Chapter

# Growth Promoting and Stabilizing of Cucumber Plants Cultivated in Soilless Cultivation Systems Using Biostimulators

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## Abstract

Cultivation of cucumber in greenhouses is predominantly carried out in soilless cultivation systems mainly in substrate culture. The use of organic and completely compostable substrates is of rising interest in such systems, unclean sheep wool was formed as substrate slabs, peat slabs and coconut fiber slabs were compared with mineral substrates rockwool and perlite. In general can be stated, intensively in greenhouses cultivated crops such as cucumbers, suffer often from inadequate abiotic or biotic growth conditions in particular in the rhizosphere. Many studies were done to find growth promoters or biostimulators to stabilize the growing condition in the rhizosphere, in case of stress situation as fluctuating salt concentration EC and pH value, but also in case of temperature stress. K-Humate, Lactate and *Bacillus subtilis* were investigated as biostimulators in such situations. Different concentration and combination of these biostimulators were investigated but also the methods of application on leaves and roots respectively. Very successful was used for the stabilization of the EC value for cucumber plants growing in substrate the application of K-Humate and *B. subtilis* (FZB24®) as single component and combined. Following the results, it can be assumed, the application of the combined biostimulators with all substances if applied over the roots was a stimulating effect visible, whereas if applied over the leaves an inhibiting effect for cucumber plant growth. Application of the biostimulators solution with all three components BS-FZB24® (0.2%), K-Humate (0.01%), and LACTOFOL "O" (0.1%) were tested regarding their effects in case of strong but short time pH and temperature stress the growth of cucumber plants. There are a strong correlation between green biomass of treated cucumber plants and their root mass. It can be assumed that one of the effects of stress prevention through the biostimulators is primarily based on increasing root growth.

**Keywords:** soilless cultivation, organic and mineral substrates, humates, lactates, *Bacillus subtilis*, abiotic stress influence

## 1. Introduction

Cucumbers (*Cucumis sativus* L.) is a vegetable originated from the rain forest of northeastern India. Therefore, they are growing in particular in temperate areas in glass-greenhouses, in order to secure the appropriate temperature and humidity whereas in sub-tropical regions mostly in plastic greenhouses to secure the suitable humidity or to safe the cucumbers from heavy rainfalls. In all cases very important are the growing conditions in the rhizosphere. In the rainforest, the cucumbers are growing in a soil with a high amount of organic matter. Therefore, substrates suitable for cucumber cultivation in greenhouse should have specific physical properties, as pore volume, air and water capacity and density of the substrates [1]. In general, substrates from three groups can be used, mineral or inert, artificial and organic (**Table 1**).

Which of the substrates is suitable, depends also from availability in the region of the basic material, the economically preparation and environmental tasks as possibilities of reuse or environmentally friendly waste disposal. All substrates have some limitations in comparison to the natural growing conditions of cucumbers:

- Strong reduction of the volume for root growth
- Different physical, chemical and biological conditions in the substrates
- Missing of a buffer for stress situation as fluctuation of the pH and salt concentration (EC) or limitations of macro- or micro nutrients
- Under natural soil or organic growing media conditions, humates are available as a buffer for fluctuation of salt concentration
- Microorganisms in the rhizosphere can have different functions as mineralization of organic material, stress reduction and as growth regulator.

A proper regulation of the growing condition in the rhizosphere of cucumbers in greenhouses, is also from high importance because the wide relation of shoot and leave to the roots - 100:1, that means a comparable little root system have to secure water and nutrients for a big biomass.

Nowadays, mainly mineral or Rockwool is used as substrate in greenhouses for fruity vegetable production as tomatoes and cucumbers. Therefore, most of the studies with different substrates for cucumber cultivations are including as 'control' the Rockwool as slabs or as granules in containers.

| **Mineral - inert** | **Organic** | **Artificial** |
|---|---|---|
| Expanded Clay | Coconut fiber | Aggrofoam* |
| Gravel | Wood fiber | PU-foam |
| Perlite | Peat | Polystyren-foam |
| Pumice | Mix substrate | Polyphenol-foam |
| Rockwool | Compost | UMF-foam |
| Sand | Moss peat | |
| Zeolite | Straw | |
| | Sheepwool | |
| | Pine bark | |

*PU = Polyurethan; UMF = Urea-Methanal foam.*
**PU foam mixed with recycled PU-granules.*

**Table 1.**
*Classification of substrates for hydroponically cultivation of vegetables as cucumbers and others.*

In general can be stated, intensively in greenhouses cultivated crops such as cucumbers, suffer often from inadequate abiotic or biotic growth conditions in particular in the rhizosphere. Many studies were done to find growth promoters or biostimulators to stabilize the production process or to enhance plant growth of cucumbers under these conditions. Following such reports and own research the wide range of effects produced by Humates based on humic acids, have beneficial effects on the growing conditions in the rhizosphere. Many experiments were showing positive effects of different Humates as 'Bioregulator' in substrate culture of cucumbers. Other organic substances with a similar effect were tested as Lactates (salts of lactic acid) because these substances have proven biostimulatory effects and as an approach to improve the nutrient balance and plant vitality. Investigations have shown that Lactates have more stable bonds with several metal ions than other chelates. This effect can be very important to improve the nutrient supply of the cucumbers in soilless cultivation systems.

Furthermore, as mentioned the biological conditions in these intensive cultivation systems are completely changed, in particular in the inert and artificial substrates. Many microorganisms from the rhizosphere can influence plant growth and plant health positively, and are therefore often referred to as "plant growth promoting rhizobacteria" [2]. Different microorganisms are tested regarding these effects best result could be induced by *Bacillus subtilis* (syn. *B. amyloliquefaciens ssp. plantarum*) regarding these growth and plant health promoting mechanisms as well as the interactions between them.

In several studies, cucumber growth in different substrates was investigated using mineral nutrient solution and with organic nutrients. Furthermore, plant growth-promoting agencies as the mentioned Humates, Lactates and Bacillus subtilis were investigated single or in combination regarding their effect as Bioregulator in the rhizosphere as well on the epigean part of the plants.

Following these investigations, different effects in relation to plant growth factors or conditions could find out in particular under stress conditions for plant growth (**Figure 1**):

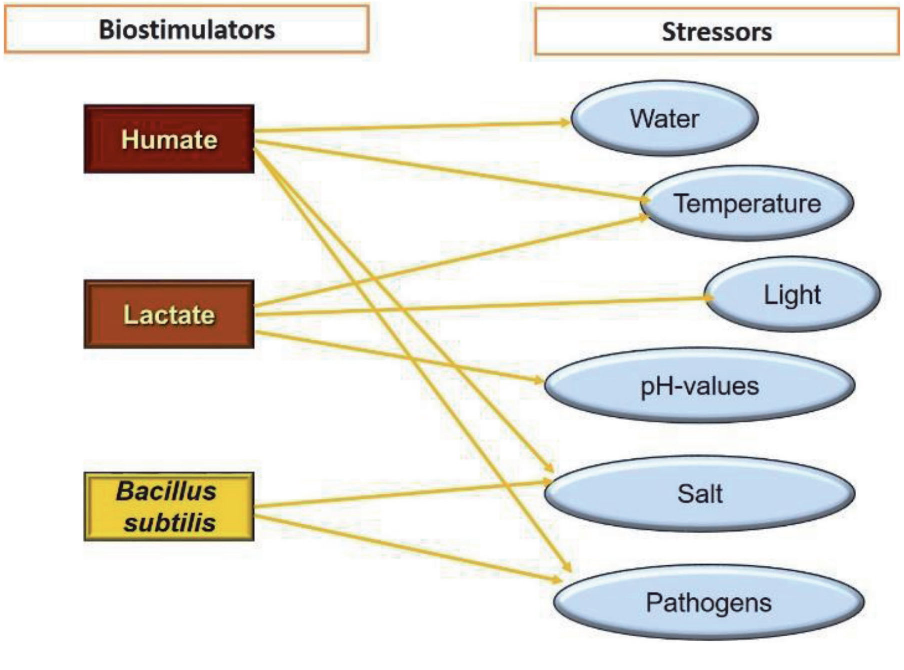


**Figure 1.**
*Effect of biostimulators humate, lactate and* Bacillus subtilis *on stressors during growth of cucumbers under protected cultivation.*

## 2. Cultivation systems for cucumber growing in greenhouses

In the last 45 years different hydroponic systems are developed in the world, the different systems can be subdivide in four groups [3]. Whereas, cucumbers are cultivated mostly in the first and second system.

### 2.1 Substrate culture

Hydroponic systems in a small amount of substrate (3 to 15 l per plant) filled in containers, bags, slabs or channels. Slabs of rockwool, peat or coconut fiber are used. This system is combined with different types of trickle irrigation. There are 'open systems' in which the drainwater flows out of the substrate slabs or cultivation container in the ground. Preferable used are the 'closed systems' in which the growing medium is lying in channels and the nutrient solution is recirculating.

