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# Living Mulch as Sustainable Tool to Improve Leaf Biomass and Phytochemical Yield of *Cynara cardunculus* var. *altilis*

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**Abstract:** Living mulch (LM) provides many beneficial agro-ecological services by reducing soil evaporation, conserving moisture, controlling soil temperature, reducing weed growth, increasing organic matter and nutrient availability, and improving microbial activities. Legumes, commonly used as living mulches, can affect the yield and quality of main crops. We hypothesized that Egyptian clover co-cultivated with *Cynara cardunculus* var. *altilis* (cultivated cardoon) can positively affect both leaf biomass and phytochemical yield. The study was performed on two growing seasons of field experiments to evaluate the potential variation in biomass and phytochemical yields of cardoon leaves. In addition, the leaves were collected at 90, 120, and 150 days after transplanting to evaluate the possible effect of the harvest time. LM improved the fresh and air-dried leaves biomass yields, total phenolic, and dry matter content, while it had no effect on the content of crude fibre, total sugars, L-ascorbic acid, total chlorophylls, and antioxidant activity. Except for luteolin-7-*O*-glucoside content, no negative effect of LM was observed on the polyphenol profile. Behind the cultivation system, the parameters were also affected by both the harvest time and growing season. According to our data, the proposed intercropping of cultivated cardoon with LM could represent an innovative cultivation system to increase both leaf biomass and the health-promoting compounds of cultivated cardoon leaves by sustainable soil management.

**Keywords:** living mulch; cultivated cardoon; biomass yield; polyphenol compounds; antioxidant activity



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## 1. Introduction

Living mulches (LM) are cover crops grown simultaneously with or in close proximity to cash crops. Advantages of LM may include protecting the soil from erosion and compaction, limiting nitrate leaching, enhancing microbial activity and animal biodiversity, increasing weed suppression and soil organic matter, as well as improving the nitrogen availability by including leguminous species as LM [1–5]. The effect of including LM in vegetable cultivation techniques depends on a number of factors, including soil and weather conditions, type of cover plant, sowing date, as well as the varietal characteristics of the LM and catch crops [6–9].

Genus *Cynara*, belonging to the family Asteraceae, comprises seven species, which in their natural environment are found only in the Mediterranean regions [10]. The species *C. cardunculus* L. includes only two domesticated forms, globe artichoke (var. *scolymus* (L.) Fiori)) and cultivated cardoon (var. *altilis* DC.), along with their common ancestor, the wild cardoon (var. *sylvestris*) [11]. In Central Europe, cultivated cardoon is primarily a valuable leaf biomass crop. Dried leaves are pharmaceutical raw material. For example, the cardoon leaves are effective in the treatment of functional disorders of the digestive and circulatory systems, protection of the body against cancer, and stimulation of the immune system

and inhibitors against hepatocellular carcinoma [12,13]. The phenolic acids of cardoon leaves are cholagogic, through the increase in the transport of bile to the duodenum [14]. The phenolic acids strengthen and regenerate liver cells [15] and have a hepatoprotective effect [16] due to the increase in the flow of bile, which reduces the level of triglycerides in the blood serum [12] and enables the removal of harmful substances that threaten the liver [17]. A systematic use of cardoon extract caused a decrease in the total cholesterol and the low-density lipoproteins in patients with impaired lipid metabolism [18]. The presence of these phytochemicals allows the inclusion of cultivated cardoon leaves as a medicinal plant with a broad spectrum of pharmacological activity. According to previous works, cultivated cardoon leaves' pro-health and antioxidant activity is determined by the polyphenol compounds, mainly the fractions of mono- and dicaffeoylquinic acids and flavonoids [19,20]. The content of these compounds in *C. cardunculus* depends on several factors [21], among others: the cultivar characteristics [22,23], environmental conditions [24,25], plant tissues [26,27], harvest time [28,29], extraction methods [30], dry temperature [31] and storage time [32]. The current state of knowledge on cardoon cultivation techniques is based in the research performed in the Mediterranean climatic and soil conditions. For example, cardoon plants are usually cultivated in wide inter-rows from 40–50 to 100–120 cm of each other. This cultivation system increases soil erosion and organic matter mineralization. A way to counteract these processes is by intercropping with LMs.

The information available on this topic concerning a temperate climate is less favourable for this thermophilic species. The subject of the effects of LM on the level of bioactive compounds in the cardoon leaves is insufficient concerning the cultivation systems in different climatic zones. In this work, it was speculated that the Egyptian clover (*Trifolium alexandrinum* L.) in intercropping with cultivated cardoon can positively affect cardoon biomass yield, primary and secondary metabolite content of cardoon leaves, improving its biological value. In order to verify the hypothesis, the study was performed in two growing seasons of field experiments in order to evaluate the possible variation of LM's effect on the biomass and phytochemical yield, chemical properties, and polyphenol profile of cultivated cardoon leaves harvested three times during each growing season.

