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Influence of microbial fertilizers from the Microbtima group on the germination of corn hybrid Kneja 310

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Abstract: In recent years, there has been an increase in the world market of plant protection agents of a microbial nature and those that improve the health status of plants. Several biological agents (bacterial and fungal insecticides, plant biostimulators containing mycorrhizae or bacteria and fertilizers with amino acids) from different companies are registered in Bulgaria. All this became possible both because of scientific progress and because of the adopted „European Green Pact“ and the inflated prices of mineral fertilizers. Accelerated implementation of the technology is also needed. The aim of the present study was to evaluate the effect of several microbial agents from the Microbtima group on the germination percentage, root and coleoptile length in the test crop maize. The germination rate of hybrid Kneja 310 seeds recorded on the 7th day was 100 % for all variants, but a difference was observed in the vitality index, root length (cm) and coleoptile (cm). It has been confirmed that the rhizosphere soil bacteria in the agents have a beneficial effect on the initial stage of plant development.

Keywords: maize hybrid Kneja 310; microbial agent; Microbtima

INTRODUCTION

In the rhizosphere, soil microorganisms interact with the root system of plants, influencing their development. Plant-associated bacteria can have beneficial (positive interaction) or detrimental (negative) effects on plant growth (Dobbelaere et al., 2003, Wille et al. 2019). Soil bacteria that positively affect the plant are called *Plant growth-promoting rhizobacteria (PGPR)*. *Rhizobacteria* positively affect flowering and photosynthetic efficiency (Hossain et al., 2017). These microorganisms are able to improve the recycling of plant nutrients and reduce the use of chemical fertilization (Cakmakci et al., 2007). Recently, due to the pollution of soil, water and air from chemicals such as pesticides, the need for environmentally friendly fertilizers or biofertilizers is increasing (Meliani et al., 2017).

The beneficial effects of the bacteria associated with the plants (*PGPR*) are mainly expressed in the direct absorption of minerals, which leads to the correction of plant hormone levels. The most important as well as best studied symbiotic mechanisms are nitrogen fixation, solubilization of inorganic phosphate, production of auxins (IAA), cytokinins, gibberellins (ACC), deaminase and production of siderophores.

For example, the lack of the phytohormone auxin, which is a growth promoter, can cause insufficient growth in plants. Auxin is synthesized from tryptophan or indole in young leaves or buds, the apical root meristem, and developing seeds and fruits. It is responsible for cell enlargement and helps cell division, flower growth, apical dominance and tropisms, as well as developing conductive tissue. The presence of a transport signal was first noticed by Darwin, in his experi-

ments on phototropism of primary leaves, while in 1928 Went discovered auxin and called it indoleacetic acid (IAA).

Microorganisms also activate a number of indirect mechanisms. These include inhibitory actions that prevent the negative effect of pathogenic organisms in their competition for the rhizosphere environment. Some of the most common indirect mechanisms are the production of antibiotics and enzymes that are capable of attacking and degrading the cell wall of pathogens.

Genera *Arthrobacter* produce hydrolytic enzymes such as chitinases, cellulases, and proteases. This is the main reason for reducing biotic stress, stimulating growth and development (Costa et al., 2024).

Bacteria of the genus *Azotobacter* synthesize substances that regulate plant growth and protect plants from phytopathogens, improve the uptake of nutrients by plants and accelerate nitrogen fixation, i.e. fix and store nitrogen in the soil. „*Azotobacter* secretes an antibiotic pesticide into the soil and prevents the spread of soil diseases such as *Pythium* and *Phytophthora*.“ (BIOFERTILIZERS sustainable development of agriculture. COPYRIGHT © BIO-FIT PROJECT # 2015-1-BG01-KA202-014258)

Pseudomonas putida promotes plant growth and protects against pathogens. Due to its aerobic metabolism, it is used as a soil remediation agent.

Bacterias of the genus *Bacillus* convert insoluble forms of phosphorus into soluble forms that are suitable for plants, thus increasing the amount of nutrients available to plants in the soil, produce exopolysaccharides and siderophores in case of water deficit or high soil salinity, and phytohormones necessary for plant growth and development.

Bacillus subtilis synthesizes more than 220 biologically active substances (Maslennikova et al., 2022) the action of most of them is directed against pathogens of dangerous plant diseases. It is often used as the main component of microbial agents for the treatment of agricultural crops (Shternshis, 2012; Degering et al., 2010). The antibiotics synthesized by them such as surfactin, iturin and fengycin (Ahimou et al., 2000) re-

duce the effect of pathogenic microorganisms on plants. The bacterium synthesizes vitamins, amino acids and enzymes that stimulate the plants' own „immunity“ or the so-called elicitors - substances that can trigger an immune response in plants.

