

COMPOSITION OF BIOACTIVE COMPOUNDS FOR CULTIVATION OF BRASSICA JUNCEA CZERN

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Introduction. Formulation of the problem

Now in food recycling industries and, in particular, in yeast industry the problem in processing of secondary waste resources forming during manufacturing of food production is still critical.

The utilization of liquid distillery wastes on the most of enterprises is almost minimal. Often it is drained into the sewer system of cities, or into rivers and ravines, at best into storage ponds, creating a negative impact on the ecological state of the environment [1,2].

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Abstract. The article is devoted to the development of a composition of bioactive compounds designed to improve the cultivation of plant food objects, in particular, *Brassica juncea czern* (mustard seeds of the talisman variety). In addition, the issue of recycling of the most concentrated fermentation liquid, which is a waste after separation at the stage of growing a pure yeast culture, is solved. The growth-stimulating activity of "Humir-1", created on the basis of hydrated fullerenes (HyFn), (C₆₀(H₂O)_n) and humates, as well "Humir-2" in which the mixture of hydrated fullerenes and humates was added to the supernatant of the fermentation fluid, in which a pure culture of yeast (strain *Saccharomyces cerevisiae*) was grown. Concentrated aqueous solutions of hydrated C₆₀HyFn which are molecular colloidal systems of spherical fractal clusters, the structural unit of which is a strong, highly hydrophilic supramolecular complex consisting of a C₆₀ fullerene molecule enclosed in the first hydrated shell containing 24 water molecules were used as a basis in "Humir" preparations. Humates, which is also part of the developed composition, in addition to stimulating growth, has a positive effect on the plant's immunity, helping to adapt to the environment and increase protection against its negative manifestations. The supernatant of the fermentation mixture, in which pure culture yeast was grown, is rich of vitamins, micro and macro elements, yeast residues, and also contains the dry matter of yeast rich in biologically active substances. The influence of the developed compositions on the efficiency of *Brassica juncea czern* cultivation was studied in laboratory and field conditions on the basis of an experimental farm of the Dokuchaev Institute of Agriculture in Kharkiv region. As a result of research, it has been proved that the developed drug "Humir-2", in addition to increasing the yield of *Brassica juncea czern*, increases photosynthetic activity, increases germination and germination energy. As a result, plants have a powerful developed root system and a significantly increased assimilation area of the Leaf.

Keywords: hydrated fullerenes, humates, yeast production waste, *Brassica juncea czern*.

As for today, significant number of scientific publications and patents, domestic and foreign ones, show great interest and practical importance in obtaining and application of growth-stimulating preparations, either as single preparations or together with traditional organo-mineral fertilizers (in form of bioadditives for plant growth) [3,4]. It makes the development of biologically active preparations for plant growth an important scientific and technical task. Thus, elaboration of preparations based on plant growth regulators, including preparations with biostimulating agents produced by microbiological synthesis and biostimulating agents obtained by

chemical synthesis, is one of the most perspective approaches for solving this problem [5].

Because of it the search of new nonconventional compounds which increase productivity of cultures in manufacture of foodstuff is actual. Here growth regulators of new generation, that improve the germination of seed material at the initial stages of development, increase yields, improve the quality of grown products, and increase the resistance of grown crops to adverse conditions.

Analysis of recent research and publications

Fullerenes are one of the promising growth-stimulating compounds for plant food objects. Fullerenes are fine-crystalline powders of black color, odorless, practically insoluble in polar solvents, slightly soluble in alkanes of normal structure, the highest solubility of fullerenes is characterized by aromatic hydrocarbons and their derivatives [6]. Fullerenols are a mixture of polyoxyhydroxylated hydrogen derivatives of fullerenes. In 2009, with the participation of a number of authors, an original method for the synthesis of fullereneol-d with the chemical formula $C_{60}(H_2O)_{24}$, good solubility in water and a molecule diameter of 0.714 nm was developed and implemented [7,8]. Hydrated fullerene $C_{60}HyFn$ is a strong hydrophilic supramolecular complex consisting of a C_{60} fullerene molecule enclosed in the first hydrated shell containing 24 water molecules: $C_{60}(H_2O)_{24}$.

