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Microorganisms number in sorghum (*Sorghum bicolor* (L.) Moench) rhizosphere after herbicide, plant growth regulator, and a biopreparation use

Liczba mikroorganizmów w ryzosferze sorga (*Sorghum bicolor* (L.) Moench) po zastosowaniu herbicydu, regulatora wzrostu roślin i biopreparatu

Summary: In the modern agricultural production, the use of herbicides and other biologically active substances is an important part of the cultivation technologies of most cereals, including the grain sorghum. It is known that most preparations, including the chemical ones, can directly or indirectly influence the development of microorganisms in the rhizosphere of plants, but the nature of their effect on the number of microbiota of grain sorghum rhizosphere has not been studied enough, which reasoned the relevance of this research. The number of microorganisms in the grain sorghum rhizosphere (hybrid Milo W) was studied during 2019–2020 under the treatment by the herbicide Citadel 25 OD (0.6; 0.8 and 1.0 $dm^3 \cdot ha^{-1}$), plant growth regulator Endophit L1 (30 $cm^3 \cdot ha^{-1}$) and biological preparation Bioarsenal (800 $g \cdot 100\ kg^{-1}$). The analysis of the obtained experimental data showed that use of the studied preparations both separately and in different compositions had a stimulating effect on the number of grain sorghum rhizosphere microbiota, which was observed in its increase, especially in variants with the combined use of the herbicide Citadel 25 OD, plant growth regulator Endophit L1 and the biopreparation Bioarsenal (compared to the control the number of rhizosphere microbiota increased by 29.4–80.6% in average by groups).

Key words: microbiological activity, rhizosphere, grain sorghum, herbicide, plant growth regulator, biopreparation

INTRODUCTION

Soil is an extraordinarily complex, multi-component system, an important part of which are microorganisms. In particular, the microbiota plays a decisive role in the mineralization of the organic matter and the formation of soil fertility [Patyka et al. 2014].

It is known that the root system of plants and the adjacent layer of soil (rhizosphere) coexist with microorganisms in a mutually beneficial way: the root system supplies the microorganisms with the dead residues and exudates (carbohydrates, amino acids, organic acids, flavonoids, glucosinolates, auxins, etc.) [Hartmann et al. 2008, Badri and Vivanco 2009, Zhalnina et al. 2018]; the rhizosphere microbiota serves to inhibit soil pathogens and increase the availability of nutrients to the plants by producing phytohormones and other biologically active substances [Henis 1986, Treesubsuntorn et al. 2018]. Therefore, the rhizosphere microbiota can influence the resistance of plants to adverse environmental factors. It is known that various physiologically active substances, including herbicides, plant growth regulators, and biopreparations, are widely used in most crops including crops of the grain sorghum. However, scientific evidences [Khamova et al. 2016, Qian et al. 2018, Bezuglova et al. 2019, Huliaieva 2019, Shutko 2019] shows that these preparations can have different effects on the formation of number and structure of the rhizosphere microbiota. Thus, Karpenko et al. [2019] note that pre-sowing treatment of soybean seeds by microbial preparation Ryzoaktyv (*Bradirhizobium japonicum M-8*) at the rate of 2 $dm^3 \cdot t^{-1}$ and further application of herbicides Gezagard 500 FW (promethrin, 500 $g \cdot dm^{-3}$) at the rates of 3.0; 4.0; 5.0 $dm^3 \cdot ha^{-1}$, Prymekstra TZ Gold 500 SK (S-metolachlor, 312.5 $g \cdot dm^{-3}$ + terbuthylazine, 187.5 $g \cdot dm^{-3}$) at the rates of 4.0, 4.25 and 4.5 $dm^3 \cdot ha^{-1}$, and Kratos (acetochlor, 900 $g \cdot dm^{-3}$) at the rates of 2.0, 2.5 and 3.0 $dm^3 \cdot ha^{-1}$ create more favorable conditions for the formation of symbiotic apparatus of the soybean and development of certain groups of microorganisms in the rhizosphere, which number increased on average by 31.1–56.3%. At the same time, use of the herbicide Kratos at the rates of 2.0–3.0 $dm^3 \cdot ha^{-1}$ resulted in 15.3–17.3% suppression of nitrifying and cellulolytic microorganisms, as well as nodule bacteria *Bradyrhizobium japonicum*, compared to the variants where the other herbicides were used.

