Valorization of coffee wastes as plant growth promoter in mulching film production: A contribution to a circular economy

Valorization of coffee wastes as plant growth promoter in mulching film production: A contribution to a circular economy

Elia Pagliarini [a], Grazia Totaro [b], Andrea Saccani [b], Francesca Gaggìa [a], Isabella Lancellotti [c], Diana Di Gioia [a], Laura Sisti [b]

[a] Department of Agricultural and Food Sciences, University of Bologna, Via Fanin 40, Bologna, Italy

[b] Department of Civil, Chemical, Environmental and Materials Engineering, University of Bologna, Via Terracini 28, Bologna, Italy

[c] Department of Engineering "E. Ferrari", University of Modena and Reggio Emilia, Via Vivarelli 10, Modena, Italy

Abstract

Food waste valorization, considered as energy and/or chemicals source, via biorefinery or biotechnology, gained great attention in recent years, because of the fast depletion of primary resources, increased waste generation and landfilling worldwide. Coffee by-products for example (i.e. coffee pulp, coffee husks, silver skin, spent coffee, etc.) have been investigated in different forms either as a source of antioxidant and valuable chemicals and as a filler in composites. A new valorization route for coffee silver skin (CSS), up to now just sent to damping, is here investigated: particulate bio-composites based on poly(butylene succinate-*co*-adipate) (PBSA), an aliphatic biodegradable polyester commercially available, have been formulated with up to a 30 wt% of CSS, in order to prepare mulching films for agriculture. The bacterial analysis of the filler indeed, has underlined the presence of potential Plant Growth-Promoting Bacteria species, mainly ascribed to the *Bacillus* genus, which can survive both the roasting and the compounding processes. The obtained composites have been characterized mechanically and thermally and their hydrophilic nature has been investigated by measuring their contact angle. Eventually, the bacteria release from the composite films has been examined by means of in-vitro tests. The plant growth promoting capability of the films was preliminarily evaluated in pot experiments using lettuce as a model crop. The composite films were able to release the endogenous bacteria in the soil and to stimulate plant and root growth of the assayed crop. The possibility to produce functionalized biodegradable mulching films by recycling agricultural wastes can thus be forecast, highlighting potential multiple advantages in terms of soil preservation/fertilization, decrease of polymeric materials in mulching products, exploitation of a waste.

Keywords

Mulching films
Biopolymer
Coffee waste
Plant Growth-Promoting Bacteria

Highlights

- Coffee silver skin contains Plant Growth-Promoting bacteria.
- Composite can be made out of PBSA and CSS to produce mulching films.
- Bacterial release from the mulching films has been detected.
- Beneficial effects on plant growth has been observed.

1. Introduction

The steady increase in the world population and the progressive decrease in the areas available for the agriculture drive the efforts towards the rise in the yield and quality of the crops. The use of plastic materials to produce mulching films has long been established both to increase the overall amounts of products and reduce watering as well as protect against pests and weeds. This is generally included in the *plasticulture* concept (Sander, 2019; Serrano-Ruiz et al., 2021; Fennell et al., 2019). Traditional plastics exploited in this activity, mostly polyethylene (PE), are non-biodegradable materials and they must be collected after their use. This generates a large volume of wastes which are not easily recyclable because of their scanty mechanical properties and pollution (Briassoulis et al., 2013). Moreover, fragments of the films are frequently dispersed in the soil, progressively reducing gas and water permeability, and altering the stability of the ecosystem (Li et al., 2022; Kim et al., 2021). The reasons for the extensive use of PE are related to the fact that it is inexpensive, easily processed, highly durable and flexible, however, as pointed out, accumulated PE fragments can affect the soil physical structure as well as the soil microbiota and may ultimately enter the food chain (Bandopadhyay et al., 2018). Currently, China is the main user of PE mulching film: around 1.4 million tons of films are employed annually, covering about 12 % of China's farmland, corresponding to five times the total area of Switzerland (Sander, 2019). Therefore, there is an urgent need to explore alternative polymers to replace conventional plastic for mulching purposes. Biodegradable polymers such as starch, cellulose, poly(butylene succinate) (PBS), poly(ε-caprolactone) (PCL), polyhydroxyalkanoates (PHAs), poly(butylene adipate-*co*-terephthalate) (PBAT), poly(lactic acid) (PLA), and poly(butylene succinate-*co*-adipate) (PBSA), are reported to be the most promising solution and have already been used to produce mulching films (Koitabashi et al., 2012; Kyrikou and Briassoulis, 2007; Serrano-Ruiz et al., 2021; Scarascia-Mugnozza et al., 2006; Jandas et al., 2013; Chien et al., 2022). However, some drawbacks should be considered: for example PLA alone is not frequently used because it is not degradable in soil environment (Aversa et al., 2022; Rudnik and Briassoulis, 2011), PHAs lack in mechanical strength and their large-scale production is restricted by the high cost of the manufacturing process, PCL degrades very quickly and has to be replaced frequently, PBAT produces microplastics, since it is fully degradable in soil only in specific conditions (Mansoor et al., 2022). On the contrary, PBSA shows high flexibility, good thermal and mechanical properties, degradability in soil, as well as excellent processability (Sisti et al., 2021). Therefore, it is presently the most performing material. In general, the positive properties of the mulching films, such as insect repellency, soil's moisture/temperature control and weed prevention, can be further expanded by loading them with molecules or fillers providing specific benefits such as monoammonium phosphate (Nunes et al., 2022), or glycerol (Treinyte et al., 2018). One of the main drawbacks in the use of biopolymeric mulching films is presently found in their higher cost when compared to oil-derived materials. A possible strategy to reduce their price is to formulate composites based on polymer matrices. The use of agricultural wastes as fillers is again the most effective choice. Coffee silver skin (CSS), a by-product of the roasting process (Gottstein et al., 2021; Sisti et al., 2021), has attracted great interest in the

formulation of bio-composites (Janissen and Huynh, 2018; Sarasini et al., 2018; Totaro et al., 2019; Sisti et al., 2022). At the same time, it may also constitute a good source of nutrients for microorganisms and its prebiotic activity has been demonstrated by different studies, re-evaluating its role as functional ingredient for different industrial sectors, including agriculture (Thligene et al., 2019; Borrelli et al., 2004; Mussatto and Teixeira, 2010; Jiménez-Zamora et al., 2015). Coffee by-products, in particular spent coffee grounds, mucilage and pulp indeed, have been demonstrated to support the growth of microorganisms, acting as substrates and inducing the production of enzymes, such as lignocellulolytic enzymes, proteases by *Bacillus* spp. (Khelil et al., 2016; Kandasamy et al., 2016), and β-glucosidase by *Bacillus subtilis* (Dias et al., 2015). Currently, only few studies focused on the microbial composition of coffee by-products (Silva et al., 2008; Bui, 2014). In view of the above-mentioned considerations, in the present research an analysis of the aerobic bacterial content of coffee silver skin was performed at different storing times after the roasting process and cultivable aerobic bacteria were isolated and characterized. Afterwards, films of PBSA and micronized coffee silver skin, up to a 30 wt%, were compounded and thermally and mechanically characterized. The presence and release of bacteria from the film after the compounding and filming process was studied. Moreover, the potential beneficial effect on plant growth of the composite films was preliminary assessed on lettuce as a target crop. To the best of our knowledge, this is the first study in which a functional mulching film, containing microorganisms deriving from an agro-industrial waste, is prepared and characterized. This strategy represents a novelty with respect to literature and an advance in the design of biodegradable mulch films, because i) a by-product from the coffee production (currently sent to damping), is valorized; ii) the cost of the mulching film can be decreased, as up to 30 wt% of the pristine polymer can be replaced by CSS; and iii) the endogenous microbiota is further exploited to enrich the soil and promote plant growth. Considering the expansion of the global market, which is estimated to increase from 3.5 billion USD to 5.1 billion by 2027, it is important to promote sustainable practices (Sander, 2019).

