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## **ELABORATION OF NATURAL POLYFUNCTIONAL PREPARATIONS WITH ANTIPARASITIC AND BIOSTIMULATING PROPERTIES FOR PLANT GROWING**

*Producer of macrolide antibiotic avermectin *Streptomyces avermitilis* UCM Ac-2179 has been isolated from Ukrainian chernozem soil, its biosynthetic activity has been increased by the traditional selection and chemical mutagenesis methods. *Streptomyces avermitilis* UCM Ac-2179 synthesizes avermectin with the content of anti-parasitic B-components more than 40%. Addition of exogenous Na-pyruvate (1.5 mg/L) in cultural medium promotes a 2.5-fold augmentation of the avermectin synthesis. The preparation Avercom has been obtained by the method of ethanol extraction from the producer biomass. This preparation includes antibiotic avermectin and other biologically active substances: free amino acids, lipids, phytohormones. Avercom has high nematocidal activity and raises plant resistance to fungal and viral diseases. On the base of Avercom and plant growth regulators the complex preparations Actinolan and Ascoldia have been elaborated. The effectiveness of the biopreparations as nematocidal and plantstimulating means under experimental and industrial conditions was confirmed.*

*Key words: streptomycetes, cultivation, avermectin, nematocide, plant growth regulator.*

The basic regularities of agrophytocenoses functioning under the conditions of sufficient provision with nutrients was established at the end of the 20<sup>th</sup> century by the efforts of soil scientists, agronomists, microbiologists and ecologists of different countries. Crop productivity depends on phytosanitary condition (availability of weeds, pests, phytopathogens), as well as various substances secreted by plant and rhizospheric microflora [7, 16, 24].

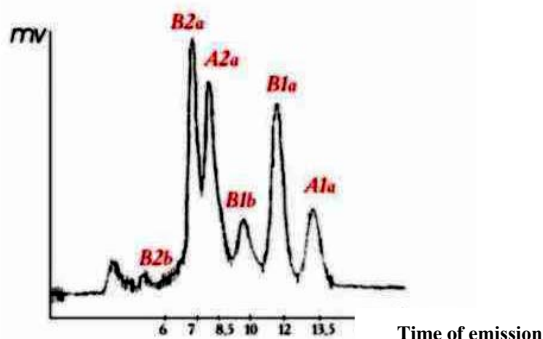
Nematodes are the one of widespread and harmful factors for plants. Crop losses from parasitic nematodes are from 25 % to 70 % in various countries; under most adverse conditions they achieve 90-100% [8, 26]. Mankind has elaborated various nematode management means: chemical, physical, biological, and agro-technical (cover crops, crop rotation, plant varieties resistant to nematode damage, soil solarization, toxic pesticides, and biological means), quarantine arrangements. All of them are directed to the decrease of nematode populations in soil to levels below the damage threshold [8, 26]

Chemical preparations are still the most effective ones for pest control at present but besides direct action they do harm to the environment, reveal negative action to crop quality, pollute the products of plant-growing, cattle-breeding, bee-keeping, fish-breeding, etc. and, at last, threaten people health. However, in recent decades the after-effects of chemicals applying such as ground water contamination, toxicity to mammals and birds, and residues in food have caused hard limitation on the use of agricultural chemicals, including nematocides in many countries [26].

Because the need of many countries with well-developed agriculture sector in economics in biopreparations is increased the professionals turn their attention more and more to the search of new biological preparations for plant growing [10, 17, 24].

Contemporary agricultural manufacture becomes more and more oriented to application of preparations with features of biopesticides for protection of agriculture plants against diseases and pests. At present preparations based on antibiotic avermectin are considered the most promising plant protectors against pests. Avermectin is the complex antiparasitic antibiotic attributed to highly efficacious antiparasitic means. The producer of macrolide antibiotic avermectin is soil streptomycete *Streptomyces avermitilis*. Avermectin is a complex of eight closely related components: four major ( $A_{1a}$ ,  $A_{2a}$ ,  $B_{1a}$ ,  $B_{2a}$ ) and four minor ( $A_{1B}$ ,  $A_{2B}$ ,  $B_{1B}$ ,  $B_{2B}$ ). The B-components have a broad spectrum of insecticide, acaricide and antihelminthic effect [9, 18].

**Isolation and cultivation technology of avermectin producer *Streptomyces avermitilis*.** Researchers of IMV of NAS of Ukraine have isolated streptomycete strains able to synthesize avermectin from the chernozem soil of the winter wheat rhizosphere. Its variant *S. avermitilis* UCM Ac-2179 with high biosynthetic activity was obtained as a result of traditional selection and chemical mutagenesis methods [21, 25]. HPLC-analysis has shown that this strain synthesises all avermectin components (Fig.1.)



