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### **BIOENRICHMENT OF POULTRY MANURE BY ASSOCIATION OF A FUNGUS TRICHODERMA HARZIANUM 128**

**The purpose.** To study features of growth of association of fungi *Trichoderma harzianum* 128 at enrichment of compost of organic matter on the basis of poultry manure and to evaluate perspectives of bio enrichment of compost. **Methods.** Laboratory (for simulation of process of bio enrichment); industrial (approbation of technique of enrichment); field (determination of efficiency of compost at growing potato); statistical. **Results.** In conditions of model experiments the optimum terms of introduction in compost substrate of agronomically valuable microorganisms depending on their predominant functions are fixed. Application of *T. harzianum* 128 for 2-nd month of enrichment of compost promotes prompt growth of strange micromycets. Efficiency of technique is shown under production conditions. The gained biocompost is characterized by heightened total carbon and nitrogen, positively influences productivity of potato and product quality. **Conclusions.** Enrichment of compost of substrate on the basis of poultry manure at add-on of association of *T. harzianum* 128 ensures intensification of mineralization processes and accumulation of agronomically valuable microorganisms — active destructors of organic matter and producers of physiologically active joints. The gained biocompost is perspective for use in farming industry.

**Key words:** compost, successions of assemblages of microorganisms, poultry manure, *Trichoderma harzianum*.

**Introduction.** As is known, the intensive use of fertilizers and pesticides in agriculture provides a significant increase of crop yields in our country and abroad. At the same time, the excessive use of agrochemicals and unbalanced plants nutrition decrease the quality of crop production, while improper storage and use of chemicals leads to the environmental pollution. In this regard, there is a need in alternative means for crops nutrition and productivity increase.

Composting of organic matter is of particular interest. The positive effect of composts is caused not only by their contribution to the accumulation of humus in soil. Their systematic use improves physical and chemical properties of soil: increase nutrients supply, reduce soil acidity, and improve soil aggregates composition. Besides, a significant number of beneficial microorganisms are also introduced to the soil with composts. At this, the receipt of composts with programmed characteristics, including microorganisms' composition criteria, is highly desirable [1]. Promising is the creation of technologies for composting of organic matter including introduction to composts of agronomically useful microorganisms. In fact, such composts could serve as microbial preparations of complex action. Important in this respect is micromyceta of *Trichoderma* genus. Some species of this taxonomic group have valuable agronomic properties, and in particular, are capable of biological destruction of organic matter, possess antagonistic properties to a number of pathogens of cultivated plants and produce plant hormones [2-5].

In this connection, present research was aimed to determine the characteristics of composting of poultry manure organic matter and evaluate the optimal period for introduction to compost of *Trichoderma harzianum* 128 fungi association.

**Materials and methods.** The study was conducted during 2013 – 2015 in the Laboratory of Soil Microbiology of Institute of Agricultural Microbiology and Agricultural Production NAAS in model and field experiments. Due to the low carbon to nitrogen ratio (9.6:1) in poultry manure, it was mixed with peat and straw (used as carbon source) in calculated amounts to preset the optimum C:N ratio of 20:1 in substrate. Plastic containers (20 l) were used in the experiments, filled with 5 kg of chicken manure (70% moisture), 0.7 kg of chopped straw and 1.9 kg of peat.

The carbon and nitrogen content in substrate was studied in dynamics using Ansten method in modification of Ponomarev and Nikolaeva [6].

The number of microorganisms in composted substrates was determined in dynamics. The number of ammonifiers was determined using meat-and-peptone agar, while microorganisms capable to metabolize mainly mineral nitrogen compounds – using starch-ammonia

agar [7]. The number of nitrogen fixing bacteria was determined by limiting dilution method in semisolid Ashby medium using acetylene test [8]. The number of microorganisms that dissolve phosphorus compounds was measured using Muromtsev medium with the addition of orthophosphate and glycerophosphate [9]. The number of micromycetes was determined on the wort agar [7].

The content of starch and ascorbic acid were determined using existing methods [10].

Pre-composted association of *Trichoderma harzianum* 128, which includes micromycetes strains *T. harzianum* 128/1 and *T. harzianum* 128/2 was used in the study of microorganisms' introduction to the substrate. This association was shown to be capable of cellulose destruction and production of physiologically active compounds, including phytohormones. Fungi association of *T. harzianum* 128 is deposited in the Depository of the D.K. Zabolotny Institute of Microbiology and Virology NAS of Ukraine and is included to the National Collection of Useful Soil Microorganisms, deposited in the Institute of Agricultural Microbiology and Agricultural Production NAAS.

