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A Study on the Potential Fertilization Effects of Microgranule Fertilizer Based on the Protein and Calcined Bones in Maize Cultivation

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Abstract: In the presented manuscript, the method of production, mechanism of action and the potential fertilizing effect of fertilizer soil microgranules, characterized by a controlled release of ingredients, that were produced from thermally processed bone waste and protein were described. The prepared fertilizer was tested in 3 doses in a pot experiment and thereafter the selected dose of 30 kg ha^{-1} was utilized in field conditions. The applied dose of fertilizer caused an average increase of maize yield of 620 kg ha^{-1} (grain moisture 14%). It was found that the obtained increase of maize did not result from the amount of supplied micro and macro elements to the soil, but it was a consequence of the fertilizer's mechanism of action. It was shown that the release of nitrogen in ammonium form from protein degradation due to the influence of fertilizer components and water had impact on the intensive growth and development of plant root system. This resulted in an increase in plant resistance to water stress during the growing season and consequently, greater yield. The developed soil fertilizer (produced mainly from the processed waste) with the proposed mechanism of action should be successfully applied in fertilizing plants that are grown in areas characterized by cyclical water shortages during the growing season

Keywords: bone waste; initial fertilizing; maize; protein

1. Introduction

The intensification of agricultural production observed in recent years results in increased consumption of fertilizers, mainly mineral ones. It was estimated that the global demand for mineral fertilizers in 2015 reached the level of 185.9 million tons in terms of pure ingredients (N, P_2O_5, K_2O [1]. The increased demand for fertilizers results in a rapidly growing fertilizer industry, and thus, an intensive exploitation of natural deposits, including phosphorites and apatites, which are the main source of phosphorus in most mineral fertilizers. The largest resources of these raw materials are in North African countries (Egypt, Algeria, Tunisia, Morocco), the United States (Florida), the PRC (People's Republic of China) and former USSR countries. Smaller deposits located in Europe are unsuitable for industrial exploitation. In addition, the depletion of phosphate rock deposits is currently one of the most serious global issues that may lead to a food production crisis. Nearly 90% of the global extraction of this raw material is used for fertilizer production [2,3]. It is estimated that at the current level of consumption of these deposits, they will be depleted within the pending century [4,5]. Therefore, the European Union (EU) emphasizes the need to look for alternative sources of obtaining raw materials for fertilizer production, including phosphorus from industrial and animal production waste. In general, it can be recovered and reused from various waste streams [6]. Sewage sludge may be one source of this raw material [7,8], as well as thermally processed bone waste [9] and bottom sediments of rivers and lakes [10,11], which can be used as renewable raw materials in the production of fertilizers under EBPR (enhanced biological phosphate removal) [12,13].

Phosphorus, next to nitrogen and potassium, is the main element necessary for the growth and development of plants. One of the basic functions of phosphorus is participation in energy transfer and accumulation and phosphorylation reactions. This element regulates the activity of many enzymes and is included in key compounds such as nucleic acids, nucleotides and phospholipids. In addition, it plays a role in the mechanism of transport of organic compounds and inorganic ions across cell membranes [14]. Plants uptake it mainly from soil through roots in the form of anions $H_2PO_4^-$ and HPO_4^{2-} [15]. In the initial phases of plant development, phosphorus is crucial for root development [16].

Aside of the phosphate fertilizers, NP fertilizers are a popular group of fertilizers. In addition to minerals, these fertilizers also contain organic additives, including raw materials rich in proteins or their hydrolysates. Patent number P.374562 describes a fertilizer product based on hydrolysates of waste proteins obtained from chicken and goose feathers [17]. Furthermore, there are commonly known products which are based on the protein biomass of various origin, converted mainly to ammonia, as a result of the impact of highly alkaline substances, such as CaO or $Ca(OH)_2$ [18]. Nitrogen in this form can be directly used by plants without nitrification, because it is released in a controlled manner, limited in time. Balawejder et al. [9] proposed a fertilizer in which the mechanism of nitrogen release is similar, but it is caused by alkaline phosphates obtained from calcinated bones.

Biochemical processes that occur in plant organisms are based on various actions related to proteins which are constructed from amino acids that include nitrogen. Sufficient rate of nitrogen assimilation results in increased rate of photosynthesis, leaf area production and assimilation of other elements. Aside from being crucial for amino acid production, chlorophyll and the stimulation of root growth, nitrogen fertilization is very important in early stages of plant development. Insufficient supply of nitrogen results in reduction of growth. Furthermore, it can cause the chlorosis which can be recognized by the change of the leaves' color from green to yellow and the appearance of red and purple spots on the leaves [19]. The uptake of nitrogen by plant roots and leaves is essential for plant growth [20].