**Fig 1. Avermectin components produced by *S. avermitilis* UCM Ac-2179 (HPLC-method)**

The first cultivation medium for avermectin producer was described by Burg et al. [9]. According to these authors' data, the best recognized source of carbon for avermectin production was glucose, the source of nitrogen was milk after peptonization. According to our results, the medium on the base of soybean meal and starch which has C/N = 30 was found the most suitable both for *S. avermitilis* UCM Ac-2179 growth and for high biosynthesis of antibiotic (730 µg/mL)[20].

We have studied the dynamics of avermectin synthesis during 9 days of cultivation. The experiments have shown that the intensive synthesis of avermectin was begun on the 4th day of cultivation and achieved maximum on the 7th day that is in correspondence with the finishing of stationary phase of growth. At the same time the strain accumulated 800 µg of avermectin per 1 mL of ethanol extract or 36.4 mg of avermectin per 1 g of dry biomass. There are other manufactured strains of *S. avermitilis* which had maximum accumulation of avermectin just after 7-8 days of cultivation and this period corresponded to the stationary phase of growth [19].

We have researched the qualitative changes of avermectin complex during cultivation of strain-producer. During 2-4 days of strain growth we observed the presence of only one avermectin A<sub>1</sub> component; then, at the stationary phase of strain growth, there appeared A<sub>2</sub> and B<sub>1</sub> components (5-9 days). Just on the 7th day of growth (at the finishing of stationary phase of growth) the B<sub>1</sub> fraction was presented in maximum quantity.

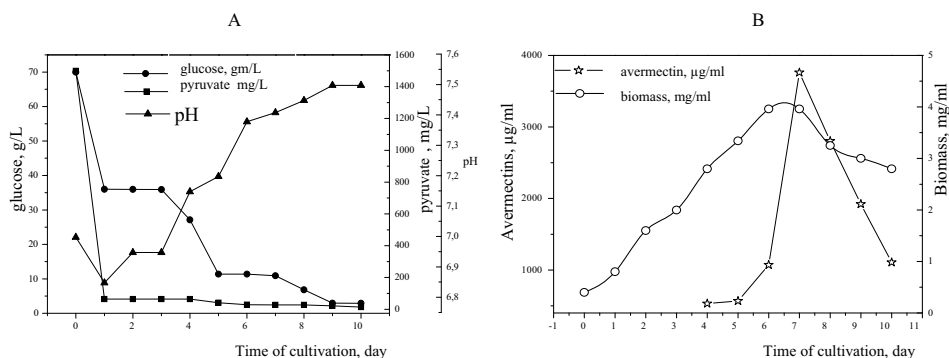
According to the literature data [9,19], glucose has a positive influence on the avermectin synthesis by various strain-producers. We have studied the effects of various glucose concentrations and the time of glucose addition to the medium upon *S. avermitilis* UCM Ac-2179 growth. The strain revealed good growth at 7 % of glucose added at the beginning of cultivation and the avermectin content was the highest – 970 µg/mL [13].

It is known that streptomycete development and biosynthesis of antibiotics take place at two phases under cultivation in liquid media: the “trophic” phase is the phase of maximum biomass accumulation, and the “idio-phase” is the phase of slowed-down growth and intensified synthesis of antibiotics [11]. It was also established that synthesis of “start” precursors – acetyl-CoA and methyl-malonyl-CoA formed under the process of glucose catabolism limiting the formation velocity of macrolid antibiotics, including avermectin. Besides this, amino acids (D,L-valine, L-iso-leucine, L-methionine and L-threonine), pyruvic acid, and Na-pyruvate may be alternative sources of acetyl-CoA. Addition of antibiotic precursors in fermentation medium is one of the means of their biosynthesis regulation [4].

Taking into account that there were no such investigations for selected producer *S. avermitilis* UCM Ac-2179 we have studied the influence of above mentioned amino acids on the ability of our strain to produce biosynthetic avermectin. Obtained results have shown that D,L-valine and L-isoleucine, irrespective of time of carrying in medium, inhibited the avermectin synthesis by *S. avermitilis* UCM

Ac-2179; at that time the addition of L-threonine in concentration of 1.0 g/L to the synthetic medium at the beginning of cultivation promoted to the highest antibiotic synthesis (160% against control without the amino acid).

The research of dynamics of the avermectin biosynthesis by *S. avermitilis* UCM Ac-2179 in liquid synthetic medium in the presence of glucose and Na-pyruvate has shown the next (Fig.2). Quantity of exogenic Na-pyruvate added in the medium was decreased 23 times just on the first day of cultivation. Glucose was also intensively utilized; pH value of cultural liquid after insignificant decrease to 6.8 was increased to 7.2. Na-pyruvate was almost not determined in the medium during idio-phase; at the same time pH value was determined on the level of 7.0 – 7.5 to the end of cultivation. Maximum biomass was marked on the 6th day and achieved almost 4 g/L. Intensive avermectin synthesis by the culture was started at the beginning of idio-phase and reached its maximum on the 7th day. There were 3760 µg per 1g of dry biomass that is 2.5 times as much as the control without Na-pyruvate [4].