## 2. Materials and Methods

### *2.1. Plant Material and Growing Conditions*

Field trials were performed at the Felin research station of the University of Life Sciences in Lublin, Poland (51.23° N, 22.56° E). The cultivated cardoon was co-cultivated with Egyptian clover as LM. Seeds cv. 'Blanco Avorio' of cultivated cardoon were provided by the Rijnsburg Seed Company (Rijnsburg, The Netherlands).

The experiment was set up in two growing seasons (2018 and 2019) under a split-plot design with three replications, where the main plot was the cultivation system (cultivated cardoon without LM as the control and cultivated cardoon with LM) and the sub-plot was the harvest time of the leaves (90 days after transplanting (DAT), the first ten days of August; 120 DAT, the first ten days of September; and 150 DAT, the first ten days of October).

Egyptian clover was sown at rate of 8 kg $ha^{-1}$ during the transplanting time of cultivated cardoon. Plants emerged 10–12 days after being sown and the soil surface was screened until the cardoon leaves were harvested. The fresh matter of plants in an area of 1 $m^2$ was from 3.7 to 5.2 kg. The clover LM initially (21–28 days after being sown) screened 35% of the soil surface and 65% at the end of the cardoon crop.

The cultivated cardoon transplants were produced in multicellular trays, each 90 mL in volume and filled with peat substrate. One seed per cell at depth of 1–2 cm. Sowing term was 10 April, one seed per cell. Transplants, 15–20 cm high with 3–5 fully developed leaves, were planted in the field on 10 May 2018 and 8 May 2019 with a spacing of 0.4 × 0.4 m and a density of 6.25 plant $m^{-2}$. Each subplot consisted of 62 plants.

The management practice was performed in accordance with the rules of local practice. In the temperate climatic zone, cardoon is grown as an annual because the temperature

below −10 °C causes plants to freeze, including the underground buds. That is why in the conditions of the present experiment, cardoon seedlings, produced in heated foil tunnels, were planted in the field in May after frosts, when the plants have 3–5 leaves and are about 10 cm high. Soil ploughing to a depth of 30 cm, harrowing and fertilization were carried out before planting: 60 kg $ha^{-1}$ N, 60 kg $ha^{-1}$ $P_2O_5$, and 120 kg $ha^{-1}$ $K_2O$ as ammonium nitrate, triple superphosphate, and potassium sulphate, respectively. During the cardoon cultivation, the inter-row weeding was carried out twice manually. Chemical plant protection was not applied, as it was not necessary. The cardoon plant showed a quick rate of growth and profuse foliage, forming bid leaves that were green on the upper side and greenish-grey and covered with tomentose hair underside. Leaf blades were situated on long, fleshy petioles.

### 2.2. Weather Conditions

In the period from April to October 2018, the total rainfall was about 7% higher than the multi-year average (1951–2010; Table 1). In 2018, October was characterized by the least rainfall of 36 mm, while July by the highest rainfall (124 mm). The same trend was observed in October 2019, followed by June and July (29, 37, and 38 mm, respectively), while the highest rainfall was registered in August (102 mm). The average air temperature from April to October was higher by 2.0 (2018) and 1.9 °C (2019), respectively, than the multi-year average (1951–2010). In the summer months (July and August), the average air temperature was higher than the multi-year average by 3.2 and 2.4 °C, respectively, in 2018 and in 2019.

**Table 1.** Average air temperature and total rainfall during the experimental trials (2018–2019)—data from the Meteorological Station in Felin, 51′13° N; 22′37° E.

| Years | Month | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **April** | **May** | **June** | **July** | **August** | **September** | **October** | **Average/ Sum** |
| | | | | Temperature (°C) | | | | |
| 2018 | 7.5 | 16.7 | 18.8 | 20.6 | 20.8 | 15.5 | 10.0 | 15.7 |
| 2019 | 9.5 | 13.4 | 21.5 | 19.4 | 20.3 | 14.5 | 11.0 | 15.6 |
| 1951–2010 | 7.4 | 13.0 | 16.2 | 17.8 | 17.1 | 12.6 | 12.4 | 13.7 |
| | | | | Rainfall (mm) | | | | |
| 2018 | 40 | 56 | 65 | 124 | 72 | 68 | 36 | 461 |
| 2019 | 49 | 93 | 37 | 38 | 102 | 52 | 29 | 400 |
| 1951–2010 | 39 | 58 | 66 | 84 | 69 | 54 | 58 | 428 |