The supply of nutrients to agricultural plants, including maize, can be done by applying fertilizers both in a standard and initial manner [21,22]. However, given the unfavorable thermal conditions prevailing just after sowing the maize, as well as the water shortages observed in recent years in Europe in this period, it is preferable to use initial fertilization. This fertilization counteracts adverse conditions for maize growth and increases the concentration of nutrients, which are missing in close proximity to the roots, necessary for proper plant growth [23–25]. The supply of nutrients, including phosphorus, is determined by the gradient of its concentration between the soil solution and the surface of semipermeable membranes in root cells. The row application of fertilizers increases significantly their concentration in the immediate vicinity of the roots. The row application of fertilizers is, therefore, beneficial in terms of plant nutrition, as it allows reduction of inputs, and also has an ecological aspect [25,26]. Lower doses of utilized fertilizers allow the lowering of fertilization costs, while at the same time, a risk of phosphorus losses to ground and surface water can be reduced.

The purpose of the work was to develop a soil fertilizer for fertilization based on an alternative source of phosphorus and protein. The fertilizer was tested in both pot and field experiments.

2. Materials and Methods

2.1. Fertilizer Components

2.1.1. Mineral Components

The bone material originated from a cattle slaughterhouse and was thermally processed at temperatures in the range of 850–950 °C. The composition of thermally processed bone waste was determined by Balawejder et al. [9] (Table 1). Lake chalk consisted mainly of calcium carbonate with a composition of 90 wt. $CaCO_3$, about 5 wt. carbonaceous matter and less than 2 wt. terrigenous components, mainly quartz [27].

Table 1. Determined mineral composition of the bone waste material.

Na	K	Mg	Ca	P	N	Fe	Mn	Zn	Cu
		[g kg^{-1}]					[mg kg^{-1}]		
4.74 ± 0.43	37.48 ± 3.50	16.77 ± 1.25	492.46 ± 9.51	161.13 ± 0.7	0.06 ± 0.01	21.03 ± 0.80	80.76 ± 2.22	10.40 ± 0.04	40.44 ± 0.97

Note. The results are presented as the mean value ± SD(standard deviation) (n = 3).

2.1.2. Protein

Whey protein isolate WPI Isolac Instant (Carbery, Ballineen, Country Cork, Ireland) with a composition: not less than 130 g kg^{-1} N, 0.2 g kg^{-1} lactose, 0.15 g kg^{-1} fat and moisture content of 6% was used.

2.2. Preparing of the Fertilizer

Raw materials, i.e., thermally processed bone waste and lake chalk for fertilizer production were subjected to grinding in a jet mill, reaching an average particle size below (<) 150 µm. Then a mixture of 79% by weight bone waste, 19% by weight lake chalk and 2% by weight protein in the form of whey protein isolate was prepared. The chalk lake sediment was used as a binder to achieve granules with adequate mechanical strength. The mix was then transferred to a fluid bed granulator using water as a binder. A granule with a regular spherical shape was obtained with a fraction distribution: 14.7% by weight larger than 3 mm, 27.5% by weight in the range 2–3 mm, 53.2% by weight in the range 1–2 mm and 4.6% by weight smaller than 1 mm. The granules were then dried in an oven at 45 °C until a moisture content of 3–4% by weight was obtained.

2.3. Analysis of the Fertilizer Composition

To dissolve mineral components, the samples of the fertilizer were treated with 70% $HClO_4$ and digested in mineralizator Tecator (FOSS, Hilleroed, Denmark) using the following temperature scheme: 165 °C for 2 h, 200 °C for 2.5 h^{-1}, 210 °C for 0.5 h^{-1}, and 230 °C for 1 h^{-1} [9]. The samples of the product were analyzed using an AAS Hitachi Z-2000 (Hitachi, Tokyo, Japan) for the determination of Fe, Mn, Zn, Cu Ca, Mg, K and Na. The content of phosphorus was determined in solution generated by digestion process, according to the vanadium-molybdenum method [28]. The concentration of phosphorus was determined using a colorimetric assay, based on the formation of colored complexes that form orthophosphate ions in the presence of vanadium and molybdenum ions. The measurements were performed using a spectrophotometer Shimadzu UV-2600 at a wavelength of 435 nm. Furthermore measurements of phosphorous soluble forms were also done according to standard: PN/EN 15956:2011 and PN/EN 15959:2011.

