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Effects of Biostimulant and Zinc (Zn) Treatment on Qualitative and Quantitative Indicators of Winter Rape (*Brassica Napus L.*)

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Winter rapeseed production has completely changed in Hungary in recent years. The use of hybrids has become commonplace, and intensive cultivation techniques are widespread. At the same time, average yields also increased, making rapeseed one of the most profitable crops. Maintaining profitability is a priority task, and in addition to further increasing average yields, improving quality parameters and crop safety is also decisive. Rapeseed production today faces significant challenges. As a result of climate change, the cultivation routine of decades is changing, and biostimulators are becoming more and more perspective in addition to foliar fertilisation with macro- and microelements. During this research, a 3-year small-plot field experiment was set up with a winter rapeseed indicator. The main objective of the experiment was to study the physiological reactions of rape plants to different doses of Zn and the combined reaction of the biostimulator and Zn. During the experiment, the reaction of the rapeseed test plant on zinc-deficient calcareous chernozem soils was investigated under the influence of different doses of Zn and combined doses of Zn and biostimulation. The aim of the study is to determine whether rapeseed plants respond to zinc supplementation and the combined effect of the bio stimulator and zinc with increased yield and improved nutritional values (protein content, oil content). In the first annual study, two treatments were performed, one in autumn (BBCH 16-18 phenological stage) and one in spring (BBCH 29 phenological stage). Zinc tetramine hydroxide was used in 4 different doses (2, 5, 10, 20 L/ha), zinc tetramine hydroxide enriched with copper in 2 different doses (5 and 10 L/ha), Quantis in the manufacturer's prescribed dose of 2 L/ha, biostimulant enriched with zinc, and a usual foliar fertiliser used in rapeseed growing with Wuxal boron at the dose recommended by the manufacturer. During the growing season, the development of root mass, root length, SPAD values, as well as the yield and oleic acid content were evaluated by mathematical statistical method. It can be stated that there was no significant difference in the root mass, root length, and SPAD values, but there was a significant yield difference in the amount of the crop in the second-year experiment (P = 10 %). Studies have shown that biostimulants have the greatest influence on the development of measured parameters.

1. Introduction

One of the biggest challenges for farmers today is to protect against abiotic environmental factors (drought, UV stress, salt concentration, water pressure), as all these factors have a detrimental effect on the growth of our cultivated plants and prevent them from reaching their genetic potential and nutrient supply potential. To prevent economic losses, more and more people regularly use biostimulators to promote physiological processes in plants. Together with changes in weather and economic environment, technologies that could significantly transform agricultural production are expected to gain ground.

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Farmers have the following options to reduce the harmful effects of climate change: irrigation and melioration, tillage, crop management programs, species and variety changes, and nutrient supply. Due to the changed weather conditions and rainless spring, the second top manure is more and more often omitted, so the harmonious nutrient supply of rapeseed should be ensured through foliar fertilisation. Not only macronutrients but especially meso- and micronutrients are delivered at the right time and in the right amount. The foliar nutrient supply of rapes, foliar treatments focusing primarily on boron and sulphur in spring, is very diverse, but in terms of foliar fertilisation in autumn, we are still lagging the significant rapeseed-producing countries. To achieve these goals, in the fall, special attention should be paid to the proper development of the root system. Phosphorus is a macronutrient, and two microelements, boron and zinc, also play a key role in this.

1.1. Biostimulators

The most effective biostimulators are usually natural substances composed of plant hormones or precursors of plant hormones. When applied correctly in plants, they will directly affect physiological processes, providing potential benefits for growth, development, crop formation and response to environmental stressors.
The most promising solution for the treatment of factors and effects of tolerance to abiotic and biotic stress may be the use of plant biostimulants (PB), which are called "substances and/or micro-organisms" whose function when applied to plants or rhizosphere is to stimulate natural processes in terms of nutrient uptake, nutrient efficiency, tolerance to abiotic stress and/or to enhance/favour crop quality regardless of its nutrient content' European Biostimulant Industry Council (EBIC, 2016).
Biostimulators, compared with their use in small doses, greatly affect the physiological processes in plants.
According to Patrick du Jardin (2015): "A plant biostimulant is any substance or microorganism applied to plants with the aim of improving nutritional efficiency, abiotic stress tolerance and/or quality properties of the crop, regardless of their nutrient content."
The main types of plant biostimulants:

1. Humic and fulvic acids
2. Protein hydrolysates and other N-containing compounds
3. Seaweed extracts and plant materials (= plant extracts)
4. Chitosan and other biopolymers
5. Inorganic compounds
6. Useful mushrooms
7. Beneficial bacteria

Biostimulant preparations are also referred to as plant enhancers. It is more correct to say that the use of these substances reduces stress on the plant, reduces the expected yield and helps preserve the genetic potential of the plant (Kubina and Nagy, 2023).

1.2. The role of zinc, zinc deficiency

According to a 2002 report by the World Health Organization, one-third (33 %) of the world's population is at risk for Zn deficiency, ranging from 4 % to 73 %, depending on the country. Almost half of the world's cereal crops are Zn deficient, leading to poor crop yields. Zn deficiency in agricultural soils is also a major global problem, affecting both yield and quality. In Hungary, the Zn content of soils is medium or rather small. Growth inhibition, damage to the nervous system, damage to organs responsible for reproduction, skin problems, immune problems, loss of appetite, hair loss and paleness are all symptoms of these syndromes due to insufficient zinc intake.Zn is an essential micronutrient that is necessary not only for humans but also for plants. There are numerous physiological impairments in Zn-deficient plant cells that delay plant growth, differentiation, and development (Cakmak, 2000), since Zn is an essential micronutrient through an enzyme component and an important enzyme activator for the plant. Zn is involved in protein metabolism and stimulates auxin production to regulate growth (Kalocsai, 2006). Zinc plays a significant role in many physiological processes, such as protein metabolism, structural and functional integrity of bio-membranes, gene expression, photosynthetic carbon metabolism and auxin synthesis (Cakmak, 2000). Due to zinc deficiency, embryonic tissues are damaged, intervascular chlorosis in the upper leaves, complete whitening of the leaf plate, and dwarfism can be observed. The leaves do not develop; the resulting auxin deficiency causes rosettes, distortion, and shortening of the stem members. The stock lags the plant height characteristic of the phenological phase. Developmental disorders also occur in generative organs, and with zinc deficiency, flower formation is delayed or even missed. In case of severe zinc deficiency, flower and crop formation disorders may reduce the yield (Kalocsai, 2006). A severe lack of zinc in the soil can cause a complete crop loss, while a loss of up to 30 % in crop yields of cereals such as wheat, rice and corn can occur even because of slight deficiencies. Maise is one of the most important crops in Hungary. It is grown in the largest area and is among the most susceptible cultures to Zn deficiency. Zn deficiency can cause serious crop damage (up to 80 %). According to Kalocsai et al. (2006), the zinc supply of Hungarian soils is also poor in international comparison. Nearly 50% of the Hungarian soils have a medium or lower zinc supply (Figure 1).

