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# An Appraisal of Biodegradable Mulch Films with Respect to Strawberry Crop Performance and Fruit Quality

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**Abstract:** *Fragaria × ananassa* is a fruit grown all over the world, appreciated for its organoleptic and nutraceutical properties. Together with other berry fruits, it is rich in bioactive molecules that make it a beneficial fruit for human health. However, strawberry cultivation is influenced by pre- and post-harvest factors. Being a small plant, its fruit comes into direct contact with the soil and, as such, can quickly decompose. To reduce this inconvenience, farmers have used different strategies to mulch the soil, and the most useful method is polyethylene mulch films that are not biodegradable. The focus on environmentally sustainable agriculture can be represented by a transition to biodegradable mulch films. In our study, ten biodegradable mulch films were used to understand their effectiveness in covering the soil during the cultivation cycle of strawberry cv. Rociera. Polyethylene film was considered the control. The best yield and the highest number of fruits with greatest size and quality were obtained on polyethylene, BioFlex<sup>®</sup> (P2), Bio 6, and Bio 7 films. On BioFlex<sup>®</sup> (P2) and Bio 3 biodegradable films, strawberries showed a higher calcium and magnesium content, respectively. These results may encourage growers toward the use of eco-sustainable agricultural practices, such as biodegradable mulch films.

**Keywords:** sustainable agriculture; marketable production; antioxidant molecules; mineral content; strawberry; weed biomass

## 1. Introduction

Strawberry belongs to the *Rosaceae* family, *Fragaria* genus, with wild and cultivated species of great agronomic importance all over the world, incorporating a wide range of ecological spread, from temperate to tropical and subtropical regions, and from sea level to high altitudes [1]. Nowadays, all cultivated varieties derive from *Fragaria × ananassa*, developed in 1700 in Europe from a hybridization of two natural hybrid species from America, *Fragaria chilosensis* and *Fragaria virginiana*. Currently, there are 11 species of *Fragaria* found in their native habitats in Europe and North and South America. The fruit of all species are dry achenes on the surface of a fleshy receptacle. The differences among the various species, or within the same species, can be seen at a morphological level, in the different color and shape of the achenes, and different morphology of the stolons and leaves [2]. Worldwide, strawberry consumption is calculated in millions of tons [3,4]. In 2018, Italy and Spain dedicated an area of 4717 ha and 7032 ha to strawberry cultivation, respectively, resulting in a production of 119,223 and 344,679 tons, respectively [5]. Strawberries are mainly consumed as a fresh product, but its organoleptic characteristics make it ideal also for processed products, such as liqueurs, syrups, jam, ice cream and essences.

Berries are a rich source of bioactive compounds with potential beneficial effects on human health [6] and have thus been extensively studied [7]. The high content of polyphenols, flavonoids, and anthocyanins makes strawberry a functional food, hence a fruit with beneficial effects on human health [8,9]. Indeed, Giampieri et al. [10] showed that extracts of transgenic lines of three strawberry cultivars contained high levels of anthocyanins, which exerted cytotoxic effects on HepG2, a human hepatic cancer cell line. The antioxidant compounds present in berries, including strawberry, reduce oxidative DNA damage and can up- or down-regulate different genes involved in the inhibition of carcinogen activation, inhibition of oncogenes and metastasis. Additionally, it can reduce the risk of neurodegenerative pathologies including Lou Gehrig's, Parkinson's, Alzheimer's, and Huntington's diseases [11]. Moreover, the protective action of anthocyanins was demonstrated by Basu et al. [12], reducing non-fatal myocardial infarction when strawberries and blueberries were consumed more than three times a week. In addition, strawberry consumption reduced blood glucose levels, obesity, inflammatory responses [13], and apoptosis in breast cancer cells [14,15]. The consumption of berry fruit, including strawberries, may mitigate the risk of type 2 diabetes (T2D). Calvano et al. [16] and Davis et al. [17] showed that dietary supplementation of freeze-dried strawberries reduced postprandial hyperglycemia and hyperinsulinemia in overweight and obese adults. The same authors showed that adult consumption of freeze-dried strawberries (50 g day<sup>-1</sup>) for eight weeks, significantly decreased total and LDL cholesterol. Strawberry extracts are potent antimicrobial agents, as in the case of *Vibrio cholerae* [18]. They are also rich in fiber, folate and manganese [8]. Furthermore, strawberry can fight cellular aging, because its polyphenols are activators of AMP-activated protein kinase (AMPK; [19,20]), molecules involved in the biogenesis of mitochondria in eukaryotic cells, and in the cellular response against ROS-induced oxidative stress damage. Giampieri et al. [20] demonstrated that the consumption of strawberries significantly increased the expression of the AMPK cascade genes in older rats. Nevertheless, high-sensitivity TNF- $\alpha$  (hs-TNF- $\alpha$ ) and soluble tumor necrosis factor receptor (sTNF-R2), two biomarkers of inflammation and lipid peroxidation, were significantly decreased in men and woman with knee osteoarthritis, when administered a dose of 50 g of strawberry powder for 12 weeks [21].

