* Schmid's Ideal) (curative spray in 2014 and 2015 year), or for a period of 16 weeks (cultivar Polastern) of spray treatments (preventive spray in 2014 year). In the case of the experiments on *R. canina* Schmid's Ideal, observations were also carried out 14 days after the end of protection. The observations were made using a rating scale where: 0 – no symptoms, 1 – 0.1 up to 25% of plant leaves with disease symptoms, 2 – over 25% leaves with disease symptoms, 3 – up to 25% of fallen leaves and rest of the leaves with disease symptoms, 4 – up to 50% of fallen leaves and rest of the leaves with disease symptoms, 5 – from 50.1 up to 90% of fallen leaves, 6 – over 90% of

fallen leaves. During the growing period, the roses were irrigated with a capillary system to prevent the natural products from being washed off the surface of the leaves. The experiments were conducted in a random block design with five bushes in four replications.

In the experiments on the germination of *D. rosae* spores, cultivar Polastern roses with visible sporulation (acervuli) of the fungus were sprayed with the test products in the morning. After 1, 7 and 14 days from the spraying, samples of leaves were taken and, in the laboratory, a drop of sterile water was applied to their surface. Next, the spores were scraped off with a scalpel onto a potato-glucose medium in Petri dishes. Another drop of water was added to the medium and the suspension of spores was spread over the surface with a glass rod. In order to limit the growth of bacteria, rose bengal at 0.5 mg/dcm^3 and 80 000 units of penicillin/dcm^3 were added prior to pouring the medium. After 24–48 hours of incubation at 18–20°C, the total numbers of spores and germinating spores were counted in the field of view (Wojdyła 2012b). Five different locations within the dish were chosen for observation, where from 30 to 60 spores were observed. Then the percentage of germinating spores was calculated. Using the Abbott formula on the basis of the infection rating of the rose bushes, the percentage efficacy of the products in limiting the development of disease symptoms and in inhibiting spore germination was calculated (Abbott 1925). In the case of spore germination, observations were made on four Petri dishes in five different areas.

The data obtained was subjected to statistical analysis. The normality of the distribution of the observed features was tested using the Shapiro-Wilk test (Shapiro and Wilk 1965). The homogeneity of variance was verified using Bartlett's test, while Box's *M* test checked the multivariate normality and the homogeneity of the variance-covariance matrices. Two-way analyses of variance (ANOVA) were carried out to determine the effects of foliar fertilizer and time of observation as well as foliar fertilizer time of observation interaction on the variability of examined traits for each trait independently. For individual traits and levels of experimental treatment mean values were calculated. Tukey's honest significant differences (HSDs) were estimated at a significance level of $\alpha = 0.05$ corrected for multiple testing using the Benjamini-Hochberg method. Homogeneous groups for the analysed traits were determined based on HSD values. The correlation of examined traits between particular times of observation was assessed based on Pearson linear correlation coefficients. The results were also analysed using multivariate methods. Euclidean distance was used as the resemblance coefficient for cluster analysis with the unweighted pair group arithmetic means method (UPGMA) (Skomra et al. 2013). A principal component analysis was applied to present a multi-trait assessment of the similarity of the foliar fertilizer in fewer dimensions with minimal information loss (Patterson et al. 2006). The influence of individual traits on the distribution of foliar fertilizer in the system of the first two principal components was tested using discriminant analysis. All the analyses were conducted using the GenStat 23 statistical software package (VSN International 2023).

Wyniki i dyskusja / Results and discussion

Actifos – the foliar fertilizer used preventively on the cultivar Polastern, which is very susceptible to black spot, showed an efficacy from 33.3% to 67.4% depending on the observation date (tab. 1). By comparison, when used curatively on *R. canina* cultivar Schmid's Ideal, the fertilizer had an efficacy from 82.1% to 88.8% (tab. 2). Used curatively in the subsequent experiment, it showed an efficacy of over 90% (tab. 3). When used for a single spray treatment of roses, Actifos showed, depending on the experiment and observation date, an efficacy from 27.5% to 83.2% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). The high efficacy of phosphites in plant protection against pathogens had been confirmed by previous studies, which resulted in the introduction of the Actifos fertilizer onto the market (Wieczorek et al. 2010). Literature data indicate that lower concentrations of phosphites induce resistance in the protected plants, and higher concentrations directly affect pathogens (Smillie et al. 1989). The effectiveness of natural and synthetic products in controlling *D. rosae* on the Polastern rose variety was characterized by proportionality observed after subsequent weeks. This relationship was confirmed by the results of correlation analysis. The correlation coefficients ranged from 0.6896 (between 2 and 16 weeks) to 0.9709 (between 4 and 8 weeks). All correlation coefficients were statistically significant.

