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Innovative growing media for horticultural nursery

Ph. D. dissertation by

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Index

ABSTRACT

Peat is a traditional growing media that is considered the best option to gain the optimum quality and yield for horticultural seedling production and ornamental plant cultivation. Efficient production of horticultural seedlings in nurseries requires that plants have to develop rapidly and uniformly. Peat results ideal for this purpose thanks to its appropriate physical, chemical and biological characteristics. However, it becomes necessary to look for alternative materials because the high cost of quality peat for horticultural use and decline in availability. Various organic residues produced in agriculture, livestock farming, forestry, industries and town, have been considered as possible alternatives to replace peat for growing media preparation. This is also in accordance with a policy of recycling and reusing.

In this study three organic waste materials were chosen to replace peat: coconut fiber, anaerobic digestate and biochar. The main chemical and physical and chemical properties of these materials were evaluated (pH, EC, BD, PSD, TGA, FT-IR, water retention, etc.).

Mixtures of these materials were tested to evaluate their ability to totally or partially replace peat as growing media. The test for their agronomical performance were seed germination and seedling growth of six vegetables species used worldwide for commercial purposes: tomato, pepper, lettuce, cauliflower, fennel in plug polystyrene trials and basil in pot.

Based on the research results, coconut fiber and digestate guarantee the better conditions for the development root systems of plantlets, both for water availability and supplying nutrient. Coconut fiber and digestate, when used in mixture with peat or between them, show physical and chemical properties, which are desirable for horticultural substrates. Plants growth on these formulated media resulted health under nutritional and agronomical profile.

Coconut fiber and digestate mixture, optimal for pepper, tomato and lettuce, appears the most innovative result because waste organic materials replace totally peat.

Mixtures containg biochar, whose properties were very different from coconut fiber and digestate, could be acceptable alternative to peat if used for plants at the advanced stage of development and in pot, as evidenced in experiment with basil.

CHAPTER 1
INTRODUCTION

1.1 Nursery plantlets producing in horticulture

Horticultural nursery is the compartment concerning the production of plantlets for horticultural, flower and ornamental cropping systems, placed both in open field and under greenhouse. It is a high-tech sector, open to innovations and new efficient solutions.

Horticultural crops cover up to 90% of cultivated area worldwide under greenhouse. The use of plantlets developed in nursery systems have a number of advantages that make more effective the field or greenhouse cultivation cycle, by including: time saving, reduction of plant replacement, improvement of plant growth homogeneity and promotion of enhanced health conditions. These beneficials have favored the development of specialized nurseries for large-scale production of plantlets, to satisfy the growing demand coming from vegetable and ornamental market, due to both economic and technical reasons. The dynamism of this particular agricultural sector involves all stakeholders, such as suppliers of seeds, the industry of growing media, containers for raising seedlings, technical equipment for planting and transplanting, automatic systems to monitor environmental parameters (air and substrate humidity, temperature, dioxide carbon), technical materials for the construction of greenhouses and tunnels, specialized products for cropping management, etc. Moreover, the knowledge of the operators in terms of cultivation techniques, nutrition, pest and growing management methods and varietal innovations, are growing continuously. However, scientific researches hardly works to find new technological solutions and innovations with the aim to improve the overall efficiency of the systems regarding the remunerative use of all economical, natural and energy resources involved. In the nursery, the control of environmental and hygrothermal factors is very important and is determinant for the qualitative and quantitative features of the yields. The sudden transition that there is between various stages of plant development, for example the passage from sowing to germination, foliar emission, root formation, etc., involves the succession of many biological and physiological changes, each favored by certain environmental conditions. Scarce attention in the management of these fundamental steps can lead to not acceptable degree of plants development for their subsequent use in productive systems. The plants, moreover, must be mature to transplantation and they will encounter conditions similar to those of the open field to avoid transplantation stress.

In the nursery, there is a long extensive use of container for the raising of vegetables. For cultivation in container, the selection of a suitable growing medium is crucial for watery and nutrient management and for technical, economic and environmental implications that it has on the productive cycle. The choice of a substrate is one of the most important questions for a nurseryman, to obtain the better standard quality and to reduce the economical incidence of the productive means on the production of growing media. The demand of suitable materials for composition of growing media is increasing and it is largely satisfied using imported peat. This organic material is suitable for agricultural uses thanks to its particular chemical, physical, and agronomical characteristics.

In recent years, excavated peat suffer an increase in prices due especially to extraction, processing, preparation and grading, blending and packaging of products and transport. The non-renewability of this resource, due to mobilization of fossil carbon and environmental changes of extraction sites, are the main drawbacks referred to the use of peat. Policies aimed to limit depletion of peat lands are expanding. In relation to this, the search and the study of other alternative materials to replace, totally or partially, peat in the growing media composition, is necessary.

1.2 Growing media

1.2.1 Chemical and physical characteristics of growing media

A growing media can be defined as a solid substrate that replace the natural soil for plant development on which roots grow regularly by extracting water and nutrients (Douglass et al., 2009). Several materials can aspire to be used for growing media preparation; however, the final choice depends by the whole of their intrinsic characteristics that may be correlated to agronomical functions, such as the ability to sustain plant growth (Parente et al., 2000). Therefore, the main objective concerning the identification of suitable substrates that could be used in nursery plantlets producing systems, is the incitation of a harmonious and balanced development of the plants (Bartolini G., 1991).

The plants grown in container, in fact, have a limited volume available to develop their root system; so, they could have an unbalanced relationship between aerial part and root.

In addition, respect to open field conditions, a greater attention for water, air, and nutrients plant consuming is necessary, because an excessive accumulation of salts, inadequate drainage and poor aeration could be detrimental for vegetative development.

In a plant nursery, the ideal growing medium must physically support the plant; promote oxygen exchange for root respiration through good porosity; allow the well drainage of exceeding water and prevent waterlogging by a high water-holding capacity. Furthermore, growing media through their ability to adsorb cations, satisfy nutritional requirements for the plants. In fact, most of the mineral plant nutrients are electrically charged ions, such as NH_4^+, K^+, Ca^{+2}, Mg^{+2}, which presence is fundamental for rapid growth of the nursery.

Additionally, pH is also a very important chemical property of a suitable growing media, because it affects nutrient availability.

The physical structure of growing media should be stable in time, standardizable for physico-chemical characteristics, both in the time and in the space, and resistant to the compaction and to volume reduction during the dehydration phase (said shrinking).

Of course, the materials used in growing media preparation must be easy available and cost-effective to satisfy requirements for sustainability. Low-cost waste materials are welcome.

Health of growing media will be ensured by the total absence of both human and plant pathogens (bacteria, nematodes, fungi, and insects). Moreover, the substrates must be free from chemical residues (pesticides) and from other potentially phytotoxic substances and weed seeds.

1.2.2 Types of growing media

There is a large variability in the potential feedstock and in physical and chemical characteristics of the substrates that may be used in horticultural systems. Moreover, new sources of natural and artificial by-products are being introduced every year in growing media industry. Substrates are primarily divided into organic and inorganic materials. The organic materials include synthetic (like phenolic resin and polyurethane) and natural organic matters (peat, coconut fiber and composted organic wastes). Inorganic substrates can be classified as natural unmodified sources (sand, tuff, and pumice), processed materials (expanded clay, perlite and vermiculite) and mineral wool (rockwool, glasswool). Based on the surface charge activity of materials, these can be distinguished in active (peat, tuff) or inert (rockwool and sand).

1.3 Peat: definition and classification

Peat is an accumulation of partially decayed vegetation or organic matter that is unique to natural areas called peatlands or mires. One of the most common component is *Sphagnum* moss, although many other plants can contribute. Peat forms a wetland, a land area that is saturated with water, either permanently or seasonally, such that it takes the characteristics as distinct ecosystem. In this environment, flooding block flows of oxygen from the atmosphere and reduce decomposition rates of organic residues.

Peatlands, also known as mires, particularly bogs, are the most important source of peat, but other less common wetland types also deposit peat, including fens, pocosins and peat swamp forests. Since organic matter accumulates over thousands of years, peat deposits also provide records of past vegetation and climates stored in plant remains, particularly pollen. Hence, they allow humans to reconstruct past environments and changes in human land use.

There are different ways to classify peat, based on:

- Decomposition rate and physical properties (according to Van Post graduation – Table 1-)
- Conditions to which organic matter decomposition occurred (high or low peats; light and dark peats),
- Botanic composition (*Sfagnum*, *Sedges*, ecc.),
- Chemical properties (eutrophic, nutrient rich; mesotrophic, moderately rich and oligotrophic, nutrient poor).

Table 1. Peat classification by Van Prost

Code	Peat description
H1	Completely undecomposed peat which, when squeezed, releases almost clear water. Plant remains easily identifiable. No amorphous material present.
H2	Almost entirely undecomposed peat which, when squeezed, releases clear or yellowish water. Plant remains still easily identifiable. No amorphous material present.
H3	Very slightly decomposed peat which, when squeezed, releases muddy brown water, but from which no peat passes between the fingers. Plant remains still identifiable, and no amorphous material present.
H4	Slightly decomposed peat which, when squeezed, releases very muddy dark water. No peat is passed between the fingers but the plant remains are slightly pasty and have lost some of their identifiable features.
H5	Moderately decomposed peat which, when squeezed, releases very "muddy" water with a very small amount of amorphous granular peat escaping between the fingers. The structure of the plant remains is quite indistinct although it is still possible to recognize certain features. The residue is very pasty.
H6	Moderately highly decomposed peat with a very indistict plant structure. When squeezed, about one-third of the peat escapes between the fingers. The residue is very pasty but shows the plant structure more distinctly than before squeezing.
H7	Highly decomposed peat. Contains a lot of amorphous material with very faintly recognizable plant structure. When squeezed, about one-half of the peat escapes between the fingers. The water, if any is released, is very dark and almost pasty.
H8	Very highly decomposed peat with a large quantity of amorphous material and very indistinct plant structure. When squeezed, about two-thirds of the peat escapes between the fingers. A small quantity of pasty water may be released. The plant material remaining in the hand consists of residues such as roots and fibres that resist decomposition
H9	Practically fully decomposed peat in which there is hardly any recognizable plant structure. When squeezed it is a fairly uniform paste.
H10	Completely decomposed peat with no discernible plant structure. When squeezed, all the wet peat escapes between the fingers.

Based on condition in which organic matter decomposition occurs, we can find high and down-placed peats.

Down-placed peat are found especially in temperate areas (Italy, West France), where are predominant vegetable species such as *Ciperacee*, *Carex*, *Phragmites*. These peats are formed in presence of water stagnation, because of groundwater.

The water, involved in this process, is rich of oxygen and salts, which allow highly decomposition and umification of dead plants. Therefore, a very dark peat was obtained, with high content of nutrients, especially N and Ca, pH, bulk density and low porosity. However, they have high susceptibility to squeeze and strain.

Between greater peat producers, we can find North Europe, Baltic Countries and Canada. They originate in cold and rainy environments, where precipitations are superior to evaporation. Mosses and vegetable residues retain rainwater, which is without salts and nutrient elements; so, it was created a saturated and anaerobic environment. In addition to different kind of *Shagnum*, suitable to live at these conditions, also *Eriophorum*, *Vaccinium*, *Erica* take part to formation of these peats.

In high-placed peatlands, two layers are distinguished: the first, deeper and more decomposed, with brown color; the second, shallow and slightly decomposed, of light color. These two layers constitute dark and light peat, respectively. Both layers are characterized by structural stability, low availability of nutritive elements and acid pH. Indeed, dark peat have higher microporosity, water holding capacity, cationic exchange capacity and buffering. Therefore, these peats seem to have suitable characteristics that meet growing medium requirements.

1.3.1 Physical and chemical characteristics of peat as growing medium

Peat is soft, homogenous, almost stable and safe under phytopathological aspects. These characteristics makes peat suitable for cultivation of all vegetable species. In Table 2 are summarized main physical and chemical characteristics of *Sphagnum* peat (Bures, 1997, Aenderek Th.G. L., 2000; Perelli et al., 2004), mostly used for growing media preparation.

Table 2. Main properties of the peat of *Sphagnum*.

Characteristic	*Sphagnum* peat
Bulk density (Kg/l)	0.07-0.30
Porosity (% vol.)	> 9
Air capacity (% vol.)	15-40
Water availability (% vol.)	25-30
Ph	3-5
Electrical conductivity (mS/cm)	0.20-0.60
Ashes (%)	1-6
Cationic Exchange capacity (meq/100 gr.)	100-170
Carbon/Nitrogen ratio	30-80

1.3.2 Why do scientists make much effort to search alternative materials for peat replacement?

The market of growing media has developed over the last thirty years, when the nurseryman have searched for new substrates with chemical-physical characteristics better than soils for plantlets production under controlled conditions. Therefore, farmers started to use peat, alone or in mixtures with blond and brown peats, and/or combined with perlite or pumice. The annual consumption of substrates, in Italy, is around 5 million m^3, consisting largely of peat imported from abroad (Pinamonti e Cementero, 1997).

The exploitation of peat in the nurseries increase energy costs linked to all phases of production, including excavation and transport of raw materials from countries manufacturers of Northern Europe to the various companies of the continent This is inciting the search for alternative materials to produce substrates known as "peat - free".