The nutraceutical and sensorial properties of strawberry can vary based on cultivar, climatic and geographical conditions, ripening stage, and conservation methods [8,22,23]. A limitation of strawberry is the quick deterioration of fruit after harvest [24]. A solution adopted in strawberry production is to keep fruit clean, dry and free from soil contact to prevent triggering the decomposition by using low density polyethylene films (LDPE) [1,25]. These films have been widely adopted for various crops including strawberry, because their use speeds up crop cycles, assures high and good quality yields, and blocks weed growth [1,3,26,27].

LDPE can be transparent, colored, or dark. Transparent films pass 50% of ultraviolet (UV) rays (220–380 nm), black ones restrain UV rays but are penetrated by short wavelengths (780–2500 nm) and longer wavelength (>2500 nm) infrared rays [3]. Polyethylene films are very resistant to degradation, thanks to their high molecular weight and their hydrophobicity. Unfortunately, these characteristics render polyethylene a slowly degrading product for possibly hundreds of years [3,25]. Due to climatic conditions and chemical products used in agriculture, plastic films can be used for one or at most two cultivation cycles and then need to be removed. Their removal from the soil at the end of a crop cycle is only partial, since they can be torn apart by harvest machinery and, consequently may be dispersed throughout the harvested area. Normally, these films should be recovered from the soil, disposed of in landfills, and recycled or even incinerated appropriately. Plastic waste disposal represents a high cost for farmers, who prefer to burn them directly in the field, thus endangering their health and the environment. Moreover, mulch films increase the serious problem of plastic dispersal in the environment that the world is now facing [3,28,29]. The use of plastic products in agriculture is estimated to be in the millions of tons per year, and at least 10% of the total amount originates from mulch films [25,28,30].

Biodegradable films can represent an excellent solution to the adverse environmental impact derived from plastic use in agriculture. They have proven to be a good alternative to polyethylene films and bare soil in short cultivation cycles as stated by Cozzolino et al. [31] and in the case of strawberry cultivation as noted by Morra et al. [32]. The main practical and ecological advantages of these films are that they can be both left on the field and buried in the soil to be degraded by microorganisms. Indeed, fungi, bacteria, and algae can transform the residues of these films into carbon dioxide, methane, water, and biomass [28,29]. The biodegradable films currently in use are mainly based on starch and cellulose, polyhydroxybutyrate/valerate copolymers, and polylactic acid polymers; molecules that are susceptible to UV- and visible light-facilitated photooxidation or thermoxidized at high temperatures [25,28,33,34]. A limitation of biodegradable films is that, similar to PE films, they are subjected to weathering and chemical substances used on crops, as well as to soil microorganism attack. Therefore, they risk incomplete soil coverage for the entire crop cycle. Moreover, Nestby and Guéry [35], in a work conducted over three years on three strawberry cultivars on mulch, showed that the marketable yield could be reduced up to 58% due to a significant mildew infestation. According to European standard values [36], PE films (dark, transparent, or thermal) used in agriculture must have particular features, such as the tensile strength at break or the tensile elongation at break of at least 16 MPa and 180–250%, respectively. Conversely, as stated by Scarascia-Mugnozza et al. [28], biodegradable films have both tensile stress and tensile elongation at break lower than those of PE set by the European Standard. However, their mechanical properties fall within the range required to ensure an adequate soil coverage during the entire strawberry growing cycle. Moreover, on the basis of ecotoxicological tests, the authors demonstrated the absence of soil ecotoxicity at the end of the crop cycle after burying the material.

To balance biodegradation and physical-mechanical properties of a mulch film, it would be essential to obtain a biodegradable film, which endures until the end of the crop cycle. Furthermore, biodegradable films should yield a satisfactory amount of a high-quality final product, together with adequate weed control, similar to PE films. According to Andrade et al. [37], biodegradable mulch films, formed by starch, allowed adequate ground cover and weed suppression during a strawberry autumn-winter cycle.

Based on the aforementioned, the aim of our work was to compare different mulch films in order to determine their ability to cover the soil during the cultivation of strawberry, control weed infestation, and distinguish their effect on berry production, quantity, and quality as compared to black PE mulch film.

## 2. Materials and Methods

### 2.1. Experimental Site, Plant Material and Growth Conditions

The experiment was conducted in a greenhouse at the experimental farm of ADESVA Technological Center, located in Lepe, Huelva-Spain (lat. 37°15' N; long 7°12' W, 48 a.s.l.). Site temperatures varied between a maximum of 30 °C and a minimum of 11 °C, generally in July and January, respectively. The soil had a sandy loam texture (66% sand, 19% silt, and 15% clay; pH 7.2, organic matter 1.9%, available P<sub>2</sub>O<sub>5</sub> of 20 mg kg<sup>-1</sup>, exchangeable K of 3270 mg kg<sup>-1</sup>). Strawberry cultivar Rociera distributed by Fresas Nuevos Materiales (Huelva, Spain), was used in the experiment. It is highly resistant to *Botrytis* and mildew, with uniform coloring and round-shaped fruit, long stems that facilitate harvest, and produces a high percentage of first category fruit.

