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BOOK OF ABSTRACTS

Un pour tous, et tous pour un !

Belgrade , 2022

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FOREWORD

The International Research Academy of Science and Art is the host of the forth IRASA International Scientific Conference "SCIENCE, EDUCATION, TECHNOLOGY AND INNOVATION – SETI IV 2022".

The conference is multidisciplinary oriented.

Thematic fields of the SETI IV 2022 conference are the following:

A. Science, technology and innovation
B. Education and knowledge for the 21 century
C. Preservation and improvement of the environment and human health
D. Governance and sustainable territorial development
E. National security and protection

The **aims** of the SETI IV 2022 Conference are the following:

- Improving knowledge basis for sustainable and resilient local, national, transnational and global development
- Support and harmonization of the implementation of sustainable development goals in society, economy, environment and urbanization
- Strengthening scientific, technological and innovative capacities at local, national and transnational level
- Recommendations for the improvement of education, research and security, and governance of the environmental, urban and territorial development
- Dissemination and intensification of professional communication and establishment of network for joint research, innovation and education
- Dissemination and intensification of professional communication and establishment of network for security challenges and risks in environment and society
- Experience Exchange Based on Best Practices

The **results** of the SETI IV 2022 Conference are presented in two publications:

- The Book of Abstracts,
- The Book of Proceedings.

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MICROBIOLOGICAL FERTILIZERS IN THE FUNCTION OF ORGANIC PRODUCTION AND HEALTH SAFE FOOD

Aleksandar Stevanović[63]; Ljubica Šarčević-Todosijević[64]; Jelena Bošković[65]; Jelena Mladenović[66]; Vladan Pešić[67]; Vera Popović[68]; Vladica Ristić[69]

Abstract

Environmental pollution is one of the main problems facing humanity on a global scale, as well as the leading cause of the growing incidence of certain human and animal diseases. Organic production as a sustainable system combines tradition and new technological solutions in the prevention of environmental pollution, both from the aspect of biological diversity of flora and fauna in the agroecosystem, and from the aspect of nutritional values and absence of pesticide residues in plant and animal products. Organic production enables the protection of biodiversity and the environment. An important feature of organic agriculture is the exclusion of the use of mineral fertilizers and pesticides, due to the potential negative effects that can be caused by their uncontrolled use. In organic production, mostly organic fertilizers and biological preparations are used, which enable the decomposition of harvest residues and the release of plant assimilates. In recent times, the possibilities of using allelopathic substances and secondary metabolites of plants as growth regulators and natural herbicides in sustainable agriculture are expanding, while the most important alternative to mineral fertilizers are microbiological fertilizers. In this paper, the importance and scope of application of microbiological fertilizers in modern agricultural production are considered.

Key words: *Organic agriculture, biodiversity, microbiological fertilizers, safe food.*

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Introduction

The increase in population on the planet has caused the intensification of agricultural production, increased and uncontrolled consumption of mineral fertilizers and pesticides, and therefore environmental pollution. Given that environmental pollution is one of the main problems facing humanity and is the leading cause of the increasing occurrence of certain human and animal diseases, organic production is one of the main ways to prevent environmental pollution and preserve human health. Organic production preserves natural resources, and the use of chemical agents in plant cultivation is reduced or completely eliminated [1, 2].

Due to the use of many chemical substances, which are applied in conventional, plant and livestock agricultural production (pesticides, hormones, antibiotics,...), and which, due to improper and uncontrolled use, accumulate in products, endangering human health, mineral fertilizers are a significant critical a point in considering the health safety of food and the impact on people's health and quality of life.

The need for a healthier environment and the numerous negatives caused by conventional agriculture have led us to turn more and more to organic agriculture, which combines the principles of ecology and agriculture, ensures sustainability, efficiency of the agro-ecosystem and new technical-technological solutions, with the aim of producing quality (nutritional and health-safe) food while protecting biodiversity and the environment [3].

