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Application of mineral carriers for immobilization of *Trichoderma viride*

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There is no doubt in the fact that preparations containing *Trichoderma* as the main component are currently a real alternative to synthetic agrochemicals as antagonists of soil-borne plant diseases and growth stimulators. The use of this kind of drugs does not always give sufficiently stable results. In addition, technological problems can often arise, which are associated with the short shelf life of liquid preparations based on *Trichoderma*. The use of the solid form of the drug often causes the problem of self-inhibition of conidia germination. And therefore a more careful calculation of the optimal dose for each specific strain is required. The development of multifunctional bio-preparations that are more stable in their effectiveness, especially preparations that include *Trichoderma*, is one of the most important tasks in the system of biotechnology development, which determined the relevance of the research.

The aim of the work is to establish the optimal carrier for the immobilization of *Trichoderma viride* cells and the effect of immobilization on the stability of drugs. It has been experimentally established that the growth and development of the immobilized fungus *Trichoderma viride* is influenced by the nature and method of modification of the carrier. It was established that when the fungus immobilized on native and modified carriers (particle size 0.5 μm – 2.5 mm) was seeded on a sterile nutrient medium, a decrease in the growth rate of *Trichoderma viride* was found, compared to the control one where the native fungus was used. It has been proven that the optimal amount of grinding of both native and modified media is – 150 μm – 1.5 mm. It has been found that saponite modified with starch is the optimal carrier for *Trichoderma viride* immobilization.

It has been proven that upon immobilization of the fungus *Trichoderma viride*, the latter becomes more resistant to the negative factors of the growth environment (antagonistic action of the natural conglomerate of microorganisms residing on spoiled alfalfa hay).

Key words: fungi, cell immobilization, carriers, modified starch, humic acids, saponite, zeolite, zeolite-containing basalt tuff.

Problem statement and analysis of recent research. Native culture or preparations of *Trichoderma viride* are widely used in agriculture. The fungus as the main component of the drug is used to struggle against a number of soil diseases, increase the yield of grain and leguminous crops. Preparations containing fungi are used for the

disposal of crop production waste, unfit for use as feed of plant origin, etc [3, 21]. The use of *Trichoderma viride* together with arbuscular mycorrhizal fungi makes it possible to replace chemical fertilizers and stimulate an increase in crop yields [16]. Stimulation of plant growth can be manifested taking into account a number of limiting factors

such as seeding rate, type of formulation, growing conditions [4, 17]. Fungi are used in the form of preparations in the form of liquid and powder. However, with different technologies that use preparations containing *Trichoderma*, various difficulties arise, under which the effectiveness of the fungi decreases. The use of native preparations of *Trichoderma* in technologies of crop production or utilization of organic waste does not always give stable results [21]. These phenomena are due to the fact that the liquid form of preparations containing fungi has a short shelf life. When using loose form of fungi preparations, the feature of autoinhibition of the process of conidia germination is manifested. Therefore, this phenomenon requires careful calculations of the necessary dose of the drug for each particular strain of fungi [21]. In addition, there is a problem of the lack of local fungal delivery systems to reduce diseases caused by soil bacterial pathogens [19].

The multi-vector use of *Trichoderma viride* in agriculture requires the development of technologies for the production of fungi preparations that are stable in their effectiveness. The issue of the effectiveness of immobilization (stabilization) of the fungi on domestic natural minerals – saponins, zeolites and zeolite-containing basalt tuffs is unexplored.

Trichoderma refers to filamentous spore fungi. These fungi produce ascomycetes and belong to the large class Deuteromycetes. These fungi are one of the types of mold that is found in almost all types of soil. *Trichoderma viride* is the most common and cultivated fungus. These fungi are widely used in the national economy [7, 20].

In world practice, a number of methods of their immobilization have been developed for more effective use of microorganisms, including fungi. Immobilization of cells of microorganisms, their parts and enzymes is a physical limitation or localization in a certain spatial location. The latest involves restricting their free movement to demonstrate hydrodynamic characteristics that differ from those of the external environment, while preserving their biological and catalytic properties. Immobilization allows repeated and continuous use of cells of microorganisms. Depending on the method and method of immobilization of microbial cells, it is possible to prolong their viability and improve their growth in adverse conditions [5, 8, 18].

