*Zeszyty Problemowe Postępów Nauk Rolniczych*
*nr 602, 2020, 35–47*
*DOI 10.22630/ZPPNR.2020.602.14*

# POSSIBILITY OF USING PREPARATIONS CONTAINING AMINO ACIDS OR PROBIOTIC, OR THEIR MIXTURES, TO PROTECT ROSES AGAINST POWDERY MILDEW

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**Summary.** One of the most common and most dangerous diseases in rose cultivation under cover and in the open field is powdery mildew caused by *Podosphaera pannosa*. Powdery mildew is also a common disease in many agricultural and horticultural plant species, contributing to significant reduction in yield and/or decorative value. In the study, preparations containing amino acids or a probiotic were used to spray plants 4 times every 7 days individually or in a mixture at various concentrations to reduce the development of powdery mildew on rose plants grown under cover. Agro-Sorb Folium at the concentrations of 0.25, 0.5, 1 and 2% was shown to have an efficacy of 77.4 to 92%, depending on the concentration. Agro-Sorb L-Amino$^+$ used in the same concentrations showed an efficacy of 73 to 91%. By comparison, Agro-Sorb Folium used at 1% in a mixture with the EmFarma Plus probiotic at 0.8 and 2% had, depending on the concentration, an efficacy of 90.5 to 100% (no symptoms of powdery mildew). Agro-Sorb L-Amino$^+$ used at 1% in a mixture with the EmFarma Plus probiotic at 0.8 and 2% showed, depending on the concentration, an efficacy of 92 to 97.8%. The study showed a significant increase in the effectiveness of the EmFarma Plus preparation containing probiotic bacteria when it was combined with a 1% dose of the growth stimulant Agro-Sorb Folium or Agro-Sorb L-Amino$^+$, compared to the use of the probiotic preparation alone. In each observation, the increase in the concentration of the tested amino acids and probiotic, used individually as well as in a mixture, was associated with a significant increase in their effectiveness. After completion of the experiment, it was found that the rose plants sprayed with Agro-Sorb Folium at a concentration of 0.5%

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or higher, and with NaturalCrop SL at a concentration of 1.0% had a significantly higher fresh weight of the aboveground parts, ranging from 12.2 to 18.3%, compared to the control plants.

**Key words:** amino acids, probiotics, mixture, protection, *Podosphaera pannosa*

## INTRODUCTION

Under greenhouse conditions, powdery mildew of rose is the most important and devastating disease, which is characterized by the appearance of white to greyish spots or patches and powdery growth on the upper surface of leaves, but it also spreads to the lower surface of leaves, young shoots, stems, buds, and flowers [Kumar and Chandel 2018]. The powdery mildew fungi occurring on rose reduce the yield by between 20 and 40%, depending on the congenial environment favourable for their growth and multiplication [Gastelum et al. 2014]. The use of chemical protection agents is still the primary method of protecting rose shrubs. However, the use of fungicides entails the risk of emergence of fungal races resistant to the applied agents, and also poses a threat to humans, animals, and the environment. Therefore, there is a need to look for other methods of plant protection, especially with the use of unconventional means such as plant extracts, plant growth stimulants, or foliar fertilizers that will limit the development of pathogens [Wojdyła 2015, 2016].

The relevant scientific literature first mentions the possibility of using amino acids in plant protection in the second half of the 20th century [Kuć et al. 1959]. The authors demonstrated that apple cultivars susceptible to *Venturia inaequalis* showed resistance to this pathogen after the amino acid phenylalanine was injected into their leaf petioles. Over the next several years, research conducted by various authors had shown the possibility of protecting plants with amino acids against: *Aphanomyces euteiches*, *Botrytis fabae*, *Cladosporium cucumerinum*, *Colletotrichum lagenarium*, *Erysiphe cichoracearum*, *Phytophthora infestans*, *Phytophthora cinnamomi*, *Puccinia recognita* f. sp. *tritici* and *Puccinia graminis* f. sp. *tritici* [Van Andel 1966] and *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium oxysporum* f. sp. *lycopersici* [Woltz and Jones 1970, Aly et al. 2010]. The research on the mechanism of action of amino acids on pathogens had demonstrated their fungicidal activity, lowering of pathogen aggressiveness (virulence), production of other compounds in soil or plant, disturbances in nitrogen metabolism, interference in the expression of symptoms, and increasing plant resistance to the pathogen [Van Andel 1966].

Hasabi et al. [2014], in a study of bacterial canker occurring on lemon (*Xanthomonas citri* subsp. *citri*), showed that, among the tested amino acids used for spraying, the highest effectiveness in reducing leaf necrosis was achieved after the application of L-methionine. The use of this amino acid was also found to result in the highest increase in the concentration of catalase, peroxidase, phenylalanine ammonia lyase, and 1,3-β-glucanase. The role of phenylalanine ammonia lyase in the induction of plant defence mechanisms is associated with the biosynthesis of phytoalexins, the transformation of phenolic compounds to lignin-like substances, and the induction of salicylic acid – a substance associated with the transmission of signals inducing local and systemic plant resistance. The level of activity of this enzyme is correlated with the degree of plant resistance to infection and with the aggressiveness of the pathogen [Gałązka 2013].