### 2.3. Biomass Yield and Primary Metabolites Analysis

Cultivated cardoon biomass leaves were harvested at 90, 120, and 150 DAT in the field from 10 plants from each treatment (control and cardoon intercropped with LM). The total yield of the aboveground biomass part was assessed. For laboratory analysis, the samples were set up in accordance with the Polish Standard PN-72/A-75050 [33]. Fresh leaves were crushed and the content of crude fibre (% FM), total sugars (g 100 $g^{-1}$ FM), L-ascorbic acid (g 100 $g^{-1}$ FM), and total chlorophylls (mg $g^{-1}$ FM) was determined and expressed as fresh weight (FM).

Simultaneously, samples of fresh plant material were dried at 40 °C to obtain an amount of 1 kg of the air-dried herb from each combination. The dry herb was ground in a grinder with a sieve diameter of 1 mm. Ground samples were stored in airtight containers for chemical analyses, comprising the contents of phenolic compounds, total phenols, caffeic acid, chlorogenic acid, cynarin, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside. Their contents were expressed as kg $ha^{-1}$.

The phytochemical analyses of the leaf samples were performed successively for three weeks. Immediately after the sampling, the plant material was ground, and the chemical analyses were performed.

The dry matter (DM) was determined after drying the plant material to a constant weight at 75 °C over 3 days [34]. Crude fibre was analysed with Hennenberg and Stohmann's method [35] based on the quantitative determination of insoluble organic matter during cooking of the defatted sample in 1.25% sulfuric acid solution and 1.25% sodium hydroxide solution.

Total sugars were determined with the Luff–Schoorl method [36] based on the reduction of $Cu^{2+}$ ions in the Luff fluid by reducing saccharides in the test solution, performed in an alkaline environment with pH 9.5 at the boiling point.

L-ascorbic acid was determined with Tillman's method, modified by Pijanowski [37] through the titration of L-ascorbic acid with 2,6-dichloroindophenol, as recommended for weakly coloured products.

The chlorophyll content was analysed according to the method described by Lichtenthaler and Wellburn [38]. Plant samples (0.1 g) were milled with 3 mg of magnesium carbonate ($MgCO_3$). The chlorophyll was extracted with 80% ($v/v$) aqueous acetone (25 mL). The absorbance was read at 663, 646, and 470 nm, respectively, with a Helios Beta spectrophotometer. The content of chlorophylls and carotenoids was calculated from the equations described by Lichtenthaler and Wellburn [38].

### *2.4. Secondary Metabolites Analysis*

Sample preparation: Ten grams of dried and ground raw material was extracted with methanol (1:10, $v$:$v$) under reflux condenser at the boiling point of the solvent for 3 h, then, after filtration, the raw material was again treated with 80% methanol and extracted twice for 2 h. The methanol extracts were combined, their solvent was evaporated, and the residue was washed with hot water (50 mL). The aqueous solutions were left in the refrigerator for 24 h. The separated tarry sediments containing ballasts were filtered off and washed with distilled water. The filtrates were degreased by shaking 3 times with petroleum ether (30 mL each). The purified aqueous solutions were extracted 10 times with diethyl ether (20 mL each). The combined ether extracts were concentrated to a volume of 100 mL and shaken 10 times with a 5% aqueous $NaHCO_3$ solution (10 mL each) in order to convert the phenolic acids into easily soluble salts in the aqueous phase. The bicarbonate fractions, containing the salts of phenolic acids, were acidified with 36% HCl to pH = 3 to obtain free phenolic acids, which were re-extracted with diethyl ether by shaking 10 times with this solvent (10 mL each). The ether extracts were combined and dried with anhydrous $Na_2SO_4$. Then, the solvent was distilled to dryness, obtaining fractions of free phenolic acids.

The total phenolic (TP) content was evaluated using the Folin–Ciocalteu test proposed by Dewanto et al. [39]. Sample extracts (0.2 mL) were mixed with 1.4 mL of freshly diluted 0.2 M Folin–Ciocalteu reagent in water. After 1 min, sodium carbonate (1.4 mL, 6% in distilled water) was added. The reaction mixture was stirred at room temperature and the absorbance of the mixture was read at 750 nm on a UV/Vis spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan). TP content was expressed as mg gallic acid equivalents kg $ha^{-1}$.

