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EFFECT OF ENTOMOPATHOGENIC FUNGI (*BEAUVERIA BASSIANA*) ON SOIL NUTRIENTS CONTENT

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Abstract

The sustainable management of agroecosystems and its resistance to pests in the context of climate change strongly depend on soil health. The soil is a living and dynamic ecosystem, maintaining nutrient cycling, providing water, oxygen, nutrients, and root support for crop plants, controlling plant diseases, insect and weed pests. One of the eco-friendly alternatives for sustainable production is the use of entomopathogenic fungi, providing multiple ecosystem services, as plant protection, promotion of plant growth, improving the nutrient uptake etc. Entomopathogenic fungi are also considered as indicators of soil health. In the present study we aim to evaluate the effect of inoculants based on Beauveria bassiana on micro- and macroelements content and soil mycoflora, in an organic tomato crop, in Buzău, România. The soil samples were taken in 2020, from 0-20 cm depth, and analyzed microbiologically and for nutrients content. The results showed that fungi inoculation has a variable, minor, influence on nutrients accumulation on short term, but higher impact on soil fungi communities. Further studies are required to establish the implications on medium and long term.

Key words: *entomopathogenic fungi, microbial inoculants, Beauveria bassiana, nutrients content, organic soil,*

INTRODUCTION

Soil is one of the most vulnerable resources that we have, but also one of the most precious. According to FAO, 2015, the soil delivers ecosystem services that enables life on Earth, as provision of food, fibre and fuels, provision of construction materials, nutrient cycling, water purification and soil contaminant reduction, source of pharmaceuticals and genetic resources, habitat for organisms, climate regulation, carbon sequestration, flood regulation, foundation for human infrastructures, cultural heritage etc. Unsustainable intensification of agriculture, including crop, livestock and forest-based systems has negatively impacted environmental and human health. While the need of increasing food production and its nutritional quality exists, this must be done without affecting the environmental health of the agroecosystems. The soil biota is particularly important in maintaining soil functions. Sustainable alternatives to synthetic fertilizers and pesticides are one of the means.

The use of microorganisms, especially entomopathogenic fungi (EPF) as biological control, is a practice that has been used more and more in recent years, to protect soil health. In natural environments, entomopathogenic fungi occur in soils and are a very important soil health indicator. They also play a role in nutrient cycling, plant growth, nutrient uptake, yield, in controlling pests and disease, plant adaptation to biotic (due to insect herbivores, plant pathogens, or parasitic nematodes), and abiotic stresses (drought or mechanical damage), interact with plant rhizosphere colonizers and biofertilizers, they are important also for environmentally friendly agriculture and sustainable management of agroecosystems (Senicovscaia 2012; Espinoza et al., 2019; Barra-Bucarei et al., 2020; Blanco-Pérez et al., 2020; González-Guzmán et al., 2020; Rajula et al., 2020; Alves et al., 2021; Li et al.,2021; Sharma et al., 2021). In soils with high organic matter content, a high biological activity and presence of antagonistic organisms can lead to a reducing EPF content, but a higher diversity and density of insects in soils with high organic matter content, implies a higher diversity of hosts for EPF having a positive effect. Also, the carbon: nitrogen-ratio can influence the EPF in soils (Uzman et al., 2019). These fungi can produce phytohormones (auxins) that improve water transport, are involved in the solubilization of phosphate, potassium, and siderophores production, activeting biologic protection mechanisms and inducing systemic resistance to phytopathogens. *B. bassiana* colonise the soil or plants as a saprophyte or an endophyte. *B. caledonica* can produce organic acids, such as oxalic and citric acids and *B. bassiana* formic, lactic, orotic, oxalic and citric acids. These organic acids change the pH of the medium and inorganic phosphorus is released. *Beauveria bassiana* also produce siderophores, which play an important role against the cellular stress caused by iron deficiency, iron being necessary for fungal cell growth and metabolism (Raya-Díaz et al., 2017; Barra-Bucarei et al., 2020; Harith-Fadzilah et al., 2021).

It is necessary to understand soil properties' relationship with EPF, soil ecology and diversity of EPF and their relationship with the plants.

Sharma et al., 2021 showed that soils that have a high content of organic matter have a higher biological activity having as results the increased EPF antagonists. For example, calcium ions promote *B. bassiana* and inhibit *Metarhizium robertsii,* another very important EPF in biocontrol, while soils with higher acidity inhibit *B. bassiana*, and higher C:N, and magnesium ions inhibit *M. robertsii* (Sánchez-Rodríguez et al., 2015; Sharma et al., 2021). Fungal microbes influence significantly plant tissue macrontrients like calcium and magnesium (Moloinyane & Nchu, 2019).