The selection of a suitable carrier (matrix) for the immobilization of cells of microorganisms is an important process for obtaining a finished product with the desired biological properties. The carriers had to be accessible, have a large surface area, which would later provide an opportunity for

the growth of microorganisms (satisfactory interstitial space). Under certain technological conditions, the matrix should be easily regenerated and reusable, not exhibit toxic effects in relation to the cells of microorganisms, and promote the manifestation of catalytic activity. Carriers should have mechanical and thermal resistance, biological and chemical stability [9, 11, 12, 14].

Immobilization of cells of microorganisms is carried out using physical and chemical methods, including the use of adsorption, encapsulation, and encapsulation in a gel [15]

To maintain the stability of *Trichoderma* in the soil during the warm season, materials (as a matrix) with sorption properties and are a source of nutrition for the culture are introduced into the soil together with fungi preparations: peat, cereal bran, tree bark, compost obtained from the manure of farm animals, substrates after seafood cultivation [10, 13].

Biotechnologies of fungi spore encapsulation with starch borate, starch xanthan or carrageenan gel have been developed to stabilize drugs.

Technologies for manufacturing alginate granules with microorganisms and alginate granules with pyrophyllite clay filler are used [13].

Immobilization of *Trichoderma* conidia and chlamydiospores was carried out on wheat bran and kaolin clay in alginate gel [13]. Both organic and inorganic carriers are also used for the immobilization of microorganisms. Among organic carriers, starch, pectin, cellulose, gelatin and their forms modified by physical and chemical methods are used [1, 2, 6]. As a matrix, calcium alginate, modified coal from animal bones is used [5, 12, 18].

The aim of the study is to establish the optimal carrier and the size of its grinding for the immobilization of *Trichoderma viride* fungi cells and its growth stability in non-sterile conditions.

Materials and methods of research. The growth of *Trichoderma viride* fungi was checked using the PDA medium – potato dextrose agar with the content of antibacterial drugs (sterile benzylpenicillin sodium salt (1000000 units) 0.5 g/dm³ and streptomycin sulfate 0.4 g/dm³). Before using the medium, it was thermally treated – autoclaved (25 minutes at a temperature of 121–123 °C).

The native culture of *Trichoderma viride* (control) and its immobilized forms were sown on PDA containing antibiotics. In addition, spoiled alfalfa hay was treated with native and immobilized drugs. Hay with a moisture content of 64.5 % was not subjected to heat treatment and treatment with antimicrobial drugs (non-sterile conditions). After 14 days of cultivation of hay treated with native and immobilized preparations of *Trichoderma viride* at a temperature of 25–26 °C, the investigat-

ed fungus was isolated from its samples on potato-dextrose agar.

The prepared PDA nutrient medium was poured into Petri dishes of 15 cm³ each. Each Petri dish was numbered, taking into account the experimental group.

Incubation of cultures of native and immobilized *Trichoderma viride* on the medium and cultures of alfalfa silage treated with these fungi was carried out in a thermostat at a temperature of 26±0.5 °C.

For the immobilization of *Trichoderma viride*, such natural minerals were used as carriers: saponite of the Tashkiv region, gray zeolite, and zeolite-containing basalt tuff with different levels of grinding (Table 1). The same grinding was used for modified saponite, modified gray zeolite and modified zeolite-containing basalt tuff. Natural carriers were modified with starch, humic acids using calcium chloride.

Table 1 – Carrier characteristics

Carrier	The amount of grinding of the native and modified form
Saponite	From 0,5 up to 150 µm
	From 150 µm up to 1,5 mm
	From 1,5 mm up to 2,5 mm
Gray zeolite	From 0,5 up to 150 µm
	From 150 µm up to 1,5 mm
	From 1,5 mm up to 2,5 mm
Zeolite-containing basalt tuff	From 0,5 up to 150 µm
	From 150 µm up to 1,5 mm
	From 1,5 mm up to 2,5 mm

Immobilization of spores and mycelium of *Trichoderma viride* on native and modified carriers was carried out by a physical method. Viridin preparations were the source of fungi biomaterial. A carrier was added to the solution of *Trichoderma viride* in the presence of 0.05 M calcium chloride and incubated for 20–22 hours at a temperature of 4.0–4.5 °C, periodically subjecting the mixture to thorough mixing every 1.5–2.0 hours.