2. Experimental

2.1. Materials

Coffee silver skin (CSS) deriving from the roasting of a mixture of *Arabica* and *Robusta* species (70 ± 5 to 30 ± 5 wt% ratio) was supplied by Cagliari Spa (Modena, Italy). Waste batches for all biological analysis were collected at different times after the roasting process, i.e., 0, 7 and 12 days. The powders had been characterized by attenuated total reflection (ATR) Fourier transform infrared (FT-IR) spectroscopy and thermogravimetric analysis (TGA), demonstrating coherence with already reported data (Ghazvini et al., 2022; Totaro et al., 2019). Briefly, ATR FT-IR spectrum of CSS (Fig. 1) shows broad band around 3300 cm^{-1} (OH and NH stretching), 2920–2850 cm^{-1} (CH stretching), 1640 cm^{-1} (carbonyl stretching) and 1030 cm^{-1} (CO stretching and CH rocking vibrations), typical of lignocellulosic materials, mainly consistent in polysaccharides (cellulose, hemicellulose), lignin, proteins, fats and minerals (Totaro et al., 2019; Ballesteros et al., 2014; Sarasini et al., 2018). No differences were highlighted from this analysis in the sample stored at different time (data not reported).

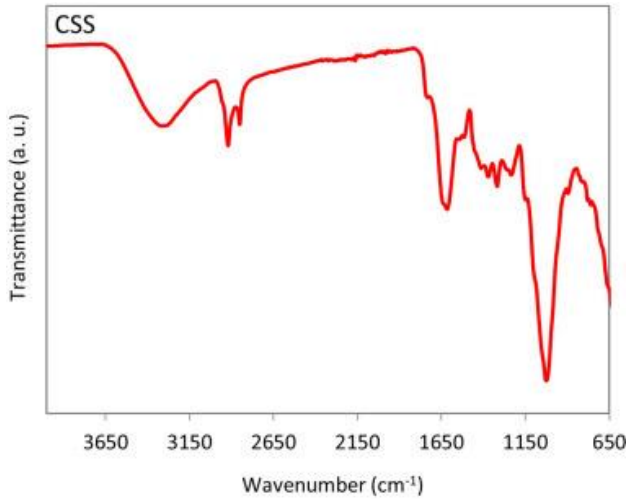

Fig. 1. ATR FT-IR profile of coffee silver skin.

The thermal behavior of CSS as well agrees with already reported data (Totaro et al., 2019; Azwa et al., 2013). It shows a first weight loss below 180 °C, corresponding to water removal (3 wt% approximately). The main loss from 180 to 430 °C (~40 wt%) refers to the decomposition of hemicellulose and cellulose, while lignin is lost after 430 °C. The final residue (23 %) corresponds to the inorganic content of CSS (Fig. 2). Fig. 1. ATR FT-IR profile of coffee silver skin.

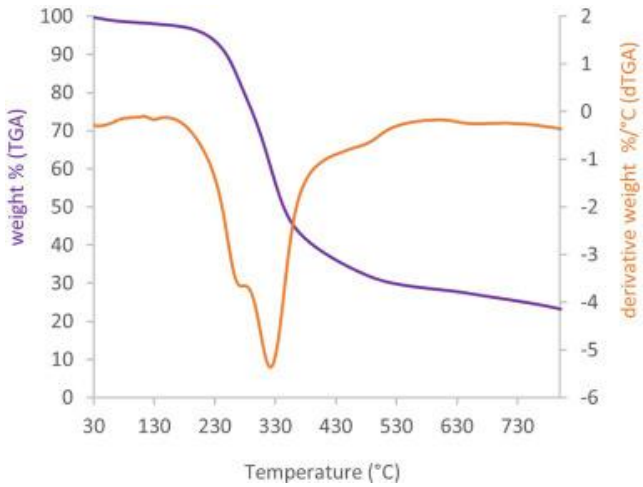

Fig. 2. TGA and dTGA profiles of coffee silver skin.

The PBSA used in this research is a commercial BioPBSTM FD92PM, PTT MCC Biochem, with a density of 1.24 g/cm^3, a MFR of 4 g/10 min (190 °C, 2.16 kg), a melting temperature of 84 °C and a suggested processing range of 130–150 °C. The ratio between butylene succinate and butylene adipate units is $(PBS)_{0.7}$–$(PBA)_{0.3}$.

2.2. Microbial characterization of CSS and strain isolation

Several bacterial strains were isolated from three CSS samples (0, 7, 12 days after the roasting process, collected to evaluate the potential variation of the cultivable microorganisms upon storage). For each sample, 1 g was collected and suspended in 99 ml of saline solution (NaCl 0.9 % wt/v) and homogenized with a Stomacher

lab blender (Colworth STOMACHER 400) for 30 min. Isolation of bacteria was made after serial dilutions of the homogenized solution using the pour plate method on plate count agar (PCA, Oxoid Milan, Italy). The plates were incubated at 37 °C for 24 h in aerobic conditions. Each analysis was performed in duplicate. The isolated colonies were counted and the number of bacteria expressed as Log of Colony Forming Unit (CFU)/g of CSS. The isolates showing a different cell and colony morphology (Optical microscope, Axioskop 40 Zeiss) were picked and purified on the same agar medium.
Isolated strains were characterized by sequencing the 16s rRNA gene. Genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). Amplification of the 16s rRNA gene was done using the universal bacterial primer 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1520R (5′-AAG GGA GGT GAT CCA GCC GCA-3′) under the following conditions: denaturation at 95 °C for 2 min followed by 35 cycles of 95 °C for 15 s, 55 °C for 1 min, 72 °C for 1 min, and final extension at 72 °C for 10 min. Amplified PCR products were analysed by electrophoresis with 1.5 % agarose gel added with Syber safe (Invitrogen, USA) and visualized with the gel documentation system Gel DocTM XR (Bio-Rad, Hercules, CA, USA). The PCR product was purified using NucleoSpin (Macherey-Nagel GmbH & Co. KG, Germany). The purified PCR products were sequenced by Eurofins MWG Operon. Sequence chromatograms were edited and analysed using the software program Finch TV version 1.4. The sequence was compared to those present in the BLAST NCBI database.

2.3. Chemical analyses on coffee silver skin

Ash content was determined by incinerating 2.5 g of CSS for 5 h at 500 °C. After cooling in the desiccator, the residue was determined gravimetrically. Total nitrogen was determined by digestion with the Kjeldahl method, followed by titration with NaOH 0.1 N. Inductive coupled plasma optical emission spectrometry (ICP-OES, Germany, Ameteck Spectro) was applied for the elemental analysis of organic samples. CSS samples were mineralized in a 4:1 solution of HNO_3 (65 % v/v): H_2O_2 (30 % v/v) in a microwave oven (START D Microwave Digestion System; Milestone Inc., Sorisole, Bergamo, Italy). The concentrations of major (P, K) and minor (Fe, Mn, Mg, Ca, B) elements was evaluated in the obtained extracts. Analyses were performed in triplicate.

2.4. Biocomposite compounding and film manufacturing

The compounding process has been carried out by means of a Brabender PL-2000 Plasti-Corder at 130 °C and 100 rpm (3 min). CSS samples, stored at different times (0, 7 and 12 d), were previously ball milled in a rotary milling equipment (MMS, Nonantola, Italy) to obtain the following particle size distribution: D_{10} 5.2 μm, D_{50} 29.9 μm and D_{90} 80.9 μm, and the sample richest in microbial entities has been used for compounding. Fig. 3 shows the morphology of the obtained powders. As can be observed, the particles have different morphology with an uneven, irregular spongy surface. If almost all the fragments have an isomorphous shape, some of them approach a fiber morphology.