Culture of *T. harzianum* 128 fungi was introduced to substrate at various stages of composting under the following experimental design:

1. Compost without micromycetes association (control);
2. Introduction of *T. harzianum* 128 to the substrate on 1-month of composting;
3. Introduction of *T. harzianum* 128 to the substrate on 2-month of composting;
4. Introduction of *T. harzianum* 128 to the substrate on 3-month of composting;
5. Introduction of *T. harzianum* 128 to the substrate on 4-month of composting;

The spore-mycelium of *T. harzianum* 128 was cultivated on wort-agar and rinsed with water. The resulting suspension had a titer of  $6.4 \times 10^6$  CFU/ml. The introduced amount was calculated at 2% rate from weight of the dry mixture of compost (128 thousand CFU/g of dry substrate).

The number of *T. harzianum* 128 was determined using dishes method on wort-agar [7].

The intensity of straw decomposition was measured during the experiment. Compost samples were taken at different periods – 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, and 7<sup>th</sup> months of composting, placed in a bowl of water and stirred. Straw was removed from the surface, while half-decayed remains were washed from compost using 0.25 mm sieve. Both fractions of plant residues were dried to the constant weight. The straw content was analyzed in the relation to the initial straw mass. The decomposition

percentage was calculated and compared to the control values (without introduction of *T. harzianum* 128 association).

The approbation of optimized version of chicken manure composting was carried out in production experiments on the fields of «Kolos, LTD» farming company (Kyiv region, Skvyrskyi district). The experiment involved composting of 200 tons of chicken manure, optimized by the carbon and nitrogen ratio, and introduction of *T. harzianum* 128 association. Spore-mycelial inoculant was obtained by fungi cultivation on the oats grain. The obtained titer was  $5.0 \times 10^8$  CFU/g of grain. Inoculant (52.6 kg) was introduced to the composted substrate using mass stirring aerator RT-120. At this the trichoderma content was accounted at 135 thousand CFU / 1 g of dry substrate.

The efficiency of experimental biological compost was tested in a field experiment on sod- mid-podzol silty sandy cultivated soil (humus content – 1.02%, easy hydrolyzed nitrogen – 57.0 - 58.0 mg (by Turin and Kononov), mobile forms of phosphorus– 330.0 mg  $P_2O_5$  (by Kirsanov), exchangeable potassium– 148 mg  $K_2O$  per 1 kg of soil (by Kirsanov);  $pH_{salt}$  – 6.2) in the Institute of Agricultural Microbiology and Agricultural Production NAAS. Mineral fertilizers were applied in in dose  $N_{60}P_{60}K_{60}$  in all test variants.

Experiments repetition was four-fold. Statistical analysis of the results was performed using analysis of variance [11], and a computer software (Microsoft Office Excel 2003 – 2007).

**Results.** The study of peculiarities of poultry manure substrates composting and determination of number of different ecological and trophic groups of microorganisms indicates a gradual decomposition of complex organic compounds by one microorganisms and creation of energy sources for others.

One of the indicators of the intensity level of mineralization processes in composted substrates is number of ammonifiers, which are characterized with two activation periods (Table 1). Thus, in control variant (without C:N optimization) a slight increase in the number of ammonifiers was observed during the initial composting stages (1 month), followed by a decrease in performance and some recovery starting after the sixth month of composting (168 days).

**Table 1. Developmental dynamics of microorganisms capable of ammonification in composted substrate, billion CFU / g**

Variant	Days after composting							
	21	42	84	105	147	168	231	252
Poultry manure, control	0.64 ± 0.06	0.52 ± 0.09	0.19 ± 0.01	0.23 ± 0.01	0.26 ± 0.05	1.50 ± 0.11	1.26 ± 0.04	0.25 ± 0.01
Compost substrate (poultry manure with straw and peat)	3.03 ± 0.02	0.33 ± 0.06	0.47 ± 0.02	1.65 ± 0.02	1.24 ± 0.06	0.22 ± 0.01	0.67 ± 0.02	0.05 ± 0.01

