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Physicochemical, Agronomical and Microbiological Evaluation of Alternative Growing Media for the Production of Rapini (*Brassica rapa* L.) Microgreens

Francesco Di Gioia,[ab*§] Palmira De Bellis,[c§] Carlo Mininni,[c] Pietro Santamaria,[a] and Francesco Serio [c]

[a] Department of Agricultural and Environmental Science, University of Bari Aldo Moro, via Amendola 165/A, 70126 Bari, Italy

[b] University of Florida, Institute of Food and Agricultural Sciences, South West Florida Research and Education Center, Immokalee, FL 34142

[c] Institute of Sciences of Food Production, National Research Council, via Amendola 122/O, 70126 Bari Italy

* Correspondence to: Francesco Di Gioia, University of Florida, Institute of Food and Agricultural Sciences, South West Florida Research and Education Center, Immokalee, FL 34142. Email: fdigioia@ufl.edu

[§]These authors contributed equally to this work.

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Abstract

BACKGROUND: Peat-based mixes and synthetic mats are the main substrates used for microgreens production. However, both are expensive and non-renewable. Recycled fibrous materials may be low-cost and renewable alternative substrates. Recycled textile-fiber (TF, polyester, cotton and polyurethane traces) and jute-kenaf-fiber (JKF, 85% jute-, 15% kenaf-fibers) mats were characterized and compared to peat and Sure-to-Grow® (STG, 100% polyethylene-terephthalate) for the production of rapini (*Brassica rapa* L.; Broccoletto group) microgreens.

RESULTS: All substrates had suitable physicochemical properties for the production of microgreens. Microgreens fresh-yield was on average 1,502 g m^{-2} in peat, TF and JKF, and was 13.1% lower with STG. Peat-grown microgreens shoots had higher concentration of K^+ and SO_4^{2-}, and two-fold higher NO_3^- concentration [1,959 *vs* 940 mg kg^{-1} of fresh weight (FW)] than those grown on STG, TF, and JKF. At harvest, substrates did not influence microgreens aerobic-bacterial populations (log 6.48 CFU g^{-1} FW). Peat- and JKF-grown microgreens had higher yeast-mould counts than TF- and STG-microgreens (log 2.64 *vs* 1.80 CFU g^{-1} FW). Peat-grown microgreens had the highest population of *Enterobacteriaceae* (log 5.46±0.82 CFU g^{-1}), and *E. coli* (log 1.46±0.15 CFU g^{-1}). *E. coli* was not detected in microgreens grown on other media.

CONCLUSION: TF and JKF may be valid alternatives to peat and STG, as both assured competitive yield, low nitrate content, and similar or higher microbiological quality.

Keywords: peat alternative, recycled fibrous materials, *Brassica rapa* L., broccoli raab microgreens, *Enterobacteriaceae*, *Escherichia coli*

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INTRODUCTION

Microgreens, an emerging category of edible greens, are tender seedlings produced from seeds of different species of vegetables, aromatic herbs, and herbaceous plants, including wild edible species.[1] Microgreens are generally harvested 7-21 days after germination, when cotyledonary leaves are fully developed, with or without the emergence of a small pair of true-leaves.[2,3] Increasingly used by chefs as edible garnish, microgreens are becoming popular also for their high content of bioactive compounds.[2,3] Moreover, with the development of the urban farming industry there is increasing interest in their commercial production.[4] Microgreens may be grown in greenhouse, or indoor, with artificial light sources,[5] in soil or most commonly in soilless systems,[6,7] using organic or inorganic solid growing media or hydroponics.[7] Despite the short growing cycle, the commercial production of microgreens requires particular attention, and the choice of the growing medium represents one of the most critical aspects of the production process. The growing medium constitutes one of the main costs of production, and plays a major role in determining yield and quality of microgreens, as well as the environmental sustainability of the production process.[7]

The ideal growing media should be locally available, relatively inexpensive, should derive from renewable sources, have an adequate ratio between micro-pores (to insure enough water holding capacity; 55-70% of total volume) and macro-pores (to ensure sufficient aeration; 20-30% of total volume).[7,8] It should have pH ranging from 5.5 to 6.5, electrical conductivity below 0.5 dS m^{-1}, and be microbiologically safe.[7]

Recent studies have focused mostly on the postharvest handling conditions and on techniques aimed to extend the shelf-life of microgreens.[9-11] However, more attention should be given to potential sources of pre-harvest microbial contamination and to the microbial quality of microgreens. Although until today no foodborne outbreaks have been associated with the consumption of microgreens, there is great concern about

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microgreen safety, given their similarity to sprouts. It was demonstrated that contaminated seeds can systematically contaminate the whole seedling.[12,13] Besides seeds, growing media could represent a potential source of microbial contamination of microgreens.

