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Antioxidant Activity, Total Phenolic Content and Morphological Traits in 1 to 4-Year-Old *Muscari comosum* Bulbs Cultivated in Three Growing Environments

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Abstract: *Muscari comosum*, commonly called "Lampascione", is an edible wild plant that grows predominantly in the Mediterranean area. In this study, qualitative characteristics (size and antioxidant properties) were investigated in "Lampascione" bulbs of different ages cultivated in three different growing environments (greenhouse, shaded greenhouse and open field). The 4-year-old bulbs grown in a greenhouse showed the best shape index. The highest total phenolic content was observed in seed bulbs for all the growing environments. The antioxidant activity expressed in terms of EC_{50} average value had a fluctuating trend. However, the best antioxidant activity was found in bulbs cultivated in open fields and in the 4-year-olds grown in the greenhouse. Therefore, all these bulbs have a high antioxidant activity and can be considered as a very good nutraceutical source useful to consumers, as well as in the pharmaceutical sector, who are more and more interested in having products for a healthy and natural diet.

Keywords: *Muscari comosum*; phenolic content; antioxidant activity; bulb age; nutraceutical plant

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1. Introduction

Among the vegetables, wild edible plants represent an important nutritional and agricultural resource. The significant presence of these plants in traditional diets is mainly due to their therapeutic virtues. Among the many wild edible plants, the "Lampascione" [*Muscari comosum* (L.) Mill.] was the object of our study. *M. comosum*, well known as tassel hyacinth, is a herbaceous, perennial, bulbous plant characterized by reddish to pinkish outer tunics, formerly classified in the Liliaceae family according to Cronquist's classification but included in the Hyacinthaceae family according to APG 2003 (Angiosperm Phylogeny Group) [1]. The edible portion is a wild hyacinth, walnut-sized bulb with a characteristic bitter taste [2]. *M. comosum* is distributed over southwestern and central Europe, the Mediterranean area and eastwards to Iran and Arabia [3]. In some regions of southern Italy (Basilicata, Apulia), *M. comosum*, commonly named "Lampascione", is very popular and is a product of traditional gastronomy [4]. A "Lampascione" crop can be established from seeds by sexual reproduction, or from bulbs and bulbils (small secondary bulbs, also called offsets, produced laterally to the main bulb) by asexual reproduction, and the commercial product (bulbs 2.5–5 cm in diameter) is obtained within 3–5 years starting from seeds [1]. Differently from other bulbous plants belonging to the Liliaceae family, such as Allium *cepa* whose marketing follows technical specifications of a procedural guideline [5,6], the "Lampascione" production has yet to be regulated.

In recent years, there has been a growing interest in the right diet, and increasing attention has been paid to plant substances that can bring beneficial properties to human health.

Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases, as well as inflammation and problems caused by cell and cutaneous aging [7,8]. This prevention effect is believed to be achieved by cumulative biological exposure to antioxidants which are able to quench the free radicals implicated in the pathology of these diseases [9]. The most abundant antioxidants in the diet are phenolic compounds, a class of secondary metabolites [10,11], whose production in plant tissue is strongly influenced by biotic/abiotic factors and it is considered as a typical plant response to the environment. Indeed, the polyphenol quantity in plant foods can vary significantly according to different factors such as plant genetics and cultivar, soil composition and growing conditions [12–15].

Keeping in mind the above and considering that "Lampascione" has diuretic, anti-inflammatory, antioxidant and anti-cancer properties, associated with their phenolic compounds [16,17], this research aimed to investigate "Lampascione" bulbs of different ages in order to identify the best from a nutraceutical point of view, also paying attention to the effect of growing environments. In detail, size (weight and shape), total phenolic content (TPC) and antioxidant activity were determined in seed bulbs and in 2 to 4-year-old bulbs, obtained from seed and "seedling bulbs", respectively, and cultivated in a greenhouse, shaded greenhouse and open fields. Thus, this study was mainly aimed at identifying the best propagation material and the best growing environmental conditions to suggest to farmers and facilitate the spread of this species.

