FIELD TESTS OF COMMERCIAL PRODUCTS EKOLIVE BIOSTIMULANTS

Mendel University in Brno, Faculty of AgroScience

1 Basic info

Requestor	Producer	Test Executor
Four Leaves Agro Herengracht 449a 1017 BR Amsterdam The Netherlands	Ekolive Germany GmbH Humperdinckweg 12 D-33102 Paderborn Germany	Mendel University in Brno (MENDELU) Faculty of AgroScience Zemědělská 1665/1 613 00 Brno Czech Republic

Duration of the test	Test no.	Location	Section	Sea level
10/2022 – 07/2023	2023_POZ_FourLeaves	Žabčice, Czech Republic	Nová obora, Hon 22b	179m

Objective of the test: measure the effects of bacteria-based stimulants in winter wheat

Hypothesis 1: replacement of the conventional fertilizers and pesticides in full

Hypothesis 2: partial replacement of conventional fertilizers – 20% less volume of conventional fertilizer applied, so additive to the conventional fertilizers/pesticides mix.

2 Methodology

Tested products:

1. Microfertile® kapalný (producers name: ekofertile® plant), UKZUZ registration no: V784
2. Microfertile® carbon (producers name: microfertile® plant), UKZUZ registration no: 5548

Crop: Winter wheat – variety Balitus (previous crop: pea (Pisum sativum))

Number of variants: 5

Number of repetitions: 3

Logic of the test: comparison of averages of the three repetitions of 5 variants (each variant had three repetitions)

Description of variants

Variant	Description	Biostimulant applied
1	**Control:** conventional fertilizers* and pesticides: herbicides, insecticides, fungicides, plant growth regulators	**0**
2	Microfertile kapalný + herbicides, insecticides – no fertilizers, no fungicides	**9L/ha**
3	Microfertile kapalný + fertilizers* 80% of standard level + pesticides (same as Control)	**3L/ha**
4	Microfertile carbon + herbicides, insecticides – no fertilizers,-no fungicides	**9L/ha**
5	Microfertile carbon + fertilizers* 80% of standard level +pesticides (same as Control)	**3L/ha**

**LAV27 - Granular nitrogen fertiliser with 27% nitrogen: ammonium nitrate granulate with finely ground limestone.*

Application dosage of the biostimulant: 300L mixture of water and stimulant/ha, 3 doses in total so 3 or 9L/ha in total according to the variant.

More details can be found in the Appendix.