Mehta et al. [1991] conducted research on the influence of amino acids on the synthesis of proteolytic enzymes (proteases) and cellulolytic enzymes. The authors showed that in the case of *Fusarium oxysporum* and *Fusarium moniliforme*, leucine and phenylalanine completely inhibited the synthesis of polygalacturonase (PG) – an enzyme responsible for the breakdown of plant cell walls. On the other hand, leucine, phenylalanine and tryptophan completely inhibited cellulase synthesis by *Fusarium moniliforme* and limited its synthesis by *Fusarium oxysporum*. Amino acids have also been shown to have a direct effect on spore germination, the lengthening of the germ hypha, and the synthesis and activity of certain enzymes responsible for the pathogenesis of *Fusarium* spp. [Bakry and Rizk 1967]. A direct effect of amino acids on *Sclerotinia sclerotiorum*, the causal agent of Sclerotinia rot (white mould), was also found by Wojdyła and Sobolewski [2016].

A probiotic was first defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the host's health" [Gibson and Roberfroid 1995]. The currently available literature mainly concerns the possibility of using probiotics containing various microorganisms as dietary supplements in human and animal nutrition [Saxelin 2008, Jach et al. 2013]. Probiotic microorganisms include mainly bacteria of the genus *Lactobacillus* and *Bifidobacterium* from the group of lactic acid bacteria, the common feature of which is the ability to anaerobically break down carbohydrates by lactic fermentation [Jach et al. 2013], as well as yeasts of the genus *Saccharomyces* [Forrsten et al. 2011]. Bacteria that are most often used in the production of functional food and probiotic preparations are those of the genera *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, and *Streptococcus* [Granato et al. 2010].

In contrast, information on the use of LAB (lactic acid bacteria) as biological control agents in agricultural production is relatively limited [Tsuda et. al. 2016]. Some LAB are known to suppress several kinds of postharvest rot [Trias et al. 2008] and disease incidence in pot-grown bean plants [Visser et al. 1986].

In a study by Wang et al. [2011], thirty-five of the 77 colonies of lactic acid bacteria isolated from koumiss showed antifungal activity against *Botrytis cinerea*, when tested by the Poison Food Technique. Of these, the most promising isolate with a broad spectrum of antifungal activity, including against *Botrytis cinerea*, *Alternaria solani*, *Phytophthora drechsleri*, *Fusarium oxysporum* and *Glomerella cingulata*, was identified as *Lactobacillus plantarum* IMAU10014. The maximum antifungal activity was observed at pH 4.0 [Wang et al. 2011]. LAB have a GRAS status (generally recognized as safe) and it has been estimated that 25% of the European diet and 60% of the diet in many developing countries consists of fermented foods (Wang et al. 2011). By *in vitro* screening, Laitila et al. [2002] showed that *Lactobacillus plantarium* cell-free extracts were effective in growth inhibition against Fusarium species including *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium oxysporum*. In an earlier study on the possibility of reducing mycotoxin production by pathogens, Ghazvini et al. [2016] had shown that *Bifidobacterium bifidum* and *Lactobacillus fermentum* significantly reduced aflatoxin production and growth rate of *Aspergillus parasiticus* in comparison with the controls. That study demonstrated the ability of *Bifidobacterium bifidum* and *Lactobacillus fermentum* to reduce aflatoxin levels and prevent the growth of mycotoxigenic moulds (*Aspergillus parasiticus*) through production of several low-molecular-weight antifungal metabolites, binding to the cell wall, or combination of acidity and microbial competition [Ghazvini et al. 2016].

The aim of the study presented here was to determine the effectiveness of preparations containing amino acids or probiotics, used individually or in a mixture at various concentrations, in reducing the development of powdery mildew of rose (*Podosphaera pannosa* (Wall.) De Bary).

