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OCCURRENCE OF ENTOMOPATHOGENIC FUNGI IN AGRICULTURAL AND NATURAL SOILS IN SOUTH-EASTERN POLAND

Summary

Soil samples from 76 sites in south-eastern Poland were collected and the presence of entomopathogenic fungi (EPF) was evaluated by the bait larvae method. EPF were detected on the average in 43.5% of samples. The soil usage method (forest, agricultural crop, garden, seminatural habitat), the date of sampling (spring, summer, autumn) and physico-chemical properties of the soil significantly differentiated the total mortality of larvae and the occurrence of EPF and their species composition. Soils of seminatural habitats and agricultural crops had significantly higher level of total mortality of larvae compared to the soils of gardens and forests. The total mortality of larvae in the soil was the highest at autumn. The share of EPF in soils due to the usage methods can be determined in a decreasing sequence: garden, agricultural crops, seminatural habitats, forest. In the soils of agricultural crops I. fumosorosea dominated and in the soils of gardens, forests and seminatural habitats B. bassiana. The share of M. anispoliae sensu lato in soils was rather similar. In the samples obtained in the summer, EPF dominate, and in the samples collected in spring and autumn, other factors dominated mortality of larvae. The species composition of EPF in the soil collected in spring and summer was similar. In the samples taken in autumn, B. bassiana species dominated.
***Key words:** entomopathogenic fungi, soil, usage method*

WYSTĘPOWANIE GRZYBÓW OWADOBÓJCZYCH W GLEBACH UŻYTKOWANYCH ROLNICZO I NATURALNYCH POLSKI POŁUDNIOWO-WSCHODNIEJ

Streszczenie

Pobrano próbki gleby z 76 stanowisk w Polsce południowo-wschodniej i oceniono w nich obecność grzybów entomopatogennych (EPF) z zastosowaniem metody owadów pułapkowych. EPF były wykrywane średnio w 43,5% próbek. Sposób użytkowania gleby (las, uprawa rolnicza, ogród, siedlisko seminaturalne), data pobrania (wiosna, lato, jesień) i właściwości fizykochemiczne gleby znacznie różnicowały ogólną śmiertelność larw oraz występowanie EPF i ich skład gatunkowy. Gleby siedlisk seminaturalnych i upraw rolniczych charakteryzował znacznie większy poziom śmiertelności larw ogółem w porównaniu z glebami ogrodów i lasów. Ogólna śmiertelność larw w glebie była najwyższa jesienią. Udział EPF ze względu na sposób użytkowania gleby można określić w malejącym ciągu: ogród, uprawy rolnicze, siedliska przyrodnicze, lasy. W glebach upraw rolniczych dominował gatunek I. fumosorosea, w glebach ogrodów, lasów i siedlisk przyrodniczych B. bassiana. Udział M. anispoliae sensu lato w glebach był wyrównany. W próbkach pobieranych latem dominowały EPF, a w próbkach zebranych wiosną i jesienią inne czynniki determinowały śmiertelność larw. Skład gatunkowy EPF w glebie zebranej wiosną i latem był zbliżony. W próbkach pobranych jesienią dominował gatunek B. bassiana.
***Słowa kluczowe**: grzyby entomopatogenne, gleba, sposób użytkowania*

1. Introduction

The interventional use of biopesticides and the protection of organisms reducing pests in the environment are the two most important strategies for biological control. The Polish biopesticides market authorized for use in organic farming is very poor. Currently, 11 biopesticides are registered on the basis of viruses or microorganisms. Five of them are fungal-based protection agents, but only one (Naturalis) is recommended for pest control [1]. Therefore, recognition and protection components of ecosystems that can reduce pest populations is very important. Entomopatphogenic fungi (EPF) are such environment component who reduces pests. Apart lethal effect infection of pests, it should be emphasized that these fungi are also effective endophytes, phytopathogens antagonists and can live in the rhizosphere, promoting the growth of plants [2, 3, 4, 5, 6]. Occurrence of EPF in soil, special in organic farming is very desirable.

Globally, fungi of the genus *Beauveria* or *Isaria* (Cordycipitaceae) and *Metarhizium* (Clavicipitaceae) are a common component of soil microbiota. The density of diasporas of these fungi in the soil may exceed 1000 $CFU \cdot g^{-1}$ [7, 8]. Extensive research carried out by Medo and Cagan [9] in Slovakia showed the presence of EPF in many case of soil samples and the dominance of *Metarhizium anisopliae* (present in 37% of trials), *Beauveria bassiana* (36%) and *Isaria fumosorosea* (9%) species. Similar results were obtained from research carried out in north-eastern Poland [10].

