Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRE-TREATMENT OF SLOW-GERMINATING APIACEAE SEEDS FOR MICROGREENS

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Abstract

The purpose of the experiment is to test the application of different fertilizers, as growth regulators, for soaking slow-germinating seeds of the family Apiaceae, in the cultivation of microgreens. A potted laboratory experiment was prepared at the University of Forestry. Four fertilizers were tested: Bioforce, Humustim, Biotor, and Algreen, with four of the species of the Apiaceae family, suitable for growing microgreens: dill, carrot, parsley, and celery. Six variants were developed: the four fertilizers and two controls - with non-soaked seeds and with seeds soaked only in water. The seeds are soaked for 24 hours before sowing. Sowing is done in plates for microgreens, on a peat substrate. Of the four tested fertilizers as growth stimulators, Biotor shows the most complex results - it accelerates germination, affects the content of dry matter, increases the level of total sugars, and affects plastid pigments.

***Key words**: Apiaceae, growth regulators, microgreens, soaked seed.*

INTRODUCTION

Micro-plants initially began to be grown as an eye-pleasing decoration and tasty addition to food. Later attention began to be paid to their nutritional and biological value and their effect on human health. Now they are gaining mass popularity as a healthy food.

Microgreens are young plants, other than sprouts, which have distinct stems, cotyledons, and the first very young true leaf. Compared to sprouts, which have germinated seeds ready in about 2-5 days and are eaten whole (along with the root), microgreens take longer (7-21 days) (Kumar et al., 2018).

The difference in time for obtaining micro-greens depends on the plant type. Vegetables, spices, herbs, etc. can be grown, and for most of them the harvest period is about 7-14 days, but there are slower-growing ones that need 21 days under optimal conditions (Stoleru et al., 2016; Kumar et al., 2018).

Stoleru et al. (2016) state that microgreens are sown at medium to high density and harvested shortly after the first true leaves appear, and according to Bulgari et al. (2021) the plants are harvested and cut above the roots.

Some of the most commonly used species are from the family Brassicaceae (broccoli, cauliflower, cabbage, turnips, radishes, arugula, etc.), Apiaceae (fennel, carrots, celery, parsley), Asteraceae (lettuce, chicory, etc.), Cucurbitaceae (melon, cucumber, pumpkin), Fabaceae (beans, chickpeas, broad beans, lentils, peas, etc.) etc. In addition to families, microplants can be divided according to their color (yellow, green, red, purple, or variegated), and aromatic species can have a very strong smell (Saleh et al., 2022).

Microgreens from the Apiaceae family are quite aromatic and rich in oils and resins, which give them specific flavors. Dill is used to flavor many dishes, but it also has therapeutic properties against inflammations, and microbial infections, for relieving insomnia, etc. (Giordano et al., 2022).

Fabek Uher et al. (2023) found that when dill, red cabbage, and alfalfa were grown as micro-greens, in hydroponic technology on perlite, with the highest levels of total carotenoids (0.196 mg/g tw), total chlorophyll (0.713 mg/g tw) and chlorophyll a (0.528 mg/g fw) were detected in dill. It has also shown the highest levels of the other investigated components: dry matter, ascorbic acid, flavonoids, etc.

The type of plants is the amount and type of carotenoids contained in them. For example, pigmented vegetables such as salad beets and carrots are rich in lycopene and beta-carotene, while green plants, such as spinach and cabbage, are rich in lutein and zeaxanthin (Saltveit, 2010, L. Yadav, 2019).

Different types of seeds require different times to sow and germinate. Some seeds require soaking, others weighting or covering with soil. (Bulgari et al., 2021).
Soaking seeds in cold water is recommended for some large-seeded crops, including peas and radishes, according to Lee. Fertilizers mixed into the growing substrate or applied after emergence through soluble fertilizers have been found to result in rapid growth and a high yield of microgreens. According to Kou, 10 ml of calcium chloride solution applied daily for 10 days resulted in an increase in the biomass of cruciferous vegetables compared to water (Li et al., 2021).
The purpose of the experiment is to test the application of different fertilizers, as growth regulators, for soaking slow-germinating seeds of the family Apiaceae, when growing them as microgreens.

