Complex biopreparation for maintaining natural fertility in agrophytocenoses

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Abstract. The effectiveness of using a new biopreparation for the decomposition of wheat straw, which consists of cellulolytic bacteria CA3 and CU6 and strains-antagonist of the phytopathogenic fungi *Pseudomonas laurentiana* ANT 17 and *Paenibacillus peoriae* ANT 13, has been studied. AND 17 and ANT 13 additionally have growth-stimulating activity and the ability to mobilize inorganic phosphates. The greatest degree of decomposition of straw was observed when combined the biopreparation and ammonium nitrate (50.8% versus 25.2% in the control). At the end of the field experiment carrots were grown in soil with decomposed straw. When biopreparation and ammonium nitrate were used for straw processing, the weight of the carrot root crop was 14.4% more than in the control, and the diameter of the root was 55.6% bigger.

1 Introduction

A necessary condition for the preservation of soil fertility is the introduction of organic fertilizers. Traditionally, agricultural producers used manure for these purposes, but in recent years its use has been steadily decreasing [1]. An alternative way to replenish the organic matter of the soil is the introduction of non-marketable parts of the grain or leguminous crops. The preservation of post-harvest plant residues in the upper part of the arable layer ensures the biological circulation of organic matter, increases the water-retaining and filtration capacity of the soil, prevents the development of erosion processes [2].

Among the reasons why the use of straw and stubble as an organic fertilizer has not yet become widespread, it is necessary to mention a rather low rate of their decomposition, especially when applying high doses. This is explained by a number of factors: weak biological activity of soils, the presence of compounds in the composition of post-harvest residues, during the decomposition of which phytotoxic substances are released. Moisture availability and temperature conditions also have a significant impact on the rate of degradation. In addition, the C:N ratio in straw is 70-90:1, therefore, at the initial stages of its destruction, soil nitrogen is immobilized, which creates a deficiency of this element and causes an additional decrease in the microbiological activity of agrobiocenosis [3].

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To stimulate the process of transformation by the native microbiota of the plant mass entering the soil, additional application of mineral fertilizers, as well as biological preparations containing highly effective consortia of microorganisms is used [4]. In order to increase the positive effect of the use of biological destructors, it is advisable to include cellulolytics and strains of microorganisms with antagonistic, nitrogen-fixing, phosphate-mobilizing activity in their composition [5].

The aim of the work was to study the effectiveness of the use of a new biological preparation based on a complex of beneficial microorganisms for the decomposition of wheat straw and to assess its effect on biological activity and soil fertility.

2 Materials and methods

The research was conducted in 2021 in a field experiment on the territory of the Republic of Bashkortostan (Russia). 20 g of crushed spring wheat straw (segments of 1-2 cm in size), brought to an air-dry state, were mixed with the soil (leached medium loamy chernozem) in a ratio of 1:100, then the mixture in an amount of 2 kg was placed in a nylon bag, which was buried at the site of soil sampling to a depth of 10-15 cm. The straw was pretreated with an aqueous solution of ammonium nitrate (NH_4NO_3) and/or a solution of the biopreparation. It included bacterial strains with high cellulolytic activity CA3 and CU6 and strains with antifungal activity against the phytopathogenic fungi, with growth-stimulating activity and the ability to mobilize inorganic phosphates *Pseudomonas laurentiana* ANT 17 and *Paenibacillus peoriae* ANT 13 (1:1:1:1). Ammonium nitrate was used at the rate of 1% by weight of straw, biopreparation – 5% by weight of straw. In the control, straw was treated with water. The duration of the experiment was five months (from May to September, 22 weeks).

The experience was laid according to the following scheme:

- Soil + straw without additional processing – control.
- Soil + straw + NH_4NO_3.
- Soil + straw + biopreparation (B).
- Soil + straw + B + NH_4NO_3.

The repetition of the experiment is fourfold. Meteorological conditions at the beginning and at the end of the experiment were at the level of long-term average data. At the end of the experiment, the bags with a mixture of soil and straw were carefully dug out and samples were taken for analysis enzymatic activity, agrochemical characteristics, phytotoxicity. The same analyses were carried out at the beginning of the experiment with the initial soil (before mixing it with straw).

Catalase activity was analyzed gasometrically by the Galstyan method, invertase – by Galstyan by determining the amount of reducing sugars, urease – colorimetrically by the Shcherbakov and Raikhinstein method, cellulase – colorimetrically by the Bagnyuk and Shchetinskaya method [6].