**Fig. 2. Changes of pH value, glucose and Na-pyruvate content (A), avermectin biosynthesis and biomass accumulation (B) by *S. avermitilis* UCM Ac-2179 cultivated in synthetic medium with the Na-pyruvate (1.5 mg/L) addition**

Determination of avermectin component content by the HPLC method has shown that in the presence of various concentrations of Na-pyruvate the ratio (percentage) of separate components (A : B) remained more or less stable during the cultivation and fluctuated in the limits of 59-61:39-41 (percentage). Velocity of A to B transition was higher and fraction B content was 40 % (in control – 44 %) in spite of considerable stimulation of avermectin synthesis in the presence of 1.5 mg/L of Na-pyruvate (Table 1.).

**Table 1**

**Components of avermectin under cultivation of *S. avermitilis* UCM Ac-2179 in synthetic medium at the Na-pyruvate addition**

Na-pyruvate concentration, mg/L	Separate avermectin component content, % to the general quantity of antibiotic					Component ratio (A:B)
	A <sub>1a</sub>	A <sub>2a</sub>	A <sub>2b</sub>	B <sub>1a</sub>	B <sub>2a</sub>	
0.5	11.9	49.6	0	18.7	19.7	61:38
1.0	10.7	49.8	0	19.3	20.2	60:40
1.5	10.3	49.1	1.48	19.8	19.1	60:39
2.5	11.2	47.9	0	20.7	20.2	59:41
Control without Na-pyruvate	9.3	46.8	0	19.6	24.3	56:44

**Biologically active compounds of preparation Avercom.** On the base of *S. avermitilis* UCM Ac-2179 we have elaborated the new preparation “Avercom”. This biopreparation is the ethanol extract from 7-days old streptomycete mycelium. It contains anti-parasitic antibiotic avermectin with more than 40 % of B-component with nematicidic activity [21].

Chemical analysis of Avercom has shown that the preparation is a complex of biologically active substances: beside avermectine the preparation contains different intracellular producer metabolism substances with different physiological features [1,6].

First of all, 21 amino acids have been found in Avercom (Table 2). Quantitatively, the highest content belongs to glutamic acid (90.4 mg/100 mL, or 28.43% of the amino acid quantity sum); tyrosine, valine, leucine, and alanine are presented in less quantity (21.6 to 37.6 mg/100 mL). Attention is drawn to the presence of “exotic”  $\gamma$ -aminobutyric amino acid (1.1286 mg/100 mL).

Table 2

Amino acid content in Avercom

Amino acid	mg/100 mL	% of $\Sigma^*$	Amino acid	mg/100 ml	% of $\Sigma$
<b>Individual amino acids</b>					
Cysteic	2.6739	0.84	Cystine	6.6818	2.13
Taurine	1.3734	0.43	Methionine	7.4487	2.34
Aspartic	11.4692	3.61	Isoleucine	14.3587	4.52
Threonine	9.6568	3.04	Tyrosine	21.6491	6.81
Serine	7.0848	2.23	Phenylalanine	12.6100	3.97
Glutamic	90.3653	28.43	$\gamma$ -aminobutyric	1.1286	0.35
Proline	11.5206	3.62	Ornithine	1.1579	0.36
Glycine	14.7956	4.65	Lysine	5.9373	1.87
Alanine	37.6130	11.83	Histidine	4.0572	1.28
Valine	23.6734	7.45	Arginine	3.0861	0.97
Leucine	29.4480	9.27			
Amino acids quantity sum				317.79	100

\*Note:  $\Sigma$  is total amino acid quantity

Avercom contains a wide range of lipids: phospholipids, mono- and diglycerides, stearins, free fatty acids, triglycerides, sterol ethers, and waxes (Fig.3). Lipid content of Avercom is the richest in phospholipids (28.3%) and sterines (21.9%). Among the free fatty acids the presence of arahidonic acid (2.4%) and linolic acid (4.6%) draws attention. It is known that arahidonic acid is derived from linolic acid; its presence in the preparation defines its elicitor properties [14].

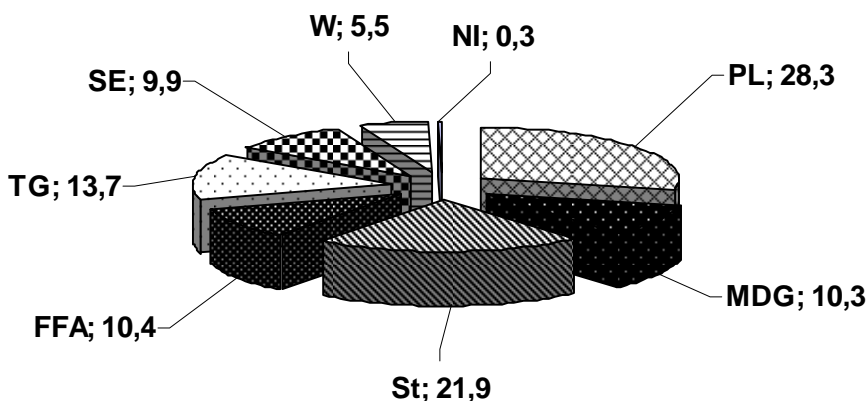


Fig.3. Lipids components of Avercom (% of total lipids quantity): PL – phosphate lipids, MDG – mono- and diglycerides, TG – triglycerides, ST – sterols, FFA – free fatty acids, SE – sterol ethers, W – waxes, NI – unidentified components