Atonik SL – when used preventively, the growth promoter showed an efficacy from 26.7% to 51.4% depending on the observation date (tab. 1). By comparison, when used curatively on *R. canina* Schmid's Ideal, it had an efficacy from 58.2% to 65.5% (tab. 2). Used curatively in the subsequent experiment, it showed an efficacy from 36.1 to 89.5% (tab. 3). The plant growth promoter used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 12% to 74.8% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). The high efficacy of Atonik SL is confirmed by the studies in which it was used to protect roses against black spot and caused a strong reduction in the development of disease symptoms (Wojdyła 2004).

Atpolan 80 EC – the mineral oil used curatively on *R. canina* Schmid's Ideal showed an efficacy from 50.7% to 65.5% (tab. 2). In the subsequent experiment, the oil used curatively showed an efficacy from 38.2% to 100% (tab. 3). The results obtained confirm previous studies in which Atpolan 80 EC showed high effectiveness in the protection of roses against black spot (Wojdyła 2016b) and caused very severe spore deformation and inhibition of spore ger-

mination (Wojdyła 2012b). A correlation was observed between the efficacy of natural and synthetic products in controlling *D. rosae* on *R. canina* Schmid's Ideal after 2 spray treatments and after 3 and 14 days from the end of the experiment. The corresponding correlation coefficients were: 0.8039 (after 2 spray treatments vs. after 3 days from the end of the experiment), 0.8333 (after 2 spray treatments vs. after 14 days from the end of the experiment) and 0.9239 (after 3 days from the end of the experiment vs. after 14 days from the end of the experiment).

Liquid Bioczos – the garlic extract used preventively showed an efficacy from 25% to 56.5% depending on the

Tabela 1. Skuteczność środków naturalnych i syntetycznych w zwalczaniu *Diplocarpon rosae* na róży odmiany Polastern po dziewięciu zabiegach opryskowych; początkowy poziom zakażenia na początku eksperymentu: 09.05.2014

Table 1. Efficacy of natural and synthetic products in the control of *Diplocarpon rosae* on rose cultivar Polastern after nine preventive spray treatments; initial infection level at the beginning of experiment: 09.05.2014

Zabiegi Treatments	Stężenie Concentration [%]	Skuteczność procentowa po tygodniach Percentage efficacy after weeks				
		2	4	8	12	16
Kontrola – Control	–	0.00 e	0.00 f	0.00 f	0.00 g	0.00 g
Domark 100 EC	0.05	100 a	100 a	97.8 a	68.0 a	46.7 a
Actifos	0.6	51.4 cd	60.0 bc	67.4 c	56.0 cd	33.3 d
Atonik SL	0.1	51.4 cd	36.0 e	50.0 e	48.0 e	26.7 ef
Liquid Bioczos	2.5	42.9 d	44.0 de	56.5 de	46.0 ef	25.0 f
Biosept Active	0.1	57.1 b-d	48.0 d	56.5 de	49.0 e	28.3 e
Huwa-San TR	0.05	57.1 b-d	52.0 cd	54.3 e	44.0 f	25.8 ef
Olejan 85 EC	1	65.7 bc	60.0 bc	65.2 cd	53.0 d	44.2 b
PronTech	0.1	71.4 b	68.0 b	71.7 c	67.0 a	40.0 c
Solfan PK	0.5	57.1 b-d	68.0 b	80.4 b	65.2 a	47.5 a
UHT Milk	4	71.4 b	68.0 b	73.9 bc	60.0 b	44.3 b