As a result of the analyses, the mineral composition of the fertilizer was determined: 313.8 g kg^{-1} P_2O_5, 5.3 g kg^{-1} Na_2O, 36.15 g kg^{-1} K_2O, 21.9 g kg^{-1} MgO, 621.1 g kg^{-1} CaO, 3.2 N g kg^{-1}, 1 mg kg^{-1} Fe, 6.5 mg kg^{-1} Mn, 0.8 mg kg^{-1} Zn and 3.2 mg kg^{-1} Cu.

Determination of pH and NH_4^+ ions activity

In order to determine the effect of fertilizer components on the solution pH, 10 g of fertilizer was suspended in 90 g of water. The suspension was transferred into a sealed bottle. After 20 min of stirring, it was found that the pH of the solution had risen to a maximum of 11. In the same solution, an ion-selective (NH_4^+) Intellical ISENH4181 electrode was immersed, which after the preparation of the suspension did not show the presence of ammonium ions. The concentration of ammonium ions should be close to activity because according to Debye-Hückel law, the activity coefficient in diluted solutions tends to one. The measurement was repeated every 24 h and a maximum activity of 0.15 g dm^{-3} of ammonium ions was observed after 5 days.

2.4. Description of the Pot Experiments

The one-way pot experiment was carried out in the glasshouse. The environmental conditions, such as temperature and air humidity, were under control and a LED based technology for lighting of plants during cultivation was utilized. In the experiment, the examined factor was the effect of the dose of developed soil fertilizer, on the growth and development of maize variety Farm Gigant maize variety (FAO 250, weight of 1000 grains = 264 g) in the initial phases of development (BBCH 0-BBCH 15). Seven levels of the tested factor were taken into account: no fertilization (control variant) and fertilization with standard composition of minerals without whey protein and the developed microgranules fortified with whey protein at a dose of 30, 40 and 60 kg ha^{-1}, taking into account the density of 70,000 plants per ha. Maize sowing in an amount of 1 seed per pot was carried out at a depth of 4 cm. Microgranules with a diameter of 2–3 mm in the tested doses were applied at a depth of 4 cm below the maize grain. The experiment was carried out in 10 replications for each variant in a completely random arrangement.

The experiments were carried out in the production pots, wherein 3 kg of soil with a granulometric composition loamy sand of lightly acidic pH (pH: KCl, 6.35; H_2O, 6.52) was placed. During the experiment a constant water content of 50% of the maximum water holding capacity (WHC) was sustained for the soil within the pots. In the five leaves phase of development (BBCH 15) a measurement of the relative amount of chlorophyll within the flag leaf was conducted using SPAD 502 (Konica-Minolta INC, Osaka, Japan) device, and then later (31 days after sowing), maize plants were harvested. After the harvest, the plants were cleaned from the soil and the dry matter of plants was determined (aboveground biomass and the biomass of the maize roots). The obtained results were statistically analyzed using the Statistica 10.1 program by the method of analysis of variance, with the significance level $\alpha = 0.05$.

2.5. Description of the Field Experiments

Verification of the dose of soil microgranules for initial fertilizing selected in the pot experiments was carried out in real (field) conditions, in field experiments conducted in 2019 at 6 locations in Poland. The field experiment scheme was designed to replace the 3-year scheme because the tested fertilizer was used parallel at several locations, characterized not only by different climatic conditions, but also by the soil conditions (Table 1) [9]. The content of mineral compounds in soil, like NPK was P 10.7–91.2 mg kg^{-1}, K 78.1–298.1 mg kg^{-1} and N 0.09–0.16%. Taking into account mineral composition content of particular field experiments, standard NPK soil fertilization was utilized, supplementing the content of these ingredients to the level required by maize grown for grain before the initial fertilization. Area of each location was 5 ha. The experiment was carried out parallel in different regions (I and II), more or less favorable for maize cultivation (Table 1). Region I covers the south-west and south-east of Poland. This area is characterized by the most favorable climatic conditions for maize cultivation due to the longest growing season as well as favorable rainfall distribution and high average air temperature, which results in a high maize grain yield. A slightly worse area in terms of maize growing is region

II, that covers the central part of Poland, which is characterized by warm summers and relatively favorable distribution of rainfall [9].