Results and discussion. It was established that the CFU index in the control (native form of the fungus) was 3.1x10⁹. Investigating the growth and development of immobilized *Trichoderma viride* fungi, it has been established that these processes are influenced by the nature and method of carrier modification.

After sowing the fungi immobilized on native saponite (particle size 0.5–150 µm), the growth rate of *Trichoderma viride* on the nutrient medium the decrease was found by 238 times compared to the control. Compared with the options where saponite modified with starch and humic acids was used, the growth of the fungus was also lower, respectively, by 8.4 and 4.2 times compared to the control (Table 2).

The lowest rate of growth of the immobilized fungus on native saponite was recorded in the variant where the size of the carrier was crushed – 1.5–2.5 mm compared to the control and variants where the modified carrier was used.

Under using modified saponite (particle size 1.5–2.5 mm) with starch and humic acids, the CFU indicator of *Trichoderma viride* decreases compared to the option where saponite with a particle size of 150 µm – 1.5 mm was used, respectively, in 8.8 and 23, 7 times.

Table 2 – The Growth of *Trichoderma viride* on PDA nutrient medium

Indicator	Native carrier	Carrier modified with starch	Carrier modified with humic acids
Control	3,2x10 ⁹	3,1x10 ⁹	3,3x10 ⁹
The drug immobilized on saponite (0,5–150 µm)	1,3 x10 ⁷	1,1 x10 ⁸	5,5 x10 ⁷
The drug immobilized on saponite (150 µm – 1,5 mm)	5,3 x10 ⁷	4,7 x10 ⁸	9,5 x10 ⁷
The drug immobilized on saponite (1,5–2,5 mm)	6,9 x10 ⁶	5,3 x10 ⁷	0,4 x10 ⁷
The drug immobilized on zeolite (0,5–150 µm)	0,9 x10 ⁶	0,5 x10 ⁷	6,2 x10 ⁶
The drug immobilized on zeolite (150 µm – 1,5 mm)	8,5 x10 ⁶	7,3 x10 ⁷	3,8 x10 ⁷
The drug immobilized on zeolite (1,5–2,5 mm)	7,7 x10 ⁵	6,1 x10 ⁶	2,0 x10 ⁶
The drug immobilized on zeolite-containing basalt tuff (0,5–150 µm)	1,7 x10 ⁶	1,0 x10 ⁷	6,0 x10 ⁶
The drug immobilized on zeolite-containing basalt tuff (150 µm – 1,5 mm)	9,3 x10 ⁶	8,2 x10 ⁷	4,3 x10 ⁷
The drug immobilized on zeolite-containing basalt tuff (1,5–2,5 mm)	4,5 x10 ⁶	5,2 x10 ⁷	1,5 x10 ⁷

Comparing the effects of saponite modification methods, it was proven that upon immobilization of *Trichoderma viride* on starch-modified saponite with a particle size of 150 μm – 1.5 mm, the CFU index of the fungus was higher than when using native saponite and modified with humic acids, respectively, by 8.8 and 4.9 times. It was found that the optimal grinding value of both native and modified saponite is – 150 microns – 1.5 mm. Thus, when using starch-modified saponite with a particle size of 150 μm to 1.5 mm, the growth of fungi on the medium was 4.2 times higher than when using such a medium with a particle size of 0.5–150 μm .

Sowing of *Trichoderma viride* immobilized on native zeolite (particle size 0.5–150 μm) showed that the number of CFU of the fungus was 3444 times less compared to the control. Relative to variants with a similar particle size of zeolite modified with starch and humic acids, it was found that the growth of *Trichoderma viride* was lower, respectively, by 55.5 and 65.9 times. The greatest growth and development of the fungus was recorded in the variant where the culture immobilized on starch-modified zeolite (150 μm – 1.5 mm) was used, compared to other experimental samples. The indicator was higher in comparison with the option where the immobilization of the fungus was carried out on the native carrier and the carrier modified with humic acids, respectively, by 8.6 times and by 92.1 %.