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

Tabela 2. Skuteczność produktów naturalnych i syntetycznych w zwalczaniu *Diplocarpon rosae* na róży *Rosa canina* odmiana Schmid's Ideal po czterech zabiegach opryskowych; początkowy poziom zakażenia na początku eksperymentu: 01.08.2014

Table 2. Efficacy of natural and synthetic products in the control of *Diplocarpon rosae* on rose *Rosa canina* Schmid's Ideal after four curative spray treatments; initial infection level at the beginning of experiment: 01.08.2014

Zabiegi Treatments	Stężenie Concentration [%]	Po dwóch zabiegach opryskowych After two spray treatments	Po kilku dniach od zakończenia doświadczenia After days from end of experiment	
			3	14
Kontrola – Control	–	0.00 e	0.00 h	0.00 f
Domark 100 EC	0.05	100 a	100 a	91.0 a
Actifos	0.6	86.2 b	88.8 b	82.1 b
Atonik SL	0.1	65.5 cd	60.5 f	58.2 d
Atpolan 80 EC	1	65.5 cd	62.8 ef	50.7 e
Liquid Bioczos	2.5	58.6 d	67.4 e	58.2 d
Biosept Active	0.1	72.4 c	62.8 ef	58.2 d
Huwa-San TR-50	0.05	58.6 d	62.8 ef	53.7 de
Olejan 85 EC	1	72.4 c	62.8 ef	44.8 g
PronTech	0.1	79.3 b	76.7 d	67.2 c
Solfan PK	0.5	79.3 b	81.4 c	68.7 c
UHT Milk 3.2%	4	72.4 c	48.8 g	52.2 e

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

observation date (tab. 1). By comparison, the garlic extract used curatively on *R. canina* Schmid's Ideal had an efficacy from 58.2% to 67.4% (tab. 2). In the subsequent experiment, it was used curatively and showed an efficacy from 65.5% to 97.4% (tab. 3). Liquid Bioczos used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 21.7% to 81.3% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6).

Tabela 3. Skuteczność produktów naturalnych i syntetycznych w zwalczaniu *Diplocarpon rosae* na róży *Rosa canina* odmiana Schmid's Ideal po czterech zabiegach opryskowych; początkowy poziom zakażenia na początku eksperymentu: 02.07.2015
Table 3. Efficacy of natural and synthetic products in the control of *Diplocarpon rosae* on rose *Rosa canina* Schmid's Ideal after four curative spray treatments; initial infection level at the beginning of experiment: 02.07.2015

Zabiegi Treatments	Stężenie Concentration [%]	Po dwóch zabiegach opryskowych After two spray treatments	Po kilku dniach od zakończenia doświadczenia After days from end of experiment	
			3	14
Kontrola – Control	–	0.00 g	0.00 e	0.00 d
Domark 100 EC	0.05	100 a	92.1 c	92.3 b
Actifos	0.6	90.9 b	90.8 cd	90.6 b
Atonik SL	0.1	36.1 f	89.5 d	84.9 c
Atpolan 80 EC	1	38.2 f	100 a	89.9 b
Liquid Bioczos	2.5	65.5 e	97.4 b	91.6 b
Biosept Active	0.1	81.8 c	95.8 b	90.6 b
Huwa-San TR-50	0.05	81.8 c	100 a	91.6 b
Olejan 85 EC	1	74.6 d	100 a	90.6 b
PronTech	0.1	100 a	100 a	95.6 a
Solfan PK	0.5	95.5 ab	95.8 b	95.0 a
UHT Milk 3.2%	4	90.9 b	89.5 d	95.0 a

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

Tabela 4. Skuteczność badanych produktów w hamowaniu kiełkowania zarodników (*Diplocarpon rosae*) na odmianie Polaster; początek doświadczenia: 25.09.2013
Table 4. Efficacy of test products in inhibiting spore germination (*Diplocarpon rosae*) on cultivar Polastern; beginning of experiment: 25.09.2013