The positive effect of nanocells based on hydrated fullerene on grain and vegetable crops under favorable conditions and under the influence of stressful factors was established, the ability of nanocells to enhance the transport of basic macronutrients to plants, intensify plant growth and transformation processes of organic and mineral compounds to the root system, increase productivity and resistance of plants to oxidative stress, improve the quality of plant products was demonstrated. Due to the antioxidant properties of these substances, they reduce the intensity of lipid peroxidation. In general, the authors [9,10] noted that fullerenes are poorly mineralized, are sorbed by humus and soil particles, and accumulate mainly in the roots when they enter plants.

In the scientific literature, there is a lot of evidence about the positive effect of humic substances and preparations created on their basis on plants [11]. The experience of using humates has shown that their presence is important for all stages of plant development, especially in the early stages [12]. For example, Humate "fertility" had a positive effect on the quality of spring wheat grain. The protein content in the grain increased by 0.6–1.3%, gluten – by 2.8–3.8%, the weight of 1000 grains increased by 2.1–3.9. All of the above is in good agreement with the data of other authors [13]. Humates ensure the efficiency of assimilation of trace elements by plants, reducing the rate of their application by 30–40%. They stimulate the development of all soil microorganisms, which contributes to the intensive assimilation and formation of humus in soils, humus and compost.

In the context of the problem of finding new non-traditional compounds that increase the productivity of plant food objects, the use of a supernatant of a fermentation mixture after growing a pure yeast culture is promising. In the supernatant of the fermentation liquid after separation, many nutrient components remain, exceeding the biological oxygen consumption (BSC) and chemical oxygen consumption (HSC) of total effluents, the content of organic and 129 mineral substances up to 20 g/l, which are in a colloidal and dissolved state, and do not settle under ordinary conditions. Effluents after separation of pure yeast culture contain up to 7% of biomass and almost all microflora, which leads to a high content of protein substances in them [14]. The colloid content ranges from 13 to 19.5% of the amount of dry matter in the liquid. Therefore, such effluents cannot be drained into the city sewer system without preliminary cleaning and decontamination. And their use for enrichment with biologically active substances (BAS) in a growth-stimulating drug is environmentally and economically profitable. The chemical composition of the supernatant of the fermentation mixture is presented in the table (Table. 1).

Table 1 – Chemical composition of the separated fermentation liquid

Chemical composition of the separated fermentation liquid	% on dry matter
Organic substances	46–67
Carbohydrate	1.95–6.20
Glycerin	0.65–5.80
Lactic acid	0.0–2.0
Volatile acids	0.0–0.50
Glutamic acid (after hydrolysis)	0.41–9.82
Betaine	8.12–20.86
Total nitrogen	4.0–6.0
Fat-like substances	0.09–4.0
Potassium	8.52–15.8
Sodium	1.61–3.81
Calcium	1.36–5.70
Magnesium	1.04–5.00
Ferrum	0.20–1.20
Phosphorus	0.23–1.60

The study of the effect of the supernatant of a yeast fermentation mixture in the composition of growth-stimulating mixtures on the efficiency of cultivation of plant food objects is relevant and poorly studied.

Purpose of the work: to study the effect of developed compositions based on humates, hydrated fullerenes and yeast production waste as growth stimulants when soaking mustard seeds of "Talisman" grade variety before planting, as well as the effect on foliar treatment during the tillering phase.

The following tasks were set to achieve the goal:

1. To explore action of the developed preparation in comparison with previously known preparations on the density of mustard seeds of "Talisman" grade variety, as well as to determine the germination energy, seed germination and influence on the pod formation phase.