2. Materials and Methods

2.1. Chemicals

Folin–Ciocalteu's phenol reagent and pure ethanol were purchased from Analyticals Carlo Erba Reagents s.r.l. (Cornaredo, Milano, Italy). DPPH 2,2-Diphenyl-1-picrylhydrazil and chlorogenic acid were purchased from Sigma-Aldrich Plc. (St. Louis, MO, USA) and from Merck Schuschard OHG (Hohenbrunn, Germany), respectively. All chemicals used were of analytical grade. The standard solutions were prepared with water plus from Carlo Erba Reagent.

2.2. Plant Material and Growth Conditions

The research began in the spring–summer of 2011 through the harvest of the *M. comosum* seeds from wild plants found in the countryside of Vaglio Basilicata (Basilicata region, Southern Italy). The collected material was cleaned, dried and suitably stored before being used for sowing.

For sowing, polypropylene hole containers, black in color, 29 cm wide, 52 cm long and with 40 cells each, were used. The containers were filled with a commercial peat-based substrate (Floradur®, Floragard Vertriebs-GmbH Co., Oldenburg, Germany), with a pH of 6.5 and containing 60% of organic matter, 210 mg L^{-1} of N, 120 mg L^{-1} of P_2O_5 and 190 mg L^{-1} of K_2O. Sowing took place on 20th October 2011 by placing two seeds in each cell of containers. Then, at seedling emergence, only one plantlet was left. On the same date, planting "seedling bulbs", obtained from seeds collected in the countryside of Vaglio Basilicata in the previous three years (2008, 2009 and 2010), were sown in the above-described containers and grown in open field conditions. Therefore, it was possible to have and compare 4 types of propagation material, i.e., seeds and 1, 2 and 3-year-old seedling bulbs, which, placed in the mentioned hole containers, were laid on the experimental farm "Pantanello" of Metaponto (Southern Italy, Basilicata Region; 40°24′ N; 16°48′ E; 10 m a.s.l.). Starting with the 2011 sowing, the propagation material was cultivated in three different environmental conditions. In detail, the cultivation was carried out in an unheated greenhouse with a metal frame covered with clear plastic (PE 200 µm thick), in a shaded greenhouse covered by a shade black net with a 30% shading effect (Monotex 30, Tenax Spa, Viganò, Italy) and in open fields. In all the cultivation, Floradur® substrate was used. In each environment, the containers were placed on wooden platforms, 15 cm tall, and they were arranged according to a randomized block design, considering 3 containers for each type of propagation material. Each plot was formed by a single container. About 225 days after planting (DAP), and at the complete senescence of the plant canopy (late May–early June 2012), the seed bulbs and 2 to 4-year-old bulbs were harvested and stored in labeled paper bags, in a dry place at RT, for the following analysis carried out on September 2012.

2.3. Climate Condition Data

For each growing environment air temperature (°C), air relative humidity (%) and solar radiance (Wm^{-2}) were recorded by using 50Y probes for the first two parameters and class A pyranometers for the last (Campbell Scientific, Inc., Logan, UT, USA). Probes were connected to a CR-10X datalogger (Campbell Scientific, Inc., Logan, UT, USA) to record data at 30 min intervals throughout the trial period. For the three environments, by periodically weighing the containers, the irrigation was made by hand using a watering can when the substrate lost 40% of its available water.

2.4. Morphological Parameters of "Lampascione" Bulbs

At harvest time, every analyzed sample was composed of 10 bulbs from each plot. The samples, properly cleaned by removing the outer tunic of the bulbs, were analyzed to obtain the following morphological parameters: mean weight (mg); diameter ("d", mm); height ("h", mm) and consequent shape index ("h/d"). Dimensional properties were measured by a hand caliper. The weight of the bulbs was measured using an electronic balance to an accuracy of 0.0001 g (TE214S, Sartorius Biotech, Goettingen, Germany).

2.5. Samples Preparation and Extraction

The bulbs were dried in an oven at 50 °C and their dry matter content, expressed in percentage, was determined. Finally, dried bulbs were grounded using an electric grinder and the obtained meal, passed through a sieve of 200 μm, was stored in screw-top test tubes in a dark place at room temperature (RT) for a few days until analysis. In total, 2.5 mL of ethanol was added to 0.5 g of meal of each investigated bulb. The mixture was stirred continuously for 24 h at 4 °C. The samples were centrifuged for 1 h at 10,000 rpm. Subsequently, the pellet was recovered and re-suspended in 2.5 mL of ethanol. The mixture was stirred again for 12 h and then centrifuged for 1 h at 10,000 rpm. The obtained supernatant was pooled with the previous one. For each investigated sample, extractions were performed in duplicate.

