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Effect of preparations containing humic substances and pure humic acids on colony growth and spore germination of entomopathogenic fungi from the *Beauveria* genus

Wpływ preparatów zawierających substancje humusowe i czystych kwasów humusowych na wzrost i kiełkowanie zarodników grzybów entomopatogenicznych z rodzaju Beauveria

Abstract. The experiment examined the effect of commercially available preparations containing humic substances in comparison with pure humic acids on the growth and germination of spores of entomopathogenic fungi (EPF) of the genus *Beauveria in vitro*. AmiAGRA, HumiAGRA, Algo-HUM (recommended field dose) and pure humic acids extracted from peat, brown coal and mushroom substrate were added to Sabouraud's culture substrate. Observation of the growth of colonies of the tested of EPF was carried out every 5 days until day 20, measuring their diameter (mm). In the second stage of the experiment, the culture medium with the addition of preparations and pure humic acids was applied in a thin layer to the surface of glass slides and 0.1 ml of an aqueous solution with spores was introduced. Observation was carried out after 24 h and 48 h, and the results obtained were expressed as percentages of the control. The conducted research showed that on the 20th day of culture (on average), preparations containing humic substances had a stimulating effect, while pure acids slightly limited the growth of colonies of the tested species. The growth of the colony of the fungus *B. basianna* was most strongly stimulated by AmiAGRA, and *B. brongniartii* by HumiAGRA. The share of germinated spores of the tested species after 48 h of contact with the substrate was higher than after 24 h. It was found that more spores germinated on substrates with the addition of preparations containing humic substances than on pure humic acids.

Keywords: humic substances, humic acids, fungi of the genus *Beauveria*, colony growth, spore germination

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INTRODUCTION

The soil environment is inhabited by such living organisms as bacteria, fungi, algae, actinomycetes, earthworms, nematodes and arthropods. They stimulate plant growth and development by decomposing plant material and other organic residues [Stevenson 1972, Vikram et al. 2022], increasing water capacity of the soil and improving its structure, and they even contribute to the neutralization of soil contaminants [Canellas et al. 2015, Rutkowska 2016, Li 2020, Nardi et al. 2021, Majchrowska-Safaryan and Tkaczuk 2023]. In agriculture, products containing humic substances are increasingly used. They are active in processes that decompose organic material and release their nutrients so that they become available to plants faster [http://rosahumus.pl/wyniki-badan/2021]. Humic substances can be extracted from lignite or peat and are most often used on lighter soils on which organic fertilizers are not applied or are difficult to decompose because of a lack of water [Gałązka and Kocoń 2015, Pikuła 2016]. In turn, humic acids can be used as an adjuvant to increase the effectiveness of biocontrol agents [Holka and Kowalska 2023] and as carriers for microorganisms [da Silva et al. 2021]. In the literature they are considered to be biocompatible and environmentally friendly [Lian et al. 2020].