Ecological, biological or organic agriculture was created as a reaction to the increasingly pronounced negative consequences on the environment (soil, water, air, biodiversity) and human health, with the aim of reducing all forms of pollution, preserving natural resources, increasing soil fertility, and producing healthy food high quality and improving people's health. Organic agriculture in the world and here, in the last decade, is gaining more and more importance. In relation to conventional, organic agriculture has an advantage from the aspect of preserving biodiversity - the biological diversity of plant and animal life in the agrobiotope and from the aspect of preserving soil fertility. Organic production is one of the main ways of preventing environmental pollution, and thus preserving health.

Our country has excellent conditions for the production of health-safe, organically produced food of high quality [1, 4].

In this paper, the impact of the application of mineral, organic and microbiological fertilizers in modern agricultural production, and their impact on the environment, biodiversity and health food safety is considered.

Influence of mineral and organic fertilizers on the environment, biodiversity and food safety

In addition to the preservation of natural resources and all components of the environment, the leading challenge in environmental protection is the production of health-safe food. Given that plants form the basis of food chains in the biosphere, and

that in food production on a global scale the primary role belongs to plant production, it is extremely important to prevent contamination of plant products in all stages of production, processing, storage and preservation. Plant products are most often contaminated with pathogenic microorganisms and chemical pollutants. Very dangerous pathogenic microorganisms enter the soil and cultivated plants most often through improperly processed organic fertilizers, water used for the application of pesticides, and contaminated irrigation water. Chemical pollutants that can be found in food of plant origin originate from natural sources, as well as from all stages of plant production. These substances can have toxic, mutagenic and carcinogenic effects in the human body. Uncontrolled application of NPK fertilizers and pesticides should be highlighted as a source of very dangerous substances that reach the environment and plants in direct crop production.

Uncontrolled application of fertilizers causes eutrophication and groundwater pollution. Accumulation of nitrates and heavy metals in soil and plants leads to the formation of carcinogenic nitrosamines, while long-term use of pesticides leads to their accumulation in plants and other links of the food chain and the manifestation of toxic mutagenic and carcinogenic effects on animals and humans [5, 6, 7].

It is important to note that in crop production, the use of chemical agents, primarily mineral fertilizers and pesticides, which are necessary for achieving high yields and feeding all mankind, cannot be completely excluded. Therefore, their rational application is important in order to produce healthy food and preserve all components of the environment [7]. Soil, water and air are equally important components of the environment, which enable the maintenance of life on the planet, and environmental protection is a preventive measure in the protection of human health [8, 9].

Mineral fertilizers are inorganic substances, of natural or industrial origin, which contain macro and micro biogenic elements necessary for plant nutrition. To the greatest extent, the formulations contain basic plant nutrients: nitrogen, phosphorus and potassium, while in certain cases secondary elements (sulfur, calcium and magnesium) and microelements (Fe, Mn, Zn, Co, B, Mo) are applied. Uncontrolled and inappropriate application of mineral fertilizers represents a potential danger to the environment and food safety, and therefore to human health. The cause of the problem is the insufficiently efficient uptake of nutrients from the applied fertilizers by the cultivated plants, whereby a significant part of the fertilizer, as a source of contamination, remains unused in the soil, from where it is drained to other parts of the ecosystem through natural flows [10, 11].

In organic fertilizers, biogenic elements are found in organic form, and they have an extremely favorable and long-term effect on fertility and structure, biological activity, water, air and heat regime and a number of physical and chemical properties of the soil, and indirectly affect the mineral nutrition of cultivated plants. The following are used as organic fertilizers: manure, compost, sedge, peat, vermicompost, green manure, wood ash, vegetable solutions and other waste organic substances created as side products in food technology and industry [12]. Microbiological preparations (microbiological fertilizers, biofertilizers) belong to the group of fertilizers or substances that are used in organic agriculture in order to provide more favorable

conditions for the nutrition of cultivated plants, and whose application is also recommended in conventional agriculture [12, 13].

