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Influence of the micromycete *Fusarium culmorum* and its antagonists on the state of the antioxidant system of *Melissa officinalis* L.

Svetlana Skugoreva [a*], Polina Gushchina [b], Yana Sharipova [b], Larisa Darovskikh [b]

a: Biomonitoring Laboratory, Institute of Biology of Komi Science Centre, Ural Branch of the Russian Academy of Sciences, Syktyvkar 167982, Russia
b: Institute of Chemistry and Ecology, Vyatka State University, Kirov 610000, Russia
* Corresponding author: skugoreva@mail.ru

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Abstract

In recent years, the popularity of herbal medicine has increased. Lemon balm (*Melissa officinalis* L.) is a perennial essential oil herb that has been used as a medicinal plant for more than 2 thousand years. It is known that the productivity of plants is directly related to their resistance to phytopathogens, in particular, micromycetes of the genus *Fusarium*. One of the main mechanisms of plant damage by phytopathogens is oxidative stress. Micromycetes of the genus *Trichoderma* and soil cyanobacteria (CB) occupy an important place among the natural antagonists of fungi of the genus *Fusarium*. The aim of the work was to study the state of the antioxidant system of *Melissa officinalis* L. plants when grown on substrates contaminated with the micromycete *Fusarium culmorum* and its antagonists – the cyanobacterium *Fischerella muscicola* and the micromycete *Trichoderma viride*. It was found that the presence of the pathogenic micromycete *F. culmorum* in the soils for growing lemon balm for two months has a stressful effect on lemon balm plants: the intensity of lipid peroxidation, the content of phenolic compounds and the amount of antioxidants in the in plant leaves were significantly higher than in the control. At the same time, at elevated temperatures, the content of phenolic compounds increased, which may be due to increased metabolism and the level of oxidative stress. The introduction of microorganisms-antagonists *F. muscicole* and *T. viride* into the soil makes it possible to activate the work of the antioxidant system of plants and reduce the effects of oxidative stress almost to the level of control. The studied antagonists can be recommended as promising for the development of biological products on their basis in order to protect medicinal plants from fusarium diseases.

Keywords

lemon balm
oxidative stress
antioxidant system
phytopathogens
micromycetes
antagonists

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Key findings

- The presence of the pathogenic micromycete *Fusarium culmorum* in the soil has a strong effect on lemon balm plants: it enhances lipid peroxidation, leads to the accumulation of phenolic compounds and antioxidants.
- The introduction of *Fischerella muscicola* and *Trichoderma viride* antagonist microbes into the soil makes it possible to activate the antioxidant system of plants and reduce the consequences of oxidative stress almost to the level of control.

1. Introduction

In recent years, despite the great success in the creation of synthetic medicinal substances, the popularity of herbal medicine is growing. Interest in medicinal herbs and preparations based on them is increasing due to the unique properties of herbal preparations and rapidly developing research technologies [1]. About 300 plant species are used by modern domestic scientific medicine [2]. The volume of cultivated medicinal plants is growing every year [3].

Lemon balm (*Melissa officinalis* L.) is a perennial essential oil herb that has been used as a medicinal plant for more than 2 thousand years. It grows in the regions of Western Asia and the Eastern Mediterranean, and is also cultivated in central Europe. Oils obtained from lemon balm plants exhibit antibacterial, antidepressant, antiviral and antispasmodic activity.

The growth and development of plants is influenced by many factors. Higher plants are in direct contact with the microflora of the rhizosphere. It is known that the productivity of plants is directly related to their resistance to phytopathogens. Phytopathogenic microorganisms (MO) synthesize toxins that can inhibit and retard plant growth. These MO include micromycetes of the genus *Fusarium*, which are widespread in soils and produce more than 150 toxins.

One of the main mechanisms of plant damage by phytopathogens is oxidative stress, which is based on a sharp increase in oxidative processes in the body with insufficient functioning of the antioxidant system [4]. As a result of stress, the formation of free radicals increases, which induces the processes of lipid peroxidation (LPO) and causes the development of destructive processes both at the level of the cell, organ, and the whole organism [5].