Moreover, public concerns about the consumption of peatlands that mobilize non-renewable carbon source and deplete natural habitats with specific characteristics of flora and fauna, are increasing (UFAFP/WSL, 2002).

Peatlands, in fact, are important ecosystems on which develops a wide variety of natural habitats that maintain diversity and the survival of some endangered species that are closely related to humans under social, economic and cultural aspects. These ecosystems are also sources of fresh water, and play an important role in carbon fixation.

The European Commission in 2001 has also ruled out the release of the Community Eco-label, for agricultural substrates containing peat or derivatives (Decision 2001/688/CE).

1.4 Peat substitute for growing media formulation

1.4.1 Coconut fiber

Coconut was commercially developed in Sri Lanka, Philippines, Indonesia, Southern India and Latin America (Evans et al., 1996a).

Coconut fiber derive from the processed mesocarp tissues, or husk, of the coconut (*Cocos nucifera*) fruit. Mesocarps are harvested, dipped in water and beaked up, obtaining mainly long fibers. The long fibers are used for various industrial purpose: ropes, mats, brushes, furniture, car seat covers, mattresses, packaging, floor coverings, pots and basket liners, erosion control netting, aquarium filters and absorbent pads for cleaning up oil spills. The remaining material is constituted by pith and medium and short fiber. This material is sieved to eliminate residual fibers and to obtain a product indicated as coir dust.

Coir dust is normally air dried and compressed into blocks or bails before it is exported to reduce transport costs. Before it can be used, the bale must be broken up. For small quantities, the bale can simply be placed in water which causes the coir dust to expand and the bale to crumble. With larger quantities, the bales are broken up in a mill. This method has the advantage of being able to handle dry material which is both lighter and less bulky to transport than wet coir.

Coconut fiber has appeared recently in horticulture and floriculture for soilless cultivation; this material can be used alone or in mixture and it is a possible alternative to peat due to its intrinsic characteristics.

In fact, this material has several features useful for a suitable peat-substitute (Creswell, 1992) such as: higher water-holding capacity; excellent drainage; absence of weeds and pathogens (Meerow, 1994); acceptable pH (between 5-7), cation exchange capacity and electrical conductivity (0.3-2.9 mS/cm by Evans et al., 1996b); renewable resource, with no ecological drawbacks to its use; slower decomposition and easier wettability than peat.

However, the coconut fiber tends to have a high content of Na and K compared to peat, but the sodium lye easily from the material when irrigated (Handreck, 1993).

For these reasons, coconut fiber is now widely accepted as a peat substitute, showing results in plant growth comparable to those of the peat.

Cresswell (1992), for example, tested coconut fiber as a growing medium for broccoli, tomato and lettuce seedlings and found earlier germination and greater size and uniformity of seedlings germinated and grown in this alternative material respect to peat.

Handreck (1993), instead, tested growth of *Petunia* x *hybrida* 'Celebrity Salmon' on mixes of different coconut fiber (from Sri Lanka and Malaysian) and peat observing equal growth on the substrates by supplying plant nutrients. This study concluded that plants developed on coir dust-based media required more Ca, S, Cu and Fe, but less K, than those grown on peat. A greater immobilization of soluble nitrogen with coconut fiber than peat was also observed.

1.4.2 Digestate

Digestate is the solid residue from anaerobic digestion of different organic materials, such as manure, plant biomass, sludge, organic fraction of municipal solid waste.

This material can be considered a good fertilizer, because anaerobic digestion causes a reduction of labile organic matter, but conserving nitrogen, phosphorus and potassium concentration that were present in starting feedstock.

In an anaerobic digestor, farmer and agro-industrial residues are biologically degraded, producing biogas and digestate.

The biogas is the main product, made by CH_4 e CO_2; it is submitted to energetic valorization (electrical energy and warm).

The digestate, indeed, is a homogeneous material, that have high humidity and characterized by more stable forms of orgnaic matter, rich in nutritive elements, such as nitrogen, phosphorus and potassium. The digestate is subjected to a further separation between solid and liquid phases, producing two fractions: one shovelable and one clarified, respectively, allowing liquid fraction recirculation and a split management of two fraction in agronomic use.

Shovelable fraction have a greater concentration of organic matter and volatiles, nitrogen in organic form and a low N-to-P ratio.

The clarified fractions have nitrogen, especially under ammoniacal form, high N-to-P ratio and lower organic matter concentration than the first.

As largely demonstrated from several studies the digestate, in fact, ensures valid fertilizer effects on the main horticultural crops.

The digestate was utilized in agromomic sector as substitute of chemical products in the cultivation of *Zea mays* plants: in 2013, Riva et al., demonstrate that use of digestate replace totally mineral fertilization.

Only in recent years, these material has been proposed to replace the peat in growing media. Compton et al, in 2006, demonstrated that plants of *Pelargonium* × *hortorum* grown on substrate constituted by mixing peat and digestate, increased dry weight respect to plants growth on peat alone.

A research has recently showed that the combination of potato anaerobic digestate with wood pellet biochar increased growth of tomato plants, as compared to the peat (Vaughn et al., 2015).

1.4.3 Biochar

Biochar is a solid material obtained from the pyrolisis of biomasses occurred at very high temperatures. Under the term “biochar” are included a wide spectrum of materials with specific characteristics. These depend from the conditions of pyrolysis (mainly temperature) and on the feedstock type used. As organic materials, all agricultural waste, including forestry, crop residues and animal manures, can be transformed into biochar. Potentially, biochar utilization may give many benefits. Biochars can be used to filter pyrolysis exhaust gases (Lehmann J., 2007), to obtain agricultural fertilizers (Marris E., 2006), to generate activated carbon by steam treatment (McHenry MP., 2209). As soil fertilizers, biochar has been used to improve forest productivity (Dumroese et al., 2009). While, when the biochar was used as a soil amendment, a significant portion of the recalcitrant biochar carbon can resist degradation for hundreds to even thousands of years, thus creating stable carbon pools (Monterumici et al., 2015). Biochar can have other benefits on soil including increases in the general fertility and water-holding capacity, reduction of bulk density, provision of additional cation exchange sites and for enhancement of microbial activity due to biochar-sourced carbon (DeLuca TH et al., 2008). These potential benefits promote plant growth and increase crop productivity, so it is considered a possible material for replacing (partially or totally) peat in growing media.

A previous study showed that pH, C-to-N ratio and bulk density of growing media increased proportionally to biochar rate used in mixtures with composted pine bark, (Kandal et al., 2016).

Tian (2012) demonstrated that plants of *Calathea rotundifola* cv. Fasciata grown on a mixture of peat and biochar (50:50 v/v), increased in total biomass by 22% compared to peat alone. The results indicated that biochar may be suitable as a partial substitute of peat in plant cultivation.

Monterumici et al. (2015), demonstrated that the best performance in terms of radish plants growth were given by mixture of biochar and compost.

CHAPTER 2

RESEARCH PURPOSE

Based on the last studies on the topic of peat-free growing media, our purpose is to increase acknowledgements about the utilization of alternative organic materials in horticultural nursery system.

In particular, this study aim: (1) to choose, in a policy of recycling and reusing, various organic materials: coconut fiber, digestate and biochar; (2) to analyze the main chemical and physical properties of these materials; (3) to evaluate their ability to replace, totally or partially, peat in growing media formulation; (4) to assess the agronomical performance of mixtrures on seed germination and plantlets growth of some vegetables species used worldwide for commercial purposes, including tomato, pepper, lettuce, cauliflower, fennel and basil.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials selection

Materials used in this study are reported in Table 3.

Table 3. Materials used in this study with indications of the suppliers.

Material	Acronym	General information
Peat	P	TRAYSUBSTRAT - Klasmann-Deilmann GmbH 49744 GEESTE Germany. pH (in water) : 6.0 EC: 0.35 dS/m Bulk density: 140 Kg/m^3 Total porosity: 85 % v/v Commercial volume: L70
Coconut fiber	FC	Water Ritention: 60-75 % Air porosity: 20-35 %
Digestate	D	Biogas Establishment , Cicerale (Salerno, Italy) Feedstock: manure, corn silage, hay or straw as structuring.
Biochar	B	Estabilishment AVG Feedstock: wood poplar Superficial area (m^2 g^{-1}): 42±4 pH: 9.6 ±0,1 Ashes : 220 ±20 C: 580±40 (g/Kg) N: 14±1 (g/Kg) Na: 0,15±0.01(g/Kg) K: 1.8±0.1 (g/Kg) Ca: 34.0±0.2 (g/Kg) Fe: 0.57±0.03 (g/Kg) Cu : 0.30±0.01 (g/Kg) Mn: 0.035±0.002 (g/Kg)

3.2 Characterization of the materials

3.2.1 Moisture

Sample moisture was determinated by drying in oven at 105 °C until constant weight, according this expression: U%= (initial weight- dry weight)/initial weight *100

3.2.2 Bulk density

Bulk density (BD) was measured filling, with the materials, holes of known volume normally used in nursery, and this expression was applied: BD (ρ) = Mass/volume

3.2.3 Particle size distribution

Air-dried samples are sieved with specific test sieves (9.5 mm; 6.3 mm; 3.15 mm; 2.0 mm; 1.0 mm; 0.5 mm e 0.25 mm) using a mechanical sieving machine (Analysette 03.502 Germania-Fritsch) and weight of each fraction was annoted.

Particle size distribution (PSD) was calculated according this expression:

Fraction mass (%) = (weight of the sample of each fraction/ total weigh)*100

Coarseness Index (CI), expressed as percentage of particles >1mm was determined.

3.2.4 Water retention

A sample of material was placed in a cylinder, wet until saturation and placed in a tensiometric cassette. It was determined water content, which presents a potential of the matrix equal to the set voltage (Metodo Ufficiale Suppl. ord. G.U. n.173 del 2-9-1997). In this study, tensiometric cassette was filled with sand and were applied tensions until -10 kPa (h=1 cm).

3.2.5 pH and EC

pH and EC were determined in aqueous extract (substrate/extractant ratio: 1/5 v/v), according to UNI EN 13037 and UNI EN 13038, respectively.

3.2.6 Bicarbonate content

Carbonate were determinated in aqueous extract (substrate/extractant ratio1:2.5 v/v), reducing carbonate with chloridic acid, throught Titrando 905 rilevator. Results were expressed in meq.

3.2.7 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy was carried out to qualitatively identify the constituents of studied materials.

Samples are dried, ground into fine particles (diameter ≤ 53 µm) and then analyzed with Perkin-Elmer Spectrum One FTIR Spectrometer. To obtain FTIR spectra, ten scans were collected for wave number ranging from 4000 to 400 $cm^{-1} \pm 4$ cm^{-1}).

3.2.8 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was obtained using Perkin-Elmer STA6000, Simultaneous Thermal Analyzer, under nitrogen and air atmosphere with a flow rate of 20 ml/min and 50ml/min respectively. The mass of the sample was about 10 mg. This experiment was carried out in a temperature interval of 30-990 °C. The weight loss and its derivative (DTG) as a function of temperature, was analyzed.

3.2.9 Germination bioassay

Lepidium sativum L. (cress) was chosen for biological characterization of the materials, thanks to its ability to confirm the absence of toxicity (Zucconi *et al.*, 1985). Aqueous extracts were obtained by shaking each substrate with distilled water (ratio 1:10 vol/vol). Twenty seeds were placed in Petri dishes (diameter 90 mm) on sterile filter paper with 4 ml of each extract. For the control, the seeds were treated with sterile and bi- distilled water. Seed germination and the length of the roots were measured after 72 h of growth at 25 °C. The percentages of relative seed germination (RSG), relative root growth (RRG) and the germination index (GI) were calculated as follows:

RSG (%) = (n° of seeds germinated in aqueous extract / n° of seeds germinated in water) *100

RRG (%) = (mean radical length in aqueous extract / mean radical length in water) *100

GI (%) = (RSG*RRG) /100.

3.3 Growing media formulation and nursery trials

3.3.1 Preparation and characterization of the mixtures

Ninenteen growing media were preparated by mixing peat, coconut fiber, digestate and biochar in different proportions. A control treatment, consisting of peat alone, was also included. All the treatments considered in this study are summarized in Table 4.

For these mixtures water content, pH and electrical conductivity were measured.

Table 4. Composition of the growing media.

	Materials % (volume)			
	Peat	**Coconut fiber**	**Digestate**	**Biochar**
1	100	0	0	0
2	0	100	0	0
3	0	0	100	0
4	0	0	0	100
5	50	50	0	0
6	50	0	50	0
7	50	0	0	50
8	25	75	0	0
9	25	0	75	0
10	25	0	0	75
11	0	75	25	0
12	0	75	0	25
13	0	50	50	0
14	0	50	0	50
15	0	25	75	0
16	0	25	0	75
17	0	0	75	25
18	0	0	50	50
19	0	0	25	75

3.3.2 Nursery trials

The growing media were tested for plug plantlets production of pepper (*Caspicum annum* L.) cv Topepo Rosso, tomato (*Solanum lycopersicum* L.) cv CRX71722 F1, lettuce (*Lactuca sativa* L.) cv Anelice, cauliflower (*Brassica oleracea* L. var. botrytis) cv Trofeo F1 and fennel (*Foeniculum vulgare* Mill) cv Tiziano (trial 1). Experiments were conducted in a greenhouse at a commercial nursery located in Eboli, Province of Salerno, Southern Italy.

