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Effect of different plant bio-stimulants in improving cucumber growth under soilless culture

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ABSTRACT: There are more studies about plant bio-stimulants but no clear results about which is the best one in improving vegetable crops specially cucumber. The aim of this study is to screen the effect of various biostimulants in improving cucumber (Cucumis sativus L.) growth under soilless culture via root application by modifying coco-peat culture media substrate. In the present study, we tested fifteen treatments as follow: T1 -control (CK); T2 - 10 mM putrescine (Put); T3 - 250 ppm seaweed (Sea); T4 - 0.02 ppm meta-topolin (MT); T5 - 100 ppm naphthalene acetic acid (NAA); T6 - 400 ppm polyaspartic acid (PAS); T7 - 50 ppm sodium nitrophenolate (98% NIT); T8 - 100 ppm tryptophan (AAF); T9 - 1% fulvic acid (FUL); T10 - 10⁷ CFU/ml Bacillus subtilis (BAS); T11 - 10⁶ CFU/ml Trichoderma (TRI); T12 - 50 ppm alanine (ALa); T13 - 150 ppm salicylic acid (SA); T14 - 1 mM silicon (SiO₂) and T15 - 0.001 ppm 24-epibrassinolide (EBR). The results obviously showed that using all bio-stimulants significantly increased cucumber growth parameters (plant height, stem diameter, leaves number, leaf area, shoot fresh weight, and root fresh weight). Seedlings Vigor Index (SVI) increased multifold compared with control by all treatments. The increase in cucumber seedlings vigor had a highly significant effect compared with control and the increase was 55.9% followed by 55.2% and 53.4% by Put, MT, and EBR treatments respectively. Our study concluded that the application of plant bio-stimulants can be used to modify coco-peat substrate with a positive effect on plant growth and improvement of cucumber plants under soilless culture.

Keywords: Vegetables; Greenhouse; Root system; Artificial substrate.

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important cucurbitaceous vegetable crops grown in China after tomato. Cucumber can be cultivated around the year, which becomes an off-season crop for the markets, fetching remunerative incomes to the farmers. Moreover, China is endowed with greenhouse conditions, which are suitable for carrying out the high productivity and high quality of cucumber fruits. High yield and extra quality under open field conditions need great care of soil fertility and soil handling, especially using organic manure and deep soil preparation [1].

Almost all of the vegetables we can find on grocery store shelves are produced either directly or indirectly in open-field soil. However, the soil itself isn't essential for plant growth and development only some of its constituents. Field soil serves two basic purposes: it acts as a reservoir to retain nutrients and water, and it provides physical support for the plant through its root system. On the other hand, soilless cultivation agrees more perfect control of the root environment which offers possibilities for inducing yield and improving high quality [2]. Substrate culture media is the main popular hydroponic scheme between the numerous hydroponic systems [3]. Recently, many materials are used as growing media [4]. Using diverse organic and inorganic substrate cultures allows better nutrient uptake, adequate growth, and improvement due to adjusting water and oxygen holding [5]. The artificial substrate can also deliver these vital requirements for plant improvement with equal (and sometimes better) growth and productivity results compared with field soil, although at substantially greater expense. Well-drained, pathogen-free field soil of uniform texture is the least-expensive medium for plant growth, but the soil doesn't always occur in this perfect package. Some soils are poorly structured or shallow and provide an insufficient root environment because of restricted aeration and slow drainage. Pathogenic organisms are a common problem in field soils compared with artificial substrate [6].

