



# Combined Effect of Biocompost and Biostimulant on Root Characteristics of *Cannabis sativa* L.

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## RESEARCH ARTICLE

### Abstract

The use of earthworms in bio-composting (vermicomposting) is gaining popularity as a bio-waste management approach for producing nutrient-rich organic fertilizer. Furthermore, the use of seaweed extracts as biostimulants changes the physical, biochemical, and biological aspects of the soil, as well as the architecture of plant roots, allowing for more effective nutrient uptake and increasing plant development and yields. This study aimed to evaluate the combined effect of biocompost and biostimulant on the development of the root system of cannabis (*Cannabis sativa* L.) plant. An outdoor pot experiment was set up a completely randomized design, with four treatments including vermicompost, vermicompost amended with seaweed-based biostimulant, vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost, and vermicompost mixed with SMS+CM compost and amended with seaweed-based biostimulant. The results revealed that the highest values of root length density, root diameter, and nitrogen content in roots (1.36 cm cm<sup>-3</sup>, 1.29 mm, and 1.23%, respectively) were found in plants grown in the substrate of vermicompost mixed with SMS+CM compost and amended with seaweed-based biostimulant, pointed this out as a recommended treatment for increased biomass production, which is important in the processing of cannabis for medicinal purposes.

**Keywords:** Root length density; seaweed-based biostimulant; spent mushroom substrate; vermicompost

## INTRODUCTION

*Cannabis sativa* L. is a versatile herbaceous crop, and its cultivation is linked with numerous products in textile fiber, food, seed oil, and medicine sectors. Nowadays, there is an increasing demand for medicinal cannabis compounds in the pharmaceutical industry (Hussain et al., 2021). Cannabis contains hundreds of secondary metabolites, including terpenes, flavonoids, and cannabinoids, such as cannabidiol (CBD), a non-psychoactive component with medicinal properties, and tetrahydrocannabinol (THC), the primary psychoactive chemical ingredient (Hussain et al., 2021). Cannabis varieties with a high CBD concentration and a high CBD to THC ratio are considered suitable for the pharmaceutical industry (Andre et al., 2016; Mostafaei Dehnavi et al., 2022). CBD and THC are bioactive compounds engaged in plant adaption mechanisms to their environmental environment. As a result, the nutritional and environmental conditions that plants encounter during their growth influence the synthesis and accumulation of these specialized molecules in plant tissues (Adesina et al., 2020; Danziger and Bernstein, 2021; Kakabouki et al., 2021). Soilless culture is particularly well-

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suitable for the cultivation of medicinal plants, including cannabis, and has received considerable attention in both protected environments and outdoor conditions due to many advantages like the potential for higher yields and quality, cleaner products (no contamination problems linked to the agricultural soil), year-round production, with minimized use of water in comparison to conventional soil-based cultivation (Fussy and Papenbrock, 2022).

Soilless culture should focus on using new substrates that are profitable and do not harm natural resources, facilitating sustainable agricultural production and resource reuse (Gohardoust et al., 2020). This requires the identification of alternative organic substrates capable of supporting efficient and intensive crop production. The most crucial properties of a substrate are adequate porosity and readily available water and nutrients, all of which are required for plant growth (Ruiz and Salas, 2019; Gohardoust et al., 2020).

Sustainable soilless plant production approaches, such as the utilization of numerous organic materials that can be used as fertilizers and substrates, are becoming more common in some horticultural and medicinal plant species, including cannabis. Organic substrates, such as biocomposts, including vermicompost (a form of compost made with the use of several earthworm species that speed up the biodegradation process), were suitable for soilless culture (Truong and Wang, 2015; Pardossi, et al., 2017; Ruiz and Salas, 2019). Furthermore, vermicompost as a substrate and residuals of organic materials, such as spent mushroom substrate (SMS) and/or cattle manure (CM) composts, are viable alternatives to improve production performance in soilless cultivated plants (Rogers, 2017; Ruiz and Salas, 2019); This could be owing to the presence of N-fixing and P-solubilizing bacteria isolated from the guts of different species of earthworms, which improved the nutritional properties of the vermicompost (Hussain et al., 2016).