Among the microorganisms inhabiting the soil, entomopathogenic fungi (EPF) from the *Beauveria, Metarhizium* or *Cordyceps* genera represent a considerable group, and they all can cause natural mycosis of soil arthropods [Roy and Cottrell 2008]. According to the literature, soil arthropod pests are responsible for about 40% of crop losses on a global scale [Popp et al. 2013, Dehaliwal et al. 2015, Mantzoukas et al. 2020, FAO 2021,Verma et al. 2021]. To minimize these losses insecticides are widely used [Sharma et al. 2019, Kalogiannidis et al. 2022], which has contributed to increasing resistance of about 500 pest species to one or more active substances [Kumar and Kalita 2017]. Furthermore, insecticides have a negative impact on human health and the environment [Geiger et al. 2010, Żak 2016, Sharma et al. 2019]. Since 2014, the European Union (EU) has made it mandatory to comply with the principles of Integrated Pest Management (IPM) contained in Directive 2009/128/EC [European Commission 2009]. According to the IPM guidelines, the use of chemical plant protection products should be kept to an absolute minimum, with priority to non-chemical methods [Jamiołkowska et al. 2017]. Therefore, the application of biological plant protection agents, including EPF, instead of chemical pesticides is an alternative method of reducing pest populations effectively [Fenibo et al. 2021, Ayilara i in. 2023]. Tanzini et al. [2001] used the term entomopathogenesis referring to the activity of microorganisms that regulate insect pest populations to a level at which no serious crop damage is observed. Entomopathogenic fungi are effective in their action because of their easy distribution and easy manufacturing techniques, availability of a large number of already identified strains and over-expression of exogenous toxins and endogenous proteins [St. Leger and Wang 2010]. Unlike chemical pesticides, they do not have a negative impact on human health and the environment. At first, fungal spores spread outside the host's body, and then they moved inside it. As a result, the insect dies within 4–7 days (depending on the number of spores). Then, the dead insect is a source of new spores, which spread further and the life cycle of EPF continues on a new host [Tkaczuk 2008]. In Poland, an increasing interest in microbial insecticides is observed, which is related to the reduction of the number of active substances approved for use in the EU [European Commission 2023] and to the possibility of receiving direct payments, according to the

Biological Pest Control program introduced in 2023, if biological plant protection products are used [Runge et al. 2022]. Currently, microbiological preparations containing, among others, EPFs, constitute only 2% of the entire market of plant protection products in Poland [Ministry of Agriculture and Rural Development 2023].

The aim of the *in vitro* studies was to determine the effect of preparations containing humic substances and pure humic acids on colony growth and spore germination of EPF from the *Beauveria* genus.

MATERIALS AND METHODS

The effect of preparations containing humic substances and pure humic acids on EPF colony growth and on spore germination was investigated in laboratory conditions. Two selected species of the *Beauveria* genus were used in the research: the fungus *B. bassiana* (Bals.-Criv.) Vuill. (B03-UPH) isolated from meadow soil collected in Klimonty (Mazowieckie Voivodeship, Poland), using a selective medium and *B. brongniartii* (Sacc.) (B04-UPH) isolated from mid-field woodlots in Boćki (Podlaskie Voivodeship, Poland), using a selective medium. The cultures of the fungi used in this experiment were deposited in the fungal collection of the Institute of Agriculture and Horticulture, The University of Siedlce, Poland, and stored on SDA medium at 4°C. They were identified macroscopically using standard keys [Samson 1974, Humber 2012], and their systematic affiliation was also confirmed by molecular identification. The ITS marker, proposed as a universal DNA code marker for fungi, was chosen for identification [Schoch et al. 2012]. Molecular identification of species was carried out in the mycological laboratory of the Biological and Chemical Research Centre of the University of Warsaw, using Qiagen and Blirt tools (DNA isolation kits, PCR kit and cleaning kit). The PCR reaction was carried out according to the procedure provided by Kovač et al. [2020]. Sanger sequencing was used with single ITS2, ITS3, ITS4 and ITS5 primers and the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) containing fluorescently labeled dideoxynucleotide triphosphates (ddNTPs), deoxynucleotide triphosphates (dNTPs), Taq FS polymerase and buffer. After sequencing, product cleaning was done by molecular filtration on columns with Sephadex G-50, and the reading of the result was entrusted to Genomed (Warsaw). The sequences were compared using the BLASTN 2.2.2 algorithm [Altschul et al. 1997], while a collection of sequences was available in the NCBI databases.

Preparations containing humic substances such as: Ami AGRA, HumiAGRA and Algo HUM (recommended field dose) and humic acids extracted from peat, lignite and spent mushroom substrate (SMS) were used in the experiment. Based on the content of humic substances in the AmiAGRA preparation, the dose of pure humic acids was calculated. The characteristics of the preparations and pure humic acids used are presented in Table 1. Humic acid preparations were obtained according to the Shnitzer method. Extraction of humic acids was performed with 0.1 M NaOH. Humic acids were deashed (purified) using a solution of HF and HCl. Purified humic acids were dried in an exiccator [Becher et al. 2021, 2022].

