

Using of probiotics and associated formation water as a basic fertiliser

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ABSTRACT

The application of probiotic preparations in order to restore soil fertility is becoming increasingly important. In this aspect, it is advisable to expand the scientific search for innovative environmentally friendly means of soil restoration, in particular, the synergistic effect of formation water (FW) and probiotics and in the system of sustainable agroecosystem functioning. Therefore, the purpose was to opportunity definition of using probiotics and associated formation water as a basic fertiliser. The research on the formation of biological fertilizers – FW (in application doses of 950–2400 L ha⁻¹) and probiotics (150 L ha⁻¹, 15% dilution) was conducted during the period of 2019–2024. The best variant during the research years was the combined application of probiotic 150 L ha⁻¹ (dilution of 15%) and 950 FW L ha⁻¹, with a yield of winter wheat of 5.13 L ha⁻¹, which is 28.9% higher than the control. The best concentration of FW on corn crops was found to be 950 L ha⁻¹ and probiotics 150 L ha⁻¹ (dilution of 15%), which resulted in an average yield increase compared to the control over the years of research in the amount of 24.3%. The number of nitrogen-fixing and ammonifying bacteria increases after applying this fertilizer, and then over the next few months their number levels out to the level of control. The use of FW concentration more than 1200 L ha⁻¹ reduces these groups of bacteria. The absence of negative impact of FW and probiotic mixture on soil structure was also found when they were applied in certain doses of FW – 650 to 1250 L ha⁻¹, probiotics in a concentration of 150 L ha⁻¹ (dilution of 15%).

Keywords: fertilizer, probiotic preparations, microbiota, formation water, biological agriculture, soil fertility.

INTRODUCTION

The intensive methods of agricultural production, involving high energy consumption, allowed to achieve high crop yields. However, the world agricultural priorities are now increasingly focused on finding ways to switch to alternative, resource-saving, environmentally friendly agricultural technologies (Pysarenko and Pysarenko, 2000; Yussefi-Menzler et al., 2010; Nikitenko and Averchev, 2021; Nimets et al., 2012; Shuvar et al., 2013).

One of the priority areas of greening agriculture is the use of organic fertilisers (Patyka et al., 1993; Ryzhuk and Medvediev, 2003; Kalinichenko, 2005; Kysil, 2005; Organic

Federation of Ukraine, 2015; Minkova, 2016; Kucher, 2017; Pysarenko, 2017; Honcharuk et al., 2020; Pisarenko et al., 2022; Kulyk et al., 2022; Pysarenko et al., 2022a). Lot of scientists studied the problems of improving organic fertilisers quality (Taylor et al., 2002; Volkohon et al., 2013; Bashkin, 2022; Magomedov et al., 2022; Zolotarev and Stepanova, 2022).

Much research is focused on the production of high-quality organic fertilisers through the use of different strains of microorganisms (Delgado et al., 2010; Abdel-Dayem et al., 2012; Indriyati, 2014; Beck-Broichsitter et al., 2018).

Recently, the use of probiotic preparations in order to restore soil fertility has been actively studied (Ageev et al., 2006). In particular,

the possibilities of using probiotics for the treatment of poultry manure were investigated by many domestic scientists and recommended preparations and doses were determined (Kishko et al., 2018). Probiotic preparations consist of bacteria of the genus *Bacillus* (probiotic bacteria) and enzymes. Probiotics do not have a negative impact on soil quality, unlike chemicals, they do not contain mineral pollutants and chemical (Pysarenko et al., 2021). Research works of Baldi et al. (2007), Guo et al. (2010), Patyka et al. (2014), Cui et al. (2017), Pysarenko et al. (2021a,c, 2022a, 2022b), Mo et al. (2022), Devi et al. (2022), Xu et al. (2023), Zhao et al. (2023) were dedicated to use microbial preparations (including probiotic and bacterial) for remediation of environment.