Since of their unique bioactive components and effects, seaweed-based bioproducts are gaining momentum in crop production systems. They have phytostimulatory properties, which cause improved plant growth and yield parameters in a variety of essential crop species (Khan et al., 2009). In addition, they have phytoelicitor activity because their components trigger defense responses in plants, which aid in resistance to a variety of pests, diseases, and abiotic conditions such as drought, salinity, and cold (Ali et al., 2021). The brown, intertidal seaweed *Ascophyllum nodosum* has received the most attention as a source of industrial and commercial plant biostimulants. *A. nodosum* commercial extracts have been shown to promote plant growth, reduce some abiotic and biotic stresses and boost plant defenses through the modulation of molecular, physiological, and biochemical processes (Shukla et al., 2019; Ali et al., 2021).

This study aimed to evaluate the combined effect of biocomposts (vermicompost and vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost) as organic substrate and the application of seaweed-based (*A. nodosum*) biostimulant on the development of the root system of cannabis plant.

## MATERIALS AND METHODS

An outdoor pot experiment was set up the Agricultural University of Athens (37° 59' N and 23° 42' E; 30 m altitude) from July until September 2021. The experiment was carried out according to a completely randomized design (CRD), with 4 treatments and twenty replications (pots) for each treatment. Specifically, the treatments were as follows: Vermicompost (V); Vermicompost amended with seaweed-based (*A. nodosum*) biostimulant (V + BIO); Vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost (V + A); and, Vermicompost mixed with SMS+CM compost and amended with seaweed-based (*A. nodosum*) biostimulant (V + BIO + A). For the purpose of the current study, a commercial vermicompost (Fytonet IKE, Acharnes, Attica, Greece) was used. The spent mushroom substrate was obtained from a local mushroom industry and is the residual material after the cultivation of *Pleurotus ostreatus*, with the SMS containing sawdust (about 30–40%, by weight), straw (about 10–30%), and wheat bran (about 10–20%). Composting was carried out by constructing a rectangular pile of SMS and cattle manure, at a ratio of 70:30, with a base of 1.8 x 2.5 m and 1.5 m high (approximate volume of 2.25 m<sup>3</sup>). The pile was turned manually once a week for 140 days in order to produce compost. For the experimental procedure, the ratio of vermicompost and SMS+CM compost was 50:50. The characteristics of the organic substrates used in the experimental procedure are presented in Table 1.

Cultivation began with the formation of *Cannabis sativa* L. cv. Ferimon seedlings - in a float system - and then the seedlings were transplanted (25<sup>th</sup> July 2021) into 9.5 L pots (24 cm diameter) by hand to a depth of 2-3 cm, one plant per pot, and watered as required. The pot density was four pots per 1m<sup>2</sup>. For the experimental purpose, the biostimulant Maxicrop® was provided by Ellagret S.A. (Mandra, Attica, Greece). The formulation contained extracts of seaweed *Ascophyllum nodosum*. The label of the product claimed to contain 63.4% organic matter. In addition, it had a density of 80 g L<sup>-1</sup>. The biostimulant was used for experimentation on concentrations of 2 mL L<sup>-1</sup> biostimulant diluted on tap water. Specifically, 1 L of solution was applied on the substrate of each pot at 8 days after transplanting (DAT). Minimum and maximum air temperatures were 18/42°C and 15/60%, respectively, and the plants were subjected to a natural day length ranging between 12-15h during the experiment.

The plant growth development was determined by measuring height at 10, 17, 24, 31, 35, 45, 52, 59, and 66 DAT on five randomly selected plants from each treatment. Root measurements were made at 66 DAT using ten randomly selected plants (pots) per treatment. For each sample, the roots were separated from the soil after being

soaked in a solution of tap water + (NaPO<sub>3</sub>)<sub>6</sub> + Na<sub>2</sub>CO<sub>3</sub> for 24 h and then decanted into a 0.1% trypan blue FAA staining solution (a mixture of 10% formalin, 50% ethanol and 5% acetic acid solutions). For the determination of root length density, root surface area density and average root diameter, the root samples were placed on a high-resolution scanner (HP Scanjet 200 Flatbed Photo Scanner; Hewlett-Packard Inc., Palo Alto, CA, USA) using DT software (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burwell, Cambridge, UK) (Kokko et al., 1993). In addition, the total nitrogen (N) content was determined after oven-drying the root samples for 72 h at 64°C, grinding them to a fine powder, and finally, applying them to the Kjeldahl procedure using a Kjeltec 8400 autoanalyzer (Foss Tecator AB, Höganäs, Sweden).