Qualitative and quantitative HPLC chromatographic analysis of phenolic acids was performed in the reversed phase system using a LaChrom-Merck liquid chromatography (Merck, Darmstadt, Germany) with a DAD diode detector (L-7450), pump (L-7100), degasser (L-7612), 20 µL dosing loop, and thermostat (L-7360) with a Rheodyne feeder and a steel column LiChrospher 100 RP C 18 with dimensions of 250 × 4 mm, filled with a stationary phase with a grain diameter of dp = 5 µm. The samples were analysed at 25 °C. The mobile phase was a solution of 80% acetonitrile and water with the addition of 1% ($v/v$) acetic acid. The flow rate was 1.0 mL per min and the injection volume was 20 µL. Identification of phenolic acids was carried out by comparing their retention times with the standards

and spectroscopically determining their spectra in the UV range (220–400 nm). The content of individual phenolic acids in the tested raw materials was calculated on the basis of a calibration curve determined for each identified phenolic acid: caffeic (3,4 dihydroxycinnamic), chlorogenic (5-*O*-caffeoylquinic), cynarin (1,3-*O*-dicaffeoylquinic), apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside. Caffeoylquinic acids are listed according to the IUPAC numbering system [40].

The total antioxidant capacity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed spectrophotometrically (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) by DPPH radical scavenging tests [41]. The DPPH radical scavenging activity was estimated by analysing a mixture of 1 mL of sample with 2 mL of 0.1 mM DPPH radical at 517 nm after 30 min in the dark. Results were expressed as mM Trolox equivalents $g^{-1}$ of DM.

### *2.5. Chemicals and Standards*

Pure caffeoylquinic acids and flavonoids determined or used for calibration were purchased as certified reagents from Merck (Darmstadt, Germany). All solvents and reagents used for the preparation of standard solutions and the extraction of phenolic acids (methanol, ethanol, ethyl acetate, toluene, acetic acid, and formic acid) were of analytical grade, while the methanol for chromatographic analysis was of HPLC quality. All solvents were obtained from Sigma-Aldrich (Poznań, Poland).

### *2.6. Statistical Analysis*

Data of biomass yield, DW, antioxidant activity, and chemical compounds were subjected to the three-way (cultivation system × growing season × harvest time) analysis of variance (ANOVA) using Statistica PL ver. 13.0 (StatSof Inc., Tulsa, OK, USA). The compliance of the distribution of the determined parameters with a normal distribution was verified with the Shapiro–Wilk test. The results were considered statistically significant at $p \leq 0.05$, and homogeneous groups were identified by Tukey's test. The relationships between the parameters were assessed by calculating the Pearson correlation coefficients. The functions available in the Statistica package were tested, and the simplest function with an appropriately high coefficient of determination (minimum 0.4) was chosen.

## 3. Results and Discussion

### *3.1. Biomass Yield and Primary Metabolites*

According to ANOVA, the cultivation system, harvest time, and growing season had a significant impact on the yield and quality of cultivated cardoon leaves (Tables 2 and 3).

**Table 2.** *p*-value for all parameters under study by ANOVA.

| Parameter | CS [a] | HT [b] | GS [c] | (CS) × (HT) | (CS) × (GS) | (ST) × (GS) |
|---|---|---|---|---|---|---|
| Yield | 0.003 * | <0.001 * | 0.245 NS | 0.206 NS | 0.766 NS | 0.266 NS |
| Yield of air-dried biomass | 0.009 ** | 0.001 ** | 0.010 * | 0.008 ** | 0.031 * | 0.065 NS |
| Dry matter | <0.001 *** | 0.001 ** | 0.867 NS | 0.168 NS | 0.220 NS | 0.800 NS |
| Crude fibre | 0.267 NS | 0.009 ** | 0.345 NS | 0.684 NS | 0.160 NS | 0.055 NS |
| Total sugars | 0.345 NS | 0.442 NS | 0.234 NS | 0.343 NS | 0.534 NS | 0.715 NS |
| L-ascorbic acid | 0.206 NS | 0.260 NS | 0.001 ** | 0.978 NS | 0.007 ** | 0.268 NS |
| Total chlorophyll | 0.480 NS | <0.001 *** | 0.504 NS | 0.115 NS | 0.898 NS | 0.896 NS |
| Total phenolic compounds | 0.121 NS | 0.025 * | <0.001 *** | 0.274 NS | 0.326 NS | 0.075 NS |
| Caffeic acid | <0.001 NS | 0.010 * | <0.001 *** | 0.205 NS | 0.677 NS | 0.309 NS |
| Chlorogenic acid | 0.144 ** | 0.002 ** | <0.001 *** | 0.025 | <0.001 *** | 0.004 ** |
| Cynarin | 0.895 ** | <0.001 *** | <0.001 *** | 0.245 NS | 0.345 NS | 0.756 NS |
| Apigenin-7-*O*-glucoside | <0.001 *** | <0.001 *** | <0.001 *** | <0.001 *** | 0.946 NS | 0.417 NS |
| Luteolin-7-*O*-glucoside | <0.001 *** | 0.003 ** | <0.001 *** | <0.001 *** | 0.878 NS | 0.545 NS |
| DPPH | 0.858 NS | <0.001 *** | <0.001 *** | 0.270 NS | 0.330 NS | 0.029 * |

[a] CS: cultivation system; [b] HT: harvest time; [c] GS: growing season; * indicates significance at the 0.05 level ($p \leq 0.05$), ** indicates significance at the 0.01 level ($p \leq 0.01$), *** indicates significance at the 0.001 level ($p \leq 0.001$); NS—not significant.

