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THE INFLUENCE OF SELECTED BIOLOGICAL PRODUCTS ON LIMITATION OF THE DEVELOPMENT OF *Sclerotinia sclerotiorum* SCLEROTIA

Summary

The aim of the study was to assess the influence of biological products on the decomposition of Sclerotinia sclerotiorum sclerotia in a laboratory environment. The following products were used in the experiment: EM Naturalnie Aktywny, EM Bio and Contans WG. The experiment was conducted in Petri dishes with tissue paper (medium quality filters) or technical sand, which were used as substrates. In each case Sclerotinia sclerotiorum sclerotia were placed on a substrate and their decomposition was observed for 5 weeks. The biological preparations used in the experiment decomposed Sclerotinia sclerotiorum sclerotia very effectively. The product containing hyperparasitic Coniothyrium minitans fungi completely destroyed the spore forms of the pathogen. The results of the experiment assessing the usefulness of preparations containing effective microorganisms for limiting the content of sclerotia in soil need to be confirmed in the natural environment.
***Key words**: biological methods of plant protection, effective microorganisms, sclerotinia stem rot, rapeseed, sclerotia*

WPŁYW WYBRANYCH PRODUKTÓW BIOLOGICZNYCH NA OGRANICZANIE ROZWOJU SKLEROCJÓW *Sclerotinia Sclerotiorum*

Streszczenie

Celem badań była ocena wpływu produktów biologicznych na rozkład sklerocjów grzyba Sclerotinia sclerotiorum w warunkach laboratoryjnych. W doświadczeniu wykorzystano 3 produkty, tj. EM Naturalnie Aktywny, EM Bio oraz Contans WG. Doświadczenie prowadzono na szalkach, w których znajdowała się bibuła (sączki jakościowe średnie) lub piasek techniczny. Każdorazowo na podłoże wykładano sklerocja S. sclerotiorum i obserwowano przez 5 tygodni ich rozpad. Zastosowane w doświadczeniu biologiczne preparaty wykazały wysoką skuteczność w procesie rozkładu sklerocjów S. sclerotiorum. Środek zawierający nadpasożytniczy grzyb Coniothyrium minitans spowodował całkowite zniszczenie form przetrwalnikowych badanego patogena. Uzyskane wyniki dotyczące zastosowania preparatów zawierających efektywne mikroorganizmy w ograniczaniu obecności sklerocjów w glebie wymagają potwierdzenia w warunkach naturalnych.
***Słowa kluczowe**: metody biologiczne ochrony roślin, efektywne mikroorganizmy, zgnilizna twardzikowa, rzepak, sklerocja*

1. Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a common fungal pathogen found on more than 400 species of crops and dicotyledonous weeds [9]. It causes economic loss of the yield of sunflowers, potatoes, tomatoes, tobacco, beans, soy, lupine, lettuce, carrot and other crops [11, 12]. The fungus forms spore structures (sclerotia) during its growth in infected organs. Sclerotia are composed of aggregated clusters of hyphae and are surrounded by a thick black wall, which is resistant to environmental factors [12, 16]. These spores have different shapes, usually irregular or oval. Their size ranges from 2 to 12 mm. When sclerotia enter soil, they can survive in it for many years while waiting for the host plant [11]. The flowering period of winter rapeseed is optimal for the development of the pathogen. At the time sclerotia form sporocarps with numerous ascospores or mycelium, which cause infections. Nearly 90% of the life cycle of *S. sclerotiorum* takes place in soil in the form of spore organs. The soil temperature, pH and humidity do not influence the vitality of *S. sclerotiorum* as much as the biological composition of soil, especially the presence of hyperparasitic fungi [1]. Hyperparasitism consists in negative influence on pathogens. Their sclerotia are destroyed by lysis. Different forms of this phenomenon can be observed in various fungal species, e.g. in the following genera: *Coniothyrium, Gliocladium, Trichoderma, Aspergillus, Penicillium, Pythium, Stachybotrys* [1, 5, 12, 16]. They often inhibit the growth of pathogens, compete for important components and have positive effect on the growth of the protected plant as they stimulate its immune mechanisms. Some bacteria, such as those of the *Bacillus* and *Pseudomonas* genera, are also versatile [2]. They are very important microorganisms used for biological protection.