In the experiment, Sabouraud Dextrose Agar (SDA) produced by bioMérieux was used as culture medium with casein enzymatic hydrolyzate – 5.0 g, hydrolyzed animal tissues – 5.0 g, glucose – 40 g and agar – 15.0 g. They were all sterilized using a steam-

pressure autoclave at 121°C under a pressure of 1 atmosphere. Preparations containing humic substances and pure humic acids were added to the culture medium prepared in this way. Then, they were transferred to sterile plastic Petri dishes with a diameter of 90 mm.

Table 1. Characteristics of preparations containing humic substances and pure humic acids used in the experiment

Preparation	Chemical composition	Dose – farm crops
AmiAGRA Agraplant	pH – 7.0–9.0, humic substances – 60%, amino acids – 70%, nitrogen (N) – 6%	soil application: 0.5–1 kg ha^{-1} in 250–300 dm^3 of water; foliar aplplication: 100 g ha^{-1} in 250–300 dm^3 of water the preparation was added to the culture medium at a dose of 2.0 g in 1 dm^3
HumiAGRA Agraplant	pH – 8.0–10.0 humic substances – 60%, potassium oxide (K_2O) – 8%	soil application: 0.5–1 kg ha^{-1} in 250–300 dm^3 of water; foliar application: 100 g ha^{-1} in 250–300 dm^3 of water the preparation was added to the culture medium at a dose of 2.0 g in 1 dm^3
AlgoHUM Agraplant	pH – 7.8–9.8, humic substances – 28% including 50% humic acids, sea algae – 50%	100 g ha^{-1} in 250–300 dm^3 of water the preparation was added to the culture medium at a dose of 0.4 g in 1 dm^3
Peat	humic acids extracted from low peat – Liwiec river valley, pH – 4.5–5.0	1.2 g in 1dm^3 of water (culture medium)
Lignite	humic acids extracted from lignite – the Bełchatów mine, pH – 4.5–5.0	1.2 g in 1 dm^3 of water (culture medium)
SMS	humic acids extracted from the spent mushroom substrate – large–area mushroom farms, Siedlce district, pH – 4.5–5.3	1.2 g in 1 dm^3 of water (culture medium)

*SMS – spent mushroom substrate

In the first part of the experiment, selected fungal isolates were grown on SDA medium at 21°C ±1°C. A fragment of mycelium from 10-day-old cultures was sampled with a preparation needle, to be inoculated centrally to SDA solid medium. The plates with the inoculated species were placed in incubators, protected from light at 22°C ±1°C. Colony growth observations were made every 5 days until day 20 by measuring colony diameter in mm. The experiment was performed in four repetitions. The control consisted of cultures growing on substrates without the addition of biopreparations and pure humic acids. The results are presented as the size of the colony diameter expressed in mm and in the case of the 20th day of culture as a percentage in relation to the control.

In the second part of the experiment, the effect of preparations containing humic substances and pure humic acids on the germination of spores of the fungal species was investigated. Spores from three-week-old colonies were transferred with a scalpel to an aqueous solution. Under the microscope at 400× magnification, the titer (concentration) of spores in the solution was determined using the Fuchs-Rosenthal chamber. The spore solution was diluted to 1.0×10^7 conidia/mL., which made it easier to observe the germinating spores. The number of spores in the field of view did not exceed 20–30. Approximately 1 ml of spore solution was pipetted onto Sabouraud medium. Slides with medium and spores were placed in incubators with the above temperature ranges. Germination observations were carried out after 24 and 48 hours. A drop of lactophenol was added to the medium with spores, and it was covered with a cover glass. Then, in the field of view the number of germinating spores per 100 observed conidia was counted. For each combination, i.e. EPF isolate – preparation, three replications were performed.