**Table 1.** Characteristics of the organic materials used in the experimental procedure

<i>Parameter</i>	<b>Vermicompost</b>	<b>Vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost</b>	<b>Limits</b>
<b>Humidity (%)</b>	34	41	30 - 40 FAO <sup>1</sup>
<b>pH</b>	7,8	7,6	4 - 9 ECN <sup>2</sup>
<b>Electric Conductivity (µS/cm)</b>	2293	3490	≤ 1.9 ECN <sup>2</sup>
<b>Salinity (g/L of NaCl)</b>	1,15	1,71	< 2.0 Austrian Ministry for Agriculture and Forestry <sup>3</sup>
<b>C/N</b>	14,3	12,0	10 - 15 FAO <sup>1</sup>
<b>CaCO<sub>3</sub> (%)</b>	4,4	15,1	
<b>Organic Matter (%)</b>	58,7	55,3	≥ 15 ECN <sup>2</sup>
<b>Total C (%)</b>	32,9	39,8	≥ 15 EU Regulation <sup>4</sup>
<b>Total N (%)</b>	2,30	3,31	≥ 1 EU Regulation <sup>4</sup>
<b>Total P (%)</b>	1,78	1,83	≥ 1 EU Regulation <sup>4</sup>
<b>Total K (%)</b>	2,1	2,0	≥ 1 EU Regulation <sup>4</sup>
<b>Total N+P+K (%)</b>	6,18	7,54	≥ 4 EU Regulation <sup>4</sup>
<b>Total Ca (%)</b>	2,1	3,2	1.5 - 3.5 Oregon State University, USA <sup>5</sup>
<b>Total Mg (%)</b>	0,19	0,33	0.25 - 0.70 Oregon State University, USA <sup>5</sup>
<b>Total Na (%)</b>	0,29	0,24	<0.6 Oregon State University, USA <sup>5</sup>
<b>Total Fe (ppm)</b>	3666	4618	
<b>Total Cu (mg/kg)</b>	48	37	100 EU ECO Label <sup>6</sup>
<b>Total Zn (mg/kg)</b>	83	64	300 EU ECO Label <sup>6</sup>
<b>Total Mn(mg/kg)</b>	182	289	
<b>NO<sub>3</sub><sup>-</sup> (mg/kg)</b>	765,84	311,76	
<b>NH<sub>4</sub><sup>+</sup> (mg/kg)</b>	15,75	9,16	