The experimental treatments were assigned in a fully randomized design with four replications for each treatment and four seeds *per* replication. Seeds were sown in polystyrene plug trays (170 cells *per* tray with diameter of 2.5 cm and volume of 20 ml) filled with the substrate mixtures. The seeds were then covered with vermiculite, irrigated and, after incubation in germination chamber for 48-72 h, placed in greenhouse.

Emergence levels were checked daily and when the plants reached the commercial size, the ball consistence, seedling height, root length, number of true leaves *per* seedling and fresh and dry weights, were measured. For tomato and pepper, were also measured stem diameter (with a precision caliper ± 0.05 cm) and first internode height.

To assess the integrity of the ball in which plantlets developed, after the extraction from the holes at transplantation time, a consistency index was obtained by attributing a value in the range between 0 to 5 according to the following scale: 0 (volume of ball <19%; 1 (20-39%), 2 (40-59%), 3 (60-79%), (4) 80-99% and 5 (100 %).

Chlorophyll content was also measured on three leaves *per* plant, except for fennel, by using SPAD-meter (Minolta SPAD Chlorophyll Meter).

A further nursery experiment was conducted by using the mixtures that gave the better agronomic performances (in terms of germination index, consistency of the ball and fresh biomass *per* plant). Such mixtures were characterized for pH, EC, water content, bulk density and main elements content.

The new experiment (trial 2) was carried out in the same nursery conditions, considering four replications for each treatment and ten seeds for replication. A control treatment, consisting of peat alone, was also included for each vegetable specie. In addition to parameters checked in the first trial, for tomato, pepper and cauliflower, were also calculated Dickson's quality index (DQI) using the following formula DQI = TDW (g)/[SH (cm) / SD (mm) + ADW (g) / RDW (g)], where TDW is total dry weight, SH is seedling height, SD is stem diameter, ADW is aerial part dry weight, RDW is root dry weight

Samples dried of all vegetable species were analyzed to determine concentration of main elements.

Subsequently, ninenteen mixtures listed in table 2 were used as a cultural media for the potting production of basil (*Ocimum basilicum*, L.). Two plantlets of basil were transplanted in pots of 20 cm diameter. An experimental randomized block design was used, with three replications for each treatments (six plants for each treatment). The tests were performed under greenhouse at Experimental Farm of CREA-ORT, Battipaglia (SA).

At flowering, such parameters were checked: plant height, leaves number *per* plant, leave length, chlorophyll content, fresh biomass and dry weight of aerial part.

Aerial part of the plants, after drying in the oven until costant weight, was analyzed for cations and anions contents.

3.4 Chemical characterization of vegetables

By ionic chromatography were determinated anions (F^-, Cl^-, NO_3^-, PO_4^{--}, SO_4^{--}), in water extract in ratio 1: vol/vol and cations (NH_4^+, Na^+, K^+, Mg^+, Ca^{++}), previous digestion incineration according to Basta N.T. in *Determination of Total Potasium, Sodium, calcium and Magnesium in Plant Materials by IC* Soil Sci.Soc.Am. J., Vol 49, 76-81, 1989.

The individual analytes are diluted in subsequent times and determined by a conductivity detector after chemical suppression of the electrical conductivity of the eluent. (Dionex ICS-1500 -THERMO SCIENTIFIC). Results are expressed in mg/kg of dry matter.

3.5 Statistical Analysis

The collected data for the morphological traits of the plants and the chemical and physical characteristics of the materials and of growing media were statistically analyzed by using analysis of variance (ANOVA) to check any difference between the means. Data were subjected to Duncan's multiple range test for comparison of the means or Dunnet's test for comparison respect to control.

CHAPTER 4

RESULTS

4.1 Materials characterization

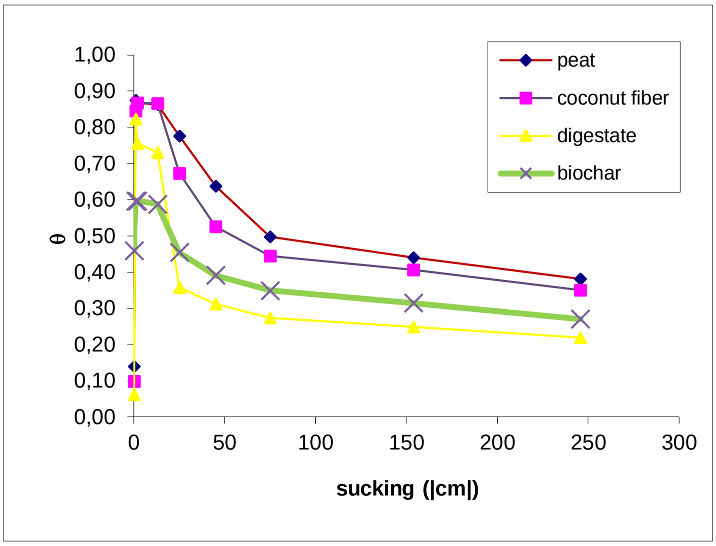
Comparison between some physical and chemical characteristics of the studied materials showed statistical differences respect to peat (Tab.5). Coconut fiber displayed higher pH respect to peat, but similar values for EC and BD and bicarbonate content; digestate is characterized for the highest bicarbonate and ashes content; biochar, at least, showed the highest values for pH, EC and BD.

Table 5. Physicochemical properties of the materials used in this study (values followed by different letters are significantly diverse ($P \leq 0.05$) according to Duncan test)

Samples	ph	Electrical Conductivity (mS/cm)	Bulk Density (gr/cm^3)	Ash (%)	Water content (%)	Bicarbonate content (meq)
Peat	6.54 d	1.92 b	0.08 b	6.66 b	69.62 b	1.54 c
Coconut fiber	7.68 c	1.89 b	0.07 b	8.60 b	81.55 a	0.89 c
Digestate	8.50 b	9.35 a	0.05 c	9.33 a	26.15 c	13.48 a
Biochar	9.65 a	9.65 a	0.26 a	3.05 c	15.78 d	5.14 b

Water retention was found high in peat, coconut fiber and digestate, lower in biochar. However, water-holding capacity of peat and coconut fiber showed similar trend in relation to suction, as well as digestate. Biochar, conversely, releases water more easily. (Fig. 1)

Figure 1. Water retention of the materials used in this study



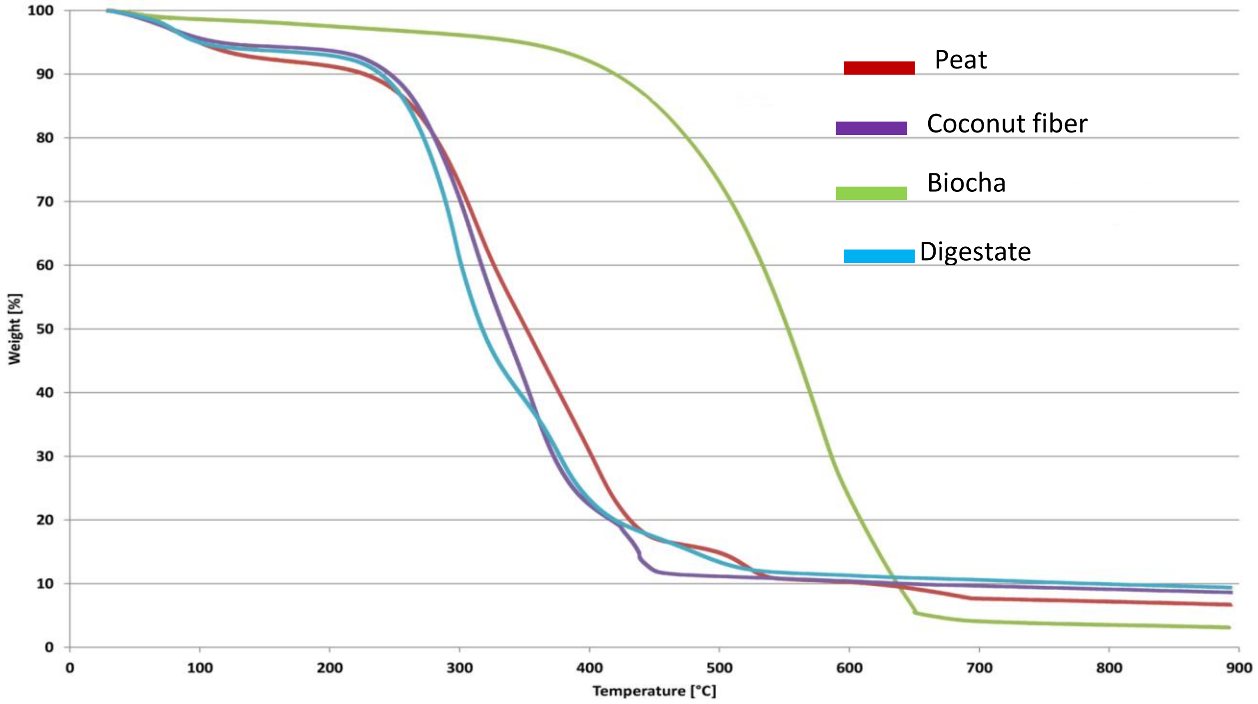
Effects of materials on germination index of cress (GI %) are showed in table 6. The GI values for all tested materials (coconut fiber, digestate and biochar) were equal or superior to 60%, showing the absence of detrimental effect derived from high salts concentrations or toxic substances. Among materials, only on biochar, root length showed significantly smaller values. Better radical elongation and higher GI values were found in cress tested on coconut fiber.

Table 6. Relative Seed Germination (RSG), Relative Root Growth (RRG) and Germination Index (GI)

	RSG	RRG	GI
Coconut fiber	93.75	87.29	81.84
Digestate	85.42	78.31	66.89
Biochar	79.17	76.38	60.47

The data obtained from thermograms of organic materials are summarized in Figure 2. The first weight decrease is related to moisture loss with a peak at 250 °C for all samples. Degradation of cellulose and lignine justifies the occurrence of the peak for coconut fiber at 450 °C. Digestate, having a greater polysaccharide and cellulosic component, degrades more rapidly, at lower temperature (about 400 °C). Biochar confirms its stable structure and it shows few and marked peaks, due to organic compounds tough to the pyrolysis.

Figure 2. Thermogravimetric results of the materials used in this study



The FTIR spectra of peat, coconut fiber, digestate and biochar are shown in figures 3-4-5-6.

Peaks reflect origin of the materials and their decomposition degree. In particular, for peat and coconut fiber, peat at 1614 nm is very marked, to underline emicellulose presence; in digestate spectrum, indeed, there is a greater diversity of polysaccharide component. Biochar show few but marked peaks due to organic compounds resistant to pyrolysis.

The FTIR spectrum of the peat displayed a number of characteristic absorbance peaks. Well-resolved peaks are seen for carbohydrate or polysaccharide (1061 cm-1), carboxylate (1520-1610 cm-1) and wax (strictly, aliphatic CH2 and CH3; 2850-2920 cm-1) as well as the broad hydroxyl band (centred at 3400 cm-1).

Absorbance between 3200 and 3600 cm^{-1} are characteristic of hydroxyl group stretching associated with cellulosic hydroxyl groups (and possibly water) while the absorbance around 2800-3000 cm^{-1} are indicative of aliphatic C-H stretching vibrations due to methylene and methyl groups. Absorbance at approximately 1730-1740 cm^{-1} and 1230 cm^{-1} are representative of carbonyl C-- O and C-O stretching vibrations, respectively.

In this study, digestate presented more peaks respect to other materials. The board band around 3400 cm^{-1} presents the H-bonded OH groups. A sharp peak at 2925 cm^{-1} and a slight shoulder at 2845 cm^{-1} show the aliphatic C-H stretching. The peak at about 1648 cm^{-1} is due to amide from polypeptides and aromatic C=C and COO^-. The peak at around 1515 cm^{-1} shows the aromatic ring vibration and represents the different substituted aromatic compounds. A small sharp peak at about 1425 cm^{-1} represent the CH_2, COO^- groups and around 1385 cm^{-1} there are the COO^- and CH_3 groups. The region of 1240- 1200 cm^{-1} due to the aromatic C-O stretching of phenols or/and the C-O of aliphatic esters. A broad peak at the 1100-1040 cm^{-1} range represents the C-O stretch of polysaccharides. The carbohydrates region is located at 1200-900 cm^{-1}.