Phases of arthropod infections by EPF are: (1) pathogenic - conidial spore germinates through the cuticles to hemocel breaking the defensive immune response, followed by passive development of hyphae or blastospores in the host's haemolymph and (2) saprophytic – after hosts death, fungi start abundant conidial sporulation. During the infection, EPF produce a number of metabolites, including secondary metabolites, which are a specific feature of a given species [11]. A lot of hydrophobic, dry, small size (2-8 μm), spherical or ovoid conidia are widely propagated in the environment, sometimes passively by other organisms on the phoresy way and these conidia are another factor limiting the number of many pests [12, 13].

The first step for the protection of beneficial organisms is to recognize their prevalence and habitat preferences. Studies on the occurrence of EPF populations in soils differing in the usage can help in their appropriate selection for agricultural or forestry applications. Also, knowledge of the species composition of EPF in different regions can be useful in assessing the potential of them to regulate the populations of important crop pests.

The aim of the performed study was to compare the species composition and the intensity of EPF occurrence in soils depending on usage method (field, forest, garden, seminatural habitats) in the south-eastern Polish voivodeships. The influence of the date of soil sampling and some physico-chemical proprieties of soils on the analyzed features was also compared.

2. Methodology

Soil samples from 76 places were collected in spring, summer and autumn. Research points were located in the south-eastern region of Poland - 50 samples from the Małopolska voivodeships and the communes: Lipnica Wielka, Skrzyszów, Słomniki, Szaflary, Trzebinia, Wielka Wieś and Zabierzów, and 24 samples from the Podkarpackie voivodeships and the communes of Baligród, Czarna and Pilzno. A uniform number of samples (19) for a given way of usage was preserved, and it was a forest, agricultural crop, garden and seminatural habitats.

The samples were taken using a shovel, to a depth of up to 20-30 cm, from soils at the temperature above 7-8°C. The mixed samples (from 5 place about 1 kg weight) were taken to neutral, plastic bags and stored up to 1 month in laboratory at the temperature of 8°C. Directly before the experiment soils samples were sieved to separate the larger particles of contamination, and dried up to a moistures level content of approximately 20–30% (optimum for fungal development). EPF were isolated from soils samples by the "Galleria bait method" [14, 15] in modification for fungi and insects species to mealworm beetle (*Tenebrio molitor* L.) larva. For each experimental soil sample, ten plastic containers of 100 ml capacity were filled with three rings of moist filter paper and soils were placed up to 2/3 of the container. Ten *T. molitor* larvae were put into each container, and then the containers were placed in an incubator in the temperature of 21C and darkness. The first mortality control was conducted after 3 days, and then at the three-days intervals until the death of all larvae. Dead larvae with symptoms of fungal infection (mycosis) were macroscopic recognized via colorization of sporulating mycelium and confirmed via identification both by low magnifying stereomicroscope (40 x magnification) of cadavers and by preparing slides for light microscopy (400 x magnification) referring to the identification key [16].

Basic analysis of physico-chemical properties of the soil was commissioned for realization in accordance with the applicable standards to the Regional Chemical-Agricultural Station in Krakow. In the case of N, P, K and Mg content, its level was estimated (low, medium, high) on the basis of soil granulometric composition.

The results were developed statistically in the Statistica 10 software using analysis of variance (ANOVA) and Duncan's test, with a significance threshold at $\alpha=0.05$.

3. Results and discussion

The bait larvae died on soil trials in various ways and this depended on the soil usage (Fig. 1) and the date of soil sampling (Fig. 2). The study found that the soil collected in agricultural crops and seminatural habitats was characterized by a higher mortality of larvae compared to soils from forests and gardens. Most larvae died on the soil collected in the autumn, significantly less in the soil collected in the summer, and the least in the soil collected in the spring.

