Effect of Biostimulators on Growth of Vegetables in Hydroponical Systems

M. Boehme, J. Schevtschenko and I. Pinker
Humboldt University of Berlin, Institute of Horticultural Sciences, Lentzeallee 75
D-14195 Berlin
Germany

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Abstract
Biostimulators could be used to stabilize hydroponical systems and to counteract stress situations. The experiment aimed to investigate the effect of lactic acid (LACTOFOL O), K-Humate and Bacillus subtilis as biostimulators in substrate culture of Cucumis sativus cv. Jessica. All substances were applied separately or in combination on leaves and roots respectively, in total 8 different treatments have been compared with the control. For each treatment 20 ml of 0.08% LACTOFOL O, 0.2% K-Humate or $10^7$ cfu/ml spore suspension of Bacillus subtilis were applied three times in weekly intervals during the 5 till 10 leaf stage. Biomass of shoots and leaves was enhanced due to the application of these substances. Also the dry matter content was higher in treated plants than in the control. After application of each biostimulator investigated to the roots the number of marketable cucumbers was enhanced in total and in relation to the non-marketable fruits. The application of K-Humate enhanced also the dry matter content of the cucumbers. The application in the root zone had a stronger effect than the application on the leaves and should be preferred. The combined application of all substances was possible but should be investigated further.

INTRODUCTION
Since about 15 years, also substances with biological origin have been used to avoid or counteract abiotic or biotic stress in horticulture. In hydroponics, investigations about effect of such biostimulators are scares until now.

In previous investigations we found beneficial effects in hydroponics of the gram-negative rhizobacterium Bacillus subtilis FZB 24® regarding the reduction of salt stress (Boehme, 1999) and its effects against fungal and bacterial diseases are also proved (Loeffler et al., 1968; Krebs et al., 1998; Schmiedeknecht et al., 1998; Grosch et al., 1999).

Beside microorganisms, also organic substances with different chemical composition can be used as biostimulators, e.g. humates and lactates. Also for these substances growth stimulating and stress-reducing effects could be shown in hydroponics (Boehme, 1999; Boehme et al.; 2000, Hoang, 2003). Humates well know, as main components of soil fertility have so far more or less no importance in hydroponics. However, some very interesting effects of humates are described concerning their stimulating effect on nutrient uptake (Fortun and Lopez, 1982; Tattini et al., 1989), counteracting salt and drought stress as well as temperature stress. The positive effect of humates on availability and uptake of nutrients like calcium, magnesium, and phosphorus due to chelating should be stressed.

Lactates, salts of lactic acid, can be also used to chelate nutrients, especially micronutrients. Stress reducing effects of lactates could be found especially in nutrient solutions with too low or too high pH values and also in stress situations because of extreme temperature (Boehme et al., 2000). Several products of a Bulgarian company ECOFOL are offered as foliar fertilizer with the brand name LACTOFOL.

All substances described have beneficial effects on plants in stress situations, however, their main effects they have in different stresses. Therefore, a combination of these substances should be investigated to stabilize the growing conditions. The experiment presented here was done with cucumber a very sensitive plant even under standard conditions. It should be find out if such combination of these substances supports their beneficial effects or if they interfere with each other. The biostimulators can be applied in the root zone or on the leaves. It should be investigated which treatment is the most effective one.
MATERIAL AND METHODS

Plant Material and Growing Conditions

Plants of *Cucumis sativus* L. cv. Jessica were used for the experiment. Cucumber seeds of cultivar Jessica were sown on 15-th of May 2003. Planting was conducted on 10-th of June.

For the investigation in large scale in greenhouse, cucumber plants were grown in substrate culture using containers with perlite. Perlite with an average dry density of 120 kg * m^-3^ was used. The grain size was between 0.06mm and 1.5mm, with 45% of all grains having been 1mm in diameter. Pore volume was 84% v/v, the water holding capacity was 45% v/v, and the air capacity was 39%.

Nutrient solution was calculated with the HYDROFER computer program to adjust the amounts of fertilisers, salts and acids required (Boehme, 1993) according the values (170 ppm N, 50 ppm P, 260 ppm K, 150 ppm Ca, 60 ppm Mg, 3 ppm Fe, 90 ppm HCO_3^-, 80 ppm S). By trickle irrigation 2 to 4 times a day 250ml per irrigation cycle was applied in period of 12-15 min.