In a study of Qian et al. [2018] application of the herbicide Diclofop-methyl at the concentration of 100 $\mu g \cdot dm^{-3}$ to soil stimulated proteobacteria development in the rhizosphere of rice by 13.3%. At the same time, there was a 13.1% and 3.0% reduction in the presence of *Firmicutes* and *Acidobacteria* relative to the control. This result indicates that the response of rhizosphere microorganisms may depend not only on the type or rate of an applied preparation but also on the species of the microorganisms.

Consequently, the development of microbiota in the rhizosphere depends on numerous factors: species of plants, nature and rates of used preparations, their combination, a period that passed after their use, etc. At the same time, the effect of preparations with different agricultural purposes on the number of the grain sorghum rhizosphere microbiota has not been sufficiently investigated yet, which determined the purpose and relevance of our study.

MATERIALS AND METHODS

The number of microbiota in the rhizosphere of grain sorghum [*Sorghum bicolor* (L.) Moench] of the hybrid Milo W was investigated in laboratory conditions of the Biology Department of Uman National University of Horticulture during 2019–2020 in soil samples from the field experiment. Field experiments were conducted in crops of grain sorghum using the herbicide Citadel 25 OD (penoxsulam 25 $g \cdot dm^{-3}$) at the rates of 0.6; 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ (manufacturer – Syngenta AG), plant growth regulator Endofit L1 (auxins, gibberellins, cytokinins – 0.26–0.52%) at the rate of 30 $cm^3 \cdot ha^{-1}$ (manufacturer – PC PCF Imptorgservice, Ukraine), and the biopreparation Bioarsenal (fungi *Beauveria bassiana*, strain MG 301 (GHA), CFU 2 × 10^{10}; *Beauveria bassiana*, strain MG 302 (DB-1), CFU 2 × 10^{10}; bacteria *Azospirillum* spp. – MG 401, CFU 1.5 × 10^{10}; and *Azotobacter* spp. – MG 402, CFU 1.5 × 10^{10} per 100 g of preparation) at the rate of 800 g per 100 kg of seeds (manufacturer – MycoGold, USA). The experiment was laid in a systematic way with series placement of variants in a threefold repetition. The experiment scheme included variants without use of the preparations (control), Citadel 25 OD at the rates of 0.6; 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ separately and in mixtures with the plant growth regulator Endofit L1 (30 $cm^3 \cdot ha^{-1}$) on the background of pre-sowing seed treatment by the biopreparation Bioarsenal (800 $g \cdot 100\ kg^{-1}$), and without it.

The herbicide Citadel 25 OD and the plant growth regulator Endofit L1 were applied at the stage of 3–6 leaves of the culture (BBCH 13–16), which corresponded to the beginning of June.

Soil of the research field – podzolized heavy-loamy chernozem on loess with 3.5% of humus content in the topsoil. Content of the mobile phosphorus and potassium compounds (according to the method of Chirikov) – 88 and 132 $mg \cdot kg^{-1}$ respectively. Content of alkaline hydrolyzed nitrogen (according to the method of Cornfild) – 103 $mg \cdot kg^{-1}$ of soil. Soil pH – 6.2, hydrolytic acidity – 2.26 $cmol \cdot kg^{-1}$ of soil [Poltoretskyi 2017].

The number of microorganisms in the rhizosphere was investigated at the stage of flowering of the culture (BBCH 63–67), which corresponded to the beginning of August.