The 30 kg ha^{-1} dose selected in the pot experiments was tested against control (standard fertilization). Maize sowing, as well as the application of the tested fertilizer were carried out in the first week of May. The sum of precipitation in this month, on which emergence and the effectiveness of the fertilizer tested were dependent (Table 2), varied and ranged from 60 mm for location D to 183 mm for location C. The sum of precipitation and the average temperature during the growing season in which the experiments were conducted were also varied (Table 2). For sowing, a precision seeder was used, equipped with a fertilizer spreader for row (start) use at the same time as sowing seeds. The fertilizer coulters were set in relation to the seed coulters in such a way that the fertilizer was placed in the soil 4 cm below the seeds. Sowing of seeds was done to a depth of about 4 cm. Maize sowing density was 70,000 ha^{-1} seeds, and weeds were controlled using standard pest control procedures for maize cultivation. Maize was harvested in all areas on November 5–20, which was followed by crop yield and moisture content determination. The area of experimental fields for harvest was 5 ha for each location and experiment variant. The obtained grain yield from individual experiment variants was converted into dry grain yield per 1 ha and grain moisture content was 14%.

Table 2. Characteristics of the location, and weather conditions (sum of rainfall, temperature) during field experiments.

Localization	Geographical Coordinates	Climatic Region of Poland for Maize Cultivation	Sum of Rainfall in May [29]	Meteorological Conditions in the Vegetative Period 2019 [29]	
				Sum of Rainfall	Average Temp.
			mm	mm	°C
A	50°49′56.3″ N 21°34′11.0″ E	II (FAO 230-260)	127	415	17.1
B	49°57′39.2″ N 20°14′38.1″ E	I (FAO to 300)	124	359	18.5
C	51°14′50.0″ N 17°32′53.4″ E	I (FAO to 300)	60	239	18.3
D	50°03′55.6″ N 21°05′11.6″ E	I (FAO to 300)	183	407	22.7
E	51°09′10.0″ N 17°39′04.5″ E	I (FAO to 300)	69	218	19.1

3. Results and Discussion

3.1. Description of the Fertilizer Functionality

Granules of the fertilizer consist mainly of mineral compounds with low solubility. It was determined that they contain 275.6 g kg^{-1} of P_2O_5 which is solubilized in strong mineral acids and 53.9 g of P_2O_5 soluble in neutral ammonium citrate. Therefore, P as a major component present in the fertilizer is released over a longer period of time due to the metabolism of microorganisms in the soil [30,31]. After this time, the plant will also absorb this element. Furthermore, the effect achieved in the early stages of plant growth and development can be associated with the degradation of the protein components of the granular fertilizer. Proteins can be degraded as a result of the exposition to strong alkalis. It was determined that ammonium nitrogen is the main product of the protein decomposition [18]. Similar conditions occur inside of fertilizer granules due to the alkaline nature of the processed bone waste. Alkaline properties of this material was confirmed by the measurements of the pH of the fertilizer suspension in water which was about 11. Furthermore the presence of ammonium ion and its level was determined using an ion-selective electrode. Initially, the analysis showed no presence of ammonium ions. Only after 24 h ammonium ions were detected and their activity was growing until it reached 0.15 g dm^{-3} after 5 days. The concentration of ammonium ions should be close to activity because according to Debye-Hückel law, the activity coefficient in diluted solutions tends to one. Due to the low solubility of the other components, the ionic strength of the solution depends mainly on the pH. At the OH-ion concentration of about 10^{-3} mol dm^{3}, the effect on the ionic strength will be small.

It is expected that a similar degradation of the protein will occur in soil environment. A stable fertilizer in a dry state after placing in soil is enriched in moisture which, in a result, is followed up by the solubilization of mineral compounds. These compounds, in turn, cause a release of the nitrogen in ammonium form which was verified by laboratory scale tests. The controlled release of the fertilizer compounds coincides with the initial stages of the plant development. Despite the fact that the nitrogen content is low, the precise placement of the granules in soil in the vicinity of plant means that this element strongly supports the initial development of plant. The fertilizer also contains phosphorus, which is insoluble at high pH. Lowering the pH of this fertilizer is possible, which would increase the availability of these fertilizer components, but would prevent protein decomposition. Most soils in Poland are an acidic, so after some time, given the low dose of fertilizer, the ingredients will be available for plants. The process of releasing these components will be supported by soil microorganisms [32].