MATERIALS AND METHODS

The experiment was carried out at the University of Forestry, Sofia, Bulgaria, in 2023.
For the experiment, seeds of the following species from the Apiaceae family were used for the production of microgreens: Dill (germination period 4-5 days); Carrot (germination period 4-7 days); Parsley (germination period 7-10 days); Celery (germination period 10-14 days). The organic fertilizers used in the experimental part, according to the manufacturers, have the following content:
Bioforce - Improves the germination of seeds, stimulates the development of the root system, and increases the resistance of plants against stress (Table 1).

Table1. Mineral and organic content of Bioforce

pH		∑ N-NH_4+NH_3 mg/kg	P_2O_5 mg/ 100 g	K_2O mg/100 g	Hummus %	
H_2O	KCL					
9.0	8.4	155.5	260	760.3	9.0	
Total micronutrient content						
N %	P %	K %	Ca %	Mg %	N %	
0.94	0.61	0.74	2.21	0.74	0.94	
Chemical analysis of "mobile" elements in EDTA						
Cu mg/kg	Zn mg/kg	Mn mg/kg	Fe mg/kg			
1.5	1720	42.8	6.2			
Total forms of heavy metals						
Zn mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Ni mg/kg	Pb mg/kg	Cd mg/kg
114	30	361	8400	14	under 6	under 2

Biotor Ruse - Organic fertilizer from red California worm, rich in useful microorganisms, enzymes, vitamins, and amino acids (Table 2).

Table 2. Mineral and organic content of Biotor Ruse

pH	Organic %	Total N mg/l	Total P mg/l	Total K mg/l	Mg mg/l
7.31	32.33	625	381.93	976.27	550.12

Humustim (potassium humate) is a growth regulator that controls the metabolism of plants and stimulates their growth and development (Table 3).

Table3. Mineral and organic content of Humustim

Total N %	P_2O_5 %	K_2O %	CaO %	MgO %	Organic C %	Organic %
2.0	1.0	6.5	2.0	0.5	23.0	45.0

Algreen - organic fertilizer, concentrated extract of fresh algae containing natural phytohormones (PGR), alginic acid, Nitrogen (N), Sulfur (S), Magnesium (Mg), and trace elements in chelated form (Table 4).

Table4. Mineral and organic content of Algreen

N g/l	S g/l	MgO g/l	B g/l	Mo g/l	Alginic acid g/l	Natural plant hormones	Organic g/l	pH
60	70	60	5	2.6	40	> 300 ppm	20	6.5-7.5

Plastic working trays with a size of 15.3 x 10.5 - approximately 161 cm^2 were used.
The used nutrient substrate "Terranovo" is the same for all variants – it is a mix of compost, coconut fiber, and perlite, with a pH of 5.5-6.5 according to the manufacturer's data (Agroflora S.A.).
Six variants have been developed. In five of them, the seeds are soaked – with water or in solutions of the selected nutrient media, and there is one without soaking - a control for comparison. The six variants are as follows:
1) control, untreated seeds (NT);
2) water treatment (WT);
3) treatment with Bioforce (BF);
4) treatment with Humustim (HT);
5) treatment with Biotor (BT);
6) treatment with Algreen (AL).

Only of the selected nutrient media, has recommendations for soaking the seeds, in which approximate norms for preparing a solution for soaking seeds are proposed. The concentration used for variants BF, HT, and BT is normally 50 ml/2 l of water. For the last treatment, because the nutrient medium is based on a concentrated extract of fresh algae, and in this case, another concentration is used - 10 ml/10 l of water.

The recommendation for Apiaceae family seeds is to soak them for nearly two days. For the experiment, they were soaked in the prepared solutions for 24 hours. After that, they are filtered, dried, and sown in the previously prepared plates, with three replications. Two sowing dates were made, and the sowings were carried out in stages: 04/04/2023 - sowing of dill and carrot; 04/28/2023 - sowing of celery and parsley.

Seeding rates are calculated according to seed size and production container size and recommendation by the seed supplier – for the celery - 0.3 g, parsley and carrot - 0.8 g each, and dill - 1 g.

The number of days until seed germination was monitored, and 20 seeds of each variant were placed in petri dishes, on a humidified medium. Counting was done on the 4th and 10th day for seed germination by variants and for plant emergence, by variants and replicates. Reported percentages of germinated plants are shown as mean per variant.