As Integrated Pest Management was introduced in the EU after 2014, biological methods of reducing pathogens became particularly important. The integrated method consists in limiting the use of chemicals to the necessary minimum and promoting environment-friendly methods of combating pathogens. Combined agrotechnical, biological and chemical methods limit the occurrence of pests, including *Sclerotinia sclerotiorum*, to the level at which they do not cause economic loss [5]. The use of products containing effective microorganisms (EM) provides a possibility of combining methods limiting diseases caused by fungal pathogens. They contain many groups of microorganisms belonging to different taxonomic units (lactic bacteria, photosynthesising bacteria, yeasts, actinobacteria, moulds and others) and characterised by a multidirectional broad-spectrum effect [10, 14]. These preparations protect plants best if they complement other agrotechnical procedures applied to reduce pests [3]. Many authors confirmed that ef-

fective microorganisms limited the development of pathogenic fungi, including those of the *Sclerotinia* genus [6, 7, 10, 13]. Naturalnie Aktywny and EM Bio are examples of such products. EM Naturalnie Aktywny contains a composition of microorganisms with probiotic and regenerative properties. According to the manufacturer, the product improves the soil structure, accelerates the decomposition of organic debris, affects the emergence of plants, improves their health and condition during growth. The EM Bio product was developed to support sewage treatment processes and organic waste composting. It contains a larger population of photosynthesising microorganisms.

The aim of the study was to assess the influence of biological products on the decomposition of *Sclerotinia sclerotiorum* sclerotia in a laboratory environment.

2. Material and methods

S. sclerotiorum spore structures which infested winter rape plants in various regions of Poland during the growing season were used as the research material. The spore structures were collected in 2016 and 2017. The sclerotia were placed in Petri dishes with a diameter of 200 mm. Two types of substrate were independently used in the dishes. The first substrate type was tissue paper (medium quality filters). The other type was technical (quartz) sand (0.1-0.3 mm), which filled the dishes up to a height of 5 mm. Both substrates were soaked in water and then its excess was removed. 2 g of the sclerotia were placed in each dish. Next, they were sprayed with products containing effective microorganisms, i.e. EM Naturalnie Aktywny. EM Bio and biofungicide – Contans WG. Table 1 lists all experimental variants, their characteristics and dosage. A higher dose (20 ml) of EM corresponds to the field dose of this product. For comparison, a control variant was also used with each type of substrate, where the sclerotia were sprayed with water only. The dishes were covered and incubated for 5 weeks at room temperature. They were exposed to light for 14 hours. There were two series of the experiment, both in four replicates. The amount of decomposed sclerotia was observed at weekly intervals. The percentage of sclerotia colonised and destroyed by the microorganisms in relation to the total amount of the pathogens placed in the dish was calculated. The results were analysed statistically.

3. Results and discussion

All the biological products used in the experiment, regardless of their doses, had statistically significant influence on the decomposition of *S. sclerotiorum* spore structures in the laboratory environment (Table 2 and 3). The amount of sclerotia destroyed in the variants with the tissue paper substrate ranged from 60.9% to 100%. The percentage of pathogens destroyed in the variants with the technical sand substrate was very similar, i.e. 62.1-100%. The Contans WG product containing hyperparasitic *C. minitans* fungi completely decomposed all the sclerotia (100%) collected in 2016 and 2017. The decomposition was observed on both substrates. So far this organism has been studied very widely both in the natural environment and in laboratories. In all cases it limited the development of *S. sclerotiorum* very effectively [12, 16, 17, 18]. There were similar results of the experiment in the variants where products with effective microorganisms, i.e. EM Naturalnie Aktywny and EM Bio, were applied on the sand substrate at a dose corresponding to the field dose, i.e. 20 ml. The products completely decomposed the sclerotia in this environment. EM Effective Microorganisms were also tested on lettuce [13]. They significantly reduced the amount of *S. sclerotiorum* spores in soil. They also limited the formation of sporocarps and hyphae on the sclerotia. There were also satisfactory results of the experiment where effective microorganisms were applied against *S. homoeocarpa* in a grass plantation [7]. The preparation containing these microorganisms also had positive effect on the colonisation of coriander seeds by *S. Sclerotiorum* [6]. EM preparations contain various bacterial species such as *Lactobacillus plantarum*. There were complex studies assessing the possible usefulness of *Propiononibacterium freudenreichii* ssp. *shermanii* and *L. plantarum* bacteria for the protection of rapeseed from major diseases [2]. The bacteria exhibited statistically significant fungistatic activity against *S. isolatinus* isolates. The growth of the pathogen mycelium was inhibited by 48.4-79.6% in an in vitro experiment. Preparations containing these bacteria were also highly effective against *S. sclerotiorum* in a field experiment, where plants were sprayed during the growing season. There were also studies on other bacterial species which could limit the development of *S. sclerotiorum* [2].

