

Review

Root Zone Management for Improving Seedling Quality of Organically Produced Horticultural Crops

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Abstract: Currently, vegetable production systems have been changed to improve yield and quality, leading to an improvement in sustainability. In horticultural cultivation, one of the most important sectors is the seedling production in nurseries. In the past, horticultural seedlings were produced directly by farmers. Nowadays, industrialized nurseries provide seedlings characterized by a uniform growth and an early and contemporaneous development. In addition, consumer concern about the impact of food production on the environment is driving an increased demand for organic vegetables with a consequent increase of agricultural land cultivated by organic methods. Hence, there is a need to produce high-quality seedlings suitable to be cultivated in the organic farming system. Root zone management, for improving seedling quality of organic horticultural crops, remains largely unexploited, such that this review highlights some of the current research and future development priorities, providing useful information to nursery growers. In particular, we reviewed all the scientific and modern knowledge on the production of organic, healthy and vigorous seedlings including the use of: (1) compost and compost tea; (2) agroindustrial byproducts; (3) microbial and non-microbial plant biostimulants; (4) beneficial microorganisms.

Keywords: compost; compost tea; organic fertilizer; plant biostimulant; biological control; transplanting; organic farming; high-quality seedlings; nursery

1. Introduction

Increasing current worldwide demand for organic food [1] is promoting research studies to obtain useful guidelines for improving organic farming sustainability. Nowadays, regarding horticultural production, farmers prefer transplant seedlings grown in a nursery, both in conventional and in organic farming systems. In modern agriculture, intensive seedling production is an essential segment, especially in horticultural production, to obtain high-quality seedlings [2]. In fact, specialized and industrialized nurseries can offer seedlings with high quality and uniform growth, more tolerant to abiotic and biotic stresses and with an early and contemporaneous development. In addition, seedling transplanting is advantageous in terms of saving energy, labor, time and seeds [3]. The cost of hybrid seeds is very important, and transplanting is an interesting method to reduce the number of seeds per square meter compared to direct sowing in the field. Nonetheless, nursery agronomic management can affect seedling quality and subsequently its field

performance [4]. In a nursery, specific expertise and competence are required to produce high-quality seedlings within a short growing period (generally, up to the third-fourth true leaf stage) satisfying the farmer's requests in term of species, environmental adaptation, and tolerance to abiotic and biotic stresses.

Minami [5] reported that a farmer says: “A good seedling is 50% of the production”. In good seedling production, one of the most important aspects regards the achievement of a well-developed and functional root system [6] able to uptake water and nutrients and give a mechanical support to the seedling [7]. Frequently, farmers consider seedlings as a “root crop” to underline the significance of good-quality root system architecture [8]. However, in the nursery, root zone management to improve seedling quality, especially of organic horticultural crops, remains largely unexploited. Hence, the main objective of the present review is to highlight some of the current research and future development priorities to enhance the root zone management for improving seedling quality of organic horticultural crops (Figure 1).

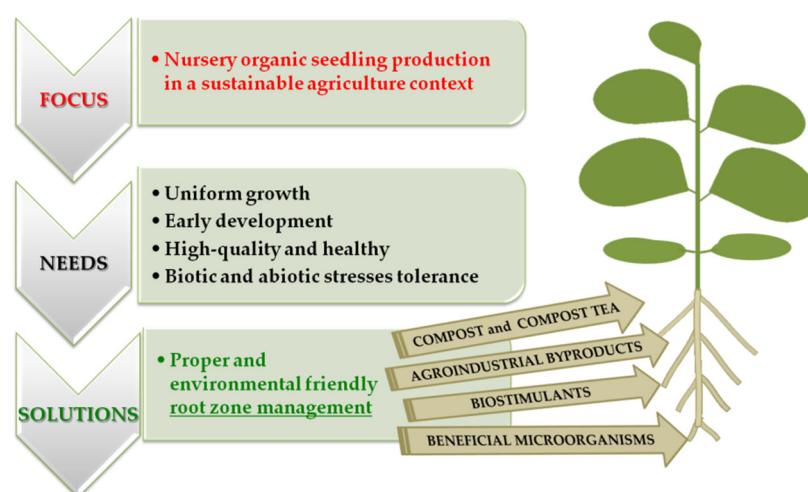


Figure 1. Root zone management for improving seedling quality of organically-produced horticultural crops.

In particular, this review explores four important and current topics about root management of horticultural seedlings: (i) compost and compost tea; (ii) agroindustrial byproducts; (iii) microbial and non-microbial plant biostimulants; (iv) beneficial microorganisms for biological control. All the information reported in the present review may allow the definition of specific guidelines for supporting nursery growers to improve the root management of seedlings suitable to be cultivated in organic farming system.