### 2.2 Water culture

The plants are cultivated without any substrate, except the substrate for propagation of the young plants. The plants are staying or hanging also in channels in recirculating nutrient solution. Quite known systems are the 'Nutrient-Film-Techniques' (NFT) and the 'Deep-Flow-Techniques' (DFT). Also gravel culture can be grouped belong to the water culture, because the gravel has only the function to stabilize the root system.

### 2.3 Aeroponics

The plants are cultivated without any substrate and staying or hanging on plastic-foam-sheets and the root system is hanging in a closed space. The nutrient solution is given by very fine nozzles as a kind of fog-system. Important is a high frequency of spraying with nutrient solution, advisably to spray every 2 to 4 minutes for 20 to 30 seconds according to the developmental stage of plants. This system is rarely used for cucumber cultivation.

### 2.4 Aquaponics

Is a combination of aquaculture with typical fish keeping in ponds and the water culture system 'nutrient-film-technique' (NFT) as a closed aquaponics, where the plants were fertilized only with the fish water. This technique becomes more and more important for the food production and has an increasing popularity, even though the system is not methodologically sound and completely scientifically investigated. Nowadays, this system is mainly used to cultivate tomatoes and leafy vegetables [4] it can be used also for cucumber cultivation.

## 3. Biostimulators to enhance cucumber growth

### 3.1 Lactate

According to previous investigations, lactates (salts of lactic acid) seem to produce bioregulatory effects. The application of lactates was tested as an approach to improve the nutrient balance and plant vitality. Investigations have shown that lactates have more stable bonds with several metal ions than other chelates.

Therefore, substances have been used as fertilizers and as bioregulators. Lactates are available under the brand name LACTOFOL® [5, 6]. That suspension fertilizer was designed mainly for foliar application and patented as a plant growth and development regulator. The composition of LACTOFOL O® is shown in **Table 2**.

### 3.2 Humates

Humates are the salts of Humic acids which are complex polymers including amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups [7]. There are different types of humates related to the organic material used and the method of extraction of the Humic Acid; in the experiments were used different types:

- Peat used by Merck
    - Na Humate
- Brown coal
    - K Humate
    - $NH_4$ Humate
- Leonardite G (Germany)
    - Fulvic Acid
    - K Humate
- Leonardite R (Russia)
    - K Humate
- Coconut fiber (Mexico)
    - K Humate

| Components | Unit | LACTOFOL O® | Components | Unit | LACTOFOL O® |
|---|---|---|---|---|---|
| Lactic acid | % | 10 | Magnesium | % | 0.1 |
| Riboflavin | mg/l | 0.5 | Iron | % | 0.4 |
| Ascorbic acid | mg/l | 3 | Boron | mg/l | 300 |
| Thiamine | mg/l | 0.1 | Copper | mg/l | 200 |
| Nitrogen | % | 30 | Manganese | mg/l | 250 |
| Phosphorus | % | 7.5 | Zinc | mg/l | 125 |
| Potassium | % | 15 | Molybdenum | mg/l | 18 |
| Calcium | % | 0.5 | Cobalt | mg/l | 6 |

**Table 2.**
*Composition of LACTOFOL O®.*

Na Humate is not very useful as Bioregulator, because the negative effects of high Na in the nutrient solution or substrates, therefore K Humates or $NH_4$ were used in the experiments.

In relation to crop growth or soil condition can be divided in Direct and Indirect effect of humates:

- Direct effects of humates
  - to increase the vitality and stimulating plant growth,
  - to increase the germination activity and accelerate the germination of seeds.
  - to improves the uptake of some nutrients and enhances the transport and availability of micronutrients in the plant
- Indirect effects of humates
  - Regulation of soil properties,
  - to improve thermal conditions and soil or substrate structure,
  - to enhance the ion - exchange capacity,
  - to stimulate development of micro-organisms.

### 3.3 Microorganisms

Intensively cultivated crops such as cucumbers in soilless cultivation systems are stressed because the growth conditions in the rhizosphere are completely different in contrast to the natural conditions. Therefore, studies were done to improve the growth condition in the rhizosphere, to enhance plant growth and to find growth promoters to stabilize the production process. Investigations were carried out to find effective biostimulators and agencies for plant protection from pest and diseases. Different bacteria species and their strains were investigated and practically used for different plants mainly in protected cultivation [8, 9].

Many microorganisms from the rhizosphere can influence plant growth and plant health positively and in case of bacteria they are often referred as "plant growth-promoting rhizobacteria" However, their effects have to be seen as the complex and as a cumulative result of various interactions between plant, pathogen, antagonists, and environmental factors [2].

Following many investigations in the soil are bacteria present in an average amount of 6 × 108 cells/g of soil, and with a live weight of about 10,000 kg/ha, they are the most common microorganisms in the soil, bacillus species are one of them. The species *Bacillus subtilis* is common in the nature and can be found in every compost pile, but it was important to find active strains promising for developing marketable products. Based on several investigations, some strains of *Bacillus subtilis* are already used in industrially and agricultural fields [10]. An effective strain is in this regard is FZB24® in particular used in agriculture also as growth promoter in the rhizosphere of the cultivated plants [11].

There are various effects induced by *Bacillus subtilis* (syn. *Amyloliquefaciens* ssp. *plantarum*), and different mechanisms of these effects as well as the interactions between them.

Following the investigations in the research and practical experiences in agriculture and horticulture, it was found out that *Bacillus subtilis* could have different effects:

- Growth promoting of plants.
    - Formation of growth hormones and enzymes for nutrient mobilization, based on better this nutrient availability and the nutrient uptake can be enhanced.
    - Improvement of seed and tuber germination.
    - Improvement of root formation of plants.
    - Improvement of plant growth, biomass production and marketable yield.
    - Improving of the earliness of generative development and vegetative growth.
- Promoting of plant health.
    - Improving plant resilience.
    - Reducing the effects of diseases in terms of intensity and frequency.
    - Achieve resistance of the plants in particular against soil born diseases.

## 4. Mineral, organic and synthetic substrates used in the substrate cultivation system of cucumbers

As mentioned in the introduction different substrates can be used for cucumber cultivation in substrate culture systems. In principle, most substrates are suitable for successful cucumber growing, if adequately supplied with water and plant nutrients.

In one experiment were compared substrates from the three groups (**Table 1**), because the ecological aspects are becoming more and more important when choosing substrates, as well the economic efficiency is important. Substrate were filled in containers or available as mats, between 8 and 9 liters of substrate were available per plant. The aim was to use substrates with different physical and chemical parameters (**Table 3**).

- Rockwool (Grodan) mats as standard substrate,
- Perlite, grain size between 0.6 mm and 1.0 mm diameter.
- Urea-methanal foam,
- Organic substrate, consisted of pine bark (40% v /v), low-bog peat (40% v/v) and manure straw mix (20% v/v).

Comparing the four substrates used, the significantly highest total yields of cucumbers were obtained in the variants ‘organic substrate’. The differences in crop

| Substrates | Physical characteristics [% v/v] | | | Chemical characteristics | | |
|---|---|---|---|---|---|---|
| | WC | AC | PV | Density [kg/m³] | pH | CEC (mval/1) |
| Rockwool | 54 | 32 | 86 | 80 | 7.5–8.8 | — |
| Perlite | 36 | 52 | 88 | 98 | 6.5–8.0 | 5 |
| UM-Foam | 45 | 45 | 90 | 25 | 6.0–6.7 | 2 |
| Organic Substrate (OM) | 50 | 40 | 90 | 350 | 6.0–6.5 | 165 |
| Target values * | 45–65 | 20–40 | 70–95 | 30–500 | 6.7–7.0 | 50 |

*WC = water capacity; AC = air capacity; PV = pore volume; CEC = cation exchange capacity.*
*[1].

**Table 3.**
*Physical and chemical characteristics of substrates used in experiments with cucumber growing in substrate culture.*

| Substrate | Months of harvest | | | | | | Average |
|---|---|---|---|---|---|---|---|
| | February | March | April | May | June | July | |
| Rockwool | 0.40 b | 4.95 b | 6.90 a | 7.78 a | 6.80 bc | 5.77 b | 5.43 |
| Perlite | 1.45 a | 4.78 b | 6.63 ab | 6.54 ab | 7.10 b | 8.48 a | 5.83 |
| UM-Foam | 1.10 a | 4.30 b | 6.10 ab | 5.65 b | 6.60 c | 8.05 a | 5.30 |
| Organic Subst. | 1.37 a | 6.24 a | 7.36 a | 7.14 a | 9.25 a | 7.27 a | 6.44 |

*Different letters indicate significant differences, LSD, $p \leq 0.05$.*

**Table 4.**
*Yield of greenhouse cucumber in mineral, artificial and organic substrate (kg/m²).*

yield of the investigated substrates in the examined months (**Table 4**) can be due, because different growing conditions, as:

- lower substrate temperatures and lower water holding capacities in urea foam and perlite
- higher temperatures and better sorption capacity in the organic substrate

The development of the leaf area of cucumber plants was examined for the variants with rockwool mats, urea foam and perlite. The following leaf areas were determined as the mean of all measuring dates: in rockwool 8215 cm$^2$, in urea foam 7889 cm$^2$, and in perlite 7438 cm$^2$.