The tomato crop has great importance worldwide for its nutritional composition and its antioxidant content. Barra-Bucarei et al., 2020 demonstrated that the greatest plant heights and biomass in the tomato crop was obtained after treatments with *B. bassiana*. Besides its activity in growth promotion, this fungus also provides important protection levels against whiteflies, one of the most spead tomato pests.

Sánchez-Rodríguez et al., 2015 showed that *B. bassiana* influences the availability of microelements as Fe, Cu, and Ag when it is directly applied to a metallic surface. On tomato and wheat crops the fungus can install, but without a negative effect on height, plant dry weight, root development in tomato or grain production in wheat. In both crops, *B.bassiana* had a positive role in preventing chlorosis, but the effect was influenced by the Fe availability. Further, a modification in plant nutrition with macroelements, mainly K, was found in wheat and tomato crops. González-Guzmán et al., 2020 showed that wheat crops inoculated with *B. bassiana* have an increase yield, being positively correlated with soil available phosphorus and with small content of Fe oxides. The increase in aerial dry matter was negatively correlated with soil available Zn in crops inoculated with *B. bassiana*, the fungi enhancing grain Zn uptake.

Purchase et al., 2009 showed that *Beauveria bassiana* grown in modified glycerol asparagine medium containing elevated levels of Zn and Pb at 30°C, accumulated up to 0.64% of available Zn and 8.44% of Pb. They also found that metal tolerance was not affected by a decrease in temperature and the mechanism of resistance in *B. bassiana* can be related to the precipitation of Pb (possibly in the form of oxalates).

Considering all the above-mentioned interrelations between EPF and chemical elements, the main objective of the present study was to elucidate the effects of *B. bassiana* on micro and macroelements accumulation in an experimental plot cultivated with tomatoes.

MATERIALS AND METHODS

Soil preparation

The soil samples were taken from topsoil (0-20 cm) in June in 2020, from two fields of an experimental plot planted with tomato, from Buzău, Romania. One field was inoculated in 2019 and the second one in 2020, respecting crop rotation scheme, the first one passing the winter in the normal conditions. The experimental variants were the following: uncultivated soil - control (C), soil cultivated with tomato, but without *B. bassiana* inoculation - NBb and soil cultivated with tomato, and treated with *B. bassiana* - Bb. Sample's preparation, physico-chemical and microbiological analyses have been performed in the Research centre for study of food quality and agricultural products, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Analysis of soil micro- and macroelements

Dried milled, homogenized, and sieved through a 250 µm soil samples were used for microwave-assisted digestion. 0.1 g soil and Suprapur solvents: 65% HNO_3 and 37% HCl were used. Elemental analysis of the samples with ICP-MS was performed, according to Dobrin et al. (2020), using an Agilent 7700x ICP-MS instrument with Mass Hunter 4.3 Workstation software for ICP-MS, version C.01.03 (Agilent Technologies, Tokyo, Japan). A single and multi-element ICP-MS calibration standard was used for the calibration curve between 0-8 µg/l for microelements and 0-800 µg/l for macroelements.

An amount of 5-10 mg of sample was used for quantification of total soil nitrogen using CHNS elemental analyser (EA3100 Elemental Analyzer). Cystine was used as standard reference material. All determinations were performed in three repetitions.

Microbial Analysis

The count of the bacteria and fungi was carried out by spread plate technique. This was done by inoculating 0.5 ml of diluted samples on PDA suitable for microbiology from Scharlau, prepared according to manufacturers' instruction. The agar plates were sealed with Parafilm® M then incubated at room temperature (28 ± 2 °C) for 24–48 h. Observed colonies were counted and expressed as colony forming units (CFUs) per gram of soil ($cfug^{-1}$) (Ameh & Kawo, 2017; Enuneku et al., 2020).

Statistical analysis

All the results for the effects of *Beauveria bassiana* on soil macro and microelements, and for bacteria and fungi count were expressed as the mean values. For statistic results we used Statistical package IBM SPSS Statistics for Windows (Version 27.0, SPSS, Ireland), using Duncan's multiple range test at significance level $P \leq 0.05$ (5%)

RESULTS AND DISCUSSIONS

Macroelements

Nitrogen content of the soil it is an important parameter that can indicate the availability and abundance of fungal species (Hallouti et al., 2020)

Regarding the influence of *B. bassiana* on distribution and accumulation of nitrogen (N) in soil, there were no significant differences between soils inoculated in 2019 and those treated in 2020 and also we didn't find any statistical differences between control, without inoculation and with inoculation experimental variants (Figure 1).

