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Combined application of microbial preparation, mineral fertilizer and bioadhesive in production of leek

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Abstract. The research deals with additional fertilizing of leek cultivars Goliath and Tango with bacterial preparation Organic-balance and mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me in combination with adhesive agent of a natural origin Liposam. Field research was performed on the experimental plots of the Department of Vegetable Growing of Uman National University of Horticulture. The research focused on microbiological processes and formation of productivity in the leek crops depending on the combination of preparations.
It has been established that the number of bacteria *Azotobacter* in the rhizosphere of leek increased 2.8 times after a four-time fertilizing with bacterial preparation Organic-balance and bioadhesive Liposam during vegetation. The maximum number of bacteria, including *Azotobacter*, in the rhizosphere of leek, was recorded after a four-time fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me in combination with Organic-balance and Liposam. The share of influence of additional fertilization on the microbiota of the rhizosphere made up 77–97%.
Leek cultivar Tango produced a larger assimilative leaf surface. Depending on the fertilizing the maximum leaf surface and photosynthetic potential of leek cultivars Tango and Goliath were recorded under combination of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam. The yielding capacity of variety Goliath was better than that one of the variety Tango regardless of fertilizing. To make the growing technology of leek more environmental friendly it is advisable to introduce in the growing technology bacterial preparation Organic-balance together with Liposam. Utilization of these preparations improves crop yield, which makes up 0.6–0.7 t ha^{-1} for variety Goliath and 1.1–2.7 t ha^{-1} for variety Tango correspondingly. The investigated leek varieties had the maximum yields under combined application of Organic-balance + Liposam with fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me, which is by 18–24% higher than provided by plants grown without fertilization.

Key words: leek growing, additional fertilization, microbial preparation, mineral fertilizer, bioadhesive, rhizosphere microbiota, assimilative leaf surface, photosynthetic potential, yield.

INTRODUCTION

Traditional vegetable growing technologies are aimed in obtaining the maximum productivity due to the intensive application of mineral fertilizers and pesticides, which inflicts considerable damage to the environment, leads to the deterioration of the biological state, physical and chemical properties of soil (Martyniuk et al., 2001). Alternative technologies of vegetable growing with the exclusion or limited utilization of chemical substances are safer, though they have a satisfactory economic efficiency (Brumfield et al., 2000; Bulluck et al., 2002). At present, research institutions are working at the introduction of preparations of natural origin, which are consistent with natural biodiversity and increase the productivity of plants (Melero et al., 2006; Sosnowski et al., 2014; Leggo, 2017).

Among such preparations, special attention is paid to the forms, that include microorganisms and products of their metabolism (Golubkina et al., 2018). Colonization of plants rhizosphere with microbiota stimulates growth and development of plants (Grant et al., 2005; Saxena et al., 2005) and promotes the adaptation to biotic and abiotic stresses (Yang et al., 2009; Grover et al., 2011). Because of unstable climatic situation (frequent droughts, increasing temperature, late spring or early autumn frosts) the relevance of using microbial preparations in agriculture is gaining in importance since other strategies of adaptation and effective resource management are money-consuming and short-term. At present bacteria of the genera *Azotobacter chroococcum* and *Bacillus subtilis* are the most wide-spread in the composition of microbial preparations. They participate in important processes of plant growth stimulation: *Azotobacter* is able to fix 60–80 kg ha^{-1} of nitrogen (Kennedy et al., 2004); *Bacillus subtilis* has fungicide properties (Kloepper, 2004).

Moreover, microbial preparations increase the availability of mineral compounds of calcium, phosphorus and iron for cultivated plants (Marra et al., 2012; Sosnowski et al., 2014; Kozhemyakov et al., 2015). Being used at the background of chemical substances they activate antioxidant processes aimed at detoxification of metabolic by-products, induced by the influence of xenobiotics (Karpenko & Prytulyak, 2014).

Under combined utilization of microbial preparations and mineral fertilizers the application rates of the latter can be reduced (Kahiluoto & Vestberg, 1998). Comparing the efficiency of using Humate and bacterial fertilizer Biostar (75% of shredded straw, 24% of mineral complex N : P : K at the ratio of 4 : 4: 4 and 1% of microorganisms *Bacillus sp.* – 5×10^6 CFU in 1 g) the preparation Biostar has the greatest influence on the yield capacity of leek. Under its use, the mass of leek increased 3.1 times compared to untreated plants (Golubkina et al., 2010).

Because of a long vegetation period high content of nutrients within a root system zone is especially urgent for leek. Thus the growing technology implies the application of up to 400 kg ha^{-1} nitrogenous fertilizers. Along with that, complex application of mineral fertilizers and biological preparations promotes a valid increase in yield capacity by 55–86%, even on the soils that have higher and high provision with mobile forms of phosphorus and exchangeable potassium (Syubayeva & Titova, 2015).