Zabiegi Treatments	Stężenie Concentration [%]	% kiełkujących zarodników po kilku dniach % germinating spores after days			% skuteczności po dniach według Abbotta (1925) % efficacy after days according to Abbott (1925)		
		1	7	14	1	7	14
Kontrola – Control	–	97.1 e	97.6 f	98.2 g	–	–	–
Domark 100 EC	0.05	8.3 b	16.7 a	13.4 a	91.46	82.9	86.4
Actifos	0.6	33.4 c	16.4 a	56.5 d	65.6	83.2	42.5
Atonik SL	0.1	24.5 c	49.5 bc	84.5 f	74.8	49.3	13.9
Liquid Bioczos	2.5	29.9 c	18.3 a	70.6 e	69.2	81.3	28.1
Biosept Active	0.05	34.5 c	76.5 de	86.0 f	64.5	21.6	12.4
Huwa-San TR-50	0.05	12.5 b	70.6 d	76.5 e	87.1	27.7	22.1
Olejan 85 EC	1	73.1 d	77.0 de	75.1 e	24.7	21.1	23.5
PronTech	0.1	1.1 a	45.4 b	45.9 c	98.9	53.5	53.3
Solfan PK	0.5	2.4 a	55.5 c	33.5 b	97.5	43.1	65.9

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

A similarly high efficacy of garlic juice in the protection of rose against black spot had been demonstrated in previous studies (Wojdyła 2001a). Particularly high effectiveness in plant protection is shown by the ajoene contained in garlic juice (Slusarenko et al. 2008). The efficacy of natural and synthetic products in controlling *D. rosae* on *R. canina* Schmid's Ideal after 2 spray treatments was correlated with the efficacy after 14 days from the end of experiment (0.7948).

Tabela 5. Skuteczność badanych produktów w hamowaniu kiełkowania zarodników (*Diplocarpon rosae*) na odmianie Polaster; początek doświadczenia: 01.10.2014

Table 5. Efficacy of test products in inhibiting spore germination (*Diplocarpon rosae*) on cultivar Polastern; beginning of experiment: 01.10.2014

Zabiegi Treatments	Stężenie Concentration [%]	% kiełkujących zarodników po kilku dniach % germinating spores after days			% skuteczności po dniach według Abbotta (1925) % efficacy after days according to Abbott (1925)		
		1	7	14	1	7	14
Kontrola – Control	–	100 g	100 j	99.7 h	–	–	–
Domark 100 EC	0.05	12.3 b	28.0 b	41.5 b	87.7	72	58.2
Actifos	0.6	72.5 d	53.0 c	64.0 c	27.5	47	35.7
Atonik SL	0.1	88.0 f	74.0 f	85.8 f	12	26	13.9
Liquid Bioczos	2.5	78.3 de	68.3 e	76.3 de	21.7	31.7	23.4
Biosept Active	0.05	79.8 g	61.8 d	78.7 e	20.2	38.2	20.9
Huwa-San TR-50	0.05	88.3 f	89.0 h	93.5 g	11.7	11	6.2
Olejan 85 EC	1	86.8 f	76.8 g	87.3 f	13.2	23.2	12.4
PronTech	0.1	0.00 a	0.00 a	0.00 a	100	100	99.7
Solfan PK	0.5	23.5 c	67.0 e	75.8 de	76.5	33	23.9
UHT Milk 3.2%	4	90.5 f	90.8 i	92.5 g	9.5	9.2	7.2

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

Tabela 6. Skuteczność badanych produktów w hamowaniu kiełkowania zarodników (*Diplocarpon rosae*) na odmianie Polaster; początek doświadczenia: 15.10.2014

Table 6. Efficacy of test products in inhibiting spore germination (*Diplocarpon rosae*) on cultivar Polastern; beginning of experiment: 15.10.2014