Because of their optimal physicochemical properties, peat and peat-based mixes represent the most common media used to produce microgreens.[7,14] Nevertheless, peat is expensive, especially in countries that do not have peat-moss resources, and its continuous extraction is posing increasing environmental concerns.[8] An alternative to peat may be coconut coir, which is an organic and renewable resource.[14] However, coir physicochemical and microbiological characteristics are not consistent, and often it may have high concentration of salts, and high fungal and bacterial counts.[15] Other available media include inorganic materials such as perlite and vermiculite (usually mixed with peat), rockwool, or synthetic fibrous materials like Sure to Grow® (STG; Sure to Grow, Beachwood, OH), specifically developed for microgreens production. However, these media are expensive, their production is energy demanding, and poses disposal problems after their use. Seeking less expensive and more sustainable growing media, microgreen growers are exploring the possibility of using organic by-products and discarded materials derived from industrial processes. Fibrous material discarded by the fiber and textile industry, such as cotton, jute and kenaf fibers,[16] may be low cost, natural and renewable growing media for microgreens production.[16]

Objective of this study was to verify two hypotheses: i) that discarded materials of the fiber and textile industry may be used as growing media for the production of microgreens; ii) that growing media may be a potential source of microbial contamination. Therefore, two recycled fibrous materials were characterized for their physicochemical, horticultural and microbiological characteristics, and tested for their effects on yield and quality of microgreens. The performances of recycled fibrous media

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were compared with those of STG and a peat mix, considered as reference media for microgreens production.

EXPERIMENTAL

Growing media

Four growing media were tested: TF: a textile-fiber mat (BCE-200, Manifattura Maiano, Capalle, Italy), constituted by a mix of recycled polyester and cotton fibers, and traces of polyurethane; JKF: GreenFelt P500 (Manifattura Maiano, Capalle, Italy), a 100% biodegradable mat, containing a mix of recycled jute (85%) and kenaf (15%) fibers; PEAT: commercial substrate Brill Type 3 Special (Gebr. Brill Substrate GmbH & Co., Georgsdorf, Germany), containing a mixture (50:50 v:v) of very fine black and white peat, and 1 kg m^{-3} of PG-MIX 14-16-18 (N-P-K) fertilizer (Yara, Grimsby, United Kingdom), containing 5.5% NO_3-N and 8.5% NH_4-N; and STG (Sure to Grow, Beachwood, OH) mats constituted of polyethylene terephthalate (PET).

Physicochemical characterization of the growing media

The main physical properties of the four growing media [dry bulk density (BD), total pore space (TPS), particle density (PD), air capacity (AC), and water-holding capacity (WC)] were determined according to the European Standard 13041[17] for peat and modified as described hereafter for the fibrous mats.

The growing media were transferred in two overlapping polyvinyl chloride cylinders (100±1 mm diameter and 50±1 mm height each). Mats of STG, TF and JKF were cut in 100 mm diameter circles and overlapped up to the edge of the cylinders. Once filled with the media the double cylinders were saturated with water for 48 h, and then transferred into a sandbox (Eijkelkamp Agrisearch Equipment, Giesbeck, The Netherlands) and kept at a pressure head of -10 cm (-1 kPa) corresponding to WC for 48 h. Thereafter the double-cylinders were removed from the sandbox and separated. Only

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the lower cylinders and the relative substrate samples were used for further measurements. The samples containing water were weighed before and after being dried at 105 °C to a constant mass to determine the sample fresh (SFW) and dry (SDW) weight. Knowing the volume (SV) of each sample (100 mL), the physical parameters were then calculated according to the following formulas:

$$BD = SDW/SV \quad \text{Eq. 1}$$

$$WC = (SFW - SDW)/SV \times 100 \quad \text{Eq. 2}$$

$$\mathrm{TPS} = [1 - (BD/PD)] \times 100 \quad \text{Eq. 3}$$

$$AC = TPS - WC \quad \text{Eq. 4}$$

Where PD was the real solid particles density, and was determined according to the following formula:

$$PD = 100/[(OM\ /\ 1.55) + (A\ /\ 2.65)] \quad \text{Eq. 5}$$

Where OM is the percentage of organic matter and A is the percentage of ash, which were determined by ashing in triplicate the samples of each medium in a muffle furnace at 450 °C for 16 hours. The values 1.55 and 2.65 (expressed in g cm^{-3}) are the average density of OM and A, respectively.

Water activity (a_w) was measured on three unprocessed samples of each medium using an AquaLab meter (Decagon Devices, Inc., Pullman, WA) following the instructions provided by the manufacturer. Electrical conductivity (EC) and pH were measured on a water-soluble extract of each growing media as proposed by Sonneveld.[18] Samples of 100 mL were obtained for each growing media filling a 100 mL cylinder. A solid standard weight corresponding to a pressure of 10 kPa was used for 10 s to obtain the same level of compression in all the samples. Mats of STG, TF and JKF were cut in pieces < 3 mm to obtain the 100 mL volume. Then, the 100 mL media sample was mixed with 1.5 times its volume of demineralized water.

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Subsequently, the suspension was shaken firmly for 15 min, and after 1 hour was filtered with paper filters to obtain the water extract and measure EC and pH.