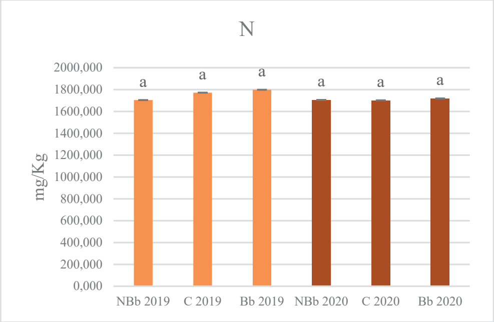

Figure 1. Effect of *B bassiana* on N soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at $P \leq 0.05$

In the plot treated in 2019 we found that between untreated variants and the one inoculated with *B. bassiana* there are not statistical differences regarding phosphorus (P) content in soil, but in 2020 we found an increase by 639.922 mg/Kg in treated variant compared to untreated variant and by 430.723 mg/Kg compared to control variant in 2020 (Figure 2). Our results are in accordance with Alves et al., 2021 who also find that the *B. bassiana* application increased soil P.

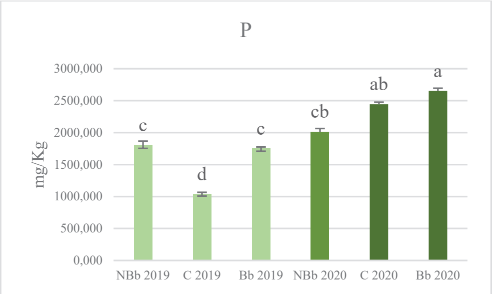

Figure 2. Effect of *B bassiana* on P soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at $P \leq 0.05$

Potassium (K) content in soil from inoculated variants had no significant variation in 2020 compared with those treated in 2019 and with untreated variants from 2019, but in 2020 it was observed an increase in K content in inoculated variants compared with control and untreated variants (Figure 3).

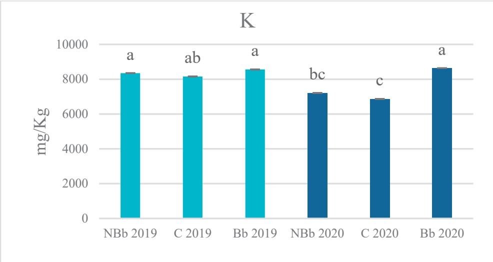

Figure 3. Effect of *B bassiana* on K soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Like K, sodium (Na), also did not had an increase in 2020 in inoculated experiments compared with those from untreated variants from 2019. In 2020 we found that there were not statistical differences between inoculated variants and control variants. Na content had a similar evolution with magnesium (Mg), in 2019 having higher values than in 2020 (Figure 4).

Figure 4. Effect of *B bassiana* on Na soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Mg is an important element for the process of photosynthesis, being a building block for chlorophyll. It also activates certain plant enzymes involved in plants growth and contributes to protein synthesis and secondary metabolite production (Moloinyane & Nchu 2019; Uzman et al., 2019). Mg had a similar accumulation in soil like Na. No statistical differences between control, untreated and treated variants for the 2020 experimental plot were found. For 2019 inoculated plots, a significant difference was found between the uncultivated control and the plots cultivated with tomatoes, regardless of their *B. bassiana* amendment (Figure 5).

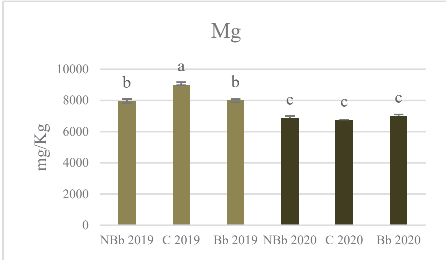

Figure 5. Effect on *B bassiana* on Mg soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Calcium (Ca) is an important element for the development of new plant tissues. It strengthens cell walls and promotes cell elongation. It has also a role in plant protection against pathogenic fungi and bacteria (Moloinyane & Nchu 2019). Ca had a similar evolution with N without significant differences between inoculated variants, control and untreated ones. Also we don't find differences between 2019 and 2020 experimental years regarding control and inoculated variants (Figure 6).