Table 1. The characteristics of biological agents used in the experiment
Tab. 1. Środki biologiczne użyte w doświadczeniu i ich charakterystyka

No.	Variants	Characteristics/content of active substance	Dose per dish
1	*Control*	-	-
2	EM Naturalnie Aktywny	water, EM effective microorganisms: lactic acid bacteria (*Lactobacillus casei, Lactobacillus plantarum)*, photosynthesising bacteria (*Rhodopseudomonas palustris)*, yeasts (*Saccharomyces cerevisiae)*, Azotobacter, sugarcane molasses, total nitrogen (at least 0.3%), potassium (calculated as K_2O, at least 0.2%)	2 ml
3	EM Naturalnie Aktywny	see above	20 ml
4	Microbial preparation – Contans WG (2 kg per ha)	*Coniothyrium minitans* ($1x10^{(9)}$ oospores per 1g of product)	4 g
5	EM Bio	water, sugarcane molasses, EM effective microorganisms: lactic acid bacteria (*Lactobacillus casei, Lactobacillus plantarum)*, photosynthesising bacteria (*Rhodopseudomonas palustris)*, yeasts (*Saccharomyces cerevisiae*) – concentration of microorganisms above 50,000,000 cfu/m	2 ml
6	EM Bio	see above	20 ml

Source: company information materials / Źródło: materiały informacyjne firm

Table 2. The influence of the products used in the experiment on the decomposition of *S. sclerotiorum* sclerotia collected in 2016 (the fifth week of observations)

Tab. 2. Wpływ zastosowanych środków na rozkład sklerocjów S. slerotiorum pochodzących z sezonu 2016 (5 tydzień obserwacji)

Variant	Dose per dish	Substrate	
		tissue paper	technical sand
Control	-	14.5 b	20.3 c
EM Naturalnie Aktywny	2 ml	95.2 a	63.8 b
EM Naturalnie Aktywny	20 ml	60.9 a	100 a
Contans WG	4 g	100 a	100 a
EM Bio	2 ml	100 a	66.7 b
EM Bio	20 ml	65.2 a	100 a

Source: own work / Źródło: opracowanie własne

Table 3. The influence of the products used in the experiment on the decomposition of *S. sclerotiorum* sclerotia collected in 2017 (the fifth week of observations)

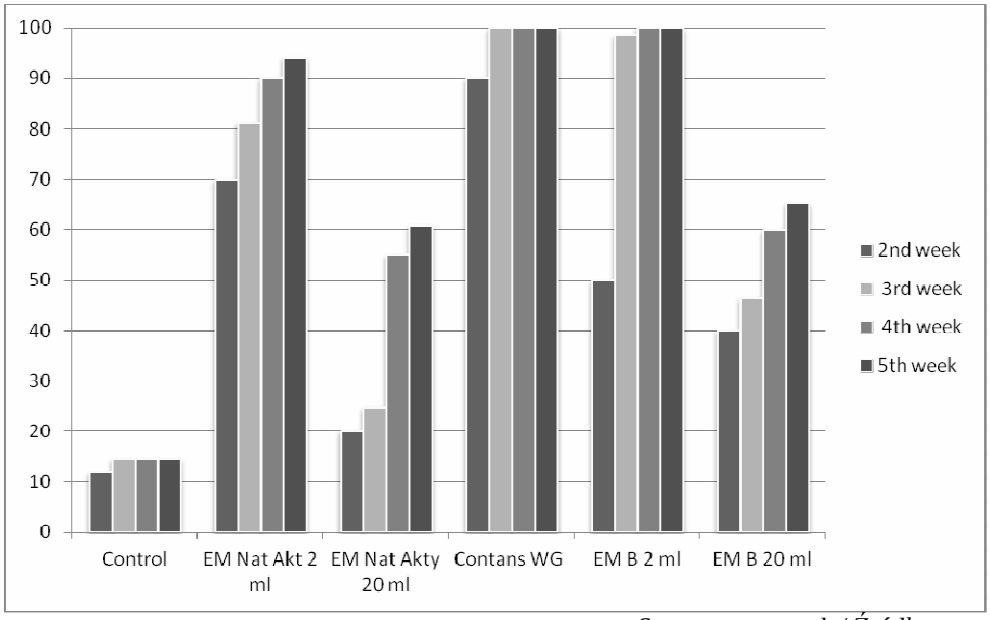
Tab. 3. Wpływ zastosowanych środków na rozkład sklerocjów S. sclerotiorum pochodzących z sezonu 2017 (5 tydzień obserwacji)

