



Article

Evaluation of Productivity Components and Antioxidant Activity of Different Types of Garlic Depending on the Morphological Organs

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Abstract: This study evaluated the elements of production, the content of bioactive compounds, and the antioxidant activity of some types of garlic: softneck—*Allium sativum* L. var. *sativum*, Rocambole—*A. sativum* var. *ophioscorodon* (Link) Döll, and chives—*A. schoenoprasum* L. The mean yields and the distribution of bioactive compounds in the bulb, in the cloves of the bulb, as well as in the bulbils formed in the inflorescence, were determined. The average production/ha in the case of chives highlighted a yield of 27.83 t ha^{−1} of mature bulbs. All these were determined to obtain a better understanding of the biological functionality of the different morphological parts of this species. The content of vitamin C and flavonoids was significantly higher ($p \leq 0.05$) in Rocambole than in the other varieties. The phenolic compounds were higher in the case of chives, followed by Rocambole and then by softneck garlic. The content of bioactive compounds was higher in the younger organ, depending on the morphological organ (bulbs, cloves, and bulbils on flower stalks) within the same variety. The vitamin C content was higher in the cloves (15.68 mg 100 g^{−1}), followed by the bulbils in inflorescence (14.64 mg 100 g^{−1}), and the mature bulb (13.14 mg 100 g^{−1}) in Rocambole. The bioactive profile of garlic depends on the age of the morphological organ, and the bioactive concentration decreases with its maturity. Therefore, unripe garlic has the best potential to be used for its health benefits. The versatile health effects and nutraceutical properties of garlic can be attributed to the variety of bioactive compounds, mainly polyphenolic substances with strong antioxidant properties, as well as the morphological organ (mature bulb with cloves, a one year bulb derived from bulbil, or bulbil in bloom).

Keywords: *Allium sativum*; variety; production; antioxidant and peroxidase activity



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1. Introduction

Garlic (*Allium sativum* L.) has been known since ancient times as an “aromatic” vegetable used as a food ingredient in many countries due to its specific flavor and the benefits it has on human health. It is used in many types of products, such as garlic oil, powder, salt, paste, and flakes [1]. There are numerous studies that show that garlic significantly reduces the risk of developing cardiovascular disease, obesity, diabetes, cancer, or cholesterol [2–4].

In addition to its therapeutic properties, garlic also has additional biological properties such as antibacterial, antifungal, and antioxidant characteristics [5]. Garlic is a source of various biologically active phytochemicals, including organosulfur compounds, polyphenolics (phenolic acids, flavonoids), and vitamins [6,7].

The effect of garlic may arise from its antibacterial properties [3] or from its ability to block the formation of cancer-causing substances, reduce cell proliferation, or induce cell death [6]. Epidemiological studies have found that an increase in consumption of *Allium*

spp. reduces the risk of prostate and gastric cancers, and this has been mainly related to two main classes of compounds: the apolar sulfur compounds and the polar saponins [5]. Currently, special attention is paid to polyphenols due to their very important role in the benefits they have for human health [8]. In addition to phenolic and organosulfur compounds, garlic is also rich in vitamins and minerals [9]. However, it should be noted that the content of these bioactive compounds may vary depending on genotype, environmental factors, culture technology, maturity of the morphological organ, or post-harvest conditions [9,10]. Garlic is an excellent natural source of bioactive sulfur-containing compounds and has promising applications in the development of functional foods or nutraceuticals for the prevention and management of certain diseases [5,9].

Most of the studies in the literature refer to “commercial” garlic varieties, but their particular allogamy type of reproduction has favored the development and spreading of numerous ecotypes that have been adapted to different types of climates and soils [10]. With the modernization and intensification of agriculture, the regional agrobiodiversity of vegetable crops has decreased because farmers cultivate modern varieties and hybrids and tend to abandon traditional varieties [1]. The characterization of the chemical properties of garlic varieties can add value and could be preferred by consumers.

In general, the bulbils (small aerial cloves from the flower stalks) of Rocambole garlic are treated as by-products because they are used only for vegetative propagation and less for consumption.

The available information on the phytochemical composition, the distribution in the morphological organs (single bulbs, cloves, and bulbils), as well as the bioactive properties of the garlic cultivated in Romania, is quite limited. Therefore, the aim of this study was to evaluate the morphological features of each variety and to generate nutritional data related to antioxidant and peroxidase activity, vitamin C content, polyphenols, and flavonoids of garlic grown in Romania, and especially the distribution of these constituents in certain morphological organs of this species according to variety. The results of this study can provide useful guidance for organic and functional food producers as well as consumers seeking products with high nutritional, bioactive, and health-promoting values.

2. Materials and Methods

2.1. The Biological Material

The biological material used in this study was represented by three types of garlic grown in Romania: Rocambole or hardneck garlic—*A. sativum* var. *ophioscorodon* (Link) Döll (V1, V2, and V3); chives, Snow Mountain, or Kashmiri garlic—*A. schoenoprasum* L. (V4, V5, and V6); and softneck or domestic garlic—*A. sativum* L. var. *sativum* (V7 and V8) (Table 1 and Figure 1). Within the softneck garlic, two local populations were evaluated: (‘De Almăj’ and ‘De Teleorman’). In order to determine the bioactive compounds and the antioxidant capacity, morphological organs (cloves from bulbs, single bulbs, and bulbils) were collected from the three garlic types, resulting in the following experimental variants:

Table 1. The specifics of the variants and the biological material used.