Today, particular attention in the development of zonal farming systems is paid to the use of local raw materials to increase effective soil fertility and biologisation of agriculture, in particular natural brines and minerals. Research conducted in (Pysarenko et al., 2022b) made it possible to establish the optimal dose of associated formation water to improve the quality of organic fertilisers.

Based on the research conducted by Obire & Amusan (2003), and Reva (2016) it was found that FW contains a significant amount of mineral elements and inorganic compounds (about 60 different micro- and macroelements), in particular sulfates and chlorides, the total mineralization is in the range of 140–180 g dm⁻³. However, the impact of FW in different doses on the soil has not been studied sufficiently. The previous research conducted by Markina (2019) established the possibility of using FW as an environmentally friendly substitute for agrochemicals on the crops of cereals in order to increase their yields. The phytosanitary impact of FW on the crops of cereals was studied as well (Pysarenko et al., 2021).

Thus, it can be stated that the study of the probiotics use in the agriculture is innovative and requires further research. However, it should be noted that microbial preparations, despite the undeniable environmental feasibility of their use, have the disadvantage of being unstable and dependent on external factors. Therefore, in view of the perspectives of previous research on the use of associated formation water to improve the quality of organic fertilizers (Pysarenko et al., 2021b), which is also a source of macro- and microelements and can act as a nutrient medium for beneficial microorganisms, it is advisable to expand the scientific search for innovative

environmentally friendly means of soil restoration, in particular, the synergistic effect of associated formation water and probiotics in the system of sustainable functioning of agroecosystems.

The purpose is to opportunity definition of using probiotics and associated formation water as a basic fertiliser for crops.

MATERIAL AND METHODS

During the period of 2019–2024, the research on the use of mixture of probiotic preparations (probiotics) and FW as a basic fertiliser on crops was carried out on the fields of PSAU – Agrarian University in Poltava. Probiotic preparations preparations *Sviteko* (*Sviteko-Agrobiotic-01* – produced by research and production enterprise “Eco-Kraiina”, Tereshky village, Poltava region, Ukraine) were used in this research, the main microorganisms of which are *Bacillus subtilis*.

For the research we used FW Suhodoliv gas and oil field, Poltava region of Ukraine, and gas field located in the center of Ukraine (Poltava region), which according to the mineralisation criterion belongs to highly mineralised water. In terms of ionic composition, FW is of the calcium chloride type and contains up to 5% of organic substances, i.e. refers to water with a low organic content.

Methods for determining the effect of probiotics and FW on microbiota of soil

For analyses, 15 g of soil was taken from each variant of experiment. The experiments were performed out in 3 replicates.

Tenfold dilutions of the soil suspension were exploited for inoculation onto selective media. The examples were transferred to sterile mortars and they dispersed according to the Zvyagintsev (1991) method. The value of trophic and ecological groups of microorganisms of soil was determined sowing cultivations of soil suspensions on relevant nutrient media (Andreiuk et al., 1988; Romero-Olivares et al., 2017; Iutynska, 2017; Liuta and Kononov, 2018).

The microorganisms number was identified on standard nutrient media by sowing the suspension of soil: ammonifying bacteria – on MPA (meat-peptone agar); pedotrophic bacteria – on SA (soil agar); bacteria that use mineral nitrogen and streptomycetes – on SAA (starch-ammonia

agar); nitrifiers were determined on Vinogradsky liquid medium (1 ml of suspension, 2–4 dilutions) and on leached starvation agar with 2.5 ml of 20% $MgNH_4_6H_2O$ (sowing on the surface); denitrifiers – on MPA medium with 0.1% ammonium nitrate; microorganisms oligotrophic – on SA (starvation agar); the microscopic fungi number – on agarified Chapek medium; the number of spore forms of microorganisms – after pasteurisation (70°) on MPA with carbohydrates, or on wort-agar (SA) medium; the number of pathogenic forms of microorganisms, respectively (Filon et al., 2017).