With the reference to above mentioned, it is important to develop such elements of leek growing technologies, that would combine the application of microbial preparations and mineral fertilizers and provide high productivity of a plant under the minimal negative influence on the agrocenosis and environment. It was this pertinent issue that determined the aim and the tasks of our research.

MATERIALS AND METHODS

The experimental part of the research was performed in the fields (experimental plots) and in the laboratories of the Department of Vegetable Growing of Uman National University of Horticulture in 2016–2018. The soil on the experimental plots is a podzolic heavy clay black soil with 1.9% of humus content with pH value – 6.3, nitrogen content in the arable top soil layer 103 mg kg^{-1}; mobile forms of phosphorus and potassium make up 122 and 135 mg kg^{-1}. The climate of the region where the experiment was conducted is temperate continental with unstable moistening.

Sixty-day seedlings of leek varieties Goliath and Tango were planted in the experimental plots in the second decade of April, the crops were planted according to the scheme 70×15 cm. The aim of the experiment was to study the efficiency of root additional fertilizing of leek with mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me and bacterial preparation Organic-balance, applied separately and together with bioadhesive Liposam.

Mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me (DripFert™, Turkey, 'Sabera' private limited company', a representative office in Ukraine) is a water soluble fertilizer without chlorine, including balanced complex of microelements: B – 0.03%; Fe – 0.04; Mn – 0.03; Zn – 0.04; Cu – 0.06% on chelate base of EDTA (ethylene diamine tetraacetic acid).

Bacterial preparation Organic-balance (BTU-Center (Ukrainian Biotechnology), Ukraine) is a concentrate of viable and inactivated microorganisms of different taxonomic groups and their active metabolites, namely: cells of bacteria *Bacillus subtilis 221* – 40 ± 10%, *Azotobacter* – 30 ± 10%, *Paenibacillus polymyxa* – 10 ± 5%, *Enterococcus* – 10 ± 5%, *Lactobacillus* – 10 ± 5%, titre $1\times10^8 – 1\times10^9$ CFU in cm^3 macro- and microelements, biologically active metabolites of bacteria.

Liposam (BTU-Center, Ukraine) is a composition of biopolymers of natural origin, applied as a biological adhesive agent for crop protection agents and fertilizers. It provides a close contact with a treated surface, creates a flexible protective film preserving moisture and applied solutions in the root system.

Variety Goliath (Rijk Zwaan, Holland) is a medium-early leek variety with a vegetation period of 150 days. According to morphological features it belongs to autumn variety and is not frost-resistant. Plants have 80 cm long leaves, pseudo-stems reach 30 cm. This variety is resistant to diseases.

Tango (Moravo seed, Czech Republic) is a mid-season ripening leek variety. According to its morphological features and frost resistance, it belongs to the winter variety. Plants are of medium height, grayish-green leaves are wide. The pseudo-stem has the height of 12 cm and is 5 cm in diameter. The taste is semi-spicy. The variety is recommended for long winter storage.

Two-factor experiment included leek varieties (factor A) Goliath and Tango (control) and bacterial preparation and mineral fertilizer applied separately or together with bioadhesive (factor B): Organic-balance (0.5 L ha^{-1}); Organic-balance (0.5 L ha^{-1}) + Liposam (0.5 L ha^{-1}); DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}); DripFert $N_{20}P_{20}K_{20}$ +

Me (50 kg ha^{-1}) + Liposam (0.5 L ha^{-1}); Organic-balance (0.5 L ha^{-1}) + DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}); Organic-balance (0.5 L ha^{-1}) + DripFert $N_{20}P_{20}K_{20}$ + Me (50 kg ha^{-1}) + Liposam (0.5 L ha^{-1}); 7) without fertilization (control).

Preparations were introduced into the soil as water solutions at the rate of 20 m^3 ha^{-1} per watering rate. Four additional fertilizations were performed during leek vegetation: the first fertilization was achieved three weeks after seedlings planting and the other three with 30 days interval. The experiment had four replicates. The allocation of repetitions and variants was multilevel. The plants were hilled up twice in July for the etiolation of pseudo-stem. The harvest was gathered and recorded at the end of the 1st decade of October.

The number of rhizospheric bacteria in the experiment was determined according to the method presented by Zvyagintsev et al. (1991). On the 25th day after the 1st and the 4thfertilizers supplementation the samples were taken from a root zone, from which soil suspension was prepared and sowed on meat-and-peptone agar. Microbiological inoculation was performed four times. The number of bacteria was calculated according to the formula $K = a \times P/m$, where: K – the number of colony-forming units (CFU), units in 1 g of absolutely dry soil; a – the number of microorganism colonies, that were grown on nutritious medium, units; P – dilution of soil extract; m – the mass of absolutely dry soil in 1g of wet soil, g.