Soil sampling was performed on a diagonal of the experimental plots from the dug up plants by removing the adjacent (rhizosphere) layer of soil from their root system. The obtained soil samples were sifted through a sieve with 2 mm holes and then were used to make average samples [Volkohon 2010]. Simultaneously, we determined the moisture content in the obtained samples by gravimetric method, drying the samples in a drying cabinet at a temperature of 105°C to a stable mass [Hrytsaienko et al. 2003].

The total number of microorganisms that use mainly organic forms of nitrogen as a source of nutrition was investigated by sowing a soil suspension of a suitable dilution on the meat-peptone agar (MPA) by pour-plate technique, followed by counting the number of colonies that formed. This indicator was expressed in colony forming units (CFU) in 1 g of absolutely dry soil.

The quantification of the nitrifying and cellulolytic microorganisms was performed according to the method of boundary dilutions (the multiple tube method) by sowing the soil suspensions on liquid growth media with further quantification of the microorganisms in 1 g of absolutely dry soil with using the McCrady tables [Volkohon 2010].

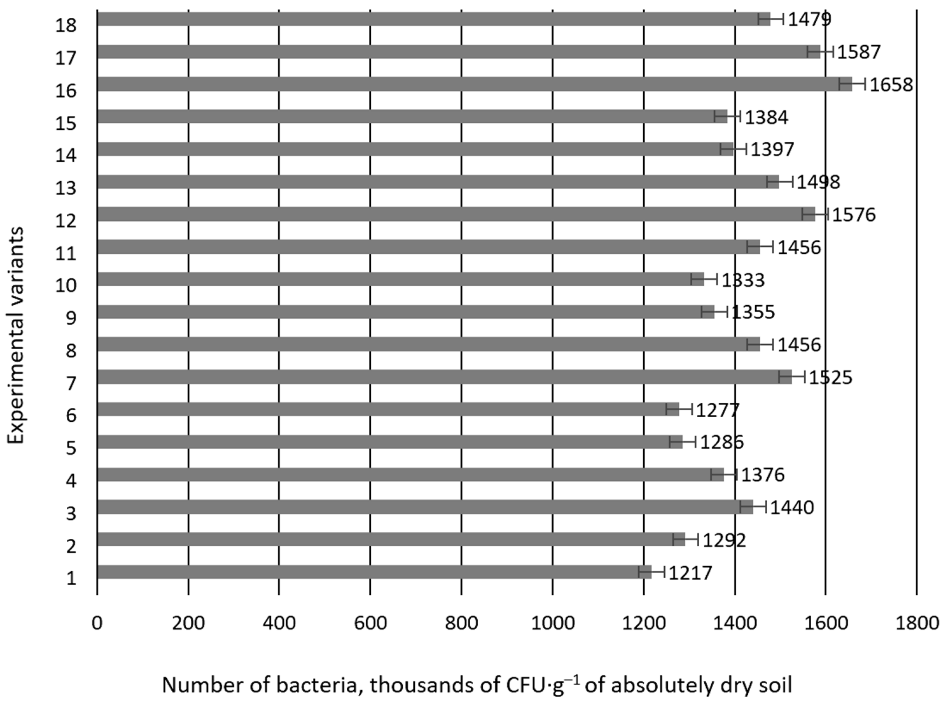
The number of nitrifying bacteria was determined on the medium of S.M. Vynohradskyi (glucose – 20.0 g; K_2HPO_4 – 0.5 g; $MgSO_4$ – 0.5 g; NaCl – 0.5 g; distilled water – bring to a volume of 1 liter), cellulolytic bacteria – on the medium of O.O. Imshenetskyi, L.I. Solntseva ($NaNH_4HPO_4$ – 1.0 g; KH_2PO_4 – 0.5 g; $MgSO_4$ – 0.4 g; NaCl – 0.1 g; $MnSO_4$ and $FeSO_4$ – 1 drop of 1% solution; peptone – 5 g; $CaCO_3$ – 2.0 g; filter paper – 15 g; pH 7.0–7.4; distilled water – bring to a volume of 1 liter). Both indicators were expressed in thousands of cells in 1 g of absolutely dry soil [Zvyagintsev 1991].