The intensity of straw decomposing process was determined by the weight method. For this purpose, at the end of the experiment soil samples (50 g) were taken from nylon bags, the undecomposed straw residues were washed on a sieve (0.25 mm), dried to a constant air-dry mass and weighed, then the percentage of straw in the soil was determined. The efficiency of straw decomposition (%) was calculated as the ratio of the difference in the weight of straw at the beginning and end of the experiment to the weight of the straw at the beginning of the experiment.

The content of organic carbon (C_{org}) was determined by wet oxidation by the Tyurin method, carbon extracted by hot water (C_{hwe}) – by the same method, but after 1 h boiling, pH_{KCl} – by the potentiometric method, mobile phosphorus and exchangeable potassium – by the Kirsanov method [7].

The influence of straw decomposition on soil toxicity was evaluated. Radish seeds (*Raphanus sativus* var. radicula Pers.) were used as a test object. 5 ml of soil extract was applied to filter paper placed in a Petri dish and 20 radish seeds were laid out. The repetition is fivefold. Petri dishes were incubated for 72 hours at 22°C. Next, the energy of seed germination and the length of the roots of the seedlings were taken into account.

An indirect assessment of soil nutrient enrichment was carried out by assessing the yield of carrots (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens). After five months of the experiment, soil containing partially decomposed straw was removed from all nylon bags of one variant and mixed. Then 450 g of soil was placed in vessels, into which 3 carrot seeds were planted. The experiment was repeated five times. The vessels were placed on a light platform. The duration of the experiment was 60 days.

Data were processed using Statistica software. In the figure and tables, data are presented as mean ± standard error (S.E.). Results from different treatments were compared using one-way ANOVA, and the Fisher-test was carried out to determinate the statistic differences. Significantly different values for each indicator are marked with different letters ($p < 0.05$).

3 Results

In our studies, when adding ammonium nitrate, the degree of decomposition of straw in the soil increased by 13.9% (Table 1), when using a biopreparation – by 22.3%. The greatest efficiency of this process was noted in the variant with the joint introduction of a compensating dose of nitrogen and a biopreparation, over the five months of the experiment, it amounted to 50.8% versus 25.2% in the control.

Table 1. Efficiency of decomposition of wheat straw in the soil.

Variants of experiment	The content of undecayed straw residues in the soil (%)	Straw decomposition efficiency (%)
Control	0.741 ± 0.030^{c}	25.2
NH_4NO_3	0.603 ± 0.023^{b}	39.1
Biopreparation	0.520 ± 0.017^{a}	47.5
Biopreparation + NH_4NO_3	0.487 ± 0.014^{a}	50.8

The addition of plant residues to the soil contributed to a noticeable increase in its enzymatic activity. The research results showed that by the end of the experiment, the invertase and catalase activity of the soil due to the introduction of straw increased by an average of 8.9-15.5% (Figure 1 a, b). The use of straw, including with additional components, led to an increase in the urease activity of the soil by about 1.5-1.6 times (Figure 1 c). The analysis of cellulase activity demonstrated that it increased within the same limits (by 1.5-1.6 times), the highest values were noted in variants with the addition of a biopreparation, which proves the effectiveness of using introduced microorganisms for the transformation of crop waste (Figure 1 d).

pH is an important chemical indicator that determines the activity of soil microbiota. In all variants of the experiment, a certain declining trend in the acidity of the soil was noted, which indicates the ongoing processes of transformation of organic compounds (Table 2). The study shows that the highest content of organic carbon compared to the beginning of the experiment was observed in variants with the introduction of microorganisms and the introduction of nitrogen. On the contrary, the amount of hot water extractable carbon in the same variants by the end of the experiment decreased by 9.9-11.8%. Thus, the biopreparation and ammonium nitrate stimulate the process of destruction of plant residues, but the substances formed in this case are actively used by microorganisms as a source of carbon and energy. It should be noted that with the introduction of straw in the soil, the phosphorus content increased by an average of 3.7-7.3 mg/100 g of soil. At the same time, it was found that the content of exchangeable potassium at the end of the experiment remained the same as at the beginning of the experiment at the level of 9.75-10.6 mg /100 g of soil (Table 2).