The obtained results were statistically processed using the Statistica program v. 13.3 (TIBCO Software Inc, Palo Alto, CA, USA). The first stage of the analysis was to check with the chi-square λ^2 test-whether the distribution of the tested trait (colony growth, spore germination) in the sample followed a normal distribution. Since the data did not have a normal distribution, the transformation $y = \log (x + 0.5)$ was applied. Then, on the transformed data, one-way ANOVA analysis was performed for each factor separately according to the following model:

$$y_{ij} = m + a_i + e_{ij}$$

where m is the population average; y_{ij} is the value of the examined trait (colony growth, spore germination); a_i is the effect of the ith level of factor A (fungal species, preparation, observation time); e_{ij} is the random error. When the factor's effect was significant, the Tukey test was used at $\alpha = 0.05$ to compare the means (posthoc analysis).

RESULTS

The research demonstrated that the preparations containing humic substances and pure humic acids extracted from peat, lignite and mushroom substrate had a various effect on colony growth and spore germination of EPF species of the *Beauveria* genus. It turned out that colony growth and spore germination were dependent on the fungal species, preparation, pure humic acid and the date of observation (Tabs 2–4).

When investigating the development of the *Beauveria bassiana* fungus, it was found during each observation that preparations containing humic substances added to the culture medium at the recommended dose had stimulated colony growth in relation to control (Tab. 2). A decrease on the medium with AlgoHUM was exceptional on day 20. On that day the fungus colony was slightly smaller (54.3 mm) than the control one, but this difference was not statistically significant. One of the preparations, AmiAGRA, had the strongest stimulating effect on *B. bassiana* fungus growth. During the next observations, colony diameters were larger than control, and the differences were statistically significant. The addition of pure humic acids to the culture medium at a dose corresponding to their content in AmiAGRA extracted from peat and lignite limited the growth of the species in relation to control on observation days 5, 15 and 20, but with a statistically significant difference only on day 15. Pure humic acids extracted from

spent mushroom substrate limited fungal growth in relation to control only on day 5. On the following observation days, the colonies were larger than control ones, and these differences were statistically significant.

Table 2. Colony diameters of the *Beauveria bassiana* during culture on media with the addition of preparations containing humic substances and pure humic acids

Preparation	Diameter of fungus colony (mm)			
	5th day	10th day	15th day	20th day
preparations containing humic substances				
AmiAGRA	18.2 ±0.95 [a]	31.0 ±0.81 [a]	44.5 ±1.29 [a]	60.7 ±2.21 [a]
HumiAGRA	16.0 ±0.50 [ab]	30.5 ±1.00 [a]	44.5 ±1.29 [a]	60.0 ±0.81 [a]
AlgoHUM	15.7 ±0.95 [b]	31.0 ±1.15 [a]	41.5 ±1.00 [ab]	54.3 ±2.87 [b]
pure humic acids				
Peat	12.7 ±0.95 [c]	26.5 ±0.58 [b]	36.7 ±0.96 [c]	53.5 ±1.29 [b]
Lignite	11.3 ±1.50 [c]	26.0 ±1.41 [b]	37.6 ±1.25 [c]	54.0 ±0.81 [b]
SMS	11.7 ±1.25 [c]	27.3 ±1.89 [b]	41.0 ±2.44 [ab]	61.0 ±0.82 [a]
Control	12.8 ±0.98 [c]	24.2 ±0.98 [c]	40.0 ±2.75 [b]	56.4 ±2.33 [b]
P – value	0.00	0.00	0.00	0.00
F – value	9158.37	9589.81	21647.08	33696.03

* SMS – spent mushroom substrate; abcd – means within columns with the same lowercase letters are not significant at α = 0.05, Tukey's HSD; ± standard deviation.