Statistical processing of the results was performed according to the methodology of Dospehov [1985] using the Microsoft Office Excel 2019, considering the indicator of the least significant difference (LSD), which characterize significance of changes in number of microorganisms between the experimental variants.

RESULTS

It was found that the total number of microorganisms in the rhizosphere of grain sorghum, which use mainly organic nitrogen compounds for nutrition, experienced significant changes under the influence of the studied preparations (Fig. 1). Thus, at the rates of herbicide Citadel 25 OD 0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ the number of these microorganisms was by 223, 159, and 69 thousand $CFU \cdot g^{-1}$ of absolutely dry soil higher than in control, which is an indicator of significant increase at the $LSD_{05} = 60.8$. At the same time, in variants of the study where the herbicide treatment was combined with application of the plant growth regulator Endofit L1, increase of the total number of microorganisms was more distinct compared to the analogous variants of the study without the growth regulator. At this combination of the preparations excess over the control was 25.3, 19.6 and 11.3%, which is averagely 5.7% more than in the variants of separate herbicide application.

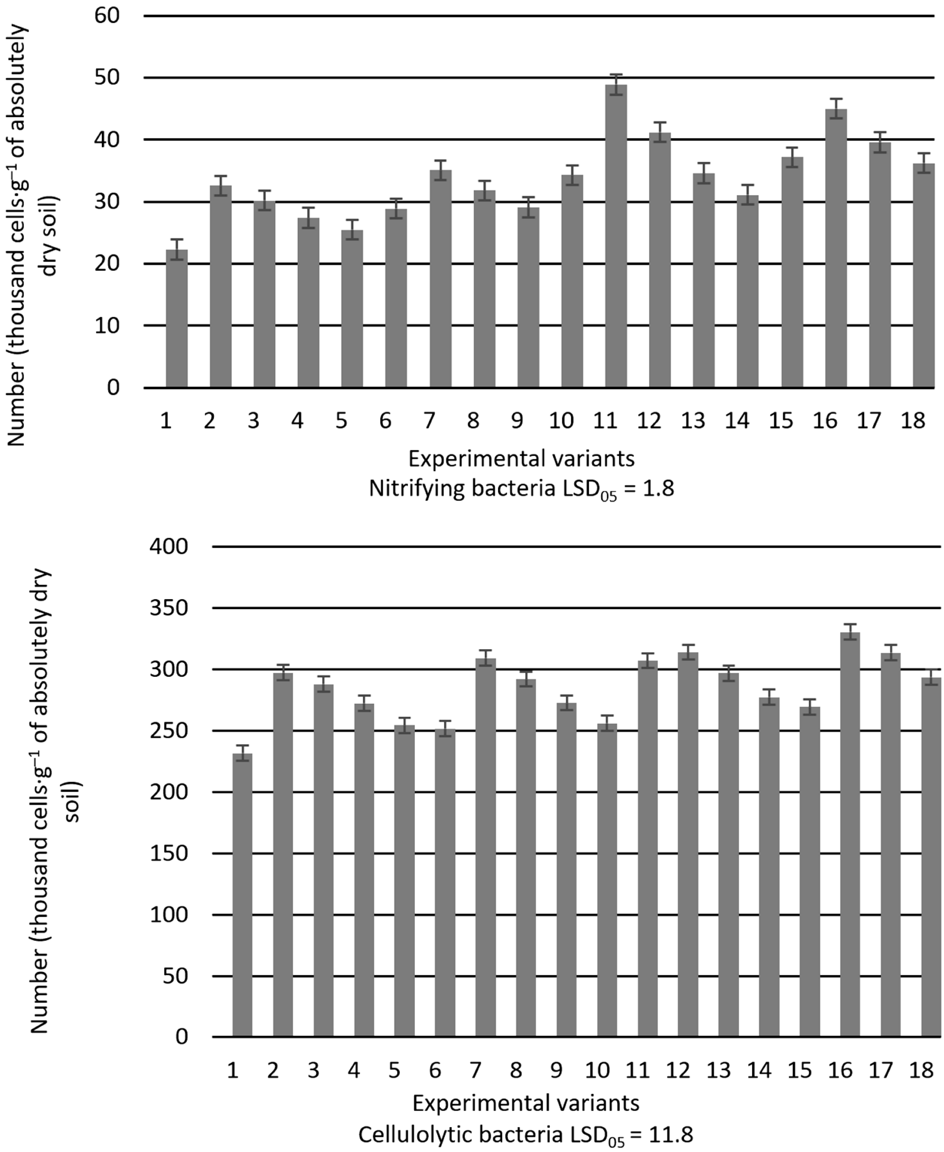
When the seeds were pretreated with Bioarsenal and the herbicide was applied on this background, the total number of microorganisms increased by 29.5, 23.1 and