Bacillus megaterium is able to convert nutrient forms inaccessible to plants into digestible ones. In particular, it releases phosphorus from organic matter and converts it into soluble salts of phosphoric acid. It has been proven that phosphorus is found in the soil as part of chemical compounds, the absorption of which is inaccessible to plants (Samsonova, 2005). There is a lot of such immobile, unexchanged phosphorus in the soil, up to 5-6 tons per hectare. The introduction of live bacteria into the soil leads to a reduction in the consumption of mineral phosphorus fertilizers by 2-3 times (Degens et al., 2000). The bacterium produces natural active substances and a wide range of vitamins. This actively helps in the germination of plant seeds and the maintenance of immature sprouts (Bobkova et al., 2020).

Bacillus thuringiensis (BT) is used to control insects of the order *Lepidoptera*, *Diptera*, *Coleoptera*, *Hymenoptera*, and nematodes. This effect is possible due to its active ingredient, a crystalline protein, which paralyzes the insect's digestive system. The infected insect stops feeding and dies.

Beauveria bassiana parasitizes various species of invertebrates, infecting them through spores and colonizing the interior of the host, leading to its death (Posada and Vega, 2005).

Trichoderma is a biocontrol agent against phytopathogenic attacks and performs bioremediation of soils contaminated with pesticides, stimulates the development of mycorrhiza, which improves plant resistance to drought.

Metarhizium anisopliae is also a biological control agent against *Hylobius pales* (Bischoff et al., 2009), which simultaneously stimulates plant growth and drought resistance by improving nitrogen assimilation.

Positive interactions benefit the plants as they assist in obtaining minerals, phytohormones, and other nutrients. These beneficial bacterial spe-

cies can also act against phytopathogen by releasing several bioactive compounds thereby helping plants to endure several stressful conditions. On the contrary, harmful interactions are inimical, since pathogens colonize the plant tissues resulting in the death of the host plants (Dolatabadian, 2020; Adedayo et al., 2022). Therefore, exploiting these beneficial microorganisms can help sustain the plants against stress that hinders their productivity (Fanai et al., 2024).

The type of plant with which the microorganism makes contact also affects the manifestation of the positive effect.

Maize is a monocot, monoecious C4 plant from the Cereal family. Characteristic of C4 plants is that they minimize photorespiration and separate the processes of initial CO₂ fixation and Calvin cycle processes into different cell types. These pathways work by ensuring that the Rubisco enzyme always encounters high concentrations of CO₂, making it less likely to bind to O₂. The corn kernel is a simple fruit with a dry, non-opening, hard pericarp firmly attached to the seed coat. The reserve nutrients are deposited in the endosperm tissue. The period from germination to the establishment of the plant as an independent autotrophic organism is the most critical phase of plant development. During this period the plant is most vulnerable and any injury, drought or attack by pests, fungi or bacteria can prove fatal.

According to Vessey (2003), the definition of "biofertilizer" (which in this study is referred to as bacterial mix) is associated with a substance that contains living microorganisms that, when added to the seeds, plant or soil, colonize the rhizosphere or interior of the plant and improve growth by supplying the essential nutrients to the treated plant. It has been proven that biofertilizers create a suitable environment from which plants benefit. They contain beneficial microorganisms, which when applied to the soil, roots or seeds help to build a microflora that renews soil vitality, improves soil fertility by enriching the soil with nitrogen and phosphorus and leads to increased yields. They also provide substances that help plants growth and development as biofertilizers have the property of activating important nutri-

ents by converting them from unusable to usable for the plant.

The aim of the present study is to evaluate the effect of several microbial agents from the Microbtima group on maize seed germination.

MATERIAL AND METHODS

Maize (*Zea mays* L.) is used as the object of the study as a test crop. Hybrid Kneja 310, selected at the Maize Research Institute - Knezha, registered in 2014, is included in a germination test carried out under laboratory conditions in the city of Sofia on the territory of the laboratory complex of the company ROMB Ltd.

Plant material

The hybrid Kneja 310 (Fig.1) is characterized by a powerful initial development, with a short vegetation period (reaches maturity for 105-110 days), resistance to lodging, diseases and pests, and drought tolerance, with good moisture release from the grain, Dent corn, yellow (www.ic-kneja.com; Scientific Products, Kneja 310).

Microbial agents „Microbtima group”

Microbial agents from the Microbtima group were used to process (treat) the seeds. They include strains of rhizosphere soil bacteria such as *Pseudomonas* sp., *Bacillus thuringiensis* and *Azotobacter vinelandii*, and microorganisms from the group of fungi such as *Trichoderma* sp., *Beauveria bassiana* and *Metarhizium anisopliae*,



Figure 1. Hybrid Kneja 310

the mycorrhizal fungus *Rhizophagus intraradices* and the unicellular yeast *Saccharomyces cerevisiae*. *Plantagra* contains humic acids. All agents are registered with the BFSA (Bulgarian Food Safety Agency).