2. Compost and Compost Tea to Produce Seedling in Nursery

Compost is a well humified organic matter obtained by composting the undecomposed organic matter deriving from different feedstock. The compost process is accomplished by inhabitant microorganisms that generate more stabilized and mature molecular forms of organic wastes. Chemical and biological features, such as pH, electrical conductivity, and microorganisms, influence the beneficial properties of compost [9]. Compost is a renewable resource that can contribute to reduce the peat rate in the growing media composition, improving their physical and chemical properties and providing suppressive effects against plant pathogens [10]. Meng et al. [11] recommended 20–50% as the right rate to replace peat with composted biogas residues and spent mushroom substrate, in order to cultivate tomato and pepper seedlings with better morphological traits. Spent coffee ground compost, used in a proportion up to 40%, enhanced the general stand of basil and tomato seedlings by affecting dry weight, leaf area and biometric parameters [12]. However, compost effects are difficult to generalize, due to drawbacks associated with the content

of salt and allelopathic compounds that may occur, partially solvable by modulating just the application rate [13]. Indeed, Carmona et al. [14] found that grapevine marc compost blended with peat-based medium only slightly affected the height and other parameters of growing lettuce, tomato, pepper and melon seedlings. However, the addition of municipal solid waste compost into peat, reduced pepper plugs morphological and physiological quality, although a priming effect on seed germination and rooting due to compost aqueous extracts was observed [15]. On the other hand, disease suppression activity of compost is the ability to affect the pathogenesis and reduce plant disease symptoms by co-operating with plant and pathogens in the telluric environment [16]. Therefore, suppressive composts have a preeminent field application in agroecological-based agricultural systems, aimed at strengthening the resilience and natural suppression of soils and growing media. In the nursery sector, they are particularly appreciated for decreasing disease incidences and dependence on synthetic fungicides in mixtures with peat as observed, for example, on *Verticillium dahliae*/eggplant [17], *Fusarium oxysporum*/cucumber [18]; *Fusarium oxysporum* f.sp. *melonis*/muskmelon [19] pathosystems. Mechanisms underlying compost suppression are essentially attributable to the microbiological components, since autoclaving can completely cancel all effects [20]; they act through antagonistic models configuring an overall effect measurable as enhanced microbial outcomes in terms of biomass and biodiversity [21], production of biocontrol-related enzymes [22] and triggering the immune response of plants [23]. Therefore, some biological parameters of composts would be able to predict the reaction to disease agents [24]. Metagenomics investigations are currently ongoing to decipher determinants of compost suppression at community levels [25,26]. De Corato et al. [27] found that suppression of *Rhizoctonia* damping-off and *Fusarium oxysporum* wilting by green composts, in container-amended media assay, was associated with population levels of the antagonistic fungus *Trichoderma*. In addition, this evidence prompted the use of promising composts in combination with microbial biocontrol agents, to enhance the suppressive results [28]. Other studies reported that some chemical properties, including N-NH₄ [29], lipid [30] and phenolic content [31], may reflect negatively effects on pathogenic communities by conferring to container media inhospitable conditions.

Composted chicken manure represents one of the traditionally soil improvers used due to its high content of organic matter and nitrogen (N); however, the large and uncontrolled use of this material can lead to eutrophication of water bodies, spread of pathogens, plant phytotoxicity and emission of greenhouse gases [32,33]. Furthermore, issues occurring during the long-period-composting process, could be related to the initial characteristics of chicken manure substrate, having a low C/N ratio, high moisture content, phytotoxic compounds, and an unpleasant odor [34]. To reduce these problems, an innovative composting process, using *Hermetia illucens* larvae, was evaluated in the management of chicken manure [34]. The larval frass obtained from a mixture of chicken manure, chabazite and water, was shown to have growing media improver properties able to sustain the production of baby-leaf lettuce, and demonstrating the possibility to be used as a substrate, if properly diluted, in a seedling nursery as well as in potted plant production [34,35].

In a recent study, the synergistic interaction between dewatering, composting and pelleting, was investigated to reduce the water content, harmful microorganisms and seed weeds in the organic fertilizers produced from livestock manure [36]. In particular, pelleting resulted in a drastic reduction of water content and harmful microorganisms in processed manure, also displaying a high nutrient availability, an improved water retention, and a reduced temperature of the growing medium. In addition, the combined effects of the three technologies reduced the total organic content, but maintained the most stable carbon, allowing a delocalization of the livestock manure from the production sites [36].

Compost tea (CT) is a liquid organic formulate obtained by extraction—from few hours to a week—of composted materials, with or without aeration during the process [37,38]. As extractive liquid, generally water is used, but other liquid extractants can be utilized, such as wastewaters from the food industry (whey, marc, molasses, etc.) with consequent different final composition of microbial populations of CT [39]. Quality of CT is determined

by different factors, such as compost type, compost-to-water ratio and aeration level [40,41]. Microorganisms of the CT are mainly bacteria, but actinomycetes, yeasts and fungi contribute to define its properties, in particular biological control of plant pathogens and biostimulation of growth, physiological performances, and productivity of the plants [42–44].

CT applications as foliar spray or soil drenching can decrease plant disease severity [39,45] involving different mechanisms mainly due to antagonistic microorganisms able to compete for space and nutrients, to produce antimicrobial compounds, to parasitize plant pathogens and to induce plant systemic resistance [46–48]. Moreover, nutrient and organic molecules such as humic and phenolic compounds, may protect the plants against diseases, by improving their nutritional status, by direct toxicity against the pathogens and by inducing systemic resistance into the plants [49]. In addition to plant protection mechanisms, several studies have indicated that cropping applications of CT may also improve yield and nutritional quality of the products [45,50–58].

CT supplies useful microorganisms, organic acids, plant hormone-like compounds and soluble mineral nutrients [39,45,51]. For these reasons, CT is proposed as a sustainable alternative to synthetic inputs for enhancing plant nutrition, protection, growth and productivity [59,60], with consequent positive effects on human health and the environment [61].

Several studies report the use of CT on crops cultivated in open fields or in greenhouses, with very interesting results on disease control and yield, emphasizing benefits due to their use as organic additive in plant cultivation and in the suppression of soil-borne diseases [37,58]. By contrast, very limited results are reported on the use of CT in nursery systems.

