

Soil health is the basis of organic agriculture

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Abstract. The biologization of agriculture is a topical issue both in Russia and in the world. The objective of the presented experiment was to confirm the positive effect biopreparations treatment of the fruit crops on the soil microbiota. As a result, in soil samples selected depend on the intensity of chemical fungicides application, from 1.15×10^4 to 1.23×10^4 CFU of micromycetes in one gram of absolutely dry soil were isolated. It was found that the largest amount of potentially pathogenic fungi was isolated in a soil sample cultivated using only chemical preparations - 20.5% of the total number of colonies. In the variant with the inclusion of biopreparation in the technology – from 7.2% to 11.0% of potentially pathogenic fungi of the total number of micromycetes. The most common among potentially pathogenic micromycetes were fungi of the genus *Fusarium* spp. Fungi of the genus *Trichoderma* were detected in all variants – not exceed 10% in the total number of micromycetes.

1 Introduction

Currently, organic agriculture is actively developing in more than 150 countries. The largest organic markets are in the USA, Germany and France. The products of Russian agricultural producers still occupy 0.2% of the world market, but in the future they can make up from 10 to 25%. Since the global demand for organic products now significantly exceeds the supply.

In 2020, the Federal Law "About Organic Products and About Amendments to Certain Legislative Acts of the Russian Federation" came into force in Russia: a national standard has been developed, harmonized with European requirements, a national certification system for organic products has been introduced.

Organic agriculture is a holistic production management system that contributes to the regeneration, conservation and development of the health of agro-ecosystems, including biodiversity, biological cycles and soil biological activity (FAO / WHO Codex Alimentarius Commission).

Many publications emphasize the need to minimize negative impact on the environment. By using elements of organic farming, traditional agriculture can be made more sustainable and environmentally friendly [1, 2].

It is possible to manage soil health by changing various agronomic practice, such as: tillage, crop rotation, fertilization, compost, manure, various pesticides. Soil quality is studied

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by various methods. For example, scientists determine enzymatic activity, study the biodiversity of organisms, etc. [3, 4, 5].

Despite the fact that the issue of soil health is very relevant and is being studied by scientists from different countries, at present there is no unambiguous understanding of how agro-technological and other methods, as well as types of farming, affect soil microorganisms. [6, 7].

Soil pathogens are becoming more harmful and difficult to control [8]. This is especially true for those crops where it is not possible to observe crop rotation, because they are perennial [9].

At present, heavy metals in the soil are studied in Russia mainly. The question of biodiversity and soil health have not been covered in the past 10 years. In this direction, scientists from Russian organizations work independently and in collaboration with foreign colleagues (Moscow State University [10-12], Russian Research Institute of Phytopathology [11, 12], Russian State Agrarian University - MAA named after K. A. Timiryazev [11], All-Russian Research Institute for Agricultural Microbiology [13], Saint Petersburg State University [13], Novosibirsk State Agricultural University [12, 14], Altai Research Institute of Agriculture [14], Chernyshevskii Saratov State University [15], A. N. Severtsov Institute of Ecology and Evolution [16], Kazan Federal University [17]).

Therefore, the study of soil microbiota and the influence of factors on the ratio of various microorganisms groups are actual. The aim of the presented experiment was to study the effect of reducing the number of chemical treatments on the soil microbiota.

2 Materials and methods

We took soil samples from an apple orchard in the Stavropol Territory of southern Russia and analyzed it for the content of fungi. Apple variety – Renet Simirenko. Soil samples were taken on October 31st after harvest.

The experimental scheme included:

Standard – traditional intensive plant protection technology of apple trees (use of only chemical insecticides and fungicides);

Bio 1 – integrated plant protection of apple trees (application of chemical and biological insecticides and fungicides);

Bio 2 – integrated plant protection of apple trees (application of chemical insecticides and fungicides, biological insecticides and a biofungicide prototype developed at the Federal Scientific Center of Biological Plant Protection).

The Standard system included 20 pesticide treatments. The integrated Bio 1 and 2 system included 13 treatments with chemical preparations and 7 treatments with microbiological preparations. The difference between the Bio 1 and Bio 2 systems was in which biofungicide the area was treated with. System Bio 2 was treated with a biofungicide based on the *Bacillus subtilis* strain IPM 215, Bio 1 – with an experimental sample of biofungicide based on the *B. velezensis* strain BZR 336g.

Soil samples were taken from each experimental site in a separate sterile paper bag and submitted to the Laboratory for the Development of Microbiological Crop Protection Products and Microorganisms Collection for further analyzes. The research used the material and technical base of the Unique Scientific Installation «Technological line for obtaining microbiological plant protection products of a new generation» (<http://ckp-rf.ru/> registered No. 671367).

Soil samples were dried on paper for 3 days, then the provided soil samples were crushed to a maximum size of aggregates of 0.5 cm. All foreign inclusions were removed manually during sieving.

After that, weighed portions of 1 g of soil of all studied samples (weighing-machine Adventurer Pro (AV4102C), Ohaus), pre-dried at room temperature and dried at 105°C to constant weight.

For mycological analysis, the prepared soil sample of 1 g (in triplicate) was thoroughly ground in a mortar, placed in a flask with sterile water (100 ml), and stirred for 1 hour on a New Brunswick Scientific Excella E25 shaker. Then the method of successive soil dilutions was used. Dilution was performed with Eppendorf variable volume pipettes. Aqueous suspension from dilutions 10², 10³ и 10⁴ dripped and carefully rubbed with a spatula over the surface of the agar medium. Inoculation was performed on Czapek's medium with pH = 4-4.5. The experiment was repeated three times. The Petri dishes was placed in a thermostat for 6 days at + 23°C. After 6 days, we counted and identified the total number of fungal colonies in each Petri dish. The identification was performed using an Axio Scope A1 microscope, Carl Zeiss with software for documenting and image processing. All cultures of micromycetes were identified to genus.