Zabiegi Treatments	Stężenie Concentration [%]	% kiełkujących zarodników po kilku dniach % germinating spores after days			% skuteczności po dniach według Abbotta (1925) % efficacy after days according to Abbott (1925)		
		1	7	14	1	7	14
Kontrola – Control	–	100 i	99.0 j	98.5 i	–	–	–
Domark 100 EC	0.05	16.0 b	17.8 b	19.0 b	84	81.2	79.5
Actifos	0.6	46.5 cd	49.5 d	40.5 c	53.5	49.5	57.9
Atonik SL	0.1	57.5 ef	64.8 g	69.0 f	42.5	34.2	29.5
Liquid Bioczos	2.5	77.5 h	53.7 e	76.5 gh	22.5	45.3	21.9
Biosept Active	0.05	44.0 c	47.5 cd	73.0 fg	56	51.5	25.5
Huwa-San TR-50	0.05	67.5 g	76.3 h	77.0 gh	32.5	26.7	21.5
Olejan 85 EC	1	51.3 de	85.5 i	55.5 e	48.7	13.5	42.9
PronTech	0.1	0.00 a	0.00 a	0.00 a	100	99	98.5
Solfan PK	0.5	54.5 ef	45.3 c	49.5 d	45.5	53.7	48.5
UHT Milk 3.2%	4	77.5 h	59.0 f	79.5 h	22.5	39.9	18.9

Średnie w kolumnach oznaczone tą samą literą, nie różnią się istotnie (p = 0,05), zgodnie z testem Duncana
Means followed by the same letter within columns are not significantly different (p = 0.05) according to Duncan's test

Biosept Active – when used preventively, the growth promoter showed an efficacy from 28.3% to 57.1% depending on the observation date (tab. 1). By comparison, when used curatively on *R. canina* Schmid's Ideal, it had an efficacy from 58.2% to 72.4% (tab. 2). In the subsequent experiment, it was used curatively and showed an efficacy from 81.8% to 95.8% (tab. 3). The growth promoter used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 12.4% to 64.5% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). The high efficacy of Biosept Active is confirmed by the studies in which the use of this product for rose protection against black spot caused a severe reduction in the development of disease symptoms (Wojdyła 2001b).

Huwa-San TR-50 – the growth promoter used preventively showed an efficacy from 25.8% to 57.1% depending on the observation date (tab. 1). By comparison, when used curatively on *R. canina* Schmid's Ideal, the product had an efficacy from 53.7% to 62.8% (tab. 2). In the subsequent experiment, it was used curatively and showed an efficacy from 81.8% to 100% (tab. 3). This plant growth promoter used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 6.2% to 87.1% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). Similar effectiveness of Huwa-San TR-50 in the protection of roses against *D. rosae* had been demonstrated in previous studies (Wojdyła 2012a). In the case of pathogens, hydrogen peroxide can cause damage to the strands of DNA nucleic acids and to cytoplasmic membranes (Imlay and Lin 1988).

Olejan 85 EC – when used preventively, the vegetable oil showed an efficacy from 44.2% to 65.7% depending on the observation date (tab. 1). By comparison, the vegetable oil used curatively on *R. canina* Schmid's Ideal had an efficacy from 44.8% to 72.4% (tab. 2). In the subsequent experiment, it was used curatively and showed an efficacy from 74.6% to 100% (tab. 3). This vegetable oil used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 12.4% to 48.7% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). The result obtained confirm the previous studies in which Olejan 85 EC showed high efficacy in the protection of roses against black spot (Wojdyła 2010, 2012c) and limited the germination of *D. rosae* spores (Wojdyła 2012b). No correlation was observed in the percentage of germinating spores on subsequent days for the 2013 observations (tab. 4).

PronTech – the growth promoter used preventively showed an efficacy from 40% to 71.7% depending on the observation date (tab. 1). By comparison, when used curatively on *R. canina* Schmid's Ideal, it had an efficacy from 67.2% to 79.3% (tab. 2). In the subsequent experiment, PronTech used curatively showed an efficacy from 95.6% to 100% (tab. 3). The plant growth promoter used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 53.3% to 100% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). In the case of fungi, the active ingredient of PronTech strongly inhibits the action of proteolytic enzymes. Therefore, all the biochemical reactions that are dependent on these enzymes are inhibited. The direct action of the agent is the destruction of cell walls of fungi and cell plasmolysis (www.upitrading.com). The active substance of PronTech belongs to the group of quaternary ammonium compounds. The mechanism of their action on microorganisms consists in:

- adsorption and penetration of the agent through the cell wall,
- reaction with cytoplasmic membrane components (proteins or lipids), which results in membrane disintegration,
- leakage of low molecular weight intracellular components,
- degradation of proteins and nucleic acids,
- lysis of cell walls caused by the action of autolytic enzymes (Salton 1968; McDonnell and Russell 1999; Obłąk and Gamian 2010).