Variant/Types of Garlic	Morphological Organs
V1-Rocambole	Mature bulb (with cloves)
V2-Rocambole	Single bulb (one year bulb)
V3-Rocambole	Bulbil from inflorescence
V4-Chives	Mature bulb (with cloves)
V5-Chives	Single bulb (one year bulb)
V6-Chives	Cloves formed at the base of the mature bulb
V7-Softneck garlic—‘De Almăj’	Mature bulb (with cloves)
V8-Softneck garlic—‘De Teleorman’	Mature bulb (with cloves)

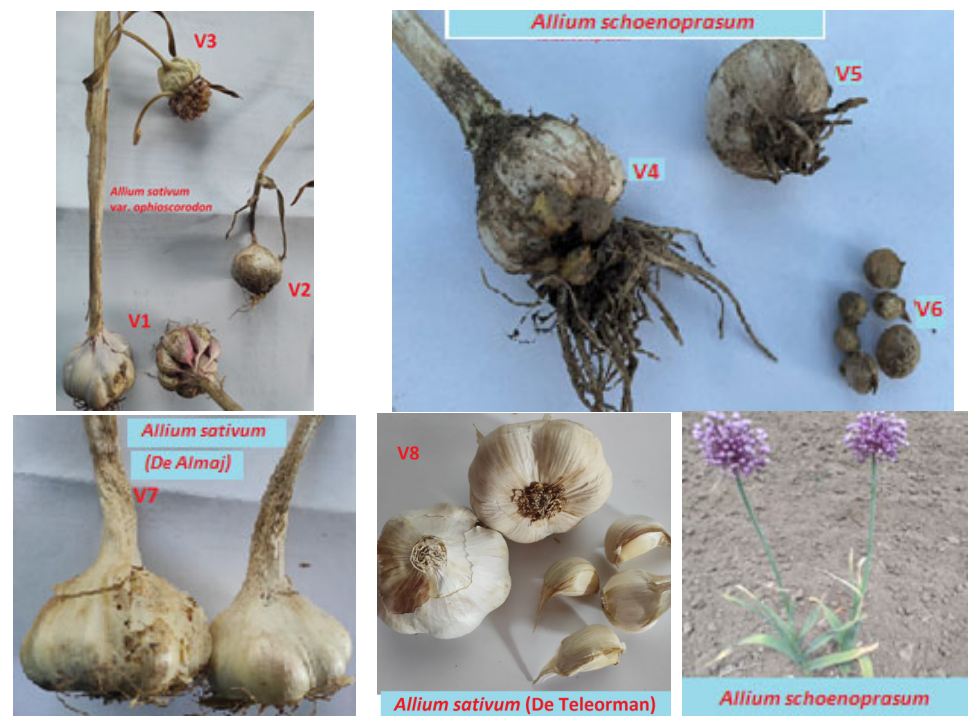


Figure 1. Garlic genotypes used in the trial. (V1-Rocambole—mature bulb with cloves; V2-Rocambole—one year old single bulb; V3-Rocambole—bulbil from inflorescence; V4-chives—mature bulb with cloves; V5-chives—one year old single bulb; V6-chives—bulbil from the base of the mature bulb; V7-softneck garlic—‘De Almaj’—mature bulb with cloves; V8-softneck garlic—‘De Teleorman’—mature bulb with cloves.

The softneck or domestic garlic has 10–30 cloves, each wrapped in white or silver-white parchment sheaths and all together in several layers of sheaths (a mature bulb). The cloves are smaller, and they are planted in spring and autumn.

Rocambole garlic has 5–10 cloves, each wrapped in reddish parchment sheaths and all together in several layers of sheaths (a mature bulb). The cloves are 2–3 times larger than those of the softneck garlic and are arranged unstratified around a central stem that, at the top, forms an inflorescence with aerial bulbils. They are planted in the fall because they have a low storage capacity. Additionally, in autumn, the bulbils from the inflorescence are planted, and in the following year, a single bulb of larger dimensions (a one year bulb) is obtained. The single bulbs are planted in the autumn of the same year and generate a head with 5–10 cloves.

Chives have 3–4 cloves in the head, each wrapped in white-yellow or reddish parchment sheaths and all together in several layers of sheaths (the mature bulb). Small cloves are formed at the base of the mature bulb that have a rounded shape with a rigid portion flattened on one side, which attaches at one point to the base of the disc of the mature bulb. The small cloves are protected by a rather hard, yellow-brown protective sheath. The cloves from the mature bulb are planted in autumn, and the mature bulb is obtained in the following year. Additionally, in autumn, the small bulbils formed in the inflorescence are planted, from which a bulb of about 2–3 cm in diameter is formed in the following year. Chives also produce inflorescence, but they are sterile.

The field trial was located in Southwest Romania, in Almaj commune (44°27' N and 23°42' E, set up on an area of 40 m², and included the 8 variants belonging to the 3 types of garlic. The garlic bulbils, of the same size, were planted at an equidistant spacing of 35 cm between rows and 10 cm between plants per row, resulting in 267,000 plants ha⁻¹. The planting took place on October 25 for both seasons. The trial was set up as a randomized block design in three replicates (n = 3), with 30 plants per replicate.

Classic farming practices were followed, including periodic weeding and NPK fertilization (17-17-17) in a dose of 200 kg ha⁻¹ after the establishment of the culture. The plants were watered by drip irrigation. No synthetic or organic pesticides were applied to control diseases, insects, or weeds. The harvest took place on 20 July 2020 and 25 July 2021.

2.2. Morphological Characteristics of Bulbs

The evaluation of the yielding characteristics was carried out after harvesting the bulbs and bulbils for each variant. Samples of 30 bulbs per variant were used to calculate the average weight of the bulb, the number of cloves per bulb, the average weight of the bulbil, and the diameter of the bulb.

2.3. Analytical Methods

2.3.1. Standards and Reagents

The methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchromon 2-carboxylic acid (Trolox), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) were purchased from Sigma-Aldrich (Steinheim, Germany). Gallic acid was purchased from Panreac (Barcelona, Spain), Quercetin was purchased from Carl Roth (Karlsruhe, Germany), and sodium carbonate anhydrous was purchased from Penta (Prague, Czech Republic). All the other chemicals used were of analytical grade.

2.3.2. Plant Material

The samples for the laboratory were obtained for each variant by selecting a representative number of cloves of garlic and bulbils well developed and without visible damage, which were washed, wiped with a soft napkin, peeled, and homogenized with an electric blender.