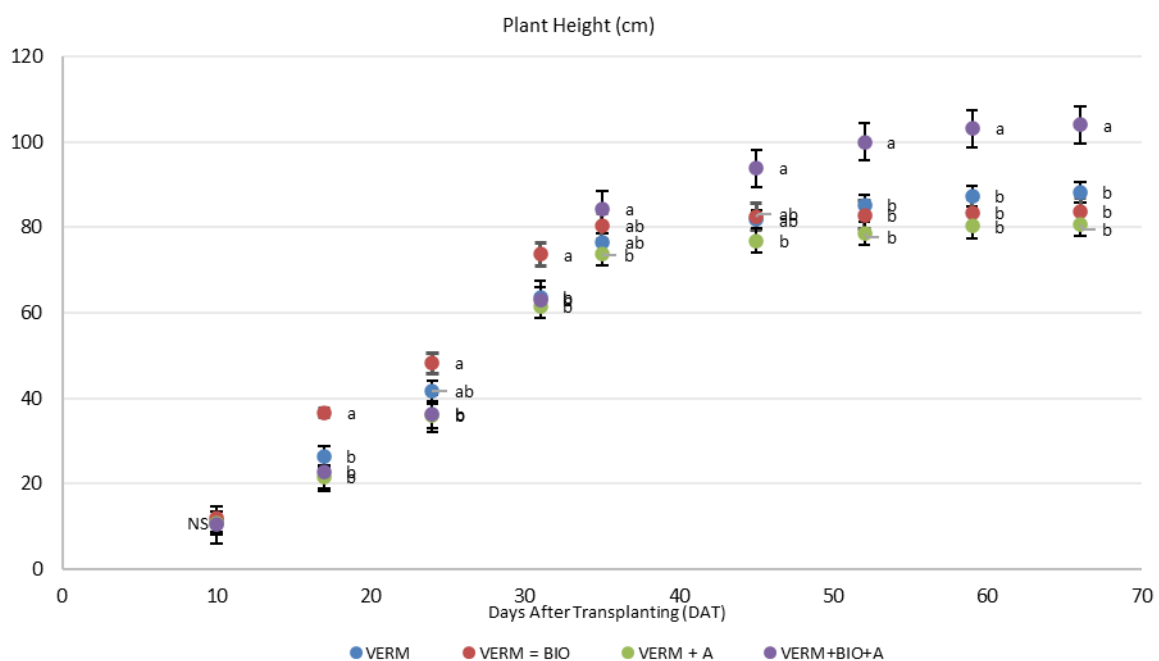
Note:<sup>1</sup>Roman, P., Martinez, M.M, Pentoja, A., 2015. Farmers Compost Handbook, Food and Agriculture Organization of the United Nations; <sup>2</sup>European Compost Network, 2018. Guidelines - Specification for the Use of Quality Compost in Growing Media, European Quality Assurance Scheme, ECN - QAS, Part D.; <sup>3</sup>Frohlich, M., Hechenbichler G., Hundsberge, S., Ortner, M., Baumgartner, A., 1993. Kompostqualität: Anwendungsfornern - Guteklassen (Compost Quality - Application Classes) in Handbuch der Kompostierung, Austrian Ministry for Agriculture and Forestry, Vienna, Austria; <sup>4</sup>European Parliament and Council, 2019. Laying down rules on the making available on the market of EU fertilizing products and amending, Regulations (EU) 2019/1009; <sup>5</sup>Sullivan, D.M., Bary, Al., Miller, R.O., Brewer, L.J., 2018. Interpreting Compost Analyses, Oregon State University, USA; <sup>6</sup>European Union, 2015. Revision of the EU Ecolabel Criteria for Soil Improvers and Growing Media [https://ec.europa.eu/environment/ecolabel/documents/gmsim/si\\_gm\\_eu\\_ecolabel\\_technical\\_report\\_june2015.pdf](https://ec.europa.eu/environment/ecolabel/documents/gmsim/si_gm_eu_ecolabel_technical_report_june2015.pdf)

Statistical analysis was performed using the SigmaPlot 12 statistical program (Systat Software Inc., San Jose, CA, USA) according to the completely randomized design (CRD). Values were compared by one-way analysis of variance (ANOVA) and differences between means were separated using the Least Significance Difference (LSD) test. All comparisons were made at the 5% level of significance ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSIONS

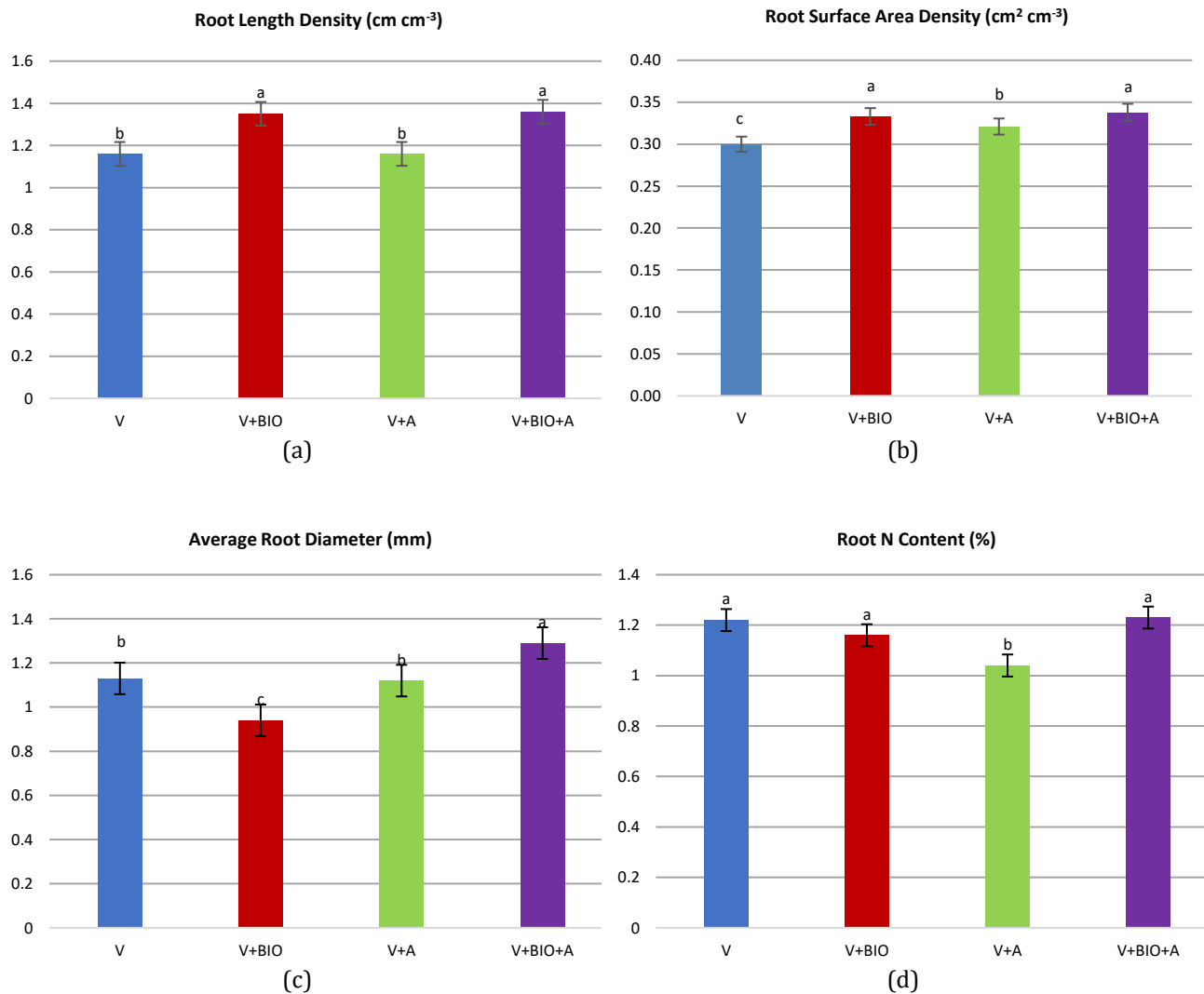
In the present study, the plant growth development is measured by plant height and the changes in this parameter are shown in Figure 1. The current study found that the plant height of cannabis was significantly influenced by the different organic substrates and the application of the seaweed-based biostimulant, from 17 to 66 days after transplanting (DAT). At 10 DAT, there were no statistically significant differences among the treatments. The

vermicompost amended with seaweed-based (*A. nodosum*) biostimulant (V + BIO) treatment had the lowest value of plant height in all measurements from 10 to 66 DAT, and the vermicompost mixed with SMS+CM compost and amended with seaweed-based (*A. nodosum*) biostimulant (V + BIO + A) presented the highest values from the first to the last measurement Figure 1. During the experimental period, the highest value of plant height (104.05 cm) was observed in V + BIO + A treatment at 66 DAT. According to Gong et al. (2019), adding spent mushroom substrate and cattle manure to the vermicompost increased the total N, P, Mg and Ca content considerably. In addition, over the last decades, seaweed extracts have been extensively researched for their potential use in crop production to improve biomass yield and produce quality. These extracts have been shown to improve seed germination and plant growth at all stages, including harvest and post-harvest. (Ali et al., 2019). Extracts of *A. nodosum* increased water uptake and nutrient uptake, which ultimately led to the promotion of the growth and development of lettuce (Crouch et al., 1990). When *A. nodosum* extracts were applied to maize plants, they were able to absorb considerably more Zn, Fe, B, Cu, Mo, S, Mg, Ca, and Mn in their leaves than controls (Ertani et al., 2018). Applications of *A. nodosum* on cottonwood substantially improved nitrogen and potassium uptake in the leaves (Fei et al., 2017). Furthermore, recent research has demonstrated that seaweed extracts and their constituents can influence the expression of genes involved in the endogenous manufacture of growth hormones such as auxin, cytokinin, and gibberellin (Ali et al., 2019).