2. To compare the preparations "Humir-1" and "Humir-2" on experimental samples and determine the effect of biologically active substances contained in the supernatant of the fermentation mixture after growing a pure yeast culture.

Research materials and methods

Hydrated fullerenes with a concentration of 100 mg/l were obtained from the developer of a water-soluble form of fullerene. C₆₀HyFn was obtained by direct oxidation of light fullerene C₆₀ with alkali in the presence of a homogeneous catalyst. It is characterized by stable composition and does not contain impurities of toxic solvents used in traditional methods of producing fullerenes. The method developed by the authors provides a high product yield (72% of the theoretically possible) and a reduction of more than an order of magnitude in its cost compared to its global counterparts. The physical and chemical properties of fullerene-d are well studied. The high lipophilicity of the carbon core of fullerenes ensures their penetration through biomembranes, nanoscale steric correspondence to biomolecules, and the "cloud" of π -electrons on the surface-participation in free radical reactions, which can be multidirectional depending on the concentration of fullerenes, the object and environmental conditions.

Gummy Max biofield dry Humate with a concentration of humic acids – 85%, K₂O – 10%, fulvic acids – 15%, solubility – 98% was used as a source of Humate for the production of the compositions "Humir-1" and "Humir-2".

Yeast production waste were collected during the separation of a pure yeast culture (*Saccharomyces cerevisiae* strain) at Kharkiv yeast plant. At cultivation stage the pure yeast culture presented in a fermenter during 20–22 hours, the yield of yeast at this stage was 70–75%. This influences the content of organic substances in molasses, and their quantity arriving from cells into medium depends on quantity of yeast in the medium and from term of their exposition within.

For use as film-forming agents in seed treatment, mainly water-soluble polymers were studied, since it is known that an increase in hydrophilicity increases the adhesion of the polymer [16] and makes the film on the seeds stronger. For the preparation of the compositions "Humir-1" and "Humir-2", it was proposed to use polymers PEG 400 and PEG 1500.

The preparation "Humir-1" contained humates, hydrated fullerenes and polymers film-forming PEG 400 and PEG 1500 in its composition.

The composition of "Humir-2" included humates, hydrated fullerenes, supernatants of the fermentation mixture after growing a pure yeast culture, PEG 400 and PEG 1500.

Research methods

Seed germination was carried out in petri dishes under the influence of various drugs, and in open areas of a pilot farm of Dokuchayev agriculture institute in Kharkov region. The germination energy was determined using the generally accepted reference method [17]. Seed germination was determined for 3–5

days, the number of normally sprouted seeds, rotten, swollen and abnormally sprouted was counted (table. 3), growth and development indicators were determined by standard reference methods [17].

Determination of nitrogen, phosphorus and potassium in experimental samples was carried out from a single Pinevich suspension in Kurkaev modification [18].

The optical density of the resulting solution of ammonium mercurium iodide, which is proportional to the composition of the reference ammonium, was determined on a photoelectric colorimeter (spectrophotometer) at a wavelength of 440 nm [18]. The optical density of a phosphorous-molybdenum blue solution is proportional to the composition of phosphorus and was determined on a photoelectric colorimeter (spectrophotometer) at a wavelength of 670 nm [18].

The potassium content in the solution was determined on a flame photometer. Radiation (emission) of potassium atoms in the burner flame, the intensity of which is proportional to the concentration of this element in the solution. Comparing the radiation intensity of the sample solution with the known concentration of potassium and the test solution, the potassium content of the reference was found [18].

Results of the research and their discussion

On the basis of a pilot farm of Dokuchayev agriculture institute in Kharkov area the humate-containing preparations under conditional name "Humir-1" and "Humir-2" were applied for processing of mustard seeds before sowing.