To induce plant growth and yield many protocols have been studied to modify plant nutrients uptake and yield such as genetic engineering, which has included allele action, gene and genome copying, and new genotype creation. Despite the improvements and the benefits in these manners, some of them may also carriage potential issues for food security and require special alertness to assurance human health protection [7, 8]. On the other hand, plant bio-stimulants usage as a protection agency to modify plant productivity and crop growth vigor. Plant bio-stimulants are well-known as friendly environment-compounds with beneficial effects on various crops [9, 10]. Bio-stimulants include different substances and micro-organisms that stimulate plant growth [11]. The description and definition of plant bio-stimulants are dependent on the natural or biological sources. It can induce plant growth when adding in a minor amount; also it can promote the adeptness of micro and macronutrients in plants, via modify nutrient capture or reduce nutrient suffers and losses or both. Bio-stimulants work on the soil by modifying soil biological activity, chemical, physical properties function and performance to develop plant growth parameters [12, 13]. Plant bio-stimulants are made up of one or more-ingredient plant extracts like; hormones, enzymes, proteins, amino acids, vitamins, microelements, and other biologically active compounds [14]. Bio-stimulants are available in diversity of formulations and with varying ingredients, which generally classified into three major groups based on their source and content [15]. These groups contain humic substances (HS), hormone-containing products (HCP), and amino acid containing products (AACP). HCPs, such as seaweed extracts, contain identifiable amounts of active plant growth substances such as auxins, cytokinins, and their derivatives. The word bio-stimulant was progressively used by the scientific researchers over the following years, expanding the range of substances and modes of actions [16, 17].

Application of various bio-stimulants such auxin, cytokinin, gibberellin and ethylene has been revealed to change the physiological and developmental processes, including plant vegetative growth, sex expression, yield, and yield quality in cucurbits. Therefore, various auxin [indole-3-acetic acid (IAA), NAA], auxin transport inhibitor (TIBA), cytokinin (KIN), gibberellin gibberellic acid (GA3), abscisic acid (ABA), ethylene [(2-chloroethylphosphonic acid (ethrel; ethephon; CEPA)] and growth retardant (MH) have been applied

exogenously to control the vegetative growth parameters and to increase fruit yield and yield components of these cucurbits [18, 19]. It was reported that application of bio-stimulants perhaps change the sex expression in cucurbits toward femaleness, mixmizing the number of pistillate flowers, number of fruits/plant, and individual fruit weight as well as yield [19]. In cucumber, using of cytokinin had useful influences on plant vegetative growth characters, resulting in highest marketable yield in the spring-summer crop and in largest fruit size in the fall-winter crop [20]. The effects of bio-stimulants on alterations of flowering, sex expression, and fruiting of cucurbits, inhibited vegetative growth, suppressed staminate and enhanced pistillate flower formation in cucumber. Early and total yields were increased by applications of cytokinin and auxin at the four-leaves stage [21]. Jadav et al. [18] conducted an experiment where the various bio-stimulants, viz., NAA, GA3, ABA, KIN, and ethrel, were used for translation from staminate flowers to pistillate flowers. Yield investigation revealed that the bio-stimulants at all concentrations significantly increased the number of fruits/plant. However, the effects of bio-stimulants are often genotype-dependent and may be environmentally adaptable [21, 22] wanting exact evaluations for single cultivars in diverse conditions. Bio-stimulants were investigated in more studies as a foliar application, but after our research, we didn't find clear studies about the effect of bio-stimulants on agriculture growth media (coco-peat) in improving cucumber root growth. Therefore, our study aimed to investigate the effect of fourteen plant bio-stimulants to choose the best three in improving cucumber grown under soilless culture in the greenhouse condition via root application mixing with coco-peat substrate culture media before transplanting.

2. MATERIALS AND METHODS

This experiment was conducted in the greenhouse condition in the department of soilless culture during October 2017, Institute of Vegetables and Flower (IVF), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China. The cucumber (*Cucumis sativus* L.) cv. [Zhong Nong No 26 (f1)] seeds were incubated in an incubator for approximately 24 hours at $28 \pm 1^{\circ}$ C. Seeds were put in Petri dishes (9 cm) on filter paper and treated with distilled water. The seedlings were planted in the trays after incubation period until it became two true leaves then the plantlets had transferred into 7 litters plastic containers after filled it with growth media. Biostimulants were mixed with coco-peat substrate. Coco-peat substrate was washed five times by tap water before use. The treatments were tested in this study as follows: T1 - control (CK); T2 - 10 mM (Put) L-1; T3 -250 ppm (Sea) L-1; T4 - 0.02 ppm (MT) L-1; T5 - 100 ppm (NAA) L-1; T6 - 400 ppm (PAS) L-1; T7 - 50 ppm (98%NIT) L-1; T8 - 100 ppm (AAF) L-1; T9 - 1% (FUL) L-1; T10 - 10⁷ CFU/ml (BAS) L-1; T11 - 10⁶ CFU/ml (TRI) L-1; T12 - 50 ppm (ALa) L-1; T13 - 150 ppm (SA) L-1); T14 - 1 mM (SiO₂) L-1 and T15 - 0.001 ppm (EBR) L-1.