The number of colony-forming units was determined by the following formula:

$$M = \frac{A \times 10^n}{V} \tag{1}$$

A – average number of colonies,

n – dilution,

V – suspension volume taken for inoculation,

M – number of CFU in 1 g of soil.

The data obtained were recalculated per 1 g of absolutely dry soil.

3 Results and discussion

In soil samples, depending on the site, from 1,15 x 10⁴ to 1,23 x 10⁴ CFU micromycetes in one gram of absolutely dry soil (table).

Table. The number of fungi in one gram of absolutely dry soil sampled from experimental plots of an apple orchard.

| Microorganisms | | Standard | | Bio 1 | | Bio 2 | |
|------------------------|----------------------------|------------------------|------|------------------------|------|------------------------|------|
| | | average titer, CFU/g | % | average titer, CFU/g | % | average titer, CFU/g | % |
| Potentially pathogenic | <i>Fusarium spp.</i> | 2.00 x 10 ³ | 17.5 | 2.20 x 10 ² | 1.8 | 1.10 x 10 ² | 0,9 |
| | <i>Verticilium spp.</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| | <i>Cladosporium spp.</i> | 1.10 x 10 ² | 1.0 | 4.40 x 10 ² | 3.7 | 0 | 0 |
| | <i>Cephalosporium spp.</i> | 1.00 x 10 ² | 1.0 | 1.00 x 10 ³ | 4.6 | 1.00 x 10 ³ | 7,2 |
| | <i>Rizopus spp.</i> | 1.10 x 10 ² | 1.0 | 0 | 0 | 0 | 0 |
| | <i>Alternaria spp.</i> | 0 | 0 | 1.10 x 10 ² | 0.9 | 0 | 0 |
| Saprophytic | <i>Trichoderma spp.</i> | 1.11 x 10 ³ | 9.7 | 7.80 x 10 ² | 6.4 | 1.11 x 10 ³ | 9,0 |
| | <i>Penicillium spp.</i> | 2.67 x 10 ³ | 23.3 | 1.44 x 10 ³ | 11.9 | 1.44 x 10 ³ | 11,7 |
| | <i>Aspergillus spp.</i> | 4.67 x 10 ³ | 40.8 | 6.78 x 10 ³ | 56.0 | 7.22 x 10 ³ | 58,6 |
| | <i>Mucor spp.</i> | 0 | 0 | 1.10 x 10 ² | 0.9 | 1.10 x 10 ² | 0,9 |
| | <i>Trichothecium spp.</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| Other | | 6,70 x 10 ² | 5.8 | 1.67 x 10 ³ | 13.8 | 1.44 x 10 ³ | 11.7 |
| <i>Total amount</i> | | 1,15 x 10 ⁴ | 100 | 1,21 x 10 ⁴ | 100 | 1,23 x 10 ⁴ | 100 |

Analyzing the data obtained, it can be concluded that the largest amount of potentially pathogenic fungi was isolated in the soil sample cultivated according to the standart intensive technology - 20.5% of the total number of colonies. In the Bio 1 variant, its number was

11.0%, and in the Bio 2 variant (using an experimental sample of biofungicide, developed at the Federal Research Center for Biological Protection) - 7.2% of potentially pathogenic fungi of the total number of micromycetes. In the Bio 2 variant was noted the least variety of fungi among this group.

Fungi of *Fusarium* spp. were the most common among potentially pathogenic micromycetes. Depending on the variant of the experiment, they were allocated from 0.9% to 17.5%. The maximum quantity of the genus *Fusarium* was observed in the variant cultivated with the standard technology. On experimental sites, where seven treatments were performed with biological products for various purposes, colonies of *Fusarium* accounted for no more than 2% of the total number of micromycetes. It should be noted that fungi of this genus not only cause fusarium wilting, but are also producers of toxins, which, in turn, can also negatively affect the physiological state of plants.

In all studied soil samples, saprophytic fungi were represented mainly by micromycetes of *Penicillium* and *Aspergillus*. Their total amount in each of the variants exceeded 60% of the total number of isolated colonies. It should be noted that some members of the genera *Penicillium*, *Aspergillus* and *Mucor* are capable of infecting agricultural crops as a result of their ability to produce phytotoxins and rapidly colonize organic substrates.

Trichoderma is one of the most important indicators. Its sufficient content in the soil reduce the accumulation of potentially pathogenic microorganisms. Colonies of *Trichoderma* were distinguished in all variants. Their percentage in the total number of micromycetes did not exceed 10%. One of the criteria for assessing the biological activity of *Trichoderma* is its ability to inhibit the growth of other microorganisms. So, in variants Bio 1 and Bio 2, 6-8% more "other" micromycetes were noted than in the Standard variant. This group includes, among other things, fungi which growth suppresses *Trichoderma*.

A significant number of potentially pathogenic fungi, genera *Penicillium* and *Aspergillus*, as well as a low content of *Trichoderma* spp. indicated that the soil is not so favorable for crop production. One of the ways to increase the suppressiveness of soils (the ability to reduce the parasitic activity of soil harmful organisms) is the introduction of biological preparations based on fungi of the genus *Trichoderma* into field.

As a result of the experiment, the relationship between the microbiological diversity of the soil and the number of chemical treatments during the growing season of the apple tree was revealed. Thus, it can be concluded that it is necessary to use biological and integrated plant protection systems to improve soil health.

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