After sowing the nutrient media, based on the rate of growth of microorganisms of certain groups, they were incubated at 28 °C for 7–15 days (Li et al., 2014).

The content of the soil sample moisture taken for the experiment was defined using the thermostat-weight method. The colonies number was enumerated taking into account the dilution of the soil suspension and moisture content. The microorganisms number was expressed in CFU (colony forming units) per 1 g of dry soil. The experiments were carried out in triplicate.

The chemical, mechanical and physical qualities of the soil were determined by the following methods

The mechanical and physical qualities of the soil (content of water-resistant aggregates, structure) were studied according to the method of Shtatnov, Savynov (Vadyunina and Korchagina, 1986). The total humus content was established according to ISO 10694:1995. Soil sampling was carried out in compliance with ISO

18400-102:2017, and preparation for analysis was carried out in compliance with the demands of ISO 23909:2008. The laboratory analysis of soil samples was carried out at accredited agro-ecological monitoring laboratory of the PSAU.

Soil chemical properties were determined by the methods: mobile sulphur according to ISO 11048:1995, soil acidity by ISO 10390:2021, determination of nitrate in soil according to ISO 14255:1998; chlorides according to DSTU 7908:2015, oil products according to ISO 11504:2017. Heavy metals in the soil were determined by the following: lead, cadmium, copper, zinc, mercury (ISO 11047:1998).

Grain crops harvesting was carried out by collecting a sheaf sample in 3 replicates, and maize - by continuous method on the experiment variant in the phase of full grain ripeness. The yield structure was determined by the method of field experiment according to Dospekhov (1985).

The mathematical processing of the experimental data was carried out according to the generally accepted methods using the MS Excel.

RESEARCH RESULTS

The study on the formation of ecologically safe fertilisers relies on biological methods – FW (in application doses of 950–2400 L ha⁻¹) and probiotic (150 L ha⁻¹, 15% dilution, according to previous studies (Nimets et al., 2022) were conducted during the period of 2019–2024 (Table 1). The research was carried out under production conditions, and associated formation water was applied with the RZHU-3.6 machine for the main soil tillage.

Table 1. The effect of probiotics and FW applying rates on yield of winter wheat (average for the research years)

Variant of the experiment	Average productivity, t ha ⁻¹	Yield increase	
		t ha ⁻¹	%
Control (without AFW and probiotic)	3.98	-	-
FW 950 L ha ⁻¹	4.38	0.40	10.1
FW 1200 L ha ⁻¹	4.83	0.85	21.4
FW 2400 L ha ⁻¹	4.60	0.62	15.6
FW 950 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 15% dilution)	5.13	1.15	28.9
FW 1200 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 15% dilution)	4.89	0.91	22.7
FW 2400 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 15% dilution)	4.29	0.31	7.8
$N_{50}P_{50}K_{50}$	4.58	0.60	15.1
LSD 0.05	2.3		

Note: statistically significant difference, $p < 0.05$.

The best variant for the research years (2019–2024) was the technology of combined application of probiotic 150 L ha⁻¹ (15% dilution) and FW 950 L ha⁻¹, which resulted in a winter wheat yield of 5.13 t ha⁻¹, which is 28.9% higher than the control. The indication of the investigated soil revealed that the introduction of probiotics and FW fostered to the creation of a specific level of biological activity in the upper layer of soil, which led to the special terms for the transformation of organic matter and agrobiocenosis productivity (Table 2).

Thus, the using of FW at 950 L ha⁻¹ and a probiotic at 150 L ha⁻¹ (15% dilution) as a fertiliser creates favourable conditions for a number of micro-organisms in the soil. The development and growth of cellulose-degrading microorganisms and microscopic fungi involved in the putrefaction of plant residues is stimulated. The activity of microorganisms oligotrophic that complete the mineralisation of organic leftovers also increases significantly. The proportion of microorganisms in microbial cenosis is significant and in control soil it was 5.9 ± 0.21 mln, when probiotics 150 L ha⁻¹ and FW 950 L ha⁻¹ was used, this meanings was 19.2 ± 0.90 mln.