Chlorophyll content: by determining chlorophylls and carotenoids in laboratory conditions according to the method of Vernon L. P. (1960), to determine the photosynthetic activity of plants; Sugar content: percentage content of sugars in the young plants (Brix, %) with a refractometer, model - Digital refractometer 32145, manufactured by B & C Germany; Dry matter content: From each variant, 50 plants were cut to determine fresh weight (FW) and dry weight (DW). After measuring the fresh weight, the samples were dried in an air dryer at 60°C until a constant dry weight was obtained. Then the data were recalculated to percent dry weight (DW × 100/FW).

RESULTS AND DISCUSSIONS

During the performance of the experiment, the temperature and humidity in the working room were monitored because it was not heated.

In the month of April, the temperature in the room ranges between 11-15°C, and a peak of 22°C was measured. On that day, the lowest atmospheric humidity for the month was measured in the room, 80%. During the rest of the time, the humidity is in the range of 90-95%

These two factors are important for creating optimal conditions for plant germination and emergence, and in this case, they slowed down the development of the first two species tested for microgreens - dill and parsley (Figure 1).

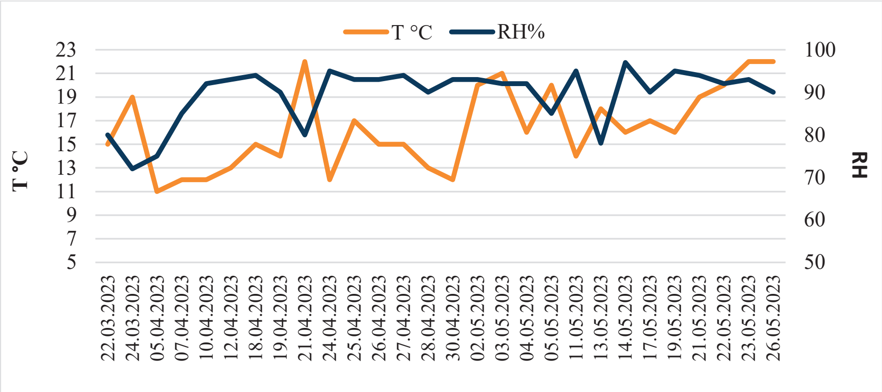

Figure 1. Temperature and air humidity in the working room

Phenology observations

Dill and carrot. In the first experiment, on the 4th day after the treatment of the seeds, the beginning of germination was reported for the dill, with the option of soaking with BT. The carrot had no sprouted seeds. On the 10th day, mass germination was observed in both species and the case of dill, the non-soaked seeds surpassed the soaked ones (Table 5).

Table 5. Seed germination and plant emergence (%) for dill and carrot

Variant	Dill			Carrots		
	Seeds		Plants	Seeds		Plants
	4th day	10th day	10th day	4th day	10th day	10th day
NT	0%	90%	60%	0%	80%	37%
WT	0%	80%	53%	0%	85%	38%
BF	0%	60%	48%	0%	75%	55%
HT	0%	70%	60%	0%	70%	50%
BT	(~5%)	80%	53%	0%	80%	50%
AL	0%	85%	42%	0%	85%	52%

On the 10th day, plant germination was recorded for all variants, with the control and the variant with BF for dill ahead of the others in %. In the case of carrots, on the 10th day, four of the variants have an average of 50% and over 50% germination of the plants (Table 5).

Celery and parsley. The second sowing was carried out with celery and parsley and, unlike the development of the previous two crops, a significant difference was found between those at the beginning. On the fourth day, the celery has no germinated seeds. This was registered on the 10th day – 10% in two of the options: soaking with BF and treating the seeds with BT (Table 6).

Table 6. Seed germination and plant emergence (%) for celery and parsley

Variant	Celery			Parsley		
	Seeds		Plants	Seeds		Plants
	4th day	10th day	10th day	4th day	10th day	10th day
NT	0%	0%	0%	0%	67%	57%
WT	0%	<10%	0%	10%	50%	70%
BF	0%	10%	0%	50%	90%	20%
HT	0%	<10%	0%	45%	90%	20%
BT	0%	10%	<10%	60%	90%	90%
AL	0%	20%	<10%	55%	95%	53%

In the last variant - soaking with AL, 20% of germinated plants were recorded. In the control variant, without soaking, there was no germination even on the 10th day. On the 10th day, germination of the seeds in the working trays was also reported. In the case of celery, there is a beginning of germination by soaking the seeds with BT and AL (Table 6).