In a vegetable nursery, one of the most used synthetic fungicides is Previcur Energy[®], that combines propamocarb and fosetyl-Al to control *Pythium* spp., and is also able to develop seedlings tolerance to *Phytophthora* spp. and a general stimulation of plants self-defense systems [62,63]. Recently, Villecco et al. [64] demonstrated that it is possible to partially replace Previcur Energy[®] with CT on tomato, pepper and melon seedlings cultivated in a nursery. Bernal-Vincente et al. [59] investigated two different types of CT obtained by citrus composts on melon seedlings in greenhouse nurseries. Both CTs demonstrated a biocontrol effect against *Fusarium oxysporum* for melon seedlings and no different bacterial biocontrol efficacy was observed between both CTs.

Restrictive legal limits about the use of agrochemicals, have stimulated researchers to work for making available new eco-friendly methods for both plant fertilization and diseases control, such as natural bioactive compounds and beneficial microorganisms. The use of CT seems to be a valid method to reduce agrochemicals not only in crop cultivation, but also in crop nurseries. In particular, the reduction of pesticides in a vegetable nursery is a necessary practice to gain the goals of sustainability in this sector. In this view, organic matter-based natural products are a valid alternative to replace agrochemicals, due to their bioefficacy, circular economy character, and negligible impacts on water and wildlife [60].

3. Valorization of Agroindustrial Byproducts in Innovative Organic Fertilizers and Growing Media Suitable for Seedling Production

Root growth and its architecture are affected by several factors, showing different performance when crops are cultivated in an open field or greenhouse. In addition, nurseries seedling production is characterized by a very short growing season, and seedling roots can explore a relatively small volume of growing medium. The consequent demand of specific nutrients means that the adoption of definite and efficient fertilization strategies, even more so in the organic nurseries, is extremely important. For example, N management requires organic fertilizers to be mineralized by microorganisms in order to become available for plant uptake [10,65]. In this regard, organic fertilizers are characterized by a slow release of nutrients, especially N, due to the rate of net N mineralization from solid organic fertilizers, which can vary in a different way (slow to fast) depending mainly on the C/N ratio. Environmentally friendly fertilizers produced using agricultural byproducts have been developed in order to improve yields and without compromising the environment

sustainability. In addition, organic fertilizers are able to provide organic matter, improve growing media fertility and reduce nutrient loss by retarding, or even controlling, the release of nutrients into growing media [66].

The current European action plan for the Circular Economy [67], together with the Bioeconomy Strategy [68] strongly incentivize the valorization of biowaste in resources. In this perspective, the European Union (EU) Regulation 2019/1009 establishes rules to make available fertilizer on the EU market, promoting biowaste recycling to produce sustainable fertilizer based on plant byproducts or plant extracts [69].

In the last decades, the anaerobic digestion (AD) process has gained great interest for the production of green energy. One of the byproducts obtained from AD is digestate [70] that recently has been exploited in agriculture as an innovative and sustainable fertilizer [71,72]. In fact, during anaerobic digestion, N, phosphorous and potassium, present in the feedstock, are retained in the digestate [73].

Humic-like substances are considered as fundamental biological active components of digestate. Natural humic-like and plant hormone-like substances were extracted from digestate using micro- and ultra-filtration, considered useful raw materials for organic fertilizers, and used as extra root feeding [74].

The application of digestate coming from anaerobic fermentation of swine manure have been considered on crops and horticultural plants under different agricultural practices and in greenhouse conditions [75,76]. The authors indicated that concentrations of liquid digestate between 2% and 3% stimulated seed germination, whereas it can be applied even at 20–30% during more advanced growth stages.

Ronga et al. [77] evaluated the effects of solid and liquid digestate as an alternative and sustainable growing medium and nutrient solution in the hydroponic cultivation of baby leaf lettuce (*Lactuca sativa* L.) by studying nine different hydroponic combinations of substrate and fertilizer in which solid and liquid digestates were used alone or together. The researchers indicated that the combined use of agriperlite (as growing medium, GM) with liquid digestate (as fertilizer, F), solid digestate (as GM) with standard solution (as F), and pelleted digestate (as GM) with standard solution (as F) displayed sustainable and alternative growing media or nutrient solutions able to enhance baby leaf lettuce growth, in turn positively affecting different parameters like root, shoot and total dry weights and SPAD index [77]. In another, study solid and liquid digestates were evaluated for the soilless production of basil and peppermint seedlings in the replacement of agriperlite and synthetic fertilizers, respectively [78]. The authors reported that the use of solid digestate as growing media and liquid digestate as fertilizer can represent a sustainable agronomic practice to valorize byproducts of biogas plants, when used in soilless production.

Greco et al. [79] demonstrated that solid digestate, can be used as valid alternative to peat for sage cultivation, when used at 40% for preparing the growing substrate.

Recently, it has been demonstrated that the good fertilizing properties of digestates sometimes may be lost if it is not completely stabilized. In order to overcome this problem, a post-treatment consisting in a further composting process could transform the digestate to a mature, stable, safe, and nutritive compost [80]. For example, very recently, it has been proven that a further maturation process of a cow slurry digestate produced more stabilized digestate-derived compost. It was able not only to increase the growth of potted seedlings, but also to induce a damping-off suppression caused by *Fusarium solani* in tomato plants, when added to soil at 5% and 10% [81]. Innovative studies about captured fish waste-derived compost and digestate from anaerobic digestion/co-digestion were considered; the applicability of such fertilizers in organic farming was evaluated [82] and biostimulant effects were reported on the growth of lettuce [83].