Experimental variants: 1 – without use of the preparations (control); 3, 4, 5 – Citadel 25 OD 0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$; 6 – Endofit L1 30 $cm^3 \cdot ha^{-1}$; 7, 8, 9 – Citadel 25 OD 0.6; 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ + Endofit L1 30 $cm^3 \cdot ha^{-1}$; 10 – Bioarsenal 800 $g \cdot 100\ kg^{-1}$ (pre-sowing seed treatment, background); 11 – background + manual weeding during the vegetation period; 12, 13, 14 – Citadel 25 OD 0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ + background; 15 – background + Endofit L1 30 $cm^3 \cdot ha^{-1}$; 16, 17, 18 – Citadel 25 OD 0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$ + Endofit L1 30 $cm^3 \cdot ha^{-1}$ + background

Fig. 1. Total number of the rhizosphere microorganisms that use mainly organic forms of nitrogen as a source of nutrition under the action of herbicide Citadel 25 OD, plant growth regulator Endofit L1 and biopreparation Bioarsenal (flowering stage, mean for 2019–2020, $LSD_{05} = 60.8$)

14.8% relative to the control and on average by 9.0% relative to the variants where the herbicide was applied alone. The highest number of the rhizosphere microorganisms that mainly consume organic forms of nitrogen was observed in variants, where the herbicide treatment was combined with application of the plant growth regulator Endofit L1 on the background of pre-sowing seed treatment by the biopreparation Bioarsenal. In these variants, at the herbicide rates of 0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$, their number increased by 441,



Explanation as in Fig. 1

Fig. 2. Number of nitrifying and cellulolytic bacteria in the grain sorghum rhizosphere under the action of herbicide Citadel 25 OD, plant growth regulator Endofit L1 and biopreparation Bioarsenal (flowering stage, mean for 2019–2020)

370 and 262 CFU$\cdot$g^{-1} of absolutely dry soil compared to the control, which is a significant indicator at the LSD_{05} = 60.8.

Research of the number of separate ecological-trophic groups of microorganisms in the grain sorghum rhizosphere (Fig. 2) showed that at the rates of herbicide Citadel 25 OD 0.6, 0.8 and 1.0 dm$^3\cdot$ha^{-1} the numbers of nitrifying and cellulolytic bacteria increased by 3.2–7.9 and 22.7–56.3 thousand of cells$\cdot$g^{-1} of absolutely dry soil at LSD_{05} =1.8 and 11.8.

At the same time, after application of the herbicide mixed with the plant growth regulator Endofit L1, the number of nitrifying bacteria exceeded control by 30.5–57.4%, cellulolytic – by 17.7–33.5%. The given ecological-trophic groups of microorganisms also showed a significant increase of their number at application of the herbicide on the background of pre-sowing seed treatment by Bioarsenal. In these conditions their number was by 39.5–84.7 and 19.7–35.6% higher than in the control. At the complex application of the herbicide Citadel 25 OD with the plant growth regulator Endofit L1 on the background of pre-sowing seed treatment by Bioarsenal, the number of nitrifying and cellulolytic bacteria increased by 62.3–101.8 and 26.7–42.7% relatively to the control.