## MATERIAL AND METHODS

The study included preparations whose main ingredients are free, natural (L-α) amino acids (histidine, serine, arginine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, cysteine, lysine, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, tryptophan), *viz.* plant growth stimulant Agro-Sorb Folium [total amino acids 12%, including free amino acids 9.3% + total nitrogen (N) 2.1% + boron (B) 0.02% + manganese (Mn) 0.05% + zinc (Zn) 0.07%], organic fertilizer Agro-Sorb L-Amino$^+$ [total amino acids 10%, including free amino acids 5% + total nitrogen (N) 2% + organic nitrogen ($N_{org}$) 2% + organic carbon ($C_{org}$) 4% + organic substances in dry weight 65%], liquid organic fertilizer Elvita Amino based on amino acids [organic nitrogen (N) 8%, organic carbon (C) 23.5%, organic matter 47%, total amino acid content 50%], liquid organic nitrogen fertilizer NaturalCrop SL with an amino acid complex (total nitrogen in organic form (N) 9%, amino acid content >56% w/w (most in the left-handed form), organic carbon (C) 24.5%), and EmFarma Plus probiotic (mother cultures of living microorganisms SCD ProBio Plus®, (organic sugar cane molasses, revitalized, non-chlorinated water, Klodawa salt, mineral complex). The tested probiotic consists of specially selected and prepared, using fermentation technology, beneficial living microorganisms naturally occurring in the environment (*Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactococcus diacetylactis*, *Lactobacillus lactis* ssp. *lactis*, *Streptococcus salivarius* ssp. *thermophilus*, *Bacillus subtilis* var. *natto*, *Saccharomyces cerevisiae*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*). The tested products were used individually or in a mixture for spraying roses grown in a greenhouse. The experiments were carried out on rose plants *cv.* 'Aga', a cultivar very susceptible to powdery mildew, planted in 1 $dm^3$ containers placed on a windowsill in a greenhouse. During the experiment the air humidity in the greenhouse was around 70%, and the temperature in the range 18–25°C. The plants were watered by directing the stream of water directly onto the substrate or the capillary mat on which the containers were placed. To prepare the working liquid, water at a temperature of about 20°C was used, into which the tested preparations or their mixtures were added. After the appearance of powdery mildew symptoms, the preparations were used individually or in a mixture (Agro-Sorb Folium, Agro-Sob L-Amino$^+$, EmFarma Plus) for spraying the plants 4 times every 7 days (Table 2), or 6 times (Table 1), at the concentrations shown in Tables 1 and 2. Domark 100 EC (100 g tetraconazole in 1 l) was used as the standard agent. The control plants were sprayed with water, while the other plants were sprayed with the test preparations at various concentrations using 100 ml of liquid per 1 $m^2$. During spraying with the liquid, the upper and lower surfaces of leaf blades were thoroughly covered. Before the start of the experiment and 3 days after the application of 2, 4 or 6 sprayings (Table 2), the severity of disease symptoms was assessed according to a 6-point scale, in which: 0 – no symptoms, 1 – up to 1% of the surface area of shoots/leaves covered with mycelium, 2 – 1.1 to 5%, 3 – 5.1 to 10%, 4 – 10.1 to 20%, 5 – more than 20% of shoot/leaf surface area covered with mycelium. Observations as to the possible phytotoxicity of the tested preparations or their mixtures were carried out 3 days after spraying according to an 8-point scale: 0 – 0% damaged or distorted leaf area, 1 – 0.1 to 1% damaged leaf area, 2 – 1.1 to 6%, 3 – 6.1 to 15%, 4 – 15.1 to 30%, 5 – 30.1 to 50%, 6 – 50.1 to 80%, 7 – more than 80% of leaf area was damaged or distorted. In addition, observations were made for signs of yellowing or growth inhibition.

After the completion of the experiments, the aboveground parts of the plants were separated from the underground parts with a scalpel and weighed to determine the fresh weight for each replication (Experiment 1). After dividing the total weight by the number of plants in each replication, mean fresh weight was obtained for a single plant. Next, the aboveground parts from the individual replications were put into envelopes and stored at 70°C for 24 hours in forced-air incubators. After the 24-hour incubation period, the plant material was again weighed with and without envelopes. To obtain the dry weight per plant, the final dry weight of aboveground parts was divided by the number of plants in the replication (15). The experiments were set up in a completely randomized block design, in 4 replications with 15 rose plants each. The data obtained were subjected to statistical analysis using Duncan's test. The percentage inhibition in the occurrence of symptoms of powdery mildew was then calculated in relation to the control plants (unprotected) using a simplified Abbott's formula [Abbott 1925].

## RESULTS AND DISCUSSION

In the first experiment, 3 days after the 2nd spraying of roses, it was observed that the highest effectiveness of 71.7 to 73.5% was shown by Agro-Sorb Folium at the concentrations of 1 and 2% (Table 1). In contrast, the lowest efficacy of 46.7% was observed for NaturalCrop SL at a conc. of 0.25%. By comparison, Agro-Sorb Folium in a mixture with the probiotic EmFarma Plus at the concentrations of 0.8 and 2% showed an efficacy of 78.4 and 86.7%, respectively. It was found that the organic fertilizer Elvita Amino at a conc. of 1% caused phytotoxicity in the form of browning up to 5% of the leaf surface area. No increase in the severity of phytotoxicity symptoms was observed in the later stages of the study. In the case of this liquid organic fertilizer based on amino acids, phytotoxicity might have been caused by too high air temperature and the concentration that was 4 times as high as that recommended by the manufacturer.

