DEVELOPING INNOVATIVE TECHNOLOGIES OF MINERAL FERTILIZERS ENRICHED MICROBIOLOGICALLY.

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BRIDGE BETWEEN SCIENCE AND ECONOMY

APPLIED RESEARCH
BIOTECHNOLOGY, CHEMISTRY, PHARMACEUTICALS, ELECTRONICS, ELECTRICAL ENGINEERING, MATERIALS ENGINEERING, PRECISE ENGINEERING, METALLURGY, OPTICS AND AVIATION

APPLICATION IN INDUSTRY
PROVIDERS OF TECHNOLOGY BASED SOLUTIONS FOR BUSINESS PROBLEMS

MORE EFFICIENT COOPERATION
UNIFIED MANAGEMENT MECHANISMS OF FINANCE, HUMAN RESOURCES, REAL ESTATE AND INTELLECTUAL PROPERTY LAWS

INTEGRATED R&D OPERATIONS
POOLING BREAKTHROUGH EXPERTISE ROOTED IN BUSINESS EXPERIENCE AND SCIENTIFIC VISION
Aim of the project

The aim of the project is to develop innovative microbiologically enriched bio fertilizers and to evaluate the effects of their use in crops and microbiological stimulation of fertility and soil productivity.

The newly developed biofertilizers were produced by combining Urea, Polifoska 4 (NPK) and Super Fos Dar 40 (P) with carriers and useful microorganisms with biostimulatory and protective effects.

Humic acids and other carriers of beneficial microorganisms, free from harmful substances, enable maintaining the high abundance and survival of beneficial microorganisms in bio-fertilizers.

Collected in SYMBIO BANK at the Institute of Horticulture in Skierniewice and new species isolated from the rhizosphere of the studied plants were used for the microbial enrichment of mineral
THE OBJECTIVES OF THE PROJECT WILL BE ACHIEVED THROUGH THE IMPLEMENTATION OF THE FOLLOWING RESEARCH TASKS:

Task 1. Technology for the production of microbially enriched fertilizers.

Task 2. Effectiveness of biofertilizers to improve the biophysical and chemical properties of degraded soils.

Task 3. Effect of biofertilizers on the growth and yielding of horticultural plants and on soil microbiology.

Task 4. The effect of biofertilizers on the growth and yielding of field crops and on the improvement of soil fertility.

Task 5. Assessment of the impact of the use of biofertilizers on the water potential and content of macro and micronutrients in soil and plants.

Task 6. Preparation for implementation, dissemination and commercialization of research results of newly developed bio-fertilizers.
WP 6.
Preparation for implementation, dissemination and commercialization of research results and newly developed biofertilizers.

WP 1
Development of new fertilizers and technologies for improvement of soil quality parameters

WP 2
Characterization of indicators of soil quality properties

WP 3
Improvement of soil quality parameters and yielding of horticultural plants

WP 4
Improvement of soil quality parameters and yielding of agricultural plants

WP 5
Improvement of water potential of soils

Fig. 1. Workpackages structure
The project is implemented by a consortium of partners:

- Institute of Horticulture (project leader)
- Institute of Agrophysics, Polish Academy of Sciences
- New Chemical Synthesis Institute
- Institute of Soil Science and Plant Cultivation State Research Institute
- Grupa Azoty Pulawy
TASK 1:

- TECHNOLOGY OF FERTILIZER ENRICHED MICROBIOLOGICALLY. (IH, INS, GA ZAP)

- Duration of task 1: 01/02/2018 - 31/01/2021
TECHNOLOGY OF FERTILIZER ENRICHED MICROBIOLOGICALLY.

Institute of Horticulture develop a technology for the industrial multiplication of microbial inoculums, necessary for the production of microbially enriched fertilizers and optimize the bio-physical-chemical conditions of the process of microorganism multiplication in industrial bioreactors.

INS, IH and Grupa Azoty PUŁAWY develop a technology for the production of microbial-enriched fertilizer lots necessary for field experiments. Lots of microbially enriched fertilizers for field testing will be produced by INSCH on a laboratory scale. The obtained biofertilizers will undergo physical and chemical tests and assess their qualitative and quantitative composition.

The key will be to develop an optimal method of introducing beneficial microorganisms to the formulation of new biofertilizers.