Variant	Dose per dish	Substrate	
		tissue paper	technical sand
Control	-	36 b	19.5 c
EM Naturalnie Aktywny	2 ml	65.2 a	62.1 b
EM Naturalnie Aktywny	20 ml	95.7 a	100 a
Contans WG	4 g	100 a	100 a
EM Bio	2 ml	91.3 a	65.2 b
EM Bio	20 ml	100 a	100 a

Source: own work / Źródło: opracowanie własne

The *Pseudomonas fluorescens* P13 strain, which was isolated from soil under rapeseed plants, also effectively inhibited the development of *S. sclerotiorum*. Morphological, physiological and biochemical tests as well as 16S rDNA analysis showed that this strain had a wide antagonistic spectrum. It reduced the growth of *S. sclerotiorum* mycelium by 84.4% and inhibited the formation of spores by 95-100% [4]. Scientific studies also confirmed that *Bacillus subtilis* effectively limited the development of *S. sclerotiorum* in soy [19].

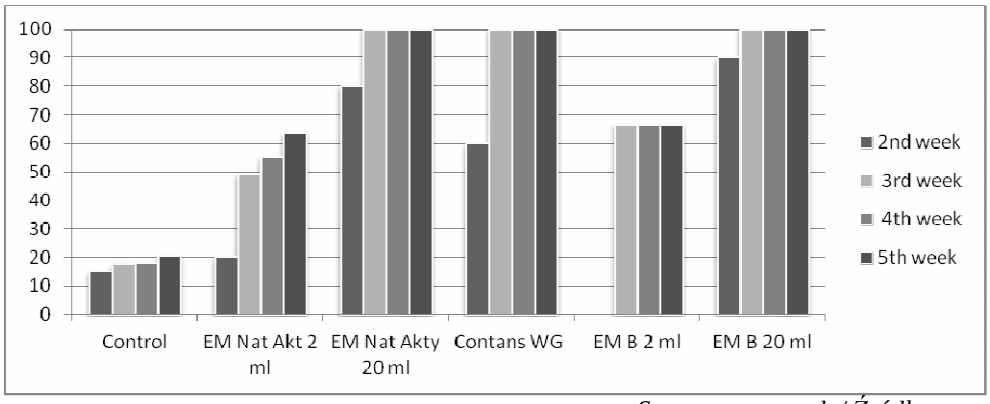
We also analysed the rate of decomposition of spore structures on individual substrates (Fig. 1-4). After only two weeks Contans WG completely destroyed the sclerotia collected in 2016 and 2017 both on the substrate with tissue paper and sand. EM Naturalnie Aktywny and EM Bio at a dose of 20 ml also completely decomposed the sclerotia but only in the variants with the sand substrate (Figs. 2 and 4). In the other variants the process was gradual and it was not always complete. In the other variants where the sclerotia were destroyed completely the decomposition took place during the fifth week of observations.



Source: own work / Źródło: opracowanie własne

Fig. 1. The rate of decomposition of *S. sclerotiorum* sclerotia [%] collected in 2016 during 4 weeks of observation of the tissue paper substrate

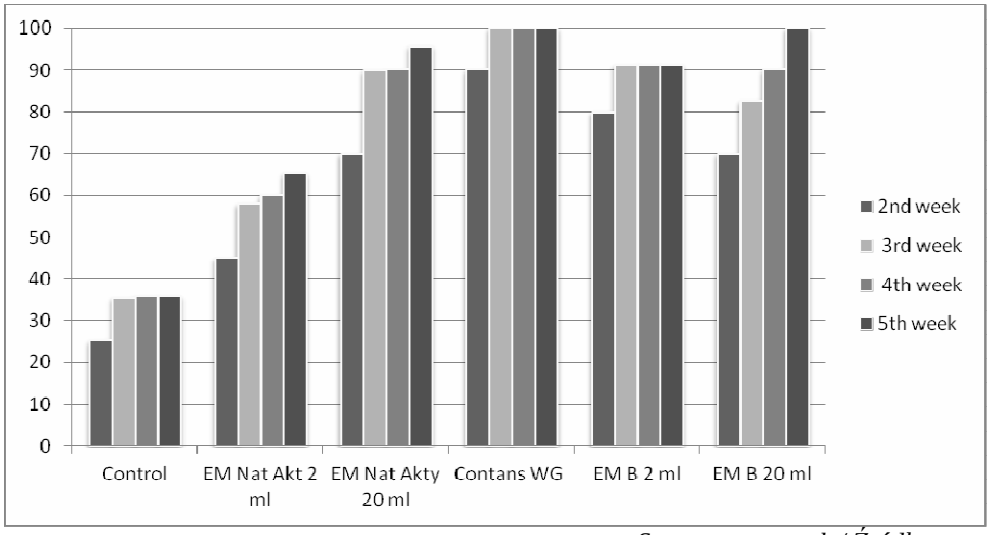
Rys. 1. Tempo rozkładu sklerocjów S. sclerotiorum [%] pochodzących z sezonu 2016 w czasie 4 tygodni obserwacji na podłożu z bibułą