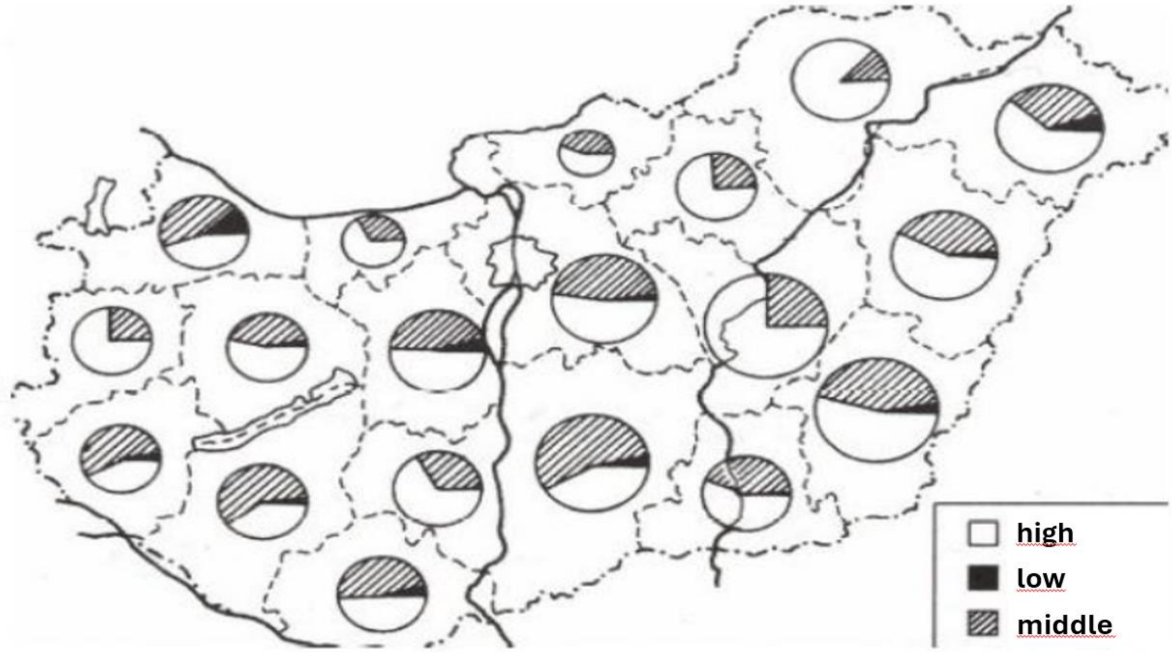

Figure 1: Zinc availability in Hungarian soils by county

2. Material and method

During this study, a 3-year small-plot field experiment was set up, with winter rapeseed as an indicator crop. The main objective of the experiment is to determine the physiological reactions of rapeseed, as well as the yield and oil content of the crop under the influence of different doses of Zn and the combined reaction of the biostimulator and Zn. The experiments were carried out on zinc-deficient calcareous soils.

The first year of the experiment was set up on the outskirts of Tarján during the farming year 2021-2022. The treatments were conducted on 21 m^2 (3x7 m) plots with 11 treatments in five replicates. Four different substances were applied in different doses: zinc tetramine hydroxide, zinc tetramine hydroxide + copper tetramine hydroxide produced from zinc and zinc-enriched industrial waste, Quantis and Wuxal boron.

The second year of the experiment (farming year 2022-2023) was set on the outskirts of Naszály. Here, 65 plots of 30 m^2 (3x10 m) were allocated in 13 treatments with 5 repetitions. Only spring treatments were used with six different substances: zinc tetramine hydroxide, zinc tetramine hydroxide + copper tetramine hydroxide produced from zinc and zinc-enriched industrial waste, Quantis, Wuxal boron, humic acid and fulvic acid.

In 2023/24, in the third year of the experiment, the experiment was set up on the outskirts of Naszály, where 65 plots of 30 m^2 (3x10 m) were treated. Quantis is an amino acid-containing biostimulator consisting of 12-19 % organic carbon (fermented sugar cane molasses) and 1.8-2.2 % amino acids (glutamic acid, alanine, glycine, etc.). 1.9 % nitrogen (urea), 0.9 % P_2O_5 (phosphoric acid), 17 % K_2O, and 2.8 % CaO (calcium oxide), which at the beginning of the experiments did not have a license certificate in Hungary. Wuxal boron is a foliar fertiliser with a high content of phosphorus and boron, which is regularly used in the cultivation of rapeseed. The two other substances included in the second year of the experiment were a preparation containing humic acid (Hymagrosol) and a preparation of fulvic acid, which has not yet been authorised.