Unsaturated lipids or free fatty acids, which are part of the phospholipids of biological membranes, are most easily oxidized. The primary products of LPO are diene conjugates and hydroperoxides; the secondary products are alcohols, ketones, aldehydes, dialdehydes, etc. Among the dialdehydes, malondialdehyde (MDA) is of particular interest, serving as a marker of the degree of endogenous intoxication. By the content of MDA in cells, one can judge the intensity of LPO.

In the plant kingdom, phenolic compounds (PC) are among the most powerful natural antioxidants. The antioxidant properties of PC – reducing agents are due to their ability to serve as “traps” for free radicals [6]. PC are able to interact with hydroxyl (LO•) and peroxyl (LOO•) lipid radicals due to their ability to donate an electron (or hydrogen atom). As a result, phenol radicals are formed – phenoxyls, which do not participate in the propagation of the oxidative process.

In general, stress reactions initiate the formation or mobilization of specialized adaptation mechanisms, and also perform operational protection of the plant organism from death under unfavorable conditions [7]. Micromycetes of the genus *Trichoderma* [8] and soil cyanobacteria (CB) [9] occupy an important place among the natural antagonists of many phytopathogenic MO, in particular, fungi of the genus Fusarium. Antimicrobial compounds of CB can suppress phytopathogens through the destruction of the cytoplasmic membrane, inhibition of protein synthesis, the activity of hydrolytic enzymes, etc. [10].

The aim of the work was to study the state of the antioxidant system of *Melissa officinalis* L. plants when grown on substrates contaminated with the micromycete *Fusarium culmorum* and its antagonists – the cyanobacterium *Fischerella muscicola* and the micromycete *Trichoderma viride*.

2. Materials and methods

2.1. Materials

The cultures of MO were taken from the collection of the Vyatka State Agricultural Academy (Kirov, Russia): micromycetes *Fusarium culmorum* and *Trichoderma viride*, cyanobacterium *Fischerella muscicola*. Sterile nutrient soil for growing plants possessed the following agrochemical characteristics: pH=5.5–6.5; N – 50–150 mg/100 g; P (P_2O_5) – 100–250 mg/100 g; K (K_2O) – 150–300 mg/100 g of soil (Tver, Russia).

The instruments used include the spectrophotometer PE-5300VI, (LLC “EKROSKHIM”, St. Petersburg, Russia) and the coulometer “Expert-006” (Econix-Expert, Moscow, Russia).

The key reagents are tris(hydroxymethyl)aminomethane, thiobarbituric acid, trichloroacetic acid (determination of MDA content in lemon balm plants); Folin-Chocalteu reagent (content of phenolic compounds in alcohol extracts from lemon balm); potassium iodide (the total content of antioxidants in alcoholic extracts from lemon balm).

2.2. Experiment preparation and conduct

Lemon balm seeds were washed with 1% potassium permanganate solution. The seeds were germinated under sterile conditions in Petri dishes on filter paper moistened with distilled water for seven days. Then the plants were transplanted into the soil. Before planting the plants, suspensions of micromycetes *F. culmorum* ($T = (5.0\pm0.1)\cdot10^9$ cells/cm^3, 1 cm^3 per 60 g of soil), *T. viride* ($T = (5.0\pm0.1)\cdot10^9$ cells/cm^3, 5 cm^3 per 60 g soil), as well as CB *F. muscicola* ($T = (3.0\pm0.1)\cdot10^9$ cells/cm^3, 5 cm^3 per 60 g soil) [11]. Experiment scheme: 1) control (without MO additives); 2) *F. culmorum*; 3) *F. culmorum* + *F. muscicola*; 4) *F. culmorum* + *T. viride*; 5) *F. culmorum* + *F. muscicola* + *T. viride*. The studies were carried out in two series of experiments, differing from each other in temperature conditions: series No. 1 – 21±1 °C, series No. 2 – 29±3 °C. In both series of experiments, the change of day and night was controlled (12 h/12 h). Two months after transplanting the plants into the soil, the content of PC

in the leaves of lemon balm was determined by the spectrophotometric method with the Folin-Ciocalteu reagent (70% ethyl alcohol was used to prepare the aqueous-alcoholic extract of lemon balm; the weighed portion of the plant was boiled with alcohol for two hours in a water bath). The MDA content was determined by spectrophotometry with thiobarbituric acid, the AA of alcohol extracts was determined by coulometric titration in extracts prepared in the same way as for the determination of PC.