It is known that endogenous phytohormones have a significant effect on growth and development of a plant, they manage vital processes in the plant at the cell level and whole organism. The use of exogenous plant growth regulators is very promising for plant metabolism optimization, to reveal the potential of varieties, to increase crop yields and improve their quality. Microorganisms of different taxonomic groups can synthesize a lot of phytohormones. However, we have found no information in the literature about the ability of streptomycete-producers of avermectin to synthesize phytohormones.

Phytohormones of three classes have been discovered in Avercom preparation namely auxins

– indole-3-acetic acid, gibberellins – gibberellic acid, cytokinins – zeatin, zeatin-riboside, isopentyladenine. Besides, the preparation contains the steroids – cholesterol, ergosterol, 24- epibrassinolid (Tabl.3) [2].

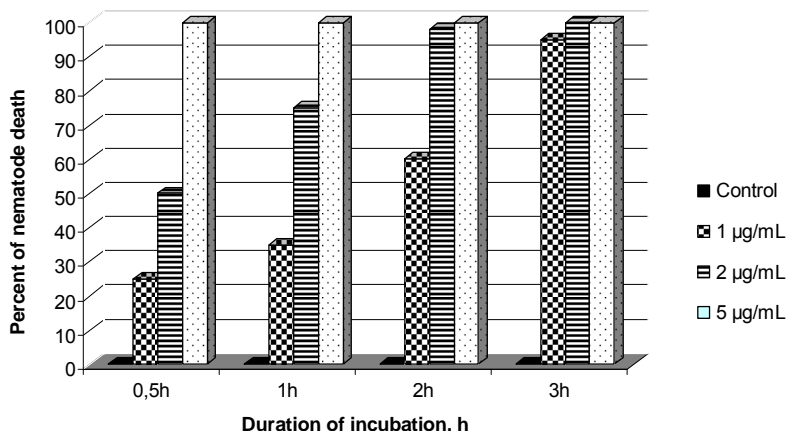
Table 3

**Amount of phytohormones in Avercom (ng/mL)**

Phytohormones		Amount of phytohormones, ng per 1 mL
Auxins	Indole-3-acetic acid	217
Cytokinins	Isopentyladenine	428
	Zeatin	149
	Zeatin-riboside	118
Gibberellins	Gibberellic acid (GA <sub>3</sub> ),	4500
Steroids	24- epibrassinolid	5660
	Ergosterol	2130
	Cholesterol	1690

**Antiparasitical, plant protective properties and toxicological risk weighting of Avercom.**

Studies of Avercom effect in different concentrations on gall root nematode *Meloidogynae incognita* under laboratory conditions have shown that the preparation in 2.0 µg/mL concentration kills 50% of species even in the first 30 minutes of action (Fig.4.); therefore, LD<sub>50</sub> for Avercom is 2.0 µg /mL in relation to nematode. Higher concentrations of Avercom induce total death of nematodes during the same period of time [3,15].



**Fig. 4 Action of Avercom on gall nematode *Meloidogynae incognita* (% of immovable individuals)**

Nematicidic activity of Avercom was investigated under laboratory conditions in the soil infected with the plant nematode groups, in particular, *Pratylenchus pratensis*, *Tylenchorhynchus dubius*, *Helicotylenchus dihystra*, *Paratylenchus nanu*, *Ditylenchus dipsaci*; among them *T. dubius* (456 species) and *P. pratensis* (128 species) were the most frequent, the quantity of the former species was 5 times exceeded the allowable rate, and the latter one – 3 times [5,12].

Soil treatment with Avercom resulted in a 4-fold decrease of the total number of plant nematodes, in particular a 2.8-fold decrease of *T. dubius* individuals. *P. pratensis* species were the most sensitive; under Avercom application they disappeared completely.

Inhibiting effect of Avercom containing 10, 15, or 20 µg of avermectin per 1 mL on development of grain aphid populations has been shown under the laboratory conditions. The quantity of insects on plants and the content of their populations in different experiment variants were determined on the 7th day after plant treatment with the preparation (Table 4.). In the control plants were treated with water and at the end of the experiment the number of insects was increased 3.5 times. The number of aphid species on the plants treated with the preparation also increased, but to much less degree. Avercom action effectiveness varied between 41.3 and 63.8%, with the highest efficiency that was marked in the variant with 20 µg /mL of the preparation. Insect fertility rate under the

preparation effect was much lesser than the control rate and varied between 0.3 and 0.5; it was the lowest when Avercom concentration was 20 µg/mL.