In parsley, on the 4th day, the seeds germinated was reported. On the 10th day, in working trays almost complete germination was recorded in the variant with BT, while in the variant BF and HT the germination was weaker (Table 6).

From the indicated data, it follows that the treatment of seeds with the BT accelerates and improves germination. The treatment of seeds with fertilizer with AL, WT, BF, and HT is ordered. According to the germination results of the plants in the working trays, the first biofertilizer (BF) outperformed the water-treated seeds in some cases.

Dry matter in %

In dill, the dry matter had the highest percentage in the second BT (3.6%), followed by the variant treated with AL (3.2%). The lowest (1.4%) was measured in the variant by soaking the seeds in water only (Figure 2).

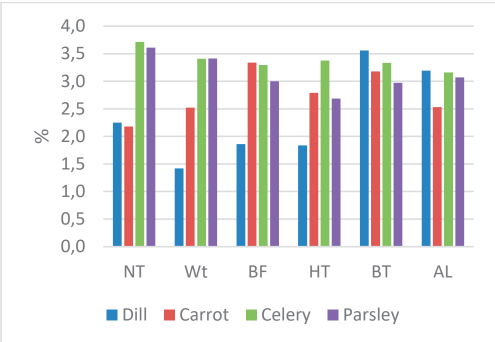

Figure 2. Dry matter (%)

The microgreens of celery had a higher dry matter content. It had approximately the same amounts for the individual variants, and the difference between the highest value (NT) and the lowest (AL) is about 0.5%. The dry matter of parsley is within the limits of around and above 3%. The highest percentage is the NT (3.6%), followed by the treating the seeds in WT (3.4%). The percentage (2.7%) is the lowest in the variant of treated seeds with HT. (Figure 2).

Sugar content % Brix

Parsley and celery microgreens showed a higher percentage of total sugars in the untreated seeds, compared to the treated variants. In the variant with soaking the seeds only in water, the percentage of total sugars also increased in the other two crops - dill and carrot. Dill was affected by seed treatment with the BF, although the values were not very high, while parsley had the lowest percentage. Treatment with HT in dill increased the content of sugars and was the highest of the tested crops. With the BT seed treatment, the percentage content of all crops was almost equalized, without a strong increase or decrease, but dill and carrot did better (Figure 3).

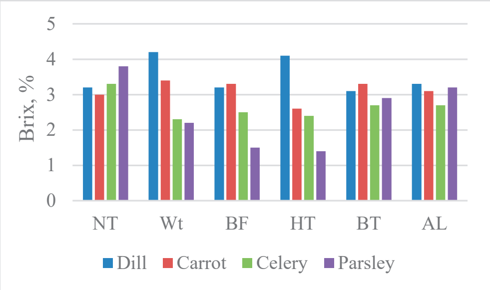

Figure 3. Content of total sugars in %

All types of microgreens had a content of total sugars above 2.5%, In the different variants, there are more pronounced peaks, and there are species with lower and higher sugar content.

Chlorophyll and carotenoid content

Compared to mature vegetables, microgreens have a higher content of vitamins and carotenoids. Dill microgreens showed the best photosynthetic activity of all plants and the level of chlorophyll A (Figure 4).

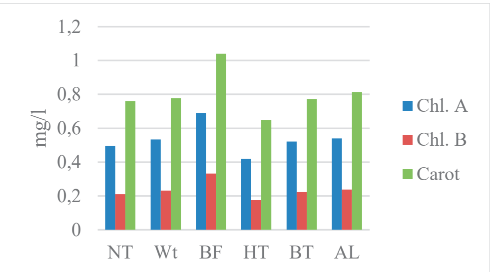

Figure 4. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in dill

It ranged from 0.419 to 0.691 mg/l, with the best performance being the variant with the BF seed treatment and the lowest for the seeds, treated with HT. The carotenoid content of dill ranges from 0.650 to 1.040 mg/l. The remaining four variants also have good indicators - treatment of seeds with AL, BT, and the control with untreated seeds, and only those treated with humate fertilizers have a lower content than the control (Figure 4).