The scheme of the tested variants in three replications is shown in Table 1.

Table 1. Experimental scheme – options

№	Variant
1	Control - H ₂ O
2	Microbtima Protect + <i>Plantagra</i>
3	Microbtima Protect + <i>Plantagra</i> + Microbtima Stop
4	Microbtima Protect + <i>Plantagra</i> + Microbtima Stop+ Microbtima Nitrosky
5	Microbtima Nitrosky
6	Microbtima Nitrosky+Microbtima Stop

Plantagra is a biomineral humic product with enzymes and cells of beneficial molds and bacteria. (ROMB's technology for extracting and producing humic acids allows the bonds in the hydrocarbons to be broken so that the humic acid molecule becomes soluble and easily absorbed when treating plants.)

Microbtima Nitrosky contains free *Azotobacter* bacteria in spore form, fixes ammonium ions from the atmosphere where nitrogen is unavailable to plants.

The mycorrhizal fungi in *Microbtima Protect* absorb, store and move hard-to-reach water and nutrients to the roots. The preparation increases the drought and heat resistance of crops, as well as to frost, and applied to the seeds creates a strong fungicidal and bactericidal protective layer.

Microbtima Stop creates a protective layer against pathogens and pests.

Methods

Evaluation of germination is conducted according to the ISTA rules for seed testing (2017, International Seed Testing Association) and the requirements of IASAS (2023, Executive Agency for Variety Testing, Approbation and Seed Control). Carrying out the experiment to determine the effect of the agents used on the germination of the grains consists in placing 30 of them between 2 layers of filter paper, previously slightly moistened with distilled water. After arranging the grains, the paper is rolled into a funnel and placed in a graduated glass with the specified liquid (variant). Each of the variants is prepared in a volume of 50 ml. The glasses are left in a thermostat at 25°C.

The results are measured on the 7th day (Figure 2).

The evaluated traits are: main root length (cm), coleoptile length (cm) and number of adventitious roots, if any. From the data obtained during the measurement, the arithmetic mean value for the sign was calculated for each individual variant and the error of the arithmetic mean, then the percentage effect of the tested product compared to the control was determined.

The **vitality index** was calculated according to the following formula (Abdul-Baki and Anderson, 1973):

$$Viability\ index = (mean\ root\ length + mean\ coleoptile\ length) \times \% \text{ germinated seeds};$$

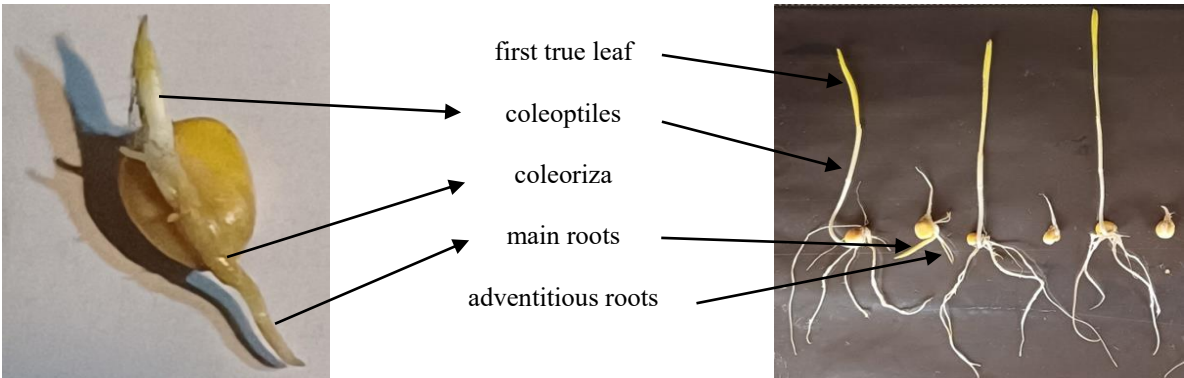


Figure 2. Germination

and Student's criterion (t), which is expressed by the ratio of the difference between the arithmetic means of the compared samples ($d = \bar{x}_1 - \bar{x}_2$), to the error of the difference (σd). The differences are significant when $t \geq t_{kr}$, and when $t < t_{kr}$ - they are insignificant.

RESULTS AND DISCUSSION

The germination of the seeds of the Kneja 310 hybrid, recorded on the 7th day (ISTA rules), was 100 % in all variants, but there was a difference in the development of the sprout. This means that the storage of the grains after the seed production of the hybrid is good and properly done.

In most kernels, in addition to the main root, two or more roots form from the cotyledon node, grow through the pericarp, and then point downward. The average value for the number of adventitious roots per individual grain was 3 for all variants tested (including the control). It can be concluded that the tested bacterial mixes do not show an effect on the formation of lateral roots.