On 7 August 2017 the greenhouse was divided into four macrotunnels (6.6 m wide) covered by plastic material, with an area of 264 m<sup>2</sup>/macrotunnel. On 13th August, in each macrotunnel, five soil beds were prepared (40 cm wide, 40 m long) 50 cm apart. In each bed, two furrows 25 cm apart were then created to accommodate two parallel lines of plants. On 20th September, the soil was disinfected with TELOPIC C35 (1,3-dichloropropene 80.3% p/v (equivalent to 60.8% p/p) + chloropicrin 44.0% p/v (equivalent to 33.3% p/p; p/v: weight/volume, w/w: weight/weight), at the rate of two irrigations

per day, for ten minutes each irrigation, for a total of 300 kg ha<sup>-1</sup>. From the vegetative period of October/December until the productive period (from January to May), the following fertilizing units were distributed: total nitrogen (234.15 kg ha<sup>-1</sup>), phosphoric anhydride (75.67 kg ha<sup>-1</sup>), potassium oxide (216.28 kg ha<sup>-1</sup>), total calcium (229.15 kg ha<sup>-1</sup>), magnesium oxide (48.61 kg ha<sup>-1</sup>), potassium chloride (52.47 kg ha<sup>-1</sup>). A drip irrigation system was adopted with 1–2 irrigations of 10–25 min daily, for a total of 4684.63 m<sup>3</sup> ha<sup>-1</sup> of water.

## 2.2. Mulch Installation, Transplanting, Experimental Design

On 18 October 2017, each soil bed was covered by mulch films. Eleven different films were used, and their thickness and black pigment percentages are shown in Table 1:

**Table 1.** Mulch film types, thickness and black pigment percentage.

Mulch film	Thickness (μm)	Black Pigment (%)
Polyethylene (P1)	35	9
BioFlex <sup>®</sup> 1130 (P2)	20	9
BioFlex <sup>®</sup> 1821 (P3)	20	9
Bio M 16 F54 (Bio 1)	20	9
Bio M 17 F53 (Bio 2)	25	9
Bio M 4b F28 (Bio 3)	20	9
Bio M 5b F28 (Bio 4)	20	9
Bio M 5b F28 (Bio 5)	25	9
Bio M 17 F53 (Bio 6)	40	5
Bio M 4b F28 (Bio 7)	40	5
Bio M 5b F28 (Bio 8)	25	5

Black pigment percentage added to the mixture.

P1 is a black low-density polyethylene (LDPE) plastic film. BioFlex<sup>®</sup> 1130 (P2) and BioFlex<sup>®</sup> 1821 (P3) (FKuR Kunststoff GmbH, Germany) are commercial biodegradable films, formed from polylactic acid (PLA)/copolyester blends, obtained from a cornstarch fermentation process. They completely degrade in the soil and are compostable according to EN 13432 certification. Furthermore, BioFlex<sup>®</sup> 1130 (P2) has a minimum biobased carbon content of 10% (calculated according to ASTM D6866 certification), tear resistance of 100/110 N / mm (according to ASTM D 1922 certification), Spencer impact test of 420 N / mm (according to ASTM D 3420 certification). BioFlex<sup>®</sup> 1821 (P3) has a minimum biobased carbon content of 10% (calculated according to ISO 16620), tear resistance of 100/180 N/mm (according to ASTM D 1922 certification), Spencer impact test of 240 N/mm (according to ASTM D 3420 certification). Biodegradable films (Bio 1–8) are based on starch, but the manufacturer has not revealed its exact concentration. The black color of all films was obtained by adding from 5 to 9% black pigment to the mixture during film preparation.

The experiment involved a completely randomized design, in which the treatments were 11 different films with three repetitions, resulting in 33 plots in total, randomly distributed in the four macrotunnels representing the greenhouse. In three macrotunnels, only the three central beds, out of total five prepared, were taken into consideration for the experiment, resulting in nine different mulch films per macrotunnel (three mulch treatments per bed). The fourth macrotunnel contained six plots with the remaining film treatment repetitions distributed on two central beds. This partition of the film treatments replicated each treatment three times and distributed the repetitions among the different macrotunnels. Each experimental plot included 25 representative plants placed in double rows. On 18th October (1st day after transplant: 1 DAT) the plants were transplanted in each bed at a plant density of 65,000 plants ha<sup>-1</sup>.

### 2.3. Environmental Control

The weather data were monitored thanks to a weather control station set up under one of the four greenhouses. It consisted of a data logger (Watermark 900M), responsible for collecting and sending, via GPRS, the data of the different sensors in use. December, January, and February were the coldest months with mean temperatures of 11.5 °C, 11.0 °C, and 10.7 °C, respectively. May and October were the hottest, with mean temperatures of 19.1 °C and 17.4 °C, respectively. Relative humidity showed an increasing trend from October, reaching the highest value of 75.7% in January. Similar values were also found in March and April. Solar radiation exhibited a decreasing trend from October to January, with the lowest values in January (9.4 MJ m<sup>-2</sup> per day). From January to May, however, the trend reversed, and the highest values were found in May with 26.0 MJ m<sup>-2</sup> per day.

### 2.4. Productive Parameters, Yield and Quality Measurements

Twice a week, or three times a week in March–April (maximum production period), the fruit was collected from all the 25 plants forming each plot, for a total of 28 harvests expressed in one cumulative value. Moldy fruit was also collected and weighed. The production was assessed by weighing the fruit and dividing them into two main categories, expressed in gram per m<sup>2</sup>. The first category (Cat. I) included fruit weighing more than 15 g, with no defects in shape or color. The second category (Cat. II) included fruit weighing less than 15 g and with few defects in shape and color. Marketable yield was determined by the sum of the total weight of the Cat. I and Cat. II fruit. As for the fruit average weight determination, once a month, or twice a month in March and April, Cat. I fruits were collected by plot and weighed, then divided by their count.