In relation to the cultivation system, the fresh and air-dried biomass yield of cultivated cardoon leaves in the co-cultivation with LM was significantly higher than those without

LM (control) (89.0 vs. 79.1 and 7.04 vs. 6.15 t ha$^{-1}$, respectively). A similar trend was observed for the leaves' DM. Hauggaard-Nielsen and Jensen [42] reported that the nutrient uptake of crops was significantly improved by intercropping legume and cereal crops. In addition, specific root–microbe interactions between the main crop and the LM root zone may also affect nutrient mobilization in the rhizosphere and contribute efficiently to plant nutrient acquisition [43]. This improved plant nutrient uptake could explain the higher fresh and air-dried biomass, as well as the DM recorded in cultivated cardoon leaves coming from the LM plot.

**Table 3.** Total fresh biomass yield (Y, t ha$^{-1}$), yield of air-dried biomass (YDM t ha$^{-1}$), dry matter (DM %), crude fibre (CF, % of FM [1]), total sugars (TS, g 100 g$^{-1}$ FM), L-ascorbic acid (LAA, mg 100 g$^{-1}$ of FM), and total chlorophylls (TCh mg g$^{-1}$ FM) in cultivated cardoon leaves as affected by main factors; for each parameter, means followed by different letters are significantly different. The values are mean ± standard deviation.

| Factor | | Y | YDM | DM | CF | TS | LAA | TCh |
|---|---|---|---|---|---|---|---|---|
| Cultivation system | nLM [2] | 79.1 ± 2.2 b | 8.33 ± 0.33 b | 10.54 ± 3.3 b | 11.19 ± 4.5 a | 0.90 ± 0.05 a | 2.58 ± 0.55 a | 9.27 ± 0.18 a |
| | LM [3] | 89.0 ± 2.3 a | 11.09 ± 0.34 a | 12.46 ± 3.2 a | 11.54 ± 4.4 a | 1.00 ± 0.05 a | 2.64 ± 0.55 a | 9.21 ± 0.18 a |
| Harvest time | 90 [4] | 79.3 ± 1.9 c | 8.93 ± 0.16 b | 11.27 ± 3.1 b | 10.80 ± 3.9 b | 0.95 ± 0.03 a | 2.63 ± 0.52 a | 8.46 ± 0.28 c |
| | 120 | 81.6 ± 1.5 b | 9.55 ± 0.20 b | 11.71 ± 3.4 a | 11.67 ± 3.8 a | 0.94 ± 0.03 a | 2.60 ± 0.53 a | 9.82 ± 0.26 a |
| | 150 | 91.3 ± 2.0 a | 10.52 ± 0.18 a | 11.52 ± 3.5 a | 11.65 ± 4.2 a | 0.95 ± 0.00 a | 2.60 ± 0.56 a | 9.43 ± 0.25 b |
| Growing season | 2018 | 86.1 ± 2.4 a | 9.91 ± 0.38 a | 11.51 ± 3.6 a | 11.58 ± 4.7 a | 1.01 ± 0.05 a | 2.98 ± 0.45 a | 9.45 ± 0.34 a |
| | 2019 | 82.0 ± 2.3 a | 9.42 ± 0.37 b | 11.49 ± 3.7 a | 11.15 ± 4.9 a | 0.89 ± 0.03 a | 2.34 ± 0.55 b | 9.03 ± 0.33 a |

[1] FM: fresh mass; [2] nLM—no living mulch; [3] LM—with living mulch; [4] days after transplanting.

With regard to primary metabolites, no differences were noted for crude fibre, total sugars, L-ascorbic acid, and total chlorophylls content between the two cultivation systems under study (Table 3). Considering that these compounds could be affected by abiotic and biotic stress [44], our hypothesis is that the competition between LM and cultivated cardoon with respect to water and soil resources had no effect on the mentioned compounds. Our results appear in contrast with the findings of Boari et al. [45], which mentioned the limiting role of the water availability in the semiarid Mediterranean region in cardoon and subterranean clover intercropping. Contrarily, Toukabri et al. [46] proved that the mixture of fenugreek and clover co-cultivated with durum wheat minimized soil water evaporation losses and mitigated the water stress of wheat.

