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Mycobiota of the rhizosphere of raspberry plants (*Rubus idaeus* L.) under the influence of varieties and new fertilizers in conditions of organic production

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Abstract. The results of studies of influence of raspberry plant varieties and new organic fertilizers on the abundance and species composition of the micromycetes in rhizospheric soil in conditions of organic production are presented. The mycobiota of Joan J and Himbo-Top raspberry varieties during plant ontogeny was analyzed and the species composition of phytopathogenic micromycetes, which are presented in the rhizospheric plant soil, was defined. It was revealed that the following fungi species prevail in the population: *Botrytis cinerea*, Pers, *Aspergillus niger*, V. Tiegh, *Alternaria alternata*, (Fr.) Keissl, *Fusarium sp*. These fungi are producers of mycotoxins that can cause dangerous diseases in animals and humans. There is a stabilizing selection of microorganisms in the phase of separation of buds in inflorescences in the mycobiota of the rhizosphere of plants of raspberry varieties under the influence of the organic fertilizer VITERI with the addition of Basil essential oil.

Key words: raspberry plant, rhizospheric soil, organic fertilizers, essential oils, micromycetes, the abundance, phytopathogens, organic production.

INTRODUCTION

A significant place in the assortment of berry products belongs to raspberries (*Rubus idaeus* L.), whose medicinal and nutritional properties are widely known (Wang & Lin, 2000; Sekizura et al., 2014). In Ukraine, plants of this culture are affected by a large number of diseases. Among them, root and grey mold rots deserve the special attention (Paulus, 1990; Markov, 2017). The causative agents of these diseases reduce the nutritional and marketable quality of berries. In addition, they are able to produce toxins that belong to the biological contamination of biocenoses (Parfeniuk, 2011; Malinovskaia et al., 2017; Dyakov & Levitin, 2018). The cultivation of berry crops, including the everbearing raspberries, while using organic technologies on industrial plantations, provides for the implementation of a set of interrelated agricultural measures. Among them, the fertilizer system and the introduction of sustainable varieties deserve the special attention (Kowalenko, 2006; Buskienė & Uselis, 2008; Emelyanova et al., 2015; Archer et al., 2016; Borodai et al., 2016; Khalil & Jamel, 2017; Sheng et al., 2019; Wendel et al., 2020). Serbian scientists Stojanov et al. (2019) found the positive effect of the organic fertilizer 'Excell Orga' (Angibaud Derome & Spécialités, France) on the growth of raspberry shoots, the productivity and quality of berries, the content of sugars, phenols and flavonoids, antioxidant abilities. New plant protection products, that have been elaborated on the basis of coniferous tree bark, showed good results (Jankevica et al., 2018). The sesame, cinnamon and neem (*Azadirachta indica A.Juss*.) oils are actively used in the practice of plant protection against powdery mildew of raspberries, spotting (*Septoria rubi*) in organic production (Archer et al., 2016). Thymol, Linalool, Limonene, Eugenol, Carvacrol are used for a long period of time - commercial preparations based on vegetable essential oils, which are used as biopesticides. They include basil oil (Archer et al., 2016). The efficiency and selective effect of essential oils of wild weed have been established: *Chenopodiastrum* murale, *Sisymbrium irio, Falcaria vulgaris, Anagallis arvensis, Crepis aspera, Sonchus oleraceus, Notobasis syriaca* against phytopathogens *Alternaria solani, Helminthosporium sativum, Rhizoctonia solani* (Durán-Lara et al., 2020). Organic-mineral fertilizer HELPROST (BTU-Center, Ukraine) has been developed in Ukraine to stimulate the growth of berries, including raspberry plants. However, there is no information about the effect of essential oils and organic fertilizers on the pathogenic mycobiota of the plant rhizosphere at different phases of the everbearing raspberry`s growth. But it is the pathogenic mycobiota that significantly reduces the quality and biological safety of raspberry products. The main indicator of the efficiency of growing raspberries is the amount of harvest, which is due to mineral organic fertilizer. With the exception of this component, the yield will decrease sharply, which is not interesting for production. Therefore, the goal of our research was to increase the biosafety of raspberry agrocenosis by reducing the number of phytopathogenic micromycetes by creating new fertilizers with the addition of essential oils and improving the quality of the crop, which will be obtained with existing technologies.