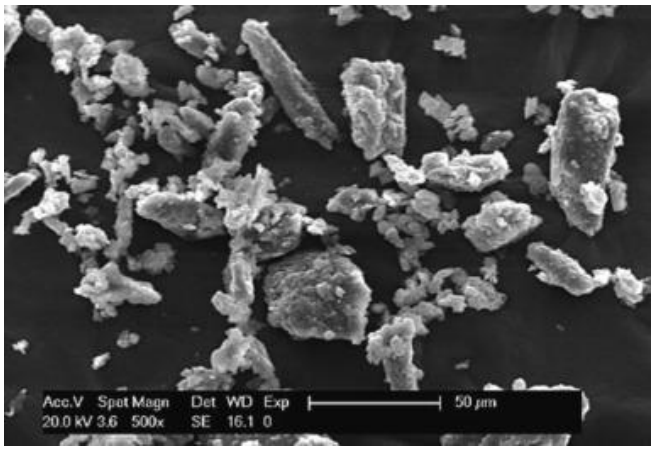

Fig. 3. Morphology of the milled silver skin.

Before compounding, the powders were previously dried for 24 h at 60 °C to eliminate moisture. Table 1 summarizes the composition and codes for the obtained composites.

Table 1
Composition and codes of the investigated samples.

Code	PBSA (wt%)	CSS (wt%)
PBSA	100	0
PBSA-10	90	10
PBSA-20	80	20
PBSA-30	70	30

Films (around 10 × 10 cm, thickness 200 ± 50 µm) to be submitted to the subsequent characterizations were obtained by compression molding for 65 s at 125 °C under a pressure of 550 bar, with a Carver equipment.

2.5. Biocomposite characterizations

All composite film samples were characterized by differential scanning calorimetry DSC (PerkinElmer Pyris DSC6) and TGA (PerkinElmer TGA7) in dry nitrogen flux. Glass transition, melting, cooling crystallization temperatures, as well as the melting enthalpies were determined from DSC. From these data, the crystallinity amount was calculated according to Eq. (1):

$$X_c(\%) = \frac{\Delta H_m}{\Delta H_m^0 \cdot f_w} 100 \quad (1)$$

where ΔH_m is the enthalpy derived from the thermograms, $\Delta H°_m$ is the melting enthalpy of the fully crystalline material (116.9 J/g) (Seggiani et al., 2019) and fw is the weight fraction of polymer in the sample.
Powders for TGA tests were previously treated at 60 °C for 24 h, to eliminate humidity and heated from 20 to 850 °C with a scan rate of 10 °C/min under nitrogen flow. Onset temperatures, residues and temperature corresponding to maximum degradation rates were determined.

Tensile test was carried out using an INSTRON 5966 machine, equipped with a 10 kN load cell (test speed 10 mm/min, ambient temperature 19 ± 1 °C and 70 ± 10 % R.H.). Four samples were made from each film. Each specimen had an initial length of 6 cm, a width of 0.5 cm and an average thickness of 200 ± 20 μm.

A DSA-30 Krüss instrument was used to determine the contact angle values. For every measurement point, the average value of contact angles measured on five/six locations on the sample was taken.

2.6. Microbial release from biocomposite films

Microbial release tests have been carried out with two different methods in order to identify both the time and the amount of micro-organisms present in the films prepared with the different CSS amounts.

The first method aimed at verifying whether the whole surface film was able to release microbial cells and the second one at quantifying the release of cells over time. Before testing, the films were treated with a 70 % ethanol water solution (v/v) for 15 min and dried under a laminar flow hood (FASTER Bio 48, Ferrara, Italy).

In the first method, the films (11 × 11 cm^2) were placed on glass PCA plates (140 mm diameter), which were incubated at 37 °C for 24 h in aerobic conditions. In the second method, the films were cut and mixed in saline solution (1 g of film in 10 ml of saline solution) and allowed to shake at 120 rpm at room temperature. Then, serial dilutions and counts on PCA plates were made after 30 min, 24 h, 48 h and 6 days. The plates were incubated at 37 °C for 24 h. Randomly, a total of 10 isolated colonies were picked up and, after purification of the strains, subjected to DNA extraction,16 s rDNA amplification and sequencing to check the identity of the released strains as described above.

2.7. Pot experiments in the greenhouse

The trial was aimed at checking potential effects of the prepared CSS mulching films on a model crop in pots. The test was carried out during spring 2022 using lettuce (*Lactuca sativa* L. var. *gentile*), chosen among other horticultural crops for its short cycle and considering that successful application of beneficial bacteria have been already reported in different cultivars (Gomes et al., 2003; Vetrano et al., 2020). The experiments were carried out in a greenhouse at the University of Bologna for 37 days. The temperature was maintained in the range 15 °C (minimum temperature) and 33 °C (maximum temperature). Two films were used: one made of PBSA (used as control sample) and the other with PBSA filled with CSS (PBSA-20). The films were tested on 10 plants each (10 plants with the control films and 10 with the 20 % CSS films). Two plants were added at the beginning and end of the lettuce plants row that were not analysed. Lettuce plants were

individually grown in 2 l plastic pots with drainage holes manually filled with potsoil (Gramoflor GmbH & Co., Manna Italia Srl, Italia), in order to avoid potsoil compacting. The films (11 × 11 cm) were drilled in the centre and positioned on the surface of topsoil. Then the lettuce plants were transplanted. Irrigation was carried out at the hole with 500 ml of groundwater every 2 days. At the end of the test the fresh leaves and roots were sampled and weighed. The roots were separated from the potsoil by gentle washing. The plant material was placed in the oven for 5 days at 70 °C and then the dried material was weighed. The percentage of water content and the percentage of dry matter were calculated from the respective weights.

2.8. Statistical analysis

Statistical analyses in the pot test were done by using STATISTICA software (version 10). A one-way analysis of the variance (growth effect on weight, fresh, dry, moisture percentage and dry matter with the application of two different mulching films) was conducted; the threshold used to determine the probability of significance was $p \leq 0.05$. The HSD Tukey test was used for the determination of the homogeneous group.

3. Results and discussion

3.1. Microbiological characterization of CSS and strain isolation

The culture-based approach was used for the characterization considering our interest in obtaining new isolates for possible use as Plant Growth-Promoting Bacteria (PGPB). The results of the bacterial count from CSS samples stored for different times after the roasting process were as follows (average of 2 counts): 5.06 ± 0.03 Log CFU/g of CSS (samples taken soon after the roasting process, 0-day samples), 5.60 ± 0.05 Log CFU/g (7-day samples), 5.80 ± 0.06 Log CFU/g (12-days samples). Several colonies were examined at the optical microscope. The presence of rod-shaped cells and spores allowed a preliminary indication of the strains belonging to the *Bacillus* genus. However, on the basis of slight differences in cell and colony morphologies, 25 strains (8 from each time of sampling) were subcultured and purified on PCA medium. Two strains from the 0-day sample failed to grow after repetitive sub-culturing. DNA was extracted from the remaining 23 samples and, basing on the amplified 16s rRNA gene sequences, the taxonomical identification reported in Table 2 was assigned. Different species of *Bacillus* were isolated from samples at different times of roasting, whereas *Mixta theicola* was isolated only in the 0-day sample. All the strains but one belonged to the *Bacillus* genus and the prevalent species in all samples was *Bacillus subtilis*. All the isolates belonging to the same species showed differences in the sequenced 16 s rRNA region.

Table 2
Best match identification phylotypes of purified PCR products deriving from the 16S rRNA amplification of the isolated strains.