3.2. Pot Experiments

The initial period of maize growth and development is one of the most important periods during plant vegetation affecting further development and the final yield of maize grain and its quality [23]. Therefore, a very important agro technical procedure applied already at the sowing stage, is the type as well as the dose of fertilization, as it determines the proper development of the root system [30]. Particularly an important and effective one is initial soil fertilization, which affects the depth of rooting, volume of roots and their distribution in soil, and thus determines the size of the zone from which plants can absorb water and nutrients [33–36].

As a result of the pot experiment, a high efficiency of the tested initial fertilization at a fertilizer dose of 30 kg ha^{-1} was found on the growth and development of maize in the early stages of development (Figures 1 and 2). This effectiveness was conditioned by the release of ammonium nitrogen from the micro granules to the zone of the maize root system, which was a product of the degradation of the protein constituting the component of the tested fertilizer. Consequently, the increased availability and quantity of ammonium nitrogen taken up by the plants influenced the efficiency of maize production at the initial stages of development through increased production of aboveground biomass and roots. The impact of initial fertilization on the increased growth of maize roots was also confirmed by Jing et al. [37]. A similar correlation was observed by Kruczek and Sulewska [38] who compared the effect of NP fertilizer (ammonium phosphate), stating that initial fertilization significantly increases the amount of nutrients taken in all the tested maize varieties, as compared to standard fertilization, and improves plant growth.

Influence of ammonium nitrogen on plants fertilized with the tested fertilizer was confirmed by measuring the relative content of chlorophyll in the flag leaf, which indicates a better degree of plant nutrition. The relative content of chlorophyll in the flag leaf of plants fertilized initially compared to the plants from the control group was much higher, especially in a case of microgranules with protein (dose 30 kg ha^{-1}) (Table 3). The impact of fertilization on the increase of chlorophyll content in various plant species was also shown in the studies by Skwaryło-Bednarz and Krzepiłko [39]. Properly selected mineral fertilization with both macro- and microelements provides the right conditions for chlorophyll synthesis and increases plant productivity. Magnesium, which is a component of the tested granule, and also a central atom in the chlorophyll molecule and participates in many physiological processes [40] played an important role here. Fertilization with the tested granule also provided plants with calcium ions (Ca^{2+}), which are necessary in plant physiology, and its deficiencies can result in the appearance of many physiological diseases. Reduced concentration of this element in plant tissues may result in the occurrence of such disorders as shape change and curling of the edges of young leaves, or weakening of the root system that covers with mucus [41]. Calcium is also a key ingredient regulating the function of the plasma membrane. Deficit of this element causes serious damage to plants, up to disappearance of membranes. It also participates in controlling the activity of many key metabolic enzymes [42].

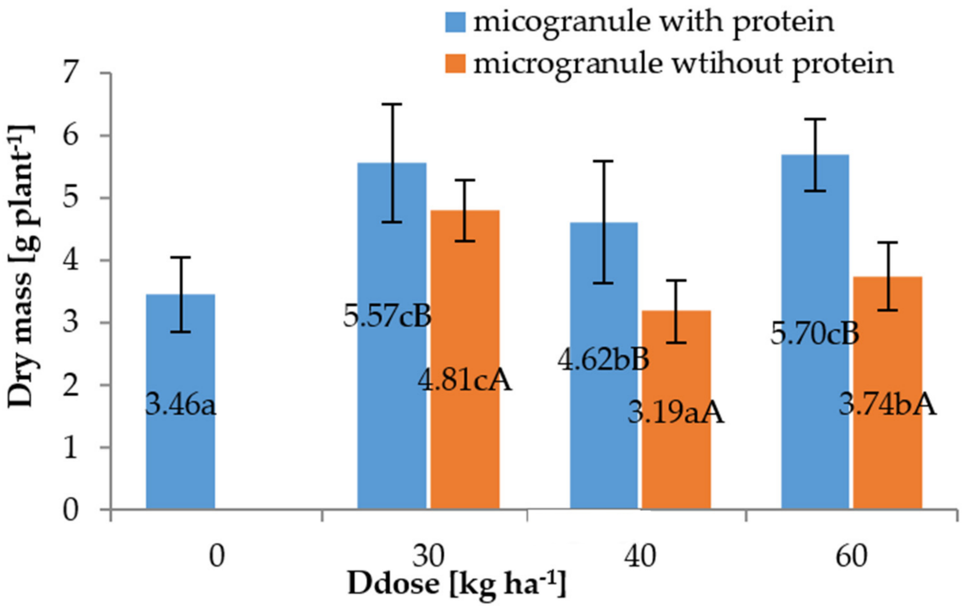

Figure 1. Dry mass of the aboveground parts of the plant.

