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Influence of various fertilizer regimes on phenological characteristics, polyphenol content, and antioxidant capacity of *Amaranthus caudatus* L.

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Keywords:

Amaranthus caudatus, Antioxidant capacity, Fertilizer

Abstract − In this study, the effects of different doses of organic (O_F), vermicompost (V_C), and chemical (C_F) fertilizers on phenological characteristics, polyphenolic compounds, and antioxidant capacity of *Amaranthus caudatus* L. were evaluated. The study was carried out using a randomized block design with four replications. The findings revealed that the fastest germination occurred in the C_F-6 L/da group, with a mean time of 7 days, while the slowest germination was observed in the O_F-1200 mL/da group, with a mean time of 16 days. Regarding flowering time, the earliest flowering occurred on the 68th day in the C_F-3 L/da group, while the latest flowering occurred on the 79th day in the O_F-1200 mL/da group. The longest vegetative period was observed in the O_F-1200 mL/da group with 215 days due to the effect of fertilizer applications and the fact that the study was carried out in the summer season. The closest vegetative period to the control group was 187-190 days in the C_F-6 L/da group. The treatment groups' total phenolic content (TPC) and total flavonoid content (TFC) exhibited a range of 0.29-2.46 mg GAE/g and 0.50-1.26 mg QE/g, respectively. The highest TPC and TFC values among the treatment groups were determined in the O_F-300 mL/ha and V_C-2 L/ha groups, respectively. The IC_{50} values of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of methanol extracts exhibited a range of 3.34 to 6.80 mg/mL, with the O_F-300 mL/da (50 mg/mL) group demonstrating the highest radical scavenging activity, exhibiting an 89.95% inhibition rate.

1. Introduction

Drought, one of our most serious environmental problems, has threatened many plant and animal species with extinction. For this reason, scientists have emphasized the search for plant species resistant to harsh environmental conditions that can grow on less fertile soils and have a high yield potential. Species of the genus Amaranthus stand out as plants with high resistance to arid climates and infertile soils, and these species can tolerate water shortages better than many common crops [1]. The Amaranthaceae family is a very large group of plants that includes 180 genera and about 2050-2500 species worldwide and is represented by 33 genera in Türkiye. Amaranthus is best known as a gluten-free pseudocereal, mostly cultivated in Mexico and South America, but can also be widely cultivated in temperate and tropical regions. It is considered a taxonomically difficult genus to classify due to the lack of clear differences between species and the difficulty distinguishing them. This plant has a structure that can easily adapt to both tropical and temperate climates. Generally herbaceous, these species can be annual or perennial and are short-lived. The plants may be monoecious or dioecious and bear flowers in spikes, compound panicles, or clusters on the main axis [2].

In recent years, interest in the Amaranthus genus has increased due to the high nutritional value of both its seeds and leaves [3, 4]. The seeds of this plant, which is particularly rich in protein, have a protein content ranging from 15-43% on a fresh weight basis, while its leaves have a protein content ranging from 14-30%.

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Amaranthus proteins stand out with their balanced amino acid profiles [5], high bioavailability [6], and functional properties. Furthermore, the Amaranthus plant is rich in important nutrients such as dietary fiber, vitamins (ascorbic acid, riboflavin, tocopherols, carotenoids), and minerals (Ca, Fe, Mg, K, Cu, Zn, and Mn). These nutrients' levels are generally higher than those found in many cereals and leafy vegetables [7]. Amaranth is receiving increasing attention due to its high nutrient content, particularly in the context of gluten-free food production. In addition to macro- and micronutrients, amaranth also contains secondary plant metabolites (unsaturated fat, squalene, phenolic compounds etc.) that may be beneficial for human health, indicating a potential role in human nutrition.

Recently, extensive research has been conducted on the phenolic compound profile of amaranth seeds [8, 9] and their functional and bioactive properties [10, 11]. However, recent studies have also highlighted that Amaranthus leaves and aerial parts are significant sources of phenolic compounds [12, 13]. Among these phenolic compounds, hydroxycinnamic acids, benzoic acids, flavonols, and glycosides have been identified in Amaranthus species' leaves, flowers, and stems [12, 14]. Betalains, especially betacyanins, are among the important antioxidant phytochemicals found in Amaranthus plants [15]. These pigments vary among Amaranthus species and genotypes [16], but betalains accumulate mainly in seedlings, leaves, and inflorescences. Research on Amaranthus has shown that this plant's seeds, oil, and leaves can lower blood pressure, cholesterol levels, and blood sugar. In addition to its antioxidant [17, 18] and anticancer [19] properties, Amaranthus is also a suitable food source for celiac patients [20].