## 5. Effects of biostimulators on the growth and cucumber plant development

### 5.1 Mineral and organic substrates used in substrate culture of cucumbers treated with biostimulators

In Europe, about 87 million sheep are produced, in Germany about 2.2 million. Often, there is a lack of capacity for cleaning the sheep wool, so unclean sheep wool is available as waste material.

The objective of this experiment was to investigate the development and yield of cucumber grown on sheep wool slabs in comparison with peat and coconut fiber slabs as well as Rockwool slabs and perlite in containers. Different sheep wool slabs in size and added components were tested, the highest stability was obtained with sheep wool slabs in combination with coconut fibers.

In this experiment following questions were investigated:

- Does sheep wool slabs have appropriate physical parameters for cucumber cultivation and how many months they can be used?
- How do the physical properties change in comparison with other organic and mineral substrates?
- How do biostimulators influence cucumber plant growth in the investigated substrates?

Sheep wool slabs used in this experiment were compared with the following substrates [12]:

- Coconut fiber (width 25 cm, length 100 cm, height after wetting 9 cm) from the company 'Dutch Plantin'.
- Peat slabs 'GroBoard' (width 20 cm, length 100 cm, height after wetting 9 cm) from the KEKKILÄ Oyj company Finland.
- Perlite, average grain size between 0.06 mm and 1.5 mm, properties see **Table 3** was filled in Container with 7 L volume.
- Rockwool slabs (width 20 cm, length 100 cm, height 7.5 cm) from the company 'Pargro' from Finland.

In this experiment fifty percent of the cucumber plants of each substrate variant were treated three times with a biostimulator solution containing 0.08% K-Humate (Fa. Humintech), 0.2% Lactofol (O) (Fa. ECOFOL) and a spore suspension ($10^7$ cfu $ml^{-1}$) of *Bacillus subtilis* FZB 24®. To the plants were applied 20 ml of this solution three times in weekly intervals starting with first treatment in 6–7 leaves stage, after transplanting. Plants of *Cucumis sativus* L. 'Indira' were used for the experiment. The experiment was conducted during two cultivation periods, first from November until April and second from June until November in the next year.

*5.1.1 Effects of biostimulators growing cucumbers in mineral and organic substrates*

For the soilless cultivation of the cucumber plants substrates was selected with different physical properties (**Table 5**). In general substrates for cucumbers should have high air capacity with a range between 20 to 40%, during the cultivation time often the air capacity is decreasing very much and water capacity increasing, therefore a certain stability is necessary in this regard. Sheep wool had the highest air capacity with about 70%, while peat had the lowest air capacities with 18%. The peat slabs were pressed to reduce the volume for the transport, the expansion of the slabs needs time. Because the low water capacity of sheep wool at the beginning, a water reserve is missing in case of low water availability. It seems that sheep wool and perlite requires a higher and more stable supply with nutrient solution than the other substrates. The physical properties after second use of the substrates

| Substrate | Before use in the experiment | | | After second use in the experiment | | |
|---|---|---|---|---|---|---|
| | AC (%) | WC (%) | PV (%) | AC (%) | WC (%) | PV (%) |
| Sheep wool slabs | 69.4 f | 22.8 a | 96.8 e | 43.1 cd | 44.1 cd | 87.2 abc |
| Peat slabs | 18.0 a | 68.0 g | 86.0 b | 30.7 b | 61.6 f | 92.3 d |
| Coconut fiber slabs | 30.6 b | 52.8 e | 83.9 a | 20.0 a | 72.3 g | 92.3 d |
| Perlite | 58.6 e | 31.6 b | 90.2 bcd | 41.4 c | 50.4 e | 91.8 d |
| Rockwool slabs | 49.2 d | 41.6 c | 90.7 cd | 17.2 a | 74.6 g | 90.1 bcd |

*AC – air capacity, WC – water capacity, PV - volume.*
*Different letters indicate significant differences (Tukey $P < 0.05$) within one parameter.*

**Table 5.**
*Physical properties of mineral and organic substrates for cucumber cultivation [12].*

were changed, in Coconut fiber and rockwool slabs the AC was below the target value.

The analyses of the mineral content of the substrates used for cucumber cultivation (**Table 6**) showed different results after the first and after second use. There was no accumulation of nutrients in the sheep wool, peat and coconut fiber slabs, but very high accumulation of $NO_3$ in perlite and and K in rockwool. Furthermore, In the coconut fiber slabs could be determined a high accumulation of $NO_3$ and Ca. In general, it can be stated that the nutrient values in the substrates do not show any unusual fluctuations. it must also be taken into account that the sorption capacity and the mineralization in the substrates are different, but the same nutrient solution was always added.

In all variants, the yield was higher in the second cultivation (**Table 7**) for all substrates tested. Furthermore, in all substrate variants the cucumber yield was

| Substrate | Nutrients | First cultivation (ppm) | Second cultivation (ppm) |
|---|---|---|---|
| Sheep wool slabs | $NO_3$ | 21.6 a | 23.6 a |
| Peat slabs | | 77.9 c | 77.1 bc |
| Coconut fiber slabs | | 68.2 c | 85.2 c |
| Perlite | | 34.7 b | 65.6 b |
| Rockwool slabs | | 345.1 d | 248.1 d |
| Sheep wool slabs | K | 28.4 a | 24.2 a |
| Peat slabs | | 115.7 d | 72.4 c |
| Coconut fiber slabs | | 113.2 d | 75.8 c |
| Perlite | | 55.3 b | 56.6 b |
| Rockwool slabs | | 70.4 c | 235.9 d |
| Sheep wool slabs | Ca | 33.8 b | 32.7 a |
| Peat slabs | | 64.9 d | 73.7 b |
| Coconut fiber slabs | | 55.7 c | 82.9 bc |
| Perlite | | 24.6 a | 92.3 cd |
| Rockwool slabs | | 115.3 e | 96.6 d |

*Different letters indicate significant differences (Tukey $P < 0.05$) within the cultivation.*

**Table 6.**
*Nutrient content in the tested substrates after the first and second cultivation of cucumbers.*

| Substrates | Yield (kg plant$^{-1}$) | | | |
|---|---|---|---|---|
| | First cultivation | | Second cultivation | |
| | Untreated | Treated | Untreated | Treated |
| Sheep wool slabs | 1.08 abcd | 1.94 d | 8.96 e | 10.07 f |
| Peat slabs | 0.57 a | 1.28 abcd | 7.49 c | 10.41 f |
| Coconut fiber slabs | 0.76 ab | 0.84 abc | 7.51 c | 8.09 d |
| Perlite | 0.77 ab | 1.16 abcd | 6.68 b | 8.95 e |
| Rockwool slabs | 1.47 bcd | 1.71 cd | 6.11 a | 9.16 e |

*Untreated – no application of biostimulators.*
*Treated – application of biostimulators (0.08% K-Humate, 0.2% Lactofol (O), spore suspension ($10^7$ cfu/ml) of* Bacillus subtilis *FZB 24® Different letters indicate significant differences (Tuckey $_{0.05}$; comparison within one cultivation).*

**Table 7.**
*Cucumber yield in organic substrates (sheep wool slabs, peat slabs, coconut fiber slabs) and mineral substrates (perlite, Rockwool slabs) not treated with biostimulators and treated with them [12].*

higher if the variants were treated with the biostimulators. The highest yield could be obtained for cucumber cultivated in the second cultivation in sheep wool slabs and peat slabs and treated with biostimulators. The lowest cucumber yield in the second year was obtained in the substrate variants rockwool slabs and perlite.

### 5.2 Effects on the nutrient supply in cucumber cultivation using biostimulators

Cucumber plants were grown in substrate culture using containers (volume 8 L) filled with perlite. The perlite from Slovakia had an average dry density of 120 kg/$m^3$ and a grain size between 0.6 and 1.5 mm diameter, with a pore volume of 84% v/v, water capacity 45% v/v and air capacity of 39%. Investigations with the organic biostimulators were undertaken regarding the effects:

- of different humate and lactate types, whereas from *Bacillus subtilis* was used the strain FZB24®,
- of concentrations and frequencies of biostimulators applications (humates, lactates and FZB24®),
- of the best method of applications (in the rhizosphere, to the growing media or in the nutrient solution, or direct to the leaves adaxial or abaxial)
- in stress situations during growth of the cucumber plants, e.g. salt stress (EC values), suboptimal pH and suboptimal temperature
- on the nutrient uptake of N, P, K, Ca, Mg and Fe

Nutrient solution was calculated with the HYDROFER program, considering the water quality and the target value during cucumber plant growth, in order to adjust the amounts of fertilizers, salts and acids required [13]. The target values for this experiment was 170 ppm N, 50 ppm P, 260 ppm K, 150 ppm Ca, 60 ppm Mg, 3 ppm Fe, S 80 ppm, the $HCO_3$ content was adjusted by 90 ppm. Nutrient solution was applied using trickle irrigation 2 to 4 times a day 250 ml per irrigation cycle in period of 12–15 min.