Observations carried out 3 days after the 4th spraying of the plants revealed the highest effectiveness of 84.6 to 87% in the case of Agro-Sorb Folium applied at the concentrations of 1.0 and 2.0% (Table 1). The lowest effectiveness of 71.4% was found on the

Table 1. Effectiveness of preparations in limiting the development of symptoms of powdery mildew on roses *cv.* 'Aga' grown in a greenhouse

Tabela 1. Skuteczność środków w ograniczaniu rozwoju objawów mączniaka prawdziwego róż na odm. 'Aga' uprawianej w szklarni

| Treatment<br>Kombinacja | Concen-<br>tration<br>Stężenie<br>[%] | Mean degree of infection after spraying 2×, 4×, and 6×<br>Stopień porażenia roślin po opryskiwaniach | | | Percentage effectiveness after spraying 2×, 4×, and 6×<br>Procentowa skuteczność po opryskiwaniach | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 2 | 4 | 6 |
| Control/Kontrola | – | 3.00$^{j}$ | 4.20$^{j}$ | 5.00$^{j}$ | – | – | – |
| Domark 100 EC | 0.05 | 0.20$^{a}$ | 0.00$^{a}$ | 0.05$^{a}$ | 93.3$^{i}$ | 100.0$^{i}$ | 99.0$^{i}$ |
| Agro-Sorb Folium | 0.25 | 1.15$^{f}$ | 0.95$^{gh}$ | 3.00$^{f–i}$ | 61.7$^{d}$ | 77.4b$^{c}$ | 40.0$^{bc}$ |
| Agro-Sorb Folium | 0.5 | 1.00$^{e}$ | 0.90$^{f–h}$ | 2.75$^{f}$ | 66.7$^{e}$ | 78.6$^{b–d}$ | 45.0$^{cd}$ |
| Agro-Sorb Folium | 1.0 | 0.85$^{d}$ | 0.65$^{e}$ | 1.95$^{d}$ | 71.7$^{f}$ | 84.6$^{e}$ | 61.0$^{f}$ |
| Agro-Sorb Folium | 2.0 | 0.79$^{d}$ | 0.55$^{d}$ | 1.70$^{cd}$ | 73.5$^{f}$ | 87.0$^{f}$ | 66.0$^{g}$ |
| Elvita Amino | 0.25 | 1.35$^{h}$ | 1.20$^{i}$ | 3.35$^{i}$ | 55.0$^{b}$ | 71.4$^{a}$ | 33.0$^{a}$ |
| Elvita Amino | 0.5 | 1.15$^{f}$ | 1.00$^{h}$ | 2.65$^{f}$ | 61.7$^{d}$ | 76.2$^{b}$ | 47.0$^{d}$ |
| Elvita Amino | 1.0 | 1.00$^{e}$ | 0.85$^{fg}$ | 1.75$^{cd}$ | 66.7$^{e}$ | 79.8$^{cd}$ | 65.0$^{fg}$ |
| EmFarma Plus | 0.8 | 1.40$^{hi}$ | 1.15$^{i}$ | 3.15$^{g–i}$ | 53.3$^{b}$ | 72.6$^{a}$ | 37.0$^{ab}$ |
| EmFarma Plus | 2.0 | 1.20$^{fg}$ | 1.00$^{h}$ | 2.85$^{f–h}$ | 60.0$^{cd}$ | 76.2$^{b}$ | 43.0$^{cd}$ |
| NaturalCropSL | 0.25 | 1.55$^{i}$ | 1.20$^{i}$ | 3.20$^{hi}$ | 46.7$^{a}$ | 71.4$^{a}$ | 36.0$^{ab}$ |
| NaturalCropSL | 0.5 | 1.40$^{i}$ | 0.95$^{gh}$ | 2.80$^{fg}$ | 53.3$^{b}$ | 77.4$^{bc}$ | 44.0$^{cd}$ |
| NaturalCropSL | 1.0 | 1.30$^{gh}$ | 0.80$^{f}$ | 2.30$^{e}$ | 56.7$^{bc}$ | 81.0$^{d}$ | 54.0$^{e}$ |
| Agro-Sorb Folium + EmFarma Plus | 1.0 + 0.8 | 0.65$^{c}$ | 0.40$^{c}$ | 1.55$^{c}$ | 78.4$^{g}$ | 90.5$^{g}$ | 69.0$^{g}$ |
| Agro-Sorb Folium + EmFarma Plus | 1.0 + 2.0 | 0.40$^{b}$ | 0.20$^{b}$ | 1.25$^{b}$ | 86.7$^{h}$ | 95.2$^{h}$ | 75.0$^{h}$ |

Experiment start date and initial degree of infection

Początek doświadczenia i porażenie wstępne 18.05. 2018 = 0.4

Note: Means followed by the same letter in a column do not differ significantly according to Duncan's test at $p = 0.05$.