A correlation was observed in the percentage of germinating spores after subsequent days for observations in first year [series I: 0.8469 (1 day vs. 7 days), 0.8506 (1 day vs. 14 days) and 0.9832 (7 days vs. 14 days)] (tab. 5).

Solfan PK – when used preventively, the foliar fertilizer showed an efficacy from 47.5% to 80.4% depending on the observation date (tab. 1). By comparison, the fertilizer used curatively on *R. canina* Schmid's Ideal had an efficacy from 68.7% to 81.4% (tab. 2). In the subsequent experiment, it was used curatively and showed an efficacy above 95% (tab. 3). Solfan PK used for a single spray treatment of roses showed, depending on the experiment and observation date, an efficacy from 23.9% to 97.5% in the inhibition of *D. rosae* spore germination (tab. 4, 5, 6). Previous studies had shown high effectiveness of Solfan PK in plant protection against rose powdery mildew (Wojdyła et al. 2010a), mallow rust (*Puccinia malvacearum* Bert. et Monty.) and willow rust (*Melampsora epitea* Thüm.) (Wojdyła et al. 2010b). Potassium bicarbonate, one of the components of Solfan PK, was highly effective in the protection of apple trees against apple scab [*Venturia inaequalis* (Cooke) Winter] (Jamar et al. 2007). The other component of the fertilizer, monopotassium phosphate, can induce local and systemic resistance in protected plants (Mucharromah and Kuć 1991).

UHT Milk 3.2% – when used preventively on the very susceptible cultivar Polastern, it showed an efficacy from 44.3% to 73.9% depending on the observation date (tab. 1). By comparison, the milk used curatively on *R. canina* cultivar Schmid's Ideal had an efficacy from 48.8% to 72.4% (tab. 2). In the subsequent experiment, the milk used cu-

ratively showed an efficacy from 89.5% to 95% (tab. 3). When used for a single spray treatment of roses, the milk showed, depending on the experiment and observation date, an efficacy from 7.2% to 39.9% in the inhibition of *D. rosae* spore germination (tab. 5, 6). A correlation was observed in the percentage of germinating spores after subsequent days for observations in first year [series II: 0.7977 (1 day vs. 7 days), 0.9268 (1 day vs. 14 days) and 0.8050 (7 days vs. 14 days)] (tab. 6).

Milk acts directly and indirectly on pathogens by inducing systemic resistance in plants (Bettiol 1999). Unsaturated fatty acids, linoleic, linolenic and oleic, in vegetable oils (rapeseed) and milk can induce systemic plant resistance to pathogens (Kuć 2001). Lactoferrin, which is contained in milk, when used for spraying, causes severe dehydration and deformation of the mycelium and spores [*Uncinula necator* (Schwein.) Burill.] (Kuć 2001).