**Table 2. Developmental dynamics of nitrogen fixing bacteria in composted substrate, million cells / g**

Variant	Days after composting							
	21	42	84	105	147	168	231	252
Poultry manure, control	0.031	0.002	0.002	0.002	0.003	1.778	909.091	830.000
Compost substrate (poultry manure with straw and peat)	0.105	0.004	0.004	0.022	0.041	2.898	1083.333	838.710

At composting of substrate that was optimized by the “carbon : nitrogen” ratio the significant development of microorganisms of studied group was observed during the first month – the number of bacteria had exceeded the control values in 2 – 5 times. The recovery period for the number of ammonifiers (the second wave of development, caused by the destruction of complex organic compounds) was also observed much earlier than in the control

– in three months, indicating on the optimization of microbial processes. Starting from the fifth month of composting (147 days) the attenuation of mineralization processes was noticed, unlike in control where it has just started.

The number of bacteria that mostly metabolize mineral compounds of nitrogen had increased after the periods of active development of ammonifiers, which is quite logical – mineralization of organic matter had resulted in accumulation of inorganic nitrogen, used by these microorganisms.

The number of nitrogen fixing bacteria was low throughout the experiment and had increased only by the end of composting period. Obviously, the presence of inorganic nitrogen compounds in the composted substrate does not promote development of diazotrophs. The reduction of nitrogen transformation processes had established optimal conditions for the nitrogen fixation and development of nitrogen fixing microorganisms (Table. 2).

Calculation of phosphorous mobilizing microorganisms indicates their low number in compost, which indirectly may indicate on the lack of appropriate conditions for their development (this mainly reflects lower phosphate content in the substrate).

Development of micromycetes was characterized with the gradual increase of their numbers during the first three months of composting with the peak period within 4<sup>th</sup> – 5<sup>th</sup> months (Fig. 1).

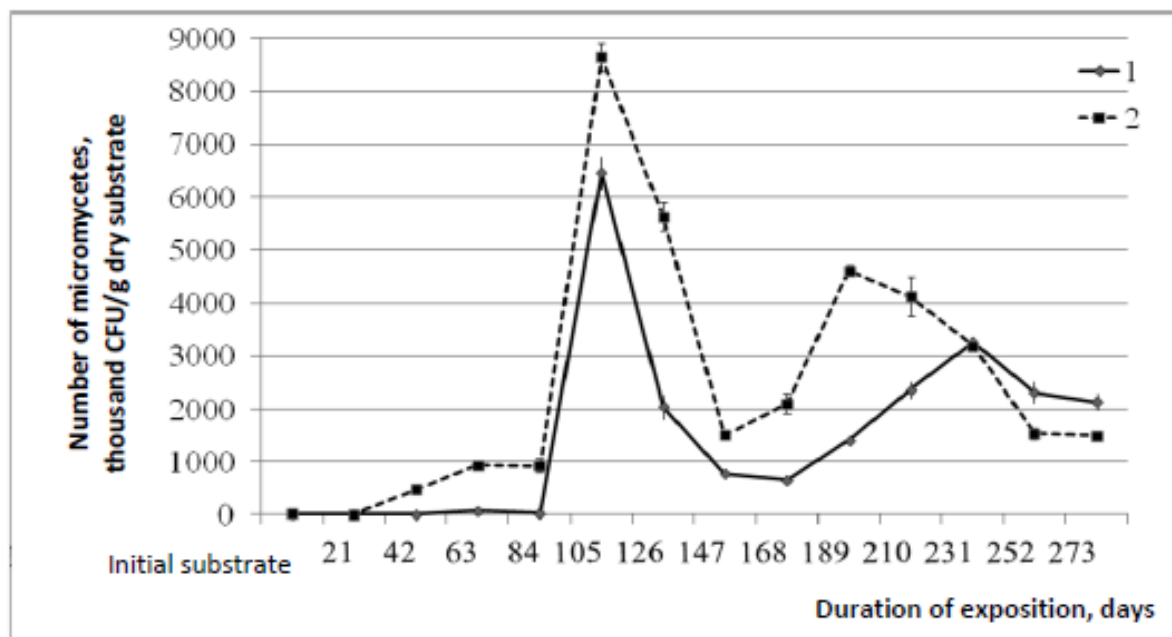
The highest number of micromycetes was observed in the optimized substrate (mixture of manure with straw and peat) – 8 649 thousand CFU / g, while in control variant the number of micromycetes was lower – 6 458 thousand CFU / g. Higher number of fungi in the substrate with optimized C:N ratio, was undoubtedly caused by the presence of additional components that contain carbon, served as an energy source and foundation for the synthesis of organic substances by microorganisms.