Fruit flesh firmness was also determined on two opposite sides at the equatorial zone, of five previously peeled fruit with similar color sampled from each plot, using a digital penetrometer (FT 327, Effegi, Milan, Italy) equipped with a 'star' plunger for strawberries (model 53207, Turoni, Forlì, Italy) and expressed as N. Measurements were performed three times throughout the cycle, at the beginning of the harvest season (February; 128 DAT) in the middle (March; 153 DAT) and at the end of the season (May; 202 DAT).

On the same five fruits used for the flesh firmness measurement, the fruit soluble solids content (° Brix) was evaluated using an Atago digital refractometer (model: PR-32) at a temperature of 20 °C.

### 2.5. Weed Biomass

Weed biomass was assessed in two steps: during the cropping season, collected only from the central part of each bed in April (178 DAT), and at the end of the season in May (202 DAT) when the plants were removed. The weeds were collected from the central part of each bed and also from the sides and above the beds. Weeds were cut and weighed, and the weed biomass was expressed as grams fresh weight m<sup>-2</sup>.

### 2.6. Quality Parameters

At the end of the last harvest, 100 g of fruits were collected per plot. They were frozen at −80 °C and subsequently freeze-dried to determine total phenol content and hydrophilic (HAA) and ABTS antioxidant activities (ABTS-AA), following use of the Folin–Ciocalteu [38], DMPD [39] and ABTS [40] methods, respectively, as previously described in Rouphael et al. [41]. Results were expressed as mg gallic acid equivalents per 100 g<sup>-1</sup> dry weight (dw), mmol Trolox per 100 g<sup>-1</sup> dw, and mmol ascorbic acid equivalents per 100 g<sup>-1</sup> dw, respectively.

Total protein content was estimated by determining first the total nitrogen content of fruit by the Kjeldahl method [42], and then by multiplying the total N value by a factor of 6.25 following official method 976.05 of the AOAC (Association of Official Analytical Chemists [43]). Data were expressed as g per 100 g<sup>-1</sup> dw.



### 2.7. Malic and Citric Acid Content and Mineral Profile

Malic and citric acid content, and minerals were determined using an ion chromatography model ICS-3000, (Dionex, Sunnyvale, CA, USA). Two different columns were used: an IonPac CS12A (4 × 250 mm) analytical column for cation determination (K, Ca, Mg, Na); and a IonPac AS11-HC analytical column (4 × 250 mm) for anion (nitrate, P, SO<sub>4</sub>) and malic and citric acid determination [41]. Data were expressed as g kg<sup>-1</sup> dw.

### 2.8. Statistical Analysis

The Shapiro–Wilk and Kolmororov–Smirnov procedures were performed to verify that the data had a normal distribution, and the Levene, O’Brien and Bartlet tests were conducted to verify the homogeneity of variances. The experimental data were subjected to analysis of variance (ANOVA) using the SPSS 20 software package (SPSS Inc., Chicago, IL). In particular, the cumulative yield for each category was calculated by adding the fruit yield at each harvest. Duncan’s multiple range test was performed for mean comparisons on each of the significant ( $p \leq 0.05$ ) variables measured.

## 3. Results

### 3.1. Marketable Production and Mulching Performance

The highest marketable yield was observed when P1 film was used (6995 g m<sup>-2</sup>; Table 2), though it was not significantly different from the biodegradable films P2, Bio 6, and Bio 7 that produced slightly less on average (−6.8%). Conversely, Bio 1, 2, and 4 showed the lowest marketable yield, with a 25% reduction compared to the P1 film. P3, Bio 3, 5, and 8 resulted in intermediate yield values. The marketable yield was divided into two categories: strawberries with a weight greater than 15 g and without defects in shape or color that belong to the first category, and strawberries with a weight less than 15 g and with slight defects that were attributed to the second category. The highest yield of Cat.I fruits was on the beds covered with LDPE film (P1; 5729 g m<sup>-2</sup>), P2, Bio 6, and Bio 7. Production of Cat. II fruit ranged between 966 g m<sup>-2</sup> (Bio 2) and 1422 g m<sup>-2</sup> (Bio 5), but no significant differences between the mulch treatments were noticed (Table 2). P1 film resulted in a minor amount of moldy fruit but was not significantly different from most of the other treatments, except for Bio 3, Bio 4, and Bio 5, which registered the greatest quantity of moldy fruit (~167 % more than P1). Mulch treatments did not affect fruit mean weight, while they had a significant effect on fruit number. The highest fruit number was harvested in P1 treatment (250 no. m<sup>-2</sup>), even though it was not significantly different from P2, P3, Bio 5, Bio 6, and Bio 7.

The resistance to degradation of mulch films can be indirectly deduced by the number of weeds they allowed to grow. LDPE P1 film did not allow the weeds to emerge throughout the crop cycle by maintaining the full coverage of the beds. Among the biodegradable films, Bio 6 and 7 had the lowest weed biomass with 220 and 225 g m<sup>-2</sup>, respectively, though not significantly different from most mulch treatments except for Bio 1 with 660 g m<sup>-2</sup>.