Number of researches with mustard seeds of grade "Talisman" which were grown in Petri dishes *in vitro* (3 repeats in each batch from 20.04 to 26.04.19) have been carried out. As control preparations known growth-stimulating "Vympel" and "Baikal" and as well as untreated seeds (dry control) were used. Besides, they were compared with the seeds processed only with hydrated fullerene (HyFn);(C₆₀(H₂O)_n). The "Humir-2" has been developed which compound contained concentrated supernatant of the fermentation mixture after growing a pure yeast culture (*Saccharomyces cerevisiae* strain). Seeds were also treated with Humir-2. The results of the study are shown in the Table (Table 2).

Table 2 – Density of mustard shoots in 2019, average indexes (350 items = 100 %)

The sample	Density of shoots, items/m ²	Ascended, %
Dry control	233.4 ± 22.1	66.69
Hydrated fullerenes	245.9 ± 25.9	70.26
Gumir - 1	280.1 ± 28.2	80.02
Gumir - 2	288 ± 27.9	82.29
Vympel	270.2 ± 26.8	77.14
Baikal	248.8 ± 20.5	71.14

It was found that the direct effect of the drug "Humir-2" was associated with the effect of not only

humic acids, but also biologically active substances introduced into the drug on the permeability of cell membranes and protein synthesis.

The density of seeds which have sprouted after their treatment with "Humir-2" containing wastes of yeast manufacture was almost by 16% higher than in dry control and more than after processing of seeds with preparations "Vympel" and "Baikal". Negligible,

but still increase in quantity of shoots in the given experiment has been found also after processing of seeds with hydrated fullerene solution only. Quantity of sprouted seeds was higher after their treatment with the preparation "Humir-2" accordingly, noted germinating ability was slightly lower after application of reference preparations "Vympel" and "Baikal" (Table 3).

Table 3 – Average indexes of activity of the preparations studied

Processing method	Energy of sprouting, %	Germinating ability %	Kind of seeds, %		
			inflated	decayed	abnormally sprouted
Control	78.9	83.4	0	6.87	6.82
Hydrated fullerenes	78.97	83.8	0.77	4.53	7.30
Vympel	80.1	88.9	1.30	4.59	7.34
Humir-1	83.8	90.1	0.63	4.20	6.10
Humir-2	85.0	92.3	0.75	4.19	5.98

Comparative analysis of the obtained data presented in table (table 3) has shown, that the best indicators of sprouting energy and germinating ability were found after treating of mustard seeds with preparations "Humir-1" and "Humir-2". At increase in sprouting energy and germinating ability the decrease in number of abnormal sprouted seeds was observed, that was more expressed after treatment of seeds with given preparations. Processing of seeds with solution contained hydrated fullerenes only has not influenced positively the indicators as showed in the table (Table 4), the number of decayed seeds appeared to be even more than in control assay. Indicators of sprouting energy and germinating ability were higher after treatment of seeds with the preparation "Humir-1" and "Humir-2" in comparison with the reference preparation "Vympel".

Data from experiments conducted in the field in 2019, showed the effectiveness of growth-stimulating drugs in comparison with the control sample, which was not processed by anything. The most important indicator in this experiment is the number of pods, the results are shown in the table (Table 5) and are clearly shown in the diagram (Fig. 1).

Table 4 – of fullerene concentration with different effect of action on mustard plantlets

Parts of plants	Fullerenes, mg / l		
	Effect of action on plant plantlets		
	No effect	Growth stimulation	Growth inhibition
Roots	0.01–1.0	5.5–10.0	≥100.0
Sprouts	0.01–10.0	25.0–100.0	>500.0

Table 5 – Mustard of 2019 at phase of pod formation, average indexes

Variant	Quantity of plants in a sheaf, items/m ²	Average quantity in 1 plant from 15 ones	
		branches	Pods
Control	216	1.78	7.3
Hydrated fullerenes	236	1.99	12
Humir-1	261	2.44	10
Humir-2	244	2.2	11.44
Vympel	252	1.82	7.82
Baikal	276	1.485	6.75