2.3. Formulas for Calculations

The concentration of MDA in lemon balm leaves was calculated by the formula [12]:

$$C = \frac{D}{\varepsilon \cdot l \cdot m}, \quad (1)$$

where C is the concentration of MDA, μmol/g fresh weight; D is the optical density; ε – the coefficient of molar extinction MDA ($1.56 \cdot 10^5$ $cm^{-1} \cdot M^{-1}$); l – the thickness of the solution layer in the cuvette, cm; m – the mass of the sample of plant material, g.

The content of intracellular PC in plant samples was calculated using the formula:

$$PC = \frac{C \cdot V_{extr.}}{m \cdot 1000}, \quad (2)$$

where PC is the total content of intracellular PC, mg gallic acid/g dry weight; C is the concentration of PC obtained from the calibration curve based on the optical density of the samples, mg of gallic acid/dm^3; $V_{extr.}$ – the total volume of the extract, cm^3; m is the weight of the sample, g; 1000 – the conversion factor dm^3 to cm^3 (extract volume).

The total antioxidant content was determined by the formula:

$$AO = \frac{M \cdot V_{extr.}}{V_{al.} \cdot m}, \quad (3)$$

where AO is the total antioxidant content, mg rutin/g dry weight; M is the concentration of phenolic compounds obtained in an aliquot of the extract, mg; $V_{extr.}$ – the total volume of the extract, cm^3; $V_{al.}$ – the volume of the aliquot, cm^3; m is the mass of the sample, g.

2.4. Statistical Analysis

The experiment was repeated four times when growing plants, the analyses – three (for determining the content of MDA and AO) and two times (for FC). The results were statistically processed in Excel. The significance of differences with the control was assessed by the Student's test.

3. Results and discussion

Medicinal plants can have a fungicidal effect due to the release of substances with allelopathic phytopathogenic properties, the so-called root rhizodeposites (essential oils, phenols, etc.) [13, 14], which reduce the effect of pathogenic MO.

The study showed that the antimicrobial activity of lemon balm was not enough to neutralize the effect of the phytopathogen. The addition of *F. culmorum* mycelium suspension to the soil initiated the development of oxidative processes in lemon balm cells (Figure 1). At a temperature of 29 °C, the concentration of MDA in lemon balm leaves in variant 2 (only with the introduction of *F. culmorum*) was 1.8 higher than in the control. In the variants with the introduction of MO antagonists into the soil, the accumulation of MDA was noted in the plants decreased almost down to the control values. To a lesser extent, this effect was manifested in the variant No. 4 with the introduction of *T. viride*, which is probably due to the relatively low antagonistic activity of this MO in relation to *F. culmorum*. To a greater extent, a decrease in the intensity of lipid peroxidation in plants caused by micromycetes was observed when *F. muscicole* was introduced into the soil (variants No. 3 and No. 5). Earlier [11] it was found that at a temperature of 21 °C the MDA content in lemon balm plants in the variant with Fusarium (No. 2) was 5.9 times higher than that in the control. The use of MO antagonists reduced the accumulation of MDA in plants.