### 3.2. Quality Analysis

The mulch films had no significant effects on fruit soluble solids content or flesh firmness (Table 3). Similarly, there was no significant effect on total protein, total phenols, ABTS antioxidant activity, or malic and citric acid content (Table 4). On the contrary, differences were evident in hydrophilic antioxidant activity levels among the treatments, with the highest values obtained on P2 film (12.80 mmol ascorbic acid eq. 100 g<sup>-1</sup> dw), which was not significantly different from P1, Bio 2, Bio 3 and Bio 4, while the lowest HAA value was on Bio 8 (10.61 mmol of ascorbic acid eq. 100 g<sup>-1</sup> dw), which was not significantly different from most mulching treatments, except for P1, P2, and Bio 3 (Table 4).

**Table 2.** Effects of different mulch films on strawberry yield, yield components, and weed biomass of strawberry.

Mulch Film	Cat. I Fruits <sup>z</sup> (g m <sup>-2</sup> )	Cat. II Fruits <sup>y</sup> (g m <sup>-2</sup> )	Moldy Fruits (g m <sup>-2</sup> )	Marketable Yield (g m <sup>-2</sup> )	Fruit Average Weight (g)	Fruit Number (no. m <sup>-2</sup> )	Weed Biomass (g m <sup>-2</sup> )
Polyethylene	5729 ± 43 a <sup>x</sup>	1265 ± 116	393 ± 128 c	6995 ± 109 a	27.98 ± 1.0	250 ± 5.8 a	0 ± 0 d
BioFlex 1	5238 ± 221 abc	1246 ± 28	620 ± 174 bc	6484 ± 239 ab	27.64 ± 1.3	236 ± 14 abc	337 ± 97 abc
BioFlex 2	4756 ± 109 bcde	1247 ± 232	654 ± 22 bc	6003 ± 326 bc	26.7 ± 1.5	226 ± 17 abcd	468 ± 95 ab
Bio 1	4253 ± 175 ef	981 ± 41	801 ± 153 abc	5234 ± 213 c	29.62 ± 2.0	178 ± 14 de	660 ± 52 a
Bio 2	4331 ± 98 ef	966 ± 155	600 ± 123 bc	5298 ± 194 c	27.67 ± 0.6	192 ± 11 cde	292 ± 32 abc
Bio 3	4410 ± 392 def	1410 ± 134	1181 ± 201 a	5820 ± 424 bc	29.28 ± 2.0	201 ± 20 bcde	588 ± 266 ab
Bio 4	3965 ± 156 f	1249 ± 164	1045 ± 74 ab	5214 ± 261 c	29.91 ± 1.4	174 ± 1.5 e	382 ± 81 abc
Bio 5	4594 ± 23 cdef	1422 ± 156	928 ± 132 ab	6016 ± 150 bc	29.15 ± 3.1	211 ± 21 abcde	280 ± 61 abc
Bio 6	5336 ± 166 ab	1231 ± 43	821 ± 151 abc	6568 ± 152 ab	26.92 ± 1.5	246 ± 19 ab	220 ± 108 bc
Bio 7	5083 ± 204 abcd	1419 ± 99	727 ± 54 bc	6502 ± 195 ab	29.91 ± 0.7	217 ± 3.5 abcde	225 ± 66 bc
Bio 8	4722 ± 359 bcde	1084 ± 120	672 ± 156 bc	5805 ± 433 bc	29.72 ± 3.2	198 ± 18 bcde	377 ± 176 abc
Significance	*** w	ns	*	***	ns	*	**

<sup>z</sup> Fruits weighing more than 15 g, with no defects in shape or color; <sup>y</sup> Fruits weighing less than 15 g and with few defects in shape and color. Marketable yield = Cat. I + Cat. II fruits.

<sup>x</sup> All data are expressed as mean ± standard error, n = 3. Different letters within each column indicate significant differences according to Duncan's multiple-range test ( $p \leq 0.05$ ). <sup>w</sup> ns,\*,\*\*,

\*\*\* Non significant or significant at  $p \leq 0.05$ , 0.01, and 0.001, respectively.

**Table 3.** Effects of different mulch films on total soluble solids (°Brix) and flesh firmness (N) of greenhouse strawberry fruit harvested at 128, 153, and 202 days after transplant (DAT).