Figure 3. FTIR spectrum of the peat

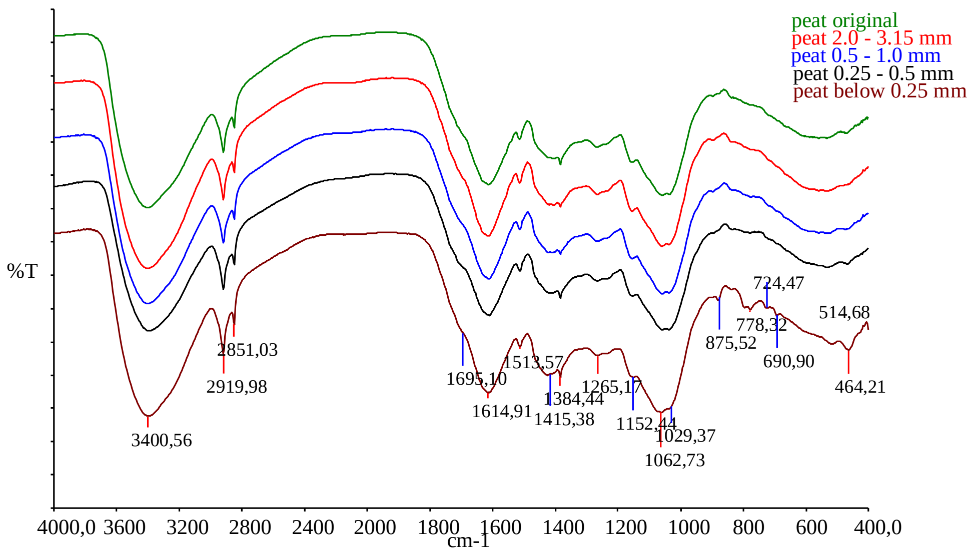


Figure 4. FTIR spectrum of the coconut fiber

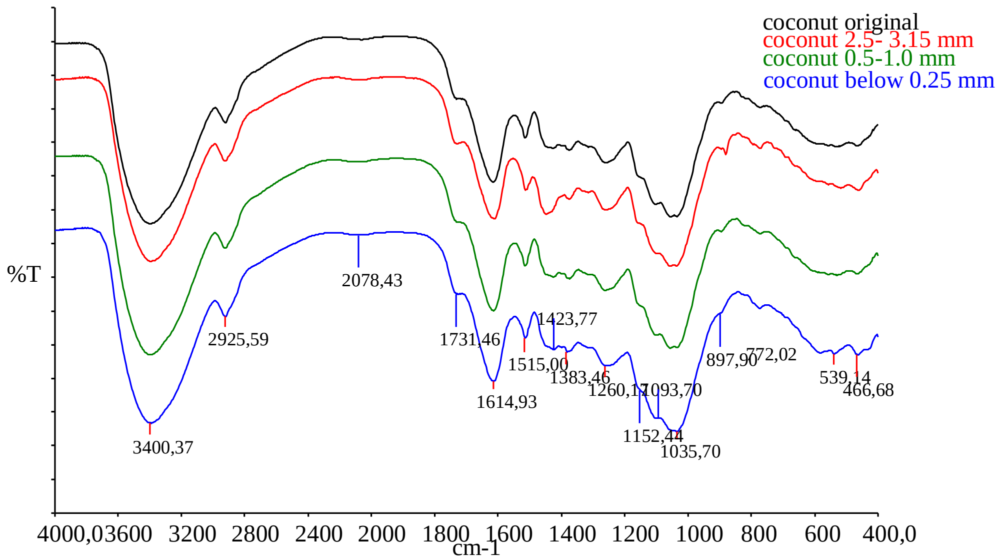


Figure 5. FTIR spectrum of solid digestate

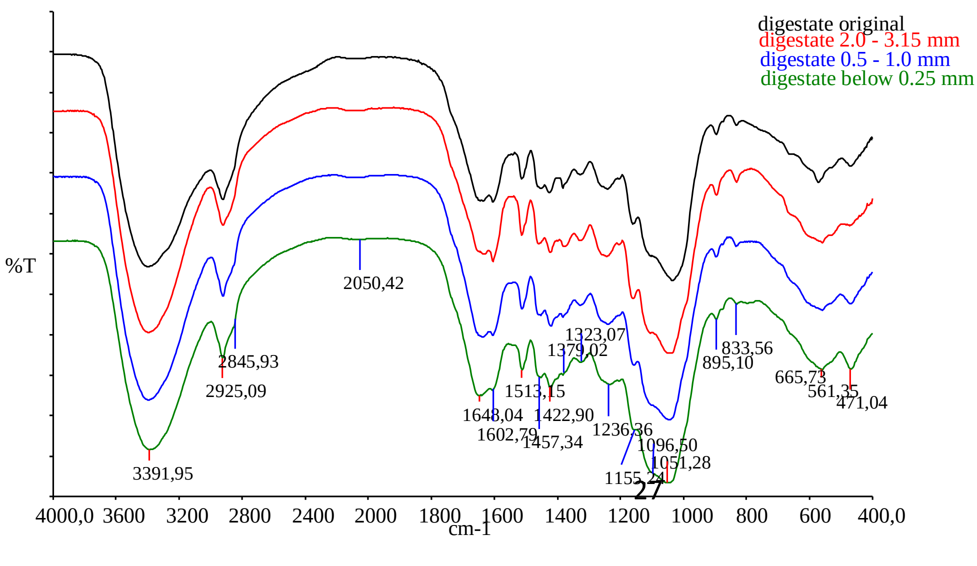
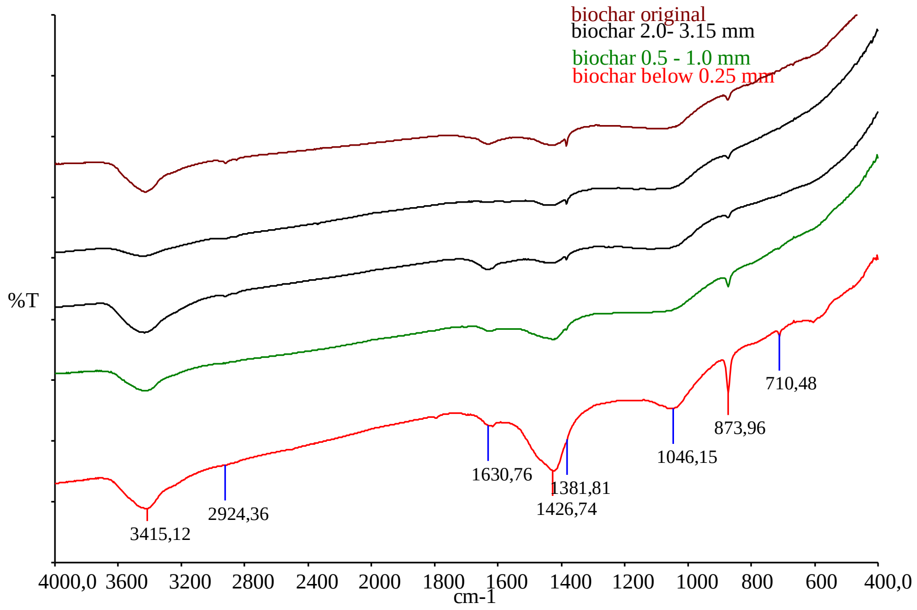


Figure 6. FTIR spectrum of the biochar



Particle size distribution is important to describe the physical quality of the substrate and its suitableness for plant growth.

In Table 7 are showed the particle size distribution of tested materials and their coarseness index. Peat and coconut fiber are made expecially by particles with diameter included between 1 and 2 mm (80% and 70% respectively), biochar by particles with diameter between 9.15 and 6.30 mm, digestate present a very variable distribution. Digestate and biochar showed high CI values: for this reasons, to make digestate and biochar more similar to peat, they were sieved before to use in agronomic trials (digestate at 5 mm, biochar at 4 mm).

Table 7. Particle size distribution of tested materials used in this study

Materials	> 9.15 (mm)	9.15- 6.30	6.30-3.15	3.15-2	2-1	1-0.5	0.5-0.25	Coarseness Index (%)
Peat	1.16	7.95	11.01	18.56	23.5	22.44	15.38	62.12
Coconut fiber	-	1.1	4.87	24.44	30.47	25.26	13.86	60.88
Digestate	20.07	19.1	18.86	17.98	12.57	8.08	3.34	88.58
Biochar	-	66.24	6.77	9.37	8.63	4.24	4.75	91.01

4.2 Nursery trials - Screening of growing media

The nineteen growing media affected differentially the emergence and plant growth of the five tested vegetable species. In detail, tomato, pepper and lettuce plantlets showed the better adaptability to variation of specificity of substrate features; cauliflower and fennel proved the most sensitive. In general, biochar gives detrimental effects on plant development against all species,also when it used in mixture with peat or other materials. Digestate and/or coconut fiber in mixtures gave well agronomic performances.

On biochar alone, the lowest levels of pepper seed germination was observed. Increasing biochar rate in the composition of growing media decreased the large part of biometric indexes. 50% coconut fiber and digestate increased plant height and fresh and dry biomass and incited values statistically similar to those observed in the control for root length, number of the leaves, length of the first leaf, stem diameter and chlorophyll content. Data are showed in table 8.

The composition of mixtures did not affected the germination of tomato seeds. However, all treatments, in comparison to peat control, incited lower values of ball consistency. 50% and/or 75% digestate increased 43%, on average, height of the plants. Moreover, with these mixtures, plants produced greater leaves number, longer first leaf and increased amount of fresh and dry biomass compared to control (Table 9).

The germination of lettuce seeds was not influenced by the type of material used in growing media preparation, as well as the number of the leaves (Table 10). Replacement of peat by aliquots of biochar gave negative effects on ball consistency. While chlorophyll content decreased in three treatments out of nineteen, such as 100B, 25FC_75D and 25FC_75B. Overall, fresh and dry weight of lettuce plantlets resulted at the same levels of the control in six digestate-based growing media. Plantlets grown on the control showed significantly higher values of height bush and root length than all mixtures.

For cauliflower, the lowest germination index and ball consistency were found on substrates made with biochar used alone or mixed with coconut fiber or digestate in the 3:1 ratio. While, there were no significant differences in the number of germinated plants for the other substrates. Number of leaves *per* plant and length of the first leaf were highest in peat control. Chlorophyll content in plants grown on peat mixed to 50% of coconut fiber, digestate and biochar, was comparable to that of control plants. Root length was positively affected by digestate and/or coconut fiber in the mixtures, giving taller plants than in the control. Finally, fresh and dry weight of the plants were reduced by all mixtures. Results are indicated in table 11.

Fennel is the highly sensitive, as well as the cauliflower, to changes in the composition of the mixtures, showing the larger number of cases with reduced germination. Plants grown on all mixtures showed a marked reduction in fresh weight and in the development of the plants. Instead, dry weight significantly decreased in only three cases respect to peat. When peat was mixed to digestate and/or coconut fiber, higher values in root length were recorded (Table 12).

Table 8. Assessment of biometric indices of pepper plantlets in different substrates (values followed by “*”were significantly different ($P \leq 0.05$) respect to control (P), according to Dunnet’s test; values followed by “()”are not analyzed statistically)

Growing medium	Germinated plants (n°)	Ball Consistency (1-5)	Height plant (cm)	Root length (cm)	Leaves (n°)	Height of 1st internode (cm)	Lenght 1st leave (cm)	Diameter stem (mm)	SPAD	Fresh biomass (gr/pt)	Dry biomass (gr/pt)
100P	3.75	5.0	5.9	5.7	4.1	5.7	3.8	1.4	23.3	3.15	0.43
100FC	3.5	4.9	4.5 *	5.4	4.7	4.1 *	2.7 *	0.2 *	22.8 *	1.49 *	0.24 *
100D	3.25	3.5 *	5.6	4.4 *	3 *	5.2	3.7	0.8 *	33.8 *	1.84 *	0.32
100B	2.5 *	0.4 ()	4.2 ()	2.8 ()	2.9()	4()	2.2()	0.7()	10.2()	0.51()	0.07 ()
50P_50FC	3.75	4.5	5.3	5.5	3.5	5.2	2.6 *	0.9 *	23.5	2.47 *	0.37
50P_50D	4	4.9	6.4	4.9	4.3	6.1	4.5	1.6	28.4	3.79	0.63 *
50P-50B	3.75	3.4 *	5.4	5.1	3.8	5.2	3.6	1.1	24.1	2.51 *	0.39
25P_75FC	4	3.4 *	6.5	4.5 *	3.5	6.0	2.7 *	1 *	18.7	2.08 *	0.32
25P_75D	4	5.0	6.7	5.0	3.9	6.4	4.2	1.7	30.4 *	4.55 *	0.73 *
25P_75B	3.75	1.9 *	6.1	4.0 *	3.3	5.2	2.9 *	0.9 *	17.4	1.53 *	0.25 *
75FC_25D	3.75	3.2 *	6.4	4.7	3.7	5.8	2.8 *	0.9 *	19.4	1.87 *	0.28 *
75FC_25B	3.75	2.4 *	5.5	5.4	2.8 *	5.1	2.4 *	1 *	17.1	1.73 *	0.22 *
50FC_50D	4	4.9	7.0 *	4.8	3.9	6.5	3.5	1.2	22.2	3.06	0.44
50FC_50B	3	3.7 *	4.1 *	4.3 *	2.5 *	3.9 *	2.6 *	0.6 *	16.8	0.81 *	0.13 *
25FC_75D	3.75	4.6	6.5	4.8	4.2	6.0	3.9	1.5	26.1	3.16	0.49
25FC_75B	4	1.4 *	4.2 *	3.6 *	3.1 *	4.0 *	2.5 *	0.6 *	9.0 *	1.08 *	0.14 *
75D_25B	3.75	2.9 *	3.7 *	5.4	2.3 *	3.5 *	1.9 *	0.5 *	7.5 *	0.66 *	0.10 *
50D_50B	3.75	2.5 *	3.5 *	2.8 *	2.1 *	3.3 *	1.7 *	0.5 *	12.4 *	0.79 *	0.10 *
25D_75B	3.5	2.5 *	3.9 *	2.9 *	2.1 *	3.8 *	1.8 *	0.5 *	9.7 *	0.70 *	0.11 *