Therefore, optimization of the C:N ratio at 20:1 in variants with mixture of chicken manure with straw and peat provides an optimal conditions for the development of the microbiota and mineralization of compounds.

Our research suggests that succession changes of the associations of microorganisms occur during the composting with the

dominance periods specific to certain ecological-trophic groups of microorganisms. Accordingly, the introduction of required bacterial strains or micromycetes to the compost during different phases will have favorable and unfavorable conditions. In our opinion, in order to ensure the best introduction conditions, microorganisms should be added to the composted substrate before phases of active development of the relevant ecological-trophic group. Thus, the best period for the introduction of ammonifiers is the first month of composting, nitrogen fixing bacteria – sixth month, micromycetes – first and second months of composting. The introduction of microorganisms during indicated time periods, we believe, will ensure composts enrichment with beneficial microbiota.

During the study of microorganisms' populations in composted substrates the development of micromycetes was of the particular interest among the other members of the microbiota as the introduction of useful forms of micromycetes could theoretically promote both acceleration of the composting timing (given the high cellulolytic activity of fungi) and compost enrichment with introduced strain and physiologically active substances.



**Figure 1.** Development of micromycetes in composted substrates depending on their composition: 1. Control (chicken manure); 2. Compost mixture (chicken manure with straw and peat).

Verification of this hypothesis on practice using *T. harzianum* 128 association indicates the possibility of its successful introduction to the composted mixture, especially on the 2nd month of composting (Fig. 2).

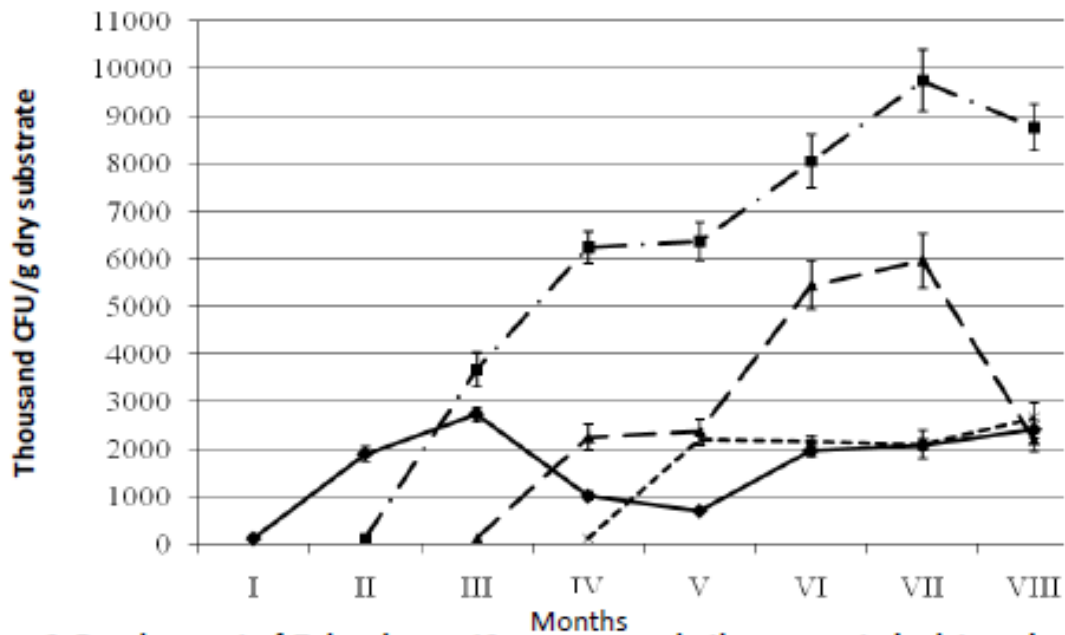


Figure 2. Development of *T. harzianum* 128 in the composted mixture depending on the introduction timing: 1 – I month; 2 – II month; 3 – III month; 4 – IV month.

The introduction of *T. harzianum* 128 to the composted mixture on the second month of composting had stimulated the rapid increase of the number of introduced microorganisms, and to the seventh month composting had resulted in 9 744 thousand CFU / g of dry compost.