Strain	CSS sample of isolation	Closest match (% similarity*)	Accession number**
CSS_0_1	0-day	*Bacillus licheniformis* (100)	OP390819
CSS_0_2	0-day	*Bacillus subtilis* (99.93)	OP390824

Strain	CSS sample of isolation	Closest match (% similarity*)	Accession number**
CSS_C1	0-day	*Bacillus subtilis* (99.93)	MZ357950
CSS_0_3	0-day	*Bacillus subtilis* (100)	OP390825
CSS_0_4	0-day	*Bacillus subtilis* (100)	OP390826
CSS_C8	0-day	*Mixta theicola* (99.98)	MZ357957
CSS_C6	0-day	*Bacillus subtilis* (100)	MZ357955
CSS_7_1	7-day	*Bacillus subtilis* (100)	OP390820
CSS_C2	7-day	*Bacillus subtilis* (99.98)	MZ357951
CSS_C5	7-day	*Bacillus amyloliquefaciens* (100)	MZ357954
CSS_C7	7-day	*Bacillus subtilis* (99.91)	MZ357956
CSS_7_2	7-day	*Bacillus licheniformis* (100)	OP390821
CSS_7_3	7-day	*Bacillus tequilensis* (100)	OP390822
CSS_7_4	7-day	*Bacillus subtilis* (100)	OP390830
CSS_7_5	7-day	*Bacillus subtilis* (100)	OP390831
CSS_12_1	12-day	*Bacillus velezensis* (100)	OP390823
CSS_12_2	12-day	*Bacillus subtilis* (100)	OP390827
CSS_12_3	12-day	*Bacillus subtilis* (100)	OP390828
CSS_12_4	12-day	*Bacillus tequilensis* (100)	OP390829
CSS_12_5	12-day	*Bacillus licheniformis* (100)	OP390832
CSS_12_6	12-day	*Bacillus halotolerans* (99.93)	OP390833
CSS_C3	12-day	*Bacillus subtilis* (100)	MZ357952
CSS_C9	12-day	*Bacillus mojavensis* (100)	MZ357958

* Similarity represents the % similarity shared with the sequences in the GenBank database.

** Provided by GeneBank (https://www.ncbi.nlm.nih.gov/genbank/).

The genus *Bacillus* has a wide distribution in different natural environments, including products derived from coffee processing (Silva et al., 2008; Bui, 2014), and has been widely used for biotechnology applications. *Bacillus* species are known to be frequent in soils, in which they are able to survive thanks to their sporulation ability. The capability of surviving in harsh environment may be the reason for their wide occurrence after the process of coffee beans roasting. In particular, the temperature value in roasting is represented by a thermal change from 80 °C to 100 °C. Some *Bacillus* species are able to produce extracellular enzymes that degrade complex polysaccharides, such as cellulose and pectin (Coughlan and Mayer, 1991). In particular, the *Bacillus subtilis* species is known to be used in the production of enzymes in the food industry (Kovács, 2019). *Bacillus licheniformis* and *Bacillus subtilis* are closely related species and act as active agents for plant growth stimulation in association with the plant root system (Rey et al., 2004). As suggested by Silva et al. (2008), who isolated mostly members belonging to this genus during natural coffee processing, the cellulolytic ability of *Bacillus* may contribute to the depolymerization of cellulose containing complexes during fermentation of coffee cherries. *Bacillus tequilensis* and *Bacillus velezensis* have also been isolated; they are known to possess properties of solubilizing macro elements and of producing secondary metabolites that promote leaf and root development in several crops. *Bacillus halotolerans*, as described by Xia et al. (2020), helps to tolerate drought and salt stresses. *Mixta theicola* was originally isolated as an endophyte from leaves of black tea and it is also a PGPB capable of producing the phytohormone indole acetic acid (Tanaka et al., 2015; Hagaggi and Mohamed, 2020). In the present study, this species has been isolated for the first time from coffee derived products. Considering its first isolation source, we can assume it may be related to plant matrixes rich in natural alkaloids, such as caffeine, which is the most widely consumed psychostimulant drug in the world (Nehlig, 2018). A recently isolated *Mixta* sp. was found in the gut of plastic-eating mealworms, suggesting a role in the interaction between the gut microbiota and worm metabolism in plastic degradation (Xia et al., 2020). The capability of adapting to a complex environment, such as the mealworm gut where many factors are involved in such a peculiar process, may explain the ability of this species to survive during the production process of CSS.

3.2. Chemical analyses of CSS

Analysis of the chemical elements is a pre-requisite for soil fertilization, in particular the content of nitrogen, phosphorus and potassium that are important for plant nutrition and growth. The mineral content in CSS is strongly influenced by the characteristics of the growing soil of *Coffea* plants, such as organic matter content and/or pH value. In addition, the application of chemicals such as fertilizers, fungicides, insecticides, and herbicides can affect element levels, as these products may contain more metals (Santos et al., 2004). The results of the chemical analyses on the different CSS samples, are given in Table 3. Ash contents of CSS are quite high with values ranging from 6.64 % to 6.98 % indicating a large mineral content. Chemical element analysis revealed a high content of macro- and micro-elements that slightly diminished increasing storage time, but not significantly. These data confirm the richness in chemical elements of CSS (Pohl et al., 2013).

Table 3
Chemical composition of CSS at different sampling times.

	0-day	7-day	12-day
Ash content (%)	6.98 ± 0.14	6.70 ± 0.67	6.64 ± 0.24
N (%)	3.40 ± 0.14	3.20 ± 0.12	2.90 ± 0.17
P_2O_5 (mg/kg)	515 ± 6.32	512 ± 4.25	507 ± 4.82
K_2O (mg/kg)	13,589 ± 54.27	13,626 ± 62.31	13,704 ± 59.39
Fe (mg/kg)	~1000	~1000	~1000
Mn (mg/kg)	165 ± 4.84	147 ± 6.32	152 ± 5.62
Mg (mg/kg)	~2000	~2000	~2000
Ca (mg/kg)	>10,000	>10,000	>10,000
B (mg/kg)	46 ± 3.84	51 ± 5.31	39 ± 2.34

In particular, the nitrogen content of 2.9 % (12-day CSS sample), although lower than the amount present in traditional fertilizers (Liu et al., 2014), may provide an additional N source to the plant and may help to reduce the amount of chemical fertilizer applied to the soil or restore N content after cropping. Nitrogen is an essential and important plant nutrient for growth and development of crop plants. In lettuce production, in particular, it exhibits marked effect on the vegetative growth, leaf, fiber and protein content (Tanaka et al., 1984).
Phosphorus, potassium, iron, manganese, magnesium, calcium and boron were detected in all CSS samples and they are also essential for plant nutritional functions (Sahin, 2022). Calcium, potassium and magnesium were the most abundant mineral elements, in agreement with other studies (Ashu and Chandravanshi, 2011; Gogoasa et al., 2013). Potassium, iron, manganese and magnesium improve photosynthetic pigment uptake capacity in addition to activating physiological and biochemical markers for stress tolerance (Roosta et al., 2018). Phosphorus is critical in cell division, crop maturation, seed formation and quality. Potassium increases the water retention capacity of plant tissues, maintenance of good conditions for a longer period (Ahammed et al., 2012) and reduces leaf burning (leaf breakage) and increased leaf yield (Laughlin, 1961). Boron promotes new cell growth and seed sterility (Katyal and Randhawa, 1983).

3.3. Thermal, mechanical and wettability characterization of bio-composites

Table 4 summarizes the results of the thermal characterization of the CSS used in this work. There are no effects on the melting and glass transition temperatures and, although the crystallization temperature decreases as the amount of CSS increases, the final amount of crystalline phase (X_c) in all the samples is almost unaffected by the filler. On the other

hand, crystallization temperature decreases with the increase of CSS (Fig. 4), highlighting an inhibition effect of CSS against the crystallization of the polymeric chains. A similar behavior is reported for poly(lactic acid) (Totaro et al., 2019) and poly(butylene adipate-*co*-terephthalate)/poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) blends (Sarasini et al., 2018). Moreover, the shape of melting changes as well: the neat PBSA presents a lower endothermic peak near T_m, highlighting the presence of two different type of crystals. With the increase of CSS, such small endothermic peak tends to disappear, highlighting the fact that the filler favors one crystal population respect to the other.