Uwaga: Średnie oznaczone tą samą literą w kolumnach nie różnią się istotnie ($p = 0,05$) według testu Duncana

Degree of infection: 0 – no symptoms; 1 – up to 1% of stem/leaf surface covered with mycelium; 2 – 1.1 to 5%; 3 – 5.1 to 10%; 4 – 10.1 to 20%; 5 – more than 20% of stem/leaf surface covered with mycelium

Skala porażenia: 0 – brak objawów, 1 – do 1% powierzchni pędów/liści pokrytej grzybnią, 2 – 1,1 do 5%, 3 – 5,1 do 10%, 4 – 10,1 do 20%, 5 – powyżej 20% powierzchni pędów/liści pokrytej grzybnią

roses protected with Elvita Amino at a conc. of 0.25% and NaturalCrop SL at 0.25%. By comparison, Agro-Sorb Folium in a mixture with the probiotic EmFarma Plus at the concentrations of 0.8 and 2% showed an efficacy of 90.5 and 95.2%, respectively (Table 1).

In the last 2 weeks of the experiment, new shoot growth was conducive to the intensification of disease symptoms. Therefore, all the preparations used individually and in a mixture showed lower effectiveness than that observed after spraying the plants 4 times (Table 1). Observations carried out 3 days after the 6th spraying of roses revealed that the highest effectiveness of 61 to 66% was shown by Agro-Sorb Folium at the concentrations of 1.0 and 2.0%, and by Elvita Amino at 1.0%. In contrast, the lowest efficacy of 33% was found in the case of Elvita Amino used at 0.25%. By comparison, Agro-Sorb Folium used in a mixture with the probiotic EmFarma Plus at the concentrations of 0.8 and 2%

showed an efficacy of 69 and 75%, respectively. In each observation, the increase in the concentration of the tested amino acids and probiotic was associated with a significant increase in their effectiveness.

In the second experiment, the highest effectiveness of 100% observed 3 days after the 2nd spraying of roses was shown by Agro-Sorb Folium at the concentrations of 1.0 and 2.0%, and by Agro-Sorb L-Amino$^+$ at a conc. of 2.0%. The lowest efficacy of 77.8% was recorded for the roses protected with EmFarma Plus applied at 0.8%. The growth stimulant Agro-Sorb Folium in a mixture with the probiotic EmFarma Plus at a conc. of 0.8% showed an efficacy of 98.8%, whereas at the probiotic's concentration of 2%, no symptoms of powdery mildew were found. By comparison, Agro-Sorb L-Amino$^+$ in a mixture with EmFarma Plus showed an effectiveness of 91.5 to 99.7%, depending on the concentration of the probiotic.

Observations carried out 3 days after the 4th spraying revealed the highest effectiveness of 91 to 92% for Agro-Sorb Folium and Agro-Sorb L-Amino$^+$ at a conc. of 2%. In contrast, the lowest efficacy of 73% was found on the plants protected with Agro-Sorb L-Amino$^+$ applied at 0.25% (Table 2). The growth stimulant Agro-Sorb Folium used in a mixture with the EmFarma Plus probiotic at a conc. of 0.8% showed an efficacy of 97.8%, whereas at the probiotic's concentration of 2%, no symptoms of powdery mildew were found. By comparison, the organic fertilizer Agro-Sorb L-Amino$^+$ used in a mixture with the EmFarma Plus probiotic at 0.8 and 2% showed an efficacy of 92 to 97.8%, depending on the concentration.

Observations carried out 14 days after the 4th spraying of roses revealed the highest effectiveness of 84 to 86% after the treatments with Agro-Sorb Folium at a conc. of 2% and Agro-Sorb L-Amino$^+$ at 2% (Table 2). In contrast, the lowest efficacy of 60% was found on the roses protected with Agro-Sorb L-Amino$^+$ at a conc. of 0.25%. Agro-Sorb Folium used in a mixture with the EmFarma Plus probiotic showed an efficacy of 95.1 to 97.7%. By comparison, the organic fertilizer Agro-Sorb L-Amino$^+$ used at 1% in a mixture with the EmFarma Plus probiotic at the concentrations of 0.8 and 2% showed an efficacy of 90.1 to 94.2%, depending on the concentration. In each observation, the increase in the concentration of the tested amino acids and probiotic was associated with a significant increase in their effectiveness. The obtained results of the increase in the effectiveness of the mixture as compared to the use of the single preparation confirm previous studies in which Agro-Sorb Folium used alone had shown lower effectiveness than when it was used in a mixture with fungicides [Wojdyła 2019]. Similarly, studies conducted on hop plants had shown high effectiveness of probiotic microorganisms in the protection against powdery mildew and downy mildew, but with a low initial severity of disease symptoms [Solarska 2013]. The Author's previous research had also shown high effectiveness of preparations containing amino acids in the protection of roses against *Podosphaera pannosa* [Wojdyła 2017], pansy against *Colletotrichum violae-tricoloris* [Wojdyła 2018], and bean against *Sclerotinia sclerotiorum* [Wojdyła and Sobolewski 2016]. Živković et al. [2019], in turn, showed that *Lactobacillus plantarum*, one of the most widespread lactic acid bacteria, exerted a strong antagonistic effect against many microorganisms, including: *Aspergillus flavus*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, and *Fusarium avenaceum*, the causal agents of apple rot during storage. Results of tests conducted *in vitro* showed that *Lactobacillus plantarum* had a stronger