The number of nitrogen fixing bacteria *Azotobacter* was recorded on the nutrition medium Ezhbi according to the method of growing colonies around soil clods and were expressed in percentage to the total number of soil clods, that were placed in Petri dish (Hrycajenkoet al., 2003).

Leaf surface area was recorded and photosynthetic potential was calculated during the vegetation period of leek. The leaf surface area (cm^2) of leek was calculated according to the formula: $S = D \times L / 2$ (where D – the length of a leaf, cm, L – the width of a leaf, cm) with further re-calculation in m^2 per 1 ha (Bondarenko & Yakovenko, 2001). Photosynthetic potential (*PhP*), m^2 × days ha^{-1} was determined according to the Nichiporovich (1972) formula: $PhP = L_1 + L_2 / 200 \times T$, where L_1 – the leaf surface area on the 20th May; L_2 – leaf surface area on the 10h of October, thousand m^2 ha^{-1}; T – the duration of inter-phase period, days. We determined the share of the commercial produce relevant to the collected yield of the pseudo-stem with a diameter of more than 1.5 cm, the harvest was weighed and calculated in t per ha.

The validity of the research and significance of the differences between the mean values of the variables examined were evaluated according to the results of dispersion and correlation analysis of mathematical statistics (Ehrmantraut et al., 2000).

RESULTS AND DISCUSSION

Soil microbiocenosis considerably influences the interaction of introduced bacteria and plants (Stamenov et al., 2012). Under field conditions, the positive effect of bacteria on improving the accessibility of nutrients for plants is not always achieved since the vital activity of microbiota depends on the growing technology and properties of plants (Jarak et al., 2012). Therefore, to determine the regularities of functioning microbial cenosis is an important criterion to evaluate the expediency of any elements of technology, because the fertility of soil and yield capacity of plants depends on the rearrangement of some ecological populations of microorganisms (Avrova, et al., 2005;

Sherstoboyeva et al., 2007). In this respect, special attention should be paid to nitrogen-fixing bacteria *Azotobacter*, that can enrich soil with nitrogen as well as synthesize growth-stimulating compounds and antagonistic compounds to the pathogenic microbiota (McSpadden-Gardener, 2004).

The data of the conducted microbiological analysis of the soil showed that application of the investigated preparations in the technology of growing leek influences considerably the growth of rhizospheric bacteria (Table 1).

Table 1. The number of microbiota in the rhizosphere of leek (mean over three years)

Additional fertilizing (B)	Bacteria, 10^6 CFU in 1 g of soil			
	Variety (Factor A)			
	Goliath	Tango (control)	*Mean value by factor B*	± compared to control
I period of recording (at the beginning of June)[1]				
Without fertilization (comparison variant, control)	1.33	1.92	*1.63*	-
Organic-balance	2.44	2.41	*2.43*	0.80
Organic-balance + Liposam	3.05	4.07	*3.56*	1.94
DripFert $N_{20}P_{20}K_{20}$ + Me	1.82	2.63	*2.23*	0.60
DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	2.15	2.91	*2.53*	0.90
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	3.17	3.76	*3.47*	1.84
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	5.31	4.84	*5.08*	3.45
Mean value by factor A	*2.75*	*3.22*	-	*0.47*
LSD_{05} – 0.25; LSD_{05A} – 0.1; LSD_{05B} – 0.18; Factor influence, % – A – 14; B – 82; AB – 4				
II period of recording (at the beginning of September)[2]				
Without fertilization (comparison variant, control)	3.54	1.68	*2.61*	-
Organic-balance	5.19	4.28	*4.74*	2,13
Organic-balance +Liposam	6.57	6.24	*6.41*	3.80
DripFert $N_{20}P_{20}K_{20}$ + Me	3.15	2.56	*2.86*	0.25
DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	3.87	2.50	*3.19*	0.58
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	5.84	4.69	*5.27*	2.66
Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	7.09	7.35	*7.22*	4.61
Mean value by factor A	*5.03*	*4.19*	-	*0.84*
LSD_{05} – 0.33; LSD_{05A} – 0.13; LSD_{05B} – 0.23; Factor influence, % – A – 20; B – 77; AB – 3				

[1]after 25 days since the first additional fertilizing; [2]after 25 days since the fourth additional fertilizing.