Table 4
DSC and TGA characterization of PBSA and biocomposites.

Sample	DSC						TGA		
	Cooling		2nd heating				T_{onset}	T_{max}	Residue (800 °C)
	T_c	ΔH_c	T_g	T_m	ΔH_m	X_c			
	°C	J/g	°C	°C	J/g	%	°C	°C	%
PBSA	43	36	−46	86	29	24.8	379	408	0
PBSA-10	38	34	−45	85	27	25.7	372	398	3.9
PBSA-20	35	31	−46	85	24	25.7	364	396	6.8
PBSA-30	32	28	−46	84	22	26.9	359	394	9.6

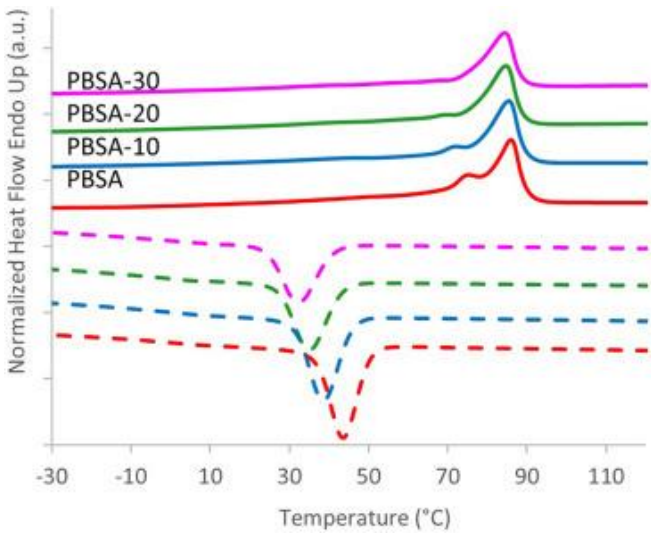

Fig. 4. DSC curves of PBSA and biocomposites.

As for the TGA results (Fig. 5), CSS addition progressively increases the residue amount and decreases the T_{onset} and T_{max} as already reported (Totaro et al., 2019). However, even at the highest CSS amount, the onset temperature is still well above the compounding one, a feature that should rule out remarkable degradation effects on the matrix. Similar data were also reported for PBS composites with spent coffee ground (Gaidukova et al., 2021).

The residue, coherent with the loading amount, is mostly due to the ash content and mineral composition of CSS (Gottstein et al., 2021) as also confirmed by the loss on ignition value.

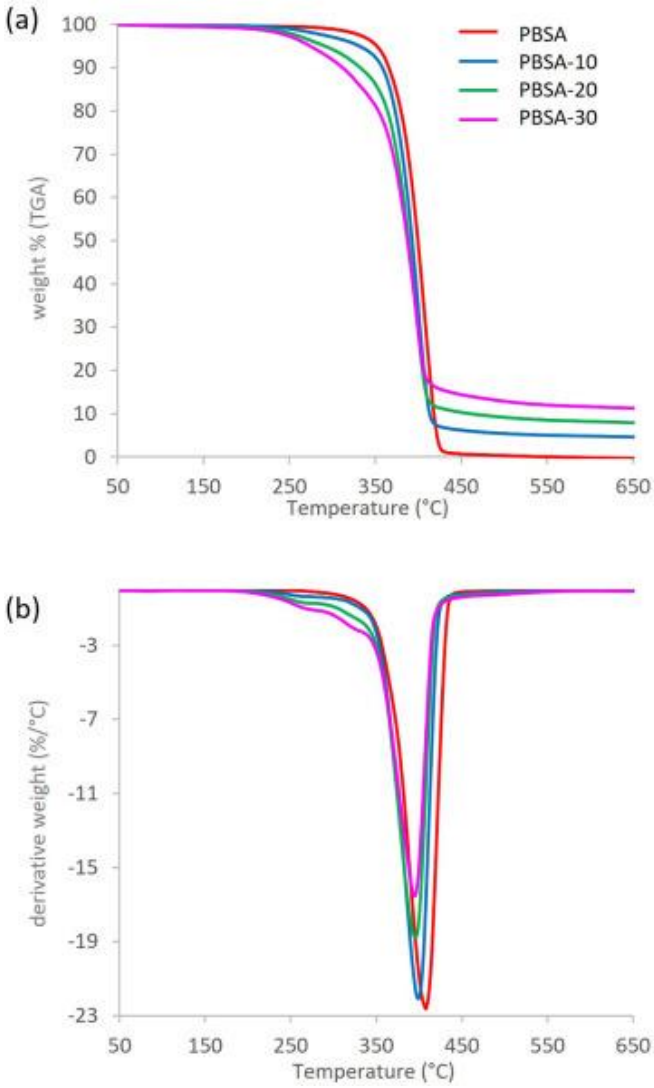

Fig. 5. (a) TGA and (b) dTGA profiles of PBSA and biocomposites.

Table 5 shows the results of the mechanical characterization of the composites. CSS addition progressively increases the value of the modulus but decreases all the other properties, leading to a more brittle behavior than the one of the matrix above the 20 wt% amount. This trend is similar to what has been reported for similar composites (Totaro et al., 2019; Ghazvini et al., 2022; Arrigo et al., 2020), particularly when no coupling agents are used in materials formulation (Picard et al., 2020). Garcia and Kim (2021) recently reviewed the properties of CSS and spent coffee ground composites and reported that chemical modification and the use of coupling agents is often required to reach improved mechanical properties.

Table 5
Mechanical characterization of PBSA and biocomposites.

Sample	E (MPa)	σ_{max} (MPa)	ε_{max} (%)	ε_{break} (%)
PBSA	276.7 ± 30.4	13.8 ± 0.8	11.6 ± 1.5	66.7 ± 20.7
PBSA-10	345.2 ± 19.3	11.0 ± 0.1	8.5 ± 0.8	69.1 ± 31.7
PBSA-20	475.8 ± 11.0	7.2 ± 0.2	3.5 ± 0.3	59.6 ± 20.4
PBSA-30	540.5 ± 28.8	5.4 ± 0.4	2.0 ± 0.1	27.3 ± 5.3

In Table 6 the contact angle values measured on the films are summarized. The CSS addition converts the initial hydrophilic character of the matrix to a hydrophobic one above 20 wt% of CSS content. The effect is higher than the one recorded in other biopolymers, such as PLA and PBS (Totaro et al., 2019; Ghazvini et al., 2022) and should be evaluated as to what concerns the degradation rates of the films. This could turn out to be beneficial for mulching films since they should prevent the soil evaporation.

Table 6
Contact angle values.

Sample	Contact angle θ (°)
PBSA	79.4 ± 2.85
PBSA-10	85.6 ± 2.82
PBSA-20	97.9 ± 2.90
PBSA-30	102.3 ± 1.92

3.4. Microbial release from biocomposite films

All biocomposite films (processed both with Methods 1 and 2) showed release of the microbial components from the entire surface after 24 h of incubation (Fig. 6). Therefore, the whole surface is able to release microbial cells even if the film is not cut into pieces.