2.6. Spectrophotometric Analysis

The absorbance measurements for determining the total phenolic content (TPC) and the EC50 were performed using a UV–Vis spectrophotometer (Ultrospec 4000, Amersham Pharmacia Biotech, Milan, Italy).

2.6.1. Spectrophotometric Analysis

The total phenolic content was determined using the Folin–Ciocalteu micro-method according to Cicco et al. (2009) [18]. Briefly, an amount of 100 μL of properly diluted samples, calibration solutions or blanks, were introduced into separate test tubes and 100 μL of the Folin–Ciocalteu reagent was added to each. The mixtures were mixed well and allowed to equilibrate. After 2–3 min, 800 μL of a 5% sodium carbonate solution was added in a synchronized way. The mixtures were swirled and put in a water bath at 40 °C for 30 min. Then, the tubes were rapidly cooled on the rocks and the color generated was read at 740 nm. The absorbances were measured in 1 cm cuvettes by the spectrophotometer UV–visible Ultrospec 4000. For the calibration curve, chlorogenic acid was chosen as the standard phenolic [19]. Standard solutions at concentrations of 20, 40, 60, 80 and 100 ppm were freshly prepared from the 1000 ppm stock solution containing pure ethanol. The total phenolic content was expressed as ppm of chlorogenic acid equivalents. The measurements of the total phenolic content were carried out in triplicate for each investigated sample.

2.6.2. Determination of Antioxidant Activity

Antioxidant activity was determined using a DPPH assay, with some changes to the procedure described by Loizzo et al. (2010) [14]. Briefly, a 0.15 mM DPPH solution was prepared with pure ethanol. A total of 500 µL of this solution was pipetted into microcuvettes and 500 µL of ethanol (control = A0), or 500 µL of the investigated samples, suitably diluted with ethanol, were added and mixed in each microcuvette. In total, 1 mL of ethanol was used to instrumental zero.

The reaction was monitored spectrophotometrically at 517 nm until the reading reached a plateau. Generally, 16 min were required to reach the reaction steady state. The antioxidant activity (AA), expressed in percent, was calculated according to the equation below:

$$AA\ (\%) = [(A0 - As/A0)] \times 100$$

where A0 represents the initial absorbance of the control (without extract) and As is the final absorbance obtained in the presence of extract. The antioxidant activity was replicated two times for each extract.

To compare the antioxidant activity of the investigated samples, the EC50 value was also determined. This parameter represents the antioxidant amount (µg) necessary to halve the initial DPPH concentration [20].

2.7. Graphs and Data Analysis

Morphological data were subjected to an analysis of variance (ANOVA) using the MSTAT-C software (version 2.0; Crop and Soil Department, Michigan State University, East Lansing, MI, USA). A Student–Newman–Keuls (SNK) test and Least Significant Difference (LSD) test were performed to compare the means, with significance at $p \leq 0.05$. The Plot, fittings and statistical analysis of total phenolic content and the percent of antioxidant activity were carried out using the Sigma Plot 6.0 software (Grafiti LLC, St Palo Alto, CA, USA).

3. Results

3.1. Monitoring of the Environmental Conditions

M. comosum bulbs (Figure 1) obtained from seed and "seedling bulbs" of different ages were cultivated for about one year under three environmental conditions (open field, greenhouse and shaded greenhouse) while monitoring light exposition, relative humidity and temperature.

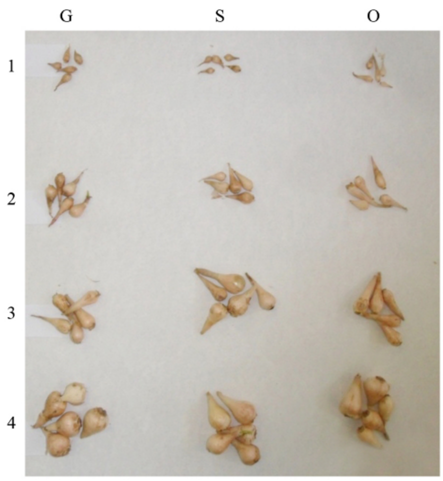

Figure 1. "Lampascione" bulbs investigated. Numbers indicate the age: 1 = seed bulbs; 2 = 2-year-old bulbs; 3 = 3-year-old bulbs; 4 = 4-year-old bulbs. Letters indicate the growing environments: G = greenhouse; S = shaded greenhouse; O = open field.