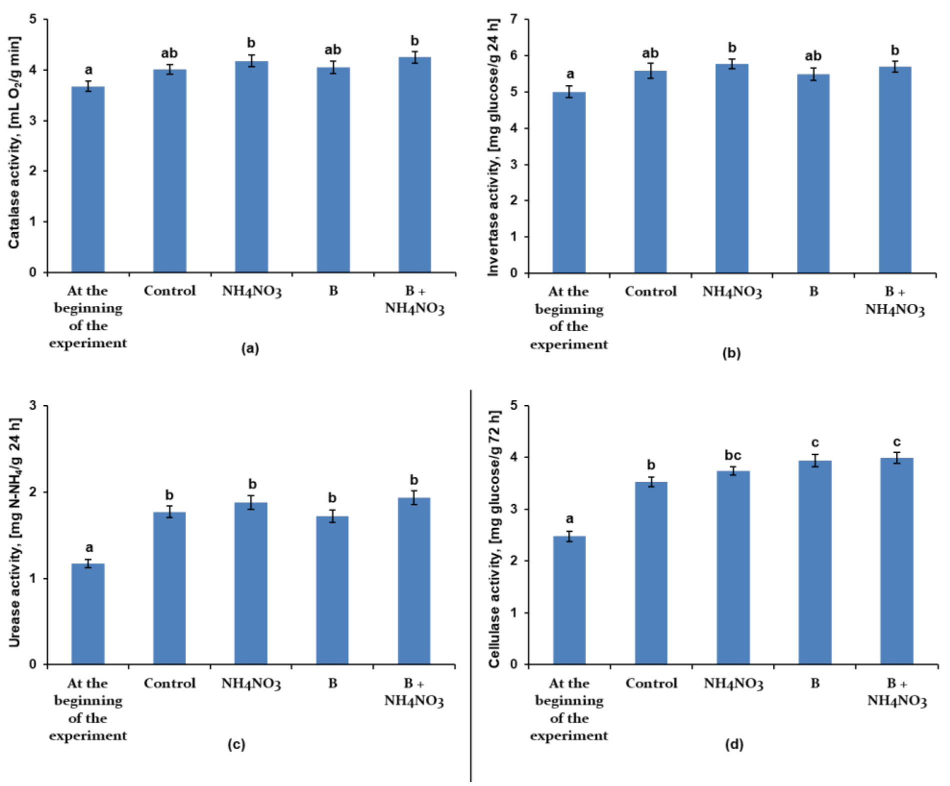

Fig. 1. Change of the enzymatic activity of the soil during the decomposition of wheat straw.

Table 2. Agrochemical characteristics of the soil containing plant residues.

Variants of experiment	**pH_{KCl}**	**C_{org}, %**	**C_{hwe}, mg/kg**	**P_2O_5, mg/100 g**	**K_2O, mg/100 g**
	At the beginning of the experiment				
	6.39 ± 0.15^a	**3.82 ± 0.11^a**	**233.0 ± 9.1^b**	**9.47 ± 0.35^a**	**10.1 ± 0.38^a**
	At the end of the experiment				
Control	6.58 ± 0.21^a	4.03 ± 0.15^{ab}	217.3 ± 8.4^{ab}	13.2 ± 0.53^b	10.6 ± 0.42^a
NH_4NO_3	6.68 ± 0.28^a	4.16 ± 0.15^{ab}	209.9 ± 8.7^a	15.2 ± 0.59^c	9.75 ± 0.39^a
Biopreparation	6.66 ± 0.20^a	4.23 ± 0.18^b	207.9 ± 9.1^a	16.4 ± 0.73^{cd}	10.5 ± 0.41^a
Biopreparation + NH_4NO_3	6.70 ± 0.18^a	4.12 ± 0.13^{ab}	205.6 ± 9.0^a	16.8 ± 0.66^d	10.5 ± 0.43^a

The analysis of soil phytotoxicity, based on the energy of seed germination and the length of radish seedlings, showed that the presence of straw residues, in general, did not have a pronounced negative effect on radish plants (Table 3). At the same time, in variants where the decomposition of straw was stimulated by the introduction of a biopreparation, the root length of radish seedlings was higher than in control by 10.6-12.9%.

Table 3. Indicators of phytotoxicity of the soil, test culture – radish.