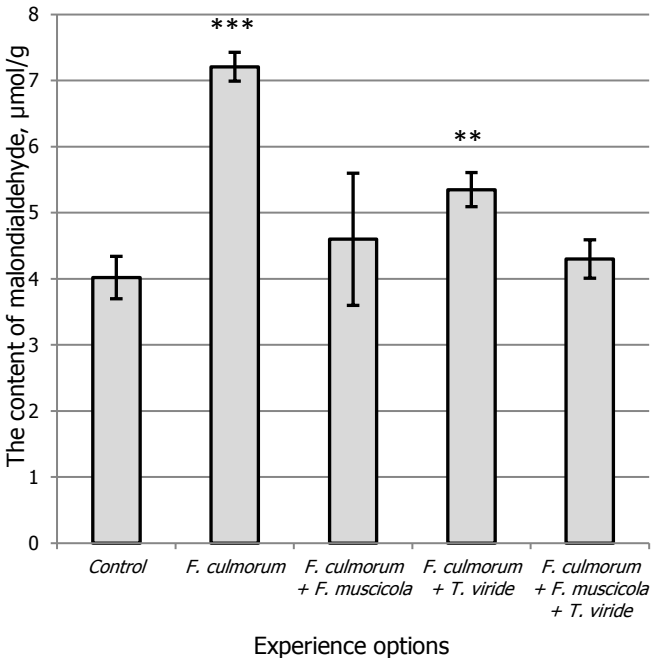

Figure 1 The content of malondialdehyde in the leaves of lemon balm plants when *F. culmorum* and its antagonists are introduced into the soil (t = 29 °C). Note: differences with control are significant at ** – p<0.01; *** – p<0.001.

In response to the action of a stress factor, the work of the body's defense system is activated, which manifests itself in the synthesis of antioxidants. The total content of antioxidants in lemon balm leaves when the suspension of *F. culmorum* mycelium (variant No. 2) was added to the soil was 1.6 times higher than that in the control (Figure 2). The use of MO antagonists significantly reduced this indicator. In the variants No. 4 and No. 5, the AA of plants slightly exceeded the control. When *F. muscicole* was added to the soil (option No. 3), the value of the total antioxidant content was 1.6 times lower than the one in the control.

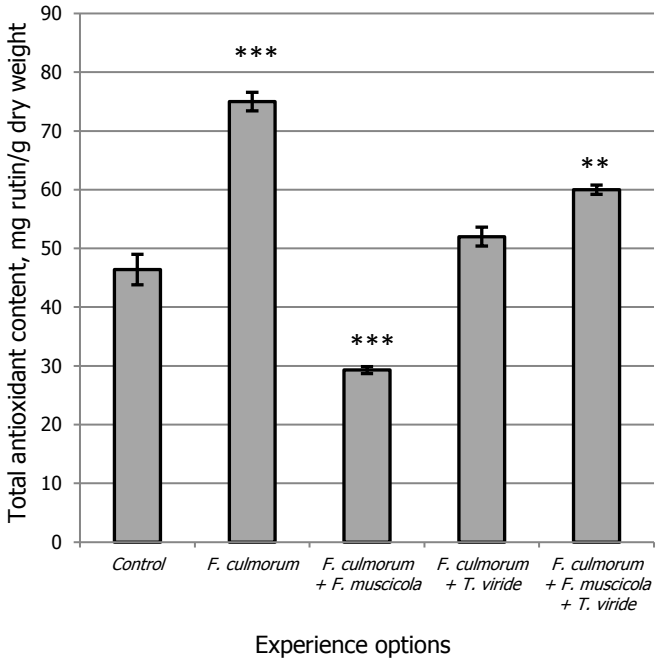

Figure 2 The total content of antioxidants in the leaves of lemon balm plants when *F. culmorum* and its antagonists are introduced into the soil (t = 29 °C). Note: differences with control are significant at ** – $p < 0.01$; *** – $p < 0.001$.