2.4. Total Soluble Peroxidase Activity (POX)

For the extraction of peroxidase, the homogenized samples were mixed with 0.1 M phosphate buffer, pH 7.0 (1:20 *w/v*), containing 0.1 mM EDTA. The resulting mixtures were centrifuged at 4515 × *g* (Hettich Rotina 38, Tuttlingen, Germany) for 20 min, and the supernatants were used for enzyme assays. Total soluble peroxidase activity (POX) was assayed by measuring the increase in absorbance at 470 nm due to guaiacol oxidation on addition of H₂O₂. To 5 mL phosphate buffer (pH 7.0), add 0.1 mL extract, 1 mL 0.16 M H₂O₂ as substrate, and 1 mL 0.1 M guaiacol. The absorbance at 470 nm was read after 1 min, and the activity was expressed as the variation of the absorbance (ΔA) per minute per gram of fresh weight $\Delta A \text{ min}^{-1} \text{ g}^{-1}$. All the data reported for chemical analysis are on a fresh weight basis.

2.5. Vitamin C Content

The content of vitamin C was determined by the iodometric method described by Dinu et al. [11]. The extraction from the biological material was undertaken in 2% hydrochloric acid (HCl) (1:10 *w/v*) and filtered. The vitamin C was then titrated with 1 mM potassium iodide (KIO₃) in the presence of 1% potassium iodide (KI). The resulting iodide (I₂) reacts with vitamin C, oxidizing it to dehydroascorbic acid. The redox titration end-point is determined by the first iodine excess that is complexed with starch, giving a deep blue-violet color. The vitamin C content was expressed as mg 100 g⁻¹.

2.6. Total Flavonoid Content (TFC)

The extracts for the determination of total flavonoid content, total phenolic content, and antioxidant activity were prepared in 80% aqueous methanol. Two grams of homogenized sample were extracted in 20 mL of 80% methanol for 16 h on an orbital shaker at room temperature. After centrifugation at 2808 *g* (Hettich Rotina 38, Tuttlingen, Ger-

many) for 5 min, the supernatant was filtered through Whatman 1 filter paper and used for the analyses.

TFC was determined by the colorimetric method at 500 nm with 10% Al (NO₃)₃ and 5% sodium nitrite (NaNO₂) in an alkaline medium. A total of 0.5 mL of the sample extract was transferred into a 10 mL volumetric flask. Furthermore, 0.6 mL of 5% sodium nitrite (NaNO₂) was added, and the mixture was shaken and left for 6 min. Secondly, 0.5 mL of 10% Al (NO₃)₃ was added to the volumetric flask, shaken, and left to stand for 6 min. Finally, 3.0 mL of the 4.3% NaOH was added. Subsequently, water was added to the scale. The absorbance was read at 500 nm after 15 min, and the results were calculated from the quercetin calibration curve. The results were expressed as µg quercetin equivalents (QE) per gram (µg QE g⁻¹) [12].

2.7. Antioxidant Activities

The total phenolic compound content (TPC) was determined colorimetrically with the Folin–Ciocalteu reagent. A total of 2 mL Folin–Ciocalteu's phenol reagent (1:10) and 1.5 mL of 7.5 % Na₂CO₃ were added to a 0.5 mL sample extract. The mixture was allowed to stand at room temperature in the dark for 90 min, and then the absorbance was recorded at 765 nm. The total phenolic content was calculated using a standard curve prepared using gallic acid and expressed as µg of gallic acid equivalents (GAE) per gram of fresh weight µg GAE g⁻¹.

Antioxidant activities were colorimetrically evaluated using two methods:

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: A total of 2 mL of 0.075 mM DPPH solution in ethanol was mixed with 0.1 mL extract and vortexed thoroughly. After 20 min in the dark, the absorbance of the remaining DPPH radicals was measured at 517 nm. A blank reagent was used to study the stability of DPPH over the test time. The scavenging activity of extracts was evaluated according to the formula: % scavenging = $[A_0 - (A_1 - A_S)] / A_0 \times 100$, where A₀ is the absorbance of DPPH alone, A₁ is the absorbance of DPPH + extract, and A_S is the absorbance of the extract only. The standard calibration curves (Trolox—T and ascorbic acid—AsA) were plotted as a function of the percentage of DPPH radical scavenging activity. For Trolox, the calibration curve is $y = 960.5x + 7.2676$, $r^2 = 0.9938$, where y is % scavenging and x is µmol Trolox. For ascorbic acid, the calibration curve is $y = 1520x + 3.39$, $r^2 = 0.9918$, where y is % scavenging and x is µmol ascorbic acid. The final results were expressed as µmol Trolox equivalents (TE) per gram (µmol TE g⁻¹) and µmol ascorbic acid per gram (µmol AsA g⁻¹).

ABTS radical cation scavenging activity: ABTS radical cation was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The ABTS radical cation solution was diluted with methanol to an absorbance of 0.70 at 734 nm. A total of 0.1 mL of sample extract was mixed with 2.9 mL of diluted ABTS radical cation solution. After a reaction at room temperature in the dark for 6 min, the absorbance at 734 nm was measured. The standard calibration curves (Trolox and ascorbic acid) were plotted as a function of the percentage of ABTS radical cation scavenging activity. For Trolox, the calibration curve is $y = 1290.9x + 7.9224$, $r^2 = 0.9933$, where y is % scavenging and x is µmol Trolox. For ascorbic acid, the calibration curve is $y = 1605.8x + 2.3115$, $r^2 = 0.9927$, where y is % scavenging and x is µmol ascorbic acid. The final results were expressed as µmol TE g⁻¹ and µmol AsA g⁻¹. All the spectrophotometric measurements were carried out with the Evolution 600 UV-Vis spectrophotometer, Thermo Scientific, England, with VISION PRO software 9.9 SR1.

All determinations were performed in triplicate (n = 3), and all results were calculated as the mean.

2.8. Statistical Analysis

The data obtained were analyzed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), followed by the post-hoc Tukey multiple range test.

3. Results

3.1. The Morphological Characteristics of the Bulbs Depending on the Variety

The obtained results regarding the variability of the morphological characteristics of garlic (eight variants) are presented in Figures 2–6. The analyzed data shows that there is great variability from one variety to another, and even within the same variety, this could prove useful in garlic breeding programs. There were significant differences in morpho-quantitative characteristics, especially in bulb diameter, bulb weight, and number of cloves in a bulb.