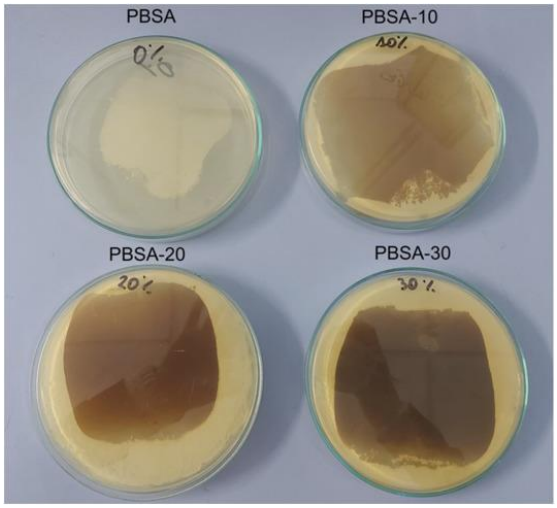

Fig. 6. Release of microorganisms in the agar plates from the films containing different percentages of coffee silver skin.

The microbial counts obtained after processing the films with Method 2 are presented in Table 7. Colonies were randomly picked up and, after processing, as described in the experimental session, they were all found to belong to the *Bacillus* genus, sequence comparison allowed the recognition of some of the previously isolated strains (Table 8). *Bacillus* strains resist at high temperatures because of the production of spores. In particular, the maximum temperature value in the compound process is 130 °C for 1 min (Margosch et al., 2006), which is sufficient to kill vegetative cells but spores can resist.

Table 7
Microbial counts in biocomposite films.

Sample	Microbial counts* (Log CFU/ml of saline solution at different processing times)			
	30 min	**24 h**	**48 h**	**6 days**
PBSA-10	2.30 ± 0.02	3.50 ± 0.02	3.60 ± 0.04	3.40 ± 0.03
PBSA-20	2.40 ± 0.04	3.60 ± 0.03	3.70 ± 0.02	4.80 ± 0.01
PBSA-30	2.30 ± 0.01	2.60 ± 0.02	2.60 ± 0.02	4.70 ± 0.06

* Average of duplicate experiments.

Table 8
Detected strain population from biocomposite films.

Strain	CSS sample of isolation	Closest match (% similarity*)
CSS_12_5	PBSA-10	*Bacillus licheniformis* (100)
CSS_12_2	PBSA-10	*Bacillus subtilis* (99.93)
CSS_12_6	PBSA-20	*Bacillus halotolerans* (99.86)
CSS_C3	PBSA-20	*Bacillus subtilis* (100)
CSS_12_3	PBSA-30	*Bacillus subtilis* (100)
CSS_12_2	PBSA-30	*Bacillus subtilis* (100)

* Similarity represents the % similarity shared with the sequences in the GenBank database.

The number of released cells generally increases with time. PBSA-10 did not show a further increase from 48 h to 6 days, whereas the other two samples showed an increase by prolonging the sampling times. No differences were observed at 6 days between PBSA-20 and PBSA-30. On the contrary, PBSA-30 showed a lower release at 24 and 48 h. This is the reason why the sample PBSA-20 was selected for pot experiments in the greenhouse: it represents a good compromise in terms of mechanical properties and microbial release over time. The results of these tests are reported in Table 9 in which the fresh and dry weight of the leaves and roots are reported.

Table 9
Fresh and dry weight of the leaves and roots, calculated as average of 10 pots.

		Fresh weight (g/plant)	Dry weight (g/plant)	Moisture content (%)	Dry matter (%)
Leaves	PBSA	35.89 ± 4.69[b]	3.41 ± 0.36[b]	90.39 ± 1.30[a]	9.61 ± 1.30[a]
	PBSA-20	42.37 ± 3.05[a]	4.23 ± 0.44[a]	89.99 ± 0.91[a]	10.01 ± 0.90[a]

		Fresh weight (g/plant)	Dry weight (g/plant)	Moisture content (%)	Dry matter (%)
	Significance	**	***	ns	ns
	PBSA	9.73 ± 1.46[b]	0.36 ± 0.03[b]	96.25 ± 1.30[a]	3.75 ± 0.65[b]
Roots	PBSA-20	12.44 ± 0.67[a]	0.57 ± 0.02[a]	95.36 ± 0.43[b]	4.63 ± 0.26[a]
	Significance	***	***	***	***

([a,b])Different letters indicate significant difference between the control and PBSA20 (HSD Tukey's test; ns: effect not significant.

**
Effect significant at $p \leq 0.05$.

***
Effect significant at $p \leq 0.01$.

Fig. 7a shows the set-up of the experiment with the control film (PBSA) and the PBSA-20 film. Biomass of fresh leaves was significantly higher in PBSA-20 than in the film without CSS, with an increase of 18.05 wt%. The dry leaf matter amount followed a similar significant trend as the fresh biomass, whereas moisture and dry matter percentages showed no significant differences. The leaves growth stimulation may be due both to the amount of macro elements present in the CSS, as shown by CSS chemical analyses, and to the presence of beneficial microorganisms that can help in nutrient absorption by the roots. The root biomass was also significantly higher in PBSA-20 compared to PBSA, as shown by the fresh weight contents. The root dry matter percentage is also higher in plants with PBSA-20 films. It is therefore possible to speculate that PBSA-20 helps in restoring the soil chemical components absorbed by plants and contributes to maintaining the beneficial soil microbiota. In particular, microorganisms are released slowly from the film, and interact with the plant root system stimulating root (Fig. 7b) and plant growth (Fig. 7c).

Fig. 7. (a) Lettuce post transplanting in the pot with the film of PBSA without CSS (left) and with 20 % CSS (right); (b) example of the aerial part of lettuce root system at the end of the growing cycle in the presence of the film without CSS (left) and with 20 % CSS (right); (c) set of plants in the agronomic trial at the end of the growing cycle.

It is possible to speculate that the bacterial present and the chemical component of CSS stimulate root growth that was expressed in higher productivity.
During the pot experiments, starting from 2 weeks after lettuce transplant, a partial degradation in the PBSA-20 film was observed and, at the end of the growth cycle, a slight external fragmentation was visually detected.

4. Conclusions

The coffee silver skin, a by-product derived from coffee roasting that represents a pollution hazard if discharged into the environment or a cost if disposed, has a rich endogenous microbiota, mainly composed of members of the *Bacillus* genus. These bacteria, as spore-formers, can survive the high temperatures reached during the roasting and the compound processing. The abundance of chemical elements, particularly N-P-K, makes CSS an important candidate for its use as a fertilizer, like other coffee by-products.
The present work shows that films based on PBSA and containing up to 30 wt% of CSS can be prepared by melt compounding at high temperature with final reliable mechanical properties. The filler presence does not alter significantly the thermal properties of the bare polymer such as the thermal stability and the crystallization process, but it converts the hydrophilic character of the PBSA to a hydrophobic one. After the compounding and filming process, the CSS in the bio-composites is still capable of releasing live endogenous microorganisms in the soil when used as mulching films. The beneficial effect has been tested positively on lettuce as a model crop, resulting in higher leaves and roots biomass. Therefore, it can be hypothesized that the chemical elements provided to the soil may help in restoring its nutritional content. In addition, the use of rhizo-stimulating bacteria is important in the light of potentially reducing the amount of water required by the plants due to an improved capability of exploring the soil with the roots.

The study points out that CSS-enriched films are a potential source of beneficial bacteria and features the possibility of producing functionalized mulching films. The addition of CSS can consequently have multiple benefits: i) to reduce the cost of the mulching material; ii) to allow a possible valorization of this by-product in the agricultural field; and iii) to exploit the endogenous microbiota to promote plants growth. Therefore, a fully biodegradable mulching film can be prepared, with benefits for the society and the environment.

Next research will be focused on different crops, with different characteristics and growth cycles, in order to assess the right film formulation and find the opportune balance between durability and degradability, still retaining adequate film processability. The mechanical behavior of films after use will be explored, as well as a soil microbial characterization, with the aim of assessing the permanence of the released microorganisms in the soil and the balance between the released microbiota and the endogenous one.

CRediT authorship contribution statement

Elia Pagliarini: Formal analysis; Investigation; Data curation; Visualization; Writing original draft.