Current experiment covers the period from 15-th of May through beginning of August 2003. Average temperature during the day was equal to 27.5°C and during the night 23.5°C.

Experimental Design

Eight different treatments were compared with the control (Table 1). K-Humate (Fa. Humintech), Lactofol (O) (Fa. ECOFOL, Table 2) and *Bacillus subtilis* FZB 24® (Fa. FZB) was applied on leaves or in the nutrient solution. Quantity and concentration of applied substances were deduced from either previous experience (Böhme, 1999) or by using application instructions of manufacturer (*Bacillus subtilis* FZB 24®). Timing of application coincided with transplanting of seedlings into big vegetation pots (Mitscherlich Pots with volume of 8 litres). Plants were treated three times in weekly intervals in following development stages: first treatment - 5-6 leaves stage; second: 7-8 leaves stage; third: 9-10 leaves stage.

Quantity of substances applied is 20 ml per pot and plant. Leaf application was conducted through spraying of the solution of given substances on surface of the leaves. Watering in substrate was conducted through direct application of solutions into the root area of the plants.

Data Collection

In the course of the experiment plant height, number of leaves, leaf area as well as EC value, pH value and nitrate content of the substrate were recorded weekly. The pH in the control dropped down from 6.8 to 5.3. All treatments started with same pH, but after the experiment the pH values had more or less the same value as in the beginning. The initial EC values ranged from 1.3 till 1.9. In the control and in the leaf treatments the EC raised to 3.7. The variant with root treatment, however, had EC values between 1.9 and 2.8.

Harvest of cucumbers started on the first of July and finished on the fourth of August. For evaluation the yield of 9 days were pooled. The quality of cucumbers was evaluated concerning number of marketable and non-marketable fruits, weight, length, and diameter of each fruit. After finishing the experiment three plants of each treatment were evaluated concerning fresh and dry matter of shoots and leaves.

Statistics

The experiment comprised 9 plants per treatment (3 x 3 replicates) randomized distributed. Data were evaluated by ANOVA (SPSS) and the statistic tests Chi square (Pearson) and LSD.

RESULTS AND DISCUSSION

A three times application of biostimulators in the growing stage (week 4, 5, and 6) affected growth and yield of cucumber plants.

Shoot Development

The application of all substances tested stimulated the shoot development represented by a higher fresh matter of shoots and leaves in most variants (Fig. 1a). Obviously the
location of application was important for the effect of the biostimulators. The application in the root zone led in each case to a higher fresh matter compared to the control. If the substances were applied over the leaves the effect on shoot fresh matter was not as strong as if they were applied in the root zone. The application of Bacillus subtilis even resulted in a lower shoot fresh matter. The effect on leaf fresh matter was also a stimulating one. It should be stressed, however, if the combination of all substances was applied the effect was opposite stimulating if applied over the roots and inhibiting if applied over the leaves.

The application of biostimulators enhanced in most cases the dry matter content of shoots and leaves (Fig. 1b). Therefore 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 (Hoang, 2003), however, in these experiments the effect on the root growth was much stronger than on the shoot growth. In this respect much more investigations are necessary and the results are only a first advice.

Comparing the ratio between shoot and leaf fresh matter (Fig. 2) it is obvious that 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. After application of biostimulators to the leaves the leaf growth was more encouraged than shoot growth resulting in a lower ratio apart from leaf treatment with the combination of all substances. In this treatment leaf development was inhibited and therefore the shoot/leaf ration increased.

**Fruit Harvest and Quality**

The total yield after one month harvesting (Fig. 3) was affected after the application of biostimulators. The fresh weight of all cucumbers with market quality was about 500g; therefore the number of fruits is representative for yield. The number of marketable fruits was higher than in the control in most variants treated with biostimulators. Especially the treatment with biostimulators on the roots resulted in a higher yield in the first harvest. Based on this the number of fruits finally harvested was considerably higher after treatment of roots with LACTOFOL and Bacillus subtilis. For the following experiments further application also during the fruit set should be taken into consideration because these additional applications could enhance the yield further. This could be especially important in long time cultivation.

