LABORATORY FOR SOIL, FERTILIZER AND PLANT MATERIAL TESTING
Department of microbiology

Test report

No.: 2000-1560/2 Sample No. 1

Date: 13.09.2022.

The client:	**Bimdi Ltd.**	**Address**	**Lole Ribara bb, 25250 Odžaci, Republic of Serbia**
		Tel:	+381 25 743 978
		Fax:	
		e-mail:	doobimdi@gmail.com
The material being tested:	Microbiological fertilizer **EKO LAME**	**Sample:**	**28/2022 МЂ**
No. of request:	**381**	Date of receipt:	13.05.2022.
		No of sample:	1
Description of the test:	Microbiological analysis of fertilizer for the purpose of registration		
Sampled:	by the client		
Note:	none		
STATEMENT: *The test results refer only to the tested sample, and cannot be duplicated. This report may not be reproduced, except in its entirety, without the consent of the Laboratory*			

Person responsible for compiling the report: Technical associate: Elena Stupavski	**Director of the Department for Agriculture and Vegetables** **Prof. Dr. Đorđe Krstić**
Person responsible for the truthfulness of the report: Head of Department: Prof. Dr. Simonida Đurić	**The Dean**
Person responsible for the truthfulness of the report: Laboratory Manager: Prof. Dr. Maja Manojlović	**Prof. Dr. Nedeljko Tica**

On the basis of the Rulebook on conditions for classifying and determining the quality of plant nutrition products "Official Gazette of RS, No. 30/17" we submit

Report on the performed testing of microbiological fertilizer

1. GENERAL INFORMATION FROM THE APPLICATION

1.1. ***Name of fertilizer***
EKO LAME - Liquid inoculum.

1.2. ***Manufacturer's name***
Bimdi Ltd
Lole Ribara bb
25250 Odzaci, Republic of Serbia

1.3. ***Microbiological composition of fertilizer:***

Total number of microorganisms - minimum 1.0 x 108 cells/ml
***Pseudomonas* spp.,**
***Bacillus* spp.,**
***Streptomyces* spp.,**
***Acinetobacter* spp.,**
***Enterobacter* spp.,**
***Absidia* spp.**
***Penicillium* spp.**

1.4. ***Proposed purpose (Sugestion for use):***
To treat:
- AGRICULTURAL CULTURES: different grains, corn, soybeans, sunflowers, sugar beets, fodder plants and tobacco;
- VEGETABLES (in the field and indoors): tomatoes, peppers, cucumbers, cabbage, root and tuber vegetables, leafy vegetables and legumes;
- FLOWERS (of all kinds, in the field and indoors);
- FRUIT (apricots, plums, cherries, apples, peaches, quinces and others);
- Evergreen trees, deciduous trees and shrubs, as well as conifers.

1.5.*Packaging*:
5,0 litter bottle.

1.6. ***Recommended amount of preparation per hectare*:**
10.0 liters of preparation per hectare dissolved in 300 liters of water.

1.7. ***Number of treatments during the growing season*:**
The number of treatments depends on the culture and method of application:
- The soil is treated once before sowing or planting
- The seeds are treated once before sowing

- Foliar: for cereals 3-4 sprays every 10-12 days, for vegetables 6-10 times and for fruits 8-12 times at the same intervals and depending on the species.

1.8. ***Method of application*****:**
- Soil treatment - watering and irrigation drop by drop
- Seed treatment
- Foliar

1.9. ***Harmful to the environment*** **- none**

1.10. Impact on human health – no impact

1.11. Storage: do not expose to direct sunlight, protect from heat sources.

1.12. Type of packaging and method of destruction: The preparation is packed in the original PET packaging.

2.1. RESULTS OF TESTS PERFORMED

2.1.1. Microbiological tests

The number and microbiological composition of the fertilizer was determined on appropriate nutrient media:

Total number of microorganisms minimum 1.0 × 108 cells/ml

Pseudomonas **spp.,**
Bacillus **spp.,**
Streptomyces **spp.,**
Acinetobacter **spp.,**
Enterobacter **spp.,**
Absidia **spp.**
Penicillium **spp.**

2.1.2. The influence of the application of the preparation on yield parameters and the yield of cabbage, corn, pepper and raspberry:

a) Effect of application of EKO LAME fertilizer on cabbage yield:

Table 1. Effect of application of **EKO LAME** fertilizer on cabbage yield:

Variant	Mass (g)
Control	700.00
EKO LAME	3.000,00
% compared to control	328.57

• Application of the EKO LAME fertilizer resulted in an increase in the yield of cabbage by more than three times (table 1)

Figure 1: Appearance of fresh heads of cabbage: top - control; below - treated with **EKO LAME**, when measuring mass (see EKO LAME annex)

б) The effect of applying the EKO LAME product on the number of corn cobs:

• By applying the preparation **EKO LAME** preparation in the corn crop, at an altitude of 800 m, an increase in the number of cobs per plant was obtained from 1 to 2-3 cobs (figure 2).