MATERIALS AND METHODS

On field research was conducted in 2017–2019 on the site of Friendsberry Ltd., located in Myronivskyi district of Kyiv region and characterized by moderate soil and climatic conditions. The microbiological, phytopathological and mycological methods were followed as mentioned in the previous studies (Dudka et al., 1982; Zvyagintsev, 1991; Parfeniuk et al., 2014).

The investigated preparations are among those recommended by Organic Standard Ltd (Ukraine) for use in organic farming. The macro- (NPK) and trace elements are main active substance of the complex organic-mineral fertilizer Viteri 8-4-5 (Zolotov, Ukraine) in an available form for plant. The additional foliar treatment with VITERI fertilizer (1% aqueous solution) and essential oils of Basil and Fennel (0.1% aqueous solution) was carried out to determine the effect of organic fertilizers on the mycobiota of the rhizospheric soil in raspberry agrocenosis during its ontogeny, using known technology (Polianchikov & Kapitanskaia, 2017). Essential oils for scientific research were produced by Arora Aromatics Pvt. Ltd (India) using original technology. The experiment included the following variants:

1. Control 1 - cultivars of raspberries that were grown in organic production by the adopted technology.
2. Control 2 - VITERY - widely known complex organo-mineral fertilizer Viteri 8-4-5 (Ukraine). The main active ingredient of which is macro- (NPK) and microelements in a form that is accessible to the plant. N.P.K fertilizer 8-4-5. The nitrogen contained in this complex organic fertilizer is in both nitrate form, which is immediately available to the plant, and in ammonium form, which is absorbed gradually and has a prolonged effect. Also, due to the ammonium form of nitrogen, VITERI 8-4-5 significantly improves the absorption of phosphorus, which promotes enhanced root growth and increases the drought resistance of the plant.
3. Composition of VITERY organic fertilizer with Basil essential oil.
4. Composition of VITERY organic fertilizer with Fennel essential oil.

Essential oils were not used separately from fertilizers because cultivars of cultivated raspberries are usually grown on complex fertilizers. If the fertilizer not added, the yield will drop sharply and its quality will suffer. This will damage the production even if the essential oil has a positive effect. It should be noted that the number of bushes in each variant averaged 35 pcs.

Studies were conducted in the following phases of raspberry development:

- Phase of growth inflorescence
- Phase of separation of buds in inflorescences of plants
- Phase of intensive fruiting.

The number of micromycetes in the rhizospheric soil of the everbearing raspberry varieties Joan J (British breeding) and Himbo-Top® (Swiss breeding) was determined in different phases of ontogeny due to the influence of the organic fertilizer VITERI and its compositions with essential oils based on commonly known methodology (Zvyagintsev, 1991; Parfeniuk et al., 2014). The number of colonies in Petri dishes was counted using an automatic counter SCAN4000 (Interscience, France). The Czapek Dox Agar was used. One-way analysis of variance (ANOVA) was used to compare the impact of all of the different treatments (Tukey's test for $p < 0.01$ and $p < 0.05$). The experimental results were processed using the STATISTICA 8.0 software package.

RESULTS AND DISCUSSION

It is known that abundance of micromycetes in the raspberry plant rhizosphere, under the influence of abiotic and anthropogenic factors, can significantly change (Kudeyarova, 1999; Nannipieri, 2003; Gadzalo, 2015). Therefore, the number of micromycetes in the rhizospheric soil depending on the raspberry variety and ontogeny phase of the plants was determined (Zvyagintsev, 1991; Parfeniuk et al., 2014).