In the first experimental year, two treatments were performed, one in the autumn (BBCH 16-18 phenological stage) and one in spring at stem start (BBCH 29 phenological stage). Zinc tetramine hydroxide was delivered in 4 different doses (2, 5,10, 20 L/ha), zinc tetramine hydroxide enriched with copper in 2 different doses (5 and 10 L/ha) and Quantis in 2 L/ha as specified by the manufacturer. In 2021, the biostimulant was not yet authorised, but this has changed today, and it will be commercially available in Hungary in 2023. The different doses were delivered with Euro Pulvé parcel sprayers. In autumn, root mass, root neck thickness, and SPAD values were measured. In the case of roots, 10-10 samples were taken per plot. In the case of SPAD measurements, 20-20 leaf test measurements were carried out per plot. During spring treatments, repeated SPAD measurements were performed, and on the eighth day after treatment, leaf samples were taken for leaf analysis. After drying and grinding, the samples were processed. Harvesting was carried out by a plot harvester in each experimental year. To avoid border effects, only the middle of the plots was harvested by leaving a 1 m wide border, and then the yield and oil content of the plots were examined. The oil content was determined using the Mininfra smart T. In the second year of the experiments, one treatment was applied before the onset of frost stress. SPAD measurements were carried out on the experimental plots on the eighth day after application, and leaf harvesting was carried out on each plot for leaf analysis. During the third-year trials, rapeseed were exposed to heat stress before and after the treatments. A statistical evaluation of third-year treatments is ongoing. In the second year of the experiment, the rapeseed crops were subjected to cold stress, with the temperature measured by the measuring station of the Albert Kázmér Faculty of the Széchenyi István University in Mosonmagyaróvár being -4 °C for two days on the fifth day after application.

3. Results and their evaluation

In the first year of trials, no significant difference in yield and oil content was found during either autumn or spring treatments. In the second year of the experiments, with the onset of the cold stress, a significant yield growth was observed in the yield of treated rapeseed (Table 1). In the experiment, Quantis, Hymagrosol and fulvic acid resulted in significant crop surpluses compared to the control treatment (Figure 2, 3). The extent of crop saving largely depends on genetics, the form of stress and its exposure, the level of intensification of the crop management programme and the time of application. In the SY Falado winter wheat variety, in 2022 (Syngenta development trial, Komárom-Esztergom County, 2022), yields increased even under moderately drought conditions with low nutrient supply levels (40 kg/ha nitrogen active ingredient). Although higher yields could be achieved with the same variety under normal weather conditions and nutrient supply, the yield loss due to stress was not significant due to the treatments. However, under normal conditions, there was no difference in yield between untreated and Quantis-treated plots. The effect of Quantis treatment on crop yield in the case of winter wheat is especially outstanding in the case of drought stress, but positive results can also be achieved with its application in the case of heat stress. Studies carried out with Quantis in rapeseed culture in Hungary by Nagy (2023) have clearly proven its effectiveness. Quantis applied in autumn at 4 - 6 leaves-stage resulted in a significant yield increase of 10 % in rapeseed, while in another study, a 4.8 % increase was obtained in the case of treatments applied at stem start. For the former, this meant a 405 kg per hectare increase in yield, while for the second, it was 170 kg per hectare.According to Alizadeh et al. (2022), the yield of humic acid-treated rapeseed increased by up to 18 % compared to control treatment if sowing was carried out at the well-time. As a result of the late planting dates, a very significant drop in yields can be observed, but even in this case, the humic acid treatment shows better yields than the control treatment; the observed increase was 49 %. Humic acid can increase crop performance, primarily due to its beneficial effects on the physiology and metabolism of the plant.