2.1. Plant growth parameters

Vegetative growth was measured three times after 10, 20, and 30 days from treatments, include plant height, leaves number, stem diameter, and leaf area. Seedlings were harvested after 6 weeks to measure shoot fresh weight and root fresh weight.

2.2. Seedling Vigor Index (SVI)

Seedling vigor was measured after 30 days by using the seedling vigor index (SVI) indicator [23]. SVI = [stem thickness (mm) / seedling length (mm) + root fresh weight (g) / shoot fresh weight (g) x [shoot fresh weight (g)] The study was carried out in Completely Randomized Design (CRD) with three replicates and 7 pots for each treatment. Five plants were randomly selected to determine all parameters. The data were analyzed statistically using CoStat version 8.1 software. The differences among means were compared with Tukey's test at level p < 0.05.

3. RESULTS

3.1. Plant height

After 10 days, the plant height had a clear significantly effect by using bio-stimulants treatments compared with the control. Plant height recorded 16.71, 16.23, and 14.1 cm by Put, MT, and EBR to cocopeat substrate respectively. The lowest plant height was 6.3 cm recorded by the control treatment (Table 1). The highest plant height after 20 days had a highly significant effect by using bio-stimulants compared with the control and it was recorded 25, 24.23 and 20.1 cm by Put, MT and EBR respectively (Table 2). After 30 days, the highest plant height had a highly effect by using bio-stimulants compared with the control. It was recorded 30.71 cm by Put application followed by MT and EBR which recorded 30.52 cm and 30 cm, respectively. The lowest plant height was 10.7 cm which recorded by the control (Table 3).

3.2. Leaves number

The greatest number of leaves had a highly significant effect by using bio-stimulants compared with the control and it recorded 3.28, 3.23 and 3.19 after 10 days by Put, MT, and EBR respectively. The lowest leaves number was 2.1 by the control treatment (Table 1). After 20 days, the Put, MT, and EBR, showed the greatest number of leaves and had a highly significant effect on leaves number compared with other bio-stimulants and control, and recorded 3.71, 3.57 and 3.88, respectively (Table 2). After 30 days the Put, MT, and EBR showed a highly significant effect of leaves number compared with other bio-stimulants and the control. The Put, MT, and EBR recorded 4.52, 4.42, and 4.88, respectively (Table 3).

3.3. Stem diameter

After 10 days the cucumber plant had a highly significant stem diameter 3.11, 2.93, and 2.67 mm under application of Put followed by MT and EBR, respectively (Table 1). After 20 days, a highly significant effect on stem diameter showed by Put followed by MT and EBR. The Put, MT and EBR treatments recorded 3.15, 3.10, and 3.01 mm, respectively (Table 2). After 30 days, a highly significant effect on stem diameter showed by Put tracked by MT and EBR and recorded 4.05, 4.03, and 3.94 mm, respectively (Table 3).

3.4. Leaf area

After 10 days, the greatest average leaf area 30.81 cm² was given by MT followed by Put and EBR, respectively (Table 1). The greatest average leaf area after 20 days was 60.31 cm² by MT followed by Put and EBR respectively (Table 2). After 30 days, the greatest average leaf area was 71.77 cm² which was recorded by MT followed by Put and EBR, respectively. These treatments showed a highly significant effect on cucumber leaf area compared with the other plant bio-stimulants and the control treatment after 10, 20, and 30 days (Table 3).

3.5. Shoot fresh weight

The results showed that the shoot fresh weight of cucumber seedlings had influenced significantly due to different bio-stimulants treatments compared with the control. In general, shoot fresh weight had

significantly increased after 30 days compared with the control and the other treatments by using Put, MT, and EBR respectively (Figure 1).