Table 9. Assessment of biometric indices of tomato plantlets in different substrates (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Germinated plants (n°)		Ball Consistency (1-5)		Height plant (cm)		Root length (cm)		Leaves (n°)		Height of 1st internode (cm)		Lenght 1st leave (cm)		Diameter stem (mm)		SPAD		Fresh biomass (gr/pt)		Dry biomass (gr/pt)	
100P	3.3		4.9		5.7		8.2		3.9		5.1		5.2		1.6		32.6		4.77		0.48	
100FC	3.8		3.2	*	4.2	*	9.1		3.2	*	3.8	*	3.5	*	1.1	*	31.3		2.20	*	0.29	
100D	3.8		3.3	*	4.9	*	4.8	*	3.4		4.3		4.3	*	1.5		24.6	*	2.74	*	0.25	
100B	3.8		0.1	*	2.9	*	1.6	*	2.3	*	2.8	*	2.4	*	0.1	*	21.2	*	0.87	*	0.11	*
50P_50FC	3.8		3.6	*	5.4		9.7		3.3	*	5.0		5.2		1.6		30.1		4.00		0.35	
50P_50D	4.0		3.3	*	6.8		5.8	*	3.8		5.8		6.4	*	2.1	*	32.6		6.91	*	0.76	*
50P-50B	3.8		2.5	*	7.7	*	5.7	*	3.9		6.8	*	5.8		1.9		26.8	*	5.48		0.48	
25P_75FC	3.5		2.5	*	5.7		6.3		3.6		5.2		5.2		1.5		30.5		4.04		0.37	
25P_75D	4.0		3.0	*	8.2	*	5.9		4.1		6.4	*	6.3	*	2.3	*	28.2		8.26	*	0.74	*
25P_75B	3.5		1.3	*	5.5		5.4	*	3.9		5.0		4.5	*	1.2	*	25.3	*	2.56	*	0.23	
75FC_25D	3.7		2.9	*	6.6		7.9		3.9		5.8		5.1		1.8		29.2		4.62		0.38	
75FC_25B	3.8		1.6	*	7.3	*	4.6	*	3.8		6.5	*	4.5		1.3		24.2	*	3.15	*	0.44	
50FC_50D	4.0		3.1	*	3.2	*	5.5	*	1.9	*	3.1	*	2.0	*	0.6	*	26.7	*	0.75	*	0.11	*
50FC_50B	3.8		0.8	*	3.7	*	7.1		2.3	*	3.2	*	2.9	*	0.7	*	29.3		0.84	*	0.12	*
25FC_75D	4.0		3.2	*	4.7		4.5	*	3.9		4.1	*	4.6		1.9		28.3		2.92	*	0.33	
25FC_75B	3.8		0.4	*	4.1	*	4.7	*	2.3	*	3.4	*	2.9	*	0.4	*	25.9	*	0.86	*	0.09	*
75D_25B	3.5		1.6	*	2.6	*	3.4	*	2.3	*	2.5	*	1.8	*	0.2	*	25.9	*	0.61	*	0.07	*
50D_50B	4.0		2.1	*	2.9	*	4.5	*	2.1	*	2.7	*	2.4	*	0.1	*	25.4	*	0.73	*	0.10	*
25D_75B	4.0		0.0	*	3.0	*	2.3	*	2.6	*	2.9	*	2.4	*	0.6	*	19.7	*	0.94	*	0.08	*

Table 10. Assessment of biometric indices of lettuce plantlets in different substrates (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test; values followed by “()”are not analyzed statistically.

Growing medium	Germinated plants (n°)		Ball consistency (1-5)		Height bush (cm)		Root length (cm)		Leaves (n°)		Lenght 1st leave (cm)		SPAD		Fresh biomass (gr/pta)		Dry biomass (gr/pta)	
100P	4.0		4.6		4.2		7.2		3.8		2.5		20.5		4.82		0.59	
100FC	4.0		4.6		2.8	*	6.9		3.8		1.4	*	18.7		3.76		0.45	*
100D	3.5		4.9		3.3	*	5.3	*	4.4	*	1.4	*	18.3		2.88	*	0.25	*
100B	4.0		2.3	*	1.8	*	1.7	*	3.5		1.4	*	11.7	*	0.94	*	0.09	*
50P_50FC	4.0		3.6	*	3.1	*	6.7		3.8		1.6	*	18.2		3.37	*	0.42	*
50P_50D	3.5		5.0		3.9		5.0	*	3.7		2.1		21.0		4.13		0.48	
50P-50B	4.0		2.4	*	4.1		6.4		3.4		2.8		18.9		4.40		0.51	
25P_75FC	4.0		4.0		3.3	*	7.0		3.8		2.1		20.5		4.43		0.53	
25P_75D	4.0		4.9		3.4	*	5.8	*	3.9		2.0		18.4		4.15		0.48	
25P_75B	4.0		2.3	*	3.3	*	5.5	*	3.9		2.0		19.8		3.53	*	0.34	*
75FC_25D	4.0		4.4		3.8		5.7	*	3.5		2.6		18.8		4.76		0.51	
75FC_25B	4.0		2.8	*	3.4	*	5.5	*	3.8		2.2		15.9		2.16	*	0.22	*
50FC_50D	4.0		4.9		3.6		4.6	*	3.6		1.9		17.7		4.24		0.50	
50FC_50B	4.0		4.1		2.7	*	4.7	*	2.9		1.2	*	19.1		1.24	*	0.12	*
25FC_75D	4.0		4.9		2.3	*	4.7	*	3.2		0.8	*	17.7	*	1.90	*	0.25	*
25FC_75B	4.0		2.9	*	2.5	*	2.7	*	3.5		0.8	*	13.2	*	1.07	*	0.08	*
75D_25B	4.0		3.4	*	1.1	*	3.4	*	3.3		1.1	*	n.d.		0.46	*	0.06	*
50D_50B	4.0		4.0		1.5	*	2.7	*	3.2		1.3	*	n.d.		0.67	*	0.06	*
25D_75B	4.0		2.1	*	1.7	*	1.5	*	3.3		0.7	*	n.d.		0.79	*	0.06	*

Table 11. Assessment of biometric indices of cauliflower plantlets in different substrates (values followed by "*"were significantly different ($P \leq 0.05$) respect to control (P), according to Dunnet's test)

Growing medium	Germinated plants (n°)		Ball consistency (1-5)		Height plant (cm)		Root length (cm)		Leaves (n°)		Lenght 1st leave (cm)		SPAD		Fresh biomass (gr/pt)		Dry biomass (gr/pt)	
100P	4		5		2.6		6.8		2.4		3.9		38.8		2.18		0.34	
100FC	4		4.9		2.3		9.1	*	1.9	*	1.8	*	30.0	*	0.72	*	0.10	*
100D	4		4.6		1.8	*	6.1		1.5	*	1.2	*	28.6	*	0.62	*	0.10	*
100B	0	*	n.d		n.d		n.d		n.d		n.d.		n.d.		n.d		n.d.	
50P_50FC	4		5		2.9		8.2		2.1		3.0	*	32.3		1.39	*	0.22	*
50P_50D	4		5		2.5		8.5	*	1.9	*	2.5	*	32.3		1.17	*	0.17	*
50P-50B	3.8		4.6		2.5		6.5		2.1		2.9	*	32.7		1.15	*	0.17	*
25P_75FC	3.5		5		2.1	*	8.4		2.0		2.1	*	28.3	*	0.86	*	0.13	*
25P_75D	4		4.9		1.7	*	10.7	*	2.0		1.7	*	25.9	*	0.78	*	0.12	*
25P_75B	3		2.5	*	1.8	*	6.4		2.1		2.0	*	27.9	*	0.52	*	0.08	*
75FC_25D	3.8		4.9		1.8	*	8.9	*	2.1		1.7	*	22.8	*	0.71	*	0.10	*
75FC_25B	4		3.3	*	1.9	*	7.6		2.0		1.7	*	26.6	*	0.75	*	0.10	*
50FC_50D	4		4.3	*	1.7	*	10	*	2.0		1.5	*	n.d.		0.78	*	0.11	*
50FC_50B	3.5		4.7		1.7	*	6.3		2.0		1.8	*	n.d.		0.62	*	0.09	*
25FC_75D	4		5		1.6	*	8.1		1.9	*	1.4	*	n.d.		0.71	*	0.11	*
25FC_75B	0	*	n.d		n.d.		n.d		n.d		n.d.		n.d.		n.d		n.d.	
75D_25B	3		2.5	*	1.3	*	4	*	3.0	*	1.1	*	n.d.		0.36	*	0.06	*
50D_50B	3		2.6	*	1.2	*	4	*	3.2	*	1.1	*	n.d.		0.32	*	0.05	*
25D_75B	0	*	n.d.		n.d.		n.d		n.d		n.d.		n.d.		n.d		n.d.	

Table 12. Assessment of biometric indices of fennel plantlets in different substrates (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test; values followed by “()”are not analyzed statistically)

Growing medium	Germinated plants (n°)		Ball consistency (1-5)		Height plant (cm)		Root length (cm)		Leaves (n°)		Fresh biomass (gr/pt)		Dry biomass (gr/pt)	
100P	3.5		4.7		10.0		4.8		2.3		2.46		0.32	
100FC	4		5		3.2	*	5.8	*	1.4	*	0.99	*	0.10	
100D	1.5	*	5 ()		3.9 ()		6.2 ()		1.3 ()		0.16 ()		0.02 ()	
100B	0.75	*	0 ()		5.5 ()		2.4 ()		2.0 ()		n.d.		n.d.	
50P_50FC	3.5		4.5		6.4	*	5.5		1.9		1.28	*	0.35	
50P_50D	4		4.7		6.3	*	5.7	*	1.4	*	1.35	*	0.14	
50P-50B	3		2.6	*	6.7	*	4.5		1.9		1.01	*	0.13	
25P_75FC	3.7		3.6		4.4	*	5.5	*	1.4	*	0.94	*	0.10	
25P_75D	3.5		4.9		3.7	*	5.7	*	1.1	*	0.83	*	0.10	
25P_75B	1	*	1 ()		6.2 ()		3.9 ()		1.8 ()		n.d.		n.d.	
75FC_25D	3.2		4.4		5.3	*	5.4		1.0	*	0.68	*	0.07	*
75FC_25B	4		3.2	*	3.1	*	5.1		1.1	*	0.84	*	0.08	
50FC_50D	3.2		4.5		4.9	*	5.3		1.0	*	0.65	*	0.07	*
50FC_50B	2.7	*	3.5 ()		5.3 ()		4.3 ()		1.2 ()		0.20 ()		0.02 ()	
25FC_75D	2	*	4.9 ()		4.1 ()		5.6 ()		1.0 ()		0.10 ()		0.02 ()	
25FC_75B	0	*	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.	
75D_25B	3.2		3.3	*	3.9	*	4.4		1.0	*	0.39	*	0.04	*
50D_50B	1	*	4 ()		4.7 ()		2.9 ()		1.0 ()		n.d.		n.d.	
25D_75B	1.7	*	4 ()		4.8 ()		2.2 ()		1.0 ()		0.10 ()		0.01 ()	

4.3 Selected mixtures

Considering germination the index,consistency of the ball and fresh biomass, some mixtures are selected for each vegetable specie (Table 13).

Table 13. Selected mixtures

Growing media	Vegetable species
100 Peat CONTROL	
50P_50FC	Cauliflower ad fennel
50P_50D	Pepper,tomato, lettuce and fennel
25P_75D	Pepper,tomato and lettuce
50FC_50D	Pepper,tomato and lettuce

Fennel grown on substrates obtained replacing peat with coconut fiber or digestate in ratio 1:1 (v/v), showed higher values of consistence of the ball, height of the plant, number of the leaves, fresh and dry weights (Tab.14). Cauliflower, instead, proved to be the most sensitive among the tested species, showing acceptable results only for one mixture (50P_50FC). However, in this case, plants displayed lower values for height and dry weight in comparison to peat control, with significantly higher length of the roots (Tab. 15). Pepper and tomato showed values for all biometric indexes similar or even higher to those recorded on peat alone (Tabb. 16-17) in mixtures with digestate. Finally, lettuce also improved growth on digestate or coconut fiber-added media (Tab.18).

These results are validated by Dickson's quality index (DQI).This parameter was calculated for plantlets whose the measures of all descriptive parameters were available : tomato pepper and cauliflower. This index slightly decreased, increasing the rate of organic matter replaced for tomato, pepper and cauliflower in the range 0.273-0.212, 0.402-0.333 and 0.125-0.106 respectively. Any statistically differences were checked between various treatments (Figg 7-8-9).