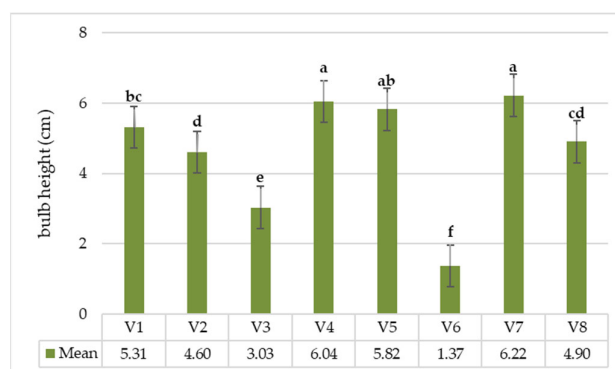


Figure 2. The height of the bulb of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

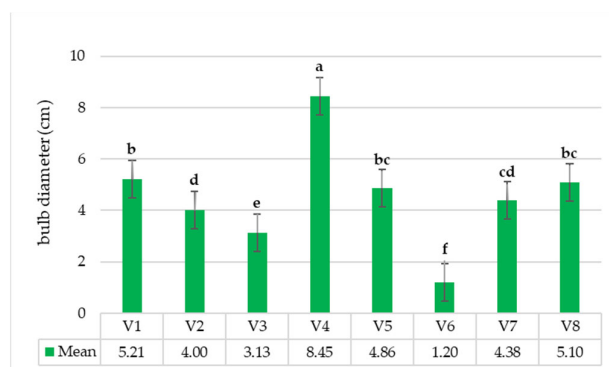


Figure 3. The diameter of the bulb of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

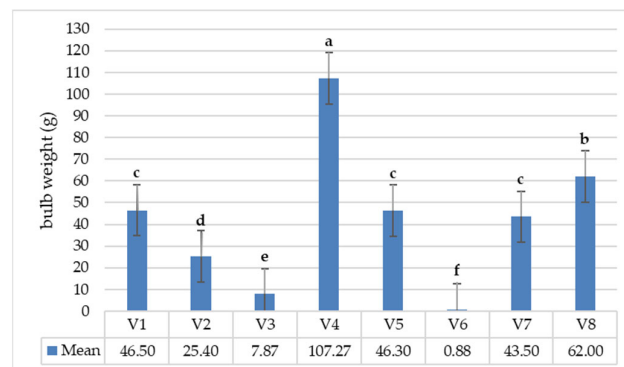


Figure 4. Bulb weight of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

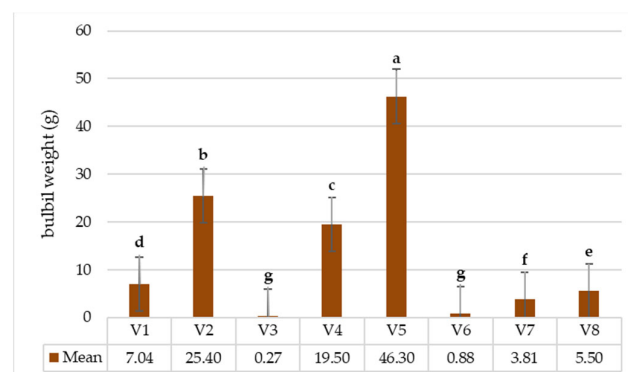


Figure 5. Cloves/bulbil weight of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

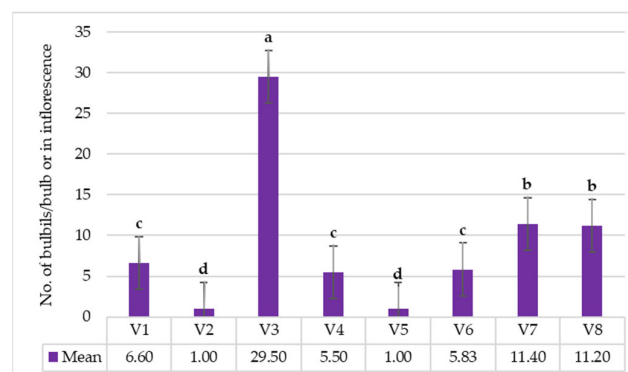


Figure 6. Number of bulbils in the inflorescence of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

The diameter of the bulb is directly proportional to the average weight of the bulb, in the sense that a bulb with a large weight also has a larger equatorial diameter than a bulb with a small weight. In terms of these characteristics, V4, V1, and V8 stand out and might be used in the future selection of new genotypes. Regarding the bulb height, the average values ranged from 6.22 cm (V7) to 1.37 cm (V6) (Figure 2), and the diameter recorded values from 8.45 cm (V4) to 3.13 cm (V3) (Figure 3).

3.2. Figures, Tables, and Schemes

As for the average bulb weight, it varied from 0.88 g per bulb at V6 to 107.27 g per bulb at V4 (Figure 4). The weight of the bulb was influenced by the average weight of the bulb and the average number of cloves in the bulb, or inflorescence. The bulbils from V3 average

0.27 g, and the largest cloves were recorded in V5 (46.30 g). This variability is obvious both between garlic types and within the same varieties depending on the morphological organ (aerial bulbil or underground bulb) (Figure 5).

Additionally, the characteristic regarding the number of cloves per bulb or bulbils per inflorescence was influenced by the garlic type, varying between 29.5 bulbils per inflorescence (V3), 1.0 single bulb (V2 and V5), and 11.40 cloves at V7 (Figure 6).

In the case of Rocambole (V2 and V3), great variability is observed within the same variant. It should be noted that garlic, in the climatic conditions of Romania, propagates only vegetatively, and the bulbils in V3 represent a material very important for the perpetuation of the species. All these morphological characteristics of the bulbs directly influenced the yield per ha, which varied from 27.8 t ha⁻¹ in V4 to 6.5 t ha⁻¹ in V2. From this point of view, V8 should also be emphasized, having a yield of 16.0 t ha⁻¹ (Figure 7).