Under the laboratory conditions, between the filter paper, with different number of grains for individual variants, there is even a first true sheet formed, reaching 90 % in variant 3.

The results for the averaged values of the traits - root and coleoptile length are presented in Table 2 and Figure 3, and for a clear account of the dif-

Table 2. Arithmetic mean ($\bar{x}$), it error and Student's test (t) versus control

Variant	$\bar{x} \pm SE$	t
Root length, cm		
Control	6,11± 0,77	-
Protect + Plantagra	8,05 ± 0,56	2,11*
Protect + Plantagra + Stop	7,71± 0,88	1,16
Protect + Plantagra + Stop + Nitrosky	8,37 ± 0,7	2,17*
Nitrosky	7,37± 0,8	1,02
Nitrosky + Stop	7,2±0,49	1,3
Coleoptile lenght¹, cm		
Control	5,84± 0,85	-
Protect + Plantagra	8,43±0,73	2,06*
Protect + Plantagra + Stop	7,23±0,81	1
Protect + Plantagra + Stop + Nitrosky	7,97±0,8	1,55
Nitrosky	6,08 ±0,83	0,17
Nitrosky + Stop	5,63±0,45	1

¹The values presented here include both the length of the coleoptile and the length of the first true leaf, if present, i.e. the entire sprout was measured.

*0.05 probability

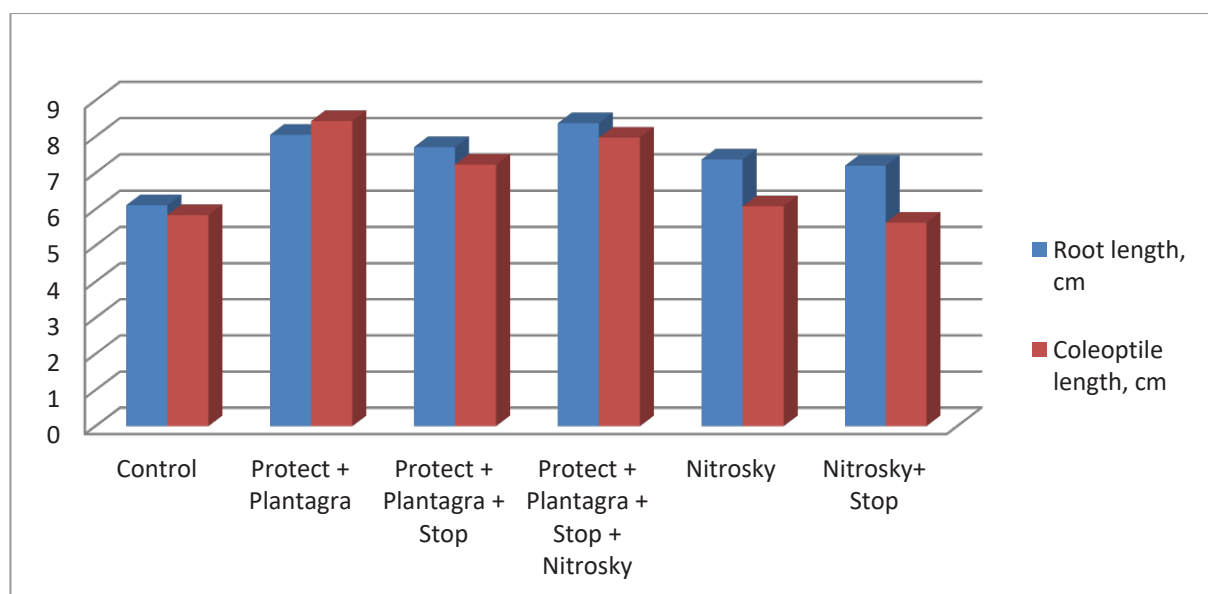


Figure 3. Average value by charectiristics

ferences, the percentage effect of each control direction variant is presented in Table 3 and Figure 4, respectively.

Root growth was positively affected in all variants, with a difference of almost 18 % (*Nitrosky* + *Stop*) to 37 % (*Protect* + *Plantagra* + *Stop* + *Nitrosky*) more than the control.

The increase in coleoptile length is up to 44 % (*Protect* + *Plantagra*) more than the untreated variant. A negative result was reported for this sign, i.e. the percentage effect was smaller compared to the control in the variant treated with the bacterial mix from *Nitrosky* + *Stop*.

To evaluate the differences between the variants and the control in terms of the two measured characteristics, the *t* criteria (Student's) was calculated, the data for which is presented in Table 2.