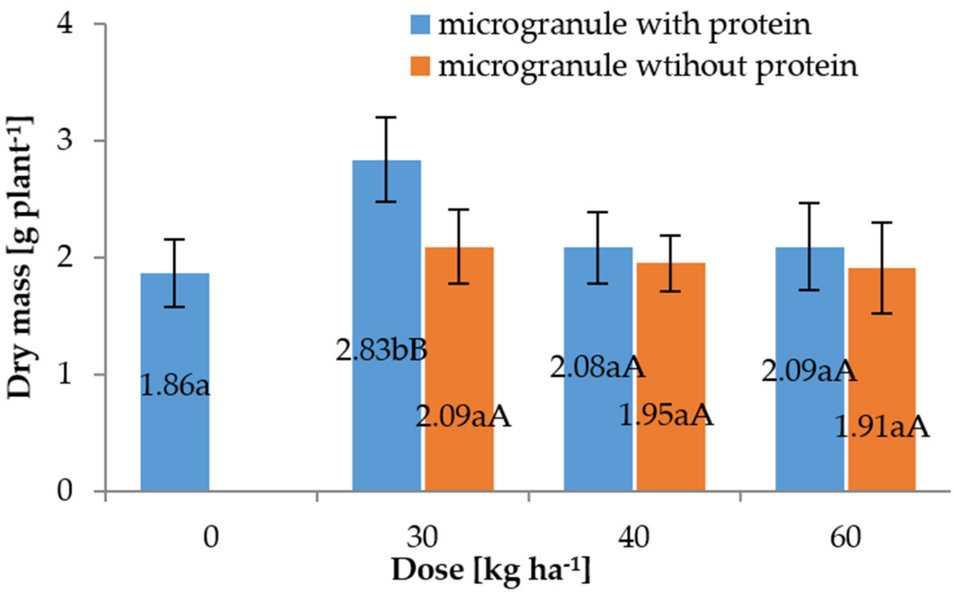

Figure 2. Dry mass of the underground parts of the plant. Note. Lowercase letters indicate statistically significant differences (α = 0.05) between control and tested doses of protein microgranules and microgranules without the addition of protein. The capital letters indicate significant differences between the tested doses of protein microgranules and no added protein (n = 20).

The doses of microgranules used in the pot experiment with a slowed release of fertilizing ingredients had a positive impact on the increase of dry matter of the aboveground and underground parts of maize plants (Figures 1 and 2). An increase in biomass production determined by the total dry matter of the plant as a result of nitrogen and phosphorus fertilization was also observed in our previous study [8]. It should be noted that in both cases, the source of the nutrients was thermally processed bone waste. The highest (more than 52%) increase in dry mass of root of maize plants compared to control plants was noted when using microgranules fortified with whey protein at a dose of 30 kg ha^{-1}. At the same time, there was a statistically significant increase in the dry weight of the roots of plants fertilized with protein fortified fertilizer compared to plants fertilized with 30 kg ha^{-1} microgranules without whey protein. This increase was the result of the action of ammonium nitrogen released from the microgranules, which influenced the intensive growth and development of the plant root system. (Figure 2). This nitrogen was released in close proximity to the germinating kernel, which, despite the low dose, allowed to accelerate and improve the development of the emerging root system. According to Möhr and Dickinson [43], the row fertilization method should be implemented primarily under conditions of using limited doses of fertilizers. As the doses used increase, the effectiveness of initial fertilization decreases.

Table 3. Relative amount of chlorophyll (SPAD) in maize plants.

Variant	Dose [kg ha^{-1}]	Leaf Peak	Leaf Center	Leaf Base
Control	0	19.97 ^{a}	18.73 ^{a}	11.43 ^{a}
Microgranules without protein	30	25.5 ^{cA}	20.6 ^{bA}	18.3 ^{dA}
	40	23.5 ^{bA}	19.0 ^{aA}	15.1 ^{bA}
	60	26.0 ^{cA}	21.9 ^{cA}	15.4 ^{bA}
Microgranules with protein	30	27.5 ^{dB}	21.40 ^{cB}	17.3 ^{cB}
	40	27.2 ^{dB}	20.8 ^{bA}	15.9 ^{bA}
	60	26.3 ^{cA}	23.17 ^{dB}	17.47 ^{cB}

Note. Lowercase letters mean statistically significant differences (α = 0.05) between control and tested doses of protein microgranules with and without added protein. The capital letters indicate significant differences between the tested doses of microgranules with and without protein. (n = 20).