Although the phenolic compound profiles and biological activities of the botanical parts of Amaranthus are well known, there is insufficient information on the effects of different fertilizers applied at different doses on the plant's growth cycle and on these phytochemicals. In this context, it is important to determine the effects of fertilizers applied to Amaranthus on growth stages and changes in total phenolic and flavonoid contents.

2. Materials and Methods

2.1. The Cultivation of *Amaranthus caudatus*

Following surface sterilization (1 minute in 1% NaClO) solution, seeds of *Amaranthus caudatus* (*A. caudatus*) (Tacir Depo, Türkiye) (Figure 1) these seeds were kept in 10% fertilizer solutions for six hours. Subsequently, the seeds were sown in pots filled with a 1:1 mixture of peat and garden soil, following a randomized block design with four replicates. The plants were irrigated at three to four-day intervals. The experiment was conducted by applying three distinct types of fertilizers (liquid synthetic, liquid organic, and vermicompost) at three varying concentrations, resulting in ten treatment groups, including a control group devoid of fertilizer. The specific types and quantities of fertilizers applied are detailed in Table 1. The germination process was observed to have commenced approximately 15 days after the sowing of the seeds. Once 80% of the plants had reached a height of 15-20 cm, the first fertilizer application was conducted. The fertilizers were applied to the soil at the manufacturer-recommended dose and at half and double this concentration. The second fertilizer application was conducted when more than 50% of the plants had reached the flowering stage. Once the seed capsules had reached maturity, the plants were harvested manually. The harvested capsules were stored at 4°C for subsequent morphological, yield, quality analysis, and extraction studies.

Table 1. Fertilizer types and treatment dosages

Treatment Groups	Chemical Content	Dosages and Abbreviations		
		Half	Recommended	Double
Control	-	-	-	-
Liquid chemical	N: P: K (7:7:7%)	C_F-1.5 L/da	C_F-3 L/da	C_F-6 L/da
Liquid organic	Total organic matter: 45%, Organic C: 19.5%, N: 3%, K_2O:7%	O_F-300 mL/da	O_F-600 mL/da	O_F-1200 mL/da
Liquid vermicompost	Total organic matter: 5%, N: 1%, Humic + fulvic acid : 10%	V_C-1 L/da	V_C-2 L/da	V_C-4 L/da

Figure 1. *A. caudatus*, grown in the greenhouse (a: Germination b: 15-20 cm length; c: Full-grown plants)

2.2. Methanol Extraction Procedure

The methanol extract of seeds was performed following the methodology outlined by Mansouri et. al. [21] with minor modifications. 10 g of pulverized seeds were extracted with 80% MeOH (200 mL) for 10 h in a Soxhlet apparatus. The *A. caudatus* seed methanol extracts were subjected to a 0.22 µm sterile filter to ensure purity after the extraction. This was followed by solvent removal through evaporation using a Rotary evaporator set at 40°C until completely dried. The percentage yield of the residue was calculated and stored at +4°C until analysis. The *A. caudatus* seed methanol extracts were used in total phenolic and flavonoid content and antioxidant analysis.

2.2.1. Total Phenolic Content Determination

The total phenolic content (TPC) of *A. caudatus* seeds was determined using the Folin-Ciocalteu colorimetric assay described by Ulusu [22]. In this procedure, varying concentrations of gallic acid (0.1–1.0 mg/mL) were utilized as the standard phenolic compound. 100 µL of methanol extract (10 mg/mL) and 600 µL of 2% (w/v) Na_2CO_3 Yuk were added and thoroughly mixed in each test tube. Subsequently, 200 µL of Folin-Ciocalteu reagent was introduced, and the final volume was adjusted to 10 mL using dH_2O. The mixture was incubated for 2 hours in the dark. Following incubation, the absorbance was measured at 750 nm. TPC was quantified based on the calibration curve generated from the gallic acid standard (y = 0.2192x+0.0713 R^2 = 0.9685).

2.3. Total Flavonoids Content Determination

The total flavonoid content (TFC) of *A. caudatus* seeds was determined with minor modifications to the method described by Barku and Mensah [23]. Quercetin solutions at varying concentrations (0.05-1 mg/mL) were prepared and used as the standard flavonoid compound.