Table 4

**Avercom influence on grain aphid populations under the laboratory conditions**

Test variant	Preparation dose, mL/ 100 mL	Insect quantity, unit/plant		Preparation efficiency, %	Insect population structure, unit/plant, 7th day		Preparation efficiency, %		Insect fertility rate
		before treatment	on the 7th day after treatment		Imago	Larvae	Imago	Larvae	
Control, water	0	38	134	0	23	111	0	0	1.0
Avercom: 10 mcg/ml	20	38	76	43.2	16	60	28.9	46.1	0.5
15 mcg/ml	30	38	78	41.3	30	48	33.3	56.9	0.4
20 mcg/ml	40	38	48	63.8	16	32	26.7	71.2	0.3

Positive results were obtained when testing Avercom under the condition of closed soil on the cucumber Angelina variety. It was observed that Avercom suppressed development of diseases induced by fungi of *Fusarium* genus (cucumber root rot and wilting agents), *Erysiphaceae* family (oidium agent), and viruses (cucumber mosaic wilting agents) under greenhouse trails. Cucumber plant damage extent was examined during three months under the closed soil conditions (Table 5).

Table 5

**Avercom effect on plant diseases in greenhouse experiment**

Test variant	Disease	Plant affection, %		
		August	September	October
Control without preparation	Mosaic wilting	28.9	45.0	57.8
	Powdery mildew	NR*	21.1	24.2
	Plant wilting	NR	5.3	21.2
	Root rot	2.8	2.8	2.8
Avercom	Mosaic wilting	23.6	50.7	5.3
	Powdery mildew	NR	20.9	28.6
	Plant wilting	NR	7.8	16.4
	Root rot	NR	NR	NR

\* Note: NR - not registered

Root rot damage of cucumber plants had not been shown during all the growing period at Avercom presence whereas 2.8% of control plants were ill. Besides, Avercom suppressed mosaic wilting agents but only in the first month of the experiment: plants were affected by viruses by 5.3% less than in the control. Plant wilting decreased by 4.8% at the end of the experiment in Avercom presence in comparison with the control. Avercom influence on oidium agent which affected control plants was weak in September.

We studied the Avercom effect on soil microbiota in the laboratory and field tests conducted on the chernozem soil. It was confirmed that the biopreparation had positive influence on quantity of microorganisms of different ecological functional groups: pedotrophic, amylolytic, ammonifying, phosphate-mobilizing [20].

Toxicological evaluation and determination of a hazard class of Avercom preparation with the help of toxicological, immunological and statistical methods have shown that, according to hygienic pesticide classification by danger degree, preparation Avercom is attributed to low-dangerous compounds by parameters of acute per-oral toxicity, skin-resorption toxicity, irritating action to skin, and allergenicity, and to moderate dangerous compounds by parameters of acute inhalation toxicity and irritating action on eyes. The maximum permitted concentration of Avercom in the air of industrial zone is 0.32 mL/m<sup>3</sup> (0.032 mg/m<sup>3</sup>).

**Avercom influence on plants and crops.** Present-day literature contains quite little data on how preparations based on avermectin influence plants. Taking into account the presence of the

wide range of biologically active compounds in Avercom, its effect on seed sprouting, growth, development, and harvest of different agriculture plants has been investigated.

Owing to biologically active substances Avercom stimulates the growth and development of various kinds of winter and spring wheat, and also cucumber (trials in greenhouses), inhibiting simultaneously the development of agents of fungus diseases [6, 15,20].

We have obtained positive results when testing Avercom under the conditions of closed soil on the cucumber culture. Avercom stimulated development of cucumber plants of Angelina variety under the production conditions of a hydroponic greenhouse. As compared to the control, plants treated with Avercom ( $2 \cdot 10^{-2}$   $\mu\text{g/mL}$ ) increased their height (by 4-5%), the number of leaves, total quantity of generative organs including pistillate ones, with the button quantity increased by 23%; at the same time, internode length reduced by 6-9%. The mass of cucumber fruits increased by 35.6% in comparison with the control [7].

On the base of Avercom and some plant growth regulators we have created two new complex preparations: Askoldia (Avercom + Radostym) [22] and Actinolan (Avercom + Biolan) [23]. Ratios between Avercom and plant growth regulators were 1:1 in each preparation.

Cucumber seedlings grew and developed under the influence of Askoldia or Actinolan almost identically with control plants. However, when the plants reached the fruit-frame end (190 cm) the difference in their development became evident; in the variants with Actinolan and Askoldia the plants had more intensive color as compared with the control, they bloomed more actively and harvest was greater. Control plants showed chlorosis on leaves. The variants with Askoldia or Actinolan showed slight plant falling; plants in the variants with Avercom grew and developed the normal way.

An analysis of influence of the tested biological preparations on cucumber plant harvest revealed the following. In the control variant which plants died 25 days before the test end only 3.85 kg/m<sup>2</sup> were gathered. On experimental fields the best harvest as compared with the control was obtained in the variant with Askoldia (6.78 kg/m<sup>2</sup>, or by 25% more in comparison with control)). Harvest of plants grown with Avercom or Actinolan was respectively by 19% and 12% higher than in the control.