*5.2.1 Effects of biostimulators on the salt concentration (EC) in the rhizosphere*

Strong fluctuation of the salt concentration (EC) can lead to an imbalance of nutrient supply in hydroponic system and can decrease plant growth and yield as it was shown for tomatoes [14]. The negative effects on cucumber plant growth if the EC values (EC 8 mS $cm^{-1}$) is very high could be positively influenced by application of humates and *B. subtilis* (FZB24®) separately or combined. This is probably an effect by the encouraging of root growth. Lactate (LACTOFOL) application had no effect in this regard. Even if the nutrient solution has the appropriate salt concentration (EC), in substrate culture, with increasing duration of the cucumber cultivation there could be an accumulation of salts mainly based on those nutrients, which are not necessary in the amount as applicate. [15]. This could lead to salt stress and reduced yield in crops like cucumber. Application of Humate and/or *Bacillus subtilis* FZB24® reduced this salt accumulation (**Figure 2**).

K-Humate showed the highest efficiency for EC stabilization this Humate was even more effective than *B. subtilis* (FZB24®). The mixture of all three compounds, however, was as effective as Humate alone and stabilized the salt concentration (EC) at about a value of 2 EC. The stabilizing effect of the salt concentration could be maintained over weeks after the last application indicating that there could be a

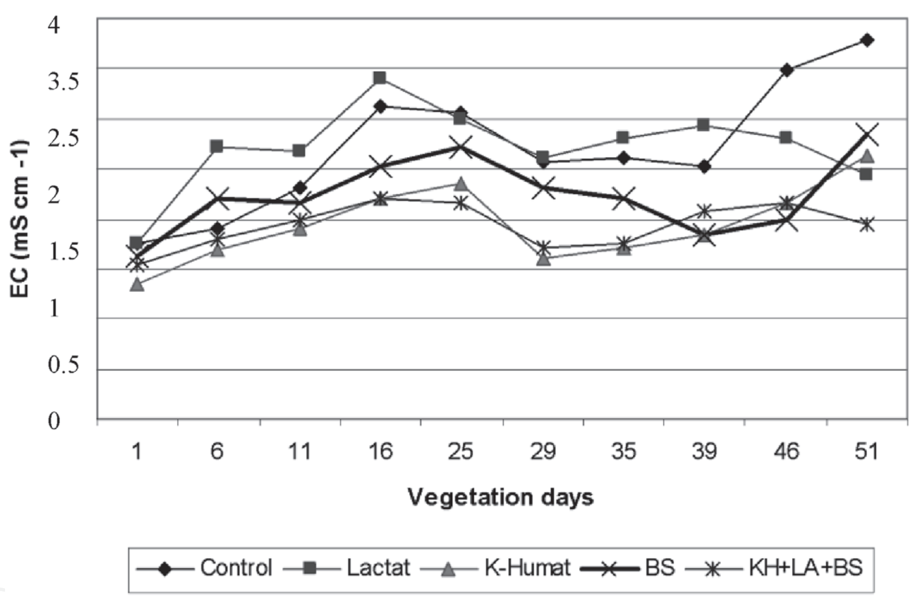


**Figure 2.**
*Effect of biostimulators (0.1% lactate, 0.01% K-Humate, 0.2%* B. subtilis *FZB24®) on EC development in the substrate during cultivation of cucumber [15].*

| Variants | Shoot weight (g $plant^{-1}$) | Leaf weight (g $plant^{-1}$) | Fruits per plant |
|---|---|---|---|
| Control | 676.00 d | 172.33 c | 7.0 ns |
| 0.1% Lactate | 817.50 bc | 203.00 b | 10.0 ns |
| 0.01% K-Humate | 776.33 c | 203.67 b | 7.8 ns |
| 0.2% B.s FZB24® | 839.33 b | 202.83 b | 10.4 ns |
| K-Humate, Lactate, B.s FZB24® | 911.83 a | 235.33 a | 7.4 ns |

*Different letters indicate significant differences, LSD, $p \leq 0.05$); ns = non-significant.*

**Table 8.**
*Growth parameters of cucumber plants treated or non-treated with biostimulators [15].*

sustainable culture of the bacteria and adsorption of the Humate on the perlite. The increase of EC from 1.5 to 3.6 was not so strong, but plants treated with biostimulators grew more vigorously and had in tendency a higher yield (**Table 8**).

### *5.2.2 Effects on pH values in the rhizosphere*

For plants, which are adopted on lower pH, like tomatoes and beans [14, 16] it is well known that, pH values higher than 5.7 in substrates can disturb plant growth. In some growing media as rockwool or perlite, the initial pH is higher than 6.5. To change the pH by additional preparation of the substrates before using with a nutrient solution having lower pH takes time and is sometimes difficult. During the cultivation of the plants to change the pH value is also not always successful. Therefore, different treatments with biostimulators were tested. Application of Lactate stimulated root growth and shoot development even at pH 7.5. In general, pH values affect the nutrient availability and uptake, in particular of micronutrients. As recorded in experiments with cucumber [15], the pH of substrates in soilless culture systems, changed with the duration of cultivation (**Figure 3**) and declined in the control to 5.2. The pH of the substrates treated with biostimulators was more stable, especially if *B. subtilis* FZB24® was added to the nutrient solution as single component or in combination with the other compounds. The nutrient uptake was positive influenced even the pH was higher than recommend.

### *5.2.3 Effects on growth of cucumber plants*

The evaluation of growth parameters of cucumber plants showed if they were treated with biostimulators then shoot and total leaf weight was significant higher than non-treated plants especially if the plants treated with all three components (**Table 8**). For the mean number of fruits harvested per plant in this short-term experiment, no significant differences between the variants, control and different treatments with biostimulators.

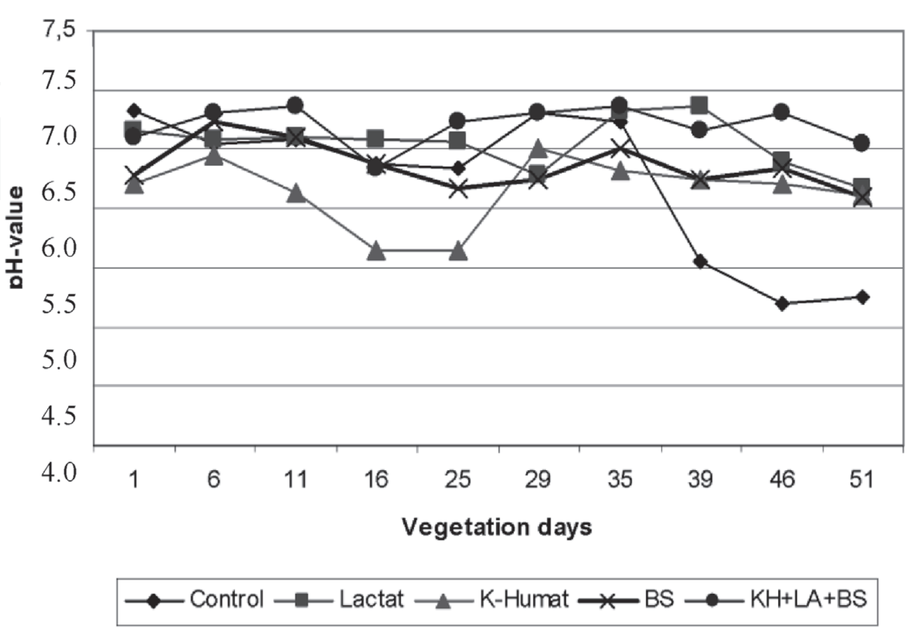


**Figure 3.**
*Effect of biostimulators (0.1% lactate, 0.01% K-Humate, 0.2%* B. subtilis *FZB24®) on pH development in the substrate during cultivation of cucumber [15].*

It can be assumed that the EC and pH stabilizing effects of the biostimulators contributed that better development of the plant, in particular if all three components are used together.

## 5.3 Effect of biostimulators and their application method on growing of cucumber plants

The bio-substances and Bacillus subtilis used as biostimulators had beneficial effects on plant growth in several experiments, also in stress situations, as inappropriate EC and pH value. The biostimulators were used in single applications or as a mix in the rhizosphere, but the lactate was used at the beginning as foliar-fertilizer [14]. Therefore, it came to the thought to use the biostimulators to the root zone and on the leaves. The aim was to investigated which treatment is the most effective one.

Experimental design is shown in **Table 9**, eight different treatments were compared with the control.