Thus, 25 days after the first fertilizing of Goliath and Tango varieties the number of bacteria exceeded 1.4 times the value typical for control. Under joint application of DripFert $N_{20}P_{20}K_{20}$ + Me, Liposam and Organic-balance it increased 1.5–1.7 and 2.0–2.4 times respectively. Contrary to Goliath variety, after the first application of preparation Organic-balance in the plots with Tango variety there was less amount of bacteria compared to the variant with mineral fertilizing. On the plots with Goliath variety after 25 days since the application of Organic-balance the number of bacteria exceeded the variant DripFert $N_{20}P_{20}K_{20}$ + Me by 34%, the variant without fertilizer by 83%. Under combined application of Organic-balance with Liposam the number of bacteria after the first fertilizing was 1.3 and 1.7 times higher compared to the option without bioadhesive on the plots with varieties Goliath and Tango respectively. After the fourth combined application of Organic-balance + Liposam the number of bacteria exceeded the option without bioadhesive 1.3 times (for variety Goliath) and 1.5 times (for variety Tango).

On average during the experiment, the greatest number of bacteria was recorded after the first fertilization with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam application where their number exceeded the options without fertilization 2.5 times (Tango) and 4.0 times (Goliath).

The number of bacteria increased during vegetation in the rhizosphere, except the control variant and DripFert $N_{20}P_{20}K_{20}$ + Me. On the plots with Goliath variety without fertilization at the beginning of September (the second period of recording) the number of bacteria in the rhizosphere of leek was 2.7 times higher compared to the data at the beginning of June (the first period of recording). After the fourth fertilization DripFert $N_{20}P_{20}K_{20}$ + Me on the plots with Goliath variety the total amount of bacteria was lower than without fertilization. However, on average according to factor B after the fourth fertilization DripFert $N_{20}P_{20}K_{20}$ + Me the number of bacteria exceeded the control by 0.25×10^6 CFU, which is significant according to the LSD_{05B} in space of soil.

Under the application of mineral fertilizer together with Liposam the higher amount of bacteria was recorded compared to the variant without bioadhesive. In the option with DripFert $N_{20}P_{20}K_{20}$ + Me together with Organic-balance at the beginning of September the number of bacteria on the plots with Tango and Goliath varieties was correspondingly 3.0 and 2.30×10^6 CFU in 1 g of soil higher than in the plots without fertilization.

Regardless of the variety after the fourth combined application of Organic balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam in the rhizosphere of, leek the number of bacteria was the highest and according to factor B, it exceeded on average the indicators of the plots without fertilizing 2.8 times.

As to nitrogen-fixing microorganisms of the genus *Azotobacter*, their number in the variant with applying only DripFert $N_{20}P_{20}K_{20}$ + Me was by 4–5% lower in the leek rhizosphere of both varieties in September (after the fourth fertilizing) compared to the indexes at the beginning of June (after the first fertilizing) (Table 2).

On average, according to factor B the number of *Azotobacter* bacteria in the option DripFert $N_{20}P_{20}K_{20}$ + Me had no significant difference compared to control. Application of Organic-balance together with mineral fertilizer increased the number of *Azotobacter* bacteria by 5–7% after the first additional fertilization and by 16–18% after the fourth fertilization compared to the variant with the application of only DripFert $N_{20}P_{20}K_{20}$ + Me. In the rhizosphere of Goliath variety, grown without additional fertilization, the number of *Azotobacter* bacteria was the lowest at the beginning of September.

In the variants with a separate application of Organic-balance and with bioadhesive, the number of *Azotobacter* bacteria was higher with maximum indexes in the variant Organic-balance + Liposam after the fourth fertilization. On average, according to factor B after the fourth fertilization with Organic-balance and with Organic-balance + Liposam the number of *Azotobacter* bacteria was 2.5 and 2.8 lower respectively.

In the variants of combined application of Organic balance + Liposam + DripFert $N_{20}P_{20}K_{20}$ + Me the share of bacteria of the genus *Azotobacter* was maximum and at the beginning of June and September it was higher by 13–14% and by 30–31% compared to the plots without additional fertilization within the variety. Similar phenomenon was recorded by other researchers (Colo et al., 2014): the total number of bacteria and the number of nitrogen fixing bacteria in the leek crops inoculated with *Azotobacter* *chroococcum* and *Bacillus subtilis* was higher. According to Tawaraya et al. (1995), leek roots secrete amino acids, sugars and organic acids, creating in this way favorable conditions for the development of different groups of microorganisms, and introduction of microbiological fertilizers into soil changes not only their number, but also their species composition. It is obvious that the increase of the total number of microbiota and *Azotobacter* bacteria in different variants of application of Organic-balance is the result of the introduction of bioagents of this preparation into the rhizosphere of plants. The increase of bacteria number in the soil under their induction causes the activation of microbiological transformations favorable for plants provision with nutrients (Jarak et al., 2006; Khadiga et al., 2015).