Agricultural wastes, such as forestry, crop residues and animal manures can be transformed to obtain biochar [84] that represents a byproduct of thermal pyrolysis [73]. Monterumici et al. [85] indicated that when the biochar is used as amendment, a stable carbon pool can be stored. Studies in the last decade have mainly focused on biochar application as a soil amendment and for soil remediation, as well as the possibility of its successfully addi-

tion in the composting processes of various agricultural waste [86]. In fact, several studies are consistent with the use of biochar combined with other products. Kaudal et al. [87] demonstrated that some properties such as density, pH, and C/N ratio of growing media, increased in a proportional manner to the biochar rate used in mixtures with composted pine bark.

Various studies have been done on the use of vermicompost as innovative growing media [88]. Atiyeh and co-authors [89] reported the first research about the beneficial use of vermicompost on the first stages of plants emergence and development. They demonstrated that the growth of marigold and tomato seedlings was enhanced using vermicompost, obtained from pig slurry or food waste, by up to 20% as growing media.

Several studies reported the innovative and successful use of spent coffee ground (SCG) in the production of innovative and sustainable biofertilizers [12,90,91]. An innovative application for SCG has concerned its use as filler for the formulation of lightweight clay ceramic aggregates for agricultural purposes [90]. More recently, the same SCGs were used alone or together with biochar as pore-forming agents in the realization of these lightweight clay ceramic aggregates and then used as sustainable fertilizers [91]. However, further studies are needed to confirm their agronomic performance on horticultural seedlings cultivated in nursery.

The first use of black soldier fly larvae processing residue as an innovative ingredient for growing media has been evaluated in the production of baby leaf lettuce, basil and tomato seedlings [35]. The study indicated that up to 20% black soldier fly larvae processing residue increased crop growth in terms of total dry weight, leaf number and leaf area without inducing any abiotic stress, demonstrating the feasibility of using black soldier fly as an interesting alternative for recycling and valuing agro-industrial by-products, and as an innovative ingredient for partially replacing commercial peat-based growing media able to provide nutrient to plants [35].

Among innovative organic fertilizers, microalgae biomasses have been investigated due to their ability to prevent nutrient losses through a gradual release of macro and micro-nutrients [92,93]. Several studies highlighted the beneficial effects of microalgae when used as biofertilizer: greater nutrient uptake, higher biomass production, and higher crop yields were displayed [94–96]. Hence, these products might be assessed also for the production of horticultural seedlings cultivated under an organic system.

Ciavatta et al. [97] reported that blood meal is an organic fertilizer containing interesting value of organic N about 10–13%. The authors displayed that about 75% of the organic carbon and of the organic N were mineralized in soil, while the remaining carbon was converted into humified compounds. In addition, the availability of the iron and its successive chelation in the humic substances increased after the soil application. Bae et al. [98] demonstrated that blood of livestock can be used as a sustainable fertilizer, instead of the synthetic fertilizer used to produce environment-friendly organic seedlings. The authors reported that the application of blood as fertilizer increased the number of leaves of hot pepper and tomato compared to the use of synthetic fertilizer. In future research, these results on the adoption of blood as sustainable fertilizer might be validated also for the production of horticultural seedlings.

4. Implications of Microbial and Non-Microbial Biostimulants for Seedling Quality

According to the Regulation (EC) N. 2019/1009 of the European Parliament and Council, microbial biostimulants include arbuscular mycorrhiza fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) belonging to the genera *Azotobacter* spp., *Azospirillum* spp. and *Rhizobium* spp. [99–101]. Both mycorrhizal fungi and rhizobacteria have a phytostimulation effect on plants through mobilization of soil nutrients, control of hormonal and nutritional balance in plants, and secretion of plant growth regulators and signal molecules [102–106]. Their effectiveness has been documented by numerous research projects that have highlighted different results depending on the interaction between a specific species (or strain) of fungus and a defined vegetable crop (or cultivar). Even

the inoculation method and the growing conditions influence the biostimulant effect on crops [107,108]. For instance, external seed treatments and early applications on seedling by soil inoculations, facilitate the microbial development in the rhizosphere, promoting interaction with the host plant. Recent research has focused on different vegetable seedlings applying AMF on seed before sowing. Watermelon seeds inoculated with *Rhizophagus intraradices* and germinated in nursery conditions showed seedlings with higher dry matter and with a root system more developed than non-inoculated seedlings [109]; inoculated seedlings revealed an increase of secretory purple acid phosphatase (PAP) genes expression and APase activity in rhizosphere soil (at the root surface) in comparison with the control, and an enhancement of phosphorus concentration in seedlings. *Rhizophagus intraradices* has been effective in onion seedlings inducing an increase of 35–40% more total biomass than the control (increased shoot and root dry matter) even if the percentage of mycorrhizal colonization in onion roots never exceeded 20% [110]. Tomato seedlings showed more leaves in response to inoculation with *Rhizophagus irregularis* compared with non-inoculated seedlings [111]. Research in greenhouses comparing different mycorrhiza species (*Glomus mosseae*, *G. clarum*, *G. caledonium*, *G. intraradices* and *G. etunicatum*) or their mixture for inoculation of eggplant revealed more vigorous seedlings before transplanting to field conditions and a significantly higher P and Zn uptake [112]. *Glomus intraradices*, *G. etunicatum*, and *Gigaspora margarita* increased the nutrient uptake in different zucchini hybrids cultivated in a growth chamber [113].