Figure 2a shows the average monthly temperatures (the average of the daily mean values for each month) in the trial period (October 2011–June 2012) for each growing environment. Although the temperature trends were similar for all the growing environments, the unheated greenhouse showed the highest temperature values. In detail, regarding the greenhouse environment, a major monthly thermal differential (DT) was observed in November with an average DT value over 9 °C. In addition, the thermal difference between the shaded greenhouse and the open field was always equal to about 1 °C for each month.

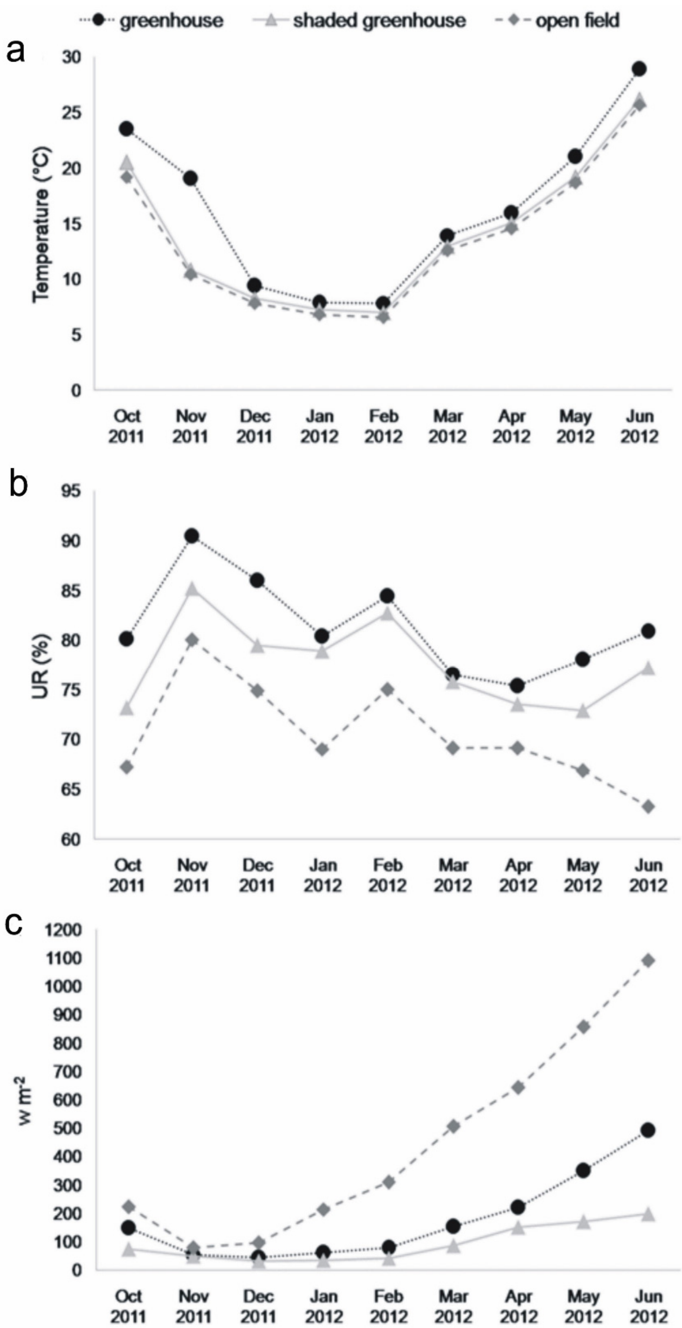

Figure 2. Monitoring of climatic parameters. Plots (**a**–**c**) show the average monthly values of air temperature, relative humidity and light intensity, respectively.

The monthly trend of the average values of air relative humidity (Figure 2b) points out that the greenhouse showed the highest values, and in contrast, the open field showed the lowest. Figure 2c shows that the light intensity of the two protected environments (plastic greenhouse and shaded greenhouse) was lower than that recorded in the open fields. Among the growing environments investigated, it is well known that the greenhouse agricultural practices that meet the farmers' demands are fostered.