Grazia Totaro: Formal analysis; Investigation; Data curation; Visualization; Writing original draft; Review and editing.

Andrea Saccani: Conceptualization; Methodology; Writing original draft; Review and Editing.

Francesca Gaggia: Investigation.

Isabella Lancellotti: Investigation.

Diana Di Gioia: Conceptualization; Methodology; Writing original draft; Review and editing.

Laura Sisti: Conceptualization; Methodology; Formal analysis; Data curation; Supervision; Writing, Review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Ahammed, G.J., Wang, M.M., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K., Yu, J.Q., 2012. The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress. Ecotox. Environ. Safe. 80, 132-139.

Arrigo, R., Bartoli, M., Malucelli, G., 2020. Poly(lactic Acid)-biochar biocomposites: Effect of processing and filler content on rheological, thermal, and mechanical properties. Polymers 12(4), 892.

Ashu, R., Chandravanshi, B.S., 2011. Concentration levels of metals in commercially available Ethiopian roasted coffee powders and their infusions. Bull. Chem. Soc. Ethiop. 25(1), 11-2.

Aversa, C., Barletta, M., Cappiello, G., Gisario, A., 2022. Compatibilization strategies and analysis of morphological features of poly (butylene adipate-co-terephthalate) (PBAT)/poly(lactic acid) PLA blends: A state-of-art review. Europ. Polymer J. 173, 111304.

Azwa, Z.N., Yousif, B.F., Manalo, A.C., Karunasena, W., 2013. A Review on the degradability of polymeric composites based on natural fibres. Mater. Des. 47, 424.

Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I., 2014. Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. Food Bioproc. Tech. 7, 3493.

Bandopadhyay, S., Martin-Closas, L., Pelacho, A.M., DeBruyn, J.M., (2018) Biodegradable Plastic Mulch Films: Impacts on Soil Microbial Communities and Ecosystem Functions. Front. Microbiol. 9:819.

Borrelli, R., Esposito, F., Napolitano, A., Ritieni, A., Fogliano V., 2004. Characterization of a new potential functional ingredient: coffee silverskin. J. Agric. Food Chem. 52, 1338–1343.

Briassoulis, D., Hiskakis, M., Babou, E., 2013. Technical specifications for mechanical recycling of agricultural plastic waste. Waste Manag. 33, 1516-1530.

Bui, H.B., 2014. Isolation of cellulolytic bacteria, including actinomycetes, from coffee exocarps in coffee-producing areas in Vietnam. Int. J. Recycl. Org. Waste Agricult. 3(1), 48.

Chien, H.-L, Tsai, Y.T.., Tseng, W.-S., Wu, J.-A., Kuo, S.-L., Chang, S.-L., Huang, S.-J. J., Liu, C-T., 2022. Biodegradation of PBSA films by elite *aspergillus* isolates and farmland soil. Polymers 14, 1320.

Coughlan, M.P., Mayer, F., 1991. The cellulose-decomposing bacteria and their enzymes systems. In: Balows, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds.), The Prokaryotes, vol.1. Springer, Berlin, pp. 460–516.

Dias, M., Melo, M.M., Schwan, R.F., Silva, C.F., 2015. A new alternative use for coffee pulp from the semi-dry process to β-glucosidase production by *Bacillus subtilis*. Lett. Appl. Microbiol. 61(6). 588–595.

Fennell, J.T., Fountain, M.T., Paul. N.D., 2019. Direct effects of protective cladding material on insect pests in crops. Crop Prot. 121. 147-156.

Gaidukova, G., Platnieks, O., Aunins, A., Barkane, A., Ingrao, C., Gaidukovs, S., 2021. Spent coffee waste as a renewable source for the production of sustainable poly(butylene succinate) biocomposites from a circular economy perspective. RSC Adv. 11, 18580.

Garcia, C.V., Kim, Y-T., 2021. Spent coffee grounds and coffee silverskin as potential materials for packaging: a review. J. Pol. Envir. 29, 2372–2384.

Ghazvini, A.K.A., Ormondroyd, G., Curling, S., Saccani, A., Sisti, L., 2022. An investigation on the possible use of coffee silverskin in PLA/PBS composites. J. Appl. Polym. Sci. 139(22). 52264.

Gogoasa, I., Pirvu, A., Alda, L. M., Velciov, A., Rada, M., Bordean, D. M., Gergen, I., 2013. The mineral content of different coffee brands. J. Hortic. Biotechnol. 17(4), 68-71.

Gomes, A., Mariano, R.L., Silveira, E. B., Mesquita, J. C., 2003. Isolation, selection of bacteria, and effect of *Bacillus* spp. in the production of organic lettuce seedlings. Hortic. Brasil. 21, 699-703.

Gottstein, V., Bernhardt, M., Dilger, E., Keller, J., Breitling-Utzmann, C.M., Schwarz, S., Kuballa, T., Lachenmeier, D.W., Bunzel, M. 2021, Coffee silver skin: chemical characterization with special consideration of dietary fiber and heat-induced contaminants. Foods 10, 1705.

Hagaggi, N.S.A., Mohamed, A.A., 2020. Enhancement of *Zea mays* (L.) growth performance using indole acetic acid producing endophyte *Mixta theicola* isolated from *Solenostemma argel* (Hayne). S. Afr. J. Bot. 134, 64-71.

Jandas, P.J., Mohanty, S., Nayak, S.K., 2013. Sustainability, Compostability, and Specific Microbial Activity on Agricultural Mulch Films Prepared from Poly(lactic acid). Ind. Eng. Chem. Res. 52, 50, 17714–17724.

Janissen, B., Huynh, T., 2018. Chemical composition and value-adding applications of coffee industry by-products: A review. Res. Cons. Rec. 128, 110-117.

Jiménez-Zamora, A., Pastoriza, S., Rufián-Henares, J. A. 2015. Revalorization of coffee by-products. Prebiotic. antimicrobial and antioxidant properties. LWT-Food Sci. Technol. 61(1), 12-18.

Khelil, O., Choubane, S., Cheba, B.A., 2016. Polyphenols content of spent coffee grounds subjected to physicochemical pretreatments influences lignocellulolytic enzymes production by *Bacillus* sp. R2. Bioresour. Technol. 211, 769–773.

Kandasamy, S., Muthusamy, G., Balakrishnan, S., Duraisamy, S., Thangasamy, S., Seralathan, K.K., Chinappan, S. 2016. Optimization of protease production from surface-modified coffee pulp waste and corncobs using *Bacillus* sp by SSF. 3 Biotech 6, 167.

Katyal, J. C., Randhawa, N. S. 1983. Micronutrientes. Food and Agriculture Organization, United Nations

Kim, S., Kim. J., Lee, H., Lee, H., 2021. Abundance and characteristics of microplastics in soils with different agricultural practices: Importance of sources with internal origin and environmental fate. J. Haz. Mat. 403,123997.

Kyrikou, I., Briassoulis, D., 2007. Biodegradation of agricultural plastic films: A critical review J. Pol. Environ. 15(2), 125-150.

Koitabashi, M., Noguchi, M.T., Sameshima-Yamashita, Y., Hiradate, S., Suzuki, K., Yoshida, S., Watanabe, T., Shinozaki, Y., Tsushima, S., Kitamoto, H.K., 2012. Degradation of biodegradable plastic mulch films in soil environment by phylloplane fungi isolated from gramineous plants. AMB Expr. 2(1), 40.

Kovács, A.T., 2019. *Bacillus Subtilis*. Trends in Microbiology, 27 (8), 724 – 725

Laughlin, W.M., 1961. Influence of potassium sprays on foliar necrosis and yields of Romaine lettuce, radishes, and potatoes. Can. J. Plant Sci. 41(2), 272-276.