**Figure 1.** Combined effect of biocompost and biostimulant on plant height of cannabis. VERM: Vermicompost, VERM=BIO: Vermicompost amended with seaweed-based (*A. nodosum*) biostimulant, VERM+A: Vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost, VERM+BIO+A: Vermicompost mixed with SMS+CM compost and amended with seaweed-based (*A. nodosum*) biostimulant. Vertical lines represent standard mean errors. Different lower-case letters denote a statistically significant difference between treatments according to the LSD test ( $p \leq 0.05$ ). NS: Not significant differences among treatments.

The results of the combined effect of organic substrates and the application of the seaweed-based biostimulant on evaluated root parameters are presented on Figure 2. According to the analysis of variance, all root growth parameters were significantly affected by the evaluated factors and the highest values of root length density, root surface area density and average root diameter (1.36 cm cm<sup>-3</sup>, 0.338 cm<sup>2</sup> cm<sup>-3</sup>, and 1.29 mm, respectively) were found in the substrate of vermicompost mixed with SMS+CM compost and amended with seaweed-based (*A. nodosum*) biostimulant (V+BIO+A). In addition, the mean value of nitrogen (N) content in roots was also greatest in the V+BIO+A treatment (1.23%) followed by vermicompost (V: 1.22%) and vermicompost amended with seaweed-based biostimulant (V+BIO: 1.16%), while the lowest mean value (1.04%) was obtained from the plants treated with vermicompost mixed with SMS+CM compost (V+A).



**Figure 2.** Combined effect of biocompost and biostimulant on (a) root length density, (b) root surface area density, (c) average root diameter and (d) root nitrogen (N) content of cannabis. V: Vermicompost, V+BIO: Vermicompost amended with seaweed-based (*A. nodosum*) biostimulant, V+A: Vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost, V+BIO+A: Vermicompost mixed with SMS+CM compost and amended with seaweed-based (*A. nodosum*) biostimulant. Vertical lines represent standard mean errors. Different lower-case letters denote a statistically significant difference between treatments according to the LSD test ( $p \leq 0.05$ ).

It is well known that vermicompost, while preserving water and nutrients, has a high amount of macro- and micro-elements that can be available to plants over the time (Pathma and Sakthivel, 2013; Wang et al., 2017). According to Atiyeh et al. (2002), it has been demonstrated that plant growth responses from vermicompost are more like to "hormone-induced activity" due to the high quantities of humic acids and humates in vermicompost rather than being increased by high levels of plant-available nutrients. In addition, Canellas et al. (2000) discovered that humic acids extracted from vermicompost increased root elongation and the production of lateral roots in maize plant. In another research study, it was reported that humic acids enhanced nutrient uptake plants by increasing the permeability of root cell membranes, boosting root growth, and enhancing root hair proliferation (Pramanik et al., 2007). As for seaweed products, there are several studies confirming their ability to cause significant plant vigor by enhancing root size and density (Crouch and van Staden, 1993; Rayorath et al., 2008).

## CONCLUSIONS

The results of the current study revealed significant combined effects of biocomposts (vermicompost and

vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost) used as organic substrates and the application of seaweed-based (*A. nodosum*) biostimulant on the development of the root system of cannabis plant. In particular, the highest values of the plant height and root growth parameters (root length density, root surface area density and average root diameter) were found after the use of vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost and amended with seaweed-based biostimulant (V + BIO + A). Regarding the nitrogen content in roots, the highest value was also observed in the treatment of V + BIO + A. It can be concluded that the use of vermicompost mixed with spent mushroom substrate (SMS) and cattle manure (CM) compost and amended with seaweed-based biostimulant is recommended for increased biomass production which is important in the processing of cannabis for medicinal purposes.

**Author Contributions:** D.B. (Dimitrios Bilalis), P.P., I.K. Conceived and designed the analysis; S.K., P.S., I.K., P.P., I.R., A.M., D.B. (Dimitrios Bilalis) Collected the data; S.K., I.K., P.P., I.R., D.B. (Dimitrios Beslemes), D.B. (Dimitrios Bilalis) Contributed data or analysis tools; S.K., I.K., P.P., I.R., D.B. (Dimitrios Beslemes), D.B. (Dimitrios Bilalis) Performed the analysis; S.K., I.K., P.P., I.R., D.B. (Dimitrios Bilalis) Wrote the paper. All authors read and approved the final manuscript.

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### Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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