Table 2. The number of *Azotobacter* bacteria in the rhizosphere of leek, % (mean over three years)

Additional fertilizing (B)	*Azotobacter*, %			
	Variety (factor A)			
	Goliath	Tango (control)	*Mean value by factor B*	± compared to control
I period of recording (at the beginning of June)				
Without fertilization (comparison variant, control)	17	13	*15.0*	-
Organic-balance	27	26	*26.5*	10.5
Organic-balance + Liposam	33	35	*34.0*	19.0
DripFert $N_{20}P_{20}K_{20}$+Me	20	16	*18.0*	3.0
DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	22	19	*20.5*	5.5
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me	25	23	*24.0*	9.0
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	30	37	*33.5*	18.5
Mean value by factor A	*25.0*	*24.1*	-	0.9
LSD_{05} – 7; LSD_{05A} – 3; LSD_{05B} – 5; Factor influence, % – A – 8; B – 90; AB – 2				
II period of recording (at the beginning of September)				
Without fertilization (comparison variant, control)	14	17	*15.5*	-
Organic-balance	35	38	*36.5*	21.0
Organic-balance + Liposam	41	47	*44.0*	28.5
DripFert $N_{20}P_{20}K_{20}$+Me	16	11	*13.5*	-2.0
DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	27	15	*21.0*	5.5
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me	32	29	*30.5*	15.0
Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam	44	48	*46.0*	30.5
Mean value by factor A	*30.0*	*29.2*	-	0.80
LSD_{05} – 8; LSD_{05A} – 3; LSD_{05B} – 6; Factor influence, % – A – 1; B – 97 ; AB – 2				

According to statistical analysis the share of factor B influence (additional fertilizing with mineral fertilizer and biological preparation) on the rhizosphere microbiota of leek made up 77–97%, and influence of the variety (factor A) and interaction of factors (AB) was not significant and made up 1–20% and 2–4% respectively. During the periods of recording, there was a strong correlation in the rhizosphere of leek between the total amount of bacteria and the share of *Azotobacter* – $r = 0.73 \pm 0.11$ (at the beginning of June) and $r = 0.92 \pm 0.04$ (at the beginning of September) (Fig. 1).

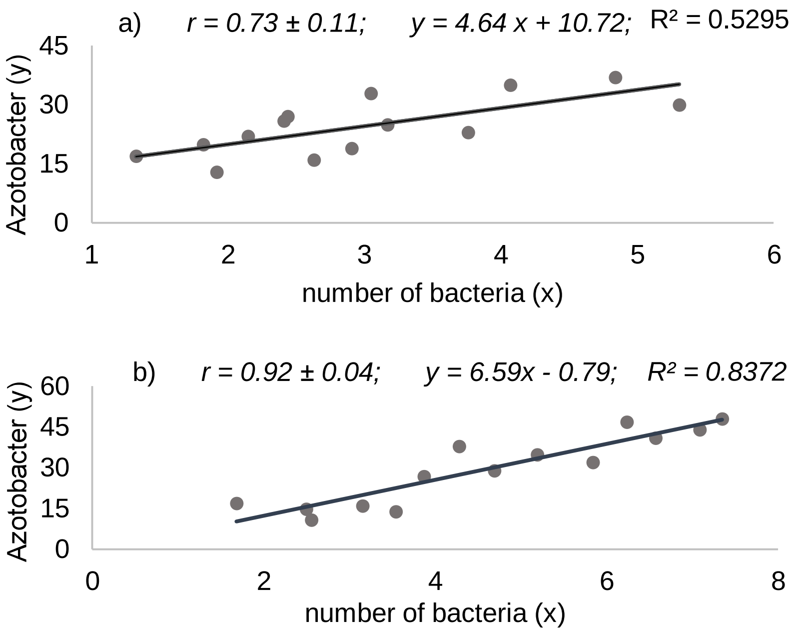

Figure 1. Correlation dependence between the total numbers of bacteria (x), 10^6 CFU in 1 g of soil and the share *Azotobacter* (y), % in the rhizosphere of leek after the first (a) and the fourth (b) additional fertilizing.

Prediction of *Azotobacter* content depending on the total number of bacteria can be calculated using regression equation $y = 4.64x + 10.72$ and $y = 6.59x – 0.79$ for the investigated periods. At the same time, in the experiments of Hajnal-Jafari et al. (2012) the introduction of *Azotobacter chroococcum* did not affect its amount in the soil, therefore correlations between the amount of *Azotobacter* and yield of corn seeds was not found. However, understanding the processes of colonization of rhizosphere by microorganisms is of great importance for the prediction of interaction of microbiota with plants towards increasing their productivity (Stephane et al., 2010).

When mineral fertilizers are applied, the increase of the efficiency of preparations containing associative nitrogen-fixing microbiota can be explained by the fact that nitrogen from the fertilizer stimulates the plant growth at the background of relatively low activity of nitrogen-fixing bacteria at the initial period of vegetation. After a while fertilized plants with rather developed root system and high level of metabolism provide optimal conditions for the activity of rhizosphere microbiota (Syubayeva & Titova, 2015), as the result, a plant gets additional amount of metabolic by-products of diazotrophs (nutrients and growth stimulators).