These water extracts were analyzed to determine the concentration of potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), nitrate (NO_3^-), phosphate (PO_4^{2-}), sulfate (SO_4^{2-}) and chlorine (Cl^-)] by ion chromatography (Dionex DX120; Dionex Corporation, CA) with a conductivity detector, using an IonPack CG12A pre-column and IonPack CS12A separation column for cations, and an IonPack AG14 pre-column and an IonPack AS14 separation column for anions.

Experimental site, plant material and growing conditions

Two experiments were conducted during the fall of 2014, on 'rapini' (*Brassica rapa* L., Broccoletto group) microgreens, also known as rappini or broccoli raab, in a polymethacrylate-covered greenhouse, located in Mola di Bari (41°03' N, 17°4' E; 24 m a.s.l.), Southern Italy. Rapini seeds landrace 'Quarantina' were purchased from Larosa Emanuele Sementi (Andria, Italy). Seeds were of high quality with 93% germination at constant 20 °C, and 360±1.20 (mean±SD, n=4) seeds per g.

To evaluate the agronomic performances of each media, on October 20th 2014 sixty 256-cm^2 (16×16 cm, 3-cm high) plastic trays (ILIP s.r.l., Valsamoggia, Italy; fifteen for each growing medium), with drainage holes, were prepared by placing a 250 cm^2 (15.8×15.8 cm) mat of STG, TF or JKF into each tray or filling the trays to a height of 2.5 cm in the case of peat. Rapini seeds previously treated with a 10 g L^{-1} NaOCl solution for 5 min under constant agitation, rinsed with drinking water for 1 min, and oven-dried on germination paper at 35 °C for 2 h, were evenly broadcasted on the surface of the growing media in each tray, at 100 g m^{-2} (35,998 seeds m^{-2}). After sowing, the trays were placed on a 2.7 m^2 (1×2.7 m) aluminum bench, and arranged in a randomized complete block design with three replications. Each experimental unit contained five trays. Sown trays were irrigated manually using a water-nozzle and

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covered with a black polyethylene film until germination was complete. Seedlings then were fertigated by subirrigation with a nutrient solution (NS) prepared with potable water containing (expressed in mg L^{-1}): nitrogen (105.1), phosphorus (15.5), potassium (117.4), magnesium (26.0), calcium (92.5), sulfur (34.6), iron (1.20), manganese (0.60), zinc (0.15), boron (0.30), copper (0.08), and molybdenum (0.03), resulting in an electrical conductivity (EC) of 1.3 dS m^{-1} and pH 6.3. The NS was delivered on the bench to each replication by a drip tape line with pressure-compensated drippers (each with a delivery rate of 8.0 L h^{-1}). Fertigation was scheduled daily to maintain a drainage percentage of minimum 30%. Drainage was collected in a reservoir tank at the base of the bench, but was not reused (open cycle management).

Following the same procedures, a second experiment was conducted to assess the growing media effects on the microbiological contamination of microgreens. On October 24th 2014, thirty-six 256-cm^2 plastic trays (nine for each growing medium), containing the four media were sown as previously described with rapini seeds. To avoid any cross-contamination, after sowing the trays of each experimental unit, constituted by three trays per media, were arranged into separate Agridrain channels (Perlite Italiana, Corsico, Italy), and placed on a 2.7 m^2 aluminum bench, arranged in a randomized complete block design with three replications. After complete germination, the trays contained in each Agridrain channel were fertigated by subirrigation as previously described. Materials and equipment used, except the growing media, were attentively washed with a 50 g L^{-1} NaOCl solution before use.

During the experiments, daily air temperature in the greenhouse averaged 16.9 °C, and daily minimum and maximum air temperatures ranged from 8.5 to 17.6 °C and from 23.5 to 32.3 °C, respectively. Relative humidity averaged 72% and ranged between a minimum of 28% and a maximum of 99%. Daily light integral (DLI)

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averaged 6.9 mol m^{-2} day^{-1} and ranged from a maximum average of 8.7 mol m^{-2} day^{-1} to a minimum average of 3.9 mol m^{-2} day^{-1}.

Microgreens harvest, yield and quality assessment

For the first experiment, at the first appearance of the first true-leaves, 11 days after sowing (DAS), microgreens of each tray were harvested by cutting the seedling just above the surface of the growing media with a sterilized knife. Harvested microgreens were weighed to determine shoot fresh weight (FW) per unit area. The FW per shoot and the actual shoot population density were calculated after counting the number of shoots on a sub-sample of 250 cm^2 constituted by a tray for each growing medium and replication. A sub-sample of approximately 120 g was dried to constant weight in a forced-draught oven at 65 °C and weighed to determine their dry weight (DW) and dry matter concentration (DM).

Dried samples were finely ground through a mill (IKA, Labortechnik, Staufen, Germany) with a 1.0-mm sieve and used for chemical analyses. Cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) were extracted from 2 g samples of dry plant tissues, ashed in a muffle furnace at 450 °C, digested with 1 M HCl in a boiling water bath for 30 min, and measured by ion chromatography (Dionex DX120). Anions (NO_3^-, Cl^-, PO_4^{2-} and SO_4^{2-}) were determined on 0.5 g of DW samples by ion chromatography (Dionex DX120) as described by Boari et al.[19]

For the microbiological experiment, 12 DAS, when microgreens reached the harvest stage, one tray per experimental unit (three for each growing medium) was packed into a plastic bag and transported to the lab for microbiological analyses.