The harvest time significantly influenced the fresh and dry biomass yield, the DM content, and some primary metabolites' (crude fibre and chlorophyll) content in cultivated cardoon leaves (Table 3). A significantly higher fresh biomass yield was obtained from plants harvested at 150 DAT, followed by those harvested at 120 DAT and 90 DAT (Table 3). Moreover, leaves harvested from plants sampled at 120 and 150 DAT were characterized by a higher DM and air-dried biomass yield. A similar trend was observed for the crude fibre content. At 120 DAT, higher chlorophyll content was recorded compared to that at 150 and 90 DAT. The total sugars and L-ascorbic acid content in cultivated cardoon leaves had no statistical differences in relation to the harvest time. On the contrary, Mandim et al. [47] reported that the cultivated cardoon leaves in intermediate maturation stages had the highest lipid content, while younger leaves were characterized by high concentrations of free sugars.

The growing season showed statistical differences only for the L-ascorbic acid content and air-dried biomass yield. In particular, they recorded the highest level in 2018 rather than in 2019 (Table 3). It could probably be explained by the water deficit linked to the less rainfall recorded in August 2018. In both growing seasons of the study, the cultivation with LM had a positive effect on the L-ascorbic acid content in cardoon leaves (data not shown).

### 3.2. Secondary Metabolites Analysis

The content of total phenolic compounds, the polyphenol profile, and the DPPH scavenging activity are presented in Table 4. The dominant compounds were chlorogenic acid and apigenin-7-*O*-glucoside, as observed in previous works [25,26].

**Table 4.** Theoretical content of total phenolic (TP), caffeic acid, chlorogenic acid, cynarin, apigenin- and luteolin-7-*O*-glucoside (kg ha$^{-1}$), DPPH assay, and µmol Trolox g$^{-1}$ in cardoon leaves as affected by main factors; for each parameter, means followed by different letters are significantly different. The values are mean ± standard deviation.

| Factor | | TP | CAF | CHL | CYN | API | LUT | DPPH |
|---|---|---|---|---|---|---|---|---|
| Cultivation system | nLM [1] | 366 ± 4.8 a | 1.25 ± 0.08 a | 15.08 ± 0.3 b | 5.50 ± 0.02 b | 140 ± 2.9 b | 0.83 ± 0.03 a | 119.0 ± 6.2 a |
| | LM [2] | 555 ± 4.7 a | 1.55 ± 0.09 a | 20.18 ± 0.3 a | 8.32 ± 0.02 a | 202 ± 2.5 a | 0.55 ± 0.02 b | 110.5 ± 4.4 a |
| Harvest time | 90 [3] | 385± 4.0 c | 1.07± 0.01 b | 10.54± 0.1 b | 5.98± 0.01 b | 121 ± 1.1 c | 0.36 ± 0.02 b | 96.2 ± 3.74 c |
| | 120 | 445± 4.9 b | 1.53± 0.08 a | 17.67± 0.2 b | 6.88± 0.01 a | 179 ± 1.3 b | 0.76 ± 0.01 a | 113.3 ± 1.17 b |
| | 150 | 540± 3.3 a | 1.68± 0.06 a | 25.35± 0.4 a | 7.68± 0.02 a | 213 ± 2.1 a | 0.84 ± 0.02 a | 134.8 ± 4.16 a |
| Growing season | 2018 | 509 ± 3.9 a | 1.68 ± 0.09 a | 19.52 ± 0.3 a | 7.33 ± 0.04 a | 205 ± 3.0 a | 0.99 ± 0.02 a | 150.0 ± 7.0 a |
| | 2019 | 402 ± 4.9 b | 1.22 ± 0.07 b | 15.73 ± 0.4 b | 6.40 ± 0.05 b | 135 ± 2.1 b | 0.47 ± 0.01 b | 79.5 ± 6.7 b |

[1] nLM—no living mulch; [2] LM—with living mulch; [3] days after transplanting.

According to ANOVA, the cultivation system had an effect on all considered compounds, except for caffeic acid (Table 2). The cultivation system with LM provided a significant increase in total phenolic, chlorogenic acid, cynarin, and apigenin-7-*O*-glucoside content and negatively affected the level of luteolin-7-*O*-glucoside. In detail, both the total phenolic and cynarin content increased by about 34% compared to the cultivation system without LM (Table 4). According to our data, the cultivation system with LM could represent an innovative technique to obtain biofortificated raw material for food and non-food applications [48]. Recent studies have reported several biological activities, such as antidiabetic, anti-HIV, and anti-haemorrhoidal [49,50]. Moreover, cardoon's phenolic compounds are responsible for the allelopathic potential of cardoon [51–53]. Polyphenol compounds act as free radical scavengers, and their content is usually correlated with antioxidant activity. Here, the DPPH did not show a statistical difference in relation to the cultivation system, as observed for the primary metabolites.