Studying the growth intensity of *Trichoderma viride* immobilized on zeolite-containing ba-

salt tuff revealed a decrease in CFU indicators compared to the control. Comparing between the immobilized fungi, it was established that the greatest culture growth was on the medium where zeolite-containing basalt tuff with a particle size of 150 μm – 1.5 mm modified with starch was used.

Comparing the effect of carriers on the growth of the fungus, it has been found that immobilization on saponite, zeolite, and zeolite-containing basalt tuff leads to a significant decrease in the CFU index of *Trichoderma viride* compared to the control. Saponite modified with starch was found to be the most effective carrier for immobilization of the fungus. The most optimal particle size of natural minerals, both native and modified, was 150 μm – 1.5 mm.

During the treatment of spoiled hay with the native preparation (control) *Trichoderma viride*, it was established that during 14 days of cultivation, the CFU indicator was 4.4×10^3 . It was found that the growth indicators of the immobilized fungi on the hay were influenced by the carrier and the size of the particles of the carrier. During the treatment of spoiled alfalfa silage with a fungus immobilized on saponite, it was found that the growth of fungi was 75 times greater than in the control when using preparations with native saponite with a particle size of 0.5–150 μm . When using *Trichoderma viride* immobilized on saponite modified with starch with a similar size of particles, the growth of the fungus was 3.9 times greater compared to the version where native saponite was used (Table 3).

Table 3 – The Growth of *Trichoderma viride* on spoiled hay

Indicator	Native carrier	Carrier modified with starch	Carrier modified with humic acids
Control	$4,4 \times 10^3$	$4,0 \times 10^3$	$3,9 \times 10^3$
The drug immobilized on saponite (0,5–150 μm)	$3,3 \times 10^5$	$1,3 \times 10^6$	$4,4 \times 10^5$
The drug immobilized on saponite (150 μm – 1,5 mm)	$9,7 \times 10^5$	$4,3 \times 10^6$	$0,8 \times 10^6$
The drug immobilized on saponite (1,5–2,5 mm)	$7,8 \times 10^4$	$6,7 \times 10^5$	$9,9 \times 10^4$
The drug immobilized on zeolite (0,5–150 μm)	$2,8 \times 10^4$	$5,8 \times 10^5$	$3,9 \times 10^4$
The drug immobilized on zeolite (150 μm – 1,5 mm)	$7,9 \times 10^4$	$9,9 \times 10^5$	$8,9 \times 10^4$
The drug immobilized on zeolite (1,5–2,5 mm)	$9,7 \times 10^3$	$8,9 \times 10^4$	$0,7 \times 10^4$
The drug immobilized on zeolite-containing basalt tuff (0,5–150 μm)	$0,8 \times 10^4$	$2,9 \times 10^5$	$2,8 \times 10^4$
The drug immobilized on zeolite-containing basalt tuff (150 μm – 1,5 mm)	$7,5 \times 10^4$	$9,6 \times 10^5$	$8,8 \times 10^4$
The drug immobilized on zeolite-containing basalt tuff (1,5–2,5 mm)	$5,5 \times 10^4$	$5,9 \times 10^5$	$6,2 \times 10^4$

Comparing the growth of the fungus immobilized on saponite with different sizes of particles and different methods of modification, it was found that the highest growth of *Trichoderma viride* on spoiled alfalfa hay was recorded in the variant where the fungus was immobilized on saponite modified with starch with a particle size of 150 μm – 1.5 mm.

When hay was treated with preparations of a fungus immobilized on native zeolite with a particle size of 0.5–150 μm , the growth of the latter was 6.3 times greater than in the control. When using a fungus immobilized on both native and modified zeolite, the whitest growth of microorganisms was recorded when using carriers with a particle size of 150 μm – 1.5 mm. Comparing the effect of immobilization of *Trichoderma viride* on different media on the growth of the fungus, it was established that the highest CFU indicators were recorded on treated hay in the variant where drugs immobilized on zeolite modified with starch were used.