K-Humate (Fa. Humintech), Lactate (Fa. ECOFOL, **Table 2**) and *B. subtilis* FZB24® (Fa. ABITEP GmbH) was applied on leaves or on the substrate used in this experiment. Quantity and concentration of applied substances were deduces from previous experience [14].

Cucumber plants were planted and cultivated in containers (volume of 7–8 liters) filled with Perlite. Nutrient solution was calculated following the HYDROFER program [13] to adjust the amounts of fertilizers, salts and acids required according the values (170 ppm N, 50 ppm P, 260 ppm K, 150 ppm Ca, 60 ppm Mg, 3 ppm Fe, 90 ppm HCO3). By trickle irrigation 2 to 4 times a day 250 ml per irrigation cycle was applied in period of 12–15 min.

Additional the plants were treated with one of the treatments, 20 ml per container and plant, three times in weekly intervals in following development stages: the first treatment in 5–6 leafs stage; second in 7–8 leafs stage; third in 9–10 leafs stage. For the variant -Leaf application- the different treatments were sprayed on the surface of leafs. In case of variant -Root application- the different treatments were given to the substrate and thereby into the rhizosphere of the plants, in the same amount and frequency.

### *5.3.1 Shoot development*

The application of biostimulators three times in the growing stage (week 4, 5, and 6) affected development and yield of cucumber plants. The application of the biostimulators stimulated the growth represented by a higher fresh matter of shoots and leaves in most variants (**Figure 4**). Obviously, the location of application was important for the effect of the biostimulators. The application to the root zone

| Treatment | Concentration of substances | Leaf application | Root application |
|---|---|---|---|
| Control | — | | |
| Lactate | 0,08% | X | X |
| K-Humate | 0,2% | X | X |
| *Bacillus subtilis* (FZB24) | Spore suspension (10/cfu/ml) | X | X |
| Lactate +K-Humate + *Bacillus subtilis* | Above mentioned concentration | X | X |

**Table 9.**
*Concentrations and application patterns of biostimulators used in the experiment [17].*

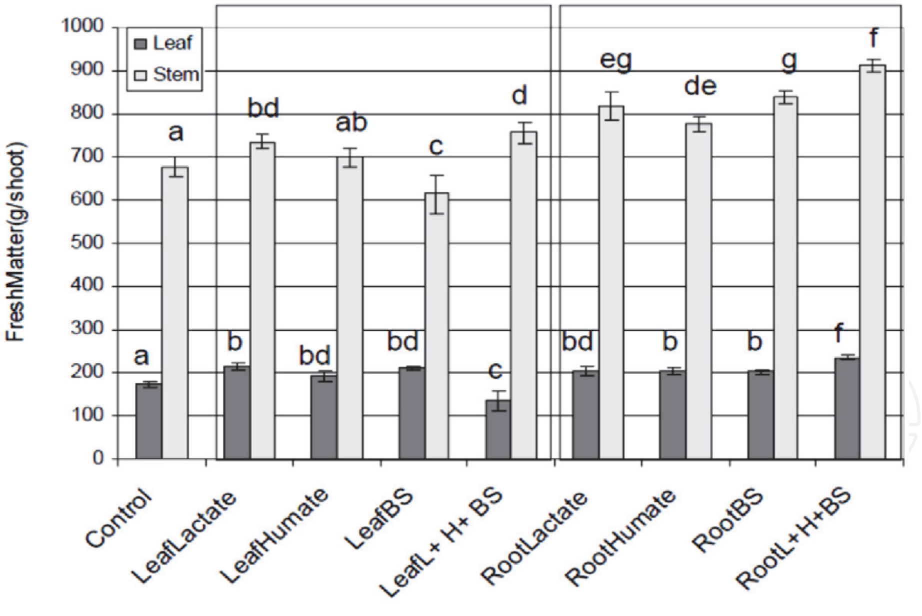


**Figure 4.**
*Fresh matter of leaves and shoots of cucumber plants after biostimulator application (lactate, K-Humate, B.subtilis BS) on leaves and roots respectively [17]. Different letters indicate significant differences (LSD, P = 0.05).*

induced in each case to a higher fresh matter compared to the control. If the Biostimulators were applied on the leaves, the effect on shoot fresh matter was not as strong in comparison to the application in the root zone. When *B. subtilis* (FZB24®) was used, the fresh mass of the shoot was even lower. It can be stated, if the combined biostimulator with all substances was applied the effect was different if applied over the roots was a stimulating effect visible, whereas if applied over the leaves an inhibiting effect for cucumber plant growth.

Comparing the effect of the treatments, also the quality of shoots and leaves seems to be different and effects on the weakness against fungi's could be expected. This effect was also found in experiments with Water spinach [18] however, in these experiments the effect on the root growth was much stronger than on the shoot growth.

Comparing the ratio between shoot and leaf fresh matter (**Figure 5**) there are no significant differences between all variants, but it seems the leaf application stronger stimulateed the leaf growth than shoot growth resulting in a lower ratio.

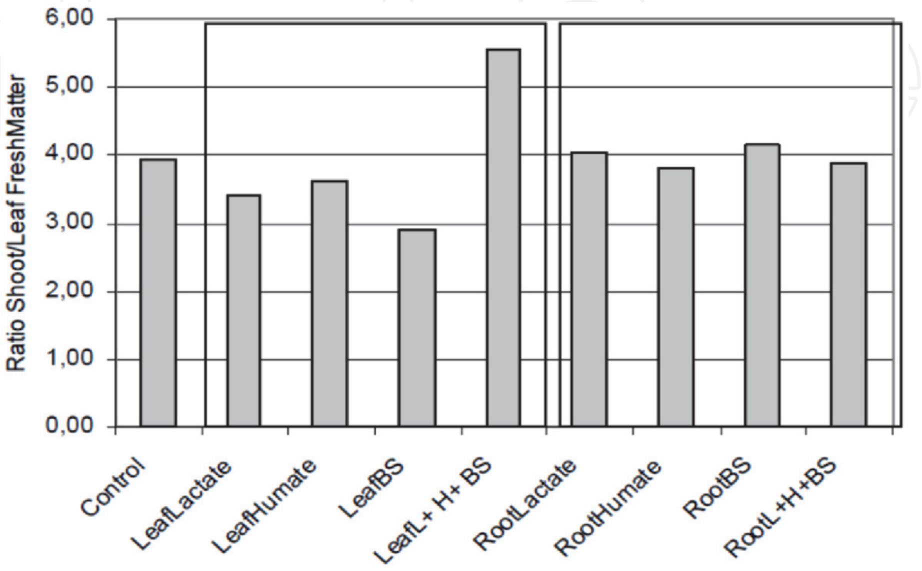


**Figure 5.**
*Effect of application biostimulators (lactate, K-Humate, FZB24®) on leaves and roots respectively on the ratio of shoots and leaf biomass after finishing the experiment [17]. No significant differences.*

However, the leaf treatment with the combination of all substances led to a reduction in leaf growth. After application of biostimulators via roots more or less the same ratio was found as in the control indicating the shoot and leaf growth was stimulated in the same manner.

### *5.3.2 Quantity of fruit harvest and marketable fruit quality*

The number of fruits harvested from the eight variants and the control was higher on average when the biostimulators were applied to the rhizosphere directly at the roots (**Figure 6**). The fresh weight of all cucumbers with market quality was about 500 g, therefore the number of fruits is representative for the cucumber yield. In particular, the treatment with biostimulators on the substrate that means to the root system, resulted in a higher yield at the first harvest. The number of fruits finally harvested was considerably higher after treatment of roots with Lactate and *B. subtilis* (FZB24®). The number of marketable fruits was higher than in the control in most variants treated with biostimulators.

For future experiments should include more applications also during the fruit set because these additional applications could enhance the yield further, this could be especially important in long time cultivation.

The different application methods with Biostimulators on the leafs and to the roots have not only an influence on cucumber plant growth and yield, but also on the amount of marketable and non-marketable fruits (**Figure 7**). The percentage of non-marketable fruits (C class) was more than 25% in the control and could be reduced by leaf application of each Biostimulator investigated until 20% and even until 10% if substances were applied over the roots.