There has been great interest in interactions between mycorrhiza species and plants under abiotic stress conditions. According to Ronga et al. [114] the fungus *Funnelformis mosseae* was more effective than *Rhizophagus intraradices* for sustaining the growth of tomato seedlings under reduced water regime and also *Funnelformis mosseae* increased the chilling tolerance of tomato seedlings cultivated at 8 °C/4 °C, day/night [115] and similar results were reported by Caradonia et al. [116] in cropping processing tomato at 1 °C. AMF-inoculated tomato seedlings showed higher biomass than control seedlings regardless of the presence of chilling stress but, under low temperature, AMF inoculation has boosted redox poise and antioxidant potential, as confirmed by a reduced lipid peroxidation of the plasma membrane and an efficient neutralization of H₂O₂, along with increased calcium precipitates in the apoplast and vacuole of root cells compared with the non-AMF control [115]. Watermelon seedlings are very sensitive to chilling stress, with significant alterations in chlorophyll content and electrolyte leakage, but the inoculation with *Rhizophagus irregularis* (previously known as *Glomus intraradices*) was effective by restoring the photosynthesis efficiency, reducing the oxidative stress with a lower accumulation of H₂O₂ and malondialdehyde and an increase of root and shoot dry matter [117]. Instead, Turkmen et al. [118] treated pepper seedlings with moderate salinity stress (75 ppm NaCl) and demonstrated how *Rhizophagus irregularis* and *Gigaspora margarita* were able to relieve the stress with respect to the growth and nutrient contents of seedlings.

With regard to rhizobacteria effects on vegetable seedlings, some scientific data are available for both *Azotobacter* and *Azospirillum* or *Rhizobium*. In a greenhouse pot experiment, *Azotobacter* sp. strain AC11 was able to promote the growth of tomato seedling (shoot height, root length, dry and fresh weight) and to increase chlorophyll, soluble protein and soluble sugar content by fixing N, solubilizing potassium and phosphorus, and by producing indole-3-acetic acid (IAA) and siderophores [119]. The same strain induced a significant resistance to salt stress of tomato seedlings through the promotion of antioxidant enzymes (superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)) and an increase of osmotic substances (soluble sugars and proteins) [119].

Azospirillum is known for the ability to affect early growth of different vegetable crops. Lettuce, tomato and cucumber seeds inoculated with three *Azospirillum brasilense* strains (i.e., Sp7, Sp7-S and Sp245) and using different inoculation methods (soaking or drenching before and after sowing), showed bacterium strain [differently able to produce IAA] and plant species, significantly differed in their effect on root and shoot growth of seedlings [120]; both emergence and seedling vigor were influenced by inoculation method

and *Azospirillum* strain whose IAA synthesis stimulated a faster emergence of healthy embryonic growing axis [120]. *A. brasilense* increased biomass of lettuce seedlings only in the presence of salinity stress during the germination [121], whereas in tomato the bacteria inoculation induced a stem and root elongation with and without salt stress [122]. Interesting results have been obtained considering N₂-fixing rhizobia for inoculation of vegetable plants. Seed treatments with *Rhizobium leguminosarum* promoted seedling quality of *Brassica campestris*, *B. napus* and *Lactuca sativa* by increasing early seedling root growth [123], and the co-inoculation of *Rhizobium tropici* (CIAT 899) with selected nodule endophytic strains of *Bacillus*, *Paenibacillus*, *Burkholderia*, and *Pseudomonas* on common bean plants grown in greenhouses can replace any sub-optimal N availability for seedlings [124].

In addition to microbial plant biostimulants, the use of non-microbial plant biostimulants such as humic acid, macro and microalgal extracts, protein hydrolysates, chitosan and silicon are considered as sustainable and efficient tools to improve vegetable seedling quality and post-transplant performance under both optimal and sub-optimal conditions. In particular, Piccolo et al. [125] reported the application of unfractionated humic acids (applied at 40, 100, 1000 or 5000 mg/L) and humic fractions (applied at 40, 100 or 200 mg/L) did not increase either the germination rate, or the germination percentage of lettuce and tomato seeds treated in Petri dishes. However, the growth seedlings parameters of both tested species, increased in treatments with unfractionated humic acid without reporting any signs of inhibition even at the highest dose (5000 mg/L). The authors concluded that the beneficial effects of humic substances on seedlings quality were associated with cell elongation, as well as with a better water uptake. Similarly, Lulakis and Plestas [126] showed that the intermediate concentrations (100–300 ppm) of humic substances extracted from vine canes mature compost, boosted the tomato seedling growth (in terms of plant height) whereas an inhibitory effect was observed at higher concentrations (1000–2000 ppm). Young and Chen [127] attributed the biostimulation action of humic acid on lettuce seedlings to the presence of polyamines (putrescine, spermine and spermidine) which are responsible for the hormone-like activities of humic substances. Moreover, the humic acid application as seed priming (5000, 10,000 or 20,000 mg/L) or substrate drench (2500 or 5000 mg/L) promote seed root development (in terms of lateral root growth and number) in both cucumber and squash [128]. The increase of root growth in humic acid-treated plants could be adopted as an efficient and sustainable strategy and enhance stand establishment in commercial applications such as plug production. Furthermore, the biostimulant action of humic acid on seedling growth of two important vegetable species, such as tomato and eggplant, was investigated by Dursun et al. [129]. The authors demonstrated that substrate drench application exhibited the highest leaf stem (at 50 and 100 mL/L) and root growth (at 50, 100 or 150 mL/L) for tomato and eggplant seedlings, respectively compared to the untreated control, by improving macro and micronutrients (N, P, K, Ca, Mg, Fe and Cu) uptake and translocation, leading to a better nutritional status. The use of lignite derived solid humic substances (32% humic acids, 3% fulvic acid and 24% humin) as media amendment was also found effective to improve vegetables (lettuce, pepper, tomato and watermelon) seedling quality and post-transplant performance under optimal and sub-optimal conditions (drought and heat stress; [130]). The application of humic acid modulated the shoot and root growth parameters in a species-dependent manner. The root traits were significantly increased in fruit-treated vegetables, whereas the highest leaf performance was recorded in the leafy vegetable tested (i.e., lettuce). Although drought and heat stresses had negative impact on yield of the four vegetables tested, however, the humic acid-treated plants showed improved stress tolerance/avoidance compared to the untreated control.