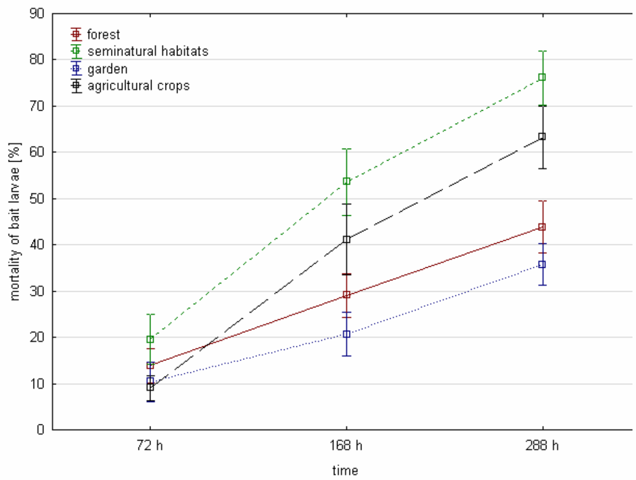

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Fig. 1. Effect of the soil usage methods on the occurrence of entomopathogenic microorganisms in total, expressed by mortality of bait larvae during the test (figure bars indicate standard errors)

Rys. 1. Wpływ sposobu użytkowania gleby na występowanie organizmów entomopatogenicznych ogółem wyrażony śmiertelnością larw pułapkowych w czasie testu (słupki wykresów wskazują błędy standardowe)

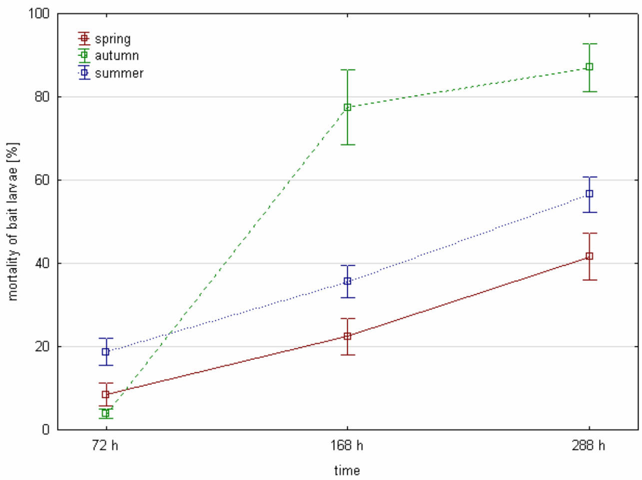

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Fig. 2. Effect of the date of soil sampling on the occurrence of entomopathogenic microorganisms in total, expressed by mortality of bait larvae during the test (figure bars indicate standard errors)

Rys. 2. Wpływ terminu poboru próby glebowej na występowanie organizmów entomopatogenicznych ogółem wyrażony śmiertelnością larw pułapkowych w czasie testu (słupki wykresów wskazują błędy standardowe)