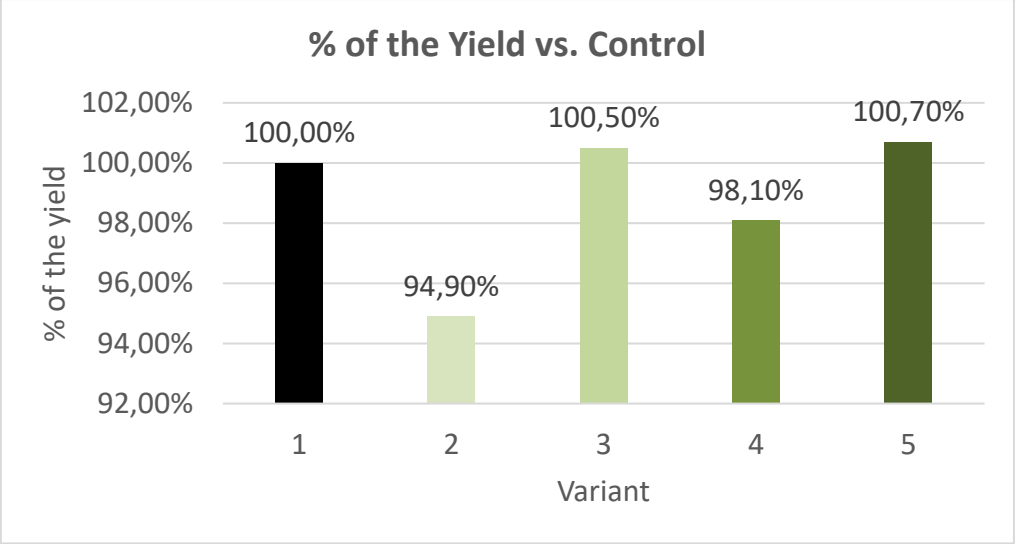
3 RESULTS

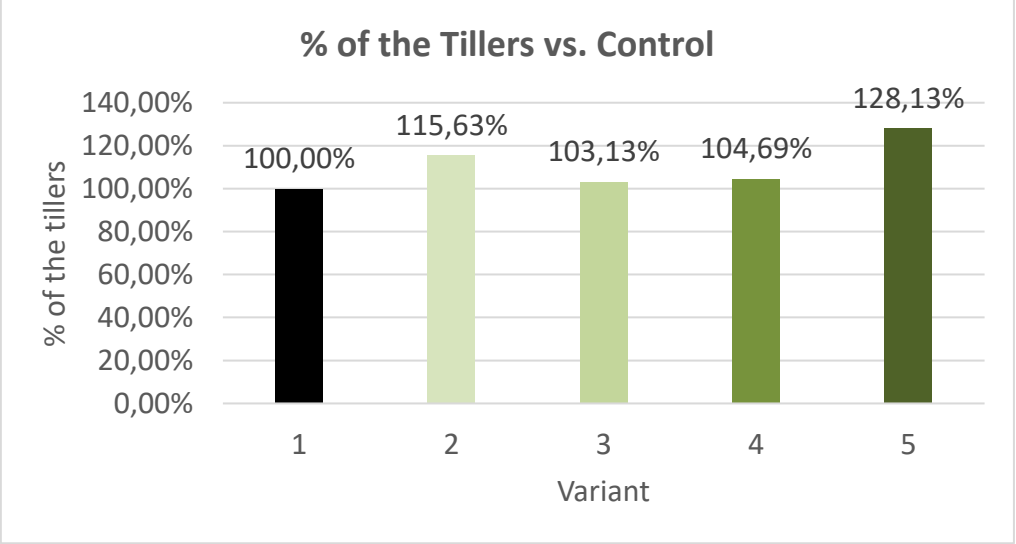
The key measured parameters were crop yield, number of tillers per plant and proportion of nitrogen content in the grain. Secondary parameters are listed in the Appendix.

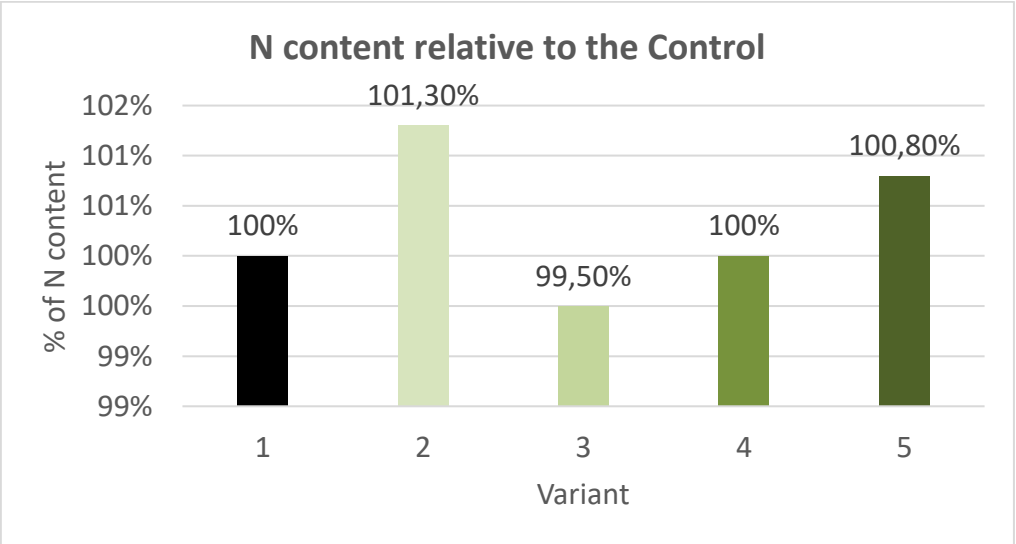
***The main hypothesis* "biostimulants have the ability to replace conventional mix of fertilizers and pesticides while delivering comparable results" has been confirmed.**
Yield is comparable in both cases (Var 2 and 4 vs Control) and number of tillers is significantly higher hence proving greater resilience of the plants with biostimulants, accompanied by the comparable proportion of N-content in the grain among the variants. This proves the ability of the biostimulants to supply the plants with the nitrogen. A slight decrease in the yield of grain in variants 2 and 4 could have been caused by the absence of fungicidal treatment. In the observed harvest year, there was a high pressure of wheat stripe rust infection (*Puccinia striiformis)*.