Source: own work / Źródło: opracowanie własne

Fig. 2. The rate of decomposition of *S. sclerotiorum* sclerotia [%] collected in 2016 during 4 weeks of observation of the sand substrate

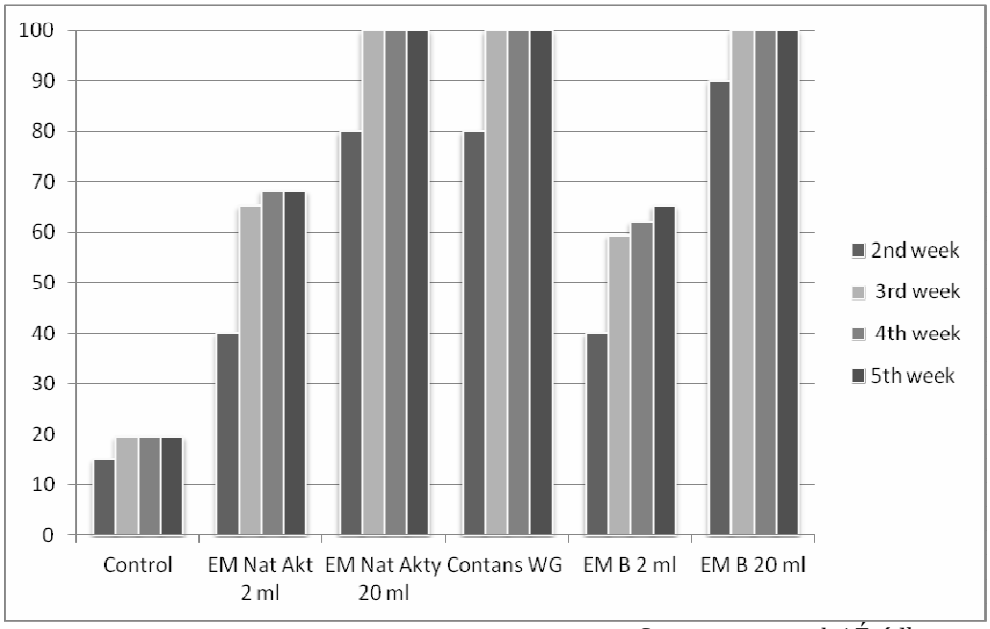
Rys. 2. Tempo rozkładu sklerocjów S. sclerotiorum [%] pochodzących z sezonu 2016 w czasie 4 tygodni obserwacji na podłożu z piaskiem



Source: own work / Źródło: opracowanie własne

Fig. 3. The rate of decomposition of *S. sclerotiorum* sclerotia [%] collected in 2017 during 4 weeks of observation of the tissue paper substrate

Rys. 3. Tempo rozkładu sklerocjów S. sclerotiorum [%] pochodzących z sezonu 2017 w czasie 4 tygodni obserwacji na podłożu z bibułą



Source: own work / Źródło: opracowanie własne

Fig. 4. The rate of decomposition of *S. sclerotiorum* sclerotia [%] collected in 2017 during 4 weeks of observation of the sand substrate

Rys. 4. Tempo rozkładu sklerocjów S. sclerotiorum [%] pochodzących z sezonu 2017 w czasie 4 tygodni obserwacji na podłożu z piaskiem

The research findings apply to the laboratory environment and the experiment needs to be tested in the natural environment. The products used in the experiments may effectively improve the phytosanitary status of soil by destroying the spores of *S. sclerotiorum*, which is an economically important pathogen. It particularly concerns soils in fields, where rapeseed and other plants hosting sclerotinia stem rot pathogens are grown. Soil is often heavily infected by *S. sclerotiorum* sclerotia [5]. The effectiveness of biological agents could be significantly modified in the natural environment. It depends on climatic conditions, soil structure, the presence of other microorganisms and the degree of soil pollution [15].

4. Conclusions

1. The biological products used in the experiment very effectively decomposed *S. sclerotiorum* sclerotia in the laboratory environment.
2. The product containing hyperparasitic *Coniothyrium minitans* fungi completely destroyed the spore forms of the pathogen.
3. The results of the experiment assessing the usefulness of preparations containing effective microorganisms for limiting the content of sclerotia in soil need to be confirmed in the natural environment.

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