The content of all compounds and the DPPH were significantly dependent on the harvest time (Table 4). The leaves collected from the plants at 150 DAT contained greater amounts of all compounds and showed higher scavenging activity as compared to those collected at 90 and 120 DAT. In particular, the total phenolic, chlorogenic acid, and apigenin-7-*O*-glucoside content increased linearly from 90 to 150 DAT (Table 4). The leaves harvested at 150 DAT accumulated about 60% more chlorogenic acid than those at 90 DAT (Table 4). Similar results were also confirmed by Pandino et al. [54].

Weather conditions during the growing season had a significant effect on the chemical composition of cardoon leaves. In 2018, the leaves reached the highest amount of all compounds and DPPH value as compared to 2019 (Table 4). In particular, the apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside content decreased by over 30% passing from 2018 to 2019 (Table 4). To our knowledge, there are few studies in the literature determining seasonal changes in globe artichoke chemical compounds globally [55,56], but scant data are available on cultivated cardoon chemical compositions depending on the growing season, although could play a crucial role in determining the raw material quality [29,56]. However, the earlier works were performed in Mediterranean regions, characterized by different weather conditions with respect to central–northern European areas, so the results are not comparable. As observed for the L-ascorbic acid, the interaction of the cultivation system × growing season also had a significant effect on the level of chlorogenic acid (data not shown). In detail, the synthesis of this compound was stimulated in both experimental growing seasons in the cultivation system with LM.

### *3.3. Correlation Coefficients*

A correlation analysis is the statistical tool that enables us to understand relationships between metabolites in a biological system [57]. The results of the analysis of the correlation of the parameters (total fresh biomass yield, DM, crude fibre, and total sugars content in cultivated cardoon leaves under different cultivation systems (with and without LM)) are presented in Table 5 as separate matrixes for experimental trials. In the case of the

cultivation system without LM, the values of the correlation coefficients indicated a positive relationship between the total fresh biomass yield and both the dry biomass yield and the content of chlorophylls in the cultivated cardoon leaves (r = 0.73, r = 0.66, respectively). The cultivation system with LM revealed a positive relationship between fresh biomass yield and both the DM and total chlorophyll content (r = 0.78, r = 0.58, respectively). The content of total sugars and chlorophyll had a positive correlation with the DM in treatments with or without LM (r = 0.64, r = 0.65, respectively). Conversely, L-ascorbic acid reportedly had a negative correlation with the DM (r = −0.58, r = −0.78, respectively, for no LM and LM). A significant value of the correlation coefficient was noted between the levels of crude fibre and chlorophyll in the cultivated cardoon leaves, but only in the cultivation with LM (r = 0.64). A negative and, at the same time, significant value of the correlation coefficient was noted between the total sugar content and L-ascorbic acid in the leaves collected at control plots (r = −0.67). A high correlation was shown between the level of total sugars and the content of chlorophyll in both cultivation systems, with LM (r = 0.85) and without LM (r = 0.74) (Table 5).

**Table 5.** Coefficient of Pearson's correlation between the fresh biomass yield (Y), yield of air-dried biomass (YDM), dry matter (DM), crude fibre (CF), total sugar (TS), L-ascorbic acid (LAA), and total chlorophyll (TCh) of cultivated cardoon leaves under different cultivation systems (*n* = 50).

| Cultivation System | Parameter | Y | YDM | DM | CF | TS | LAA | TCh |
|---|---|---|---|---|---|---|---|---|
| nLM [1] | Y | - | | | | | | |
| | YDM | 0.66 | - | | | | | |
| | DM | ns | 0.62 | - | | | | |
| | CF | ns | ns | ns | - | | | |
| | TS | ns | ns | 0.67 | ns | - | | |
| | LAA | ns | ns | −0.58 | ns | −0.67 | - | |
| | TCh | 0.73 | ns | 0.64 | ns | 0.74 | ns | - |
| LM [2] | Y | - | | | | | | |
| | YDM | ns | - | | | | | |
| | DM | 0.78 | 0.79 | - | | | | |
| | CF | ns | 0.54 | 0.44 | - | | | |
| | TS | ns | ns | 0.64 | ns | - | | |
| | LAA | ns | ns | −0.78 | ns | ns | - | |
| | TCh | 0.58 | ns | 0.65 | 0.64 | 0.85 | ns | - |

[1] nLM—no living mulch; [2] LM—with living mulch.