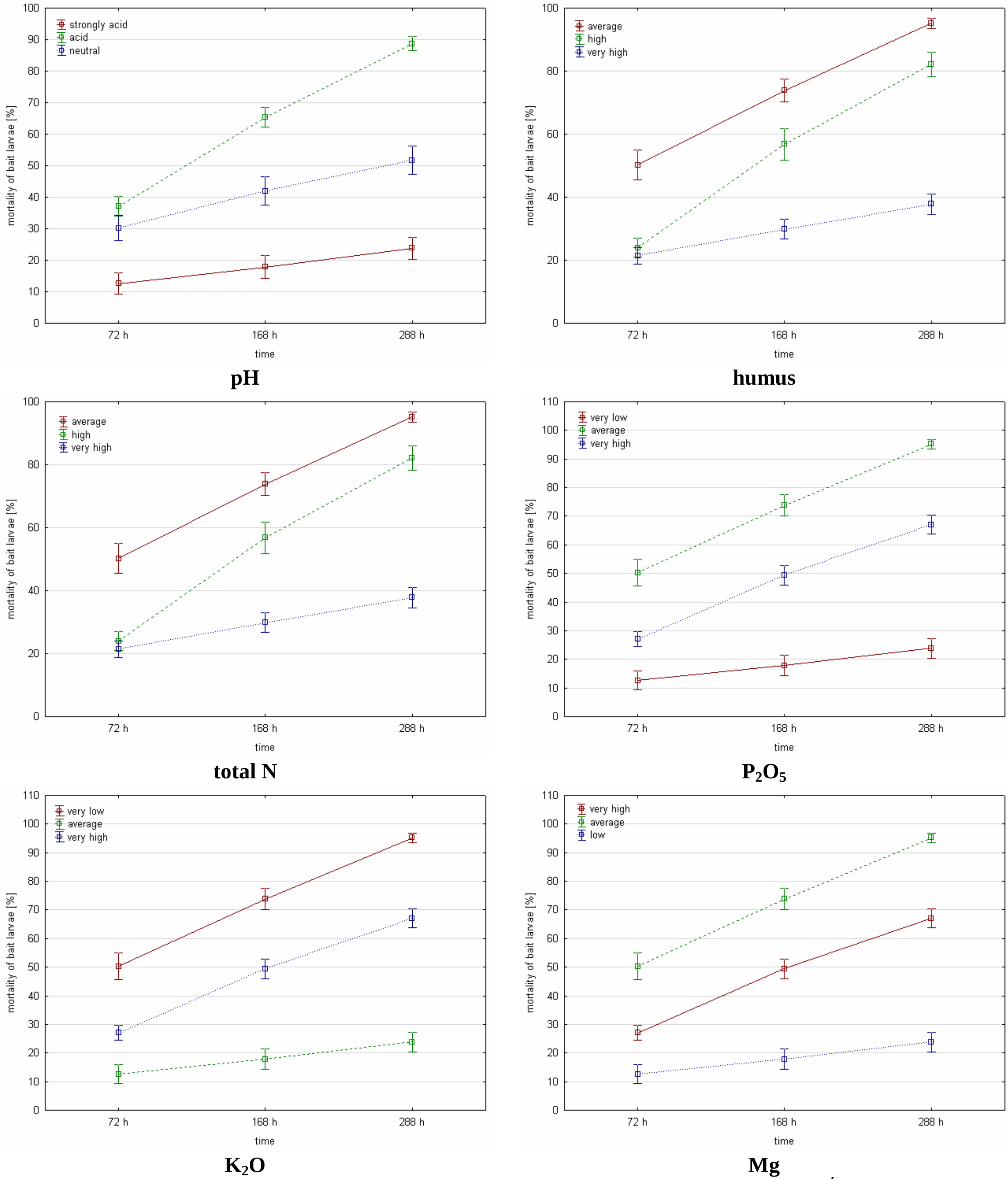

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Fig. 3. Effect of some physico-chemical properties of the soil on the occurrence of entomopathogenic microorganisms in total expressed by mortality of bait larvae during the test (figure bars indicate standard errors)

Rys. 3. Wpływ wybranych właściwości fizyko-chemicznych gleby na występowanie organizmów entomopatogenicznych ogółem wyrażony śmiertelnością larw pułapkowych w czasie testu (słupki wykresów wskazują błędy standardowe)

All assessed physico-chemical properties of the soil differentiated the occurrence of EM. Preference was given to acidic reaction, average humus, nitrogen and magnesium content as well as low levels of phosphorus and potassium (Fig. 3).

In relation to the diagnosed pool of EPF, their highest content was found in soils taken from gardens, where they accounted for 52% of EM in total. Soils of agricultural crops and seminatural habitats were characterized by a similar abundance in EPF, and their lowest share was recorded in soils collected in forests (Fig. 4). The composition of species marked with EPF was similar in agricultural soils, forests and soils of seminatural habitats. In the case of soils from gardens, *B. bassiana* was the dominant species.

EPF predominated in soils collected in the summer and accounted for 55% of total EM. In this case, the most mycoses of the bait larvae caused the *B. bassiana* species. In the soils collected in spring, the EPF accounted for slightly more than 1/3 of EM, and the species distribution was balanced. In the soils sampled in autumn, the EPF pool was significantly the smallest with respect to all terms (only 17% of total EM) and, similarly to the soils collected in the summer, the *B. bassiana* species was slightly dominant (Fig. 5).

In a extensive bait insect method study conducted in Slovakia by Medo and Cagan [9], a very similar (average 47.7%) as recorded in our own research (43.5% on average) share of EPF occurring in cultivated and natural soil was found. The *B. bassiana* was also dominant species and the *M. anisopliae* slightly more pronounced. These studies also indicated the preference of EPF to the acidic and neutral soil pH. Similarly, studies by Tkaczuk et al. [10] conducted in organic and conventional farms in the Lubelskie Voivodeship showed the widespread occurrence of EPF and the dominance of the *M. anisopliae* species. Tkaczuk et al. [10] showed that EPF were found in a larger quantity in the soils of organic farms, and this is particularly true for *B. bassiana* and *M. anisopliae* species. For the *I. fumosorosea* species, there was a greater tolerance to chemical means of production and this was confirmed by own research, because this species was isolated in the largest share from agricultural soils.

Studies showing the presence of EM including EPF in such a large number should be disseminated to a greater extent. This will contribute to the increase of awareness of the natural resistance of the environment to pests, especially those that are found permanently or at some stages of their development in soils.