## 5.4 Use of biostimulators to reduce abiotic stress in cucumber plants

Different bacteria and in particular *Bacillus subtilis* are well known for their effects against soil-born fungal and bacterial diseases. There are selected strains in this regard with good effects in the field of plant protection. The strain *Bacillus subtilis* FZB24 is in addition capable of evolving different kinds of stress protective

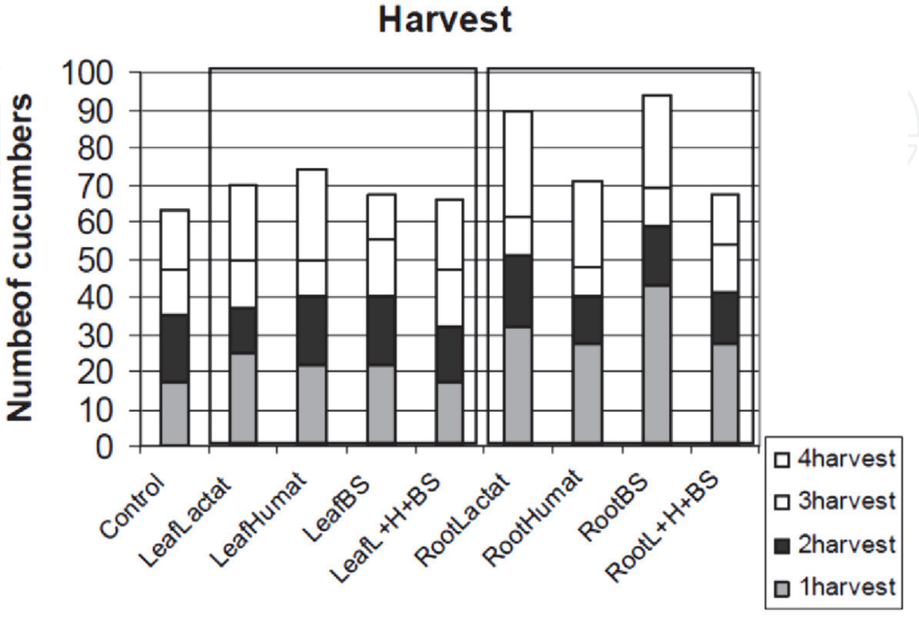


**Figure 6.**
*Effect of application biostimulators (lactate, K-Humate, FZB24®) on leaves and roots respectively on number of marketable fruits in four harvesting periods of 9 days each. No significant differences.*

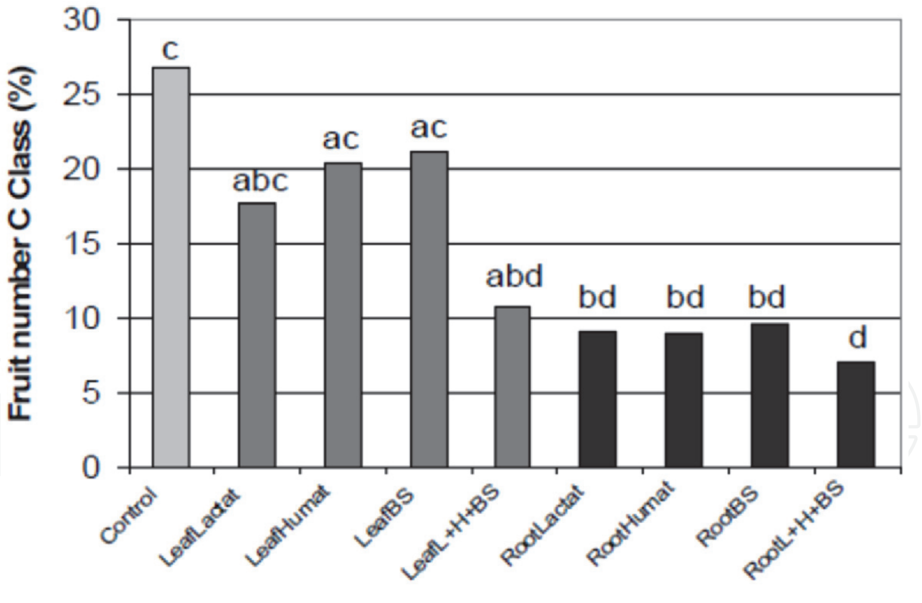


**Figure 7.**
*Effect of application biostimulators (lactate, K-Humate, FZB24®) on leaves and roots respectively on the percentage on non-marketable fruits. Different letters indicate significant differences (chi-square-test, P = 0.05).*

mechanisms including stimulation of plants' self-defense mechanisms, as it was demonstrated in chapter 5.2.1 and 5.2.2 in case of suboptimal pH, EC values. Furthermore, *Bacillus subtilis* can accelerate plant growth and stimulate the process of formation of plant organs. Furthermore, *B. subtilis* can increase the unspecific resistances of plants against stress conditions, such as extreme high temperatures, frost, drought, strong radiation, and deficiency of plant nutrients [18, 19].

Application of lactates in the form of LACTOFOL (**Table 2**) tends to reduce plant stress under suboptimal pH levels of nutrient solution (chapter 5.2.2) [6, 14, 16]. Introduction of Lactate as LACTOFOL into the growing system increases availability of micro- and macro-nutrients for plants. Investigations have shown that lactates have more stable bonds with several metal ions than other chelates do. Therefore, lactates have been used as foliar fertilizers and as well as bioregulators.

In greenhouse cultivation of cucumbers in soilless culture systems, two stress situations are of importance, the pH and the temperature stress. In an experiment was the aim to investigate the physiological effect of a biostimulating complex consisting of *B. subtilis* (FZB24®), K-Humate and Lactate as LACTOFOL (**Table 2**) on the growth of cucumber. The aim was to investigate the photosynthetic reactions to clarify first stress responses in the cucumber plants. Measuring of Chlorophyll Fluorescence is a very useful method for evaluation of plants' photosynthetic conditions and a tool in non-invasive stress detection and its subsequent evaluation [12].

### *5.4.1 Experimental design*

(*Cucumis sativus* L.) cv. Jessica was cultivated in 'Mitscherlich' container volume 8 L filled with perlite with a physical and chemical properties as described in **Table 3**. The same nutrient solution was used as described in chapter 5.3. The cucumber plants were cultivated in a climatic chamber at 25°C and 80% RH.

One half of the cucumber plants were not treated with the Biostimulator solution. The substrate of the other half of the plants was treated once a week with 300 ml of biostimulator solution (*B. subtilis* (0.2%) + K-humate (0.01%) + lactate (0.1%) per container. Treatments coincided with the following plant developmental stages, first treatment at 5–6 leaf stage (week 1); second at 7–8 leaf stage (2 weeks) and the third at 9–10 leaf stage (3 weeks).

After the last treatment with the biostimulator solution (4 weeks), the stress factor was applied. For the pH stress experiment, pH values were adjusted to a suboptimal level (pH 3.2) by adding $H_3PO_4$ to the nutrient solution. This pH stress was maintained for 1 week. For temperature stress, temperature in the growth chamber was lowered from 25 to 6°C for 3 h.

#### *5.4.2 pH stress for cucumber plants*

One week after transplanting the cucumber plants the chlorophyll fluorescence Fv/Fm-value increased from 0.760 (**Figure 8**) to 0.790 in plants treated with biostimulating complex and 0.770 in plants without treatment. A drastic decrease in electron efficiency was observed after imposition of a strong lowering the pH value. Between the 4th and 5th measurements, Fv/Fm of treated plants decreased to 0.747 and that of the non-treated ones even to 0.654.

Whereas without biostimulator treatment, the fluorescence Fv/Fm-value was slightly decreasing after second and third week. In the time of the pH stress influence in the fifths week, it is visible, the stress effect was much stronger (**Figure 8**), where the lowest Fv/Fm value was 0.620. Four weeks later the plants treated with the biostimulator treatment recovered completely from the stress, but the plants without Biostimulator were still in weak condition. The plants treated with biostimulator mixture showed a higher electron efficiency of photosystem II (0.765 Fv/Fm-value) at the end of the experiment as compared to the plants without treatment (0.670 Fv/Fm-value).

The final evaluation of cucumber plants showed that this plants treated with biostimulators had significant shorter shoot length and but heavier weight than non-treated plants (**Table 10**). The roots were longer than non-treated plants and treated plants also yielded some marketable fruits (data not shown). Obviously, the biostimulators mixture was effective for reduction of the pH-stress.