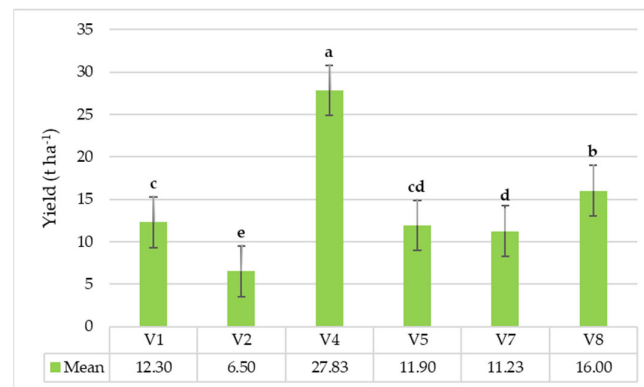


Figure 7. Yield of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

3.3. The Quality Parameters

The content of vitamin C shows a variation of this bioactive compound from 15.68 mg 100 g⁻¹ at V2 to 10.46 mg 100 g⁻¹ at V7. The highest amount of vitamin C was accumulated by Rocambole, followed by softneck garlic (V8), and then by chives. Regarding the morphological organ, an increase in the concentration of vitamin C is observed in the bulb formed from a single clove and in bulbils from inflorescence (V2—15.68 mg 100 g⁻¹ and V3—14.64 mg 100 g⁻¹, respectively), while the highest content of chives was in the mature bulb, followed by the one year old bulb, and then by the cloves at the base of the mature bulb. In the case of this variety, which is resistant to negative temperatures (−10 °C), it can be mentioned that the change in environmental conditions caused a decrease in the accumulation of this constituent because the recorded values are lower than those of Rocambole and softneck garlic (V8) (Figure 8).

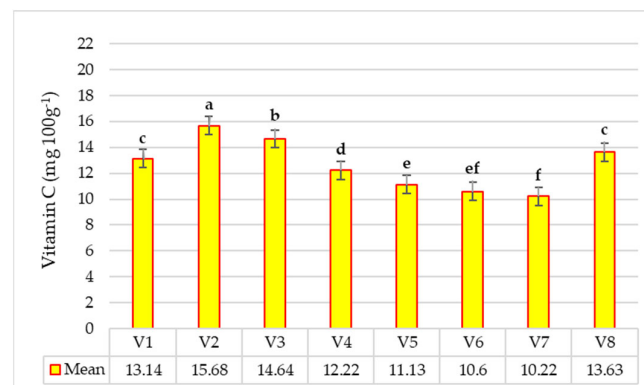


Figure 8. The content of vitamin C in garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

The total polyphenol content varied both within the garlic types and between variants. The highest content of total polyphenols was observed for V4, followed by V1, and then V7 and V8 (at the mature bulb). The total polyphenols at V4 and V1 had higher values than V7 and V8. It is noteworthy that polyphenol content had higher values in the mature bulb of the same variety with more cloves (V4—623.72 $\mu\text{g GAE g}^{-1}$ and V1—487.23 $\mu\text{g GAE g}^{-1}$) compared to the bulb with a single clove or to the bulbils (Figure 9).

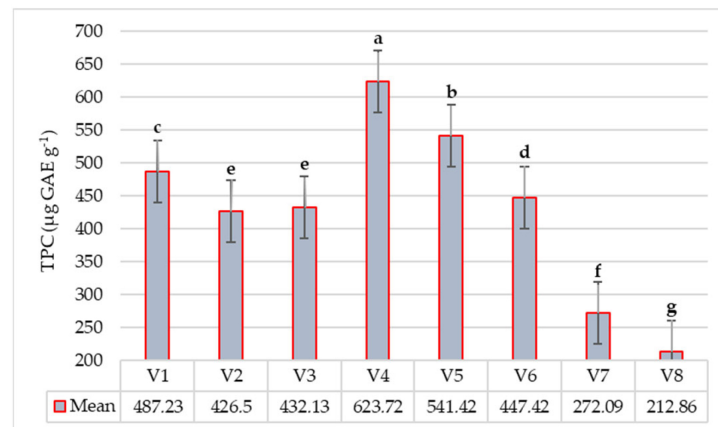


Figure 9. The total polyphenols of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

It can be mentioned that the variant very much determines the content of total polyphenols. The content of total polyphenols in V7 and V8 was 272.09 $\mu\text{g GAE g}^{-1}$ and 212.86 $\mu\text{g GAE g}^{-1}$, respectively, and was two times higher (487.23 $\mu\text{g GAE g}^{-1}$) in Rocambole (V1) and three times higher (623.72 $\mu\text{g GAE g}^{-1}$) in chives (V4). The size of the bulb or bulbils must be taken into account, as it indirectly affects the final concentration of the phenolic compounds.

The results of **the total flavonoid content** showed that V3 recorded the highest level (353.58 $\mu\text{g QE g}^{-1}$) of flavonoids, followed by V1 (223.50 $\mu\text{g QE g}^{-1}$). On the other hand, the lowest levels were recorded in V8 (119.56 $\mu\text{g QE g}^{-1}$) and V6 (138.08 $\mu\text{g QE g}^{-1}$) (Figure 10).

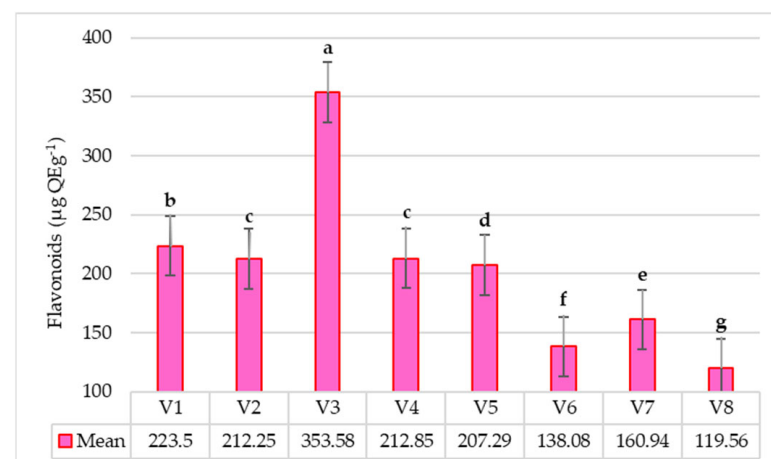


Figure 10. Flavonoids of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

The antioxidant enzymatic activity of the studied garlic varieties is presented in Figure 11. The peroxidase enzymatic antioxidant activity registered the largest variation within Rocambole. This ranged from 13.33 $\Delta\text{A g}^{-1} \text{min}^{-1}$ at (V3) to 1.26 $\Delta\text{A g}^{-1} \text{min}^{-1}$ at