Concerning the application of macro and microalgal extracts on seedlings quality, several authors [131–133] have demonstrated the biostimulation action of this important category of plant biostimulant which constitute more than one third of the total market [134]. A biostimulant product based on kelp extract (*Durvillaea potatorum* and *Ascophyllum nodosum*), applied at weekly intervals at a concentration of 4%, 1%, 0.5% or 0.2%, enhances

greenhouse broccoli establishment and growth by boosting shoot biomass, stem diameter and leaf area by 145%, 65% and 75%, respectively, compared to untreated plants [131]. Moreover, the foliar or drench application of two green (*Ulva lactuca* and *Caulerpa sertularioides*), and two brown (*Padina gymnospora* and *Sargassum liebmannii*) seaweed extracts at different concentrations (0.2, 0.4 or 1.0%) were assessed under controlled conditions on tomato seedlings [133]. The authors reported that specific seaweeds, such as *U. lactuca* and *P. gymnospora*, were the best to stimulate the tomato germination and seedlings quality (higher shoot and root length and weight) when they were applied at lower concentrations (i.e., 0.2%). The authors also demonstrated that soil drench application should be adopted among vegetable growers, since it was able to enhance tomato plant height, compared to the foliar application treatment. These results might be validated also on horticultural seedlings cultivated in a nursery under an organic system. In line with the previous research, the radical application of microalgae strains *Scenedesmus quadricauda* positively affected the growth of lettuce seedlings grown on an inert substrate, such as pumice. The authors showed that the application of microalgae two times during the growing cycle resulted in an increase in: (i) dry matter percentage and proteins content; (ii) carotenoids and chlorophyll chloroplast pigments; (iii) enzyme activities of glutamate synthase, cytrate synthase and phenylalanine ammonia lyase.

Thanks to the combination of morphological and metabolomics approaches, Lucini et al. [135] and Kim et al. [136] elucidated the hormonal effects of a vegetal-based biostimulant (containing lignosulfonates, key amino acids and peptides as well as micronutrients) on melon and tomato. In both experiments brassinosteroids and stress related compounds (carotenoids, flavonoids and glucosinolates) triggered in roots may have been involved in modulation of root system architecture by improving adventitious rooting responses observed after biostimulant root application, thus enhancing tolerance/resistance toward plant stress (i.e., transplant shock).

In another interesting study, non-microbial biostimulants based on chitosan microparticles improved both shoot and root biomass of tomato seedlings, reinforcing their performance before transplanting [137]. The contribution of chitosan microparticles seems more effective on: (i) the modulation of cytokinin and auxin hormonal signaling pathways; and (ii) the stimulation of the antioxidant enzyme activities CAT and SOD and defense protein markers. Finally, Bu and co-workers [138] demonstrated the beneficial effect of exogenous application of silicon under optimal and especially autotoxic (3-phenylpropionic acid) induced stress in cucumber seedlings by boosting the relative activities of key enzymes such as ascorbate peroxidase (APX), CAT, POD and SOD combined with the protection of the cell wall and membrane integrity (by reducing the $O_2^{\bullet-}$, H_2O_2 and proline contents and the percentage of electrolyte leakage).

Although several efforts have been carried out in the field of microbial and non-microbial plant biostimulants, more research is needed to elucidate the physiological, biochemical and molecular mechanisms/mode of actions in relation to their composition, application time, concentrations, environmental conditions and species, in order to develop a second generation of functional biostimulants with specific biostimulatory action(s).

5. Beneficial Microorganisms for Biological Control of Seedling Diseases

Damping-off, seedling blight, wilt and root rot diseases significantly decrease the quality of young seedlings of many horticultural crops such as cucumber, tomato, pepper, melon, eggplant, lettuce, etc. [139,140]. The common pathogens responsible for the spread of these diseases belong to fungi and bacteria of genera *Fusarium*, *Rhizoctonia*, *Verticillium*, *Sclerotium*, *Pythium*, *Xanthomonas*, *Ralstonia solanacearum*, etc. [140–142]. In the nursery, the spread of these pathogens is due to the use of infected potting media/trays, recycled irrigation water, or infected seeds [140]. Also, the intensification of nursery production, causing high moisture and nutrient supplies, can promote occurrence and spread of root zone pathogens [143].

Generally, in the nursery, pathogens are controlled by an intensive use of plant protection products [140]. However, in an organic system, pathogen management of root zone is based on the use of preventive measures such as certificate and resistant plant materials, endpoint inspections and quarantines [144]. When preventive measures are not enough to control pathogens, plant protection products may be applied. At the moment, the active substances registered for use in the nursery for an organic system against root zone pathogens belong mainly to microorganism groups also known as biological control agents (BCAs).