After obtaining the appropriate quality parameters, INS in cooperation with the Grupa Azoty PUŁAWY will produce pilot lots of biofertilizers for application on experimental plots.
*Paecilomyces lilacinus*, szczep WT15A, $1,58 \pm 0,04 \cdot 10^8$ u/g

*Aspergillus niger*, szczep G199AA, $2,5 \pm 0,18 \cdot 10^9$ u/g

Table 1. Amounts of oil suspension introduced into the tested fertilizers

<table>
<thead>
<tr>
<th>Fertilizer</th>
<th>Addition of a suspension [ml/kg fertilizers]</th>
<th>The amount of biopreparation introduced [g/kg fertilizers]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>5</td>
<td>0,1</td>
</tr>
<tr>
<td>Super Fosdar 40 (SSP)</td>
<td>10</td>
<td>0,2</td>
</tr>
<tr>
<td>Polifoska (NPK)</td>
<td>20</td>
<td>0,4</td>
</tr>
</tbody>
</table>
Attempts to apply spores of microscopic fungi in the form of an oil suspension.

The purpose of the conducted research was:
- a) determination of the absorption of fertilizers in relation to rapeseed oil;
- b) determination of the possibility of producing a stable oil suspension of fungal spores
- c) determining the survival rate of fungal spores in oil suspension and in fertilizers.

Laboratory tests of applying oil to Super Fosdar 40 (SSP), Polifoska 4 (NPK) and Urea fertilizers

- Designed absorption:
  - Pulrea urea: 5 ml / kg
  - Super Fosdar 40: 10 ml / kg
  - Polifoska 4: 20 ml / kg
As a result of the tests, it was found that fungal spores of the genus *Paecilomyces lilacinus* and *Aspergillus niger* introduced into fertilizers in the form of oil emulsions do not show sufficient survival. The content of live strains of the fungi tested was below the detection threshold.

- The average population of *Aspergillus niger* in the dry formulation was $28.33 \cdot 10^8$ CFU / g, whereas for the oil formulation it was $8.5 \cdot 10^7$ CFU / g. For *Paecilomyces lilacinus*, these values were $17.625 \cdot 10^8$ CFU / g and $4 \cdot 10^7$ CFU / g, respectively. It should be noted, therefore, that the fungibility of the fungi in the oil suspension decreases.

- Since the content of spores in the suspension was 20% (m / V), the activity of oil preparations should be about 5x lower than the solid formulations (assuming the density of oil suspension $d \approx 1$ g / mL. Meanwhile taking into account this dilution, the activity of oil suspensions decreased about 20 times in case of *A. niger* and about 10-fold for *P. lilacinus*.
The granulation laboratory tests were carried out using a laboratory pan granulator with the following parameters:
- diameter $\phi = 400$ mm,
- height of the edge $= 100$ mm,
- revolutions $= 16 - 17 / \text{min}$,
- inclination angle of the plate - variable $30-60^\circ$.
- A hand sprayer - type Kwazar, capacity 1 l was used for dosing (spraying) the granulation liquid.

The following fertilizers were tested:
- Polifoska 4 NPK  $(6-12-34(10))$
- Super Fosdar 40
- and Urea in the variants presented in the table.

As granulation liquids, water, an aqueous solution of sodium lignosulfonate (LsNa) and liquid humic acids provided by ZA Puławy were used.
## A List of Variants of Laboratory Experiments