Mulch Film.	Total Soluble Solids (°Brix)			Flesh Firmness (N)		
	128 DAT	153 DAT	202 DAT	128 DAT	153 DAT	202 DAT
Polyethylene	12.57 ± 0.22 <sup>z</sup>	10.53 ± 0.44	9.82 ± 0.50	2.32 ± 0.19	2.39 ± 0.16	2.19 ± 0.13
BioFlex 1	11.90 ± 0.56	10.11 ± 0.61	10.31 ± 1.25	2.40 ± 0.14	2.33 ± 0.19	2.26 ± 0.10
BioFlex 2	10.37 ± 0.63	10.99 ± 0.14	10.08 ± 0.92	2.48 ± 0.19	2.48 ± 0.22	2.25 ± 0.05
Bio 1	11.49 ± 0.74	10.53 ± 0.58	9.34 ± 0.44	2.25 ± 0.14	2.55 ± 0.06	2.20 ± 0.09
Bio 2	12.86 ± 0.30	11.04 ± 0.23	9.91 ± 0.66	2.55 ± 0.24	2.57 ± 0.10	2.37 ± 0.12
Bio 3	10.83 ± 0.08	10.52 ± 0.57	9.61 ± 0.48	2.51 ± 0.16	2.40 ± 0.15	2.15 ± 0.06
Bio 4	12.72 ± 1.36	10.79 ± 0.27	10.18 ± 0.35	2.64 ± 0.08	2.50 ± 0.13	2.26 ± 0.09
Bio 5	10.76 ± 0.24	10.91 ± 0.44	10.51 ± 0.31	2.46 ± 0.13	2.42 ± 0.19	2.30 ± 0.03
Bio 6	12.79 ± 0.50	11.19 ± 0.23	9.71 ± 0.37	2.36 ± 0.17	2.50 ± 0.17	2.29 ± 0.07
Bio 7	11.34 ± 0.55	10.60 ± 0.56	9.24 ± 0.41	2.58 ± 0.07	2.26 ± 0.07	2.17 ± 0.04
Bio 8	11.48 ± 1.26	11.88 ± 0.54	9.69 ± 0.26	2.59 ± 0.17	2.44 ± 0.09	2.24 ± 0.04
Significance	ns <sup>y</sup>	ns	ns	ns	ns	ns

<sup>z</sup> All data are expressed as mean ± standard error, n = 3. Absence of letters within each column indicate no significant differences according to Duncan's multiple-range test ( $p \leq 0.05$ ). <sup>y</sup>

ns Non-significant.

**Table 4.** Effects of different mulch films on total protein, antioxidant activities, total phenols, and malic and citric acids of greenhouse strawberry.

Mulch Film	Total Proteins (g 100 g <sup>-1</sup> DW)	LAA (mmol Trolox 100 g <sup>-1</sup> dw)	HAA (mmol ascorbic ac. eq. 100g <sup>-1</sup> dw)	Total Phenols (mg gallic ac. eq. 100g <sup>-1</sup> dw)	Malic Acid (g kg <sup>-1</sup> dw)	CITRIC ACID (g kg <sup>-1</sup> dw)
Polyethylene	7.86 ± 0.41 <sup>z</sup>	52.33 ± 0.79	12.33 ± 0.30 ab	7.22 ± 0.51	19.95 ± 0.51	72.76 ± 4.81
BioFlex 1	7.60 ± 0.48	54.00 ± 2.31	12.80 ± 0.46 a	7.50 ± 0.46	21.55 ± 0.92	74.28 ± 2.75
BioFlex 2	6.85 ± 0.28	57.74 ± 2.15	11.20 ± 0.42 bc	7.46 ± 0.19	19.59 ± 1.97	73.40 ± 3.61
Bio 1	6.98 ± 0.12	54.71 ± 2.90	11.44 ± 0.55 bc	7.31 ± 0.43	18.16 ± 1.22	69.57 ± 0.51
Bio 2	6.53 ± 0.06	56.32 ± 0.46	11.97 ± 0.23 abc	7.41 ± 0.12	17.73 ± 0.51	71.40 ± 1.76
Bio 3	7.81 ± 0.67	55.27 ± 2.67	12.19 ± 0.36 ab	7.00 ± 0.36	19.79 ± 0.99	71.51 ± 1.38
Bio 4	7.98 ± 0.28	57.18 ± 0.51	11.47 ± 0.13 abc	7.01 ± 0.21	19.35 ± 1.96	74.22 ± 2.02
Bio 5	7.58 ± 0.53	53.70 ± 0.73	11.31 ± 0.18 bc	7.29 ± 0.29	18.16 ± 0.10	68.50 ± 2.97
Bio 6	7.39 ± 0.16	55.14 ± 1.14	11.37 ± 0.42 bc	6.84 ± 0.23	18.76 ± 1.10	72.85 ± 1.44
Bio 7	7.91 ± 0.59	54.84 ± 1.66	11.15 ± 0.08 bc	7.19 ± 0.25	17.47 ± 1.06	69.56 ± 0.75
Bio 8	7.90 ± 0.36	55.08 ± 1.90	10.61 ± 0.82 c	6.96 ± 0.36	18.81 ± 0.54	74.46 ± 0.81
Significance	ns <sup>y</sup>	ns	*	ns	ns	ns

<sup>z</sup> All data are expressed as mean ± standard error, n = 3. Different letters within each column indicate significant differences according to Duncan's multiple-range test ( $p \leq 0.05$ ).

<sup>y</sup> ns,\* Non-significant or significant at  $p \leq 0.05$ , respectively.



### 3.3. Mineral Profile

Phosphorus, potassium, sulfate, and sodium content were not influenced by mulch film treatments. Significant differences among mulches were found for nitrate, calcium, and magnesium content (Table 5). The lowest nitrate content was recorded with strawberries grown on Bio1 and Bio 2 films, with 1.34 and 1.35 g kg<sup>-1</sup> dw, respectively. These results were not significantly different from most of the other film treatments, except for P2, Bio 3, and Bio 7, which had higher accumulations of nitrate in the fruits. Moreover, fruits grown in P1 and P2 film treatments had the highest calcium content (~1.74 g kg<sup>-1</sup> dw). As for magnesium, Bio 3 and Bio 4 showed the highest content of around 1.58 g kg<sup>-1</sup> dw, while Bio 2 exhibited the lowest fruit content (1.16 g kg<sup>-1</sup> dw; Table 5).