Table 3. Colony diameters of the *Beauveria brongniartii* during culture on media with the addition of preparations containing humic substances and pure humic acids

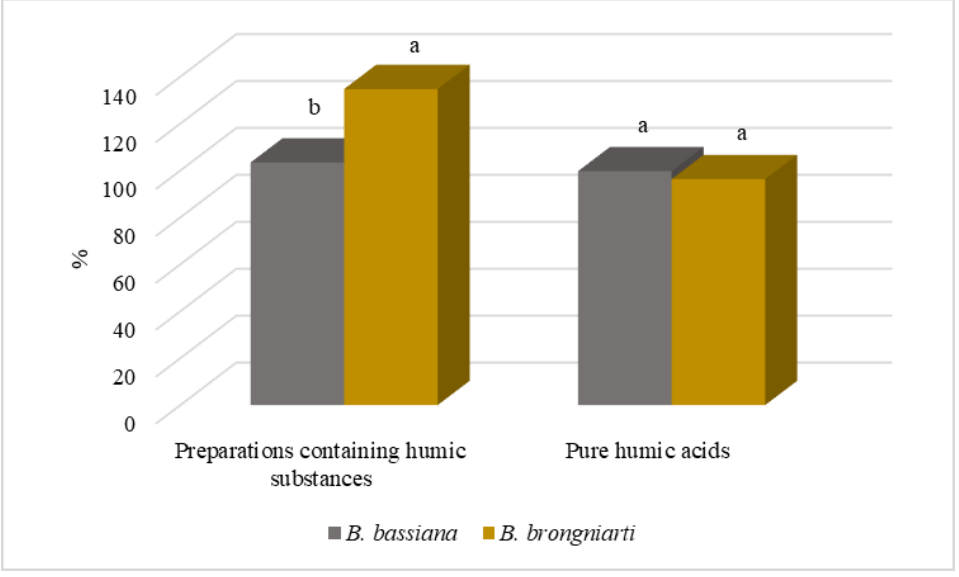
Preparation	Diameter of fungus colony (mm)			
	5th day	10th day	15th day	20th day
Preparations containing humic substances				
AmiAGRA	21.7 ±1.50 [a]	42.8 ±2.21 [a]	60.7 ±2.87 [a]	72.5 ±2.64 [a]
HumiAGRA	21.8 ±1.50 [a]	45.7 ±0.95 [a]	66.5 ±3.11 [a]	78.7 ±2.98 [a]
AlgoHUM	22.5 ±1.92 [a]	43.0 ±4.78 [a]	68.3 ±0.94 [a]	75.3 ±2.87 [a]
Pure humic acids				
Peat	17.7 ±1.29 [b]	31.0 ±1.25 [b]	43.2 ±1.73 [b]	53.5 ±1.09 [b]
Lignite	16.2 ±0.95 [b]	31.2 ±1.15 [b]	40.5 ±1.41 [b]	54.7 ±2.50 [b]
SMS	15.8 ±0.81 [b]	28.5 ±2.65 [b]	40.3 ±1.88 [b]	53.6 ±1.25 [b]
Control	17.8 ±1.16 [b]	32.3 ±3.66 [b]	43.8 ±3.54 [b]	56.0 ±2.45 [b]
P – value	0.00	0.00	0.00	0.00
F – value	20788.42	41719.01	48718.11	96370.16

* SMS – spent mushroom substrate; abcd – means within columns with the same lowercase letters are not significant at α = 0.05, Tukey's HSD; ± standard deviation

Table 4. Germination of *Beauveria bassiana* and *Beauveria brongniartii* spores on media with the addition of preparations containing humic substances and pure humic acids (in % in relation to the control)