As it was noted, soil in the greenhouse had been infected with larvae of root-knot nematode *Meloidogynae incognita*. Even in a month after sowing seedlings the root system of the control plants was covered with galls, in other words completely infected by meloidoginosis. The root system of the plants in the variants with biological preparations was infected lesser that allowed plants to vegetate actively and yield harvest.

Effectiveness of the developed preparations was studied under field conditions. When growing spring wheat Kollektivnaya 3 variety, seeds were treated with such biological preparations as Avercom, Askoldia or Actinolan [20]. During the whole experiment complex preparations stimulated wheat plant growth (Table 6).

Table 6

**Influence of biological preparations on growth of wheat Kollektivnaya 3 variety**

Preparation, ml/t seeds	Plant height		Quantity of leaves per plant, % of control	Leaf square, % of control	Dry substance content, % of control	
	cm	% of control			Leaves	Stems
Stem-extension stage						
Control without preparations	32.3	100	100	100	100	100
Avercom	40.1	124.2	113.3	124.7	101.2	107.5
Askoldia	38.9	120.4	120.0	130.5	115.9	117.4
Actinolan	49.0	151.7	121.3	151.0	116.7	120.1
Blooming stage						
Control without preparations	49.5	100	100	100	100	100
Avercom	51.6	104.2	114.3	102.2	100.7	100.0
Askoldia	55.1	111.3	151.1	147.2	109.5	113.4
Actinolan	55.9	112.9	157.2	163.3	111.8	114.2

Actinolan influence was the most active; the plant height in the stem-extension stage increased by 51.7%, and in the blooming stage – by 12.9%. The quantity of leaves and leaf surface area in this variant increased significantly: at the stem-extension stage they were increased by 21.3% and 51%; at the blooming stage their characteristics grew by 57.2% and 63,3 % respectively, as compared to the control. As a natural result of the increased quantity of leaves of one plant the leaf surface area was also increased in all the experiment variants as compared to the control.

Dry substance content in the leaves of the plants treated by biopreparations at the stem-extension stage was increased by 11.3% on the average and in the stems by 15% as compared to the control; at the blooming stage this characteristic somewhat reduced but remained higher than in the control.

All the tested biological preparations provided increase of plant photosynthetic activity (Table 7). Chlorophyll content in the wheat leaves was increased at the stem-extension stage by 20.9% at an average and at the blooming stage – by 33.3% in comparison with the control. Photosynthesis productivity (the quantity of dry biomass developed at a leaf surface unit during a day) having tight connection with the plant productivity (harvest) and agrotechnical measure effectiveness grew under the action of Actinolan by 26.5–26.7% in comparison with the control. The action of other tested preparations was weaker.

Table 7

**Influence of biological preparations on spring wheat photosynthetic apparatus efficiency**

Test variant	Chlorophyll content		Photosynthesis productivity	
	mg/g of wet weight	% of control	g/m <sup>2</sup> per day	% of control
Stem-extension stage				
Control without preparations	2.70	100	6.5	100
Avercom	3.13	115.9	6.9	106.2
Askoldia	3.21	118.9	7.6	116.9
Actinolan	3.45	127.8	8.2	126.5
Blooming stage				
Control without preparations	2.90	100	7.5	100
Avercom	3.32	114.5	8.3	110.7
Askoldia	4.10	141.4	9.2	122.7
Actinolan	4.33	149.3	9.5	126.7

Spring wheat harvest under the action of the complex biological preparations exceeded the control by 22.1%. The largest harvest (4070 kg/ha) was gathered at Actinolan application. It was somewhat less (3890 kg/ha) in the variant of the Askoldia application. The harvest in the control variant was 3200 kg/ha [7].

Therefore, we have obtained the effective producer of avermectin *Streptomyces avermitilis* UCM Ac-2179. On the base of this strain we have elaborated the new polyfunctional preparation Avercom with nematicidic and phytostimulating effect. The preparation is ecologically safe, does not show negative impact on soil microorganisms and warm-blooded. The preparation is offered for use in plant cultivation; it provides soil recovery from parasitic plant nematode, increases the plant yield, and helps to obtain ecologically safe products.

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**СОЗДАНИЕ ПРИРОДНЫХ ПОЛИФУНКЦИОНАЛЬНЫХ ПРЕПАРАТОВ  
С АНТИПАРАЗИТИЧЕСКИМИ И ФИТОСТИМУЛИРУЮЩИМИ  
СВОЙСТВАМИ ДЛЯ РАСТЕНИЕВОДСТВА**

**Резюме**

Из черноземной почвы Украины выделен стрептомицет – продуцент макролидного антибиотика авермектина, биосинтетическая активность которого повышена методами аналитической селекции и химического мутагенеза. *Streptomyces avermitilis* УКМ Ac-2179 синтезирует авермектиновый комплекс, в котором содержание антипаразитарных В-компонентов составляет не менее 40%. При добавлении в



среду культивування екзогенного пірувата натрія (1,5 мг/л) біосинтез авермектина збільшується в 2,5 рази. Из биомассы *Streptomyces avermitilis* УКМ-Ас 2179 методом этанольной экстракции получен препарат Аверком, в составе которого, кроме авермектинов, присутствуют также биологически активные вещества – свободные аминокислоты, липиды, фитогормоны. Аверком проявляет высокую нематодцидную активность, повышает устойчивость растений к заболеваниям грибной и вирусной этиологии. На основе Аверкома и регуляторов роста растений созданы комплексные препараты Актинолан и Аскольдия. В опытных и производственных условиях подтверждена эффективность применения этих биопрепаратов в качестве нематодцидных и фитостимулирующих средств.