3.6. Root fresh weight

Regarding root fresh weight, all the treatments had a significant influence on root fresh weight. In general, Put, MT, and EBR had a significantly increased root fresh weight after 30 days compared with the control and the other treatments (Figure 2).

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Treatments	Plant high (cm)	Leaves number	Stem diameter (mm)	Leaf area (cm ²)
СК	6.3±0.11 ^f	2.10 ± 0.08^{d}	1.70 ± 0.07^{g}	7.56 ± 0.84^{f}
Put	16.7±0.74 ^a	3.29±0.14 ^a	3.10±0.08 ^a	29.69 ± 1.94^{ab}
Sea	13.4±0.29 ^b	3.05 ± 0.09^{ab}	2.62±0.18 ^{bcd}	27.45 ± 0.97^{bc}
MT	16.2±0.08 ^a	3.24±0.01 ^{ab}	2.93±0.11 ^{ab}	30.81±0.21ª
NAA	12.2±0.35°	2.71±0.16 ^c	2.29 ± 0.23^{def}	25.77±0.38°
PAS	13.5±0.31 ^b	3.19±0.07 ^{ab}	2.79±0.07 ^{bc}	28.57±0.10 ^{abc}
98% NIT	13.5±0.07 ^b	3.10±0.06 ^{ab}	2.67±0.15 ^{bcd}	28.57±0.27 ^{abc}
AAF	13.3±0.09 ^b	2.81±0.01°	2.39±0.17 ^{cdef}	28.01±1.99 ^{abc}
FUL	13.5±0.08 ^b	3.00±0.10 ^b	2.64±0.15 ^{bcd}	27.45±0.99 ^{bc}
BAS	12.1±0.05 ^c	2.62±0.21°	2.53±0.24 ^{bcde}	21.85±0.23 ^d
TRI	10.3±0.28 ^d	2.29±0.18 ^d	2.25±0.21 ^{ef}	21.29±1.70 ^d
ALa	10.4 ± 0.16^{d}	2.29 ± 0.32^{d}	2.10±0.10 ^f	18.70±0.81 ^e
SA	12.0±0.20 ^c	2.33±0.17 ^d	2.17±0.19 ^{ef}	21.85±0.97 ^d
SiO ₂	9.7±0.64 ^e	2.29±0.08 ^d	2.14±0.09 ^{ef}	21.85±1.40 ^{de}
EBR	14.0±0.09 ^b	3.19±0.04 ^{ab.}	2.67±0.18 ^{bcd}	29.13±0.51 ^b

Table 1. Effect of bio-stimulants on cucumber seedlings vegetative growth after 10 days.

Values followed by the same letters are not significantly different at 0.05 levels.

Table 2. Effect of bio-stimulants on cucumber seedlings vegetative growth after 20 days.

Treatments	Plant high (cm)	Leaves number	Stem diameter (mm)	Leaf area (cm ²)
СК	9.0±0.21 ^k	2.33 ± 0.08^{f}	1.90 ± 0.07^{f}	12.89±1.55 ^e
Put	25.0±0.14 ^a	3.70±0.10 ^a	3.20±0.06 ^a	56.40±0.16 ^{ab}
Sea	17.1±0.50 ^g	3.24±0.21 ^{abcd}	2.71±0.20 ^{bcde}	39.22±1.96°
MT	24.2±0.14 ^b	3.57±0.34 ^{ab}	3.10±0.10 ^{ab}	60.32±4.12 ^a
NAA	15.1±0.16 ^h	3.00±0.14 ^{cde}	2.70±0.18 ^{bcde}	35.95±1.13°
PAS	20.0 ± 0.20^{d}	3.33±0.11 ^{abc}	3.02±0.24 ^{abc}	41.83±1.96°
98% NIT	18.4 ± 0.45^{f}	3.24±0.25 ^{abcd}	2.91±0.19 ^{abcd}	41.18±2.26 ^c
AAF	16.7±0.37 ^g	3.10±0.86 ^{bcd}	2.62±0.17 ^{cde}	37.91±1.96°
FUL	19.3±0.71 ^e	3.19±0.16 ^{bcd}	2.89±0.15 ^{abcd}	38.56±1.96°
BAS	14.6±0.28 ⁱ	2.76 ± 0.21^{def}	2.60±0.11 ^{de}	23.81±0.80 ^d
TRI	13.3±0.21 ^j	2.57 ± 0.28^{ef}	2.57±0.18 ^{de}	22.88±1.40 ^d
ALa	12.7 ± 0.45^{j}	2.48 ± 0.43^{f}	2.37±0.24 ^e	20.82 ± 0.16^{d}
SA	14.2 ± 0.29^{i}	2.81 ± 0.85^{def}	2.52±0.07 ^{de}	22.41±0.21 ^d
SiO ₂	13.2 ± 0.08^{j}	2.57±0.37 ^{ef}	2.58±0.06 ^{de}	20.82±0.61 ^d
EBR	21.1±0.14 ^c	3.38±0.91 ^{abc}	3.01±0.12 ^{abc}	54.15±4.77 ^b