3.2. Morphological Parameters of Bulbs

As shown in Table 1, all morphological parameters of harvested bulbs were significantly affected by both the growing environments (open field, greenhouse and shaded greenhouse) and the propagation materials (seed and different-year-old "seedling bulbs").

Table 1. Influence of the growing environments and propagation material on morphological parameters of *M. comosum* bulbs.

	Age (Years)	Mean Weight (mg)	Diameter (mm)	Height (mm)	Shape Index
Environments (E) [1]					
Open field		2965 b	13.6 b	29.0 b	2.19 c
Greenhouse		3511 a	14.5 a	32.9 a	2.45 a
Shaded greenhouse		3396 a	14.6 a	33.5 a	2.33 b
Significance [2]		*	*	*	**
Propagation material (P) [1]					
Seed	1	463 d	7.6 d	18.8 d	2.48 b
1-year-old "seedling bulbs"	2	1808 c	12.0 c	32.1 c	2.67 a
2-year-old "seedling bulbs"	3	4232 b	16.7 b	38.7 b	2.31 c
3-year-old "seedling bulbs"	4	6660 a	20.7 a	37.5 a	1.84 d
Significance [2]		**	**	**	**
"E x P" Interaction					
Open field	1	435	7.6	16.7	2.20
Greenhouse	1	520	7.5	22.7	3.03
Shaded greenhouse	1	433	7.8	17.0	2.20
Open field	2	1500	11.2	28.6	2.57
Greenhouse	2	2032	12.2	33.4	2.73
Shaded greenhouse	2	1893	12.7	34.4	2.70
Open field	3	3738	16.1	33.7	2.07
Greenhouse	3	4308	16.7	40.0	2.40
Shaded greenhouse	3	4650	17.3	42.3	2.47
Open field	4	6186	19.4	36.8	1.93
Greenhouse	4	7185	21.8	35.0	1.63
Shaded greenhouse	4	6608	20.8	40.3	1.98
Significance [2]		*	**	**	**
LSD [3]		41.2	0.60	1.02	0.13

[1] The values in the columns that do not have any letters in common are significantly different ($p \leq 0.05$) according to the Student–Newman–Keuls (SNK) test. Each value was expressed as means (n = 10).
[2] * = Significance at $p \leq 0.05$; ** = Significance at $p \leq 0.01$.
[3] Least Significant Difference (LSD) at $p \leq 0.05$.

In particular, the bulbs obtained under protected shelters (greenhouse and shaded greenhouse) had higher mean weight, height and diameter values, and these morphological characteristics increased significantly with age. A significant effect on these parameters from the interaction, "Environments x Propagation materials" (E x P), was also observed. As shown in Table 1, the 4-year-old bulbs grown in the greenhouse are those with a shape index nearest to roundness (SI = 1.63).

3.3. Dry Matter Content

Regarding the growing environments, the bulbs grown in the greenhouse showed the lowest dry matter value equal to 25.4%. Instead, in the other two environments (open field and shaded greenhouse) an average percent value of 28.1 ± 0.6 was achieved and a significant difference with respect to greenhouse value was observed. Regarding the propagation material, seed bulbs showed the lowest dry matter value equal to 22.8% compared to the other bulbs that showed statistically similar percent values, on average, equal to 28.6 ± 0.87. No significant differences in this parameter were found in the interaction between growing conditions and propagation materials.

3.4. Total Phenolic Content

The average total phenolic contents in "Lampascione" bulb extracts were reported in Table 2.

Table 2. Total phenolic content (TPC) of investigated bulbs.

TPC (μg Chlorogenic Acid Equivalents mL^{-1} of Extract)					
Age of Bulbs (A)	**Growing Environments (E)**				
	Greenhouse	**Shaded Greenhouse**	**Open Field**	**Means [1]**	**Significance [2]**
Seed bulbs	489	461	459	470 a	**
2-year-old bulbs	298	356	282	312 bc	**
3-year-old bulbs	182	313	305	267 c	**
4-year-old bulbs	259	393	367	340 b	**
Significance [2] "A x E" Interaction ** LSD [3] = 43.7					
Means [1]	307 b	381 a	353 ab		
Significance [2]	**	**	**		

[1] Values in the column and row that do not have any letters in common are significantly different ($p \leq 0.05$) according to the Student–Newman–Keuls (SNK) test. Each value is expressed as mean (n = 6).
[2] ** = Significance at $p \leq 0.01$.
[3] Least Significant Difference (LSD) at $p \leq 0.05$.