Application of microbiological fertilizer bioprepares - microbiological fertilizers

Plant protection technologies that are being pursued today include the creation of unfavorable conditions for the development of plant diseases by applying agrotechnical measures and biopreparations. The active components of biopreparations are living organisms or products of their life activity, they are safe compared to chemical preparations and their application achieves the change of environmental pollution [8, 14, 15, 16].

There are a large number of microbial fertilization biopreparations, that is, microbiological fertilizers, as an alternative to the application of mineral fertilizers. They also act as plant growth stimulators. In the composition of this group of fertilizers, bacteria from the ecophysiological group of aminoheterotrophs are particularly important, as well as free nitrogen fixers of the bacterial genus Azotobacter, and symbiotic nitrogen fixers of the bacterial genera Rhizobium and Bradyrhizobium [8, 14, 15, 16].

Common to all microbiological fertilization biopreparations is that they contain live microorganisms, which, when introduced into the rhizosphere, increase the availability of nutrients and improve plant growth. Also, these preparations do not contain chemical additives, and in addition to plants, they also have a positive effect on the soil and the environment. Microorganisms in the composition of microbiological fertilizers produce a variety of biologically active substances, amino acids, polysaccharides, organic acids, enzymes, vitamins, which positively influence the yield of cultivated plants. The living components of microbiological fertilizers consist of various taxa of bacteria, fungi and algae [15].

It is important to note that the total number of microorganisms is the most important indicator of soil fertility and varies, depending on the type of soil and the effect of environmental factors. Based on the results of two years of research, Šarčević - Todosijević et al. (2016) point out that the total number of microorganisms in the chernozem of Zemun Polje ranged widely, from 53.3 - 501.2x105g-1. The total number of microorganisms is in significant and very significant correlative relationships with representatives of different ecophysiological groups of microorganisms [18].

Given the importance of nitrogen in plant nutrition, bacteria-based fertilizers are most often used in practice, which enable the supply of nitrogen assimilatives. These are bacterial species, which simultaneously participate in the biogeochemical cycles of nitrogen circulation [19, 20].

Nitragin (rhizotrophin) is a biopreparation based on nodular bacteria of the genus Rhizobiumi Bradyrhizobium. It is used in order to encourage the formation of nodules and increase symbiotic nitrogen fixation, especially in legumes [15].

Microbial biofertilization biopreparations or microbiological fertilizers play a significant role in reducing soil and agroecosystem pollution in agricultural

production. They represent a biological alternative to the application of mineral fertilizers in agriculture, especially nitrogen, which reduces environmental pollution [15, 21, 22].

The mentioned natural products exhibit an active biological effect, allelopathic, herbicidal and toxic, and are mostly isolated from lower organisms (algae, bacteria, fungi), commercially produced and applied. Thus, a number of plant protection products with bactericidal and fungicidal effects were isolated from bacteria (Blasticidin-S, Harpin protein, Kasugamicin, Mildiomycin, Oxytetracycline, Polyoxin, Streptomycin, Validamycin) method [23]. The occurrence of allelopathy is responsible for preventing the development of phytopathogenic microorganisms [15, 19]. Certain plant species produce different metabolites (essential oils and plant extracts), which exhibit pesticidal properties and are called botanical pesticides [22].

Milić et al. (2001) point out that in Serbia, for many years, research has been carried out into the justification of the use of inoculation in the production of vegetable and fodder legumes. They also point out that the application of these fertilizers achieves extremely good results in the cultivation of other plant species, and therefore they are recommended for wide production. Fertilizers produced at the Scientific Institute for Agriculture and Vegetables in Novi Sad (NS Nitragin) demonstrated the justification of the application. Application of inoculation is particularly justified in the production of beans, peas and alfalfa. Symbiotic nitrogen-fixing bacteria of beans and alfalfa are few, especially in soils with a lower pH value. Thus, when sowing beans and alfalfa, inoculation with effective and acid-resistant strains of these bacteria increases nitrogen fixation and enables their cultivation even on less fertile soils. By applying the mentioned nitrogen-fixing strains, large and reddish nodules are formed on the central roots of the inoculated plants, and the yield and nitrogen content in the grain increases. By inoculation with symbiotic nitrogen-fixing bacteria, legumes are independently provided with the necessary amounts of nitrogen, which has economic and ecological significance because the need to apply larger amounts of nitrogenous mineral fertilizers is reduced [24].