According to the results, the number of colony-forming units in the rhizospheric soil of Joan J plants before treatment with preparations, in the phase of growth inflorescence, was at the level of control and was in averaged 1,012 thousand CFU g^{-1} of soil regardless of the variant (Fig. 1).

In the phase of separation of buds in inflorescences, the number of CFU in the control variant sharply increased and reached 2,23 thousand CFU g^{-1} of soil. In the same period of plant growth, the number of CFUs in the studied treatments significantly exceeded the control and ranged from 3,03 thousand to 3,84 thousand CFU g^{-1} of soil (Fig. 1).s

It should be noted that the highest number of CFUs was observed in variants, where foliar treatment with VITERI fertilizer was applied with the addition of Fennel essential oil, which indicates about stimulatory activity of this combination in relation to the mycobiota in the rhizosphere of raspberry plants. The highest inhibitory properties in relation to the mycobiota of the rhizosphere of the plants of the variety raspberry Joan J characterized by foliar treatment with VITERI fertilizer in the phase of separation of buds in inflorescences.

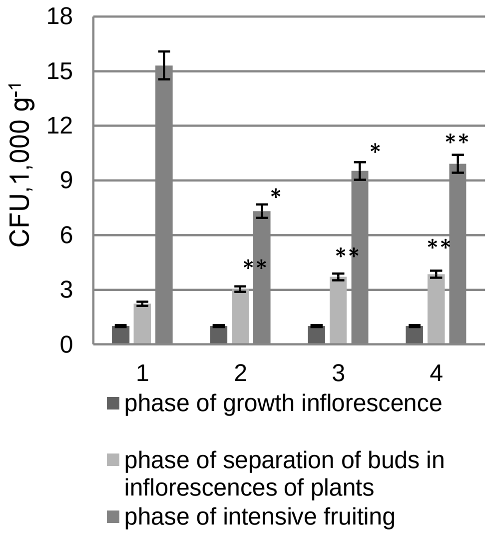

Figure 1. Influence of organic fertilizer VITERI and its compositions with essential oils on the number of micromycetes of rhizospheric soil of Joan J raspberry plants in different phases of ontogeny (1-control, 2-foliar treatment with VITERI fertilizer, 3-foliar treatment with VITERI fertilizer + essential Basil oil, 4-foliar treatment with VITERI fertilizer + essential Fennel oil).

Note: significance of differences compared to the control was a ssessed by one-way ANOVA; * – signific ant differences at $p < 0.05$, ** – significant differences at $p < 0.01$ (Tukey's test).

The highest density of mycobiota in the rhizosphere of plants was observed in the phase of intensive fruiting of raspberries (Fig. 1). During this period, the number of CFUs amounted to 15,3 thousand CFU g^{-1} of soil in the control variant. According to the data, presented in the figure, the greatest inhibition of mycobiota in this period occurred in variants 2 (foliar treatment with VITERI fertilizer) and 3 (foliar treatment with VITERI fertilizer+Basil essential oil), where the number of CFU g^{-1} of soil was in average on 8 and 6 thousand respectively lower than the control.

According to the results of the studies, presented in Fig. 2, it was found that in the rhizosphere of the raspberry plants Himbo -Top, before the tillage, in the budding phase, the number of CFUs was at the level of control and was in averaged 0,787 thousand g^{-1} of soil, which is significantly lower in compared to Joan J (Fig. 1) in the same period of time. But in the phase of separation of buds from inflorescences in control, the number of CFUs sharply increased and had averaged 5,7 thousand CFU g^{-1} of soil. In the variants 2 (foliar treatment with VITERI fertilizer) and 3 (foliar treatment with VITERI fertilizer+Basil essential oil) in the specified period their number was respectively higher on 2 and 1 thousand CFU g^{-1} of soil respectively than at the control. At the same time, in variant 4 (foliar treatment with VITERI fertilizer+Fennel essential oil) in the rhizosphere of Himbo-Top raspberry plants the number of CFUs was significantly lower compared to control and variants 2 and 3.

