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IMPORTANCE OF BIOLOGICAL SUPPLEMENTS BASED ON *BACILLUS SUBTILIS*

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Abstract: *In order to fully upgrade the ecological state of the environment, we must resort to alternative agricultural production systems that protect, maintain and improve the processes of pedogenesis and soil bioproductivity, implementing organic feritlizers and microbiological supplements. Factors unducing plant diseases may incur significant damage to cultivated species, by diminishing the quality and destroying the yield. Due to negative impact of chemical pesticides on the environment and human health, the possibility of implementing biopesticides in intensive plant protection has lately become the object of intensive studies. This study points out the anti-microbe effect of biological suplementproduced on the basis of Bacillus subtilis.*

Key words*: Bacillus subtilis, microbiological supplements, plant pathogenes*

INTRODUCTION

Numerous technogenous and anthropogenous factors exert highly negative impact on the environment (Đukić and Mandić, 2000; Đukić et al., 2007a), accordingly the issue of diminishing the impact is being raised. According to Solanka et al. (2015), microorganisms that exert antagonist properties to pathogenes parasitizing the cultivated plant species, have the ability to substitute chemical pesticides with negative environmental impact. Ecological state improvement may be contributed to by transition of certain types of agricultural production to harmless methods, including partial or full substitution of chemical plant protection agents for biological ones, such as microbiologically originated supplements (Đukić et al., 2007b). Here not only

the live cultures of microorganisms-introducents, but the products of their metablism may also act as basis for the supplement.

Microorganism-based biopesticides may represent an alternative course of crop protection, due to its safety for people and untargeted organisms, both in individual applications and within an integrated pest management (IPM) system. Gašić and Tanović, 2013; Seiber et al., 2014; Mnif and Ghribi, 2015). Marketing share of biopesticides in the global market of pests and pathogenes eradication products is still low, but there is a constant increase in their imoplementation and production (Damalas and Koutroubas, 2018). For a biopesticide to be accepted in broad application, it has to fulfill several conditions: to be economical to produce, be consistently stable in warehousing, have high residual activity, be easily managed, mixed and applied, and provide efficient control of targeted pests (Gašić and Tanović, 2013).

The aim of this study is to point to great potential of alternative systems of managing agricultural producition that lead to increase in biological productivity of plants and to protection of the environment.

Advantages of biological supplements

Advantages of microorganism-based biological supplements are numerous: they are ecologically harmless, do not pollute the environment, are neither harmful to humans and animals nor phytotoxic, and do not act as a mutagen. Live microorganism cultures, constituting ingredients of biological supplements, are able to fixate the atmospheric nitrogen, synthesize plant growth stimulators and vitamins, convert certain chemical compounds (e.g. phosphates) to forms that are better absorbed by plants, as well as colonize the root system or plant phyllosphere.

The foundation of anti-microbe activity of biological supplements based on microorganisms lies in their ability to synthisize biologically active compounds, such as antibiotics and similar compounds, pigments, siderophores (catecholates and hydroxamate type), etc.

Practically speaking, the most important group of biological supplements is the one intended for protection of plants against bacterial and fungal infections. Diseases of thess types are the most common in agricultural plants and do not affect only vegetable crops of open and closed spaces, but also the cereal grains, leguminosae and a large number of other plants, causing significant reduction in quality and yield loss (Đukić et al., 2007c).

A spectrum of biological supplements for struggle against fungal diseases is still narrow. The most well known among them are: Trihodermin, Trihocetin, Fitolavin-100, Mikolitin, Baktofit, Rizoplan, Bacifit. As for the group of supplements active against diseases of plant bacterial nature, there are only a few of them, most prominent of which are: Agrofil – biological

supplement based on bacteria *Agrobacterium tumefaciens* and Mikolin – based on *Bacillus mycoides*. Reviewing the Register of pesticides in agriculture and forestry of the Republic of Serbia for 2020, biofungicides that have been registered and licensed for the market are the following: Ekatrasol F (*B. subtilis* Č 13), F stop (*B. subtilis* ST 1/III), Bacillomix aurum B (*B. subtilis* BS10), Erwix (*B. subtilis* BS10) and Polyversum (*Pythium oligandrum*) (Tim priređivača, 2020):

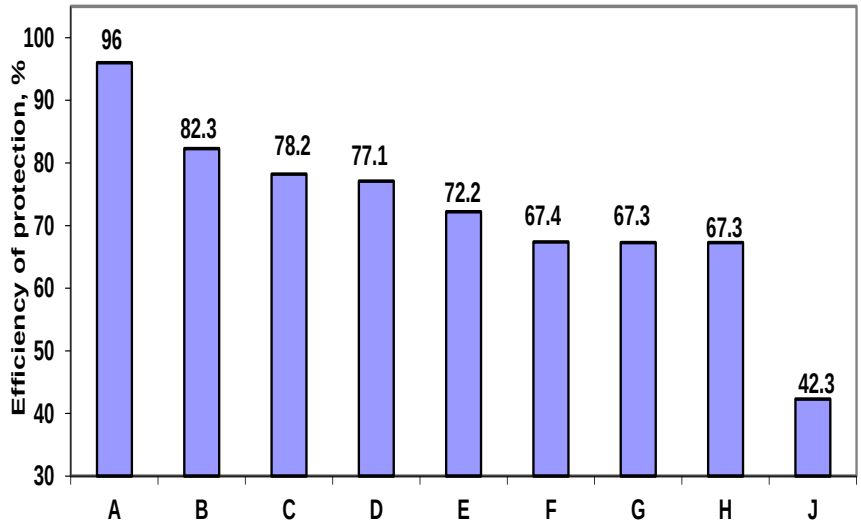
Suppressing of pathogenic bacteria and fungi by implementing biological agent *Bacillus subtilis*