Govedarica et al. (2002) investigated the effectiveness of a mixture of bacterial species Azotobacter chroococcum, Azotobacter vinelandii, Azospirillum lipoferum, Bacillus megaterium and Bacillus subtilis in three maize hybrids (NSSC-640, Balkan, NSSC-666), three sunflower hybrids (NS-H-111, NS -H-17, NS-H-45), three sugar beet varieties (Dana, NS-HY-11, Alina) and three wheat varieties (Pobeda, Evropa-90, NS-Rana-5). The tested plant species were grown on carbonate chernozem type soil. Unlike the control variant, the seeds of corn, sunflower, sugar beet and wheat were inoculated with a mixture of bacteria before sowing. At the end of the growing season, the yields and microbiological activity in the rhizosphere soil of corn, sunflower, sugar beet and wheat were determined. The high effectiveness of the examined mixture of bacteria was determined by these studies. Bacterial inoculation caused a high yield increase in all three maize-sunflower hybrids and all three varieties of sugar beet and wheat. In the rhizosphere soil of all inoculated plant species, compared to the control, a statistically significant increase of all useful ecophysiological groups of microorganisms was found: the total number of microorganisms, abundance of ammonifiers, actinomycetes, azotobacter, oligonitrophilic bacteria, as well as

dehydrogenase activity. In contrast, a decrease in the number of fungi was observed. Therefore, the application of bacteriization increases the yield of cultivated plants, and their production is more economical and environmentally friendly [25].

Mrkovački et al. (2016) studied the effect of inoculation with the bacterial taxon Azotobacter chroococcum on sugar beet yield parameters and soil microbiological activity. As an inoculum, a mixture of three strains of the bacterial species Azotobacter chroococcum was used. Inoculation was performed twice: (A) by introducing the strains into the soil before sowing; and (B) reintroducing the strains into the soil two weeks after sowing. Biochemical analyzes of isolated strains of microorganisms from treated soil confirmed their ability to produce auxin, a plant growth hormone. In addition to the positive effects on the tested sugar beet yield parameters, the inoculation had a stimulating effect on the abundance of azotobacter and free nitrogen fixers in the rhizosphere, the ability to dissolve phosphorus and the availability of phosphate to plants, that is, on the overall fertility of the soil [26].

In a Polish trial, Dozet et al. (2018b), under conditions of organic production, the influence of different genotypes and applied microbiological fertilizers on yield and yield components of garden peas was observed. The influence of applied microbiological fertilizers was statistically significant. Significantly more technologically mature pea pods were observed in the variant with the application of Nitragin (15.56), compared to the control (13.32), without the application of fertilizers [27, 28].

Although in practice it has been shown that they are most effectively applied on vegetable and fodder crops [15], fertilization biopreparations are characterized by a wide range of effects.

Thus, in the experiment Mijović et al. (2016), carried out at the Experimental Farm of the Faculty of Biotechnology in Podgorica, one of the variants of testing the effect of fertilizers on the Kardinal grape variety was a variant fed with Slavol fertilizer (a bio-organic fertilizer that contains bacteria and vitamins, enzymes and growth stimulators). The yield of grapes per vine in this variant was 4.20 kg, that is, it was 1.25 kg higher than the control (without foliar application). Fertilizer microorganisms colonize the root system, where they perform the processes of nitrogen fixation, phosphomineralization, humification and directly supply plants with assimilatives [14].