The results of the research show, that essential oils and their compositions with organic fertilizer VITERI can both suppress and stimulate the formation of mycobiota density in the rhizosphere of raspberry plants (Figs 1, 2).

The given results indicate a significant difference between the influence of different essential oils and their compositions with organic fertilizer VITERI on the density of micromycetes in the mycobiota of the rhizosphere soil of plants of different raspberry varieties. According to the results (Figs 1, 2), a statistically significant difference is particularly evident between control and foliar treatment via essential oils and their compositions with organic fertilizer VITERI in the phase of intensive fruiting. Therefore, the treatment plays a major role in fungal culturable communities.

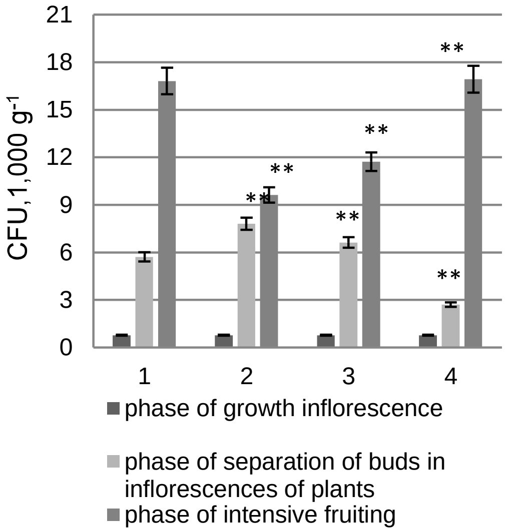

Figure 2. Influence of organic fertilizer VITERI and its compositions with essential oils on the number of micromycetes of rhizospheric soil of Himbo-Top raspberry plants in different phases of ontogeny (1-control, 2-foliar treatment with VITERI fertilizer, 3-foliar treatment with VITERI fertilizer+essential Basil oil, 4-foliar treatment with VITERI fertilizer+essential Fennel oil).

Note: significance of differences compared to the control was a ssessed by one-way ANOVA; * – signific ant differences at $p < 0.05$, ** – significant differences at $p < 0.01$ (Tukey's test).

This process depends on both the variety and the phase of ontogeny of plants. After all, while the number of CFU g^{-1} of soil in the rhizosphere of the Joan J plant varied from 7,3 thousand to 9,9 thousand CFU g^{-1} of soil, depending on the variant and phase of plant development, their number in the rhizosphere of the plant variety Himbo-Top was significantly higher and ranged from 9,6 thousand to 16,9 thousand CFU g^{-1} of soil. It indicates a significant differentiation of raspberry plant varieties according to the mechanisms of synecological relations in the plant-nourishment - microorganism - environment triangle (Figs 1, 2).

The species composition of phytopathogenic micromycetes presented in the rhizospheric soil of the studied varieties was established. The overwhelming majority of pathogens that affected the raspberry plants belonged to known producers of mycotoxins, among which special attention deserves: *Botrytis cinerea, Aspergillus niger, Fusarium grameniarum,* which are producers of mycotoxins (Table 1). These toxins can cause dangerous diseases for humans and animals (Levitin, 2009).

According to the data presented in the Table 2, the phytopathogenic mycobiota in the rhizospheric soil of the raspberry cultivar of the Joan J raspberry plant was represented by following fungi: *Alternaria alternata, Fusarium grameniarum, Aspergillus niger*. The abundance of these micromycetes was in averaged 19% regardless of the variant. Among them, *Aspergillus niger* was the dominant species.