**Table 5.** Effects of different mulch films on mineral profile of greenhouse strawberry.

Mulch Film	NO <sub>3</sub> (g kg <sup>-1</sup> dw)	P (g kg <sup>-1</sup> dw)	K (g kg <sup>-1</sup> dw)	Ca (g kg <sup>-1</sup> dw)	Mg (g kg <sup>-1</sup> dw)	SO <sub>4</sub> (g kg <sup>-1</sup> dw)	Na (g kg <sup>-1</sup> dw)
Polyethylene	2.38 ± 0.37 abc <sup>z</sup>	4.45 ± 0.23	16.11 ± 0.94	1.68 ± 0.07 ab	1.35 ± 0.06 de	2.33 ± 0.21	0.25 ± 0.05
BioFlex 1	2.71 ± 0.36 ab	4.52 ± 0.45	17.05 ± 0.69	1.79 ± 0.09 a	1.49 ± 0.05 bc	2.12 ± 0.38	0.61 ± 0.12
BioFlex 2	1.71 ± 0.05 bc	4.11 ± 0.20	15.52 ± 0.19	1.51 ± 0.05 c	1.34 ± 0.01 de	1.95 ± 0.46	0.29 ± 0.01
Bio 1	1.34 ± 0.10 c	3.95 ± 0.16	14.95 ± 0.43	1.41 ± 0.01 c	1.26 ± 0.01 ef	1.39 ± 0.03	0.37 ± 0.07
Bio 2	1.35 ± 0.13 c	3.75 ± 0.25	14.68 ± 0.87	1.41 ± 0.06 c	1.16 ± 0.05 f	1.61 ± 0.50	0.23 ± 0.06
Bio 3	2.74 ± 0.67 ab	4.66 ± 0.13	17.13 ± 0.69	1.38 ± 0.05 c	1.62 ± 0.04 a	1.57 ± 0.12	0.43 ± 0.13
Bio 4	2.24 ± 0.37 abc	5.08 ± 0.32	16.92 ± 0.98	1.40 ± 0.04 c	1.54 ± 0.06 ab	1.58 ± 0.17	0.59 ± 0.12
Bio 5	2.07 ± 0.28 abc	4.47 ± 0.51	16.22 ± 0.58	1.48 ± 0.05 c	1.49 ± 0.05 bc	1.71 ± 0.24	0.38 ± 0.04
Bio 6	2.04 ± 0.20 abc	4.66 ± 0.21	15.77 ± 0.17	1.43 ± 0.05 c	1.38 ± 0.05 cde	1.60 ± 0.22	0.27 ± 0.04
Bio 7	2.83 ± 0.28 a	4.89 ± 0.47	17.53 ± 0.65	1.53 ± 0.06 bc	1.39 ± 0.01 cd	1.48 ± 0.20	0.44 ± 0.29
Bio 8	2.23 ± 0.14 abc	4.60 ± 0.26	17.18 ± 0.36	1.51 ± 0.01 c	1.34 ± 0.02 de	1.68 ± 0.07	0.24 ± 0.06
Significance	* y	ns	ns	***	***	ns	ns

<sup>z</sup> All data are expressed as mean ± standard error, n = 3. Different letters within each column indicate significant differences according to Duncan's multiple-range test ( $p \leq 0.05$ ). y ns, \*, \*\*\* Non-significant or significant at  $p \leq 0.05$  and 0.001, respectively.

## 4. Discussion

Mulching is a common practice in horticulture that embodies a prime necessity because fruit in contact with the soil tends to perish in short notice, such as strawberry. Among the different ways of soil mulching in strawberry cultivation, PE film is the most widely used all over the world. In our study, strawberry grown on PE film had the highest marketable yield and the highest number of fruits belonging to the first category, encompassing berries weighing greater than 15 g and exempt of any defects. These results are comparable to some biodegradable films such as P2, Bio 6, and Bio7. Other biodegradable films (Bio 1, 2, and 4) led to a reduction in production of up to 25% compared to PE film. The different yields obtained among the mulch films may reflect the specific effect they have on the soil hydrothermal regime. Generally, PE film generates higher soil temperatures, since it can absorb more solar radiation, and retains the heat between the soil and the film. Furthermore, PE film is made of high molecular weight molecules and is hydrophobic [3,30], therefore acting as a waterproof barrier that slows down or blocks the evaporation of water from the soil. Thus, it maintains a constant soil moisture, and allows steam to condense and infiltrate into the soil. By measuring the water vapor permeability of PE films, Bilck et al. [3] found that it was up to 250 times lower than the biodegradable films they compared. The better soil hydrothermal conditions achieved under PE films, compared to other types of mulch films, are considered responsible for better flowering and fruiting of strawberry plants [1]. Biodegradable films have been found to be more porous and, therefore, less efficient in reducing evaporation [24]. Many authors have confirmed the greater growth of strawberry plants when mulched with black PE compared to other types of mulch [1,44–46]. Others such as Scarascia-Mugnozza et al. [28] and Filippi et al. [47], reported the opposite results.