Differences between samples can be random (implausible, insignificant, inconsequential) or reliable (certain, significant, substantial). Random are due to the influence of random causes and are statistically unproven. In this case, the null hypothesis (H_0) is accepted, i.e. that the general parameters of the compared samples do not differ from each other. Credible differences are due to the influence of a certain factor and are statistically proven. Accordingly, the alternative hypothesis (H_1) is accepted here, i.e. that the general parameters of the compared samples differ.

The obtained result indicates that the differences are reliable for the trait root length in the variants in which treatment with mix 2) *Protect* + *Plantagra* and 4) *Protect* + *Plantagra* + *Stop*

Table 3. Percentage effect of the bacterial mixes

Percentage effect	Control	Protect + Plantagra	Protect + Plantagra + Stop	Protect + Plantagra + Stop + Nitrosky	Nitrosky	Nitrosky+ Stop
Root length	100	131,75	126,19	136,99	120,62	117,84
Coleoptile length	100	144,35	123,8	136,47	104,11	96,4

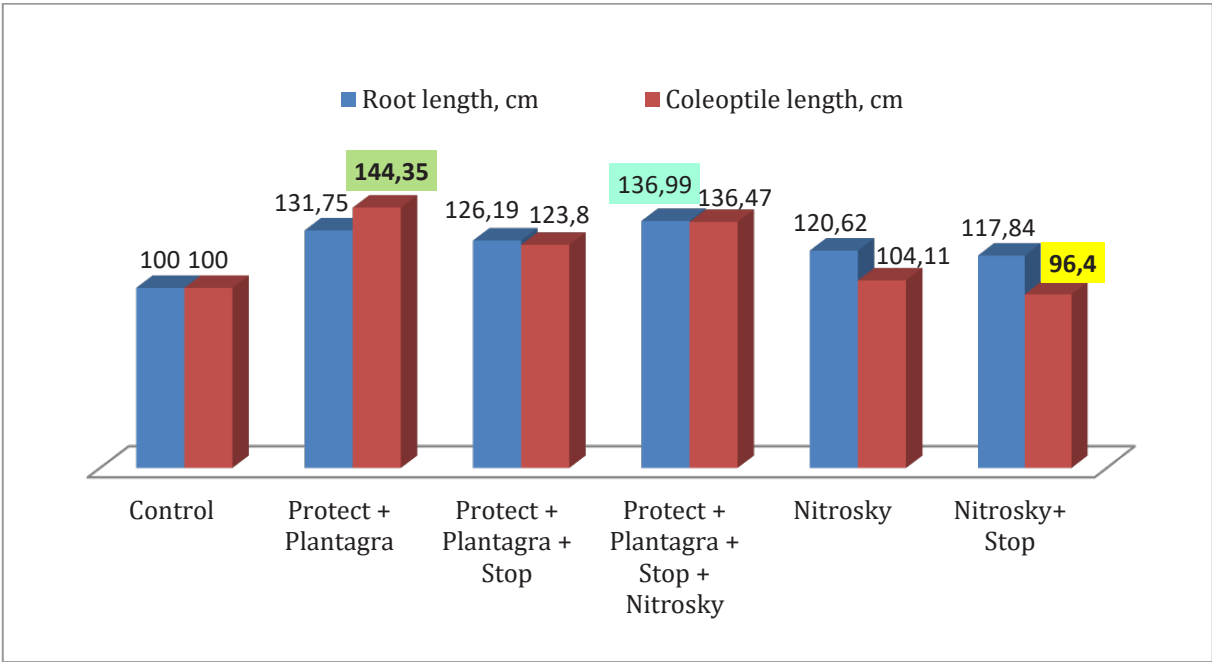


Figure 4. Percentage effect relative to the control (%)

+ *Nitrosky* was used and for the trait coleoptile length in the variant mix 2) *Protect* + *Plantagra*.

The significance of the differences between the other mean arithmetic values compared to the control has not been proven. This means that despite the reported differences between the respective mean values, they are small and therefore statistically invalid.

Table 4 lists the *Vitality Index*. The best result is for the variant treated with *Protect* + *Plantagra* (16.48), followed by a very small difference

Table 4. Vitality index and percentage effect relative to control

Variant	Vitality index	Percentage effect relative to control
Control	11,95	100
<i>Protect</i> + <i>Plantagra</i>	16,48	137,90
<i>Protect</i> + <i>Plantagra</i> + <i>Stop</i>	14,94	125,02
<i>Protect</i> + <i>Plantagra</i> + <i>Stop</i> + <i>Nitrosky</i>	16,34	136,74
<i>Nitrosky</i>	13,45	112,55
<i>Nitrosky</i> + <i>Stop</i>	12,83	107,36

(16.34) from the one treated with the mix *Protect* + *Plantagra* + *Stop* + *Nitrosky*. In the control, the index is 11.95. The percentage effect relative to the control is also indicated. This can also be seen in Figures 5 and 6. Differences were reported above and statistically proven for these two variants.