According to the data of leaf surface area of leek it was found that additional fertilization has a positive effect on the formation of indexes of assimilation activity. Application of Organic-balance alone or its application in the mixture with Liposam promoted the formation of leaf surface during the harvesting period of Goliath and Tango varieties by 3.18–4.21 thousand m^2 ha^{-1} and 0.84–2.25 thousand m^2 ha^{-1} more than plants that were not treated with the preparation (Fig. 2).

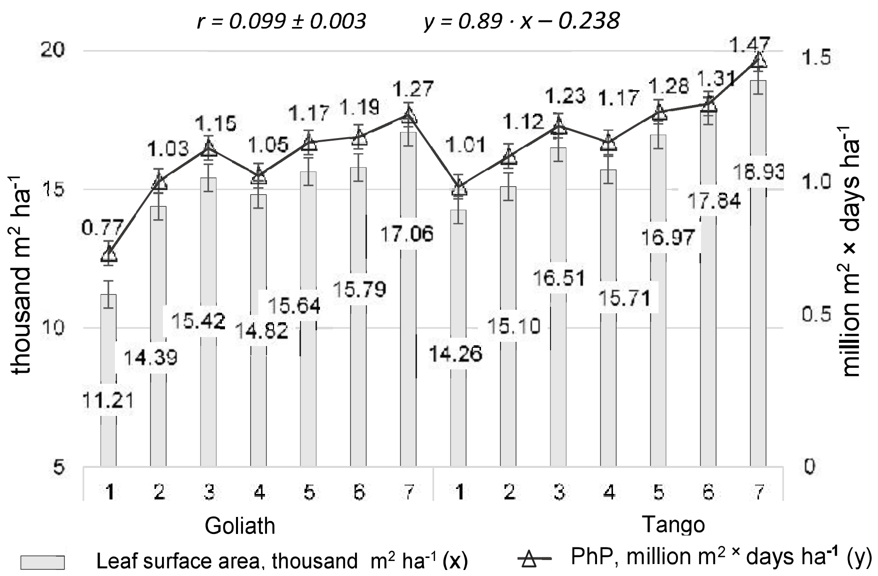

Figure 2. Indexes of assimilation activity of leek, average over 2016–2018:

1) without fertilization (comparison variant - Goliath, control-Tango);
2) Organic-balance;
3) Organic-balance + Liposam;
4) DripFert $N_{20}P_{20}K_{20}$+Me;
5) DripFert $N_{20}P_{20}K_{20}$+Me + Liposam;
6) Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me;
7) Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam

Results of statistical analysis

Leaf area (as of 10.X) (thousand m^2 ha^{-1})					
The year of research					
2016		2017		2018	
$LSD_{0.5}$	*CV^1*, %	*$LSD_{0.5}$*	*CV^1*, %	*$LSD_{0.5}$*	*CV^1*, %
1.72	*11*	*0.95*	*15*	*1.41*	*15*

^{1} – coefficient of variation.

After additional fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me on the 10th of October the average increase of leaf surface of Goliath variety made up 3.61 thousand m^2 ha^{-1}, and of Tango variety made up 1.45 thousand m^2 ha^{-1} compared to the variants without fertilization. Fertilization with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam turned out to be more efficient than fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me alone, the photosynthetic potential was higher by 0.11–0.14 m^2 × days ha^{-1}. For both varieties the maximum indexes of assimilative leaf surface were recorded when there was a combined application of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam and they were by 52% (Goliath) and 33% (Tango) higher than in the options without fertilization.

Obtained indexes of the photosynthetic potential of leek indicate correlation dependence with leaf surface area: $r = 0.992 \pm 0.004$. On average over the period from 20th May to 10th October Tango variety had the highest values of photosynthetic potential in the variants Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and Organic-balance + DripFert $N_{20}P_{20}K_{20}$+Me + Liposam: 1.3–1.5 times higher than in control. The photosynthetic potential of Goliath variety under similar variants of growing was lower than that one of Tango variety. According to dispersion analysis, the power of influence on the assimilative surface of factor A (variety) was 48%, factor B (fertilizing) – 47%. The level of indexes variation was average and made up 11–15%.

The obtained data of assimilation activity of leek depending on additional fertilization with biological or mineral fertilizer were in good agreement with the results of other researchers (Kloepper, 2004; Hosam et al., 2013), who indicated that inoculation with *Pseudomonas* isolates had a stimulating effect on the plant growth. In the research of Khadiga et al. (2015) the maximum leaf surface and dry matter of leek were formed under combined application of *B. subtilis + Ps. fluorescens + Yeast*. According to the data in Abou & Yousry (2012), after inoculation with *Glomus intraradices* biometric parameters of leek were by 25–28% higher compared to the variants without inoculation.