Table 14. Plant growth and developmental characteristics of fennel plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Consistency ball (1-5)	Height plant (cm)	Root length (cm)	Leaves (n°)	Fresh biomass (gr/pt)	Dry biomass (gr/pt)
100P	5	16.47	7.59	3.50	1.94	0.23
50P_50D	5	18.86 *	8.97 *	4.08 *	2.87 *	0.31 *
50P_50FC	5	18.55 *	8.65 *	3.80 *	2.71 *	0.31 *

Table 15. Plant growth and developmental characteristics of cauliflower plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Consistency ball (1-5)	Height plant (cm)	Root length (cm)	Leaves (n°)	Height of 1°internode (cm)	Lenght 1st leave (cm)	Diameter stem (mm)	SPAD	Fresh biomass (gr/pt)	Dry biomass (gr/pt)
100P	5	2.99	10.65	4.3	2.29	4.21	1.71	34.58	1.4	0.26
50P_50FC	5	2.65 *	12.31 *	4.6	1.94 *	3.79 *	1.66	36.48	1.33	0.21 *

Table 16. Plant growth and developmental characteristics of tomato plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Consistency ball (1-5)	Height plant (cm)	Root length (cm)	Leaves (n°)	Height of 1st internode (cm)	Lenght 1st leave (cm)	Diameter stem (mm)	SPAD	Fresh biomass (gr/pt)	Dry biomass (gr/pt)
100P	5	10.15	14.06	4.78	7.02	5.40	2.57	28.88	1.51	0.18
50P_50D	5	10.25	14.65	4.68	6.67	4.94 *	2.51	28.73	1.41	0.18
25P_75D	5	11.91 *	9.08 *	6.28 *	4.91 *	4.26 *	2.44	32.55 *	1.89 *	0.19
50D_50FC	5	12.25 *	7.18 *	4.58	6.81	6.26 *	2.99 *	31.40 *	2.06 *	0.19

Table 17. Plant growth and developmental characteristics of pepper plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Consistency ball (1-5)	Height plant (cm)	Root length (cm)	Leaves (n°)	Height of 1st internode (cm)	Lenght 1st leave (cm)	Diameter stem (mm)	SPAD	Fresh biomass (gr/pt)	Dry biomass (gr/pt)
100P	5.0	10.0	5.4	6.1	7.8	6.0	2.0	39.63	1.91	0.28
50P_50D	5.0	10.6	5.3	6.2	7.1 *	5.1 *	2.0	42.69 *	2.04	0.34 *
25P_75D	5.0	9.5	5.5	6.2	6.6 *	4.6 *	1.9	35.63 *	1.52 *	0.27
50FC_50D	4.9 *	9.6	5.6	8.0 *	5.8 *	4.3 *	1.9	45.10 *	1.97	0.28

Table 18. Plant growth and developmental characteristics of lettuce plants (values followed by "*"were significantly different ($P \leq 0.05$) respect to control (P), according to Dunnet's test)

Growing media	Consistency ball (1-5)	Height bush (cm)		Root length (cm)		Leaves (n°)		Lenght 1st leave (cm)		SPAD		Fresh biomass (gr/pta)		Dry biomass (gr/pta)
100P	5	3.8		8.77		5.68	c	2.87		20.7		1.12		0.53
50P_50D	5	3.29	*	7.63	*	7	*	2.37	*	19.99		1.15	*	0.47
25P_75D	5	3.04	*	6.61	*	6.44	*	2.26	*	17.13	*	1.25		0.68
50FC_50D	5	2.99	*	6.1	*	6.73	*	2.14	*	16.71	*	1.15	*	0.61

Figure 7. Dickson quality index (DQI) of the seedlings of tomato with different compositions of the substrate

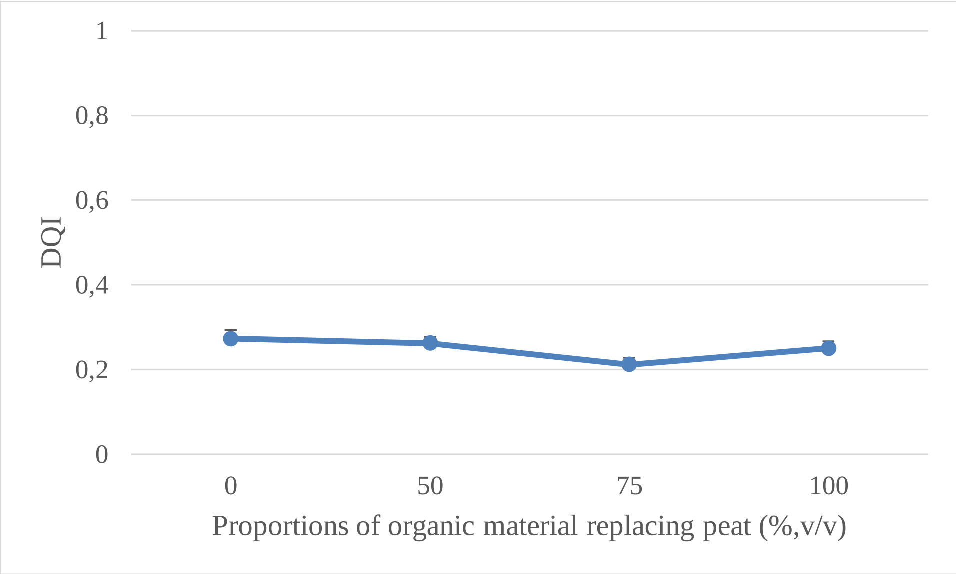


Figure 8. Dickson quality index (DQI) of the seedlings of pepper with different compositions of the substrate

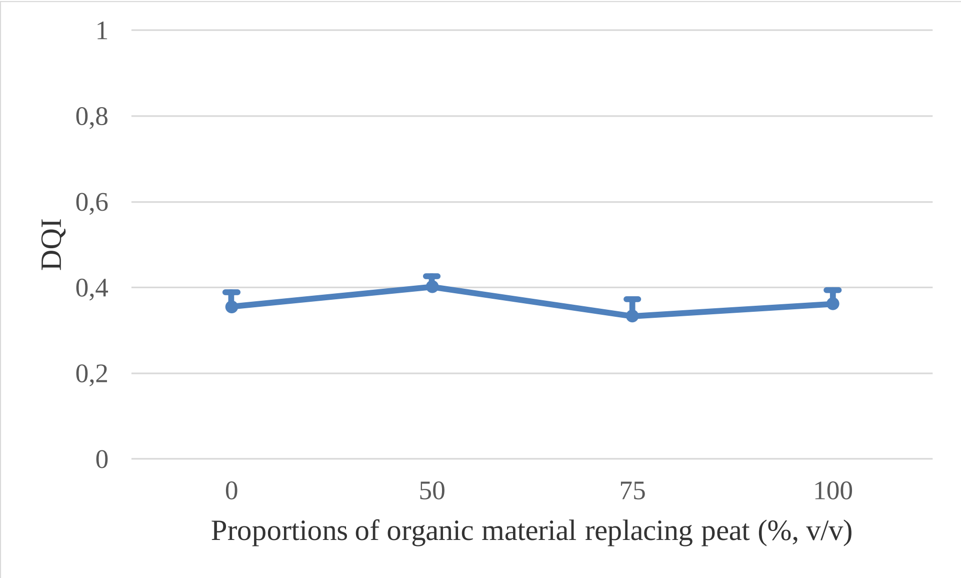
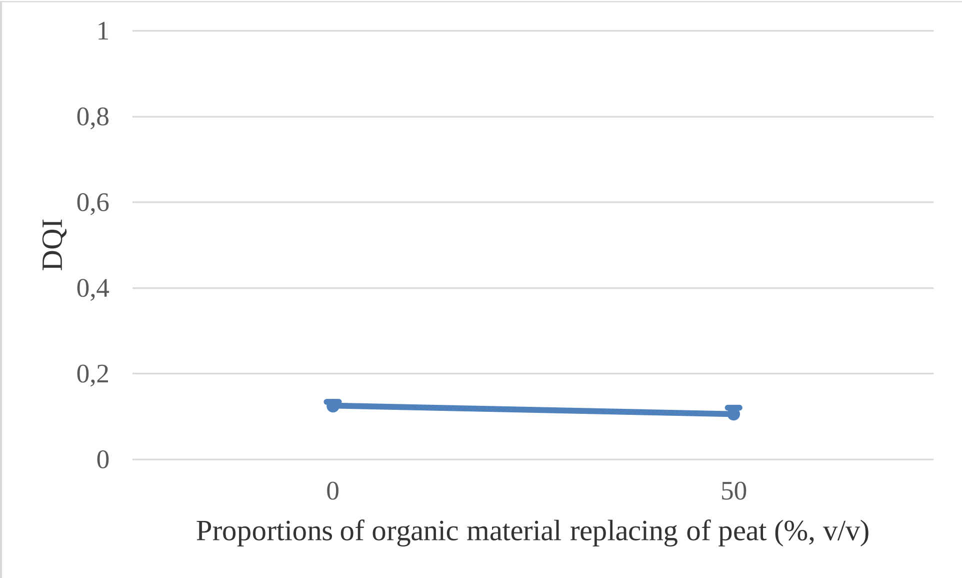


Figure 9. Dickson quality index (DQI) of the seedlings of cauliflower with different compositions of the substrate



4.4 Chemical characterization of the selected mixtures

pH, EC and HCO_3^- content of mixtures were significantly influenced by the amount of digestate and/or coconut fiber used (Tab. 19). Replacing peat, especially with 75% of digestate, pH and EC increased, showing the higher values (8.04 and 4390 µS/cm for pH and EC, respectively). Presence of digestate in the mixture led to a significantly increase of HCO_3^- content.

Table 19. pH, electrical conductivity (EC) and HCO_3^- in different substrates (values followed by "*"were significantly differfent ($P \leq 0.05$) respect to control (P), according to Dunnet's test)

Growing medium	pH		EC (µS/cm)		HCO_3^- (meq)	
100P	6.00		2034		1.540	
50P_50FC	6.69		569	*	1.590	
50P_50D	6.90	*	3988	*	9.710	*
25P_75D	8.04	*	4390	*	8.955	*
50FC_50D	6.92	*	4106	*	9.658	*

Replacing peat with digestate and/or coconut fiber had led to a greater availability of macroelements N, P and K. Nitrate ion showed significantly higher concentration only in 50P and 50D. On other side, it was recorded a greater concentration of sodium and chlorine, in ionic form. Mg and PO_4^{2-} increased in peat-replaced media, reverse trend was recorded for Ca and SO_4^{2-} (Tab.20).

Table 20. Nutrient concentration of the studied materials and selected mixture (values followed by "*"were significantly different ($P \leq 0.05$) respect to control (P), according to Dunnet's test)

Growing medium	Na		NH_4^+		K		Mg		Ca		Cl^-		NO_3^-		PO_4^{2-}		SO_4^{2-}	
	Meq																	
100P	0.624		0.279		1.381		2.681		7.304		0.237		0.223		0.117		0.283	
100FC	1.740	*	0.201		3.971		1.081		3.227	*	0.483		0.080		0.010		0.183	*
100D	3.890	*	1.447	*	38.638	*	5.636	*	3.649	*	1.880	*	0.583	*	0.417	*	0.207	*
100B	0.168		0.246		6.543	*	2.672		2.295	*	0.047		0.077		0.093		0.187	*
50P_50FC	1.036		0.169		2.664		1.714		4.245	*	0.200		0.077		0.103		0.227	*
50P_50D	3.217	*	1.627	*	28.336	*	6.939	*	5.298	*	1.183	*	0.777	*	0.393	*	0.180	*
25P_75D	3.388	*	1.533	*	28.133	*	4.110		4.298	*	1.713	*	0.270		0.347	*	0.180	*
50FC_50D	3.396	*	1.722	*	30.291	*	5.034	*	4.448	*	1.303	*	0.267		0.377	*	0.170	

4.5 Chemical characterization of vegetable tissues

Mg, Ca and SO_4^{2-} contents of underground part of fennel increased in peat-replaced media. Treatments did not gave differences for elements in the leaves, with the exception for K, that was double concentrated in plants grown on 50P_50D respect to control. Roots grown on peat and coconut fiber mixed media (50:50 v/v) showed higher values of Na, K, Mg and Ca (Tab. 21).

For cauliflower, the rate of coconut fiber used in the media affected the concentration of only K and SO_4^{2-} in shoots and roots. The concentration of all other elements was comparable with those recorded for the plants grown on peat alone (Tab. 22).

Pepper grown on mixture peat-free (50FC_50D) presented higher concentrations of K and Mg in the leaves, as showed in table 23.

Increasing the rate of peat replacement, lower concentration of NO_3^- and Mg were observed in the roots of tomato plants (Table 24).

For all treatments, lettuce plants were richest in K, Cl and PO_4^{2-}, especially in aerial parts. Higher concentration of NO_3^- was found in roots of plants grown on media formulated by mixing peat and digestate (25:75 v/v) and coconut fiber and digestate (50:50 v/v). These results are pointed out in table 25.