Ключевые слова: стрептомицеты, культивование, авермектин, нематодцид, фитогормоны.

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## **СТВОРЕННЯ ПРИРОДНИХ ПОЛІФУНКЦІОНАЛЬНИХ ПРЕПАРАТІВ З АНТИПАРАЗИТИЧНИМИ І ФІТОСТИМУЛЮЮЧИМИ ВЛАСТИВОСТЯМИ ДЛЯ РОСЛИННИЦТВА**

### **Резюме**

Из черноземного ґрунту України виділено стрептоміцет – продуцент макролідного антибіотика авермектина, біосинтетична активність якого підвищена методами аналітичної селекції і хімічного мутагенезу. *Streptomyces avermitilis* УКМ Ас-2179 синтезує авермектиновий комплекс, в якому вміст антипаразитарних В-компонентів складає не менше 40%. За умов додавання у середовище культивування екзогенного пірувата натрія (1,5 мг/л) біосинтез авермектину збільшується у 2,5 рази. Из біомаси *Streptomyces avermitilis* УКМ Ас-2179 методом етанольної екстракції отримано препарат Аверком, у складі якого, крім авермектинів, наявні біологічно активні речовини – вільні амінокислоти, ліпіди, фітогормони. Аверком характеризується високою нематодцидною активністю, підвищує стійкість рослин до захворювань грибної і вірусної етіології. На основі Аверкома і регуляторів росту рослин створені комплексні препарати Актинолан та Аскольдія. В дослідних і виробничих умовах підтверджена ефективність застосування цих біопрепаратів як нематодцидних і фітостимулюючих засобів.

Ключові слова: стрептоміцети, культивування, авермектин, нематодцид, фітогормони

1. Biliavska L. Complex of biologically active substances of *Streptomyces avermitilis* UCM Ac-2179 – avermectin producer // Intern. Scientific Conf. “S.P. Kostychev and contemporary agricultural microbiology” (Yalta, 8-12 October 2007). – Chernihiv: CSTEEL, 2007. – P. 41.
2. Biliavska L., Dragovoz I., Volkogon M., Kozyr's'ka V., Valaghurova H., Petruk T., Iutynska G. Research the plant growth stimulating activity and phytohormone content in the preparation Avercom obtained from *Streptomyces avermitilis* UCM Ac-2179 // 2<sup>nd</sup> Intern. Symp “Plant growth substances: intracellular hormonal signaling and applying in agriculture”: Proc. (Kyiv, 8-12 October, 2007). – Kyiv, 2007. – P. 125.
3. Biliavska L.A., Galagan T.A., Boltovska E.V., Kozyr's'ka V.E., Valagurova E.V., Sygareva D.D., Iutynska G.A. Anti-nematode properties of *Streptomyces avermitilis* UCM Ac-2179 and its avermectin complex // Stūnta Agricola. – – 2009. – N 1. – P. 29-33. (In Russian)
4. Biliavska L., Kozyr's'ka V., Valaghurova H., Iutynska G. Effect of pyruvate and valine on avermectin biosintesis *Streptomyces avermitilis* UCM Ac-2179// Microbiol. Zhurn. – 2007. – 69, N45 – P. 10-17. (in Ukrainian)
5. Biliavska L.A., Kozyr's'ka V.E., Valagurova E.V., Iutynska G.A. Avercom – the new native preparation with nematocidic and plant stimulating action // Agricultural Microbiology. – 2008. – N 7. – P. 22-29. (In Ukrainian)
6. Biliavska L.A., Kozyr's'ka V.E., Valagurova E.V., Iutynska G.A. Avercom – complex of biologically active compounds of avermectin synthesizing strain *Streptomyces avermitilis* UCM Ac-2179 // Agricultural Microbiology. – 2008. – N 8. – P. 36-41. (In Ukrainian)
7. Bioregulation of microbial-plant systems /Editors G.O.Iutynska, S.P.Ponomarenko, - Kyiv: Nichlava, 2010.– 464 p.
8. Bird D. McK., Opperman Ch. H., Davies K.G. Interactions between bacteria and plant-parasitic nematodes: now and then // Intern. J. Parasit. – 2003. – 33. – P. 1269-1276.
9. Burg R.W., Miller B.M., Baker E.E. et al. Avermectins, new family of potent anthelmintic agents, producing organism and fermentation // Antimicrob. Agents and Chemother. – 1979. – N 15. – P. 361-367.