The best photosynthetic activity in carrots was shown by plants from the control variant - without seed soaking, with the content of chlorophyll A reaching 1.982 mg/l. In all variants with seed treatment, it is weaker, compared to the control, with vitamin A content ranging from 0.281 to 0.577 mg/l. The values are lowest when plants are treated with BF Carotenoid values in carrots ranged between 0.455 and 1.982 mg/l, being the highest in the control variant without seed soaking. In all variants with seed soaking, they were lower compared to the control and ranged from 0.455 to 0.871 mg/l (Figure 5).

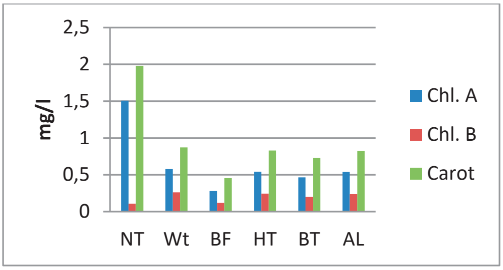

Figure 5. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in carrot

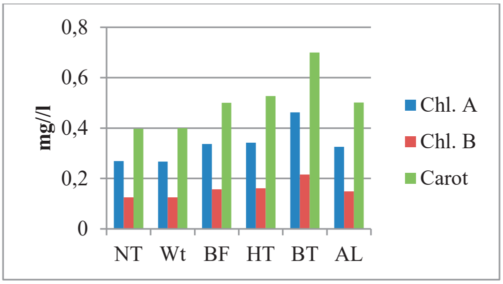

Figure 6. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in celery

Celery showed lower physiological activity in the NT and the variant with WT compared to the treated variants. and the level of chlorophyll A in it ranged from 0.267 to 0.462 mg/l, being the highest in the treated variant with BT. The

levels of carotenoids in this variant range between 0.400 and 0.700 mg/l, and after the variant is treated with BT is the variant HT (Figure 6).
Parsley photosynthetic activity is better and in three of the variants, the values are almost equal. Chlorophyll A levels ranged from 0.299 to 0.535 mg/l, with the highest values, similar to carrot, being the untreated control (Figure 7).

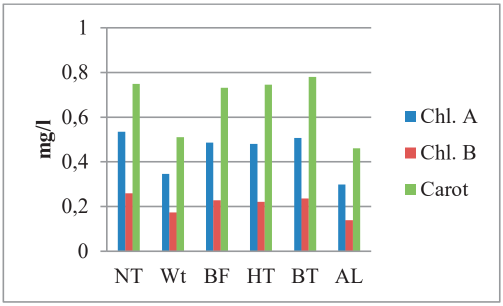

Figure 7. Content of chlorophyll A, chlorophyll B, and carotenoids (mg/l) in parsley

With almost equal values, are the variants treated with BF and HT. The level of carotenoids in parsley is also higher, ranging from 0.461 to 0.780 mg/l. The variant with soaked seeds in the BT has the highest values, closely followed by the NT, HT, and BF (Figure 7).
Photosynthetically active chlorophyll A in all variants ranges from 0.256 to 0.691 mg/l, and the ratio between chlorophyll A and chlorophyll B (1:2) is respected and is an indicator of good photosynthetic activity. Data for carotenoids show higher values and are in the range of 0.400 - 1.040 mg/l.

CONCLUSIONS

Three of the nutrient media used to lead to an acceleration of seed germination and plant emergence – first is treatment with Biofertilizer, followed by organic fertilizer with algae extract Algreen and biofertilizer Bioforce. Good results are also obtained by just soaking the seeds in water.
Soaking the seeds in Biofertilizer, Bioforce or Algreen organic fertilizer affects the dry matter content of the microgreens. When treating the seeds with Biotor, all types of microplants have a content of total sugars above 2.5%.
Both - Bioforce and Biotor - have an impact on the accumulation of chlorophyll and carotenoids.
Dill and carrots have a higher content of total sugars than the others. Carrot, dill, and parsley are distinguished by a higher content of carotenoids.

ACKNOWLEDGEMENTS

The research work was carried out with financial support from the SRC Project "Comparative study of technologies for growing vegetables and spices in urban conditions", No. B-1217/27.04.2022.

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