Table 1: Effect of treatments on rapeseed yield (t/ha) during the 2nd year of treatments

Serial number	Treatments	Dose	Average	%
1	Untreated		2.200	100
2	Zn 2 L	2 L/ha	2.384	108.4
3	Zn 5 L	5 L/ha	2.484	112.9
4	Zn 10 L	10 L/ha	2.482	112.8
5	Zn 20 L/ha	20 L/ha	2.462	111.9
6	Zn enriched 5 L/ha	5 L/ha	2.386	108.5
7	Zn enriched 10 L/ha	10 L/ha	2.486	113.0
8	Quantis 2 L/ha	2 L/ha	2.638	119.9
9	Quantis + Zn	2 L/ha + 10 L/ha	2.544	115.6
10	Quantis + Zn enriched	2 L/ha + 5 L/ha	2.446	111.2
11	Wuxal Boron	2.5 L/ha	2.476	112.5
12	Hymagrosol	2 L/ha	2.488	113.1
13	Fulvic acid	2 L/ha	2.666	121.2
SzD 10 % *			**0.200**	**9.1**
SzD 5 % **			**0.239**	**10.9**
SzD 1 % *			**0.319**	**14.5**
CV%= 7.6				

The treatments resulted in a significant increase in rapeseed yield (p<0.1) despite the frost stress effect, except for the Zn 2 L and Zn enriched 5 L treatments. Among the three types of biostimulants used, the highest increment was obtained with Fulvic acid (+460 kg/ha), the increment with Quantis 2 L was 438 kg/ha, while the increment with Hymagrosol was 280 kg/ha (Figure 2).

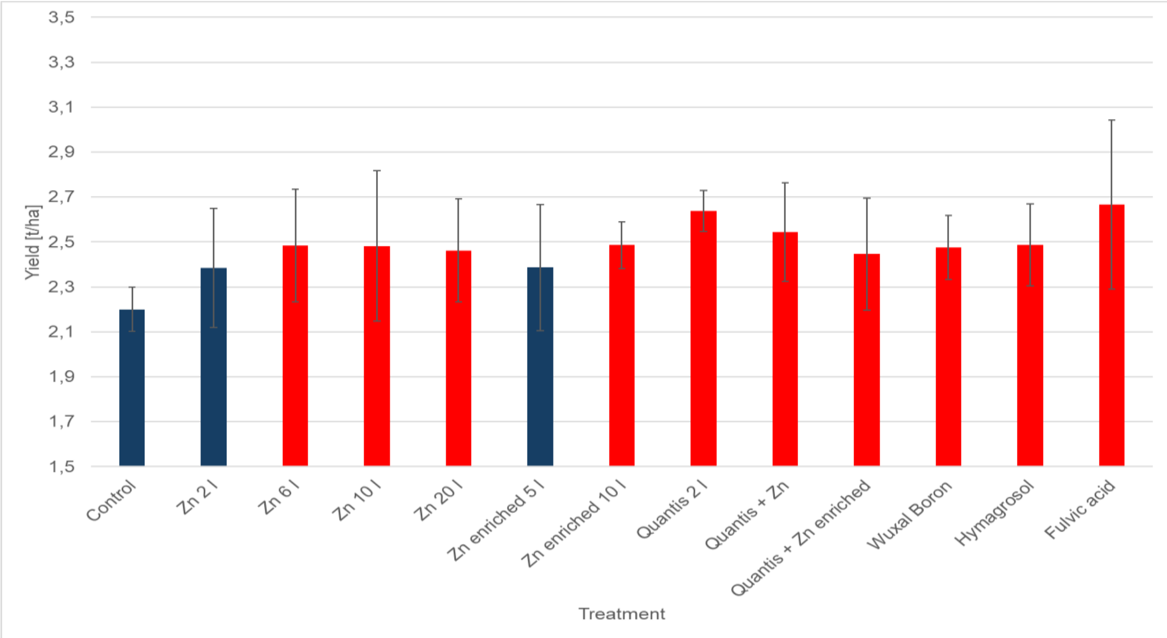

Figure 2: Effect of treatments on rapeseed yield (t/ha) during the 2nd year of treatments. Red bars show statistically confirmed differences (p<0.1)

Most treatments also resulted in an increase in the oil yield (Figure 3), although the increment was not significant (p>0.1). The oil yield showed a slight decrease after treatments Zn 2 L and Zn enriched 5 L. Among the three types of biostimulants used, the highest increment was obtained with Quantis (+218 kg/ha), the increment with Fulvic acid was 213 kg/ha, while the increment with Hymagrosol was 137 kg/ha.