Figure 2: The appearance of the corn crop at 800 m above sea level, the appearance of 3 cobs on the corn stem after the application of **EKO LAME** (see EKO LAME annex).

c) Effect of application of **EKO LAME** fertilizer on pepper yield:

- By using the **EKO LAME** preparation in the production of industrial pepper in the open field, a yield of 66 t/ha was obtained, with 80% of first class peppers (figure 3)

Figure 3: Field of pepper (up) and pepper size (below) when treated with **EKO LAME**

d) Effect of application of **EKO LAME** fertilizer on raspberry yield parameters:

• By using the **EKO LAME** fertilizer in raspberry production, the sugar content was increased by 4-6%. The shelf life of harvested ripe fruit is at least 50% longer. The ripening period is shortened by 7 to 10 days. The production is completely independent of classical chemicals (figure 4.)

Figure 4: Appearance of raspberry plants and fruits treated with **EKO LAME** fertilizer

2.1.3. Physical properties of fertilizers

Appearance: LIQUID
Color: intense yellow, transparent.
pH: 8,5

3. TESTING METHODS

3.1. Microbiological methods:

The number of cells of *Pseudomonas spp*., and *Acinetobacter spp*. were determined on TSA (tryptone-soy agar) nutrient medium, the number of cells of *Bacillus spp*. on L-agar, *Enterobacter spp.* on Hi-Chrome agar. The presence of the genus *Streptomyces* was determined on synthetic agar, and the fungus *Absidia spp*. and *Penicillium spp*. on potato dextrose agar (Jarak and Đurić, 2006).

3.2. Effects on plants:

Experiment on cabbage: it was carried out on the production plots of Vozar farm, from Kisač. The experiment was conducted as part of organic vegetable production. The cabbage plant was treated with the microbiological preparation **EKO LAME**.

Corn experiment: it was compared on the plots of the Ivanjica region, at an altitude of 800 m. The corn crop was treated with the preparation **EKO LAME**.
Experiment on pepper: it was carried out in the production plots of the Municipality of Gospodjinci. The pepper plant was treated with the **EKO LAME**, twice during the growing season, in May and August 2020.
Experiment on raspberries: it was carried out on a raspberry orchard in Ivanjica. Raspberries were foliary treated with the **EKO LAME**.

4. RECOMMENDATIONS FOR THE APPLICATION OF PREPARATIONS BASED ON TESTS PERFORMED

4.1. Plant species and soil type:

It is recommended for use in crops of arable and vegetable plants, fruit trees and vines, flowers, evergreen trees and deciduous trees and shrubs, as well as conifers.
It can be applied on all types of soil
It can be applied in conventional and organic agricultural production.

4.2. Amount of fertilizer

10.0 liters of fertilizer per hectare dissolved in 300 liters of water.

4.3. Application time:

Application of the preparation can be during the entire growing season of any plant species.

Field crops: spraying the soil before sowing, as well as seed treatment before sowing.
Then the plant culture is sprayed twice in the early phase of the growing season: the first time with the emergence of two leaves and the second spraying follows after 10 days.
For fruit, spraying is recommended after each heavy rain, and for conventional production, after the use of chemical agents.

4.4. Method of application:

Soil treatment - watering and irrigation drop by drop
Seed treatment
Foliar

4.5. Number of treatments during the year

The number of treatments depends on the culture and method of application:
- The soil is treated once before sowing or planting
- Seeds are treated once before sowing
- Foliar: for cereals 3-4 sprays every 10-12 days, for vegetables 6-10 times and for fruits 8-12 times at the same intervals and depending on the species.

4.6. Possible adverse effects

The proposed method of application has no adverse effects on plants and the environment.

4.7. Restrictions on application: *none*

***4.8. Type of packaging and method of destruction*:** The preparation is packed in the original PET packaging.

5. DATA ON NUTRITIONAL VALUE

• The following microorganisms are present in optimal numbers in the fertilizer:
***Pseudomonas* spp., *Bacillus* spp., *Streptomyces* spp., *Acinetobacter* spp., *Enterobacter* spp., *Absidia* spp. и *Penicillium* spp.**