As a result of all this, it can be said that the rhizosphere soil bacteria in the agents have a beneficial effect on the initial stage of plant development. In all variants, higher values than the control were recorded for the measured signs. The only exception is the slightly lower number for the length of the coleoptile in the treatment with the *Nitrosky* + *Stop* mix variant. These agents are responsible for nitrogen fixation and protection against pathogens and pests.

Cereal plants have a highly differentiated germ. The coleorhiza, which covers the radicle, is the first structure to sprout through the seed coat and fruit coat. Coleorhiza is followed by the germinal root, which rapidly elongates and pierces it. After the germinal root emerges from the grain, the coleoptile is pushed to the soil surface as a result of mesocotyl elongation. Shortly after breaking through the soil surface and emerging above it, the coleoptile stops growing and the next true leaves emerge through the opening at its apex.

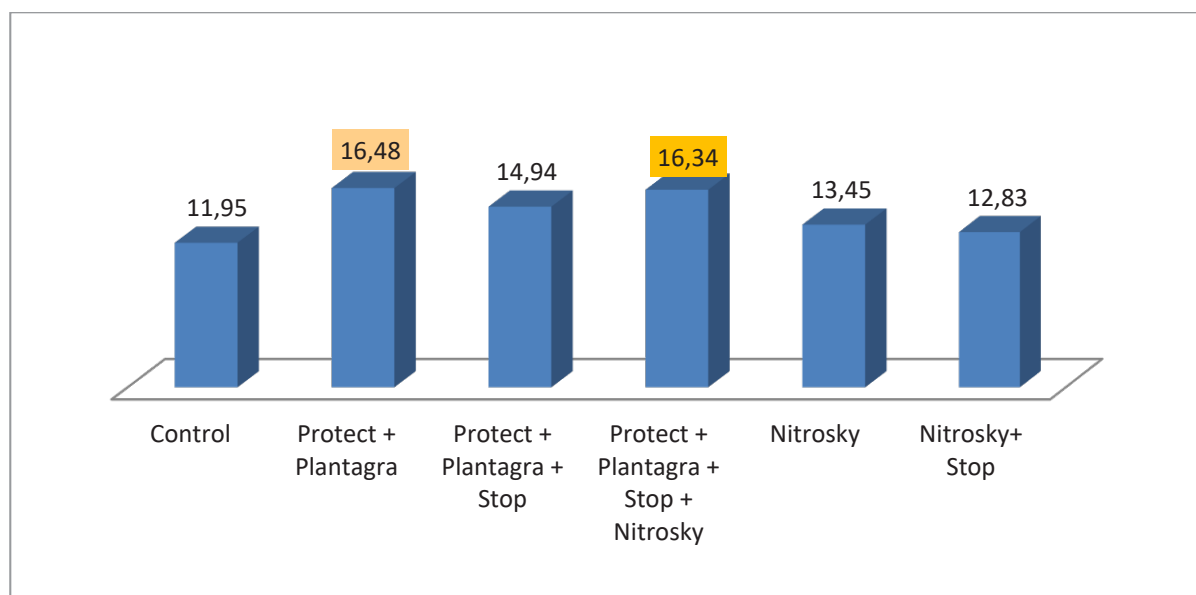


Figure 5. Vitality index

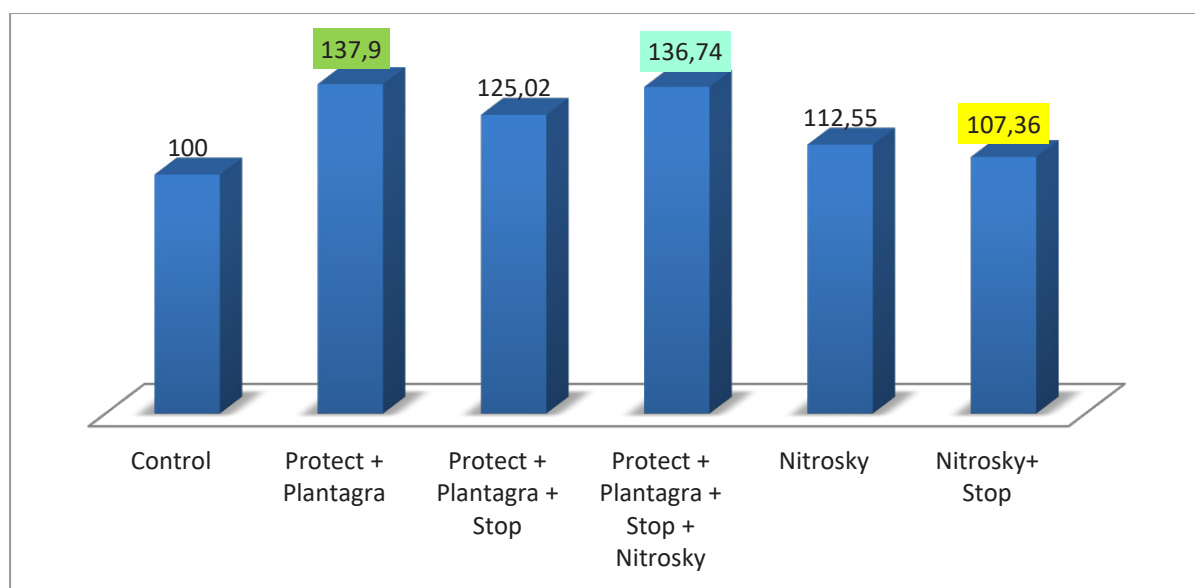


Figure 6. Percentage effect relative to the control (%)

The germ of the corn seed is not very large and is located among the endosperm tissue. The endosperm is highly developed and completely replaces the nucellus. The grains are thin and their function is to absorb the stored nutrients and pass them on to the emerging germ.

Figure 7 show photos of the seeds for each variant. Variant 3 shows the best results with 90 % of the germinated seeds having a well formed first true leaf.