Table 1. Species composition of phytopathogenic micromycetes of mycobiota of raspberry plants of the Joan J and Himbo-Top varieties

Disease	Pathogen	Mycotoxins
Septoriosis	*Septoria rubi*	Only properties
Grey rot	*Botrytis cinerea*	Botrydial
Black mold	*Aspergillus niger*	Aflatoxin
Cladosporiosis	*Cladosporium herbarum*	Only properties
Alternariosis	*Alternaria alternata*	Tentotoxin

Table 2. Spectrum of phytopathogenic micromycetes of the raspberry plants` rhizosphere soil of the Joan J variety

Variants	The proportion of phytopathogenic micromycetes in the mycobiota of the rhizosphere, %		
	phase of growth inflorescence	phase of separation of buds in inflorescences	phase of intensive fruiting
Control	*Alternaria alternata (3)* *Fusarium grameniarum (4)* *Aspergillus niger (12)*	*Botrytis cinerea (14)* *Alternaria alternata (20)* *Fusarium grameniarum (12)*	---
VITERI fertilizer	*Alternaria alternata (3)* *Fusarium grameniarum (4)* *Aspergillus niger (12)*	*Botrytis cinerea (10)* *Alternaria alternata (4)* *Fusarium grameniarum (6)* *Aspergillus niger (60)*	*Aspergillus niger (10)*
VITERI fertilizer + essential Basil oil	*Alternaria alternata (3)* *Fusarium grameniarum (4)* *Aspergillus niger (12)*	*Botrytis cinerea (16)* *Alternaria alternata (12)* *Fusarium grameniarum (14)*	*Fusarium* grameniarum *(10)*
VITERI fertilizer + essential Fennel oil	*Alternaria alternata (3)* *Fusarium grameniarum (4)* *Aspergillus niger (12)*	*Botrytis cinerea (6)* *Alternaria alternata (8)* *Fusarium grameniarum (4)* *Aspergillus niger (48)*	*Fusarium grameniarum (4)* *Aspergillus niger (10)*

Saprotrophic micromycetes were dominating, which include *Penicillium herquei, Penicillium terrestre and Trichoderma viride.* The obtained results give reason to consider that populations of phytopathogenic micromycetes in the rhizosphere soil of plants of these varieties are under conditions of rigid anthropogenic pressure, which in the future may lead to activation of homeostatic processes (Table 2, 3). That is confirmed by the data obtained during the study of mycobiota in the rhizosphere of the Joan J raspberry plants in the budding phase of inflorescences. During this period, the population of phytopathogenic micromycetes ranged from 42 to 80%. While in the mycobiota of the rhizospheric soil of raspberry plants in the control variant, the proportion of phytopathogenic micromycetes was 42% that indicate about stabilizing selection in the population, in the variant with organic fertilizer VITERI it reached 80% that indicate about rigid directed selection of phytopathogenic micromycetes. Foliar treatment of VITERI fertilizer with the addition of Fennel essential oil the number of CFUs of phytopathogenic micromycetes was significantly lower compared to VITERI organic fertilizer and had averaged 66% (Table 2). At the same time, foliar treatment with organic fertilizer VITERI with the addition of essential oil of Basil inhibited the development of phytopathogenic micromycetes in the rhizosphere of plants of the studied variety. The most environmentally safe combination was VITERI+Basil essential oil. In this embodiment, the number of phytopathogenic micromycetes in the rhizospheric mycobiota was 42%, which is close to the stabilizing selection in the mycobiota of the rhizosphere of Joan J raspberry plants.

It should be noted that in the phase of intensive fruiting of raspberry plants in the control variant of the mycobiota of the rhizospheric soil was represented only by saprophytic fungi. The number of phytopathogenic micromycetes on the Joan J variety during this period significantly decreased compared to the phase of separation of buds in the inflorescences and reached an average of 10% in the VITERI variant and 14% in the VITERI variant with the addition of Fennel essential oil (Table 2).

In the mycobiota of the rhizosphere of plants of the Himbo-Top variety of raspberries, the phytopathogenic mycobiota, as in the rhizosphere of the Joan J plants, was represented by following fungi: *Alternaria alternata, Fusarium grameniarum, Aspergillus niger.* But the abundance of these micromycetes was slightly lower, in averaging 14% regardless of the variant (Table 3). Among them, as well as the cultivar of the Joan J variety, the species *Aspergillus niger* dominated. The saprophytic part of the mycobiota was represented by following fungi: *Penicillium herquei, Penicillium terrestre, Trichoderma viride*, whose density reached an average of 86%.