3.3. Field Experiments

The conducted field experiments confirmed the effectiveness of the tested soil fertilizer for initial fertilization of maize cultivation. After considering standard fertilization to the minimum nutrient levels required for maize growth, the use of the test fertilizer was the only variable in the experiment. The application of the developed fertilizer resulted in an increase of grain yield of 620 kg ha^{-1} (grain moisture 14%) on average, for designated locations. The highest increase in yield was recorded for location D (1000 kg ha^{-1}, 11.9% compared to the control variant) (Figure 3). This increase, however, was observed, not due to the amount of nutrients supplied to plants, but resulted from the mechanism of action of the fertilizer, whose effectiveness depended on the amount of precipitation in the month of sowing and application of the fertilizer (May). In that month, the highest rainfall was 183 mm, which resulted in faster emergence, and parallel, controlled release of ammonium nitrogen from the tested granule, resulting in an intense growth and development of the plant root system. The highly developed plant root system plays a key protective role from stress, and above all drought stress, which has been observed in Europe in the growing season in recent years [9,34,36]. It should be noted that the right root system makes it possible to increase the absorption of other nutrients from the soil.

The increased tolerance of plants fertilized with the fertilizer tested during the growing season resulted in increased yields (Figure 3).

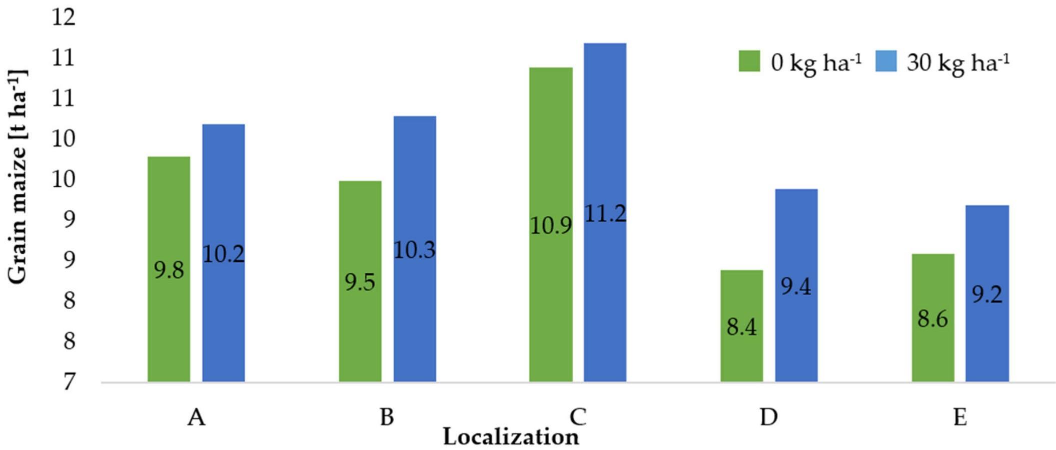

Figure 3. Grain yield (moisture 14%) of maize fertilized with the tested fertilizer dose depending on localization.

4. Conclusions

Initial fertilization combined with seed sowing introduces small amounts of fertilizer into the soil, but its location means that fertilizer components are available to plants at the right time and concentration. A 30 kg ha^{-1} dose has been shown to result in an average increase in maize grain yield by 620 kg ha^{-1} (6.6% compared to the control variant). It should be noted that the obtained increase in yields is not directly related to the quantity of supplied micro and macro elements, but to the mechanism of fertilizer action. It was shown that the release of ammonium nitrogen had impact on the intensive growth and development of the plant root system, which translated into an increase in the resistance of plants to water stress during the growing season and resulted in higher yields. Therefore, P as a major component present in the fertilizer is released over a longer period of time due to the metabolism of microorganisms in the soil. After this time, the plant will also absorb this element. Bearing in mind the climatic situation in Europe related to water shortage and depleted mineral resources, the developed soil fertilizer (produced mainly from processed waste) with the proposed mechanism of action should find wide application in fertilizing crop plants, such as maize or sugar beet.

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