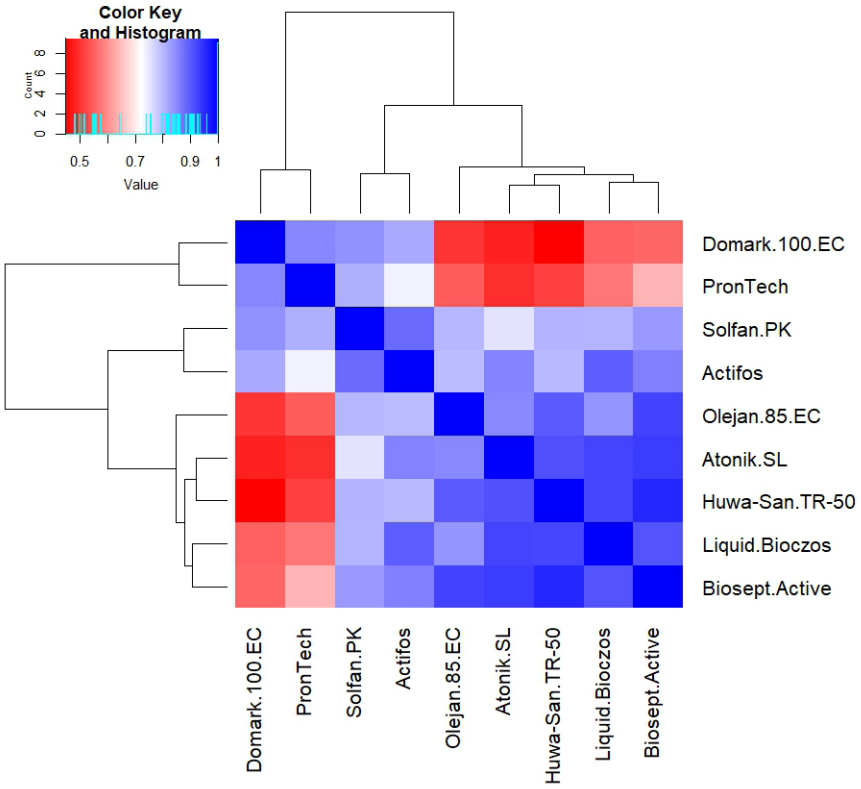
The effectiveness of the test products in the protection of roses against black spot was closely dependent on the species or variety, and also on the initial severity of symptoms and weather conditions. In first year, in the first weeks of the experiment on the very susceptible cultivar Polastern, the severity of disease symptoms on the control plants was low, and only occasional spots were observed on the lower leaves of the protected roses. In the following weeks (week 12 and 16) of the experiment there was a sharp increase in the severity of symptoms, which was associated with a decrease in the effectiveness of the test products (tab. 1). In the case of using the products curatively, only a 14-day discontinuation in the use of the products resulted in increased severity of symptoms on the control roses and a decrease in the effectiveness of the products tested (tab. 2). In the subsequent experiment, the effectiveness of the products was still very high even after 14 days from the end of the experiment, which could have been caused by the weather, characterized by lack of precipitation during that period (tab. 3).

The Domark 100 EC fungicide showed higher or similar effectiveness in the protection of rose against black spot compared with the products tested. The undoubted advantage of the discussed agents, apart from the wide range of pathogens combated, is the possibility of their repeated use during the vegetation period without the risk of pathogen resistance. They can be used to protect not only ornamental plants but also other crop species, where they are approved for use as plant growth stimulants or foliar fertilizers (Wojdyła et al. 2023).