Table 2. Effectiveness of preparations and their mixtures in limiting the development of symptoms of powdery mildew on roses *cv.* 'Aga' grown in a greenhouse

Tabela 2. Skuteczność środków w ograniczaniu rozwoju objawów mączniaka prawdziwego róż na odm. 'Aga' uprawianej w szklarni

| Treatment<br>Kombinacja | Concentration<br>Stężenie<br>[%] | Mean degree of plant infection after spraying 2× and 4×<br>Stopień porażenia roślin po opryskiwaniach | | Degree of infection 14 days after spraying 4×<br>Stopień porażenia po dniach od wykonania 4-krotnego opryskiwania | Percentage effectiveness after spraying 2× and 4× (%)<br>Procentowa skuteczność po opryskiwaniach | | Percentage effectiveness 14 days after spraying 4×<br>Procentowa skuteczność po dniach od wykonania 4-krotnego opryskiwania |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 14 | 2 | 4 | 14 |
| Control / Kontrola | – | $4.05^{g}$ | $5.00^{h}$ | $5.00^{k}$ | – | – | – |
| Domark 100 EC | 0.05 | $0.00^{a}$ | $0.00^{a}$ | $0.00^{a}$ | $100.0^{f}$ | $100.0^{g}$ | $100.0^{j}$ |
| Agro-Sorb Folium | 0.25 | $0.80^{ef}$ | $1.00^{ef}$ | $1.80^{ij}$ | $80.3^{ab}$ | $80.0^{bc}$ | $64.0^{ab}$ |
| Agro-Sorb Folium | 0.5 | $0.65^{d–f}$ | $0.84^{de}$ | $1.40^{h}$ | $84.0^{bc}$ | $83.0^{cd}$ | $72.0^{c}$ |
| Agro-Sorb Folium | 1.0 | $0.00^{a}$ | $0.65^{d}$ | $1.00^{fg}$ | $100.0^{f}$ | $87.0^{d}$ | $80.0^{de}$ |
| Agro-Sorb Folium | 2.0 | $0.00^{a}$ | $0.40^{c}$ | $0.70^{e}$ | $100.0^{f}$ | $92.0^{e}$ | $86.1^{f}$ |
| Agro-Sorb L-Amino+ | 0.25 | $0.80^{ef}$ | $1.35^{g}$ | $2.00^{j}$ | $80.3^{ab}$ | $73.0^{a}$ | $60.0^{a}$ |
| Agro-Sorb L-Amino+ | 0.5 | $0.60^{de}$ | $1.05^{e–g}$ | $1.65^{hi}$ | $85.2^{bc}$ | $79.0^{bc}$ | $67.0^{bc}$ |
| Agro-Sorb L-Amino+ | 1.0 | $0.34^{c}$ | $0.85^{de}$ | $1.10^{g}$ | $91.6^{d}$ | $83.0^{cd}$ | $78.0^{d}$ |
| Agro-Sorb L-Amino+ | 2.0 | $0.00^{a}$ | $0.45^{c}$ | $0.80^{ef}$ | $100.0^{f}$ | $91.0^{e}$ | $84.0^{ef}$ |
| EmFarma Plus | 0.8 | $0.90^{f}$ | $1{,}25^{fg}$ | $1.80^{ij}$ | $77.8^{a}$ | $75.0^{ab}$ | $64.0^{ab}$ |
| EmFarma Plus | 2.0 | $0.45^{cd}$ | $0.85^{de}$ | $1.50^{h}$ | $88.9^{cd}$ | $83.0^{cd}$ | $70.0^{c}$ |
| Agro-Sorb Folium + EmFarma Plus | 1.0 +0.8 | $0.05^{b}$ | $0.11^{b}$ | $0.24^{c}$ | $98.8^{e}$ | $97.8^{f}$ | $95.1^{h}$ |
| Agro-Sorb Folium + EmFarma Plus | 1.0 + 2 | $0.00^{a}$ | $0.00^{a}$ | $0.11^{b}$ | $100.0^{f}$ | $100.0^{g}$ | $97.7^{i}$ |
| Agro-Sorb L-Amino+ + EmFarma Plus | 1.0 + 0.8 | $0.35^{c}$ | $0.40^{c}$ | $0.50^{d}$ | $91.5^{d}$ | $92.0^{e}$ | $90.1^{g}$ |
| Agro-Sorb L-Amino+ + EmFarma Plus | 1.0 + 2 | $0.01^{b}$ | $0.11^{b}$ | $0.30^{c}$ | $99.7^{ef}$ | $97.8^{f}$ | $94.2^{h}$ |