Table 21. Nutrient concentration, in above and underground part of fennel plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl	NO_3^-	PO_4^{2-}	SO_4^{2-}
					mg/Kg (dry matter)				
Above ground part									
100P	5.772	0	28.903	5.861	16.84	4.552	4.434	23.136	12.062
50P_50FC	6.834	0	28.399	7.597	24.24	4.654	4.659	22.138	12.281
50P_50D	6.862	0	45 *	7.837	19.68	9.263 *	4.693	25.626	12.478
Underground part									
100P	3.34	0.532	6.66	1.26	14.36	10.185	4.784	25.134	9.743
50P_50FC	10.551 *	0	40.57 *	13.718 *	26.71 *	10.204	4.703	25.465	9.944 *
50P_50D	4.809	0	6.126	4.08 *	20.76 *	11.522	4.92	25.219	9.098 *

Table 22. Nutrient concentration, in above and underground part of cauliflower plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl	NO_3^-	PO_4^{2-}	SO_4^{2-}
					mg/Kg (dry matter)				
Above ground part									
100P	3.332	0	18.47	6.547	17.914	1.838	4.318	12.8	11.3
50P_50FC	2.429	0	28.15 *	7.422	18.045	1.783	4,393	12.5	17.2 *
Underground part									
100P	2.118	0,000	9.365	7.286	21.801	4.668	4.680	16.1	8.81
50P_50FC	2.419	0.394	11.440	6.581	20.743	4.894	4.703	15.4	9.73 *

Table 23. Nutrient concentration, in above and underground part of pepper plants (values followed by “*”were significantly different ($P \leq 0.05$) respect to control (P), according to Dunnet’s test)

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl	NO_3^-	PO_4^{2-}	SO_4^{2-}
	mg/Kg (dry matter)								
Above ground part									
100P	2.108	0	27.672	7.159	15.224	6.910	3.561	16.558	9.059
50P_50D	1.897	0	22.255	6.301	14.838	3.964	5.779	12.590 *	4.454 *
25P_75D	2.812	0	27.835	8.492	18.721	4.872	4.960	14.219	7.173 *
50FC_50D	2.963	0	34.474 *	9.904 *	16.558	4.720	3.040	10.410 *	3.790 *
Underground part									
100P	2.076	0.363	15.265	6.388	19.682	5.483	4.712	18.490	10.727
50P_50D	2.712	0.448	10.741 *	7.518	18.595	6.635 *	4.465	14.241 *	10.352
25P_75D	1.870	0 *	9.391 *	5.235	18.236	5.419	5.624 *	13.789 *	8.585 *
50FC_50D	2.670	0.365	16.243	4.617 *	15.323	6.857 *	4.944	19.631	8.270 *

Table 24. Nutrient concentration, in above and underground part of tomato plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test)

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl	NO_3^-	PO_4^{2-}	SO_4^{2-}
	mg/Kg (dry matter)								
Above ground part									
100P	2.306	0	14.587	11.020	30.103	12.001	3.523	18.507	24.642
50P_50D	2.061	0	17.024	9.732	18.539 *	9.070 *	0 *	22.499	22.283
25P_75D	2.510	0	26.377 *	12.550	23.721	15.600	4.947 *	24.516	14.427 *
50FC_50D	3.063	0	26.301 *	19.830 *	17.378 *	8.580 *	2.241 *	28.845	12.692 *
Underground part									
100P	4.382	0.797	15.354	9.318	17.810	8.090	6.574	32.805	8.659
50P_50D	4.976	0.829	29.846	6.561 *	17.775	6.220	5.444 *	26.896	12.054
25P_75D	3.616	0 *	30.498 *	5.772 *	21.290	8.160	0 *	28.562	13.983 *
50FC_50D	3.426	0.449 *	31.721 *	7.988	20.067	11.300 *	4.858 *	33.385	10.980

Table 25. Nutrient concentration, in above and underground part of lettuce plants (values followed by "*"were significantly different (P≤0.05) respect to control (P), according to Dunnet's test)

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl	NO_3^-	PO_4^{2-}	SO_4^{2-}
	mg/Kg (dry matter)								
Above ground part									
100P	2.901	0	13.396	4.179	10.736	3.917	2.630	5.149	7.215
50P_50D	3.780	0	38.075 *	4.747	14.102	7.704 *	2.988	10.439 *	7.570
25P_75D	3.607	0	27.887 *	3.730	11.425	9.648 *	4.390 *	14.301 *	8.503
50FC_50D	3.964 *	0	31.856 *	4.158	9.694	10.062 *	2.292	12.437 *	6.169
Underground part									
100P	3.919	0.664	7.879	5.747	16.011	5.059	3.197	23.965	8.744
50P_50D	2.674	0.386 *	27.655 *	4.845	20.691	4.352	0 *	18.816	8.604
25P_75D	3.036	0.567	38.043 *	6.138	22.124	4.773	5.245 *	13.870 *	8.859
50FC_50D	3.562	0.176 *	26.396 *	3.495 *	18.734	5.465	4.470 *	24.389	8.228 *

4.6 Effects of different growing media on growth attributes of basil

The partial replacement of the peat with coconut fiber, digestate or biochar, did not affect fresh and dry basil biomass, according to statistical analysis (Tab 26). Total replacement of the peat, conversely, had decrease fresh biomass in all treatments, except for 75FC_25B and 25D_75B, while only 50FC_50D, 75FC_25D, 75FC_25B, 50D_50B and 75D_25B were lower for dry weight.

Number of the leaves and content in chlorophyll was not influenced by the type of substrate, while in some cases (50P_50D, 50FC_50B) higher plants respect to control were observed. Similarly leave length, slightly was affected by five treatments out of 19.

In table 27 content of the main anions and cations in basil leaves was displayed.

These values range between 0.92-4.01 mg/Kg (Na), 0.01-0.68 mg/Kg (NH4+), 21.69-46.82 mg/Kg (K), 2.42-8.44 mg/Kg (Mg), 12.42-57.73 mg/Kg (Ca), 5.39-13.53 mg/Kg (Cl^-), 4.41-9.22 mg/Kg (NO_3^-), 13.93-19.29 mg/Kg (PO_4^{2-}), 9.09-10.24 mg/Kg (SO_4^{2-}). K, Mg, Ca, Cl^-, PO_4^{2-}, SO_4^{2-}. Concentration increased in many treatments by partial or total replacement of the peat, while Na NH_4^+ and NO_3^- are an exception. It should be emphasized that there were not effects on Na concentration, which could be negative for development of the plants. Increase of other elements concentration is correlated to better nutritional state of the plants.

Table 26. Basil fresh and dry weight, chlorophyll content (SPAD), plant height, number of leaves and length of the first leave (values followed by "*"were significantly different (P≤0.05) respect to control (P), according to Dunnet's test).

Growing medium	Height plant (cm)		Leaves (n°)		Lenght 1st leave (cm)		SPAD		Fresh biomass (gr/pt)		Dry biomass (gr/pt)	
100P	21.8		34.2		11.9		40.2		27.24		3.59	
100FC	23.8		38.5		11.5		41.1		28.09		3.15	
100D	25.9		29.7		10.5		38.2		27.04		3.64	
100B	25.7		33.2		10.2		38.3		24.81		3.20	
50P_50FC	23.7		37.7		10.4		40.9		29.88		3.69	
50P_50D	26.5	*	32.5		10.6		42.0		29.41		3.80	
50P-50B	25.3		27.2		10.1	*	39.7		24.87		3.21	
25P_75FC	24.3		32.3		10.5		42.7		28.50		3.50	
25P_75D	23.7		38.8		10.9		40.3		24.03		3.22	
25P_75B	24.0		27.8		10.1	*	39.2		23.03		2.97	
75FC_25D	21.7		34.7		10.5		41.4		21.90	*	2.79	*
75FC_25B	27.0	*	31.3		10.7		37.5		22.38	*	3.00	
50FC_50D	26.0		33.2		9.9	*	38.2		22.02	*	2.92	
50FC_50B	23.7		31.5		10.1	*	38.7		22.62	*	2.86	
25FC_75D	22.5		29.2		10.3		38.9		22.46	*	2.56	*
25FC_75B	23.3		33.5		10.6		39.3		24.20		2.62	*
75D_25B	25.7		33.3		10.4		37.9		24.02		3.33	
50D_50B	22.7		27.8		10.3		38.3		22.07	*	2.78	*
25D_75B	22.0		29.0		9.7	*	37.6		21.86	*	2.71	*

Table 27. Nutrient concentration in basil plants (values followed by “*”were significantly different (P≤0.05) respect to control (P), according to Dunnet’s test).

Growing medium	Na	NH_4^+	K	Mg	Ca	Cl^-	NO_3^-	PO_4^{2-}	SO_4^{2-}
	mg/Kg (dry matter)								
100P	2,85	0,42	21,69	2,93	29,66	5,39	7,24	13,93	9,09
100FC	1,51	0,32	34,38	3,23	12,42 *	7,90	9,22	16,98 *	10,03 *
100D	1,71	0,65	26,80	4,27	32,27	12,33 *	1,37	15,42	9,20
100B	3,32	0,45	30,70	3,91	31,67	9,0,1 *	4,63 *	11,98	9,23
50P_50FC	1,02	0,19	23,25	2,42	36,67	9,98 *	6,96	16,48	9,73
50P_50D	0,73	0,26	36,54	4,60	34,40	13,53 *	6,11	18,11 *	9,60
50P-50B	1,60	0,18	33,25	3,99	40,49	7,04	5,17	17,26 *	10,13 *
25P_75FC	1,55	0,17	34,30	6,06 *	53,53 *	10,38 *	7,98	18,69 *	10,19 *
25P_75D	1,40	0,63	43,91 *	4,98	27,81	10,05 *	4,71	15,58	9,17
25P_75B	1,74	0,16	29,45	5,14	57,73 *	6,22	4,73	13,32	9,58
75FC_25D	4,01	0,58	44,90 *	4,19	32,37	11,26 *	6,46	19,29 *	10,24 *
75FC_25B	2,17	0,17	36,97	5,91 *	48,52 *	8,65 *	4,62	13,54	8,93
50FC_50D	2,68	0,00	34,79	7,36 *	40,74	10,76 *	4,98	16,27	9,17
50FC_50B	3,49	0,38	33,96	5,66	41,30	7,79	4,98	13,75	9,52
25FC_75D	1,93	0,56	46,82 *	7,54 *	41,93	9,87 *	7,85	18,83 *	9,99 *
25FC_75B	3,43	0,42	37,38 *	8,44 *	38,97	9,67 *	6,11	16,45	10,08 *
75D_25B	3,39	0,68	32,28	6,66 *	47,30 *	9,09 *	4,72	14,01	9,42
50D_50B	2,04	0,39	45,99 *	5,30	48,24 *	10,07 *	4,41 *	15,43	9,62
25D_75B	0,92	0,19	35,21	4,18	41,25	11,96 *	4,56	17,06 *	9,57

CHAPTER 5
DISCUSSION

Peat is considered worldwide a traditional growing media on the basis of its suitable agronomical characteristics that confer quality to production of horticultural seedling and ornamental plants. However, alternative materials for substrate composition are strongly searched. Efforts to find new solutions that could allow to solve this important issue are done by growers and nurserymen, linked to the whole industrial chain, and by researchers. This problematic has became more urgent by the fact that peat is a non-renewable resource and it is a scarce and expensive cultural substrates (Lazcano et.al, 2009). Several studies have focused their attention on set up of new low-cost organic materials, which could replace peat in the formulation of growing media for the production of vegetables plug transplants (Mazuela and Urrestarazu, 2005).

Properties of materials can differently affect the growth and the development of the plants. Therefore, the selection of the materials between various alternatives is important for plant productivity. A good growing media have to provide sufficient anchorage or support to the plant, to serve as pool for nutrients and water, to allow oxygen diffusion to the roots and to permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad et al., 2001). In addition, growing media also play an important role for seed germination and for source of nutrient and quality of seedlings (Wilson et al., 2001).

In order to assess the applicability of new materials for growing media formulation, the evaluation of the suitability of agronomical performances of the substrates is the first requirement to be satisfied. The choice is supported by scientists through the utilization of tools based on high-technologies coming from different fields. In particular, in several recent studies, these peculiar investigations are entrusted to Nuclear Magnetic Resonance Analysis (NMR), Fourier Transform Infrared Spectroscopy (FTIR), Thermal Analysis and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), as the most advanced methods. Therefore, chemical fine analysis (including the determination of elemental composition to assess nutritional and/or anti-germinative aspects) and molecular spectroscopic analysis (to know the actual composition of organic matter components) are largely used. Moreover, physical and hydrological characteristics are taking more into account because of the crucial influence of these properties on the functionality of substrates for cultivation. These characterizations served to individuate the material or the best mixture that could replace, totally or in part, peat in the media growing preparation.

In this study, we selected new mixtures, alternative to peat, to be used as growing media for nursery horticultural plants among organic materials such as coconut fiber, digestate and biochar on the basis of their characteristics and agronomical performances. The choice of these materials, obtained by recycling organic feedstock, allowed to achieve two objectives: organic residues re-utilization and reduction of the environmental impact of the use of peat.

Agronomic responses of some mixtures proved better than the others. In particular, biochar, in most of the cases, affecting negatively the plant growth (with exception of basil), even when it occurs at low concentrations in mixtures. Findings of this work confirmed that materials readily available and non-expensive, such as coconut fiber, digestate and limitedly biochar, are suitable for plantlets production under nursery conditions. In fact, firstly, no fitotoxicity was observed. Overall, a peat-free substrate obtained by mixing coconut fiber with digestate appeared comparable to peat and to peat with coconut fiber growing media for plantlets production. This study reveals a strong effect of growing media on the tested vegetable species, both in terms of germination and plant development. Further, the percentage of alternative materials used to replace peat strongly affects the observed properties of growing media, indicating a quantity-depending effect.