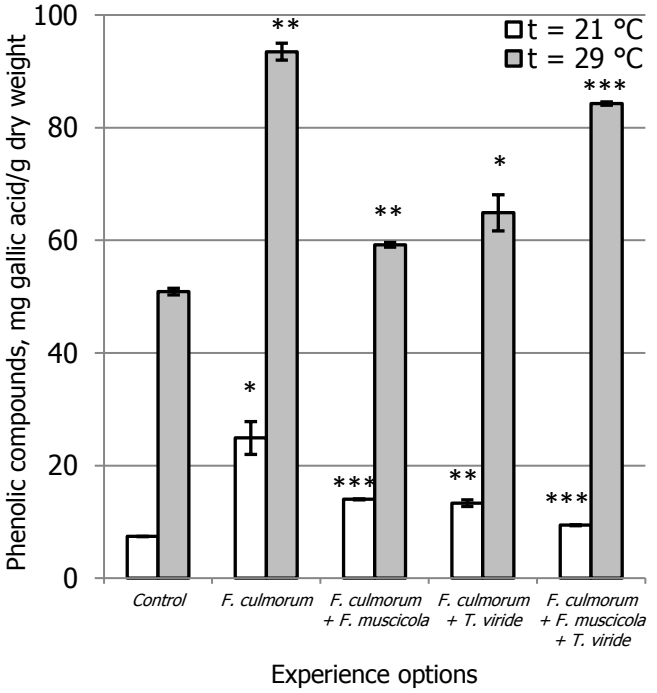

Figure 3 The content of phenolic compounds in the leaves of lemon balm plants when *F. culmorum* and its antagonists are introduced into the soil. Note: differences with control are significant at * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$.

The activation of LPO processes in plant cells led to the accumulation of PS. With an increase in temperature, plant metabolism intensified, which led to an increase in the PS content. Thus, at a temperature of 29 °C, the values of the PC concentration in lemon balm plants were 3.8–9.0 times higher than at a temperature of 21 °C (Figure 3). With an increase in temperature, an increase in the concentration of MDA in lemon balm plants was also noted [11]. There is evidence in the literature that temperature stress affects the antioxidant system of plants. It was found that under the action of elevated temperatures there is an increase in activity and the appearance of multiple forms of enzymes in plants [15], which is explained by an increase in metabolism.

In the variants with MO addition, a greater accumulation of PC was noted than in the control (by 1.3–3.4 times), which indicates a more active work of the plant defense system in response to the stress factor. The maximum content of these compounds was determined in the option No. 2 with the addition of a suspension of the mycelium of the phytopathogenic micromycete *F. culmorum*. In the other variants, lower values of this indicator were noted, which may be due to the antagonistic effect of *F. muscicola* and *T. viride*.

There is a direct correlation between the content of PC and MDA (r=0.69), PC and AO (r=0.84), MDA and AO (r=0.69), which indicates the activation of the plant antioxidant system (accumulation of antioxidants and PC) under conditions of oxidative stress.

4. Conclusion

The presence of the pathogenic micromycete *F. culmorum* in the cultivation grounds has a stressful effect on lemon balm plants: the LPO intensity, the content of PC and AO in the leaves were significantly higher than those in the control. At the same time, at elevated temperatures, the PC content increased, which may be due to the increase in the metabolism and the level of oxidative stress.

The introduction of microorganisms-antagonists *F. muscicole* and *T. viride* into the soil makes it possible to activate the work of the antioxidant system of plants and reduce the effects of oxidative stress almost to the level of control. The studied antagonists can be recommended as promising for the development of biological products on their basis in order to protect medicinal plants from fusarium diseases.

Supplementary materials

No supplementary materials are available.

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Author contributions

Conceptualization: S.S.
Data curation: L.D.
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Investigation: Ya.B., S.S.
Methodology: L.D.
Project administration: S.S.
Resources: L.D.
Software: P.G.
Supervision: S.S.
Validation: L.D.
Visualization: P.G.
Writing – original draft: S.S., P.G.
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Conflict of interest

The authors declare no conflict of interest.

Additional information

Author IDs:

Svetlana Skugoreva, Scopus ID 57195525210;

Larisa Darovskikh, Scopus ID 57201724246.

Websites:

Institute of Biology of Komi Science Centre, UB RAS, https://ib.komisc.ru/rus;

Vyatka State University, https://www.vyatsu.ru.

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