New BCAs are isolated from different substrates such as soil, vegetable material, compost, peat, etc. [145,146]. The BCAs can be applied alone or in consortia directly on seeds, sprayed to soil or used to enriched peat and compost. BCAs can able to establish antagonistic interactions against pathogens through specific mechanisms, such as parasitism, antibiosis or competition for nutrients or sites of infection and/or, indirectly, inducing the host plant's defenses [147]. These properties can be exploited in the management of nursery diseases [148]. Biocontrol definition also includes the use of living natural enemies of pathogens, and/or their products such as antibiotics, secondary metabolites, lytic enzymes, etc. [149]. The main potential BCAs studied belong to antagonistic fungal groups, such as *Trichoderma* spp., arbuscular mycorrhizal fungi and bacteria, such as *Bacillus* spp. and *Pseudomonas* spp. [150,151].

Trichoderma spp. are ubiquitous and free-living fungi that are among the most well-known and globally commercialized biocontrol fungi for agricultural applications. Their winning strategy relies on the exploitation of different mechanisms of action [152], strong rhizosphere competence [153] and endophytic status [154]. In the vegetable nursery sector, *Trichoderma* antagonistic strains may have versatile uses ranging from growing media bioactivation to the direct suppression of pathogens administered by drenching [155,156] and/or spraying [157]. *Trichoderma* contribute both to health management, reducing dependence on chemical fungicides, and to an increase of seedling quality associated with growth promotion [158]. Several plant protection biocontrol studies have confirmed the effectiveness of *Trichoderma* spp. in increasing tolerance against pathogenic fungi, bacteria and viruses. The increased tolerance was due to several mechanism of action such as mycoparasitism, the induction of hypersensitive response (HR), systemic acquired resistance (SAR) and/or induced systemic resistance (ISR), effects on plant nutrient condition, changes in plant transcripts by plant gene modulation, competition for nutrients and space, fungistasis, antibiosis and modification of the rhizosphere [159–161].

On a set of *Solanaceous* and *Brassicaceous* vegetable seedlings, *T. viride*, *T. harzianum*, and *T. koningii*, applied as seed treatment, proved to be able to control *Pythium aphanidermatum*, as well as the synthetic fungicides [162]. *T. virens*, *T. harzianum* and *T. asperullum* selected strains enrich organic media and effectively enhance the growth of *Abelmoschus esculentus* and *Amaranthus viridis* [163]. Nursery management with *T. harzianum* improved eggplant seedling performance and reduced damping-off disease [164] giving a significant advantage to the improved seedlings in the successive transplant [165].

A study on a single treatment with *T. harzianum* strain T22 on seeds of tomato (genotype 'Jubilee'), exposed to *Pythium ultimum*, reported that the seed treatment avoided losses (–15%) due to post-emergence damping-off [166].

Combined inoculation resulted in a general synergistic effect on disease control. Giarlardi et al. [141] tested some *Trichoderma* strains in consortia to reduce the severity of *Fusarium wilt* in lettuce (genotype 'Crispilla') and rocket (genotype 'Coltivata'). The BCAs were applied four times by spraying the soil in each plug tray with a high volume of water (1800 L/ha). The treatments reduced the spread of disease compared to the untreated control, with an average efficacy of 40% in both crops. Synergistic effects on control of *Fusarium wilt* incidence were found also in inoculations of *T. harzianum* combined with arbuscular mycorrhizal fungi, such as *Rhizophagus intraradices*, *Funneliformis constrictus* and *Claroideoglomus claroideum*, on Giotto melon (C. melo L.) [167]. Pascual et al. [28] has

studied the use of *T. harzianum* T-78 to enrich compost reporting a lesser pathogen incidence (*Fusarium oxysporum* f. sp. *melonis*) on muskmelon seedlings (genotype 'Giotto').

Beyond the *Trichoderma* group, research is proposed for the biocontrol of other fungi such as *Coniotirium minitans*, non-pathogenic *Fusarium oxysporum* and the oomycete *Pythium oligandrum*. *C. minitans* is a specific hyperparasitic fungus: it feeds on the sclerotia of *Sclerotinia* spp. For this reason, it is very useful in the defense of lettuce and other species susceptible to this rotting agent [168]. On the other hand, recent studies focused on the biocontrol ability of non-pathogenic *F. oxysporum* strains, resulting in the reduction of phytopathogenic attacks by occupying the infection sites or by triggering the immune response in the plant [169]. *P. oligandrum* is also proposed for the protection of nursery vegetables from the attacks of some of the most feared soil pathogens; it exerts antagonistic action through competition and production of enzymes and antifungal metabolites [170,171].

Other than fungi, bacteria can be used as a plant growth stimulator (plant growth promoting rhizobacteria, PGPR) or for controlling plant disease (biocontrol agents, BCA) [172–177]. These useful bacteria are largely used in agriculture, both in open field and in greenhouse [178–182]; they also can be used in nursery to improve growth and protect seedlings. The increasing application of useful bacteria in agriculture is due to the growing public demand for organic and chemical-free foods and for the recent restrictive regulations about the use of agrochemicals to reduce negative effects on environment and human health [183] and to reduce the development of pathogen resistance, due to repeated use of agrochemicals [184].