Species/ Preparation	Entomopathogenic fungi			
	Beauveria bassiana		*Beauveria brongniarti*	
	after 24 h	after 48 h	after 24 h	after 48 h
preparations containing humic substances				
AmiAGRA	90.2 ^{b}	94.4 ^{a}	92.3 ^{b}	96.4 ^{a}
HumiAGRA	89.8 ^{b}	92.6 ^{ab}	94.7 ^{b}	98.3 ^{a}
AlgoHUM	78.9 ^{c}	89.2 ^{ab}	90.8 ^{b}	97.2 ^{a}
pure humic acids				
Peat	74.4 ^{cd}	78.2 ^{d}	66.6 ^{d}	70.2 ^{c}
Lignite	70.2 ^{d}	80.4 ^{c}	72.4 ^{c}	80.8 ^{b}
SMS	79.7 ^{c}	86.4 ^{c}	73.2 ^{c}	80.4 ^{b}
Control	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}
P – value	0.00	0.00	0.00	0.00
F – value	274953.40	157622.60	174724.70	561228.80

*SMS – spent mushroom substrate; abcd – means within columns with the same lowercase letters are not significant at α = 0.05, Tukey's HSD; ± standard deviation

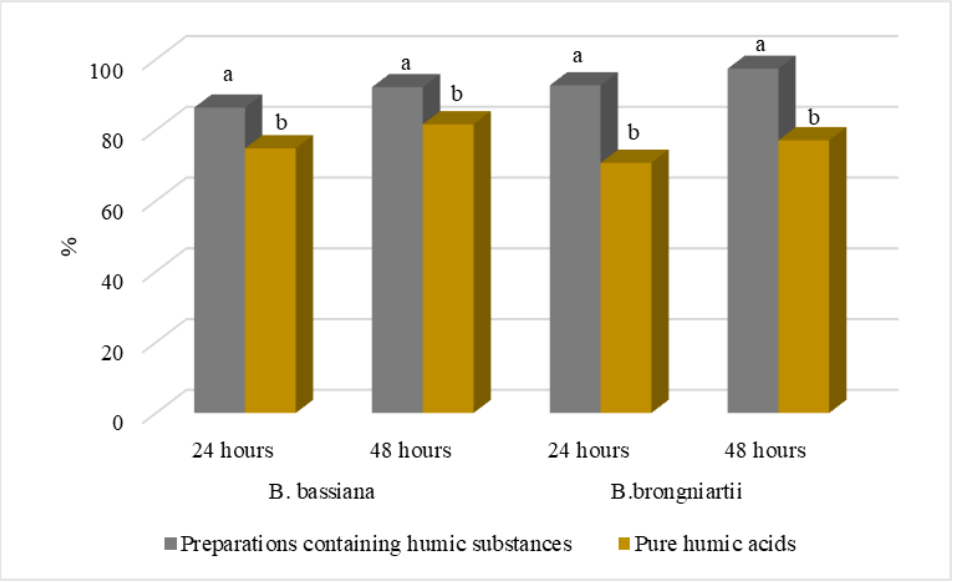


a – means with the same lowercase letters are not significant at α = 0.05, Tukey's HSD

Fig. 1. Colony size of entomopathogenic fungi on the 20th day of cultivation on media with the addition of preparations containing humic substances and pure humic acids (in % in relation to the control)

During each observation it was found that the preparations containing humic acids added to the culture medium had stimulated the growth of *Beauveria brongniarti* colonies in relation to control in a statistically significant way (Tab. 3). AlgoHUM stimulation of *B. brongniarti* strain growth was noted on days 5 and 15, while HumiAGRA on days 10 and 20. Colonies of *B. brongniarti* reached 78.7 mm on day 20. Each time it was observed that pure humic acids had had an inhibitory effect, limiting isolate growth, but the differences were not statistically significant. Throughout the observation cycle, pure humic acids extracted from spent mushroom substrate inhibited the growth of *B. brongniarti* colonies the most.