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Fertilizers</th>
<th>Mass of fertilizers [kg]</th>
<th>Fungal spores [g]</th>
<th>Potato starch [g]</th>
<th>Granulation liquid</th>
<th>The amount of granulation liquid [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polifoska 4</td>
<td>1</td>
<td>225</td>
<td>25</td>
<td>water</td>
<td>155</td>
</tr>
<tr>
<td>2</td>
<td>Polifoska 4</td>
<td>1</td>
<td>200</td>
<td>50</td>
<td>water</td>
<td>153</td>
</tr>
<tr>
<td>3</td>
<td>Polifoska 4</td>
<td>1</td>
<td>250</td>
<td></td>
<td>LsNa r-r 10%</td>
<td>260</td>
</tr>
<tr>
<td>4</td>
<td>Polifoska 4</td>
<td>1</td>
<td>200</td>
<td>50</td>
<td>LsNa r-r 10%</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>Super Fosdar 40</td>
<td>1</td>
<td>200</td>
<td>50</td>
<td>LsNa r-r 10%</td>
<td>170</td>
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<tr>
<td>6</td>
<td>Super Fosdar 40</td>
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<td>225</td>
<td>25</td>
<td>LsNa r-r 10%</td>
<td>181</td>
</tr>
<tr>
<td>7</td>
<td>Super Fosdar 40</td>
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<td>250</td>
<td></td>
<td>LsNa r-r 10%</td>
<td>179</td>
</tr>
<tr>
<td>8</td>
<td>Polifoska 4</td>
<td>1</td>
<td>250</td>
<td></td>
<td>LsNa r-r 10%</td>
<td>223</td>
</tr>
<tr>
<td>9</td>
<td>Mocznik</td>
<td>1</td>
<td>250</td>
<td></td>
<td>LsNa r-r 10%</td>
<td>188,9</td>
</tr>
<tr>
<td>10</td>
<td>Mocznik</td>
<td>1</td>
<td>225</td>
<td>25</td>
<td>LsNa r-r 10%</td>
<td>132,4</td>
</tr>
<tr>
<td>11</td>
<td>Mocznik</td>
<td>1</td>
<td>200</td>
<td>50</td>
<td>LsNa r-r 10%</td>
<td>144</td>
</tr>
<tr>
<td>12</td>
<td>Polifoska 4</td>
<td>1</td>
<td>250</td>
<td></td>
<td>Humic acids liquid</td>
<td>237,4</td>
</tr>
<tr>
<td>13</td>
<td>Polifoska 4</td>
<td>1</td>
<td>200</td>
<td>50</td>
<td>Humic acids liquid</td>
<td>274,8</td>
</tr>
<tr>
<td>14</td>
<td>Super Fosdar 40</td>
<td>1</td>
<td>250</td>
<td></td>
<td>Humic acids liquid</td>
<td>254</td>
</tr>
<tr>
<td>15</td>
<td>Super Fosdar 40</td>
<td>1</td>
<td>200</td>
<td>20</td>
<td>Humic acids liquid</td>
<td>269</td>
</tr>
</tbody>
</table>
The highest quality of the coatings (the lowest abrasiveness) was obtained using fungi spores with the addition of potato starch, using liquid humic acids as granulation liquid.

The described method failed to obtain urea biofertilizers.
The results of the survival of bacteria in the prepared samples bio fertilizer

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample weight [g]</th>
<th>bacterial population ([x \times 10^8 u/g])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polifoska +15% Bacillus (pelet)</td>
<td>0.545</td>
<td>0.0001</td>
</tr>
<tr>
<td>Foscar +12% Bacillus + PEG + Gly</td>
<td>0.805</td>
<td>0.23</td>
</tr>
<tr>
<td>Polifoska +15% Bacillus + PEG + Stearin</td>
<td>0.775</td>
<td>0.000013</td>
</tr>
<tr>
<td>Polifoska +15% Bacillus + PEG</td>
<td>0.618</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Results of field experiments - maize fertilized with fertilizer and microbially enriched fertilizer.
The production of biofertilizers by the method of coating the mineral fertilizer granules with an external layer containing a neutral carrier seems to be the most appropriate direction for the production of this type of products.

During the production process, it is advantageous to use low temperatures and to avoid using water as much as possible, because in the presence of moisture, especially at elevated temperatures, as during drying, rapid growth of live bacteria from their spore forms took place. It is also beneficial to physically separate the bacteria from the fertilizer granules, so that they are not exposed to high local concentrations of mineral salts formed during the dissolution of the fertilizer in the soil under the influence of moisture. Diversification of the dissolution rate of both these layers by creating a readily soluble outer coating containing microorganisms may favourably affect the effectiveness of the use of biofertilizers.

In the carried out laboratory tests, the method of incorporation of bacteria into granulated fertilizers was developed by applying the coating in the form of an external layer of bacteria deposited on an organic carrier (carbohydrate) using different binder formulations. The bacterial survival in the manufactured biofertilizer products was about 1 month and was probably reduced by the hygroscopicity of the product. Biofertilizers, due to their hygroscopicity, were sensitive to moisture and lost their microbiological activity when they were stored improperly.

In the further stage of research, the quality of the coatings should be improved by modifying their composition or production method, with particular emphasis on the high survival rate of microorganisms and the effectiveness of their activity.
THANK YOU FOR YOUR ATTENTION