In the bacterial complexes of *Pantagra* and *Microbtima Protect*, *Stop* and *Nitrosky*, in addition to the fixation and protection of the grain, the agents are responsible for increasing the root system and for a good balance of the microfertilizer composition in the root zone and are a natural anti-stress agent, too. It is recommended to treat the corn kernels with this joint mix before sowing as this solution is not only financially accessible to producers but also leads to having uniform germination timing among the seeds.

Overall, it has been identified that the factor most positively influencing and stimulating the biological effect on phytohormonal activity already in the seeds is the combination, found in the microbial agents, of the microorganisms and the synthesis of hormone-like metabolites from them.

After evaluating the impact of the agents on germination, the following *recommendation* can be made. *For a quick start and uniform growth of corn seeds before sowing, treat with a bacterial combination from Pantagra and Microbtima Protect, Stop and Nitrosky.* They act as components of the soil and improve its nutritional properties by using microorganisms that enter into symbiosis with the plants.

At the moment, several biological agents (bacterial and fungal insecticides, plant biostimulators containing mycorrhizae or bacteria and fertilizers with amino acids) from different companies are registered in Bulgaria. One of them is the microbial agents from the *Microbtima* group of the company ROMB Ltd.

It can be said that the microbial fertilizers of the *Microbtima* group fix the atmospheric nitrogen, found in the soil (*Microbtima Nitrosky*) and make it more available to plants. The bacterial mix as *Microbtima Protect* produces hormones and anti-metabolites that stimulate root growth. The biofertilizer dissolves insoluble forms of phosphates such as tricalcium, iron and aluminum phosphates, bringing them into an absorbable form. They can clean waste phosphates from soil layers and break down organic matter, help-