Comparing the average colony sizes of both EPF species on day 20 (percentage in relation to control), it was found that preparations containing humic substances increased the size of *B. brongniarti* colony more than that of *B. bassiana*, and the difference was statistically significant. Pure humic acids limited their growth, but this difference was statistically insignificant, with *B. brongniarti* being more affected (Fig. 1).



a – means between preparations and pure humic acids in the hours with the same lowercase letters are not significant at $\alpha = 0.05$, Tukey's HSD

Fig. 2. Germination of entomopathogenic fungi spores on media with the addition of preparations containing humic substances and pure humic acids (in % in relation to the control)

The share of germinated spores of EPF species was higher 48 h after the inoculation than after 24 h (Tab. 4). In a statistically significant way, pure humic acids reduced spore germination more than preparations containing humic substances (Tab. 4, Fig. 2). After 24 h and 48 h, *B. bassiana* spore germination was decreased the least by AmiAGRA and *B. brongniarti* germination by HumiAGRA. In turn, in relation to control the number of germinated spores of the *B. bassiana* fungus was significantly decreased by humic acids extracted from lignite and from peat 24 h after the inoculation

in the case of the former and 48 h in the case of the latter. Germination of the *B. brongniarti* spores after both 24 h and 48 h was limited the most by humic acids extracted from peat. The addition of humic acids extracted from spent mushroom substrate to the culture medium was the least effective towards germination of both EPF species (Tab. 4).

DISCUSSION

Due to rising prices of mineral fertilizers, global warming and the related restrictions on production means, modern agriculture is looking for innovative solutions leading to stable and high-quality yields, without overburdening natural ecosystems. One of such solutions may be increasingly used bioproducts, including humic biostimulants [Rutkowska 2016, Dara 2019, Baker et al. 2020, Kuźmiar et al. 2021, Majchrowska-Safaryan and Tkaczuk 2023], which improve soil structure, increase water retention and nutrient absorption, stimulate root system growth and activate the development of soil microbial populations [Ulukan 2008, de Jardin 2015, Rutkowska 2016, Majchrowska-Safaryan and Tkaczuk 2023]. In order to reduce production costs, effectively grow plants and protect them against pests, these products can be used together with bioinsecticides, which are increasingly becoming a necessary alternative for farmers due to the requirements limiting the use of chemical plant protection products. Bioinsecticides approved for use in agriculture are based, among others, on EPF of the *Beauveria*, *Metarhizium* and *Cordyceps* genera, which can effectively reduce the population of arthropods in the soil environment [Tkaczuk 2008, Sosnowska 2013, 2018, 2019, Grzyb et al. 2019, Majchrowska-Safaryan and Tkaczuk 2023, Holka and Kowalska 2023]. In Poland, biopreparations based on strains of the *B. bassiana* fungus are registered, such as: Mycotrol 22 WP – *B. bassiana* strain GHA, Mycotrol OD – *B. bassiana* strain GHA, Naturalis – *B. bassiana* strain ATCC 74040 and Velifer – *B. bassiana* strain PPRI 5339 [Ministry of Agriculture and Rural Development 2023], but there are no preparations containing the *B. brongniartii* fungus.

During each observation it was noted that the preparations containing humic substances stimulated the growth of *B. bassiana* and *B. brongniarti* colonies, while pure humic acids extracted from peat and lignite had an inhibitory effect. The effect of mushroom substrate humic acids varied. According to Fedoseeva et al. [2021], humic substances directly affect cells, causing various biological effects stimulating or inhibiting fungal development. Investigating the effect of three biofertilizers (EM-4, Multibion and Supermagro) on the growth and germination of EPF, Hirose et al. [2001] found that Multibion slightly limited *B. bassiana* development. In turn, studying an effect of biofertilizers containing humic substances on the development of *Beauveria* and *Metarhizium* genera, Majchrowska-Safarayn and Tkaczuk [2023] found on the 20th day that those products stimulated their growth. Among them, Rosahumus was the most effective. It was added to the culture medium in the recommended field dose and stimulated fungal species growth throughout the experiment, which indicates that it can be applied together with EPF in plant cultivation and protection. Moino and Alves [1998] and Quintela and McCoy [1998] confirm that products improving soil properties such as biofertilizers are compatible with EPF, increasing their effectiveness. In turn, humic acids present in organic matter promote resistance of fungi to high temperature and UV radiation, and they