Ammonifiers and nitrogen fixers are very important microorganisms in the cycle of nutrients. The population dynamics of these groups of soil microorganisms is shown in Figure 1–2. The number of nitrogen-fixing and ammonifying bacteria expand just after application, and then over the next few months their number levels out to the level of control. The use of FW concentration more than 1200 L ha⁻¹ result in reduction in these groups of bacteria. In 2019–2024, we also studied changes in the physical and chemical soil qualities in the outcome of using FW and probiotic as an organic fertiliser on winter wheat crops. The most valuable structure is one that has aggregates between 10 mm and 0.25 mm in size, which do not break down in water for long periods of time (Patyka et al., 1993; Kaminskyi et al., 2020).

FW and probiotic were applied as a basic fertiliser for the main tillage in the following rates: AFW 950, 1200, 2400 L ha⁻¹; probiotic – 150 L ha⁻¹ (15% dilution). The structure of soil or aggregate state and the content of waterproof units were determined in dissimilar layers of soil. Soil samples were taken one month after the application. The plots without AFW and probiotic application, as well as plots with full mineral

Table 2. Main groups microorganisms number in the soil, average value Actinomycetes

Variant of the experiment	Ammonifying bacteria (mln)*	Bacteria nitrogen-fixing (mln)*	Microorganisms oligotrophic (mln)*	Pedotrophic bacteria (mln)*	Actinomycetes (mln)*	Total number of bacteria (mln)*	The microscopic fungi number (ths.)
Control	13.9±0.40	19.3±0.23	3.5±0.15	12.2 ±0.57	0.6±0.12	5.9±0.21	36.4±1.10
FW 950 L ha ⁻¹	22.9±1.15	26.2±0.60	3.7±0.06	36.9±1.77	1.2±0.00	11.7±0.13	40.2±0.60
FW 950 L ha ⁻¹ + probiotic (150 L ha ⁻¹)	24.7±0.29	28.8±1.15	8.6±0.10	38.6±0.03	1.4±0.03	19.2±0.90	39.5±1.20

Note: amount of cells in 1 gram of soil; statistically significant difference, p < 0.05.

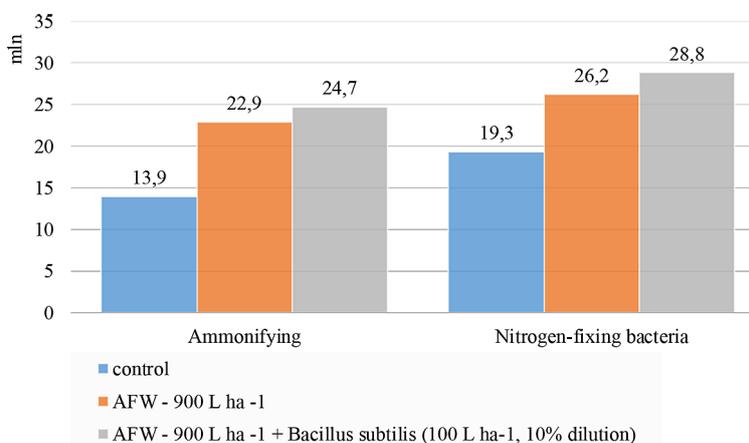


Figure 1. The number of bacteria nitrogen-fixing and ammonifying under the use of different systems of basic fertiliser on the 30th day after application (averaged data for 2019–2024, control – without FW and probiotic)