The results of the correlation analysis of the content of phenolic compounds in cardoon leaves in the cultivation systems with LM and without LM are presented in Table 6. Significant correlations were found between the level of total phenolic compounds and the content of caffeic acid in cultivation without LM (r = 0.77) and in cultivation with LM (r = 0.98). In the cultivation with LM, there was a relationship between the total phenolic content and the luteolin-7-*O*-glucoside content (r = 0.78). The relationship between the content of apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside as well as the content of caffeic acid turned out to be significant, and it was a negative relationship (r = −0.78 and r = −0.58, respectively). This means that with the increase in apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside in the cultivated cardoon leaves, the content of caffeic acid decreased. Pandino et al. [58] also found that the content of flavonoids (apigenin and luteolin-7-*O*-glucoside) decreases with the increase in the amounts of phenolic acids in cultivated cardoon. Drought stress inhibits plant growth, and the carbon produced by photosynthesis is used up in the formation of secondary metabolites [59]. Under stress, phenolic compounds protect cell organelles against damage and participate in maintaining ion homeostasis. Polyphenolic compounds neutralize free radicals and control the flow of ions and water in the plant [60]. In our research, cultivation with LM showed a significantly strong correlation between the content of caffeic acid and the contents of chlorogenic acid (r = 0.88) and cynarin (r = 0.77). High correlation coefficients were found between the cynarin content and the luteolin-7-*O*-glucoside content in cardoon leaves extracts for all experimental treatments (no LM, LM). In cultivation with LM, a high correlation coefficient

was also found between the content of cynarin and the level of luteolin-7-*O*-glucoside in the cardoon leaves (r = 0.85). Changes in the amount of caffeic acid and chlorogenic acid in cultivated cardoon may result from their transformations caused by hydrolysis, oxidation, and transesterification reactions [48]. Esters, i.e., a group of chlorogenic acids, are formed from the combination of the carboxyl group of caffeic acid with the phenol group of quinic acid [61].

**Table 6.** Coefficient of Pearson's correlation between the total phenolic (TP), caffeic acid (CAF), chlorogenic acid (CHL), cynarin (CYN), apigenin-7-*O*-glucoside (API), and luteolin-7-*O*-glucoside (LUT) of cultivated cardoon leaves under different cultivation systems ($n$ = 50).

| Cultivation System | Parameter | TP | CAF | CHL | CYN | API | LUT |
|---|---|---|---|---|---|---|---|
| nLM [1] | TP | - | | | | | |
| | CAF | 0.77 | - | | | | |
| | CHL | 0.67 | ns | - | | | |
| | CYN | ns | ns | ns | - | | |
| | API | ns | −0.64 | ns | ns | - | |
| | LUT | ns | −0.83 | ns | 0.74 | ns | - |
| LM [2] | TP | - | | | | | |
| | CAF | 0.98 | - | | | | |
| | CHL | ns | 0.88 | - | | | |
| | CYN | ns | 0.77 | ns | - | | |
| | API | ns | −0.78 | ns | ns | - | |
| | LUT | 0.78 | −0.58 | 0.64 | 0.85 | ns | - |

[1] nLM—no living mulch; [2] LM—with living mulch.

## 4. Conclusions

The present research provides important insight regarding the biomass yield chemical composition of cultivated cardoon leaves in relation to the cultivation system, growing season, and harvest time under temperate zone conditions. The use of LM for cultivation had a positive effect on the yield and quality of cultivated cardoon crop leaves as a raw material for food and the pharmaceutical industry. In cultivation with LM, higher yields of fresh biomass and air-dried biomass were obtained. As result of cultivation with LM, cultivated cardoon leaves were characterized by a higher dry matter, along with a higher content of total phenolic compounds, apigenin-7-*O*-glucoside, and cynarin. Growing cultivated cardoon with LM did not significantly affect the total sugars, L-ascorbic acid, and total chlorophylls. Overall, our data indicated that Egyptian clover co-cultivated with cultivated cardoon could be considered a promising cultivation system to improve both the biomass and phytochemical leaf yield of cultivated cardoon. Growing cultivated cardoon in wide rows risks the leaching of nutrients from the soil and erosion. In this context, the proposed cultivation system could represent an eco-friendly agronomic management system. The level of bioactive compounds also depended on the growing season and harvest time. The most favourable time for achieving the highest biological value of leaves was in the first growing season and 150 days after transplanting. In our opinion, even though the results obtained are very promising, further research is needed under different environments to better set up the cultivation system and evaluate the residual effects of the LM on the soil characteristics.

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