are beneficial for soil and plants [Tomaszewski et al. 2011, De Melo et al. 2016]. Felizatti et al. [2021], studying the effect of biopolymers, including humin and humic acids, on the growth and germination of *B. bassianas* spores, found a slight inhibitory effect. Applying EPF together with humic substances in field conditions, Kaiser et al. [2019] observed a significant increase in relation to control in the survival of *B. bassiana* spores after 7 and 14 days.

In the present research, preparations containing humic substances limited fungal spores germination only slightly, while pure humic acids were more toxic. To the greatest extent, spore germination was inhibited by pure humic acids extracted from peat. Felizatti et al. [2021] indicate that the inhibitory effect of humic acids is probably related to a decrease in the pH value of the culture medium. Humic acids lowered its pH values to 5.3 and 4.5 (1% and 2% w/v, respectively). In contrast, according to Padmavathi et al. [2003] and Luo et al. [2015], the *B. bassiana* fungus is a relatively resistant species and can adapt to pH values ranging from 5 to 10. Therefore, the limitation of growth and germination of *B. bassiana* spores in response to pure humic acids added to the substrate may be related to pH changes resulting in unfavorable chemical conditions and in inadequate nutrient availability. Hirose et al. [2001] found that EM-4 and Multibion added to the culture medium at the recommended dose reduced the germination rate and limited the vegetative growth of the *M. anisopliae* EPF. According to Kaiser et al. [2019], humic acids used in vitro had high potential (above 90%) in protecting *B. bassiana* fungus spores from UV radiation that hinders the germination of EPF conidia [Fargues et al. 1996, Fernandes et al. 2007]. Quesada-Moraga et al. [2023] concluded that one of the main factors limiting *B. bassiana* fungus effectiveness in controlling soil pests is exposure to UV-A and UV-B radiation, resulting in a reduction in the number of its germinating spores.

The effect of humic substances and pure humic acids on the growth and germination of EPF spores has not been reported in the literature to any great extent. However, they are both increasingly used as fertilizers as they improve the condition of arable soils. In turn, EPF inhabiting the soil environment form very complex relationships with other microorganisms and with plants, including crops [Pava-Ripoll et al. 2011, Jaber and Enkerli 2017, Mantzoukas and Grammatikopoulos 2020, Canfora et al. 2023]. Therefore, together with humic stimulants, they can become an alternative preserving biodiversity in crop cultivation and protection in accordance with the European Green Deal strategy. Humic substances and humic acids can play an important role in improving the action of EPF, by providing better coverage of plant surfaces and thus increasing effective pest control [Holka and Kowalska 2023, Majchrowska-Safaryan and Tkaczuk 2023].

CONCLUSIONS

The conducted research showed that on the 20th day of cultivation of the tested EPF of the genus *Beauveria*, (on average) preparations containing humic substances had a stimulating effect, while pure acids slightly limited their growth. The growth of the colony of the fungus *B. basianna* was most strongly stimulated by AmiAGRA, and *B. brongniartii* by HumiAGRA. The share of germinated spores after 48 hours of contact with the substrate was higher than after 24 h, and more spores germinated on substrates with the addition of preparations containing humic substances than on pure humic acids.

Statistical analysis showed that preparations containing humic substances added to the culture medium had a significantly positive effect on the growth of the colonies of the tested fungi. After 48 hours of contact with the substrate of spores of the tested fungal species, pure humic acids significantly limited their germination. The obtained results indicate the potential possibilities of combined use of preparations containing humic substances and pure humic acids with tested species of EPF in the context of integrated and ecological agriculture.

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