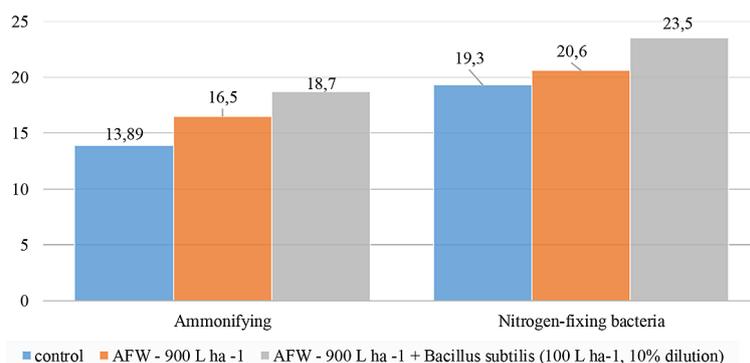


Figure 2. The number of bacteria nitrogen-fixing and ammonifying under the use of different systems of basic fertiliser on the 60th day after application (averaged data for 2019–2024, control – without FW and probiotic).

fertilizer $N_{50}P_{50}K_{50}$ were taken as control. The experimentally obtained data is shown in Table 3.

It was found that the enlarge of a dose of FW above 1200 L ha^{-1} had a negative effect on the soil structure, especially the upper layer – 0–10 cm (when FW was applied at 2400 L ha^{-1} , the air-dry aggregates content in the 0–10 cm layer of soil was 77.1). Instead, when FW was applied in a concentration of 950 to 1200 L ha^{-1} in a mixture FW and probiotic, there was no significant deterioration. The resistance of water of soil aggregates also significantly depended on the dose of FW application. The water resilience of aggregates of soil decreased sharply when a concentration of 2400 L ha^{-1} was used (with the applying of 2400 L ha^{-1} , the content of water-proof aggregates in the layer of soil of 0–10 cm decreased to 75.5 against 84.1 in the control). Thus, we can make preliminary conclusions about the absence of a negative impact of FW and probiotic on the structure of soil when applied in concentration of FW – from 600 to 1200 L ha^{-1} , probiotics in a dose of 150 L ha^{-1} (15% dilution).

A number of chemical parameters are among the indicators of soil system stability. Therefore, during the period of 2019–2024, we investigated the chemical parameters of the soil after adding of FW and probiotic (Table 4). The addition of probiotics and FW in the soil solution does not contribute to an increase in the content of nitrates, but, on the contrary, lowers it. This can be explained by the fact that FW and probiotic preparations in the suggested doses stimulate the development and growth of not only plants, but also soil biota, which is a direct consumer of anions and cations. The application of FW in concentrations of 950– 2400 L ha^{-1} does not foster to the storage of heavy metals and oil in the soil. On the contrary, the content of petroleum products in the soil solution of the upper soil layer changes significantly due to the optimisation of the vital activity of soil microflora. The study of the proposed basic fertiliser in the form of a mixture of FW and probiotic was also carried out on maize crops during 2019–2024. It was discovered that the best concentration of probiotics 150 L ha^{-1} on maize crops and FW is 950

Table 3. Structural state of the soil after application of probiotics and FW on winter wheat crops (averaged data for 2018–2023)

Variant	Aggregate content 0.25–10 mm, % by weight in the soil layer					
	0–10		10–20		20–30	
	Air-dry	Water-resistant	Air-dry	Water-resistant	Air-dry	Water-resistant
Control (without AFW and probiotic)	82.3	84.1	86.3	78.2	80.7	96.5
AFW 950 L ha^{-1} + probiotic (150 L ha^{-1} , 15% dilution)	89.6	84.5	85.2	75.5	84.3	89.3
AFW 1250 L ha^{-1} + probiotic (150 L ha^{-1} , 15% dilution)	79.6	87.8	85.8	89.6	84.1	92.5
AFW 2450 L ha^{-1} + probiotic (150 L ha^{-1} , 10% dilution)	77.1	75.5	85.1	64.9	81.4	73.1
$N_{50}P_{50}K_{50}$	82.4	64.8	90.9	73.7	88.3	64.6

Note: statistically significant difference, $p < 0.05$.