Li., S., Ding. F., Flury, M., Jones, D.L., Wang, J., 2022. Macro- and microplastic accumulation in soil after 32 years of plastic film mulching. Environ. Pollut. 300,118945.

Liu, C.W., Sung, Y., Chen, B.C., Lai, H.Y., 2014, Effects of nitrogen fertilizers on the growth and nitrate content of lettuce (*Lactuca sativa L.*). Int. J. Environ. Res. Public Health, 11(4), 4427-4440.

Mansoor, Z., Tchuenbou-Magaia, F., Kowalczuk, M., Adamus, G., Manning, G., Parati, M., Radecka, I., Khan, H., 2022. Polymers Use as Mulch Films in Agriculture-A Review of History, Problems and Current Trends. Polymers, 14, 5062.

Margosch, D., Ehrmann, M.A., Buckow, R., Heinz, V., Vogel, R. F., Gänzle, M.G., 2006. High-pressure-mediated survival of *Clostridium botulinum* and *Bacillus amyloliquefaciens* endospores at high temperature. Appl. Environ. Microbiol. 72(5), 3476-3481.

Mussatto. S.I., & Teixeira. J.A., 2010. Increase in the fructo-oligosaccharides yield and productivity by solid-state fermentation with *Aspergillus japonicus* using agro-industrial residues as support and nutrient source. Biochem. Eng. J. 53(1), 154-157.

Nehlig, A., 2018. Interindividual differences in caffeine metabolism and factors driving caffeine consumption. Pharmacol. Rev. 70(2), 384-411.

Nunes, A.P.P., Ferreira Santos, C., Guelfi, D., 2022. Interfaces between biodegradable organic matrices coating and MAP fertilizer for improve use efficiency. Sci. Total Environ. 804, 15, 149896

Picard, M.C., Rodriguez-Uribe, A., Thimmanagari, M., Misra, M., Mohanty, A.K., 2020. Sustainable biocomposites from Poly(butylene succinate) and apple pomace: A Study on compatibilization performance. Waste Biomass Valor. 11(7), 3775-3787

Pohl, P., Stelmach, E., Welna, M., Szymczycha-Madeja, A., 2013. Determination of the elemental composition of coffee using instrumental methods. Food Anal. Methods, 6(2), 598-613.

Rey, M.W., Ramaiya, P., Nelson, B.A., Brody-Karpin, S.D., Zaretsky, E.J., Tang, M., Lopez de Leon, A., Xiang, H., Gusti, V., Clausen, G., Olsen, P.B. Rasmussen, M.C., Andersen, J.T., Jørgensen, P.L., Larsen, T.S., Sorokin, A., Bolotin, A., Lapidus, A., Galleron, N., Ehrlich, S.D., Berka, R.M., 2004. Complete genome sequence of the industrial bacterium *Bacillus Licheniformis* and comparisons with closely related *bacillus* Species. Genome Biol. 5, r77.

Roosta, H.R., Estaji, A., Niknam, F., 2018. Effect of iron, zinc and manganese shortage-induced change on photosyntetic pigments, some osmoregulators and chlorophyll fluorescence parameters in lettuce. Photosynthetica, 56(2), 606-615.

Rudnik, E., Briassoulis, D., 2011. Degradation behaviour of poly(lactic acid) films and fibres in soil under Mediterranean field conditions and laboratory simulations testing Ind. Crops Prod. 33, 648–658.

Sander, M., 2019. Biodegradation of polymeric mulch films in agricultural soils: concepts. knowledge gaps. and future research directions. Environ. Sci. Technol. 53, 2304–2315.

Santos, E.E., Lauria, D.C., Da Silveira, C.P., 2004. Assessment of daily intake of trace elements due to consumption of foodstuffs by adult inhabitants of Rio de Janeiro city. Sci. Total Environ. 327(1-3), 69-79.

Sarasini, F., Luzi, F., Dominici, G., Maffei, A., Iannone, A. Zuorro, R., Lavecchia, L. Torre, A., Carbonell-Verdu, R., Balart, D., Puglia. 2018 Effect of Different Compatibilizers on Sustainable Composites Based on a PHBV/PBAT Matrix Filled with Coffee Silverskin. Polymer 10, 1256.

Scarascia-Mugnozza, G., Schettini, E., Vox, G., Malinconico, M., Immirzi, B., Pagliara, S., 2006. Mechanical properties decay and morphological behaviour of biodegradable films for agricultural mulching in real scale experiment. Polym. Degrad. Stab. 91, 2801-2808.

Seggiani, M., Gigante, V., Cinelli, P., Coltelli, M.-B., Sandroni, M., Anguillesi, I., Lazzeri, A., 2019. Processing and Mechanical Performances of Poly(butylene succinate-co-adipate)

(PBSA) and Raw Hydrolyzed Collagen (HC) Thermoplastic Blends. Polym. Test. 77, 105900.

Serrano-Ruiz, H., Martin-Closas, L., Pelacho, A.M., 2021, Biodegradable plastic mulches: Impact on the agricultural biotic environment. Sci. Total Environ. 750, 141228.

Silva, C.F., Batista, L.R., Abreu, L.M., Dias, E.S., Schwan, R.F. 2008. Succession of bacterial and fungal communities during natural coffee (*Coffea arabica*) fermentation. Food Microbiology, 25(8), 951-957.

Sisti, L., Totaro, G., Rosato, A., Di Gioia, D., Barbieri, L., Saccani, A., 2022. Durability of biopolymeric composites formulated with fillers from a by-product of coffee roasting. Polym. Comp. 43(3), 1485-1493.

Sisti, L., Celli, A., Totaro, G., Cinelli, P., Signori, F., Lazzeri, A., Bikaki, M., Corvini, P., Ferri, M., Tassoni, A., et al. 2021. Monomers, Materials and Energy from Coffee By-Products: A Review. Sustainability, 13, 6921.

Sahin, O., 2022. Growing media effect on essential and non-essential mineral composition of lettuce. J. Plant Nutrition, 1-10.

Tanaka, A., Yamaguchi, J., Miura, S., & Tamaru, H., 1984. Comparison of fertilizer nitrogen efficiency among field crops. Soil Sci. Plant Nutr. 30(2), 199-208.

Tanaka, Y.K., Horie, N., Mochida, K., Yoshida, Y., Okugawa, E., Nanjo, F., 2015. *Pantoea theicola* sp. nov., isolated from black tea. Int. J. Syst. Evol. Microbiol, 65(10), 3313-3319.

Thligene, N., Mezzapesa, G.N., Mondelli, D., Veronico, P., Melillo, M.T., Dumontet, S., Miano, T., Sasanelli, N., 2019. Effect of coffee silver skin and brewers' spent grain in the control of root-knot nematodes. Helminthologia, 56(1), 30- 41.

Totaro, G., Sisti, L., Fiorini, M., Andreola, F.N., Saccani, A. 2019. Formulation of Green Particulate Composites from PLA and PBS Matrix and Wastes Deriving from the Coffee Production. J. Polym. Environ. 27(7) 1488-1496.

Treinyte, J., Cesoniene, L., Bridziuviene, D., Ostrauskaite, J., Bucinskas, A., Rainosalo, E., Grazuleviciene, V., 2018. Applicability of crude glycerol as the multifunctional additive for the preparation of mulching coatings. Waste Biomass Valorization 9(10), 1855-1865.

Xia, M., Wang, J., Huo, Y. X., Yang, Y., 2020. *Mixta tenebrionis* sp. nov., isolated from the gut of the plastic-eating mealworm *Tenebrio molitor* L., Int. J. Syst. Evol. Microbiol., 70(2), 790-796.

Vetrano, F., Miceli, C., Angileri, V., Frangipane, B., Moncada, A., Miceli, A., 2020. Effect of bacterial inoculum and fertigation management on nursery and field production of lettuce plants. Agronomy, 10, 1477.