(V2). Additionally, activity was recorded the highest in young morphological organs (V3 with $13.33 \Delta A g^{-1} min^{-1}$) within this biocomponent, while peroxidase activity was high in the mature bulb of chives, which was also observed in softneck garlic in the two local populations (V7 with $8.81 \Delta A g^{-1} min^{-1}$ and V8 with $6.08 \Delta A g^{-1} min^{-1}$).

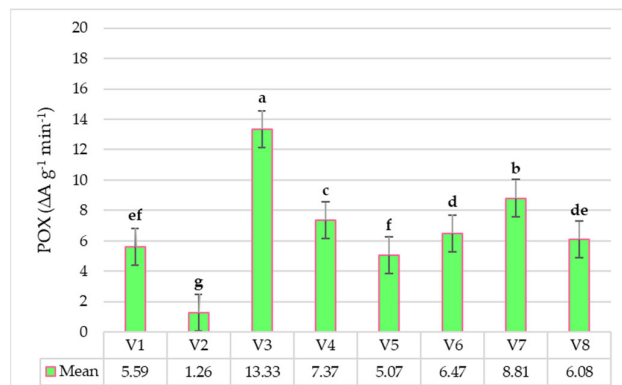


Figure 11. The antioxidant activity (POX) of garlic variants. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

The peroxidase activity is influenced by the variety, the morphological organ of the species, and the way in which the edible organ was formed (in the soil or on the flower stalk). The bulbils in V3 are formed in the inflorescence (in the air), and the morphological organs are formed in the soil in all other variants.

In order to accurately evaluate the antioxidant activity of garlic according to variety and morphological organ, two distinct measurements of antioxidant activity were performed, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. In our study, the antioxidant activity determined by DPPH showed different values between varieties; they ranged from $1.578 \mu mol TE g^{-1}$ at V4 to $0.664 \mu mol TE g^{-1}$ at V8 (Figure 12).

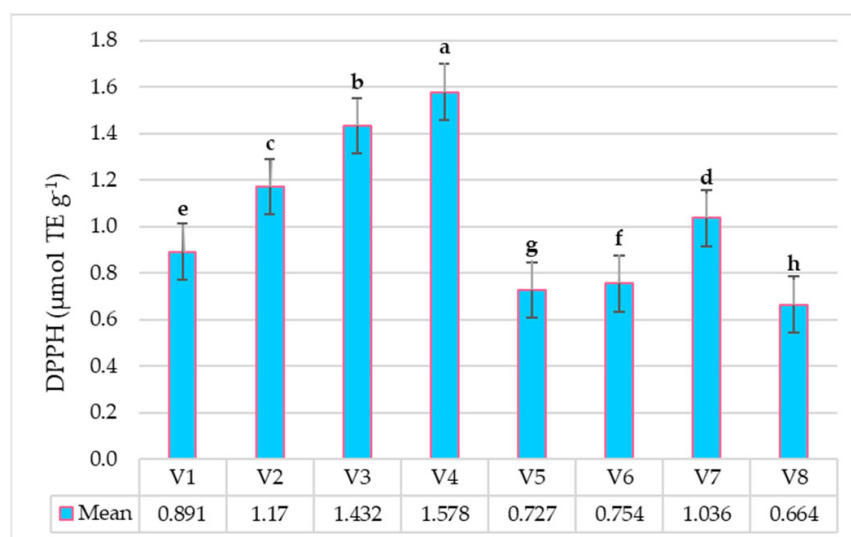


Figure 12. The antioxidant activity (DPPH) of garlic variants, expressed as Trolox equivalents. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

The DPPH activity was higher within Rocambole variants in younger morphological organs (bulbils), compared to that in mature bulbs (with cloves) (Figures 12 and 13), which was also observed in chives using the ABTS methods (Figures 14 and 15).

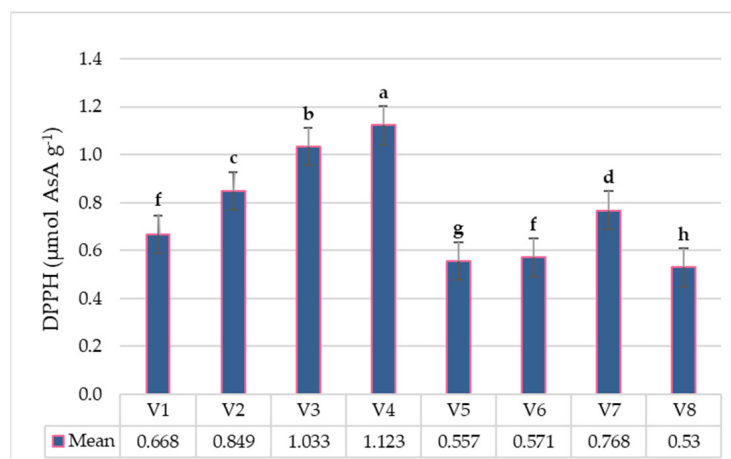


Figure 13. The antioxidant activity (DPPH) of garlic variants, expressed as ascorbic acid. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