Bacteria used as PGPR and BCA principally belong to *Pseudomonas* and *Bacillus* genera [185–192]. Several authors have described the ability of many *Pseudomonas* spp. and *Bacillus* spp. rhizosphere strains to promote plant growth and control a plethora of fungal plant pathogens. Plant growth promotion is due to different mechanisms, such as biosynthesis of IAA, cytokinins or gibberellins [193–197], the induction, *in planta*, of these phytohormones or repression of synthesis of phytohormones that promote plant senescence, such as ethylene [194]. Other mechanisms that can promote plant growth are nitrogen-fixing or phosphate solubilizing properties of some strains [198,199]. Disease control is due to different direct mechanisms such as antibiosis, competition for nutrients and space, production of siderophores and toxic volatile compounds [200–208] and by indirect mechanisms, such as the activation of ISR [209–214]. In many cases, BCA have plant growth promotion properties as they can reduce density, on the roots, of phytopathogenic and deleterious bacteria [215,216] by the aforementioned mechanisms.

Among bacteria used in agriculture, *Bacillus* spp. are particularly suitable for commercial purposes. In fact, they are able to produce endospores, structures that confer resistance to desiccation, higher temperature and extreme pH and osmotic conditions, allowing bacterial cells to overcome adverse environmental situations.

Regarding BCAs based on bacteria, Sahu et al. [217] found that tomato seeds (genotype 'Selection-22') inoculated with endophyte *Bacillus* sp. 2P2 showed protection against *Sclerotium rolfsii* by reducing disease incidence by 67% in a nursery. The authors ascribed the activities of the antagonistic bacteria both to the inhibition of growth of *S. rolfsii* and induction of systemic resistance in seedlings. *Paenibacillus polymyxa* applications (BIO formulation) in the seedling nursery soil resulted in the lowest *Fusarium* wilt disease incidence on watermelon [*Citrullus lanatus* (Trunb.) Matsum and Nakai] genotype 'Kang-bing Jingxin' with an average biocontrol efficacy of 55% in comparison with untreated control [218].

Bacteria could be good antagonists against bacteria pathogens such as *Ralstonia solanacearum* and *Xanthomonas* spp. A previous study, in fact, showed as *Pseudomonas putida* A1 delayed wilt symptoms for 4 days and reduced disease severity [146].

The success of the biological control agents depends on many different factors, including the pathogen infestation level and the type of inoculum [219]. Also, the formulation of BCAs can play a key role in the effectiveness of products [220] as it can influence the survival of microorganisms on peat [221]. In the latter study [221], the authors reported

that, after 8 weeks, a formulated form containing oat, bentonite, vermiculite and water, kept the inoculation level of *T. harzianum* colony-forming units two times higher than the non-formulated *T. harzianum* inoculum in nursery plants grown in polystyrene containers. Bernal-Vicente et al. [222] assessed different types of solid (bentonite, vermiculite, river sand and wheat bran) and liquid (water, guar gum, carboxymethylcellulose) carriers for application of *Trichoderma harzianum* T-78 for improving its peat survival and promoting biocontrol activity against *Fusarium wilt* of melon in seedling nursery conditions. The liquid carrier was incorporated directly into the peat or used to soak seeds, whereas the solid carriers were mixed homogeneously with peat or superficially applied. Interestingly, the most effective treatments that gained the lowest percentage of infected plants and the greatest *T. harzianum* population were the solid treatments based on bentonite mixed with peat or vermiculite superficially applied, partially confirming the results obtained by Martínez-Medina et al. [221].

6. Conclusions

To improve nursery sustainability, a reduction of external inputs, especially in terms of synthetic fertilizers, pesticides and fungicides is required. From this point of view, an organic farming system might be an interesting sector to improve food quality and nursery sustainability. In horticultural production, a high quality of seedling is a goal. In fact, farmers require a seedling with high quality to overcome transplanting stress in a shorter time. In addition, seedlings with high quality are characterized by tolerance to abiotic and biotic stresses, guaranteeing high yield and good quality of the harvest products. These concepts are fundamental, especially for seedlings suitable to be cultivated under organic farming systems, where synthetic products are not allowed to overcome abiotic and biotic stresses. Until now, not much research has dealt with issues related to horticultural seedlings and only few studies have analyzed the organic production of seedlings in nurseries. Several strategies to improve the sustainable root zone management of seedlings grown in nurseries, are presented in the present review. All this information might allow the definition of precise protocols for helping nursery growers in the production of seedlings suitable to be cultivated in organic farming systems and in the correct applications of the strategies here reported. However, physiological, environmental, genetic and agronomic factors can form a complex network of interactions and synergies contributing to influence root growth of the seedlings.

7. Future Directions

Most nursery growers are worried about the future, and they not only aim to modernize equipment, but are interested in “what can happen tomorrow”. In fact, legislation, business and crop tendencies, new technologies and products, politics and environmental changes are all aspects to be considered. The nursery growers have to be prepared for the new changes, and this review aims to provide useful information on current opportunities and future directions on root zone management for improving seedling quality of organically produced horticultural crops. Some interesting key parameters should be monitored by nursery growers and investigated by researchers like: (i) architectural root traits (rooting depth and root length density); (ii) morphological root traits (root diameter and specific root length); and (iii) physiological root traits (root respiration, root nutrient uptake, root exudates). Finally, further studies with a multi-disciplinary approach are required to understand the seedlings’ root growth in terms of its integrity, allowing the development of new and sustainable strategies effectively able to increase the sustainability of organic production of vegetable seedlings in nurseries.

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