10. Chet I., Ordentlich A., Shapira R., Oppenheim A. Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria // *Plant and Soil*. – 1990. – **129**, N 1. – P. 85-92.
11. Egorov N.S. Principles of antibiotic doctrine. – M.: Publ. House of Moscow State Univ., 2004. – 512 p. (in Russian)
12. Galagan T.A., Sygareva D.D., Iutynska G.A., Boltovska E.V., Biliavska L.A., Kozyrytska V.E. Avercom applying against gall nematode *Meloidogyne incognita* in cucumber under hot-house // *Information Bulletin of VPRS MNP*. – 2009. – N 39. – P. 80-85. (In Russian)
13. Iutynska G. Cultivation technology of *Streptomyces avermitilis* strains – producers of avermectin / Scientific conf. “Problems of modern microbiology and biotechnology” : Proc. (Tashkent, 23 – 25 October, 2009).- Tashkent, 2003.- P.8 (In Russian)
14. Iutynska G.A., Petruk T.V., Kozyrytska V.E., Valagurova E.V. Lipids of avermectin-synthesizing strain *Streptomyces avermitilis* UCM Ac-2161 // *Scientific Herald of Agrarian Academy of Sciences of Ukraine*. – 2005. – **56**. – P. 42-49. (In Ukrainian)
15. Kozyrytska V.E., Valagurova E.V., Petruk T.V., Biliavska L.A., Iutynska G.A. Nematocidal and plant regulating properties of *Streptomyces avermitilis* UCM Ac-2179 avermectin complex // *Stūnta Agricola*. – 2007. – N 2. – P. 21-29. (In Russian)
16. Kurdish I.K., Bega Z.T., Gordienko A.S., Dyrenko D.I. The effect of *Azotobacter vinelandii* on plant seed germination and adhesion of these bacteria to cucumber roots // *Appl. Biochem. and Microbiol.* – 2006. – **42**, N4.- P. 438-442. (In Russian)
17. Kurdish I.K. Introduction of microorganisms in agroecosystems. – Kiev:Naukova Dumka, 2010. – 253 p. (In Ukrainian)
18. McCann-McCormik P.A., Monaghan R.L., Baker E.E. Avermectins // *Adv. Biotechnol.* – 1981. – **1**. – P. 69-74.
19. Mironov V.A., Sergeeva A.V., Voronkova V.V., Danilenko V.N. Biosynthesis of avermectins: physiological and technological aspects // *Antibiotics and Chemical Therapy*. – 1997. – **42**, N 3. – P.31-36. (in Russian)
20. New plant growth regulators: basic research and technologies of application / Editors S.P.Ponomarenko, G.O.Iutynska, – Kyiv: Nichlava, 2010.– 211 p.
21. Pat. 69639 Ukraine, MKII C 12N 1/20 C 12P 17/02, C 12P 17/18, C 12P 19/62, C 12R 1/465. Strain *Streptomyces avermitilis* – producer of avermectins, compounds with antiparasitic action / G.A. Iutynska, V.E Kozyrytska, E.V. Valagurova, M.S. Mukvich, L.A. Biliavska, T.V. Petruk // *Publ.* 15.08.2006. – *Bul. N* 8. (in Ukrainian)
22. Pat. 53253 UA, MPK A01N 63/02 C 12 N 1/20 A01P 21/00 Complex biopreparation “Actinolan” for treatment of plants /Ponomarenko S.P., Anishin L.A., G.A. Iutynska, V.E Kozyrytska, E.V. Valagurova, L.A. Biliavska, T.V. Petruk // *Publ.* 27.09.2010. – *Bul.N* 18 (in Ukrainian)
23. Pat. 95557 UA, MPK A01N 63/02 C 12 N 1/20 A01P 21/00 Complex biopreparation “Askoldia” for treatment of plants / G.A. Iutynska, V.E Kozyrytska, E.V. Valagurova, L.A. Biliavska, T.V. Petruk, S.P. Ponomarenko, Anishin L.A // *Publ.* 10.08.2011. – *Bul.N* 15 (in Ukrainian)
24. Patyka V.F., Tykhonovitch I.A., Filipiev G.D., Gamajunova V.V., Andrusenko G.I. Microorganisms and alternative agriculture. – Kyiv: Urozhay, 1993. – 174 p. (In Ukrainian)
25. Petruk T.V., Biliavska L.A., Kozyrytska V.E., Mukvich M.S. Increase of *Streptomyces avermitilis* UCM Ac-2161 avermectin biosynthesis under N-methyl-N'-nitro-N-nitrosoguanidine // *Microbiol. Zhurn.* – 2004. – **66**, N 5 – P. 24-29. (in Ukrainian)
26. Schmitt D. P., Sipes B. S. Plant-parasitic nematodes and their management // *Plant Disease*. – CTAHR. – 1998. – PD-15. – 4 p.

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