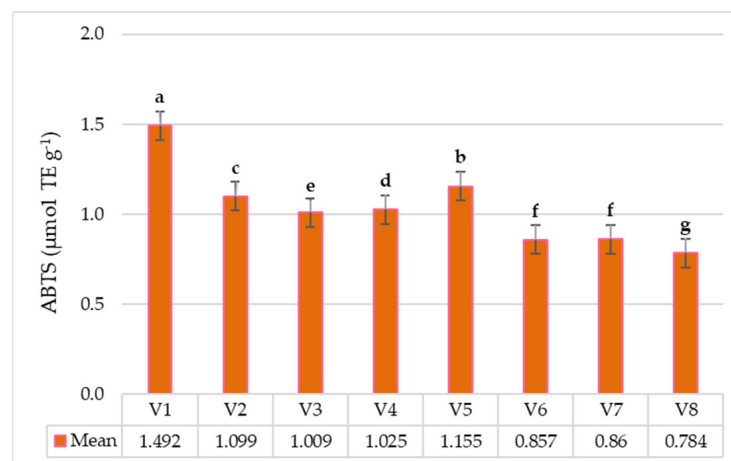


Figure 14. The antioxidant activity (ABTS) of garlic variants, expressed as Trolox equivalents. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

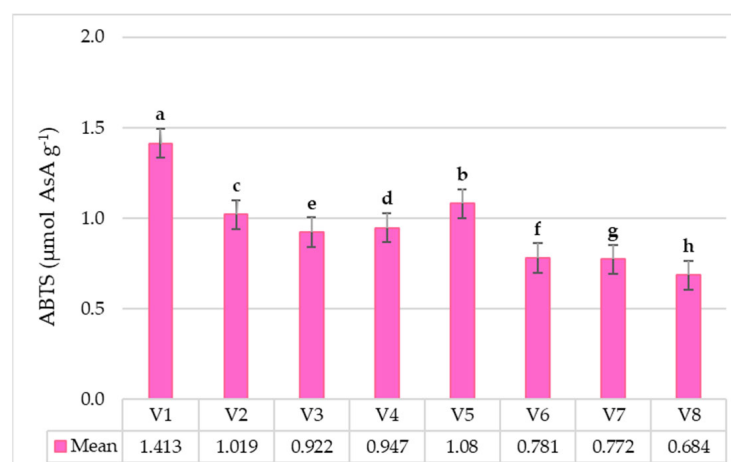


Figure 15. The antioxidant activity (ABTS) of garlic variants, expressed as ascorbic acid. Bars represent standard errors. Means followed by the same letter are not significantly different (Tukey multiple range test, $p < 0.05$).

V4, which has the highest content of total polyphenols, also has the highest antioxidant activity (DPPH), $1.578 \mu\text{mol TE g}^{-1}$ and $1.123 \mu\text{mol AsA g}^{-1}$, respectively, compared to the other variants. The antioxidant activity of the eight variants is very different depending on the method by which it was determined and also on the morphological organ from which the extract was made.

4. Discussion

The diversity of morphological characteristics of crop germplasm resources plays a significant role in breeding programs. The variations found in the qualitative traits are useful in the identification of germplasm and the development of new varieties, and the quantitative traits have a direct agronomic interest.

The bulb and bulbil are the two main organs of garlic used for consumption. Thus, bulb-related traits such as average bulb weight, bulb diameter, bulb height, number of cloves per bulb, and average clove weight were very important to help evaluate the bulb and cloves quality.

The results of the current study, regarding the diameter of the bulb, the average weight of the bulb, and the number of cloves per bulb, are similar to the results of previous research works, which reported a wide range of variability in the morphological characteristics of the bulb in the studied garlic genotypes [13,14].

The selection of genotypes with regard to the highest bulb weight, number of cloves/bulb and bulb equatorial diameter should be recommended as some of the best breeding traits for garlic genetic improvement. Considering that a variable is feasible for the direct selection of garlic cultivars, it must have a direct effect on the yield and a high correlation leading in the same direction with the yield of garlic bulbs. In this sense, variables such as the diameter of the bulb, the average weight of the bulb, and its height are most suitable for the direct selection of the most productive garlic genotypes because they have a cause–effect relationship with the crop yield.

The bulb yield is the most important trait for garlic and has been evaluated by numerous researchers in previous studies [15–18]. Mishra et al. (2013) [15] found by studying 20 promising garlic genotypes that they differed significantly in terms of various morphological characteristics. In this experiment, bulb yield was found to be strongly positively correlated with bulb weight and bulb diameter. Raju et al. (2013) [16] obtained similar results on 56 garlic genotypes.

Studying the diversity of 31 local garlic genotypes in Tunisia found that yield was strongly influenced by bulb weight and diameter, clove weight, number of leaves per plant, and stem length [17]. Baghalian et al. (2005) [18] found a significant positive correlation between average clove and bulb weight and a negative correlation between average clove weight and number of cloves.

The interest in natural antioxidants and especially in dietary antioxidants, which are present in vegetables and contribute to protection against oxidative stress in humans, is increasing [6]. Currently, vegetables are frequently used in natural medicine to treat many diseases instead of synthetic products. Some vegetable crops, such as softneck garlic, due to their ingredients, including γ -glutamyl-S-allyl-L-cysteine and S-allyl-L-cysteine sulfoxide and L-Alliin, have antitoxic, anticancer, antiviral, and antioxidant properties and fight free radicals in the body. Garlic is one of the vegetables that contain a wide variety of minerals and vitamins; the chemical composition is influenced by genotype, environmental, and cultural conditions [19]. One of the vitamins contained in black and white garlic is vitamin C. Vitamin C acts as an antioxidant and effectively defeats free radicals that damage cells or cultures [20]. In a study conducted by Gambelli et al. (2021) [10], regarding the vitamin content of some garlic ecotypes, they showed that vitamin C turned out to be the most represented vitamin, with an amount that varied from 9.7 to $15.6 \text{ mg}^{-1} 100 \text{ g f.w.}$

The results of the present study regarding the vitamin C content are similar to those reported by Azzini et al. (2014) [21] in Italian garlic varieties, but they are superior to those reported [22,23].

Garlic is not only a vegetable with high nutritional value, but it is also a vegetable with medicinal value, and from this point of view, total phenolic compounds (TPC) play an important role as powerful antioxidants. It is important that garlic genotypes contain higher levels of TPC [22]. Furthermore, this species has also been proposed as one of the richest sources of total phenolic compounds among commonly consumed vegetables and has been highly valued in terms of the contribution of the phenolic compounds to the human diet [9].