***Secondary hypothesis* of partial (20% less) replacement of the fertilizers has been confirmed too. Variants with biostimulant** delivered slightly increased yield (Var 3 and 5, 100,5 % and 100,7 % respectively) compared to Control (100%). In terms of the tillers, it is even better than the Control (103% and 128%!). **Hence confirming increased resilience of the plants while applying biostimulant.**

The following graphs and table show the main results.


% of the Yield vs. Control
% of the yield
102,00%
100,00%
98,00%
96,00%
94,00%
92,00%
100,00%
94,90%
100,50%
98,10%
100,70%
1
2
3
4
5
Variant


% of the Tillers vs. Control
% of the tillers
140,00%
120,00%
100,00%
80,00%
60,00%
40,00%
20,00%
0,00%
100,00%
115,63%
103,13%
104,69%
128,13%
1
2
3
4
5
Variant


N content relative to the Control
% of N content
102%
101%
101%
100%
100%
99%
99%
100%
101,30%
99,50%
100%
100,80%
1
2
3
4
5
Variant

Variant	Description	Average yield of 3 repetitions (t/ha at 14 % moisture)	% of the average yield	Average number of tillers per plant	% of the tillers	N-content (%) average	% of the N content
1	Control	12,85	**100,00%**	2,13	**100,00%**	13,23	**100,0%**
2	Microfertile kapalný + herbicides, insecticides – no fertilizers, no fungicides	12,2	**94,90%**	2,47	**115,63%**	13,40	**101,3%**
3	Microfertile kapalný + fertilizers 80% of standard level + pesticides (same as Control)	12,92	**100,50%**	2,20	**103,13%**	13,17	**99,5%**
4	Microfertile carbon + herbicides, insecticides – no fertilizers, no fungicides	12,61	**98,10%**	2,23	**104,69%**	13,23	**100,0%**
5	Microfertile carbon + fertilizers 80% of standard level + pesticides (same as Control)	12,94	**100,70%**	2,73	**128,13%**	13,33	**100,8%**

4 DISCUSSION

Additional level of the information would be obtained if another test would be effectuated where the same volumes of conventional fertilizers and pesticides would be applied.

At least one additional test should be conducted in a drier location, i.e., if the plant were to receive less water, to verify the validity of the conclusions. It can be assumed that the influence of biostimulants on improving crop water management, as demonstrated in other experiments, exists. It would also be appropriate to conduct long-term tests to monitor further effects biostimulants on plants and soil.

The timing of biostimulant application should also be verified according to a new logic tested by the manufacturer elsewhere:

- 1st application in BBCH 21 – 29 (autumn, from the 3rd leaf stage)
- 2nd application in BBCH 30 – 31 (spring, offshoots to columnisation stage)
- 4th application in BBCH 39 – 49 (on the flag leaf up to the booting)
- Also, an application in BBCH 00 (seed staining) possible.

Soil analysis shall be effectuated in the next test as to determinate the impact on the soil microfauna/microflora as also in the long term.

In further experiments, it would be appropriate to test additional qualitative parameters of the grain to see if they are also influenced. It would also be advisable to monitor nutrient uptake by plants at several intervals during the growing season.