Values followed by the same letters are not significantly different at 0.05 levels.

Treatments	Plant high (cm)	Leaves number	Stem diameter (mm)	Leaf area (cm ²)
СК	10.70 ± 0.42^{k}	2.76 ± 0.24^{g}	2.37 ± 0.07^{f}	15.33±1.85 ^e
Put	30.70±0.14 ^a	4.52±0.78 ^a	4.06±0.08 ^a	67.11±0.19 ^{ab}
Sea	25.50±0.21 ^e	4.14±0.26 ^{bcd}	3.26±0.09 ^{bc}	46.67±2.33°
MT	30.50±0.23 ^{ab}	4.43±0.38 ^{ab}	4.03±0.11 ^a	71.78±4.91ª
NAA	24.50 ± 0.08^{f}	4.05±0.61 ^d	3.03±0.23 ^{bcd}	42.78±1.34 ^c
PAS	27.40±0.50°	4.19 ± 0.47^{bcd}	3.40±0.07 ^b	49.78±2.33°
98%NIT	26.10 ± 0.24^{d}	4.19 ± 0.18^{bcd}	3.17±0.15 ^{bc}	49.00±2.89°
AAF	24.90 ± 0.41^{f}	4.10±0.16 ^{cd}	3.17±017 ^{bc}	45.11±2.33°
FUL	25.40±0.09 ^e	4.14 ± 0.17^{bcd}	3.09±0.15 ^{bc}	45.89±2.54°
BAS	21.00±0.21 ^g	3.71±0.29 ^d	3.17±0.24 ^{bc}	28.33±3.11 ^d
TRI	17.90±0.24 ⁱ	3.57±0.67 ^e	2.72±0.20 ^{de}	27.22±0.96 ^d
ALa	13.20±0.43 ^j	3.29 ± 0.43^{f}	2.94±0.10 ^e	24.78±1.66 ^d
SA	20.10±0.37 ^h	3.62±0.22 ^e	2.77±0.19 ^{cde}	26.67±0.11 ^d
SiO ₂	17.50±0.43 ⁱ	3.48±0.68 ^{ef}	2.66±0.09 ^{de}	24.78±1.33 ^d
EBR	30.00±0.29 ^b	4.38±0.47 ^{abc}	3.94±0.18 ^a	64.44±0.26 ^b

Table 3. Effect of bio-stimulants on cucumber seedlings vegetative growth after 30 days.

Values followed by the same letters are not significantly different at 0.05 levels.



Figure 1. Effects of bio-stimulants on cucumber shoot fresh weight after 30 days. Values followed by the same letters are not significantly different at 0.05 levels.



Figure 2. Effects of bio-stimulants on cucumber root fresh weight after 30 days. Values followed by the same letters are not significantly different at 0.05 levels.



Figure 3. Effects of bio-stimulants on cucumber seedlings vigor after 30 days. Values followed by the same letters are not significantly different at 0.05 levels.