There are several characteristics of the substrates that can influence their utilization as growing media. Accessibility and availability of nutrients for plants in growing media are related with the pH of media, so increase or decrease in pH could have direct effects on plant growth and development;

moreover, pH outside the optimum range can adversely affect plants by damaging roots (Mehmood et al., 2013). Different authors suggested, for pH values, acceptable limits for an ideal substrate ranging among 5.3-6.5 (Abad et al. 1993; Bunt, 1988; Noguera et al., 2003; Sanchez- Mondero et al., 2004). Only peat showed value included in this range (6.54), whilst the studied substrates ranged from 6.54 to 9.65, this last recorded for the biochar, probably due to low acid functionality on surface and characteristics of the ashes.

Soluble salt level, estimated by measuring the electrical conductivity (EC), is an important parameter for materials used as growing media. Salinity, in fact, represents the main limiting factor for seed germination and plant growth (Bustamante et al. 2008; Gasco et al., 2005). Among the components contributing to salinity there are Na, K, Cl^-, ammonia, nitrate and sulfate.

The suggested reference EC level for growing media is <550 µS/cm as reported by Abad et al., 2005, Robbins and Evans, 2013 and Yeager et al., 1997. High values indicate a large amount of soluble salts that may inhibit biological activity. Only for biochar and digestate, the recorded EC levels are over the suggested reference indicating an excess of soluble salts. It has been mentioned in different articles that the excess of soluble salts and/or of $NH4^+$ are responsible for the lower percentages of germination and the delays in development observed in seedlings of tomato, lettuce, pepper and other vegetables species, on substrates based on mushroom compost (Lohr et al., 1984), biosolid composts (Vavrina, 1994; Roe et al., 1997) and composts of urbane wastes (Roe and Kostewicz, 1992). In any case, some researches have proposed to replace peat with other nutrient-rich and complex organic material, such as composts, in order to increase significantly electrical conductivity of the media, remaining in the acceptable range.

However, as demonstrated by Noguera et al. 1997, 2000 a-b, the excess of soluble salts is easily and effectively leached from the material under customary irrigation regimes when used for ornamental plants in containers or “grow-bags” for tomatoes, flowers, etc., in garden greenhouses.

In our work, coconut fiber displayed EC value similar to that of peat, both under the reference level, as confirmed by Abad et al., (2001). These researches have selected 13 coconut coir dust samples from Asia, America and Africa to evaluate their physico-chemical and chemical characteristics: two samples showed a very low salt content, similar to that of peat.

The FT-IR spectra gained insight about types of chemical functional groups that were present in materials tested in this study.

Similar spectra of peats were found in literature, in which slight shifts of peaks have been related to the extraction area and the decomposition rate of the peats (Chapman et al., 2001).

Selected coconut fiber in this work showed FT-IR spectra similar to those found in other studies. As reported by Brigida et al. (2010), spectra of a green coconut fiber coming from Brazilian northeast coast, revealed the strong presence of cellulose and lignin structure of the fiber as well as of hemicellulose. Further, typical peaks of esters, ethers and phenols groups attributed mainly to waxes from the epidermal tissues have been detected (Herrera-Franco & Valadarez- Gonzàlea, 2005).

In literature, little has been found about digestate composition, in terms of functional groups. However, the used digestate shows surface polar characteristics in all dimensional fractions as well or probably higher (mainly in terms of polysaccharides and polypeptides) in comparison to peat and coconut.

Findings about FT-IT spectra of biochar in this study agree with Kloss et al. (2012), who characterized biochars derived from wheat straw, poplar and spruce woods that were slowly pyrolyzed. Their biochars were highly heterogeneous and the FT-IR suggested the loss from the original materials of volatile aliphatic compounds with the concomitant formation of aromatic structures during the pyrolysis. As compared with peat and the other alternative materials, the FT-IR analysis of the used biochar indicates very lower polar functional groups on the surface for all dimensional fractions.

Bulk density (BD) is an indicator of compaction in media that have importance for growth of plant roots (Kevin et al., 2010). BD of containerized substrates gives a good indication of porosity, which determines the rate at which air and oxygen can move through the substrate. In this study, the bulk density of coconut fiber is similar to that of peat, whilst BD of biochar and digestate is significantly different. Abad et al., (2001) defined that the bulk density requirement of an ideal substrate should be < 0.40 g cm^{-3}. The range of BD value for all tested materials is under that limit value. Digestate has the lowest bulk density, while biochar showed the highest. High bulk density values have the disadvantage of increasing the transportation cost and reducing porosity and the water-holding capacity (Corti et al., 1998). Progressive addition of biochar pellets in growing media to replace peat showed a slight increase in BD (Dumroese et al., 2011); however, in soil, Basso et al., 2013 showed that addition of biochar decreased BD values. The particle size distribution (PSD) is important to describe the physical quality of the substrate and its suitableness for plant growth because affects the balance between water and air content (Raviv et al., 1986). In fact, PSD of a growth medium determines pore space, gas exchange and water-holding capacity (Abad et al., 2001). Handreck (1983) studied the particle size and physical properties of container media and concluded that particles smaller than 0.55 mm, and in particular smaller than 0.25 mm, have the highest influence on porosity and water retention. Researchers have reported that particles between 0.25 and 2.00 mm are optimal for a plant growth medium because they retain sufficient water and provide sufficient gas exchange to support vigorous plant growth (Benito et al., 2006; Jayasinghe et al., 2010). Jayasinghe (2012) reported that an excess of larger particles may lead to excessive aeration and inadequate water retention and that an excess of fine particles may clog pores and decrease air-filled porosity. Richards et al. (1986), as well as Jayasinghe (2012), established that coarseness index (CI) for ideal medium should be between 30% and 45% by weight. In this study, all the purchased materials, in particular digestate and biochar showed CI values that overcome the upper limit of the range. In fact, for these last, an improvement of CI index was obtained by sieving at 5 mm and 4 mm respectively.

The three materials, tested for the growth of all selected vegetable species, were not comparable to peat performance, although they showed some biometric parameters similar to those of peat.

Therefore, these materials appeared not convenient as growing media for horticultural crops; while, some mixtures with these materials appeared suitable.

Some of the screened mixtures composed by coconut fiber, digestate and biochar together or with peat, gave comparable or better results as compared to peat. Agronomical trials performed for lettuce, tomato, pepper, cauliflower and fennel allowed to select four best combinations: 50P_50D for lettuce, tomato, pepper and cauliflower; 25P_75D and 50FC_50D for lettuce, tomato, pepper; 50P_50FC for cauliflower and fennel. None of all mixtures formulated with biochar gave results worse than peat for all vegetable species.

There are very few reports concerning the use of anaerobic digestate as horticultural substrates.

Although digestate have been directly applied to agricultural fields (Charmley et al., 2006), few studies have been performed in the area of nursery plant production (Vaugh, 2013). The use of substrate made from waste of animal and/or plant origin, such as digestate, could be an alternative for increasing the nutrient content reducing so the use of fertilizers, in addition to the ability to increase the water-holding capacity. Our results agree with those of Dede et al. (2012), in which residue of hazelnut husk and municipal biosolid was tested to evaluate their suitability as potential growing media for palm tree young plants (*Washingtonia robusta*). These materials showed the physical and physic-chemical properties in the optimum range, except in mixtures with biosolid at 75%. Also good results were obtained for the plant growth, suggesting the use of these materials for the cultivation of ornamental palm tree. The main factor affecting plant growth was the higher dose of nitrogen in biosolid application, which is positively correlated with leaf N and plant dry weight. Vaughn et al. (2013) examined new growing media used in production of containerized greenhouse and nursery crops, such as tomato (*Solanum lycopersicum* L.) and marigold (*Tagetes erecta* L.) formulated with an acidified biochar, produced from several feedstock. The combination of this biochar with a dried anaerobic digestate, resulting from fermentation of potato processing wastes, gives an increased growth of tomato plants, respect to the peat: vermiculite control.

However, there are few papers that show good performances of biochar used as growing media. For example, Vaughn et al., (2015) examined biochar in replacement of peat moss in soilless substrates: only when low level of biochar has substituted peat (5%, 10% and 15%), plant heights increased, while, at higher concentration, detrimental effects on plant growth were observed. The authors attributed these last detrimental effects, to high volatiles present in the mixture and to overly high pH values. Because biochar is inherently resistant to decomposition, the initial values for physical properties of biochars should remain stable over time, similarly to vermiculite, perlite and sand. This suggests the potential to develop "designer biochar" with desired attributes for specific uses (Novak et al., 2009; Spokas et al., 2012).

The performance of the selected mixtures was confirmed by repeating agronomic trial, further supporting the suitability of the materials in growing media preparations. Results of this study suggest that nutritional and anti-nutritional properties of materials used in mixture composition, may affect plant development. In fact, plants cultivated on growing media rich in useful elements (Ca, Mg, N, P, K, PO_4^{2-} and SO_4^{2-}), such as digestate and coconut fiber, have showed better biometric characteristics. Whereas, slightly shunted growth was observed for plants developed in the cases of substitute material (such as biochar) carrying phytotoxic elements, such as Na and Cl^-.

Composts are largely used to search alternative materials for growing media preparation. Therefore, acknowledgements on the topic of peat replacement are given mainly from study concerning the use of these organic materials. The combination of peat and compost in growing media can be synergistic: peat often enhances aeration and water retention and compost improves the fertilizing capacity of a substrate. In addition, organic by-products and compost tend to have porosity and aeration properties comparable to those of peat and, as such, are ideal substitutes in propagating media (Chong, 2005). However, composts frequently have a high salt content, which may be the most important limitation in their use for replacing peat in growing media formulation. Other constraints for use include possible presence of contaminants (trace elements, organic elements, glass), potential phytotoxicity (immaturity and/or salt level, pH) and differences in species responses.

Bustamante et al. (2008) demonstrated that lettuce and broccoli seed germination was not influenced by the type of compost used and the compost proportion incorporated in the media elaborated. The use of composts in the growing media, indeed, influenced significantly the nutritional status of the aerial parts of the plants.

Brito et al. (2015) reported that the use of *Acacia* waste compost, as component for horticultural substrate, did not negatively affect either lettuce emergence or lettuce growth.

The materials selected in this study, as digestate, coconut fiber and biochar, are very different from compost, both for physical and chemical characteristics. So, agronomical response registered here for the tested vegetable species can be ascribed to properties of the media. However, also the development stage of growing plants is an important factor: germination proved more sensitive than the transplanted plants to substrate influence. In fact, basil-potting experiments, as matter of the fact, gave less variable responses among all tested different mixtures, obtained by combining coconut fiber, digestate and biochar. Fresh and dry basil biomass were not affected by the partial replacement of the peat with other tested materials. However, in this trial shoots nutritional status, for some elements, seems influenced by growing media composition. Mixtures of biochar with peat, coconut fiber and digestate showed agronomical performances similar to other combinations; so, this suggest that the stage of plants development, as well as the use of bigger containers that could assure a greater growth of the plants thanks to a better expansion of radical apparatus, are an important aspect.

The "functional equilibrium" between shoot and root growth varies widely between species and is strongly modified by internal and external factors. The root functions and the shoot/root dry weight ratio mainly depend on the concentration of nutrients in the substrate, and the physical, chemical and microbiological conditions for root activity and formation on new roots.

CONCLUSIONS

The selection of proper growing medium is one of the most important goal in nursery plants production. Peat is still the most popular substrate for those working in the sector. This determined an intensive exploitation of peatlands that are limited resources. Therefore, during the last years, the peat is decreasing in quality and increasing in prices because the progressive depletion of source of supply.

The aim of this work has been to study new materials able to replace totally or in part peat as growing media. Attention has been focused on three waste organic materials that are easily available and at low cost: coconut fiber, digestate and biochar. The evaluation of the agronomic performance of these materials was supported by measuring their main physical and chemical properties. Their ability to replace peat as growing media was validated through nursery trials for the plantlets production of tomato, pepper, lettuce, cauliflower and fennel, and through potting production of basil. Nursery trials for the plantlets production were conducted following the traditional nursery practices, particularly irrigation, fertilization and the type of plug trial for the growth of the plants.

The results indicate that the ability of such materials to replace peat is little influenced by their studied different characteristics. This could depend by different factors, among which:

- the abundance of irrigation and fertilization, usually adopted in nurseries, attenuates the effects of substrates; in fact pH and EC of the substrates do not seem to affect their performance;
- water retention is an important factor: in fact, for the biochar, it is the lowest and a limited production was obtained for tomato, pepper, lettuce, cauliflower and fennel.

Overall, our findings indicate that coconut fiber and digestate guarantee better conditions at which plantlets can develop root systems, both for water availability and supplying nutrient, without any release of phytotoxicity factors that inhibit germination also for more sensitive species as shown by the tested *Lepidium sativum*, and mine also the vitality of the plants.

Comparing our medium mixtures with ideal media, as peat, most of their properties match or exceed specifications for growing media. Coconut fiber and digestate in mixture with peat or between them possess physical and chemical properties, which are desirable for horticultural substrates. Plants growth on these formulated media resulted health under the nutritional and agronomical profile.
Mixture obtained with coconut fiber and digestate, optimal for pepper, tomato and lettuce, appears the most innovative result because waste organic materials replace totally peat.
Growing media, obtaining mixing also biochar, result inefficient for horticultural seedling production, although they resulted acceptable alternative to peat for basil plants in pot.

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