5 CONCLUSION

It can be concluded that biostimulants may partially function as a substitute for fertilizers, as all the main measured parameters were very comparable to the control variant.

Using biostimulants as a replacement of the pesticides is promising, however, more tests need to be conducted with different weather conditions and longer duration.

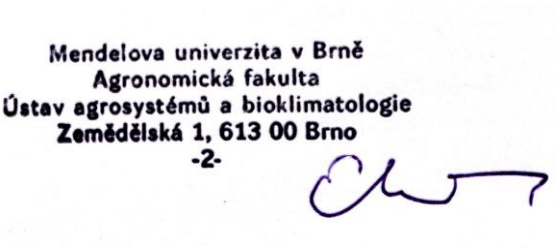
Main outcomes:

1) Replacement of the fertilizers – both products (microfertile kapalný and microfertile carbon) provided the crop yield very comparable to the Control version.

2) Biostimulant microfertile carbon provides significantly more tillers (variant 5 by 28%). Hence showing very positive probability of improved resilience and immunity of the plants.

3) Biostimulants were able to provide the plants with the same or comparable volume of the nitrogen content in the grains. This proves substitution of nitrogen-based industrial fertilizers as the stimulants ability to attract nitrogen from various sources has been confirmed (air, soil, other fertilizers).

The field test was done in a small area (in total 120m2 of the area where biostimulants were applied, more details are in the Appendix), hence it is a limited proof. However, given the other tests effectuated by other universities in different countries (Ondokuz Mayıs University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Samsun, Türkiye, Neubrandenburg University of Applied Sciences, Germany) and other larger scale field tests on large range of plants, it is reasonable to conclude that the measured effects are likely to be extrapolated as generally valid.



Ing. Petr Elzner, PhD.
Brno, April 2024

APPENDIX

The following **parameters** were measured according to the University laboratory standards.

- Number of plants after emergence (2-3 weeks after sowing)
- Number of tillers
- Above and below ground dry matter (2 dates during the growing season)
- Number of ears
- Plant height
- Grain yield, grain moisture
- Volume weight
- Nitrogen content in the grain
- Weight of thousand grains
- Proportion in the sieves

All variants were effectuated in the same **location**, therefore weather and soil conditions were the same. The location of the test is often used for tests for major producers such as BASF, Syngenta.

Size of one plot: 10m2 (1,36 x 7,3m)

The agronomic treatment (timing, sowing, harvest etc.) was the same for all variants, except application of the fertilizers and agro-chemical protection.

The applied fertilizers and protection were standard industrial mineral type (LAV 27 – 27% of N and MgO), herbicides (sekator OD), growth regulator (Moddus and Retacel extra R68), insecticide (decis forte, voodo), fungicide (delaro). Herbicides (iodosulfuronmethyl Na + amidosulfuron), growth regulator (chlormequat chloride; trinexapac-ethyl), insecicides (deltamethrin; esfenvalerate), fungicide (prothioconazole + trifloxystrobin).

Timeline of the test:

Timing	Activity
11 October 2022	Sowing
14 October 2022	1st biostimulant application
31 October 2022	2nd biostimulant application
21 November 2022	3rd biostimulant application
February – May 2023	Application of fertilizers, herbicides, insecticides
July 2023	Harvest

Impact of the Weather

Overall, no major impact of the weather was observed.

Levels of rain (cumulative monthly water amount in mm per m2) were 5 months (out of 10 months in total of the test) very under average and 2 months above average. Hence rather negative impact of the rains as early in the vegetation cycle there was a lack of water (Oct – Nov and March) and also late vegetation phase in June and July. Moreover, the rain volumes in January and April were very above average which was too high, especially the April volume of 2,5x more than average.

Temperatures were rather normal except 4 months: the only extreme difference was January with very above average temperatures (3,5C more) which did not have significant impact as not active

vegetation period, while April and May with slightly below average temperatures (1,6 and 2,7C). This combined with very high rains in April had rather negative impact.