A very important result of this experiment is that the relative amount of marketable and non-marketable fruits is affected by treatments with biostimulators (Fig. 4). The ratio of non-marketable fruits (C class) was more than 25% in the control and could be reduced by leaf application of each substance investigated till 20% and even till 10% if substances were applied over the roots.

The application of humates affected also the quality of cucumbers as indicated by the dry matter content (Fig. 5). The dry matter content increased after the treatment with humates in the root zone or on leaves and also in combination with the other substances.
CONCLUSIONS
Based on the results the application of biostimulators in hydroponics seems to be useful and should investigate further. It should be stressed that all very different biostimulators had in most cases a stimulating effect on shoot development and number of marketable fruits.

Effects on shoot growth and yield were dependent on the biostimulator used. It is surprising, that all substances tested stimulated the vegetative growth while yield was higher if LACTOFOL and *Bacillus subtilis* was applied. In former experiments, however, a combination of all substances was even more effective than separate use (Boehme, 1999), in this experiment a combination only in some cases was better than single substances. For causal explanations much more experiments are necessary.

Literature Cited
Prosorovskaya, A.A. 1936. The Effect of humic acid and its derivatives on the uptake of Nitrogen, phosphorus, potassium, and iron by plants. Trudy nauch mogo Instituta Udobreniy Insektoufugitisidan, 127.
# Tables

Table 1. Concentrations and application patterns of biostimulators used in the experiment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration of substances</th>
<th>Leaf application</th>
<th>Root application</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LACTOFOL</td>
<td>0.08%</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>K-Humate</td>
<td>0.2%</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Spore suspension (10^7 cfu/ml)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LACTOFOL + K-Humate + <em>Bacillus subtilis</em></td>
<td>concentration is in effect respectively</td>
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<td>X</td>
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</table>

Table 2. Composition of LACTOFOL ®.

<table>
<thead>
<tr>
<th>Components</th>
<th>Unit</th>
<th>LACTOFOL®</th>
<th>Components</th>
<th>Unit</th>
<th>LACTOFOL®</th>
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</thead>
<tbody>
<tr>
<td>Lactic acid</td>
<td>%</td>
<td>10</td>
<td>Magnesium</td>
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<tr>
<td>Riboflavin</td>
<td>mg/l</td>
<td>0.5</td>
<td>Iron</td>
<td>%</td>
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<td>Ascorbic acid</td>
<td>mg/l</td>
<td>3</td>
<td>Boron</td>
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<tr>
<td>Thiamine</td>
<td>mg/l</td>
<td>0.1</td>
<td>Copper</td>
<td>mg/l</td>
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<tr>
<td>Nitrogen</td>
<td>%</td>
<td>30</td>
<td>Manganese</td>
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<td>Phosphorus</td>
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<td>Zinc</td>
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<td>Potassium</td>
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<td>15</td>
<td>Molybdenum</td>
<td>mg/l</td>
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<tr>
<td>Calcium</td>
<td>%</td>
<td>0.5</td>
<td>Cobalt</td>
<td>mg/l</td>
<td>6</td>
</tr>
</tbody>
</table>
Figures

Fig. 1a.

Fig. 1b

Fig. 1. Effect of application biostimulators (Lactate, K-Humate, Bacillus subtilis) on leaves and roots respectively on biomass of shoots and leaves after finishing the experiment (Fig. 1a = FM; Fig. 1b = DM). Different letters indicate significant differences (LSD, P=0.05).
Fig. 2. Effect of application biostimulators (Lactate, K-Humate, *Bacillus subtilis*) on leaves and roots respectively on the ratio of shoots and leaf biomass after finishing the experiment. No significant differences.

Fig. 3. Effect of application biostimulators (Lactate, K-Humate, *Bacillus subtilis*) on leaves and roots respectively on number of marketable fruits in four harvesting periods of 9 days each. No significant differences.
Fig. 4. Effect of application biostimulators (Lactate, K-Humate, Bacillus subtilis) on leaves and roots respectively on the percentage on non-marketable fruits. Different letters indicate significant differences (Chi-square-test, P=0.05).

Fig. 5. Effect of application biostimulators (Lactate, K-Humate, Bacillus subtilis) on leaves and roots respectively on dry matter content of marketable cucumbers. Different letters indicate significant differences (LSD, P=0.05).