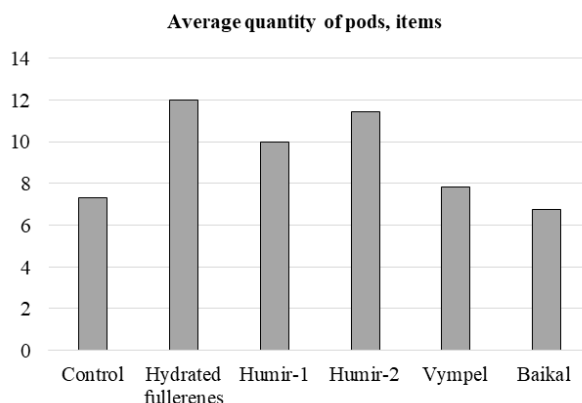


Fig. 1. The average quantity of mustard pods

On the fig. 1 the average quantity of mustard pods is presented. The higher results were obtained after processing with the preparation Gumir-2 and fullerenes however crop increase was different (Table 6).

Table 6 – The mustard crop account in 2018-2019 years, average indexes

Variant	Weight of seeds from 1 lot, centner/hectare	Increase in crop, %
Control	0.550	---
Hydrated fullerenes	0.803	46
Humir-1	0.996	81.1
Vympel	0.846	53.8
Humir-2	1.028	86.9
Baikal	0.944	71.6

As shown in the table (Table 6), increase in the mustard crop after treatment with the preparation Gumir-2 was higher, than after processing with the preparation Humir-1 and other growth-stimulating preparations that can be explained by presence of microelements from waste of yeast cell material.

The obtained results made the basis for including of solution of fullerenes, a solution of a supernatant of a fermentation mixture, as well as a mixture of fermentation liquid and hydrated fullerenes were used for foliar treatment of mustard. The obtained results clearly showed the effect of the prepared mixture on the raw mass of the plant (Table 7).

Thus, the results of studies under regulated conditions showed the ability of the solution with fullerene and yeast biomass waste in a certain concentration range to have a positive growth-stimulating and anti-stress effect on plants. The reasons for improving the physiological state of plants and

increasing their resistance to oxidative stress under the influence of the developed nano composition are the activation of metabolic processes, metabolism, and, as a result, an increase in the intake of necessary macro- and microelements into plants, which are contained in sufficient quantities in the waste of yeast production.

Table 7 – Biomass of mustard leaf plants during foliar treatment

Out-of-root processing	Crude mass of the plant	
	g	deviation from control, %
Water (control)	160.8 ± 11.3	-
Aqueous fullerene solution	188.4 ± 14.0*	+27.6 ± 2.9*
Solution of the fermentation mixture supernatant	198.7 ± 16.3*	+37.9 ± 4.2*
Fullerene solution + fermentation mixture supernatant	240.3 ± 15.4*	+79.5 ± 3.1*

* – value statistically differs from control in 5 % significance level ($P \geq 95\%$)

Comparison of the micro- and macro-element composition (Table 8) in the aboveground part of mustard with Root and foliar top dressing with preparations "Humir-1" and "Humir-2" was conducted.

Results of the experiment showed that macro and microelements are better absorbed after processing with Humir-2. Thus, in the developed composition, biologically active substances that were present in yeast production waste affected the content of basic elements in the aboveground part of mustard, and humates and hydrated fullerenes increased their effectiveness.