Microbiological analyses

Microbiological analyses were performed to evaluate the microbiological contamination of the four growing media and microbial growth levels on microgreens

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grown on each substrate, as well as to assess preliminarily the microbiological characteristics of the NS used for fertigation and of the rapini seeds.

Ten g of each growing medium (three replicates), added to 90 mL of sterile Buffered Peptone Water (BPW, Biolife), and 3 g of microgreens, randomly selected from each tray and cut with a pair of sterilized scissors, added to 27 mL of BPW were homogenized in a Stomacher (Seward, London, United Kingdom) for 2 min. The resulting suspensions were serially diluted in the same diluent and plated in duplicate on the following agar media for the detection and enumeration of microorganisms: Plate Count Agar (PCA, Difco) incubated at 30 °C for 24 h to determine the total aerobic mesophilic bacterial counts (AMB); Potato Dextrose Agar (PDA, Difco) supplemented with 200 mg L^{-1} chloramphenicol (Sigma) for yeast and mould (YM) count, incubated at 25 °C for 72 h; Violet Red Bile Glucose agar (VRBGA, Difco) for total *Enterobacteriaceae*, incubated aerobically at 37 °C for 24 h; Tryptone Bile X-GLUC (TBX) agar (Biolife) for *Escherichia coli*, incubated aerobically at 44 °C for 24 h. Each sample was tested in triplicate. AMB, YM, and *Enterobacteriaceae* counts were used as indicators of the overall microbiological quality of growing media and microgreens, and only *E. coli* was considered as a potential human pathogen and an indicator of the product microbiological safety.

Following the same method, microbiological analyses were preliminarily performed in triplicate on samples of the NS used for the fertigation, and on samples of rapini seeds either treated with a NaOCl solution or untreated. Three samples (10 g each) of seeds were either washed (under constant agitation) for 5 min in 100 mL distilled water (untreated), or treated (under constant agitation) for 5 min with 100 mL of 10 g L^{-1} NaOCl solution, and then rinsed with drinking water for 1 min, and oven-dried on germination paper at 35 °C for 2 h.

Statistical analysis

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Data were subject to analysis of variance using the General Linear Models procedure of the Statistical Analysis System software (SAS Institute, Cary, NC, USA). Significant differences between treatments were analyzed by Duncan's multiple range test at $P = 0.05$.

RESULTS AND DISCUSSION

Physical and chemical properties of the four growing media

All growing media had BD values below the maximum value (400 kg m^{-3}) established for an ideal substrate (Table 1).[8] Peat had the highest BD (130.2 kg m^{-3}) followed by JKF mats, while TF and STG mats had the lowest BD (mean 33.9 kg m^{-3}). The value of BD provides a good indication of the porosity of the growing medium. High BD values imply higher transportation costs and a reduction in porosity,[20] therefore a relatively low BD is highly desirable.

All four growing media had TPS values between 92.4% and 97.9%. Excluding peat, with an average AC value within the ideal range for soilless media (20-30% of the total volume), the other media had AC values substantially higher (> 67.6%).

High TPS and AC values may represent positive traits for a substrate because may assure suitable gaseous exchange for the root system. However, growing media characterized by high AC values have also a low water holding capacity, which implies that water should be applied more frequently, and in small amounts, to avoid excessive drying or drainage.[8]

The WC of STG, JKF and TF was on average 58%, 63% and 87% lower than in peat, respectively. Therefore, excluding peat, all growing media had WC values below the ideal range (55-70% of the total volume).[8] In the case of living microgreens commercialized directly in trays with growing medium, a low water holding capacity may limit the product shelf life.

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Peat and TF had pH values within the optimal range (5.5-6.6), while JKF and STG had average pH values slightly above the ideal maximum value, but still suitable for microgreen production. Except for peat, all growing media had EC values substantially lower than 500 μS cm^{-1}, a value demonstrated to be ideal for microgreens production.

Macronutrients concentration of water extracts were significantly influenced by the type of growing media. Peat had the highest concentration of NO_3^-, PO_4^{2-}, Ca^{2+}, Mg^{2+}, SO_4^{2-} and Cl^-. Jute-kenaf-fiber mats had the highest concentration of K^+ and Cl^-, and a Na^+ concentration similar to that of peat. Textile-fiber mats had a higher NH_4^+ concentration than other media. Excluding the peat mix that contained 1 kg m^{-3} of N-P-K (14-16-18) fertilizer, and JKF that contained K^+, the growing media had low macronutrients concentration, which implies that their use for the production of microgreens would require supplemental applications of nutrients through the NS. Nevertheless, the low nutrient concentration of these growing media may be considered an advantage, as it may allow better control and management of the microgreens nutrient contents. Also, because of their short growing cycle, microgreens require relatively low amounts of nutrients.[7,21]

Microgreens population density, fresh weight yield, fresh weight per shoot, and dry matter accumulation