The data reported in Table 2 shows that the age of the bulbs had a significant influence on the total phenolic content (TCP), with the maximum value recorded in seed bulbs and the minimum for the 3-year-old bulbs. On the other hand, with regard to the growing environment, bulbs cultivated in the shaded greenhouse had the highest TCP, whilst the greenhouse had bulbs with the lowest phenolic content (Table 2). The interaction of the "Age of Bulbs x Growing environments" (A x E) had values with significant differences (Table 2).

Nevertheless, the trend of the total phenolic content versus the age of bulbs, grown in the three different environments, is shown in Figure 3 in order to obtain an overview and for an easier comparison.

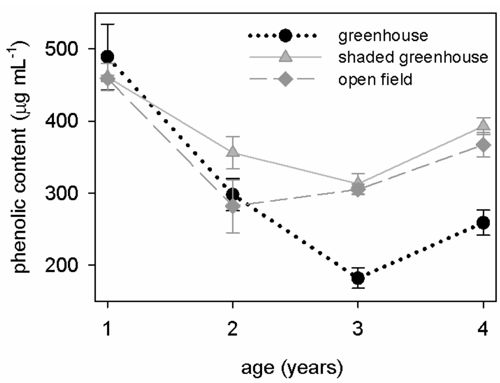

Figure 3. Trend of total phenolic content in investigated bulbs. Vertical bars indicate standard deviation (SD; n = 6).

The trend is similar for the bulbs cultivated in the greenhouse and in the shaded greenhouse. Indeed, the total phenolic content decreases in the 2 and 3-year-old bulbs and increases in the 4-year-old bulbs. Instead, for the bulbs grown in open fields, an increase in phenolic content was found in the 2-year-old bulbs.

3.5. Antioxidant Activity

In our experiment, dilutions from 1:2 to 1:40 (v/v) of each "Lampascione" extract were analyzed using a DPPH assay for the determination of free radical scavenging potential.

The plot of the antioxidant activity in percent versus relative phenolic amount, derived from investigated samples, is shown in Figure 4.

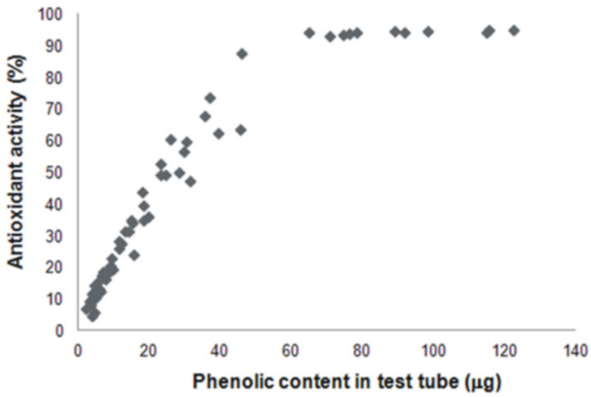

Figure 4. Correlation between phenolic content and antioxidant activity of investigated bulbs (grey diamonds indicate data relative to all the dilutions of the samples investigated).

In our experiment, a linear range (R2 = 0.94) was observed only up to a phenolic content equal to about 40 µg.

Figure 5 shows the EC50 values in the bulb extracts investigated.

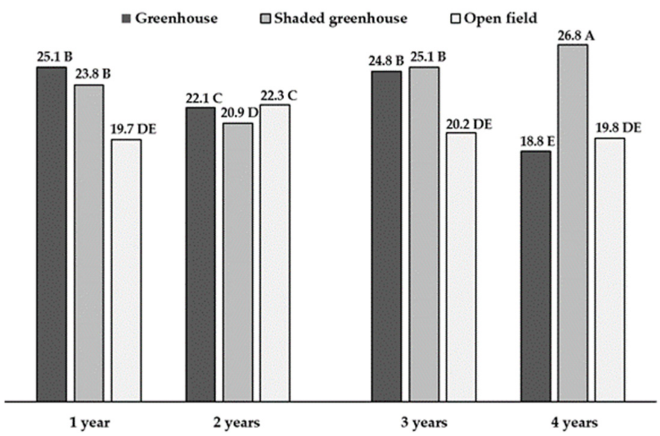

Figure 5. EC50 values expressed as phenolic amount (µg) were required to halve the initial DPPH concentration. Mean values (n = 4) followed by different letters are significantly different at $p \leq 0.01$, according to the SNK test.