Experiment start date and initial degree of infection

Początek doświadczenia i porażenie wstępne 13.09. 2018 = 0.3

Note see Table 1

Uwaga: patrz Tabela 1

inhibitory effect on spore germination than on mycelium growth in all the fungi tested. Similarly, in a study by Kumar and Chandel [2018], *Bacillus subtilis*, one of the components of the probiotic tested in the present study, when maintained on a medium *in vitro*, caused a 77.19% reduction in the germination of *Podosphaera pannosa* spores. When used in the protection of roses in the form of spraying, it reduced the development of powdery mildew on rose by about 74%. *Bacillus* spp. strain BS061 potently inhibited the mycelial growth of *Botrytis cinerea*, and under glasshouse conditions significantly reduced disease incidence of powdery mildew on cucumber and strawberry. Kim et al. [2013] also found that the culture filtrate of BS061 inhibited the mycelial growth of various plant pathogens. *Bacillus subtilis* UMAF6639 is an antagonistic strain specifically selected for the efficient control of the cucurbit powdery mildew fungus *Podosphaera fusca*, which is a major threat to cucurbits worldwide. The antagonistic activity relies on the production of the antifungal compounds iturin and fengycin [García-Gutiérrez et al. 2013]. The antagonistic strain *Bacillus subtilis* UMAF6639 also confers protection to melon plants against cucurbit powdery mildew by activation of jasmonate and salicylic acid-dependent defence responses [García-Gutiérrez et al. 2013]. *Bacillus subtillis* (Serenade MAX) applied every 14 days provided significant control of rose powdery mildew, which was comparable to that provided by muclobutanil [Elmhirst et al. 2011]. Unfortunately, the available literature data, apart from some exceptions [Solarska 2011], usually concern the possibility of using single types of microorganisms included in probiotics for the protection of plants against diseases. The data published so far indicate a higher effectiveness of multi-strain preparations compared to single-strain ones, including those in which bacterial strains are components of a mixture [Chapman et al. 2011]. It should also be noted that individual strains, even those belonging to the same species, can have different probiotic effects [Jach et al. 2013]. The standard fungicide Domark 100 EC showed, in most of the observations, significantly higher efficacy compared to the tested preparations or their mixtures.

In the first experiment, after the protection of roses had ended, it was found that the plants sprayed with Agro-Sorb Folium at a conc. of 0.5% or higher, and with NaturalCrop SL at 1.0% had a significantly higher fresh weight of the aboveground parts, ranging from 12.2 to 18.3%, in comparison with the control plants (Table 3). A similar relationship was found for the dry weight of the aboveground parts of the rose plants (Table 3). The plants sprayed with Agro-Sorb Folium at 0.5% or higher, and with EmFarma Plus at 0.8% had from 9.61 to 17.3% higher dry weight of the aboveground parts than the control plants (Table 3). The highest fresh and dry weight of the aboveground parts was found in the plants sprayed with Agro-Sorb Folium at a conc. of 1.0%. The remaining preparations tested gave a similar or lower fresh and dry weight compared to the control plants. The obtained results confirm the Author's previously conducted research in which the preparations containing amino acids (Agro-Sorb Folium and Agro-Sorb L-Amino$^{+}$) used for spraying or watering caused significant stimulation of the growth of rose plants [Wojdyła 2017] and pansy plants [Wojdyła 2018]. Also Mukta et al. [2017] demonstrated the stimulation of growth and fruiting of strawberry plants as a result of using probiotic bacteria of the genus *Stenotrophomonas* and *Ochrobacterum*. The tested amino acids or foliar fertilizers, due to their action as stimulants of plant growth and development, as well as inhibitors of the development of many foliar pathogens, can be used in the inte-

Table 3. Effect of preparations on the fresh and dry weight of the aboveground plant parts. Experiment 1 – observation 14 days after the last spraying

Tabela 3. Wpływ środków na świeżą i suchą masę części nadziemnych roślin. Doświadczenie I obserwacja po 14 dniach od wykonania ostatniego opryskiwania