In the rhizosphere of Himbo-Top raspberry plants, a significant increase in phytopathogenic micromycetes was observed, both in the control variant and under the influence of foliar treatment with VITERI organic fertilizer compared to the Joan J variety (Table 3).

Table 3. Spectrum of phytopathogenic micromycetes of the raspberry plants` rhizosphere soil of the Himbo-Top variety

Variants	The proportion of phytopathogenic micromycetes in the mycobiota of the rhizosphere, %		
	phase of growth inflorescence	phase of separation of buds in inflorescences	phase of intensive fruiting
Control	*Alternaria alternata (2)* *Fusarium grameniarum(2)* *Aspergillus niger (10)*	*Botrytis cinerea (10)* *Alternaria alternata (6)* *Fusarium grameniarum (4)* *Aspergillus niger (40)*	---
VITERI fertilizer	*Alternaria alternata (2)* *Fusarium grameniarum(2)* *Aspergillus niger (10)*	*Botrytis cinerea (10)* *Alternaria alternata (4)* *Fusarium grameniarum (6)* *Aspergillus niger (60)*	*Alternaria alternata (20)* *Aspergillus niger (30)*
VITERI fertilizer + essential Basil oil	*Alternaria alternata (2)* *Fusarium grameniarum (2)* *Aspergillus niger (10)*	*Botrytis cinerea (9)* *Alternaria alternata (6)* *Fusarium grameniarum (5)* *Aspergillus niger (35)*	*Botrytis cinerea (10)* *Alternaria alternata (10)*
VITERI fertilizer + essential Fennel oil	*Alternaria alternata (2)* *Fusarium grameniarum (2)* *Aspergillus niger (10)*	*Botrytis cinerea (14)* *Alternaria alternate (10)* *Fusarium grameniarum (4)* *Aspergillus niger (4)*	*Alternaria alternata (10)*

Their number in the variants indicated varied in average from 36 to 80% respectively. In VITERI+Basil essential oil, equilibrium between saprotrophs and phytopathogens was observed, and in VITERI+Fennel essential oil, the number of saprotrophs was almost three times larger than the number of phytopathogenic micromycetes. The results of the studies show that in the phase of separation of buds in inflorescences by foliar treatment with organic fertilizer VITERI with the addition of Basil essential oil in the rhizosphere of plants of both investigated raspberries there is a stabilizing selection in the mycobiota of the rhizosphere.

It should be noted that in the phase of intensive fruiting, almost 80% of the mycobiota of the raspberry plant rhizosphere of the studied varieties were saprotrophic fungi of the genus *Penicillium (Penicillium terrestre, Penicillium breviocompactum and Penicillium ciplicissimum) and Trichoderma viride*. The significant inhibition of phytopathogenic mycobiota in the plant rhizosphere in all variants of the Himbo-Top variety was observed during this period. The control variant was dominated by saprotrophs. At the same time, the number of phytopathogenic micromycetes averaged 50% for the foliar VITERI treatment, while the essential compositions of Basil and Fennel were significantly reduced (up to 20% and 10%, respectively).

CONCLUSIONS

Essential oils and their compositions with organic fertilizer VITERI significantly affect the abundance and biodiversity of micromycetes in the rhizosphere soil of raspberry plants. Foliar treatment with fungal micromycetes in the mycobiota of the rhizosphere of plants of the studied raspberry cultivars is observed during foliar treatment with VITERI organic fertilizer with the addition of Basil essential oil in the phase of bud separation in inflorescences. The number of phytopathogenic micromycetes in the rhizosphere mycobiota of the Joan Jay variety was 42%, and in the mycobiota of the rhizosphere of raspberry plants of the Himbo-Top variety -55%. This indicates the high prospects of this composition for stabilizing the agrocenosis of raspberries and improving the quality and biosafety of growing crops in organic production.

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