Growing media did not affect microgreen population density, that averaged 30,680 shoots m^{-2}, but did affect mean shoot FW, fresh yield and dry matter content (Table 2). No significant fresh weight yield differences were observed between peat, TF and JKF. STG provided a fresh weight yield on average 13.1% lower compared to the other growing media. Peat produced microgreens with the highest FW per shoot (54.1 mg), followed by TF, JKF and STG that produced shoots with a 10.1%, 11.5% and 19.2% lower FW, respectively. Microgreens grown on STG, TF and JKF had similar

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values of DM (on average 65.3 g kg^{-1} of FW), while those grown on peat had a DM accumulation 11.4% lower. The lower DM accumulation observed in peat-grown microgreens may be explained by the higher water holding capacity of this medium as compared to the other fibrous mats. From a quality standpoint a higher DM accumulation may be associated with longer shelf life,[22] and is therefore highly desirable.

Microgreen shoot mineral concentration

Microgreen shoots grown on peat had the highest concentration of NO_3^-, K^+, SO_4^{2-} and Na^+, and the lowest Cl^- concentration (Table 3). Microgreens grown on fibrous mats had similar mineral concentration. The higher mineral concentration of peat-grown microgreen shoots may be explained by the presence of PG-MIX 14-16-18 (N-P-K) and by the generally higher nutrient concentration of the peat-mix, as compared to the fibrous mats (Table 1), suggesting that the microgreen shoot mineral concentration is highly influenced by the media nutrient concentration. Nevertheless, despite peat had a high concentration of Cl^-, peat-grown microgreens had the lowest Cl^- concentration, which may be explained by a higher interference of peat with the nutrient availability and accumulation in the small seedlings. Peat-grown microgreens had a higher mineral concentration, and thus, a higher nutritional value than those grown on fibrous mats. However, peat-grown microgreens had 108% higher NO_3^- concentration than microgreens grown on fibrous mats. In contrast with the findings of Pinto et al.,[23] who observed relatively low NO_3^- concentration in lettuce microgreens as compared to mature lettuce, these results suggest that, like other leafy vegetables, microgreens can accumulate conspicuous amount of nitrates.[24] While there is evidence that nitrates may be beneficial for human health,[25] nitrates are still considered anti-nutrients, and a reduction of their dietary intake is recommended as a preventive health care measure.[26,27] The results of this study also suggest that using growing media with low

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NO_3^- concentration, and maintaining an adequate NO_3^- level in the NS, it would be possible to limit microgreens NO_3^- accumulation.

Microbial populations on growing media, seeds, nutrient solution and microgreens

The preliminary microbiological analyses performed on the NS and on Rapini seeds treated with NaOCl solution, revealed that both were not contaminated with microorganisms (detection limits were log 1.48 CFU g^{-1} for AMB and YM, and log 0.48 CFU g^{-1} for *Enterobacteriaceae* and *E. coli*) (Data not shown). Instead, untreated seeds had a total bacterial population of log 4.36±0.10 CFU g^{-1} and *Enterobacteriaceae* of log 1±0.00 CFU g^{-1}. In agreement with the findings of other studies,[28] these results demonstrate that the initial seed disinfection treatment with NaOCl was effective in eliminating or reducing microorganisms from the seed surface.

On growing media, total viable counts enumerated on PCA, indicated that peat had the highest AMB populations (log 5.93±0.03 CFU g^{-1}), while the lowest were observed on STG (log 2.00±0.04 CFU g^{-1}) (Fig. 1). Yeasts and moulds were also present with the higher counts in peat, and were 2 log-cycles lower in TF and JKF, while were absent in STG ($P \leq 0.0001$). *Enterobacteriaceae* were present only in peat and STG ($P \leq 0.0001$), while *E. coli* was not detected in any substrate. Overall, despite its alleged antimicrobial properties,[29] peat had the highest microbial populations, which may be explained by its organic origin, its higher a_w and nutrient concentration, as compared to the other media.

At harvest, the AMB population associated with the microgreen shoots was not influenced by growing media (Fig. 2). Microgreens grown on TF and STG had a lower ($P \leq 0.05$) YM population as compared to those grown on peat and JKF. *Enterobacteriaceae* were absent on microgreens grown on TF and STG, while those grown on peat had the highest concentration of *Enterobacteriaceae* (log 5.46±0.82 CFU

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g^{-1}) and the presence of *E. coli* (log 1.46±0.15 CFU g^{-1}), which was not present on microgreen shoots grown on the other media.

Such results support the hypothesis of Chandra et al.,[9] that bacterial populations can easily grow on the delicate and immature tissue structure of microgreens. Moreover, the growth of bacteria may be stimulated by sugars and other organic molecules deriving from the breakdown of the endosperm during germination.[30] On 12-day-old rapini microgreen shoots the total AMB populations (on average log 6.9±0.42 CFU g^{-1}) were similar to those found on daikon radish (*Raphanus sativus* L. var. *longipinnatus*) microgreen shoots after seven days of storage,[11] on buckwheat (*Fagopyrum esculentum* Moench CV. Manner) microgreens,[10] and on baby spinach (*Spinacia oleracea* L.) leaves after washing,[31] while were 1 log-cycle lower than those found on shoots of “Tah Tasai” Chinese cabbage (*Brassica campestris* var. narinosa) microgreens.[9] The lower bacterial populations observed in this study as compared to other studies may be explained by the fact that we used seeds treated with NaOCl and NS that was not contaminated.