- Certain species of the genus ***Pseudomonas*** produce metabolites such as antibiotics and hydrogen cyanide (HCN), some produce siderophores with a high affinity for Fe^{3+} absorption, and some produce the plant hormone auxin. All these metabolites strongly influence the growth of certain harmful microorganisms, and on the other hand, they increase the availability of nutrients to plants. In addition to these metabolites, *Pseudomonas* produces a phytohormone, indole-acetic acid, which positively affects the growth and development of the roots of various plants, increasing the length of the roots by 35-50 % compared to plants that were not inoculated. The species *Pseudomonas mendocina* is capable of breaking down the polymers PBAT (polybutylene adipote co-telephalate) and PLA (polylactic acid), which are an integral part of biodegradable mulch films in agricultural production (Kwiecien et al., 2018).
- ***Bacillus* spp**. shows a wide range of mechanisms that can stimulate plant growth, but also mobilize nutrients from sources unavailable to plants through the production of phytohormones and extracellular enzymes (Yao et al., 2006; Forchetti et al., 2007; Lee et al., 2008; Swain and Ruy, 2009). It rapidly colonizes the roots of plants and has the ability to multiply on the roots (Dijkstra et al., 1987).
- ***Streptomyces* spp**, are bacteria that belongs to the group of *Actomycetes* that produces the antibiotic streptomycin and with its presence in the soil protects plants from attacks by pathogenic microorganisms. At the same time, it is the producer of a whole range of extracellular enzymes that mineralize organic matter in the soil, thus providing plants with easily accessible nutrients and stimulating their growth.
- ***Acinetobacter* spp.** are ubiquitous Gram-negative non-motile coccobacilli (exhibit "twitch motility" probably due to polar fimbriae). ***Acinetobacter* spp**. are widely distributed in nature, in soil and water, as free-living saprophytes. They are found in almost 100% of soil and fresh water samples. Many ***Acinetobacter*** isolates resemble saprophytic pseudomonas capable of utilizing a wide range of organic compounds as the sole source of carbon and energy. Accordingly, *Acinetobacteria* degrade various organic pollutants. Most strains do

not reduce nitrate to nitrite, but they can use both nitrate and nitrite as nitrogen sources via assimilatory nitrate reductase (Kampfer, 2014).

- Bacteria belonging to the genus ***Enterobacter***, originating from the soil, are excellent decomposers of cellobiose and rhamnose and thus actively participate in the mineralization of organic matter in the soil. In addition, the species isolated from the rhizosphere of wheat, sorghum and rice are capable of binding atmospheric nitrogen and thus converting it into a form accessible to plants.
- Fungi from the genus ***Absidia*** belong to the class *Zygomycota* (*Mucor spp., Rhizopus sp*p.), which are intensive decomposers of organic matter in the soil. Plants grown in this way provide accessible nutrients and thus stimulate their growth. The species *Absidia cylindrospora*, which has the ability to biodegrade pollutants, can be effective in the treatment of soil contaminated with a wide range of hydrophobic compounds including chlorinated anilines and benzenes, thiophenes and polycyclic aromatic hydrocarbons.
- ***Penicillium* spp.** is a widespread soil fungus. An excellent mineralizer of organic matter, a producer of antibiotics, a competitor of pathogenic fungi and therefore an excellent biostimulator for all cultivated agricultural crops.

EKO LAME product enables production according to the principle of "absolute ecology" and placement on markets that have a higher value (organic products). Approaching the efficiency of conventional production and alternative production enables greater production of healthy food and reduces the tendency of import dependence that is currently being imposed. This fertilizer is easy to use, the production process is economically and ecologically acceptable, and the application is flexible. The use of **EKO LAME** with plants can be planned-preventive or as needed.

6. CONCLUSION

The applicant submitted a detailed microbiological composition, physical characteristics, instructions and method of application, type of packaging, method of storage, as well as the term of use of the microbiological fertilizer **EKO LAME**.

In connection with Art. 2. point 3, art. 3 point 4 and art. 14, Paragraph 3, item 1, and Art. 35 and Art. 54 of the Rulebook on the conditions for classifying and determining the quality of plant nutrition products, nutrient content deviations and the minimum and maximum values of permitted nutrient content deviations and on the content of the declaration and the method of labeling plant nutrition products, («Official Gazette of the RS», no. .30/2017, we propose that **EKO LAME** can be classified as **Microbiological fertilizer** for the purpose of registration in the Register of plant nutrition and soil conditioners.

a. Sorting and labeling:

EKO LAME / liquid microbiological fertilizer / microbiological fertilizers (preparations) containing plant growth stimulator microorganisms as well as growth stimulators of microbial origin / microbiological fertilizers (preparations) containing plant growth stimulator microorganisms that produce gibberellins, auxins, cytokinins, vitamins and other growth substances.

Trade name of the fertilizer: **EKO LAME**

Type of fertilizer: Liquid microbiological fertilizer

Type: / microbiological fertilizers (preparations) that contain plant growth stimulators and microbiological growth stimulators

Name of fertilizer within the type: microbiological fertilizers (preparations) containing plant growth stimulator microorganisms that produce gibberellins, auxins, cytokinins, vitamins and other growth substances.

7. REFERENCES

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Authenticated
Head of the microbiology department

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