#### *5.4.3 Temperature stress for cucumber plants*

Temperature stress can be happens in greenhouses if the heating system is not working or the ventilation is not proper functioning. Therefore, this stress was

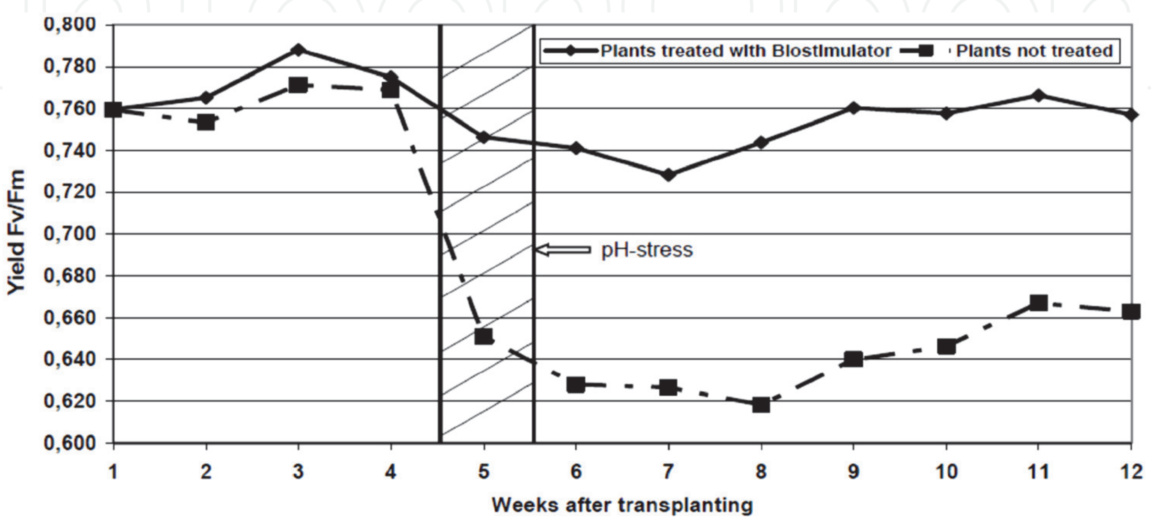


**Figure 8.**
*Electron efficiency in photosystem II of cucumber plants treated with biostimulators mixture before and after pH-stress (pH 3.2) for one week. [12].*

| Variants | Shoot length (cm) | Shoot weight (g/ plant) | Leaf weight (g/plant) | Leaf area ($cm^2$/plant) | Root weight (g/plant) | Root length (m/plant) |
|---|---|---|---|---|---|---|
| Not-treated | 325.75 b | 230,25 b | 460 ns | 7002.3 ns | 275.75 ns | 45.08 ns |
| Treated with biostimulator | 315.25 a | 350 a | 477.75 ns | 7105.2 ns | 265.5 ns | 52.93 ns |

*Different letters indicate significant differences (LSD, p = 0.05); and ns = non-significant).*

**Table 10.**
*Growth parameters of cucumber plants treated with biostimulators mixture prior to pH- stress (pH 3.2) for one week [12].*

given for a short time from three hours and the lowest temperature was 6°C. This stress was applied by lowering the air temperature in the climate chamber right after the third treatment with the biostimulating mixture.

The Fv/Fm parameter had the same pattern as in case of pH stress. However, Fv/Fm development showed its peculiarities (**Figure 9**). Measurement 1 to 4 gave equal electron efficiency levels. After temperature stress, Fv/Fm values decreased considerably indicating a reduction in photosystem II efficiency. Only the treated plants were able to reach higher levels of Fv/Fm after stress and could recover much better than non-treated plants.

At the end of this temperature stress experiment, the plant growth parameters were also determined (**Table 11**). The effect of the biostimulator mixture led to a

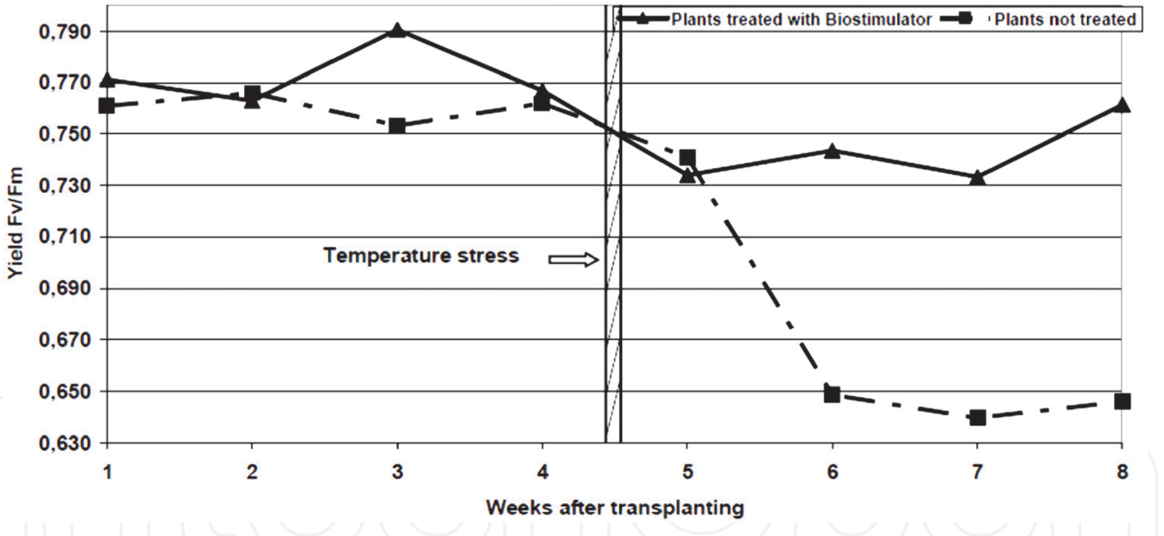


**Figure 9.**
*Electron efficiency in photosystem II of cucumber plants treated with biostimulators mixture before and after low temperature treatment with 6°C for 3 h. [12].*

| Variants | Shoot length (cm) | Shoot weight (g/plant) | Leaf weight (g/plant) | Leaf area ($cm^2$/plant) | Root weight (g/plant) | Root length (m/plant) |
|---|---|---|---|---|---|---|
| Not-treated | 341,75 b | 244,5 b | 477,75 ns | 7333,7 b | 206 b | 4,86 b |
| Treated with biostimulator | 390 a | 358,5 a | 548,25 ns | 9637,4 a | 321,25 a | 6,98 a |

*Different letters indicate significant differences (LSD, p = 0.05); and ns = non-significant.*

**Table 11.**
*Growth parameters of cucumber plants treated with biostimulator mix prior to temperature - stress at 6°C for 3 h [12].*

significant difference in all parameters as compared to the non-treated plants, except for the leaf area.

The question is what could be the reason, that the temperature stress was less disturbing the cucumber plant if the plants were several time treated with Biostimulator. The biostimulators used in this experiment had shown also in previous experiments a positive reaction on the root growth [14]. It can be assumed plants with well-developed root systems have higher resistance against different stress situations. Therefore, a correlation between the green biomass of the cucumber plants and the root mass were calculated. In the experiments without treatments with biostimulators, no correlation could be found (**Figure 10**).

On the other hand, those cucumber plants treated with biostimulators showed a very close correlation ($R^2$ linear =0.949) between green biomass and mass of roots (**Figure 11**). This close relationship confirms the hypothesis that increases in root mass lead to formation of larger shoots and leaf mass even under stress conditions if treated with some biostimulators.

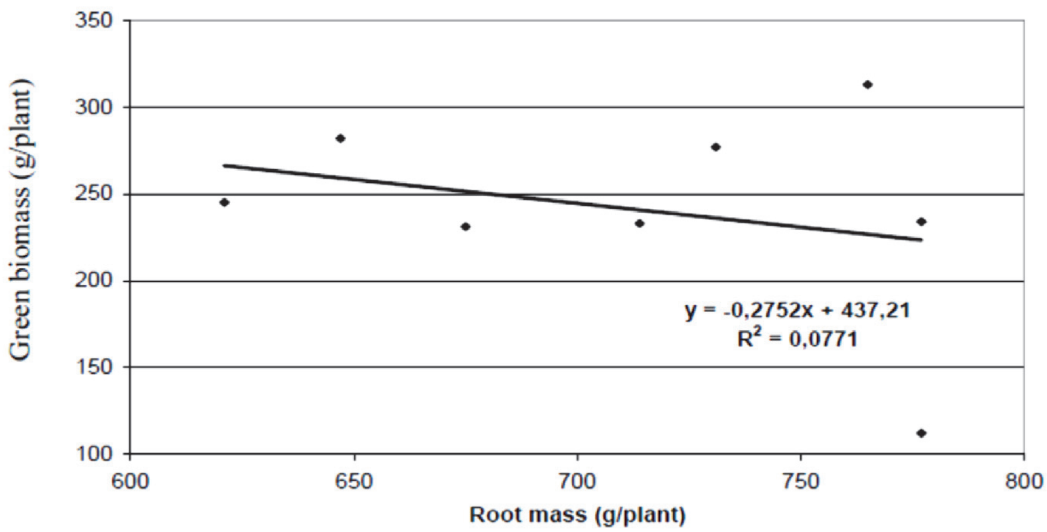


**Figure 10.**
*Correlation between green biomass and root mass of cucumbers in plants exposed to pH and temperature stress condition, without biostimulators treatment [12].*