Interestingly, YM counts observed on rapini microgreens (from 1.80 to 2.75 log CFU g^{-1}) were much lower than those reported by Xiao et al.[11] on unwashed radish microgreens (7.1 log CFU g^{-1}), and by Allende et al.[31] on baby spinach leaves after washing (6.1 log CFU g^{-1} only for yeasts). Probably, the antagonistic activity of the contaminant bacterial populations, and the production system adopted, were not favorable for the growth of fungi. While *Enterobacteriaceae* were detected in both peat and STG, they reached high counts only on microgreens grown on peat, and were not present on microgreens grown on STG. It could be that, *Enterobacteriaceae*, present in low counts on STG were not transferred on microgreens, remaining on the growth medium or on portions of the seedlings not harvested, such as the seed coats, roots and the lower portion of the hypocotyls. Such results may be due to the physical-chemical

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properties of the medium, to the presence of other microorganisms more competitive in the colonization of the seedlings, as well as to the spatial distribution of bacteria on different portions of the microgreens.[12] *Vice versa*, *Enterobacteriaceae* were in large numbers on shoots of microgreens grown on JKF, while they were not detected in JKF mats. Likely, *Enterobacteriaceae* were present in JKF below the detection limit, and then were transferred to the seedlings, where they increased. Therefore, in this case the medium with the associate microbiota favored the transfer and the growth of *Enterobacteriaceae* on microgreens. These results suggest that TF and STG should be preferred over the two organic media, because *Enterobacteriaceae* include species that can cause soft-rot, affecting the shelf-life of fresh vegetables,[32] and promoting the growth of human pathogens.[33]

E. coli was observed only on microgreens grown on peat. Likely, also in this case, *E. coli* was present below the detection limit in this medium, and the physical, chemical and microbial characteristics of the peat favored the growth of *E. coli* and the *Enterobacteriaceae* on the microgreens. Previous studies by Xiao et al.[11,12] indicated that *E. coli* strains could proliferate significantly during microgreens growth reaching different levels depending on the initial inoculation level of the seeds.

The results of this study demonstrate that fibrous materials discarded by the fiber and textile industry may be recycled as growing media for the production of microgreens, and suggest that the growing medium may represent a source of microbial contamination, and the selection of media with suitable microbiological characteristics is an important aspect to consider to ensure high quality and safe microgreens.

CONCLUSIONS

The results of this study demonstrate that: i) microgreens yield and quality, including the microbiological quality, are highly influenced by the growing medium; ii) recycled fibrous materials discarded from the textile-fiber industry can be used as low-

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cost and renewable alternative substrates for the production of microgreens, ensuring high yield and quality; and iii) growing media represent a potential source of microbial contamination for microgreens. Therefore, great attention should be placed in the selection of the growing media, and particular care should be given to the microbiological characteristics of the substrate to assure the production of high quality microgreens.

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Table 1. Physical and chemical characterization of the four growing media tested for the production of microgreens.[†]

Physical chemical parameters[††]	Growing media								*Significance*[†††]
	Peat		Sure-to-Grow®		Textile fibers		Jute-kenaf fibers		
BD (kg m^{-3})	130.2±0.77	a	33.4±0.48	c	34.3±0.64	c	76.1±0.11	b	****
TPS (%)	92.4±0.04	c	97.9±0.03	a	97.8±0.04	a	95.1±0.01	b	****
AC (%)	20.1±0.77	c	67.6±2.39	b	88.1±0.04	a	68.6±0.71	b	****
WC (%)	72.3±0.74	a	30.3±2.41	b	9.7±0.06	c	26.5±0.71	b	****
Ash (%)	9.10±0.09	a	1.27±0.01	b	0.82±0.10	c	1.15±0.06	b	****
pH	5.75±0.01	c	6.94 ± 0.04	a	6.58±0.02	b	6.95±0.01	a	****
EC (μS cm^{-1})	546±49.70	a	29±12.12	d	250±5.78	b	127±4.04	c	****
a_w	0.99±0.001	a	0.51±0.005	d	0.67±0.00	b	0.64±0.003	c	****
NO_3^- (mg L^{-1})	1.38±0.06	a	0.48±0.21	c	0.85±0.02	b	0.18±0.03	c	***
NH_4^- (mg L^{-1})	0.00±0.00	b	0.23±0.11	b	2.87±0.43	a	0.00±0.00	b	***
PO_4^{2-} (mg L^{-1})	3.35±0.24	a	0.45±0.06	b	0.35±0.01	b	0.25±0.03	b	****
K^+ (mg L^{-1})	31.28±3.40	a	3.22±1.50	b	8.61±0.56	b	40.80±3.58	a	***
Ca^{2+} (mg L^{-1})	52.21±4.42	a	1.98±0.16	b	4.05±0.10	b	6.73±1.10	b	****
Mg^{2+} (mg L^{-1})	6.03±0.36	a	0.29±0.03	c	0.72±0.06	bc	1.03±0.10	b	****
SO_4^{2-} (mg L^{-1})	4.66±0.93	a	0.70±0.46	b	1.25±0.01	b	1.33±0.05	b	**
Na^+ (mg L^{-1})	5.73±0.63	b	1.52±0.51	c	5.60±0.30	b	15.78±0.87	a	****
Cl^- (mg L^{-1})	9.90±0.48	a	1.44±0.15	c	5.91±0.06	b	9.09±0.50	a	****