Both the different chemical composition and the physical characteristics, such as thickness and color, favor the best performance of PE film, compared to the other films. The PE film in our work had a thickness of 35 µm and was black in color. Bio 6 and Bio 7 films were more transparent than PE but were 40 µm thick, which might have led to their comparable performance to the control P1 and with less weed biomass. Moreover, P2 also performed comparably to P1, which could be attributed

to the fact that has a high Spencer impact test making it different from P3 which is characterized by a lower one. The other biodegradable films were black as well but were thinner than PE. These characteristics probably allowed the PE film to remain intact until the end of the crop cycle, and to completely block weed growth. The biodegradable films remained intact until March / April, the middle of the production season. Then, they showed a degradation that increased in the following months, especially with Bio 1, 6, and 7 films. However, Bio 6 and Bio 7 films resulted in a lower weed total biomass than the other biodegradable films (with 220 and 225 g m<sup>-2</sup> of weeds), which may be attributed to their thickness. The films that resulted in the greatest weed biomass were Bio 1, Bio 3, and BioFlex® (P3), films that were characterized by a low thickness. Gupta and Acharya [48], Tarara [49], and Singh et al. [44] attributed the best yield of strawberries cultivated on PE film to the complete suppression of weeds. In our study, the microbial degradation of the biodegradable films may have been accelerated by water supplied through irrigation, as it was also shown in the work of Costa et al. [29]. Varying growth of weeds in the presence of four types of biodegradable mulch films was also shown in the cultivation of tomato plants in the work of Cowan et al. [50]. A good mulching effect with biodegradable films (Mater Bi) was shown by Filippi et al. [47] in melon crop.

In support to our results, infrared spectroscopy analysis carried out by Scarascia-Mugnozza et al. [28] on biodegradable starch-based films for the cultivation of strawberry plants showed that 59 days after contact with the soil, the peak corresponding to starch -OH groups were very small. Moreover, an electron microscopic analysis of the films showed holes corresponding to starch particle disappearance or reduction by soil microorganisms. By analyzing mechanical properties of biodegradable films, such as elongation at break and stress at break, after 124 days of strawberry cultivation, the above-mentioned authors also found that these properties decreased significantly with biodegradable films. Similarly, Bilck et al. [3], by analyzing various mechanical parameters of starch biodegradable and PE films such as strength, elasticity, and rigidity, found that PE films remained flexible and without breaks at the end of the strawberry plant cultivation cycle. Instead, the biodegradable films showed a 50% reduction in their tensile strength and a 120% reduction in their elasticity. Their stiffness increased by 50%, and there was a reduction in deformation, showing them to be more fragile than PE films.

Strawberry, like other berry fruits (raspberries, blackberries, cranberries, and blueberries), is rich in nutraceutical molecules, such as ascorbic acid, phenols, anthocyanins, and flavonoids, which content can vary with the cultivar choice and fruit ripening stage [9,51]. These molecules have strong antioxidant power, capable of preventing or blocking already triggered radical reactions. Another important characteristic of strawberry is its flavor, which is due to the percentage of TSS, mainly glucose, and then fructose and sucrose [9]. According to Singh et al. [1], the best microclimate, together with weed suppression with a PE film, may lead to a better quality strawberry fruit in terms of a higher TSS and ascorbic acid content, and reduced acidity, compared to fruit mulched with other material (clear PE mulch or with straw). However, in our study, neither TSS nor the firmness of the fruit was influenced by the type of mulch film. This TSS data is supported by the results obtained using various mulch films by Costa et al. [29]. As well, the results concerning total protein, phenols, ABTS antioxidant activity, and malic and citric acid content were not influenced by the mulch treatment. Differences were found only in the hydrophilic antioxidant activity. This latter increased in the presence of biodegradable films used in the cultivation of two strawberry cultivars [32]. A higher content of antioxidants may be related to a condition of abiotic or biotic stress, following climatic conditions or pathogenic attacks or diseases.

The effect of the different mulch films on fruit mineral content in our study was evident for nitrate, calcium, and magnesium content. Both calcium and magnesium are essential nutrients for plant growth and development. Their accumulation in strawberries increases their nutraceutical properties. The different composition of nitrate, calcium, and magnesium may depend on different penetration and proliferation of roots in the soil, which can influence the absorption of these nutrients. Kumar and Dey [24], for example, found that strawberry root length, volume, and weight were higher in the

presence of both PE and biodegradable film, compared to bare soil. The authors found higher N, P, and K absorption in strawberries grown with PE and biodegradable films, compared to plants grown in bare soil.

## 5. Conclusions

Currently, there is a great effort to reduce the use of plastic, due to the pollution it is causing. Agriculture is becoming more aware of this dramatic problem. Therefore, adoption of environmentally sustainable practices is increasing, such as the replacement of classic polyethylene films used for soil mulch with biodegradable films that offer similar results. Our study shed light on some biodegradable films, such as BioFlex<sup>®</sup> (P2), Bio 6, and Bio 7, that were capable of maintaining fruit production comparable to that of black polyethylene film. These films also made it possible to have a greater total number of fruits, including Cat.I fruits, and an overall high marketable yield and greater weed suppression (especially for Bio 6 and Bio 7) than the other biodegradable films. Such results represent sustainable alternatives to polyethylene film use, encouraging farmers towards the adoption of biodegradable films, where production and quality are maintained and pollution is reduced. Nonetheless, more in-depth focus on the thickness of biodegradable films could improve the performance of this green practice.

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