The values obtained in the present study are also supported by Chen et al. (2013) [6], who found a notable variation in TPC among the garlic cultivars collected from China. Our results, however, are superior to those obtained by Choi et al. (2014) [24] in black garlic, which is supposed to have a higher polyphenol content than white garlic. Naji et al. (2017) [4] found that in single-clove garlic, the content of phenolic compounds and antioxidant activity was higher than in multicloves garlic. Total polyphenols vary significantly between garlic varieties, a claim also made by Chen et al. (2013) [6] in a study of 43 garlic varieties or by Lu X et al. (2011) [25]. The variability of total polyphenols between these varieties can be attributed to their different characteristics, specifically related to genotype or variety.

There is increasing evidence that TPC, which is present in natural foods like garlic, can reduce the risk of serious health disorders due to its antioxidant activity. Polyphenols are reducing agents and, together with other dietary reducing agents such as vitamin C, protect tissues against oxidative stress [23].

Garlic is also characterized by the presence of phenolic compounds, and the main group consists of phenolic acids (mainly caffeic acids), followed by flavonoids. Quercetin (quercetin) has important functional benefits, including anti-inflammatory activity, an antihistamine effect, allergy medication, as well as anticancer and antiviral activities [1].

Numerous researchers have stated that the pharmacological effects of flavonoids are correlated with their antioxidant activities. Moreover, it is suggested that the overall antioxidant effect of flavonoids on lipid peroxidation may be related to their purifying properties of OH⁻ and -O₂ and their reaction with peroxyl radicals. Flavonoids are complex structures belonging to the division of polyphenols and have many functions in plants: they are compounds of defense against insects and pathogens and ideal natural antioxidants (Franco et al. 2007) [26].

According to some authors, the biosynthesis of phenols/flavonoids is regulated in response to a wide range of stress/abiotic factors, such as drought, cold, or a low supply of water and nutrients [1,27]. Based on these considerations, garlic growers should focus their efforts on selection programs based on the morphological characteristics, yield, and biochemical composition of available garlic genotypes.

The results obtained in this study are superior to those reported by Gadel-Hak et al. (2011) [23]. As a general conclusion, it can be said that this compound content was higher in Rocambole, followed by chives, and then by softneck garlic, as in the case of vitamin C. In this respect, garlic growers should focus their efforts on selection programs based on horticultural characteristics and chemical composition.

Peroxidases are oxidoreductases present in plants, microorganisms, and animals. They have multiple applications in enzymatic immunoassays, medical diagnostics, wastewater detoxification, industrial waste treatment, and biosensors for the determination of glucose, alcohols, or phenolic compounds [28].

Csiszár et al. (2007) [29] reported that a slight water deficit (a 40% decrease in soil water content) during the growing season, for a week in the 3–5 leaf stage, causes significant changes in antioxidants and antioxidant enzyme activities such as catalase, glutathione reductase, glutathione S-transferase, peroxidase, and superoxide dismutase. The results of the study by Naji et al. (2017) [4] are consistent with those obtained in the current study because the multi-bulb garlic bulb showed lower antioxidant activity compared to the single-bulb extract.

The antioxidant enzyme activity plays an important role in plants that are subject to water or heat stress. The enzyme activity, such as peroxidase, was the highest in V3 (morphological organs formed at the aerial part of the plant), which indicates a thermal stress and emphasizes the importance of peroxidase because it mitigates oxidative stress and damage that can appear. Due to increased ROS levels, these findings have been reported in many plants under various stresses [30–33]. Particular attention must be paid to garlic varieties that are resistant to heat stress, which has increased in recent years.

Our results, in the case of chives (DPPH method) and Rocambole (ABTS method), are also supported by Jang et al. (2018) [34], who showed that the antioxidant properties of garlic in mature bulbs proved to be higher than those of garlic bulbils, results obtained by DPPH, ABTS, FRAP, H₂O₂, and Fe²⁺ chelating tests. The phenolic compounds and flavonoids have a positive correlation with DPPH and ABTS radical scavenging activities due to the donation of hydrogen and electrons from the hydroxyl groups of these compounds, results also supported by [35,36]. In particular, the ABTS method is considered to be potentially more efficient than the DPPH method because ABTS can measure both hydrophilic and hydrophobic substances [37].

These results are consistent with those obtained by Chen et al. 2013 [6] in a study conducted on 43 varieties of garlic. The variety or genotype and culture conditions, as well as their interaction, can influence the phytochemical composition and antioxidant capacity of garlic bulbils [38].

5. Conclusions

The variety of garlic has a significant effect on the chemical composition; therefore, when choosing a variety, this should be made in accordance with climatic requirements and market needs, focusing mainly on the quality of the final product. The fact that garlic, in many parts of the world, multiplies vegetatively, the use of bulbils from the previous crop year by farmers, as well as the existence of different ecotypes grown in certain areas for decades, can create quality problems in terms of uniformity and content of bioactive compounds. Chives are adapted to negative temperatures, and when changing environmental conditions, they recorded an increase in TPC and antioxidant activity compared to other varieties. The bulbils from inflorescence as well as the one year old ones (V3, V5, and V6) had a remarkable content in bioactive constituents (vitamin C, total polyphenols, flavonoid content, and antioxidant activity through ABTS), which was even higher than the records of the two local cultivars. It should be noted that the bioactive profile of garlic depends on the age of the morphological organ, and the bioactive concentration decreases with maturity. Therefore, immature garlic of this type has the best potential to be used for its health benefits. The versatile health effects and nutraceutical properties of garlic can be attributed to the variety of bioactive compounds, mainly polyphenolic substances with strong antioxidant properties, as well as the morphological organ (a mature bulb with cloves, a one year single bulb, or bulbil in the inflorescence).

From this point of view, garlic varieties can be cultivated and used in breeding programs in order to obtain genotypes with superior added value. Our study demonstrates the relevance of genotype and environmental conditions (culture areas and pedoclimatic factors) as well as their interaction, thus influencing the production, phytochemical composition, and antioxidant properties of garlic varieties.

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