[†] Means of three replications ± standard error. [††] BD, bulk density; TPS, total pore space; AC, air capacity; WC, water capacity; EC, electrical conductivity, a_w, water activity. [†††] Significance of F: **, ***, ****. Significant at $P \leq 0.01$, 0.001, and 0.0001, respectively. Means followed by different letters within each row are significantly different at $P = 0.05$ by Duncan's multiple range test.

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Table 2. Growing media effect on microgreen shoot population density, fresh weight yield, fresh weight per shoot and dry matter accumulation of *Brassica rapa* microgreens.†

Growing media	Shoot Population Density	Fresh Weight Yield	Shoot fresh weight	Dry matter
	(shoot m^{-2})	(g m^{-2})	(mg $shoot^{-1}$)	(g kg^{-1} FW)
Peat	30,716	1,580 a	54.1 a	57.8 b
Sure to Grow®	29,831	1,306 b	43.7 c	67.6 a
Textile fibers	31,276	1,470 a	48.6 b	63.8 a
Jute-kenaf fibers	30,899	1,457 a	47.9 b	64.4 a
Significance††	NS	**	***	**

† Means of three replications. †† Significance of F: NS, **, ***. Not significant, or significant at $P \leq 0.01$, or 0.001, respectively. Means followed by different letters within each column are significantly different at $P = 0.05$ by Duncan's multiple range test.

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Table 3. Mineral concentration of *Brassica rapa* microgreen shoots grown on four growing media. †

Growing media	NO_3^-		HPO_4^{2-}	SO_4^{2-}		Cl^-		K^+		Ca^{2+}	Mg^{2+}	Na^+	
	mg kg^{-1} of fresh weight												
Peat	1,959	a	1,280	1,757	a	286	b	2,731	a	1,028	270	116	a
Sure to Grow®	986	b	1,441	895	b	520	a	1,341	b	907	288	75	b
Textile fibers	940	b	1,061	854	b	510	a	1,407	b	1,003	293	85	b
Jute-kenaf fibers	892	b	1,171	765	b	468	a	1,399	b	942	290	84	b
Significance††	****		NS	****		***		****		NS	NS	*	

† Means of three replications. †† Significance of F: NS, *, ***, ****. Not significant, or significant at $P \leq 0.05$, 0.001, and 0.0001, respectively. Means followed by different letters within each column are significantly different at $P = 0.05$ by Duncan's multiple range test.

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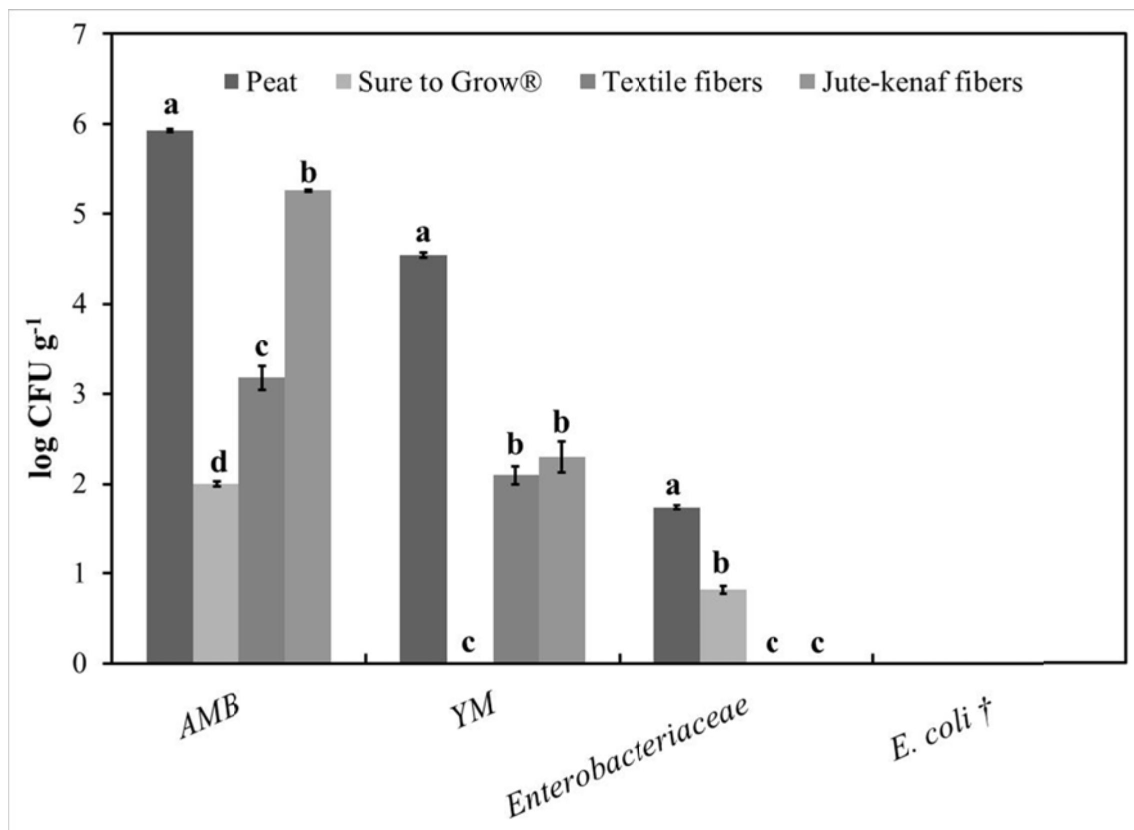