There's been an ever increasing use of biopesticides lately in control of phytopathogenic bacteria (Agrios, 2005; Alabouvette et al., 2006). Assis et al. (1996) have studied the antagonism between 32 epiphyte isolates of *Bacillus* spp. from cabbage, kale and radish, 13 of which have reduced by 100 % the occurrence of black rot in kale in greenhouse conditions. In field experiments the occurrence of black rot in cabbage has been reduced by 48 - 78 % by applying the same 13 isolates of *Bacillus* spp. (Assis et al., 1997). Representatives of the *Bacillus* spp. genus have ben involved in control of plant diseases, through various mechanisms of operation, such as competition over food and space, induction of systemic resistability, production of antiboitics (Monteiro et al., 2005). This genus consists of diverse group of gram-positive, aerobic or facultative anaerobic bacteria, with extreme ability to quickly adapt to various environmental and nutritive conditions, and to produce a wide spectrum of bioactive metabolites (Perez-Garcia et al., 2011). Among the well-known representatives of the mentioned genus, *Bacillus subtilis* stands out, being able to produce numerous antibiotics (bacilisin i fengimicin, dificidin i oxidificidin, bacitracin, bacilin i balilomicin b and iturin) affecting numerous types of aerobic and anaerobic bacteria (Grahovac et al., 2009). Certain strains of *B. subtilis* genus, the cultures of which synthesize antibiotics, exert 100% anti-microbe activity in relation to a high number of phytopathogenic bacteria, such as *Erwinia*, *Pseudomonas*, *Xanthomonas* (Vlajiæ et al., 2016), *Clavibacter* (Milijaševiæ-Marèiæ et al., 2018), but aslo of the fythopatogenic fungi of genera: *Sclerotinia* (Radujkov et al., 2015), *Verticillium* (Milijaševiæ-Marèiæ et al., 2018), *Fusarium* (Feldeždi et al., 2016), *Trichoderma pleuroti and Trichoderma pleuroticola* (Potoènik et al., 2019), *Rhizoctonia solani* (Solanaki et al., 2015), *Alternaria*, *Ascochita*, *Botrytis*, *Phytophtora*, etc. Here it was determined that both the vegetative and generative functions of mold are suppressed.

Selected strains of this bacteria genus synthesize an antibiotic of aromatic nature, with aminoglycoside component. The antibiotic is resistant to proteolysis, temperature and pH of the environment. The metabolism products,

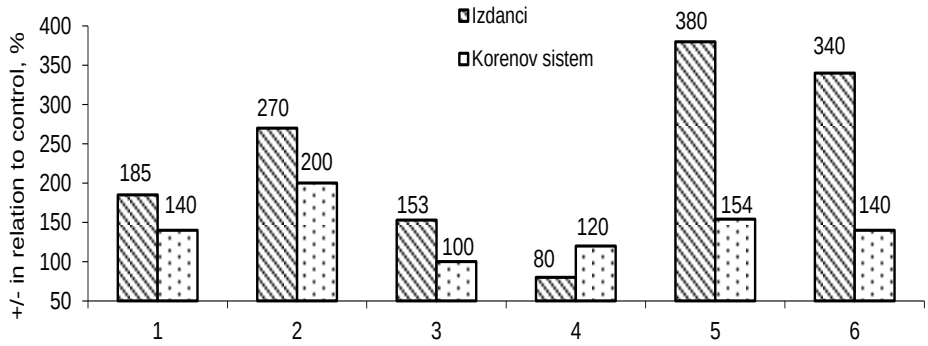
lipopeptides, affect various components of the cell wall, preventing adhesion to plant organs, while the enzym *subtilin* obstructs the development of pathogenes (Klokoèar - Šmit et al., 2003). However, toxine production slowing down the growth of pathogenes - the antibiosis mechanism, is one of the most important mechanisms (Thomashow and Weller, 1996).

Biological supplements, produced on the basis of this bacteria, that is, some of its strains, such as Bactogen, for example, exert high anti-microbe activity in closed space conditions, and can therefore be considered as highly effficient in protection of plants against bacteriosis, fungal infections and nematodes (Graph. 1).



Graph. 1. Efficiency of bactogen application in protection of vegetables against bacterial and fungal infections: A-Bacteriosis; B- Root rot; C- Powdery mildew; D- Ascochitosis; E-Peronosporosis; F- Cladosporosis; G- White rot; H- Black rot; J- Nematodes

Apart from the anti-microbe activity, this supplement is capable of stimulating plant growth and development (Jemcev and Đukić, 2000). Its effect is particularly evident in early stages of plant development (Graph. 2). The supplement helps increase the vegetable crop yield (1,1 - 1,2 kg/m^2 in cucumber; 2 kg/m^2 – in tomato, for example) – Maksimova et al. (2000).



Graph. 2. Phytostumulating effect of bacterial supplement produced on the basis of *Bacillus subtilis* (bactogen): 1- cucumber; 2- wheat; 3- corn; 4- barley; 5- flax; 6-rye

CONCLUSION

Biological supplements, produced on the basis of *Bacillus subtilis*, exert manifest a multifold positive effect :

- protect plants against bacterial and fungal infections;
- stimulate plant growth and development;
- to a certain extent represent an alternative to chemical means of plant protection, contributing to protection of the environment.

Due to all of the above, if used correctly and timely, the supplements based on the mentioned biological agent can be an adequate substitute for chemical pesticides. This will respectively lead to production of health-safe food and preservation of the environment.

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