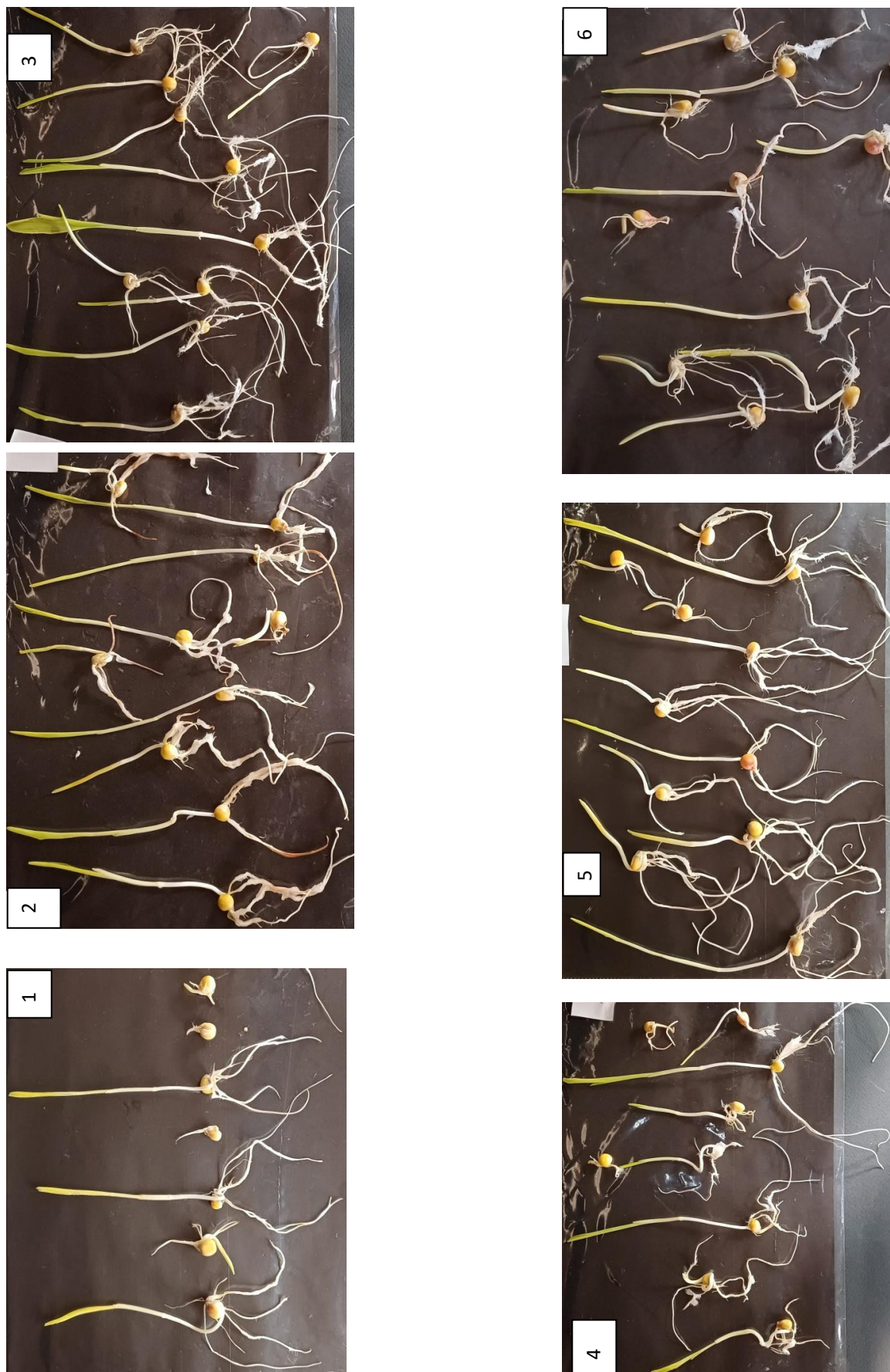


Figure 7. Options:

1. Control - H2O; 2. Microbtima Protect + Plantagra; 3. Microbtima Protect + Microbtima Stop; 4. Microbtima Protect + Plantagra + Microbtima Stop + Microbtima Nitrosky; 5. Microbtima Nitrosky; 6. Microbtima Nitrosky + Microbtima Stop.

ing to mineralize the soil. The microbial fertilizer increases the availability of nutrients and increases crop yields by 10-20%, without adverse effects or pollution of the soil and the environment.

For example, Microbtima *Protect* contains *Pseudomonas putida*. It is a gram-negative, rod-shaped, saprophytic soil bacterium with proven plant growth-promoting capacity (Georgieva et al., 2023). According to Khan et al. (2020) *Pseudomonas* sp. have fungicidal ability. De Andrade et al. (2023) and Sivakumar et al. (2020) describe a large number of microorganisms with their diverse abilities such as phytohormone production, hydrogen cyanide or nitrogen fixation, etc.

After assessing the impact on germination, it is necessary to check how these agents would affect plant vegetation and, above all, whether their use would lead to increased yields. High yields are the main goal of farmers and, if the result is positive, they will have an incentive to use these preparations. Another reason for their use is their ability to improve and heal the soil by regulating soil pH (For example *Plantagra*). To obtain good results, it is important to use the agents at the right vegetation time and in the right concentration.

Microbial communities in the rhizosphere as an alternative to chemical fertilizers have become the subject of enormous interest in sustainable agriculture and bioconservation programs. Biofertilizers are a natural component of healthy soil that they enrich, not pollute (Vessey 2003).

Microbial agents from the Microbtima group are a good example of a healthy product, an innovative solution in agriculture (instead of chemical preparations for pest control and fertilization), industrial biotechnology and a green biobased economy. They are suitable for maintaining fertile soil. Further studies are needed to determine the mechanisms of nutrient absorption in plants and whether different plant genotypes influence the effectiveness of the preparations.

CONCLUSION

The germination rate of hybrid Kneja 310 seeds recorded on the 7th day was 100 % for all

variants, but a difference was observed in the vitality index, root length (cm) and coleoptile (cm). Rhizosphere soil bacteria in the agents have a beneficial effect on the initial stage of plant development.

The comparative test showed that maize responded favorably to treatment with the tested bacterial mixes. The difference between two variants (1. *Protect* + *Plantagra* and 2. *Protect* + *Plantagra* + *Stop* + *Nitrosky*) for the trait root length and one variant (*Protect* + *Plantagra*) for the trait coleoptile length was statistically proven.

As a result of this study, joint treatment of corn grains before sowing with the bacterial mix of *Pantagra* and Microbtima *Protect*, *Stop* and *Nitrosky* can be recommended.

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Conflict of interest: none.

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