4. Conclusions

1. The soil usage method, the date of sampling and the assessed physico-chemical properties of the soil significantly differentiate the mortality of bait insects and occurrence of fungi and their species composition.
2. Soils of seminatural habitats and agricultural crops are characterized by a significantly higher number of factors causing insects mortality compared to the soils of gardens and forests.
3. The amount and activity of factors causing insects mortality in the soil is the highest in autumn.
4. The share of fungi in the pool of soils factors causing insects mortality due to the usage methods can be determined in a decreasing sequence: garden> agricultural farming = wasteland> forest.
5. In the soils of agricultural crops, *I. fumosorosea* dominates, and in the soils of gardens, forests and seminatural habitats - *B. bassiana*. The share of *M. anispoliae sensu lato* in soils used in various ways is similar.
6. In the samples taken in the summer, the fungi dominate, and in the samples collected in spring and autumn, other factors causing insects mortality dominate.
7. The species composition of fungi in the soil collected in spring and summer is equal and similar. In the samples taken in autumn, *B. bassiana* dominates.

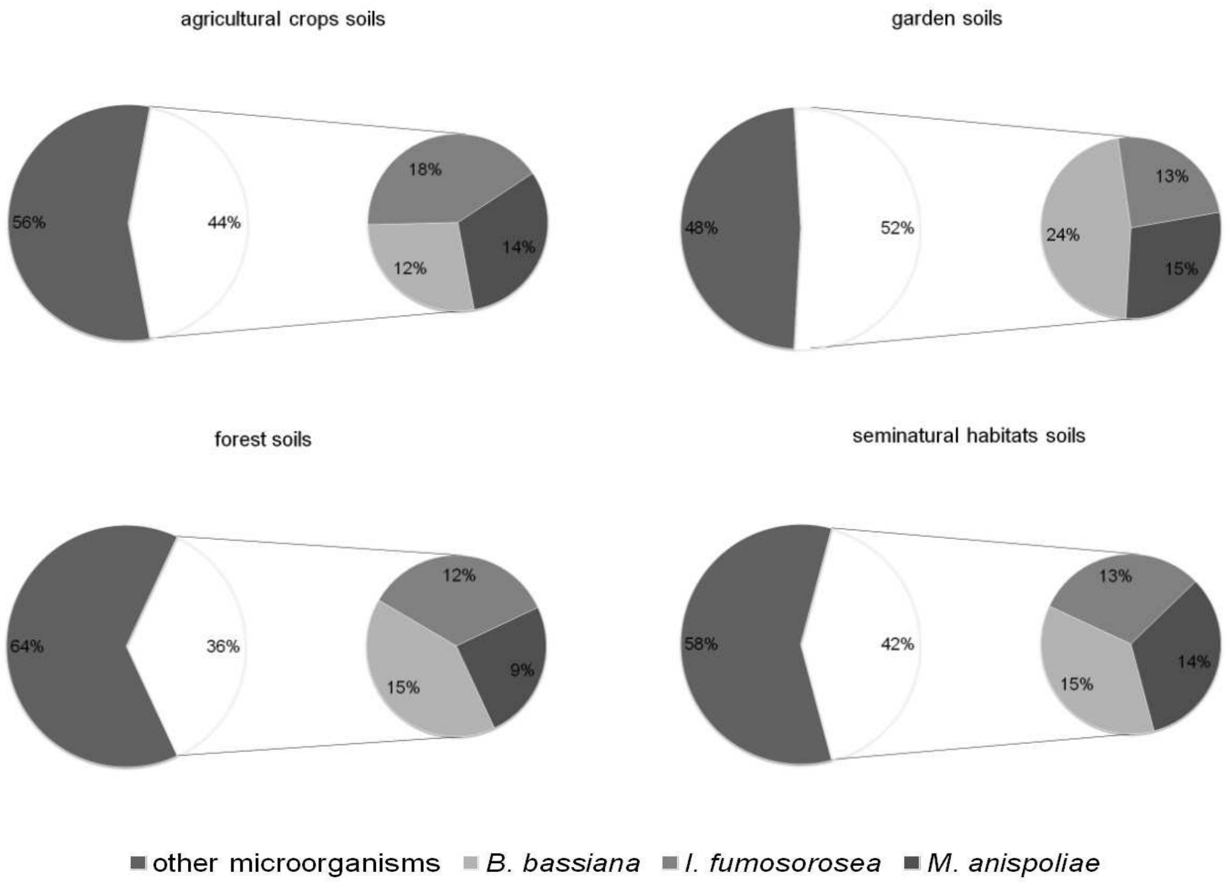

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Fig. 4. Effect of the soil usage methods on the quantity and species composition of entomopathogenic fungi isolated from the soil using the trap insect method