Variants of experiment	Germination energy, %	Root length, mm
	At the beginning of the experiment	
	92.6 ± 3.8^{ab}	**24.5 ± 0.8^{b}**
	At the end of the experiment	
Control	88.9 ± 3.5^{a}	20.8 ± 0.8^{a}
NH_4NO_3	97.8 ± 4.0^{b}	22.1 ± 1.0^{ab}
Biopreparation	95.6 ± 3.4^{ab}	23.5 ± 0.9^{b}
Biopreparation + NH_4NO_3	95.6 ± 3.7^{ab}	23.0 ± 1.1^{b}

At the end of the field experiment, a vegetation experiment was set up, the purpose of which was to assess how the decomposition of wheat straw contributed to increasing soil fertility and the growth of subsequent plants in the crop rotation. It was found that carrots grown on soil with a higher degree of straw decomposition surpassed control plants in the growth rate of root crops (Table 4). The best results were obtained in the variant with additional treatment of the straw with a biopreparation and nitrogen: the weight of the carrot root was greater than in the control by 14.4%, the diameter of the root crop – by 55.6%.

Table 4. Morphometric indicators of carrot root crops.

Variants of experiment	Weight, g	Diameter, cm
Control	11.1 ± 0.48^{a}	0.9 ± 0.04^{a}
NH_4NO_3	12.5 ± 0.51^{b}	1.3 ± 0.05^{c}
Biopreparation	12.3 ± 0.45^{b}	1.1 ± 0.04^{b}
Biopreparation + NH_4NO_3	12.7 ± 0.58^{b}	1.4 ± 0.05^{c}

4 Discussion

The mineralization of organic matter includes two periods of unequal duration. After a relatively short initial stage of active decomposition of organic residues, a slowdown occurs caused by the consumption of easily accessible power sources and the accumulation of hard-to-decompose components and inhibitory compounds [8].

A decrease in the intensity of decomposition of plant mass may also occur due to the fact that straw is characterized by a high value of the ratio C : N. Over time, nitrogen deficiency only increases, and the metabolic activity of microorganisms begins to slow down [19]. It is obvious that one of the main ways to increase the efficiency of straw mineralization is the addition of mineral nitrogen to the soil [9]. Moreover, since the number of cellulolytic microorganisms in the soil is usually small [10], the process of straw destruction can be accelerated by introducing active strains of cellulolytics [11]. The results of the study showed that in the presence of ammonium nitrate (1% of the mass of plant residues), the process of decomposition of straw is activated (Table 1). The combined introduction of nitrogen and a biological preparation allowed to increase the efficiency of this process by additional 11.7%.

Soil biogenicity is determined by a pool of microorganisms and enzyme systems, including previously immobilized enzymes that can be activated when an additional substrate appears for their action ("substrate effect") [12]. The introduction of organic matter into the soil caused an increase in the number of aerobic microbiota that determines catalase activity, the increase in invertase activity was primarily due to the sugar content in straw (Figure 1). The presence of additional mineral nitrogen also contributed to an increase in the activity of the soil enzymes. Microorganisms use nitrogen for the synthesis of protein substances of cells, and in the process of their vital activity biologically fixed nitrogen enters the soil in the form of various organic compounds that are transformed with the participation of urease [13].

Soil organic matter includes active, easily decomposable and passive, stable components. Easily transformable components contained in plant residues are the main source of organic matter of microorganisms, due to its assimilation in microbial biomass, the carbon content in the soil increases, which is further transformed into humus carbon [14]. Changes in the content of organic carbon and its easily decomposable forms characterize the activity of microbiological processes occurring in the soil (Table 2). When applying organic fertilizers, an accumulation of such an important element for plants as phosphorus in an accessible form can be observed in the soil [15] (Table 2). This fact is explained by the activation of the entire soil microflora, including microorganisms involved in the transformation of phosphorus compounds.

The soil containing straw may have shown some toxicity at the first stages of decomposition [16] (Table 3), but in the future there was a steady positive effect of straw decomposition products on soil fertility. Thus, improving the growth and development of carrot plants (Table 4), apparently, was due to the fact that as a result of the effective decomposition of straw, there was an accumulation of reserves of organic matter, as well as available phosphorus. In addition, one of the possible explanations for the established effect is the ability of the bacteria that make up the biopreparation to suppress the development of phytopathogenic soil micromycetes, i.e. to improve the phytosanitary condition of the soil [17].

5 Conclusion

As a result of the research, the effectiveness of the use of a new complex microbiological preparation for the decomposition of wheat straw has been established. During the five months of the experiment, the degree of destruction of straw during the introduction of a biological preparation was 47.5%, with the addition of a biological preparation and a compensating dose of nitrogen – 50.8% versus 25.2% in the control.

When assessing the effect that straw decomposition has on soil toxicity (radish was used as a test object), it was found that in variants where straw decomposition was stimulated by the introduction of a biological preparation, the root length of radish seedlings was higher than in the control by 10.6-12.9%.