Table 4. Changes in soil chemical parameters after application of probiotics and FW as the basic fertiliser (average for 2018–2023)

Variant of the experiment	pH of the soil solution	Anions, cations, mg kg ⁻¹			Oil product, mg/kg	Heavy metals, mg kg ⁻¹				
		Nitrates	Chlorides	Mobile sulphur		Hg	Cu	Pb	Zn	Kd
Control (without AFW and probiotic)	7.6	9.8	131	42.0	330	0.091	0.6	2	28	-
AFW 950 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 15% dilution)	6.5	8.7	149	40.2	200	0.052	1.0	4	15	-
AFW 1200 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 105% dilution)	6.5	8.7	149	42.8	200	0.065	0.7	4	18	-
AFW 2400 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 15% dilution)	6.2	8.7	149	58.6	200	0.060	0.7	6	18	-
N ₅₀ P ₅₀ K ₅₀	6.4	30.5	149	34.4	340	0.090	0.8	6	23	-

Table 5. Impact of FW and probiotic treatment on the productivity of maize crops (average for the research years)

Variant of experiment	Average productivity, t/ha	Increase in productivity	
		t/ha	%
Without FW and probiotic -control	4.02	-	-
FW 950 L ha ⁻¹	6.88	2.86	71.1
FW 1250 L ha ⁻¹	6.11	2.09	51.9
FW 2450 L ha ⁻¹	5.62	1.60	39.8
FW 950 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 10% dilution)	7.22	3.20	79.6
FW 1200 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 10% dilution)	6.20	2.18	54.2
FW 2400 L ha ⁻¹ + probiotic (150 L ha ⁻¹ , 10% dilution)	5.76	1.74	43.2
N ₅₀ P ₅₀ K ₅₀	6.08	2.06	51.3
LSD 0.05	2.3		

L ha⁻¹ (15% dilution). which resulted in an average yield increase of 24.3% over the research years.

As shown in Table 5, the application of FW of various concentrations and probiotics on maize crops leads to a significant increase in yield. Thus, the use FW concentrations of 950, 1250 and 2450 L ha⁻¹ increased the yield by 2.86 t/ha, 2.09 t/ha and 1.60 t ha⁻¹ (by 71.1%, 51.9% and 39.8% - compared to control). However, the combined use of these concentrations of FW (950, 1200 and 2400 L ha⁻¹) with probiotics (150 L ha⁻¹, 15% dilution) increased this efficiency: 3.20 t ha⁻¹, 2.18 09 t/ha and 1.74 09 t/ha (by 79.6%, 54.2% and 43.2% - compared to control). The utilize of mineral fertilisers N₅₀P₅₀K₅₀ allowed to increase the yield by 2.06 t/ha, by 51.3% compared to the control, which is slightly lower (by 0.5%) compared to the best variant with the use of FW 950 L ha⁻¹ and probiotic 150 L ha⁻¹ (15% dilution). Thus, the best effect was obtained as in the variant on winter wheat using FW 950 L ha⁻¹ and probiotics (150 L ha⁻¹, 15% dilution) as a basic fertilizer. The favourable effect of FW and probiotic as the main fertiliser was observed not only in winter wheat crops, but also in maize.

CONCLUSIONS

The conducted research on the complex application of probiotics (150 L ha⁻¹, 15% dilution) and AFW at application rates of 950–2400 L ha⁻¹ made it possible to determine the optimal concentration of FW – 950 L ha⁻¹, at which the enhance in winter wheat yield was 28.9% compared to the control, and the enhance in corn yield was 79.6% compared to the control. This is because such concentrations of FW and probiotic create favourable conditions for the number of soil microorganisms, including stimulating the growth and development of decomposing-cellulose microorganisms and microscopic fungi involved in the decomposition of plant residues.

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