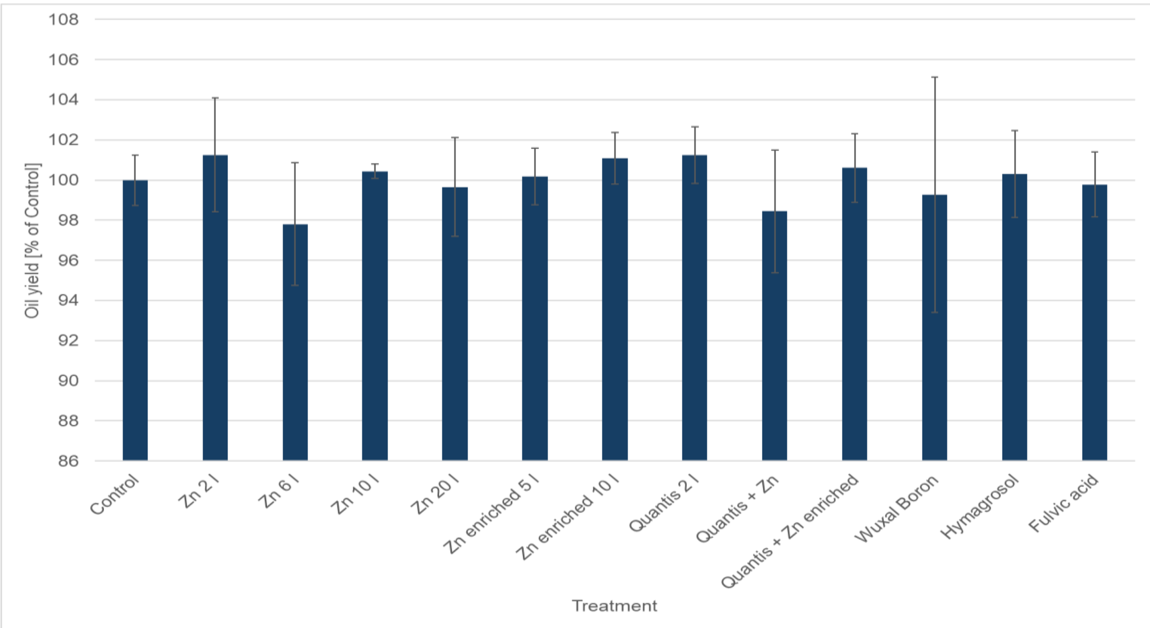

Figure 3: Effect of treatments on rapeseed oil yield in % (control = 100 %) during the 2nd year of treatments

4. Conclusions

According to Nagy (2023), 85 % of 80 experiments in winter wheat showed an increase in yield after single-flag leaf or flowering treatment with Quantis. The yield increase was, on average, 6 %, which amounted to 183 kg of crop per hectare. In a Hungarian development study conducted in 2019, a single treatment of Quantis applied to flag leaves at a dose of 2 L/ha was able to cause a yield increase of 5.7 %. A development experiment with sunflowers with Quantis showed a yield surplus of 5.9 %, which meant an excess of 192 kg/ha. The Hungarian results are consistent with foreign data. Studies in Bulgaria showed a 6 % increase in single and 13 % after double application of Quantis. According to the results presented, the improvement in quantitative and qualitative parameters after application can be confirmed.

As a result of the experiments, it can be concluded that areas treated with zinc, zinc enrichment and biostimulants led to excessive yields. Per hectare, the preparation containing fulvic acid reported a yield of +460 kg. The oil content per hectare also resulted in a significant surplus of +218 kg/ha of oil, which means additional income for the farmer.

The experiments carried out confirmed that biostimulants have a positive effect on the yield and oil yield compared to control after cold stress. As a technological proposal, the practical period of application of the product is before the stress event.

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