In the vegetative experiment, it was shown that when growing carrots in soil containing decomposed straw, the best results were obtained in the variant with additional straw treatment with a biological preparation and nitrogen: the weight of the carrot root was 14.4% greater than in the control, the diameter of the root crop was 55.6%.

In general, the use of this biological product-destructor seems to be particularly effective in crop rotations saturated with grain crops, as well as on soils where intensive anthropogenic load has negatively affected the diversity and abundance of the soil-forming microbiota. Further study and introduction of such microbiological preparations undoubtedly deserves the most careful attention, since their low cost and small application rates allow us to consider the technology associated with their use as an economically advantageous approach to the disposal of unused plant biomass.

References

1. J. Timsina, Can organic sources of nutrients increase crop yields to meet global food demand?, Agronomy, **8, 214** (2018)
2. M.S. Turmel, A. Speratti, F. Baudron, N. Verhulst, B. Govaerts, Crop residue management and soil health: A systems analysis, Agric. Syst., **134**, 6-16 (2015)
3. F. Yan, Y. Sun, X. Hui, M. Jiang, K. Xiang, The effect of straw mulch on nitrogen, phosphorus and potassium uptake and use in hybrid rice, Paddy Water Environ., **17**, 23-33 (2019)
4. S. Qin, K. Jiao, D. Lyu, L. Shi, L. Liu, Effects of maize residue and cellulose-decomposing bacteria inocula on soil microbial community, functional diversity, organic fractions, and growth of *Malus hupehensis* Rehd., Arch. Agron. Soil Sci., **61**, 173-184 (2015)
5. I.E. Smirnova, A.K. Sadanov, Cellulolytic bacteria and association of effective microorganisms for biocontrol of root rot infections in sugar beet (*Beta vulgaris* L.), Agricult. Biol., **54**, 1041-1052 (2019)
6. F.H. Haziev, Methods of soil enzymology, Moscow, Nauka, 252 (2005)
7. Workshop on agrochemistry, Moscow, MSU, 689 (2001)
8. A. Grzyb, A. Wolna-Maruwka, A. Niewiadomska, Environmental factors affecting the mineralization of crop residues. Agronomy, **10, 1951** (2020)
9. G. Mühlbachová, P. Růžek, H. Kusá, R. Vavera, M. Káš, Winter wheat straw decomposition under different nitrogen fertilizers. Agriculture, **11, 83** (2021)
10. T. Melnichuk, A. Egovtseva, S. Abdurashytov, E. Abdurashytova, I. Kameneva et al., Associative to *Triticum aestivum* L. bacteria as a source of strains for biotechnology of the rhizosphere, J. Microbiol. Biotech. Food Sci., **8**, 1194-1197 (2021)
11. A. Panfilova, Influence of stubble biodestructor on soil microbiological activity and grain yield of winter wheat (*Triticum aestivum* L.), Notulae Scientia Biologicae, **13, 11035** (2021)
12. A.T. Adetunji, F.B. Lewu, R. Mulidzi, B. Ncube, The biological activities of β-glucosidase, phosphatase and urease as soil quality indicators: a review, J. Soil Sci. Plant Nutr., **17**, 794-807 (2017)
13. B. Sawicka, B. Krochmal-Marczak, P. Pszczółkowski, E.J. Bielińska, A. Wójcikowska-Kapusta, Effect of differentiated nitrogen fertilization on the enzymatic activity of the soil for sweet potato (*Ipomoea batatas* L.[lam.]) cultivation, Agronomy, **10, 1970** (2020)
14. A. Vidal, F. Watteau, L. Remusat, C.W. Mueller, T.T. Nguyen Tu, Earthworm cast formation and development: a shift from plant litter to mineral associated organic matter, Front. Environ. Sci., **7, 55** (2019)
15. J. Lehmann, C.M. Hansel, C. Kaiser, M. Kleber, K. Maher, Persistence of soil organic carbon caused by functional complexity, Nat. Geosci., **13**, 529-534 (2020)
16. Y. Lou, A.S. Davis, A.C. Yannarell, Interactions between allelochemicals and the microbial community affect weed suppression following cover crop residue incorporation into soil, Plant Soil., **399**, 357-371 (2016)
17. E. Patkowska, Biostimulants managed fungal phytopathogens and enhanced activity of beneficial microorganisms in rhizosphere of scorzonera (*Scorzonera hispanica* L.), Agriculture, **11**, 347 (2021)