3.7. Seedling Vigor Index (SVI)

Regarding the cucumber seedlings vigor, the seedlings treated with 10 mM Put, 0.02 ppm MT, and 0.001 ppm EBR, mixed with coco-peat substrate culture media before transplanting had extra significant seedlings vigor compared with other plant bio-stimulants and with control (Figure 3). The increase in cucumber seedlings vigor was 55.9%, 55.2% and 53.4% by Put, MT, and EBR, respectively.

4. DISCUSSION

Plant bio-stimulants are usually applied in addition to standard fertilization usages to induce the nutrient uptake or the quality of the product [24]. The positive effect of plant bio-stimulants on plant growth under normal and stress conditions [25, 26]. As shown in this study all of the bio-stimulants promoted cucumber seedlings growth in greenhouse conditions via root application when mixed with coco-peat substrate before transplanting. It is well-known that stimulation of plant roots is the first step to promote all of the plant organs. The plant root is involved in the uptake of water and nutrients, synthesis of plant hormones, and storage function [27, 28]. In our study, we found that all of the plant bio-stimulants we used had a significant effect compared with control in influencing vegetative growth (plant length, leaves number, stem diameter, leaf area, shoot fresh weight, and root fresh weight). In our study, we applied small quantities from these additives to roots via mixing them with coco-peat substrate before transplanting to promote substrate structure, function, and improve plant response [16]. The best three bio-stimulants revealed highly significant results compared with others and with control were Put, MT and EBR respectively. The Put, MT, and EBR applied to coco-peat substrate influenced cucumber seedlings vigor (Figure 3) and shoot fresh weight (Figure 1). Our suggestion that plant growth regulators can make alterations in the phenotypes of plants and affect growth either by enhancing or by stimulating the natural growth regulatory systems from seed germination to senescence [29]. On the same hand, plant growth regulators may be improved the physiological efficiency of plants including photosynthetic capacity and effective partitioning of assimilates [30]. The productivity in field crops can be increased by stimulating the translocation of photo-assimilates. In this study, the increase of shoot fresh weight by using bio-stimulants (Figure 2), maybe also due to the influence of the photosynthetic process, which can support the translocation from source-to-sink and the opposite way [31]. It's well known that photosynthesis is a process that can convert light energy to chemical energy, sucrose and starch are the

main products of photosynthesis [32]. The cucumber plant is a good source of soluble sugar and vitamin c, which are extremely improved by increasing of photosynthetic process and light capture [33], photosynthetic can involve in the synthesis of ethylene, gibberellins, as well as in the control of cell growth and the reduction of oxidative stress. The effect of plant bio-stimulants on the photosynthetic process, soluble sugar, and vitamin c in cucumber organs need more investigation.

The increase in root fresh weight due to that bio-stimulants can enhance phenolic compounds that were toxic to soil bacteria and protozoa that were unfriendly around *Rhizobium* species. The role of plant phenolic acid in protection and communication during *Agrobacterium* and *Rhizobium* infection was tested by Yang et al. [34]. Other suggestion is bio-stimulants combined with coco-peat increased the proportion of phospholipids in root cells by creating phosphor element that is unavailable in the culture media for the plant, moreover bio-stimulants application has a useful influence on the substrate culture bacterial community [35]. In our study, we tested the effect of 14 plant bio-stimulants mixed with coco-peat substrate on the cucumber to choose the best 3 treatments in effecting of seedlings vegetative growth to investigate them in bio-physiology and biochemical parameters in the other future study under normal and stress conditions.

5. CONCLUSION

The results clearly showed that using all bio-stimulants significantly increased cucumber growth parameters (plant height, stems diameter, leaves number, leaf area, shoot fresh weight, root fresh weight, and seedlings vigor index). Our study suggests that the application of all plant bio-stimulants used in this study can be safely and environmentally friendly with a positive effect on plant growth and improvement of cucumber plants productivity. The best three bio-stimulants that showed a highly significant effect on the above-mentioned parameters were Put, MT, and EBR respectively compared with control.

Authors' Contributions: WJ, SHA and EA conceived and designed the experiment. SHA, HY, and PL studied and analyzed the data. SHA and BNS helped sample preparation and data collection. SHA, EA, and WJ wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This study was approved by the soilless culture department, institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.

Availability of data and materials: The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

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