The obtained results give a reason to claim that relatively to the control, where none of the preparations were used, the herbicide had no negative influence on the researched groups of bacteria, although their number decreased simultaneously with the increasement of the herbicide rate. At the maximal rate of Citadel 25 OD 1.0 dm$^3\cdot$ha^{-1} the number of microorganisms decreased by 10.7% compared to the indicators at the minimal rate of the herbicide 0.6 dm$^3\cdot$ha^{-1}. A similar tendency also appeared in the number of nitrifying and cellulolytic bacteria – at the maximal rate they were by 15.6 and 11.7% lower than at the minimal rate of the preparation. Meanwhile, application of the herbicide in combination with the plant growth regulator Endofit L1, and also its application on the background of pre-sowing seed treatment by the biopreparation Bioarsenal led to a significant increasement of the number of researched ecological-trophic groups of microorganisms compared to the variants where the herbicide was applied alone. The most favorable development conditions for the microorganisms were observed in the variants of research with the complex use of the preparations.

DISCUSSION

It is evident from the results of the study that the herbicide Citadel 25 OD had no negative influence on the development of the grain sorghum's rhizosphere microorganisms, although with its rate increasement to the maximal, their number decreased. Growth of the rhizosphere microbiota on the background of herbicide action is presumably connected to the decreased weed influence on the grain sorghum plants, which creates more favorable conditions for developing additional root area and more intensive secretion of the root exudates [Karpenko 2012, Singh et al. 2018]. At the same time, some suppressive effect of the higher herbicide rates on the development of rhizosphere microbiota may be explained by changes in the exudate composition and general decrease of their secretion. Moreover, it can be explained by the probable presence of herbicide metabolism products which produce by plants and impair the life conditions of the rhizosphere microbiota [Jennifer et al. 2007].

Application of the herbicide jointly with the plant growth regulator provided increasement of the number of researched groups of microorganisms. Such result is a consequence of metabolism activation in the organisms of plants, which led to the general intensification of the growth processes and the process of the root exudates secretion [Karpenko 2012, Bezuglova et al. 2019]. A similar effect was described in studies of other scientists. For example, according to the research of Prytuliak et al. [2016], application of herbicides Hrand (15, 20, 25 30 $g \cdot ha^{-1}$) and Zernovii (0.5, 0.7, 0.9, 1.1 $dm^3 \cdot ha^{-1}$) in crops of winter triticale led to increasement of the total number of rhizosphere microbiota by 3–13% and 2–8% compared to the control. Meanwhile, after application of the mentioned herbicides in mixtures with the plant growth regulator Biolan the total number of bacteria increased by 4–10% compared to the variants where the herbicides were applied alone.

Research of Hrytsaienko and Karpenko [2012] also proves the positive influence of a plant growth regulator applied in mixtures with herbicides on development of the spring barley rhizosphere microbiota. After application of a herbicide Granstar 75 (tribenuron methyl) at rates of 10–25 $g \cdot ha^{-1}$ along with a herbicide 2,4-DA 500 (2,4 dichlorophenoxyacetic acid) at a rate of 1.0 $dm^3 \cdot ha^{-1}$ and a plant growth regulator Emistym C, a significant increase of the number of nitrifying and cellulolytic bacteria occurred. These indicators were higher than in the similar variants without use of the plant growth regulator in average by 37.6 and 7.9%.

Application of the herbicide Citadel 25 OD on the background of pre-sowing seed treatment by Bioarsenal had also a significant positive influence on the development of the rhizosphere microorganisms compared to the variants of separate herbicide use. A similar tendency was also observed by Holodryha et al. [2015] in crops of soybean, where inoculation of seeds by the biological preparation Ryzobofit and further treatment of the plants by the herbicide Diesiliet (0.6–0.8 $dm^3 \cdot ha^{-1}$) led to intensified development of the rhizosphere microbiota. Thus, in the variants where the herbicide was applied on the background of pre-sowing seed treatment by Ryzobofit, the total number of bacteria was in average by 19.4% higher than in the variants of separate herbicide use.