| Treatment<br>Kombinacja | Concentration<br>Stężenie<br>[%] | Mean fresh weight of aboveground parts<br>Średnia masa części nadziemnych<br>[g] | Mean dry weight of aboveground parts<br>Sucha masa części nadziemnych<br>[g] | Percentage increase in fresh weight of aboveground parts<br>Procentowy wzrost świeżej masy części nadziemnych | Percentage increase in dry weight of aboveground parts<br>Procentowy wzrost suchej masy części nadziemnych |
|---|---|---|---|---|---|
| Control/Kontrola | – | $5.67^{a–c}$ | $1.56^{b–d}$ | – | – |
| Domark 100 EC | 0.05 | $6.27^{d–f}$ | $1.75^{fg}$ | 10,6 | 12.2 |
| Agro-Sorb Folium 0,25% | 0.25 | $5.40^{a}$ | $1.51^{b}$ | –4,76 | –3.21 |
| Agro-Sorb Folium 0,5% | 0.5 | $6.59^{f}$ | $1.80^{gh}$ | 16,2 | 15.4 |
| Agro-Sorb Folium 1% | 1.0 | $6.71^{f}$ | $1.83^{h}$ | 18,3 | 17.3 |
| Agro-Sorb Folium 2% | 2.0 | $6.44^{ef}$ | $1.71^{f}$ | 13,6 | 9.61 |
| Elvita Amino | 0.25 | $6.08^{c–e}$ | $1.61^{de}$ | 7,23 | 3.20 |
| Elvita Amino | 0.5 | $5.86^{a–d}$ | $1.53^{bc}$ | 3,35 | –1.92 |
| Elvita Amino | 1.0 | $5.57^{ab}$ | $1.41^{a}$ | –1,76 | –9.62 |
| EmFarma Plus 0,8% | 0.8 | $5.99^{b–e}$ | $1.75^{fg}$ | 5.64 | 12.2 |
| EmFarma Plus 2% | 2.0 | $5.41^{a}$ | $1.53^{bc}$ | –4.59 | –1.92 |
| NaturalCropSL | 0.25 | $5.68^{a–c}$ | $1.58^{cd}$ | 0.18 | 1.28 |
| NaturalCropSL | 0.5 | $5.74^{a–c}$ | $1.53^{bc}$ | 1.23 | –1.92 |
| NaturalCropSL | 1.0 | $6.36^{ef}$ | $1.64^{e}$ | 12.2 | 5.13 |
| Agro-Sorb Folium + EmFarma Plus | 1.0 + 0.8 | $5.81^{a–d}$ | $1.61^{de}$ | 2.47 | 3.21 |
| Agro-Sorb Folium + EmFarma Plus | 1.0 + 2.0 | $5.70^{a–c}$ | $1.58^{cd}$ | 0.53 | 1.28 |

Note see Table 1
Uwaga: patrz Tabela 1

grated protection of rose against diseases. Out of the tested preparations, only the organic fertilizer Elvita Amino at a concentration of 1% showed phytotoxicity resulting in the browning of up to 5% of leaf surface area.

## CONCLUSIONS

When used for spraying roses, the plant growth stimulant Agro-Sorb Folium, the fertilizers Agro-Sorb L-Amino$^+$, Elvita Amino and NaturalCrop SL, the main components of which are amino acids, and the probiotic EmFarma Plus showed high effectiveness in limiting the development of powdery mildew. With the increase in the concentration of the tested preparations, they were found to be more effective. Among the tested preparations, Agro-Sorb Folium and Agro-Sorb L-Amino$^+$ used in a mixture with the EmFarma Plus probiotic showed significantly higher effectiveness than when used individually. It was demonstrated that Agro-Sorb Folium, NaturalCrop SL and the probiotic EmFarma Plus caused significant stimulation of the growth of rose plants. The obtained results show promising possibilities of using preparations containing amino acids and probiotics, individually or combined, in plant protection.

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## MOŻLIWOŚĆ WYKORZYSTANIA ŚRODKÓW ZAWIERAJACYCH AMINOKWASY I PROBIOTYKU ORAZ ICH MIESZANIN W OCHRONIE RÓŻ PRZED MĄCZNIAKIEM PRAWDZIWYM

**Streszczenie.** Stymulator wzrostu roślin Agro-Sorb Folium, nawozy Agro-Sorb L-Amino$^+$, Elvita Amino, NaturalCrop SL, których głównym składnikiem są aminokwasy oraz probiotyk EmFarma Plus użyte do opryskiwania róż wykazywały wysoką skuteczność w ograniczaniu rozwoju mączniaka prawdziwego. Wraz ze wzrostem stężenia badanych środków stwierdzono ich wyższą skuteczności. Z pośród badanych środków Agro-Sorb Folium i Agro-Sorb L-Amino$^+$ stosowane w mieszaninie z probiotykiem EmFarma Plus wykazywały istotnie wyższą skuteczność aniżeli stosowane pojedynczo. Wykazano, że Agro-Sorb Folium, NaturalCrop SL oraz probiotyk EmFarma Plus powodowały istotną stymulację wzrostu krzewów róży. Otrzymane wyniki badań dają obiecujące możliwości wykorzystania środków zawierających aminokwasy oraz probiotyków stosowanych pojedynczo lub w mieszaninie w ochronie roślin.

**Słowa kluczowe:** aminokwasy, probiotyki, mieszanina, ochrona, *Podosphaera pannosa*