The highest antioxidant activity was found in seed bulbs grown in open fields and in the 4-year-old bulbs, except for those grown in the shaded greenhouse. Indeed, the latter also showed the lowest antioxidant activity with respect to the different growing environments and all the other bulb ages considered. The highest variability among the EC50 values was observed in the bulbs grown in the greenhouse and the lowest was found in the bulbs from the open fields. The best antioxidant activity was found in bulbs cultivated in open fields, which showed the highest average value, calculated considering the different ages investigated, than those from the other growing environments (Figure 5). However, for all the growing environments, although the highest total phenolic content was observed in seed bulbs, the antioxidant activity had a changeable trend in different aged bulbs.

4. Discussion

In order to foster the diffusion of wild edible plants, such as "Lampascione" rich in phenolic antioxidants, such as low molecular weight phenols, phenolic acids and flavonoids (especially flavanones derivatives [16,21–23]), some qualitative and nutraceutical parameters, besides the morphological ones, were investigated in this study.

The shape index (height/diameter), considered a commercial qualitative index, is a useful parameter to evaluate the roundness/elongation of "Lampascione" bulbs. Indeed, round bulbs are generally preferred by consumers than elongated bulbs [1]. Therefore, bulbs with a shape index closer to the unit value are qualitatively better. As shown in Table 1, considering the "Environments x Propagation materials" (E x P) interaction, the 4-year-old bulbs grown in the greenhouse are those more next to the roundness (SI = 1.63). Thus, they can be considered a good commercial product to be earmarked for both fresh market and processing use. Confirming what was already discussed in a previous paper [24], the kind of propagation material had a strong effect on the achievement of an optimal size of the *M. comosum* bulbs at the end of the vegetative cycle. In particular, the mean weight, diameter and height of the 4-year-old bulbs, obtained from the 3-year-old "seedling bulbs", increased drastically. This occurred in all the growing environments investigated (greenhouse, shaded greenhouse and open field). These parameters were useful for commercial purposes, which in turn takes into account the renewed interest in this edible wild plant due to economic, ecological, historic-cultural, nutraceutical and gastronomic (culinary tradition) aspects [25–27]. Although the effect of the three different growing conditions on the morphological traits of the *M. comosum* bulbs and on the 1-year-old seedling's life cycle traits was already discussed in the previous paper [24], it was still unclear as to how the size of the bulbs obtained from seed and from different-year-old "seedling bulbs" could influence the growth and the quality of the bulbs in the following years of cultivation in the three different growing conditions. Therefore, besides the morphological traits, here we also investigated the TPC and antioxidant activity.

It is known that the total phenolic content is generally correlated with antioxidant activity. Therefore, it could be considered a very important quality index for human health.

In regard to the phenolic content in the "Lampascione" samples, the highest value was always found in the seed bulbs obtained directly by seed. This is probably due to the fact that the metabolites responsible for the total phenolic content could be mostly synthesized in smaller bulbs being younger. This aspect was investigated in the "Lampascione" bulbs and was revealed for the first time in this work. Nevertheless, additional investigations in this direction are desirable. Furthermore, the results showed substantial differences in total phenolic content, both in the different-aged bulbs and those from the different growing environments ($p \leq 0.01$). Therefore, we can assert that the total phenolic content of the investigated *M. comosum* bulbs is affected both by bulb age and the growing environment, in agreement with the literature [15,28].

Considering that light exposure has the ability to change the plant's metabolite composition [29,30], the increase in phenolic content, found already in the 2-year-old bulbs, is probably due to the effect of the higher light exposure on the plants grown in open fields with respect to the those cultivated in the other growing environments.