Mazur et al. (2019) reported that the preparation containing *Rhizohumin* bacteria in combination with plant growth regulator Emistym C stimulated the development of leaf apparatus and promoted the increase of the photosynthetic potential of lupine in compared to control by 8.1 m^2 ha^{-1} (27%). The increase of biological indexes of plants under the treatment of microorganisms is considered to reflect the growing activity of rhizosphere microbiota (Mantelin & Touraine, 2004; Tilak et al., 2006).

The prospects of the investigated methods of growing leek under the application of additional fertilizing are evaluated according to the yield capacity and marketability of etiolated pseudo-stem. According to the data of Syubayeva & Titova (2015), the introduction of biological preparation Azophobakterin-AF into soil provides the maximum and valid yield increase of leek at the background of mineral additional fertilizing. Application of *Azotobacter* bacteria and other beneficial bacteria is recommended for the increase of leek, pepper, tomatoes, cucumber, wheat, corn and other crops yield (Kumar et al., 2001; Hajnal-Jafari et al., 2012; Colo et al., 2014). After the inoculation of sugar beet seeds with *Azotobacter chroococcum* the increase of yield of root crops made up 23% (Mrkovački et al., 2016). According to the data of Geel et al. (2006), in the variant without the application of mineral fertilizers leek yield reached 39–45 t ha^{-1}, and after the application of N_{120} it was higher by 3.0–5.5 t ha^{-1}. The maximum yield of dry weight of leek was achieved in the variant when split fertilizing with mineral nitrogen was applied (Savic, 2012).

According to the obtained results regardless of fertilizing the yielding capacity of Tango variety was lower in comparison with Goliath variety (Table 3). In the option without fertilization mean difference was 7.7 t ha^{-1} over three years. Fertilization of Tango variety with DripFert $N_{20}P_{20}K_{20}$+Me alone provided mean yield increase by 7%, whereas fertilizing together with Liposam or Organic-balance – by 14–16%.

In the option where Tango variety was fertilized with Organic-balance the yield was by 1.1 t ha^{-1} higher, or by 80% compared to control. Combined application of bacterial preparation with bioadhesive was more effective, the average yield capacity of Tango variety was by 0.7 t ha^{-1} higher than without Liposam and by 13% higher than in control.

The yield capacity of Tango variety in 2016 was lower than in control when Organic-balance and Organic balance + Liposam were applied, though with insignificant smaller difference (by 0.2–0.5 t ha^{-1}) according to the statistical analysis (LSD_{05B} – *1.4* t ha^{-1}). The yield capacity of Tango variety in 2018 was insignificantly higher than control in the variant with DripFert $N_{20}P_{20}K_{20}$ + Me. Throughout the experiment period the yield capacity of Tango variety was significantly higher under the application of Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam by 2.1–4.6 t ha^{-1} according to the LSD_{05B} – *2.0–2.1* t ha^{-1}. On average over three years complex fertilization of Tango variety with mineral fertilizer and bacterial preparation together with Liposam provided the yield which was by 22% higher than in control.

During the experimental period a considerable increase of yield within Goliath variety was achieved in the variants with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam, and Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me. Goliath variety had the highest yield capacity in 2018 under combined fertilizing with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam with 3.7 t ha^{-1} yield increase compared comparison to variant and 1.9 times higher than in control. Average increase of yield of Goliath variety over three years under fertilizing with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam made up 18% compared to the comparison variant. Under the application of DripFert $N_{20}P_{20}K_{20}$ + Me the yield of Goliath variety was by 0.5–1.0 t ha^{-1} lower compared to additional fertilization with mineral fertilizer together with Liposam or Organic-balance. Yield increase of this variety was lower under the application of Organic balance alone and made up 0.6 t ha^{-1}.

In a whole the yielding capacity of leek was lower under the application of Organic balance separately or together with bioadhesive compared to the variants where it was applied with DripFert $N_{20}P_{20}K_{20}$ + Me. The maximum yield increase was achieved in the variant where Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam were applied: by 18% (Goliath) and by 24% (Tango).