The highest number of the investigated microbial groups was found when the herbicide Citadel 25 OD was applied in combination with the plant growth regulator Endofit L1 on the background of pre-sowing seed treatment by the biopreparation Bioarsenal. This combination of the preparations stimulated development of the rhizosphere microorganisms, which conforms with the results obtained by Hrytsaienko and Voloshyna [2014]. According to their study, joint use of the herbicide Lantselot (13, 23, 33 $g \cdot ha^{-1}$) with the plant growth regulator Biolan (20 $cm^3 \cdot ha^{-1}$) on the background of pre-sowing seed treatment by the plant growth regulator Radostym (250 $cm^3 \cdot t^{-1}$) led to increasement of the total number of bacteria and micromycetes in average by 45.7 and 39.9% compared to the variants of separate herbicide use.

Voloshyna [2014] also admit increase of the number of nitrifying and cellulolytic microorganisms at the complex application of the herbicide Lantselot 450 WG along with the plant growth regulator Biolan on the background of pre-sowing seed treatment by the plant growth regulator Radostym. In these variants there was increase in the number of indicated groups of microorganisms by 40.6 and 17.3% on average in comparison with the variants where only herbicide was used.

Similar results are also reported by Pidan [2015]. Application of the herbicide Fiuzylad forte 150 (0.5, 0.75, 1.0 $dm^3 \cdot ha^{-1}$) and the plant growth regulator Radostym (20 $cm^3 \cdot ha^{-1}$) on the background of pre-sowing seed treatment by the Radostym (250 $cm^3 \cdot t^{-1}$) in crops of sunflower led to increasement of the total number of bacteria, actinomycetes and micromycetes by 16.7, 15.6, 12.1% respectively.

Consequently, the results of our research are consistent with the literature regarding the effects of chemical and biological preparates on microbiological processes in the rhizosphere of cultivated plants. In particular, summary demonstrates that the herbicide Citadel 25 OD has no negative influence on development of the studied ecological-trophic groups of microorganisms. Meantime at the application of the herbicide with the plant growth regulator Endofit L1 on the background of pre-sowing seed treatment by Bioarsenal the microorganism development conditions in the grain sorghum rhizosphere significantly improve. It proves by increasement of their number by 48.3% in average by groups relatively to the control.

CONCLUSIONS

1. The herbicide Citadel 25 OD at the rates of 0.6, 0.8, 1.0 $dm^3 \cdot ha^{-1}$, plant growth regulator Endofit L1 at the rate of 30 $cm^3 \cdot ha^{-1}$, and the biopreparation Bioarsenal at the rate of 800 $g \cdot 100\ kg^{-1}$ are able to significantly affect the development of particular ecological-trophic groups of microorganisms in the rhizosphere of grain sorghum.

2. It is proven that the number of microorganisms in the rhizosphere of grain sorghum depends on the rates of the herbicide application: as the herbicide rate increases to the maximum (1.0 $dm^3 \cdot ha^{-1}$), the number of microbiota decreases in average by 12.7% relative to the variants with the minimal rate of the preparation.

3. Application of the herbicide Citadel 25 OD (0.6, 0.8 and 1.0 $dm^3 \cdot ha^{-1}$) in mixtures with the plant growth regulator Endofit L1 (30 $cm^3 \cdot ha^{-1}$), and also its application on the background of pre-sowing seed treatment by Bioarsenal (800 $g \cdot 100\ kg^{-1}$) creates more favorable conditions for development of the rhizosphere microbiota compared to the variants of separate use of the herbicide. At these combinations of the preparations the number of the rhizosphere microbiota increase in average by groups by 29.3–36.7%.

4. The highest number of the rhizosphere microorganisms (bacteria that use mainly organic forms of nitrogen as a source of nutrition, nitrifying and cellulolytic bacteria) observes in the variants with the complex application of the preparates where excess to the control is 29.4–80.6% in average by groups.

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