Table 8 – Content of main elements in the aerial parts of mustard after root and out-of-root feeding

Experimental variants	Content of substances						
	Nitrogen, % d.w.*	Phosphorus, % d.w.	Potassium % d.w.	Protein nitrogen % d.w.	Nonprotein nitrogen, % d.w.	Ammonium nitrogen, % d.w.	Crude ash, % d.w.
Plants (aerial part) after root supplementary feeding							
Humir-1	1.75	0.25	4.84	0.205	1.55	0	14.18
Humir-2	2.0	0.32	5.01	0.224	1.78	0	15.28
Plants (aerial part) after out-of-root supplementary feeding							
Humir-1	2.04	0.28	5.61	0.196	1.84	0	16.21
Humir-2	2.65	0.40	6.3	0.317	2.33	0.049	18.56

*d.w.– dry weight

Conclusion

Experiments conducted in 2019 at Sudislavsky GSU of Kharkiv region showed a significant effect of the drug "Humir-2" on the yield of mustard of the "talisman" variety. On average, over 2 years, seed treatment provided a yield increase of 6.7 centners/ha, which is 31.2% more than in the control. In addition, a positive effect was obtained when processing vegetative plants in the tillering phase against the background of processing plants in the growing season.

The increase in the amount of grain was 13.6 centners / ha and 63.2% higher than the control.

Treatment of mustard seeds with Humir-2 significantly increases germination and germination energy. The result is plants with a powerful developed root system and a significantly increased assimilation area of the Leaf.

Over the past 2 years, the average increase in the yield of mustard grain could theoretically amount to 2.52 centners / ha, and in practice, when grown in the field in 2019, it was several percent more. Profitability

by year in our conditions ranged from 128 to 150% (due to an increase in the vegetative component of the plant). In addition, on average, over 2 years, the protein content in grain increased by 1.42% and the weight of 1000 grains – by 1.3%.

The drug "Humir-2" is an innovative drug of a complex nature of action, and at the same time a regulator of growth, generative and root-forming processes, an inducer of disease resistance and a stress adaptogen drug.

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КОМПОЗИЦІЯ БІОАКТИВНИХ СПОЛУК ДЛЯ КУЛЬТИВУВАННЯ BRASSICA JUNCEA CZERN

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Анотація. Роботу присвячено розробленню композиції біоактивних сполук, призначених для поліпшення культивування рослинних харчових об'єктів, зокрема, *brassica juncea czern* (насіння гірчиці сорту «Талісман»). Крім цього вирішується питання утилізації концентрованої бродильної рідини, яка є відходом після сепарації на етапі вирощування чистої культури дріжджів. Досліджено рістестимулювальну активність препарату «Гумір-1», створеного на основі гідратованих фулеренів (hyfn);(C₆₀(H₂O)_n) і гуматів, а також препарату «Гумір-2» в який до суміші гідратованих фулеренів та гуматів додавали супернатант бродильної суміші, в якій вирощувалась чиста культура дріжджів (штам *Saccharomyces cerevisiae*). Для основи в препаратах «Гумір» використовували концентровані водні розчини гідратованого C₆₀HyFn, що є молекулярно-колоїдними системами сферичних фрактальних кластерів, структурною одиницею яких є міцний, високогідрофільний супрамолекулярний комплекс, що складається з молекули фулерену C₆₀, укладеної в першу гідратну оболонку, котра містить 24 молекули води. Гумати, які також входять до складу розробленої композиції окрім стимулювання росту позитивно впливає на імунітет рослини, допомагаючи адаптуватись до навколишнього середовища і збільшити захист від його негативних проявів. Супернатант бродильної суміші, в якій вирощували дріжджі чистої культури, багатий на вітаміни, мікро та макро елементи, а також містить суху речовину дріжджів багату біологічно активними речовинами. Вплив розроблених композицій на ефективність

культивування *brassica juncea czern* досліджено в лабораторних та польових умовах на базі дослідного господарства інституту сільського господарства ім. Докучаєва Харківської області. В результаті досліджень доведено, що розроблений препарат «Гумір-2», окрім підвищення врожайності *Brassica juncea czern*, збільшує фотосинтетичну активність, підвищує схожість і енергію проростання. Як результат рослини з потужною розвинутою кореневою системою та істотно збільшеною асиміляційною площею листа.

Ключові слова: гідратовані фулерени, гумати, відходи дріжджового виробництва, *Brassica juncea czern*.

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