Figure 1. Aerobic mesophilic bacteria (AMB), yeast and mould (YM), *Enterobacteriaceae* and *Escherichia coli* (log CFU g^{-1}) associated to the four growing media tested for the production of microgreens. Means of three replications. Vertical bars represent ± standard error, means followed by different letters within a microbial grouping are significantly different at $P = 0.05$ by Duncan's multiple range test. † *E. coli* was not detected in all the media at detection limit of log 0.48 CFU g^{-1}.

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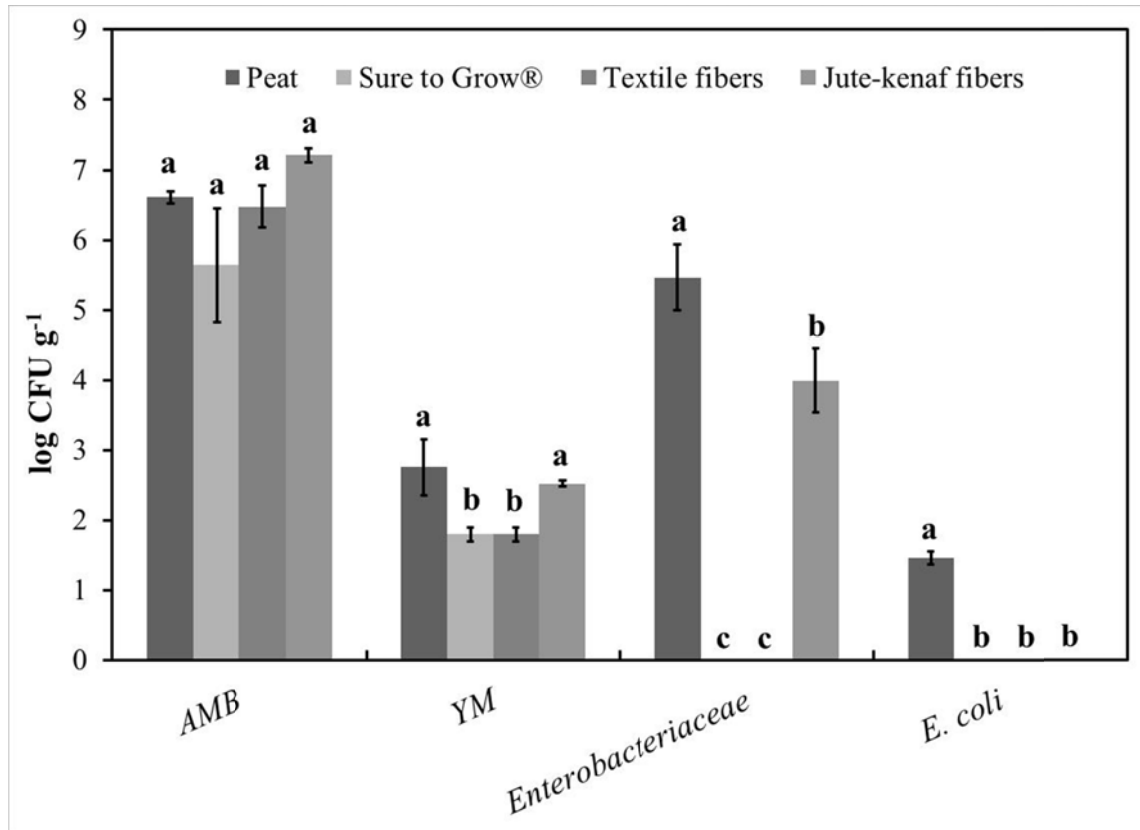

Figure 2. Aerobic mesophilic bacteria (AMB), yeast and mould (YM), *Enterobacteriaceae* and *Escherichia coli* (log CFU g^{-1}) observed on *Brassica rapa* microgreen shoots grown on four growing media at harvest. Means of three replications. Vertical bars represent ± standard error, means followed by different letters within a microbial grouping are significantly different at P = 0.05 by Duncan's multiple range test.

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