*T. harzianum* 128 association had actively affected the mineralization rates of organic substances included in the compost (see Table 3 for the decomposition intensity rates of composted straw substrate).

According to the received data the intensity of straw decomposition was significantly (in 1.8-2.5 times) higher in compost substrates with *T. harzianum* 128 association starting from the third month of composting (time of intensive growth of introduced microorganisms). By the end of the 8<sup>th</sup> month of composting the decomposition of organic matter was complete in the substrates with introduced fungi association. Straw mineralization in the control variant was not fully done by that time.

**Table 3. Intensity of straw decomposition in composts under the influence of microorganisms**

Variant	Time of composting (months)						
	II	III	IV	V	VI	VII	VIII
Without introduction of microorganisms, <i>control</i>	5,1	11,6	22,4	34,8	42,7	54,3	60,4
With introduction of <i>T. harzianum</i> 128	5,1	24,5	57,3	71,2	83,5	98,1	100

Basically, poultry manure substrate composting can be terminated on the seventh month, when the highest number of *T. harzianum* 128 was observed. Nevertheless, the final decision is limited by the other parameters of compost status. In particular, the full maturity of composted mixture in experiment (no specific smell, full straw decomposition) was noted on eight month, which also was characterized with high levels of introduced microorganisms.

Our findings were proved in other model experiment with two composting options for substrates based on chicken manure with optimized C:N ratio – without introduction of microorganisms and using the micromycetes *T. harzianum* 128 association.

The efficiency of technological aspects of composting identified in the laboratory was verified in production experiments. It was established that the number of introduced micromycetes had increased from 135 thousand CFU / g of dry compost to 8 150 thousand CFU / g of dry compost. Besides, it has prevented carbon and nitrogen losses, which can be caused by the accumulation of these elements in the newly formed humic compounds and/or their use for micromycetes mycelium formation (Tab. 4).

Hence, chicken manure composting substrate should be optimized for carbon-nitrogen ratio, thus ensuring active progress of microbiological processes. Introduction of *T. harzianum* 128 association is appropriate during the second month of manure composting mixed with peat and straw. Under these conditions the maximum number of introduced microorganism was observed on the seventh month of composting – nine million CFU / g of dry substrate. On the eighth month of composting this number was still high. Besides, the completion of organic matter composting was also observed at this time. The proposed technological methods reduce composting terms and nutrient losses while providing compost substrate enriched with agronomically valuable microorganisms.

**Table 4. Changes in compost quality indicators under the impact of *T. harzianum* 128.**

(Production experiments)

Version	Carbon content, %		Nitrogen content, %		Number of <i>T. harzianum</i> 128, tsnd. CFU / g dry compost	
	Start of experiment	End of experiment	Start of experiment	End of experiment	Start of experiment	End of experiment
Without introduction of microorganisms, <i>control</i>	45.1	36.2	3.04	1.77	Not determined	Not determined
With introduction of <i>T. harzianum</i> 128	45.1	40.6	3.04	2.02	135 ± 0	8150 ± 620
LSD <sub>05</sub>	2.3	1.8	0.1	0.1		

Developed technology for biological composting of poultry manure in practice act as the microbial preparation, but instead of microorganism cultivation in fermenters provides conditions for active development of microorganisms in the natural substrate.

The effectiveness of compost enriched with *T. harzianum* 128, was verified in field experiments on potatoes. Previously, it was found that biological composts possess high growth stimulating activity and can be used locally in limited quantities. In particular, the highest efficiency of biological compost was observed upon its application in doses of 10 – 15 pellets / plant at planting potatoes (one pellet weight – 0.5 g).

Field data indicates that use of biological composts in specified doses had promoted active sprouting of potato tubers. Physiologically active substances in compost ensure rapid growth of vegetative mass and stimulate the formation of generative organs of potato plants. In addition, the active development of root system and increase of root mass and their volume was observed, providing an increase in general and active adsorption roots surface. This can contribute to the improvement water and nutrients uptake from the soil. These effects have positively affected the crop yield and output quality.

Obtained results on biological compost efficiency are summarized in Table 5. The scheme of the experiment was expanded for comparison with the variant of compost obtained without microorganisms' introduction.

The received data suggest that local application of biological compost

had increased crop productivity by 30% compared to the control. Side to side comparison of composts obtained with and without introduced microorganisms had shown the significant yield increase under the use of proposed technology.