Azotobacterin is a biopreparation, which is based on the ability of species of the genus Azotobacter to reproduce in the rhizosphere of field crops and improve the nitrogen nutrition of plants through nitrogen fixation [15].

Azotobacter sp. it belongs to the group of free nitrogen fixers, a group of microorganisms that absorb elemental nitrogen and reduce it to ammonia form.

It is represented in a large number of soil types, but it is particularly rich in chernozems, which belong to soils of pronounced biogenicity and fertility. In the research of Šarčević–Todosijević et al. (2017), the abundance of Azotobacter sp. in the chernozem at the Zemun Polje site, the seod ranged from 100.4 to 182.7 (102 g-1), and in the chernozem at the Rača Kragujevacka site from 45.7 to 119.2 (102 g-1).

The process of nitrogen reduction consists of a series of biochemical reactions, catalyzed by the enzyme nitrogenase, and is characterized by exceptional sensitivity

to molecular nitrogen, which is an energetic hydrogen acceptor and depresses the creation of reduced forms of nitrogen [29]. Studying the influence of increasing amounts of nitrogen fertilizers on the abundance of Azotobacter sp., Šarčević - Todosijević et al. (2017) found that increasing amounts of nitrogen fertilizers significantly and very significantly reduced abundance compared to the control (variant without fertilizer application), which is in line with other research [30, 18, 31].

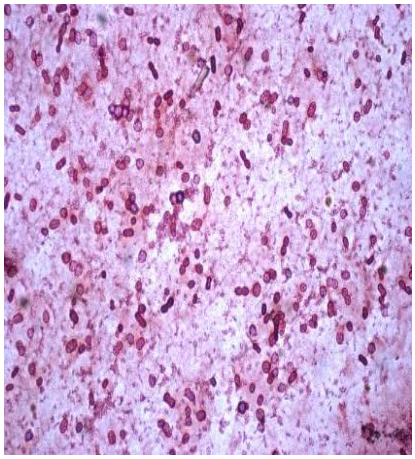

Figure 1. Azotobacter sp., isolated from soil samples of the chernozem type, on the nutrient medium of Fyodorov (Šarčević - Todosijević et al., 2017)

Zivanović et al. (2020) examined the productivity of beans depending on NPK fertilization, nitrogen fertilization and seed inoculation with nitrogen-fixing bacteria in the agroecological conditions of central Šumadija, on gajnajča-type soil. Bean productivity parameters were more strongly influenced by fertilization with mineral nutrients compared to seed inoculation with nodule bacteria. Compared to the variant without fertilizing, enhanced by supplementary nutrition with mineral fertilizers, the number of pods per plant increased from 27.9% (AN) to 52.9% (NPK+AN), the number of grains per plant from 11.9% (AN) up to 31.1% (NPK+AN), as well as grain yield from 8.6% (AN) to 32.1% (NPK). The highest yield of bean grains (1.97 t ha-1) was achieved in the combination of NPK fertilizer application and seed inoculation, which is the author's recommendation to bean producers when choosing a fertilization system on the soil of the seeder type (Živanović et al., 2020). Bean (Phaseolus vulgaris L.) is the most important leguminous plant grown for food purposes (Figure 2). [33]
Mineral fertilizers, applied in rational and optimal amounts for plant nutrition, are key to increasing the yield of cultivated crops and intensifying biological processes in the soil, beneficial for ecosystems. In contrast, high doses of applied nitrogen mineral fertilizers show opposite tendencies, cause increased mineralization of humus and other nitrogenous compounds, accumulation of nitrates, reduce the share of biological nitrogen in plant nutrition, which leads to deterioration of soil properties and drop in crop yields. At the same time, heavy metals are introduced and accumulated in the soil, enter the food chain and threaten the health safety of food. Therefore, the rational

use of mineral fertilizers is a priority that should be pursued in plant production [1, 15].