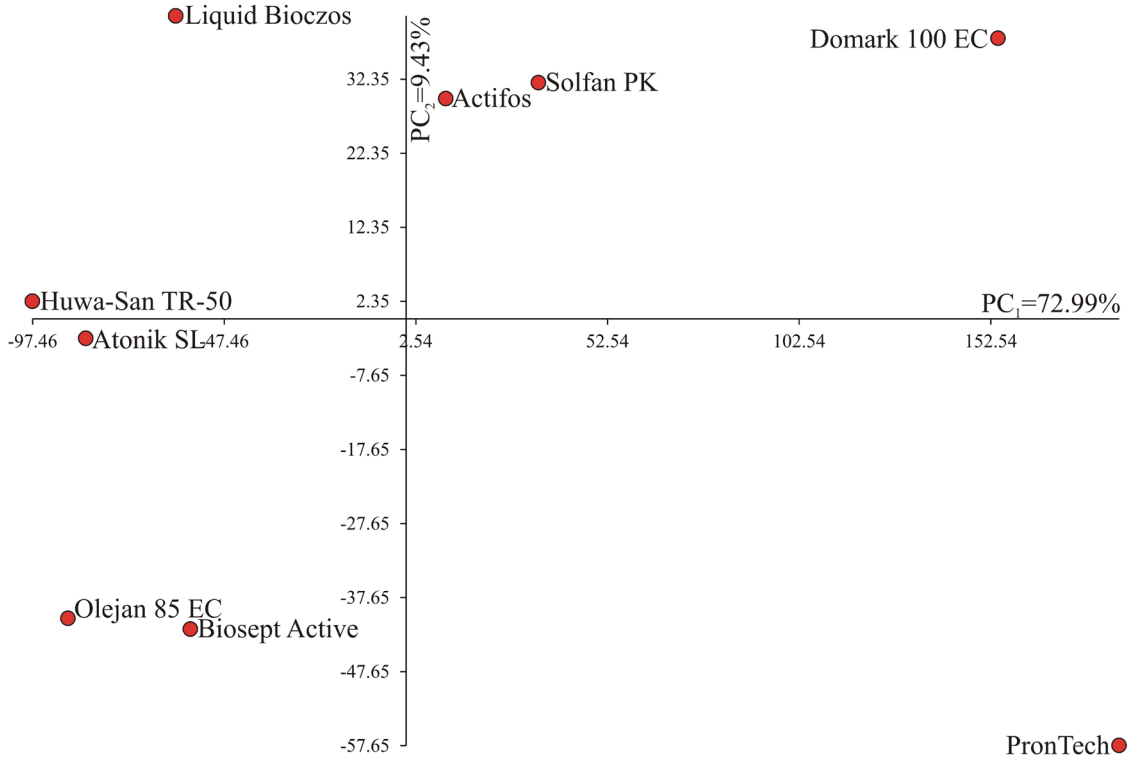
Considering similarities/differences in each characteristic can lead to different interpretations, especially when no statistically significant correlations are observed. Multivariate methods address this issue. Mean clustering and principal component analysis were used to assess similarities/differences in the effects of the treatments. The Euclidean similarity between foliar fertilizers calculated based on all observed characteristics (traits and time of observation) ranged from 0.4469 (between Domark 100 EC and Huwa-San TR-50) to 0.9575 (between Biosept Active and Huwa-San TR-50) (tab. 7). The calculated similarity values were used to construct a similarity dendrogram, which distinguished three main clusters. One of them was Domark 100 EC and PronTech, the second – Solfan PK and Actifos, and the third one – the remaining foliar fertilizers (fig. 1). The first two principal components explained 82.42% of the total variability between foliar fertilizers calculated based on all observed traits. Three groups consisting of two foliar fertilizers were distinguished: (1) Actifos and Solfan PK, (2) Atonik SL and Huwa-San TR-50, (3) Biosept Active and Olejan 85 EC (fig. 2). The remaining three foliar fertilizers were separate single-element groups (fig. 2). The obtained results allowed us to group treatments that produce similar effects across all considered characteristics. This may facilitate practical decision-making regarding the selection of the appropriate treatment.

Tabela 7. Podobieństwo euklidesowe pomiędzy nawozami dolistnymi
Table 7. The Euclidean similarity between foliar fertilizers

Nawozy dolistne Foliar fertilizers	Domark 100 EC	Actifos	Atonik SL	Liquid Bioczos	Biosept Active	Huwa-San TR-50	Olejan 85 EC	PronTech
Actifos	0.817	–	–	–	–	–	–	–
Atonik SL	0.4818	0.8579	–	–	–	–	–	–
Liquid Bioczos	0.5526	0.8979	0.9258	–	–	–	–	–
Biosept Active	0.5584	0.861	0.9342	0.9079	–	–	–	–
Huwa-San TR-50	0.4469	0.8004	0.9129	0.9238	0.9575	–	–	–
Olejan 85 EC	0.5045	0.7986	0.8529	0.8382	0.9277	0.9023	–	–
PronTech	0.8549	0.7407	0.4977	0.5746	0.6418	0.5161	0.5471	–
Solfan PK	0.843	0.8845	0.7545	0.8055	0.8354	0.8059	0.8018	0.8108



Rys. 1. Mapa cieplna podobieństwa euklidesowego pomiędzy nawozami dolistnymi
Fig. 1. Heat map of Euclidean similarity between foliar fertilizers



Rys. 2. Rozkład nawozów dolistnych w układzie dwóch pierwszych składowych głównych
Fig. 2. Distribution of foliar fertilizers in the system of the first two principal components

Wnioski / Conclusions

Actifos, Atpolan 80 EC, Olejan 85 EC, PronTech, Solfan PK and UHT Milk used to protect rose showed in most experiments very high effectiveness in reducing the development of black spot symptoms. PronTech, Actifos and Solfan PK showed the highest efficacy in inhibiting germination of *D. rosae* spores. All of the natural and synthetic products tested can be used in the cultivation of roses to protect against black spot. None of the tested products was phytotoxic to the rose cultivars used in the experiments.

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