Biological compost has positively influenced the starch content in tubers – by 2.9% comparing to control and 2.1% comparing to the compost obtained without introduction of microorganisms.

**Table 5. Impact of bioorganic fertilizer on potato yield. Latona variety, field experiment**

Version	Yield, t/ha	Increment to control, %	Starch content, %	Ascorbic acid content, mg-%
Control	16.7	-	12.9	13.8
Chicken manure	17.2	2.9	13.1	15.6
Compost without introduced microorganisms	19.8	18.6	13.7	16.6
Compost With introduction of <i>T. harzianum</i> 128	21.7	29.9	15.8	17.7
LSD <sub>05</sub>	1.57		0.1	0.19

**Note:** mineral fertilizers in N<sub>60</sub>P<sub>60</sub>K<sub>60</sub> dose were applied in all variants

Biocompost had also influenced the growth of ascorbic acid content in tubers. Thus, its content was significantly increased by 3.9 mg-% compared to the control and 1.1 mg-% compared to the compost not enriched with trichoderma association (Table 5).

### Conclusions.

The introduction of *T. harzianum* 128 association to composted substrate based on poultry manure with straw and peat ensures shortening of composting timing, accumulation in compost of agronomically valuable microorganisms – active destructors of organic matter and producers of physiologically active substances. Received biocompost received upon the introduction of selected micromycetes to the substrate was shown to be promising technology in agricultural production.

## Bibliography

1. Gautam S.P. Composting of municipal solid waste of Jabalpur city/S.P. Gautam, P.S. Bundela, A.K. Pandey, M.K. Awasthi, S. Sarsaiya//Global J Environ Res – 2010 – Vol.4, № 1. – P. 43–46.
2. Bernal-Vicente A. *Trichoderma harzianum* T-78 supplementation of compost stimulates the antioxidant defense system in melon plants/A. Bernal Vicente, J.A. Pascual, F. Tittarelli, J.A. Hernández, P. Diaz-Vivancos//J. Sci Food Agric. – 2015 – Vol. 95, № 11. – P. 2208–2214.
3. Bernal-Vicente A. Inoculation of *Trichoderma harzianum* during maturation of vineyard waste compost to control muskmelon Fusarium wilt/A. Bernal Vicente, M. Ros, J. Antonio Pascual//BioResources –2012. – Vol. 7, № 2. – P. 1948–1960.
4. Blaya J. Changes induced by *Trichoderma harzianum* in suppressive compost controlling Fusarium wilt/J. Blaya, R. Lopez-Mondejar, E. Lloret, J.A. Pascual, M. Ros//Pestic Biochem Physiol. –2013 – Vol. 107, № 1. – P. 112–119.
5. Gneusheva I.A. Biological activity of fungi of *Trichoderma* genus and their industrial application/I.A. Gneusheva, N.E. Pavlovskaya, I.V. Yakovleva//Bulletin of Orel NAU. – 2010. – №3 (24). – P. 36-38.
6. Agrochemical analysis/[M.M. Gorodniy, A.P. Lisoval, A.V. Bykin et al.] ed. M.M. Gorodniy 2 edition. — K.: Aristey, 2005. —476 p.
7. Methods of soil microbiology and biochemistry/[I.V. Aseeva, I.P. Babieva, B.A. Byzov et al. ]; ed. D.G. Zvyagintsev. – M.: MSU, 1991. – 304 p.
8. Villemin G. Utilization du test de reduction de l'acetylene pour la numeration des bacteries libres fixatrices d'azote/G. Villemin, J. Balandreau, Y. Dommergues//Ann. Microbiol. Enzimol. – 1974. – 24, №2. – P. 87-94.
9. Volkogon V.V. Experimental soil microbiology/[V.V. Volkogon, O.V. Nadkernychna, L.M. Tokmakova et al.] ed. V.V. Volkogon. – K.: Agrarna nauka, 2010. – 464 p.
10. Methods of state scientific-technical expertise of plant varieties. Methods for determination of crop production quality parameters/ed. Tkachik S.O. – 4 edition. – Vinnytsa: «Nilan, LTD», 2015. – 160 p.
11. Dospekhov B.A. Field experiments methods/B.A. Dospekhov. — M.: Kolos, 1979. — 376 p.