Figure 2. Phaseolus vulgaris L., Fabaceae [24]

The most important goals of sustainable crop production include reducing the use of pesticides, using biological protection measures and biopesticides (biological preparations for protecting plants from pests). Biopesticides and microbial fertilization biopreparations represent one of the most significant discoveries of biotechnology. Their application, as an alternative to chemical pest control and the application of mineral fertilizers, achieves a change in environmental pollution and preserves the health and safety of food [1, 15, 16, 36]. The use of microbial antagonists in the control of plant pathogens is particularly significant, considering that they do not show negative effects on the environment [1, 37].

In healthy plant production, in addition to chemical pollution of the environment, it is necessary to prevent biological pollution, that is, the spread of human and animal diseases through pathogenic microorganisms that can be found in the soil and on cultivated plants [15]. In this way, diseases caused by very dangerous taxa of microorganisms such as: Salmonella sp., Shigella sp., can be transmitted. Klebsiella pneumoniae, Bacillus cereus, Clostridium botulinum, Escherichia coli, Pseudomonas sp., Yersinia sp., Listeria monocytogenes, Vibrio cholerae, etc., and most often reach the soil through water and animals [2, 36, 38]. From the aspect of health-safe plant production, it is important to mention the possibility of contamination of plant products with mycotoxins originating from molds of the genera Aspergillus, Penicillium and Fusarium. It has been scientifically proven that the mentioned toxins exert a carcinogenic effect on humans and animals, whereby aflatoxin B1 (AFB1), a product of the fungal species Aspergillus flavus, represents the most carcinogenic substance of biological origin. The mentioned taxa of fungi often contaminate cereals, almonds, walnuts, peanuts and similar plant products, and develop as a result of food damage by insects, inadequate drying and storage in humid conditions. Therefore,

proper storage and preservation of plant products is the final, but equally important stage in the process of health-safe plant production [41, 42, 43].

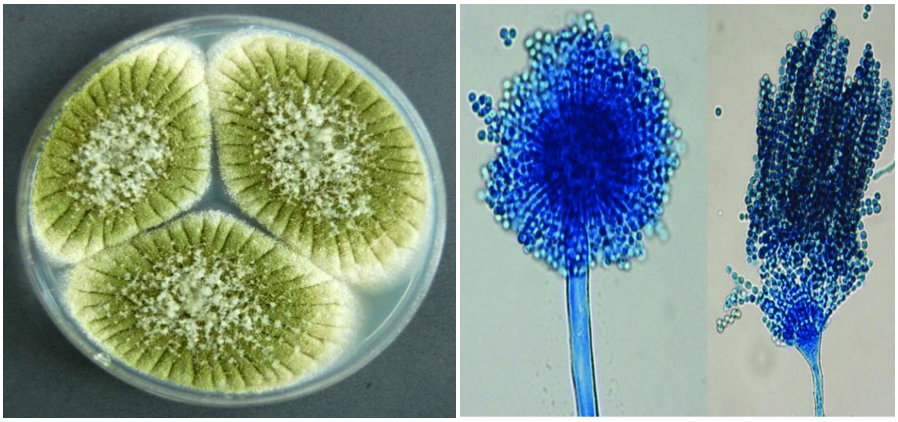

Figure 3. Aspergillus flavus

Conclusion

The most important goals of sustainable plant production are the reduction of pesticide use, the use of biological protection measures and biopesticides. Biopesticides and biopreparations for microbial fertilization represent one of the most significant discoveries of biotechnology. The application of microbial antagonists in the control of plant pathogens is particularly significant, given that they do not have negative effects on the environment [8, 16, 27, 34, 37]. In organic production, one of the basic aspirations is "biologization". First of all, this is achieved by using biopreparations for microbiological fertilization. As active components, these preparations contain microorganisms from different ecophysiological groups, which participate in the cycles of matter circulation on the planet, and are responsible for the formation and maintenance of soil fertility. [32, 35, 44].

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