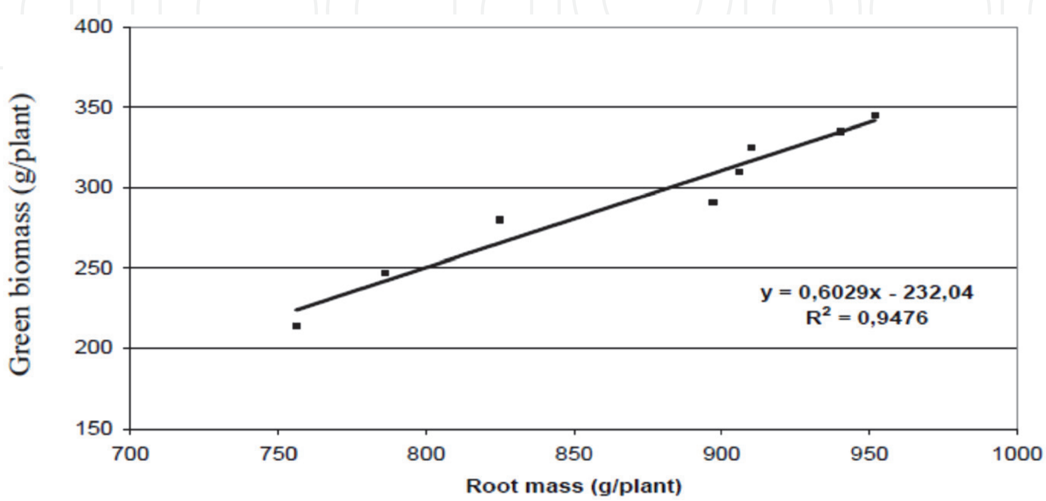


**Figure 11.**
*Correlation between green biomass and root mass of cucumbers in the experiment with pH and temperature stress condition, with biostimulators treatments [12].*

## 6. Conclusions

In general can be stated, intensively in greenhouses cultivated crops such as cucumbers, suffer often from inadequate abiotic or biotic growth conditions in particular in the rhizosphere. Many studies were done to find growth promoters or biostimulators to stabilize the production process or to enhance plant growth of cucumbers under these conditions. One task was to find substrates with appropriate physical and chemical properties for root growth of cucumbers desirable from organic materials. Sheep wool slabs, peat slabs and coconut fiber slabs were therefore successful tested with and without Biostimulator treatments and compared with perlite and rockwool slabs. It was to decide which of the biostimulators and their modifications should be tested and could be recommended. Based on the research with different Humates the K-Humate was selected. From the different lactates the LACTOFOL"O" (CO. ECOFOL) a foliar fertilizer was chosen after many previous experiments. From the different strains of Bacillus subtilis the strain FZB24® was successful tested and can be recommended. Under the growing conditions in soilless culture, using different substrates a buffer is missing completely or partly for regulation of the nutrient availability and regulation of the sorption capacity as in the natural soil. Therefore, different stress situations can occur, the Biostimulators used in all investigations as single component or in treatments combined in order to reduce such stress situation. Very successful was used for cucumber plants growing in substrate with a high EC value the application of K-Humate and *B. subtilis* (FZB24®) as single component and combined the salt concentration could be stabilized convenient for cucumbers. The mixture of all three components, however, was as effective as Humate alone and stabilized the salt concentration (EC) at about a value of 2 EC.

In general, pH values affect the nutrient availability and uptake, in particular of micronutrients. The pH of substrates in soilless culture systems, changed with the duration of cultivation. Application of Lactate (LACTOFOL) and *B. subtilis* (FZB24®) stimulated root growth and shoot development even at pH 7.5 and the pH of the substrates treated with biostimulators was more stable.

Application of the biostimulators solution with all three components BS-FZB24® (0.2%), K-Humate (0.01%), and LACTOFOL "O" (0.1%) were tested regarding their effects in case of strong but short time pH and temperature stress the growth of cucumber plants. The chlorophyll fluorescence Fv/Fm value showed a positive effect of the curative biostimulator treatments under the stress counteraction in plants. Results showed that there was strong correlation between green biomass of treated cucumber plants and their root mass. It can be assumed that the effect of stress prevention by the biostimulator was based mainly on enhancing the root growth.

In the experiments using the biostimulator for stabilization of cucumber plant growth, the biostimulators were applicate only in the rhizosphere that means direct to the roots. Lactate was originally developed and used as foliar-fertilizer. Therefore, it came to the thought to use the biostimulators to the root zone and on the leaves. Following the experimental results, it can be assumed, the application of the combined biostimulators with all substances if applied over the roots was a stimulating effect visible, whereas if applied over the leaves an inhibiting effect for cucumber plant growth. The number of fruits harvested from all variants and the control was higher on average when the biostimulators were applied to the rhizosphere directly at the roots in comparison to the leaf application.

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## References

[1] Geissler T. Böhme, M. Lankow (1991). Gemüseproduktion unter Glas und Plasten -Produktionsverfahren - Deutscher Landwirtschaftsverlag Berlin, Hochschullehrbuch; 6 Auflage.

[2] Schippers, B. 1992. Prospects for management of natural suppressiveness to control soilborne pathogenes. In E. C. Tjamos, G. C. Papavizas, & R. J. Cook (Eds.), Biological control of plant diseases, progress and challenges for the future (NATO ASI Series A: Life Sciences), Vol. 230, pp. 21–34). New York: Plenum Press.

[3] Kavetskiy, S.; M. Kraynyuk; G. Hofman; M. Böhme and O. Cleemput (2003). Soil Quality and fertilization. Tempus Tacis, NAUU, Kiev. ISBN 966-8006-20-8, p. 103–167

[4] Böhme, M., M. Dewenter and A. Gohlke (2020). Aquaponics using Asian leafy vegetables – potential and challenge. Acta Hortic. 1273. ISHS 2020. DOI 10.17660/ActaHortic1273 p. 155-122

[5] Pavlova, A., Batschvarov, P. 1992. Listno Podchranvane na Rastenijata cac Suspensioni torove LACTOFOL. Priloshenie na Suspensionite Torove, Proceedings: 1-28, Sofia

[6] Shaban, N., Manolov, I., Khadum, E., Rankov, V. (1995). Complex Assesment of the Effect of Suspension Foliar Fertilizer LACTOFOL 'O' on Cucumber. Agroeco, Proceedings, Plovdiv, 2: 33-41

[7] Frimmel F.H. and R.F. Christman (1988). Humin Substances and Their Role in the Environment. 1st ed. Vol.1., Wiley-Interscience Publication: New York.

[8] Krebs, B., Höding, B., Kübart, S., Alemayehu, M. W., Junge, H., Schmiedeknecht, G., Grosch, R., Bochow, H., & Hevesi, M. (1998). Use of Bacillus subtilis as biocontrol agent. I. Activities and characterization of Bacillus subtilis strains. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 105, 181–197.

[9] Elsorra, E., Idris, H., Bochow, H., Ross, H., & Borriss, R. (2004). Use of Bacillus subtilis as bio- control agent. VI. Phytohormonelike action of culture filtrates prepared from plant growth promoting Bacillus amyloliquefaciens FZB24, FZB42, FZB45 and Bacillus subtilis FZB37. Journal of Plant Diseases and Protection, 111(6), 583–597, ISSN 0340-8159.

[10] Junge, H., Krebs, B., & Kilian, M. (2000). Strain selection, production, and formulation of the biological plant vitality enhancing agent FZB24® Bacillus subtilis. Pflanzenschutz-Nachrichten Bayer, 1(1), 94–104.

[11] Kilian, M., Steiner, U., Krebs, B., Junge, H., Schmiedeknecht, G., & Hain, R. (2000). FZB24® Bacillus subtilis – mode of action of a microbial agent enhancing plant vitality. Pflanzenschutz- Nachrichten Bayer, 1 (1), 72–93.

[12] Böhme, M., J. Schevchenko, I. Pinker , S. Herfort (2008). Cucumber grown in sheepwool slabs treated with biostimulator compared to other organic and mineral substrates. Acta Hortic. 779, p. 299–306,

[13] Böhme, M. (1993). Parameters for calculating nutrient solution for hydroponics. Eighth international congress on soilless culture, Hunters Rest, Proceedings, Wageningen, 85–96.

[14] Böhme, M. (1999). Effects of Lactate, Humate and *Bacillus subtilis* on the growth of tomato plants in hydroponic systems, Symposium on growing media & hydroponics, Windsor, Canada, Acta Hortic. (ISHS) 465, p. 231-239

[15] Böhme, M., I. Pinker, Y. Schevchenko (2007). Effects of biostimulators on nutrient supply in vegetables.: Procc Gent.. pp. 6:

[16] Böhme, M., Shaban, N. and Abdelaziz, O. (2000). Reaction of Some Vegetable Crops to treatments with Lactat as Bioregulator and Fertilizer. Acta Hortic. 514, 33–40.

[17] Böhme, M., Schevtschenko, J. and Pinker, I. (2005). Effect of biostimulators on growth of vegetables in hydroponical systems. Acta Hortic. 697:337–344.

[18] Hoang, T. L. (2003). Untersuchungen zur Wirkung von Huminsäure auf das Wachstum und die Nährstoffaufnahme von Tomaten (*Lycopersicon esculentum* MILL) und Wasserspinat (*Ipomoea aquatica* NORSSK) [PhD thesis], Humboldt-Universität zu Berlin, Landwirtschaftlich-Gärtnerische Fakultät.

[19] Hoang, T. L., & Böhme, M. (2001). Influence of humic acid on the growth of tomato in hydroponic systems. Acta Horticulturae, 548, 451–458.