Table 3. The yield of leek pseudo-stem of and marketability of output, 2016–2018

Variety (A)	Additional fertilizing (B)	Yield (t ha^{-1})				Marke-tability (% from total plant mass)
		Years of research			Average over three years	
		2016	2017	2018		
Goliath	Without fertilization (comparison variant)	19.9	20.4	24.6	21.6	62
	Organic-balance	19.2	21.0	26.3	22.2	58
	Organic-balance + Liposam	21.7	21.7	27.0	23.5	60
	DripFert $N_{20}P_{20}K_{20}$ + Me	21.8	22.2	25.7	23.2	55
	DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	22.3	22.5	26.2	23.7	51
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	22.0	23.2	27.4	24.2	57
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	24.1	23.8	28.3	25.4	64
Tango	Without fertilization (control)	12.7	14.2	15.0	13.9	49
	Organic-balance	12.1	15.8	17.1	15.0	61
	Organic-balance + Liposam	12.5	16.2	18.4	15.7	56
	DripFert $N_{20}P_{20}K_{20}$ + Me	13.0	15.0	16.4	14.8	50
	DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	1.34	16.8	17.3	15.8	56
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me	13.6	17.0	17.6	16.1	55
	Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam	14.8	17.6	19.6	17.3	54
LSD_{05A}		*0.8*	*0.9*	*0.8*		–
LSD_{05B}		*1.4*	*1.5*	*1.5*		
LSD_{05}		*2.0*	*2.1*	*2.1*		
CV^1, %		*26*	*18*	*22*		

[1] – coefficient of variation.

The share of commercial product referred to the harvested yield was determined (Table 3). On average over three years Tango variety had the lowest share of commercial yield in control and under fertilizing with DripFert $N_{20}P_{20}K_{20}$ + Me. The higher yielding capacity of Goliath variety was caused by the higher mass of pseudo-stem than that of Tango variety. Thus, the marketability of Goliath variety without fertilizing was 13% higher than control. Fertilizing of Goliath variety with DripFert $N_{20}P_{20}K_{20}$ + Me did not improve marketabilitys, which was by 7% lower than variant without fertilization. The best marketability of Goliath variety was achieved after combined application of DripFert $N_{20}P_{20}K_{20}$ + Me, Organic-balance and Liposam.

Obtained results as to the efficiency of the application of microbiological preparations are in good agreement with the data of Colo et al. (2014): the mass of leek and output of standard products were significantly higher in the variants with *Azotobacter chroococcum, Bacillus subtilis* treatment. The yielding capacity of leek after the treatment of seeds with *Azotobacter chroococcum, Bacillus subtilis and Pseudomonas fluorescens* increased by 6.7–17.1 t ha^{-1} compared to control. The treatment of seeds with a mixture of these strains turned out to be less effective.

In the experiments of Choudhary & Paliwal (2017) the highest yield of broccoli was also achieved under the application of mineral fertilizers together with biological preparations that contain *Azosprillum* and worm compost (2.5 times higher than control). In general, the application of microbial preparations and mineral fertilizers increases the yielding capacity of vegetables and creates the conditions for their effective environmental friendly growth.

It has been established that the application of biological bacterial preparation Organic-balance with Liposam promotes the formation of the optimal microbial cenosis in the rhizosphere of leek at the background of mineral fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me. It proved by the statistically valid increase of the total number of microbiota, including *Azotobacter* bacteria. After a four-time application of Organic-balance + Liposam or together with DripFert $N_{20}P_{20}K_{20}$ + Me the total number of bacteria increased 2.5–2.8 times, the total number of *Azotobacter* bacteria increased 2.8–3.0 times in the rhizosphere of leek compared to the option without fertilization.

The leaf surface area and photosynthetic potential of leek were determined by variety properties as well as by the use of additional mineral fertilization and biological bacterial preparation. Over the period from the 10^{th} of May to the 10^{th} of October the photosynthetic potential of Tango variety was higher than that of Goliath variety, its maximum indexes were recorded in the options with fertilization Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me and Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam, on average the excess made up 18–46% compared to control.

The yield capacity of the investigated varieties of leek increased by 6–7% at the background of mineral fertilization DripFert $N_{20}P_{20}K_{20}$ + Me alone. Under the application of the bacterial preparation Organic-balance separately and together with Liposam the yield increase of Goliath variety was 3–9% and 8–13% of Tango variety compared to the options without fertilization. Application of Liposam or Organic-balance increased the efficiency of mineral fertilizer. Fertilization with DripFert $N_{20}P_{20}K_{20}$ + Me together with Organic-balance provided higher yield capacity of investigated leek varieties by 12–16%. The highest level of yield capacity was recorded under combined fertilization of leek with Organic-balance + DripFert $N_{20}P_{20}K_{20}$ + Me + Liposam – yield increase was 18% (Goliath) and 24% (Tango).

CONCLUSIONS

To summarize, it can be stated that in order to increase ecological safety of growing technology of leek it is reasonable to apply additional fertilization with biological preparations Organic-balance + Liposam throughout vegetation that promotes the increase in the number of soil microbiota, including nitrogen-fixing *Azotobacter* bacteria, and provides the increase of yield capacity and marketability of a pseudo-stem.

These preparations demonstrate the best efficiency when they are combined with mineral fertilizer DripFert $N_{20}P_{20}K_{20}$ + Me (a four-time additional fertilization). Regardless of fertilization the highest yield capacity and marketability of a pseudo-stem was manifested by leek variety Goliath.

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