The growth of the fungus immobilized on native zeolite-containing basalt tuff with a particle size of 0.5–150 μm on the hay was more intense than in the control. The difference according to the CFU indicator was 1.8 times. Immobilization of the fungus on zeolite-containing basalt tuff (particle size 150 μm – 1.5 mm) modified with starch and humic acids allows to obtain preparations with better growth on alfalfa hay compared to preparations immobilized on the native mineral, respectively, by 12.8 times and by 17.3 %. Comparing the growth indicators of *Trichoderma viride* immobilized on native and modified zeolite-containing basalt tuff on alfalfa hay, it was established that the most intense growth was obtained with the use of the fungus immobilized on a natural mineral modified with starch.

Analyzing the growth of native and immobilized preparations of *Trichoderma viride* on alfalfa hay it has been revealed that the highest rates of CFU of the fungus were obtained using preparations immobilized on modified saponite (particle size 150 μm – 1.5 mm).

Thus, we have proved that with the help of immobilization, the fungus *Trichoderma viride* becomes more resistant to negative factors of the external environment (the antagonistic effect of the natural conglomerate of microorganisms of spoiled alfalfa hay), which confirms the data of researchers [5, 13].

Conclusion. 1. When *Trichoderma viride* is immobilized on modified saponite with a particle size of 150 μm – 1.5 mm, the growth intensity of the fungus on a sterile nutrient medium PDA decreases by 238 times compared to the native preparation.

2. Among natural minerals (saponite, gray zeolite and zeolite-containing basalt tuff) and their modified forms, saponite modified with starch is the optimal carrier for immobilization of *Trichoderma viride* fungus.

3. Immobilization of *Trichoderma viride* on starch-modified saponite with the participation of calcium ions allows to maintain 977 times greater growth of the fungus in an environment with unfavorable external conditions compared to its native form.

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Застосування мінеральних носіїв для іммобілізації *Trichoderma viride*

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Препарати, що містять *Trichoderma* як основний компонент, нині є реальною альтернативою синтетичним агрохімікатам як антагоністи ґрунтових хвороб рослин й стимулятори росту. Застосування таких препаратів не завжди дають стабільні результати. Окрім того, часто можуть виникати технологічні проблеми, які пов'язані із коротким терміном придатності рідкої форми препаратів на основі *Trichoderma*. Застосування твердої форми препарату часто викликає проблему самогальмування проростання конідій. І тому потрібний більш ретельний розрахунок оптимальної дози для кожного конкретного штаму. Розроблення більш стабільних за своєю ефективністю поліфункціональних біопрепаратів, особливо препаратів, до складу яких входить *Trichoderma*, є одним із найважливіших завдань біотехнології, що зумовило актуальність дослідження.

Метою роботи є встановлення оптимального носія для іммобілізації клітин *Trichoderma viride* та впливу іммобілізації на стабільність препаратів. Експериментально встановлено, що на ріст і розвиток іммобілізованого гриба *Trichoderma viride* впливає природа і спосіб модифікації носія. За посіву на стерильному поживному середовищі іммобілізованого на нативних і модифікованих носіях гриба (величина часточок 0,5 мкм-2,5 мм) виявлено зниження показника росту *Trichoderma viride* порівняно з контролем, де використовували нативний гриб. Доведено, що оптимальною величиною подрібнення як нативних так і модифікованих носіїв є 150 мкм – 1,5 мм. Виявлено, що оптимальним носієм для іммобілізації *Trichoderma viride* є модифікований крохмалем сапоніт.

Доведено, що за іммобілізації гриба *Trichoderma viride* останій стає стійкішим до негативних факторів середовища росту (антагоністична дія природнього конгломерату мікроорганізмів, що перебуває на зіпсованому сінажі люцерни).

Ключові слова: гриби, іммобілізація клітин, носії, модифікований крохмаль, гумінові кислоти, сапоніт, цеоліт, цеолітовмісний базальтовий туф.



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