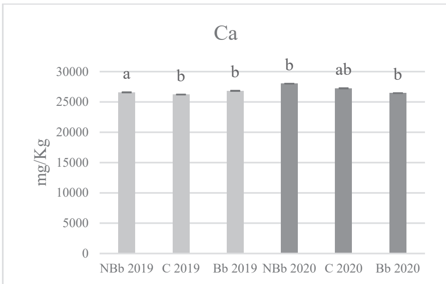

Figure 6. Effect of *B bassiana* on Ca soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

Microelements

The analysed microelements are iron, copper, zinc, manganese, molybdenum, chromium, and cobalt. Iron content have also the same accumulation in experimental variants like Ca, with no differences between years and experimental variants (Figure 7).

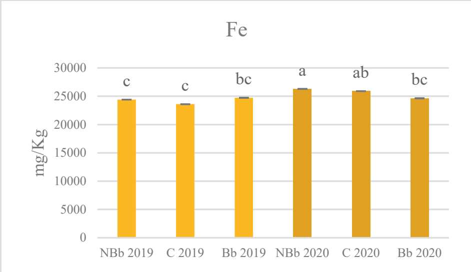

Figure 7. Effect on *B bassiana* on Fe soil content. Data are means ± SE of six replications. Bars with the same letters are not significantly different at P≤0.05

For the other analysed microelements, as Cu, Zn, Mg, Mo, Cr, Co, (Table 1), the following discussions could be made: for Cu there is difference between inoculation years, but not between experimental variants, following the same trend like Mg, higher content in 2019 than in 2020; Zn content in 2019 hadn't a significant difference between treated and untreated variant, but in 2020 the inoculated sol had the highest content, similar to P; Mn and Mo content had no statistically assured variations; Cr content was higher in treated variants compared with control and untreated variants in both years.

Table 1. Effects of *B.bassiana* treatments on microelements content in soil. Data are means ± SE. Values with different letters are significantly different according to Duncan's multiple range test (P≤0.05)

		Cu (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)	Mo (mg/Kg)	Cr (mg/Kg)	Co (mg/Kg)
2019	NBb	39.673±0.542 b	99.720±3.436 ab	717.427±7.940 bc	2.866±0.088 a	51.417±2.145 ab	11.468±0.427 bc
	C	46.199±0.665 a	83.837±3.323 b	698.384±4.359 c	2.753±0.062 a	54.991±1.829 a	12.747±0.291 a
	Bb	41.388±0.931 b	98.846±2.354 ab	729.133±7.167 abc	2.719±0.070 a	52.483±1.004 ab	11.561±0.097 b
2020	NBb	33.668±0.931 c	93.524±3.473 ab	757.681±9.524 a	2.887±0.111 a	46.935±0.315 a	11.060±0.338 bcd
	C	32.058±0.809 c	85.633±1.206 b	739.272±10.779 ab	2.860±0.046 a	46.659±0.604 a	10.593±0.237 cd
	Bb	34.415±0.684 c	113.556±3.969 a	717.009±11.896 bc	3.051±0.164 a	50.759±0.869 b	10.319±0.260 d

Regarding the soil biota, in soil samples from 2020 inoculated plot, both the number of bacteria and fungi from inoculated variants were higher than in control ones, and higher compared with soil from 2019 plots (table 2). The total bacterial counts are in accordance with Ameh & Kawo, 2017, that found 5.94 x 10^6 cfu g^{-1} in untreated soils, and Jaskulska et al., 2020 found that bacteria CFUs ranging between 4.3-12.9 × 10^6 cfu g^{-1} soil, and for fungi ranging between 26.3-35.0 × 10^4cfu g^{-1}.

Table 2. Effect of inoculation treatments on total bacteria and fungi (cfu/g) in soil. Data are means ± SE. Values with different letters are significantly different according to Duncan's multiple range test (P≤0.05)

	Bacterial count (10^6 cfu g^{-1})				Fungi counts (10^4 cfu g^{-1})			
	2019		**2020**		**2019**		**2020**	
NBb	5.651±0.070	b	6.962± 0.238	ab	8.679 ±0.450	c	21.858±1.859	b
C	8.245±0.579	a	5.778±0.487	b	6.195±0.605	c	12.079±0.269	c
Bb	4.579±0.077	b	6.468± 0.138	ab	6.889±0.110	c	42.945±0.995	a

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CONCLUSIONS

B. bassiana had an influence on P, Na and Zn. Also, the highest CFU of fungi was found in the inoculated variants, in both years. These soil indicators can be used to predict the soil microbial quality based on soil chemical analysis, useful for integrated pest management, in the context of sustainable agriculture.

Further investigations are necessary to understand the ecology of the EPF, the relationships of bacteria and fungi with the habitat, as well as among coexisting species. Further studies are foreseen on other beneficial soil microbes.

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