Many wild edible plants can be defined as food-medicine due to the presence of different classes of natural products or active compounds, among them phenolics [31]. Many of these compounds have important antioxidant properties depending on different factors of environmental conditions such as temperature, rainfall and relative humidity, as well as the planting parameters or growing conditions [30,32].

Although a correlation between antioxidant activity and the total phenolic content is already reported in the literature [33,34], the antioxidant capacity might not always linearly correlate with the amount of phenolics [35].

In light of this, and considering that antioxidant activity in the percentage of an extract cannot be predicted on the basis of its total phenolic content [36], the antioxidant activity was expressed as EC50 in order to carry out a comparative study between the investigated samples. This parameter, calculated from different dilutions of the sample, provides more important and reliable information when compared to antioxidant activity in percentage, since it takes the rate-reaction into account, that is to say, the kinetic behavior of each sample [20]. EC50 can be defined as the sample amount, expressed in micrograms, required to halve the initial DPPH concentration. Therefore, when the EC50 value is lower, the antioxidant capacity is higher.

It is known that there are many aspects/factors involved in the antioxidant activity of plant extracts. Indeed, chemical structure, stability and bioavailability should be considered overall when the research is focused on antioxidant compounds [37], and in order to evaluate their action mechanisms, many methodologies should be considered too. In general, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing/antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) and peroxyl radical scavenging capacity (PSC) are some among the many chemical assays used to study antioxidant activity [37,38]. In this study, the DPPH assay was used to determine the antioxidant activity, evaluating the direct scavenging of free radicals by their ability to transfer hydrogen atoms [20].

The best antioxidant activity was found in bulbs cultivated in open fields. This result can be due to the fact that the higher light exposure in the open fields can increase the production of free radicals causing oxidative stress and, as a result, the production of antioxidants increases for self-defense against environmental stress [29,30]. The antioxidant activity, expressed as EC50 (Figure 5), does not reflect the trend of total phenolic content observed in Figure 3. This behavior is most likely due to the different chemical compositions of the phenols in the extracts investigated. Therefore, considering that the total phenolic content and antioxidant activity are affected by different factors, of which some are intrinsic (genus, species, cultivar) and others extrinsic (environmental, agronomic, storage, handling) [39], we can assert that the synergic effect of the above-mentioned factors on "Lampascione" antioxidants can play a very important role and should be further investigated. In fact, only a synergic action between the ages (intrinsic factor) and the growing environments (extrinsic factor) could explain the results of our experiment. In this view, the "Lampascione" bulbs grown in open fields, being a richer source of antioxidants, can be considered with better qualities than those derived from the other two growing environments. Moreover, although the younger bulbs have a smaller size, and thus they are not suitable for commercial purposes, those from the open fields can be considered useful for extracting bioactive or nutraceutical compounds important in the pharmaceutical sector. The *M. comosum* bulbs have a high price on the market. Sadly, the high costs, ever more escalating, for harvesting *M. comosum* bulbs have favored their importation, mainly from northern Africa [1]. The results discussed here, as well as any other future information, may promote the cultivation of this profitable species, especially in certain regions of southern Italy (particularly Puglia and Basilicata), where *M. comosum* bulbs are widely used in traditional gastronomy [40].

Summarizing, the phenolic content of the bulbs was affected more by age than by growing conditions. Instead, the antioxidant activity varied significantly both by age and growing condition. However, the best antioxidant activity was found in bulbs cultivated in open fields, where no substantial difference was observed for this parameter within the ages investigated, and in the 4-year-old bulbs grown in the greenhouse. This means that the environments of cultivation and bulb ages have an impact on the antioxidant activity in *M. comosum* bulbs, probably due to their different chemical composition. Further investigations should be carried out to identify the *M. comosum* bulb phenolics that mainly contribute to antioxidant properties.

5. Conclusions

We can conclude that, among the bulbs investigated, the 4-year-old "Lampascione" from the greenhouse and all those grown in open fields can be considered as a food with better nutraceutical properties as they are a rich source of phenolics with high antioxidant activity and meet the need of fresh-market processing use, as well as the pharmaceutical sector. It would be desirable to spread the "Lampascione" cultivation in marginal areas, both to prevent their disappearance in nature and to enhance rural areas, all with a view to safeguarding biodiversity and a resource that enriches our diet with healthy biocomponents.

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