Rys. 4. Wpływ sposobu użytkowania gleby na ilość i skład gatunkowy grzybów entomopatogenicznych izolowanych z gleby metodą owadów pułapkowych

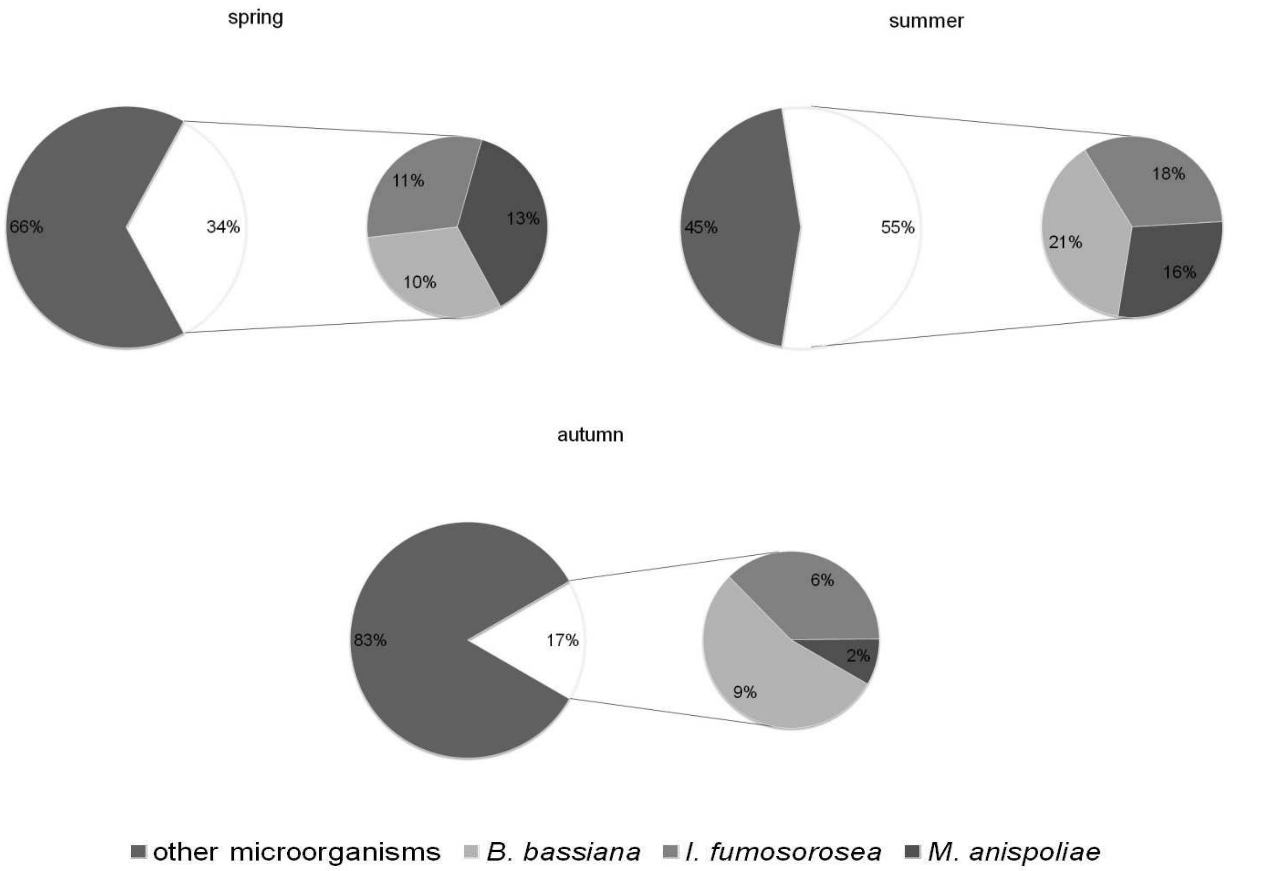

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Fig. 5. Effect of the date of soil sampling on the quantity and species composition of entomopathogenic fungi isolated from the soil using the trap insect method

Rys. 5. Wpływ terminu poboru próby glebowej na ilość i skład gatunkowy grzybów entomopatogenicznych izolowanych z gleby metodą owadów pułapkowych

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