To 250 µL of either methanol extract or quercetin standard solution, 1.25 mL of dH_2O and 75 µL of 5% (w/v) $NaNO_3$ were added and thoroughly mixed. After 6 minutes, 150 µL of 10% (w/v) $Al(NO_3)_3$ was introduced, after 5 minutes by adding 0.5 mL of 1 M NaOH. The final volume was adjusted to 2.5 mL with dH_2O and mixed thoroughly. The absorbance was then measured at 415 nm. TFC was calculated based on the calibration curve of the quercetin standard (y = 1.3268x +0.02045 R^2 = 0.9606).

2.4. Determination of Antioxidant Activity

The radical scavenging activity of methanol extracts obtained from *A. caudatus* seeds was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, with slight adjustments to the method described by Ulusu and Ulusu [24]. According to the method, different concentrations of methanol extracts (12.5, 25, 50, 100, 200, 400 µg/mL) were prepared alongside ascorbic acid, which served as a positive control. A 180 µL solution of DPPH (1 mM in methanol) was added to 20 µL of the methanol extract and mixed thoroughly by vortexing. The mixtures were then incubated for 30 minutes in the dark at room temperature. Following incubation, absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Shimadzu, UV-1800). The assay included a control consisting solely of DPPH (without extract) and a blank containing methanol. The DPPH radical scavenging activity was expressed as percentage inhibition, calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control absorbance} - \text{Extract absorbance}}{\text{Control absorbance}} 100$$

2.5. Statistical Analysis

Differences between the control and treatment groups were assessed using one-way analysis of variance (ANOVA) [25]. Post-hoc comparisons were conducted with Duncan's multiple range test, with statistical significance set at $p < 0.05$ across four replicates (n = 4). All statistical analyses were carried out using the IBM SPSS Statistics software, Version 24.

3. Results and Discussion

3.1. Time of Germination (day)

The term "germination or emergence time" is the period between the sowing of seeds and the point at which a specified percentage (commonly 50 and 80%) of seedlings emerge above the soil surface and become visible. This parameter assesses seed vigor and the environmental conditions influencing germination and early plant development, such as soil temperature, moisture, and nutrient availability [26]. In the study, *A. caudatus* seeds sown on 18.04.2022 showed emergence between 25.04.2022 and 04.05.2022, indicating that the germination period ranged from 7 to 16 days. Among the treatment groups, the fastest germination was observed in the C_F-6 L/da (7 days), followed by the V_C-4 L/da and C_F-3 L/da groups (8 days). The slowest germination occurred in the O_F-1200 mL/da, lasting 16 days. The variation in the nutrient content of the applied fertilizers significantly influenced the emergence performance, leading to statistically significant differences. The average emergence times for each group are presented in Figure 2a. A study evaluating the effect of temperature on the emergence time of nine different Amaranthus species determined that germination was completed within 4 to 8 days at 30°C.

Furthermore, for the majority of species across all treatment groups, germination was observed to be fully completed within 8 to 9 days [27]. In a study investigating the impact of different fertilizer treatments on seed germination and seedling growth in *Helianthus annuus* L., it was observed that organic fertilizer significantly reduced the germination time of seeds (10 days), promoting faster development. In contrast, the synthetic fertilizer treatment group exhibited a longer germination period (14 days), indicating a delayed response in seed germination under synthetic fertilizer conditions [28]. The fertilization process has a marked effect on the emergence of seeds, modifying the soil conditions that regulate germination and the early growth of seedlings. Appropriate fertilization increases the availability of essential nutrients, including nitrogen, phosphorus, and potassium, which are vital for root development and early seedling vigor [29]. Studies have demonstrated that both organic and inorganic fertilizers can improve seedling emergence by enriching the soil with vital nutrients and enhancing its physical properties, such as structure and water-holding capacity [30]. For example, using

vermicompost and other organic fertilizers has increased soil porosity and microbial activity, creating a favorable environment for seed emergence [31].

Conversely, inorganic fertilizers provide immediate nutrient availability, accelerating germination and promoting quicker emergence in many crop species [32]. However, applying excessive or imbalanced fertilizers may harm seed emergence, resulting in alterations to the soil's osmotic potential or the development of nutrient toxicity. This can impede water uptake and reduce germination rates [33]. It is, therefore, essential to optimize the type and dosage of fertilizers to promote efficient seed emergence and improve crop performance.

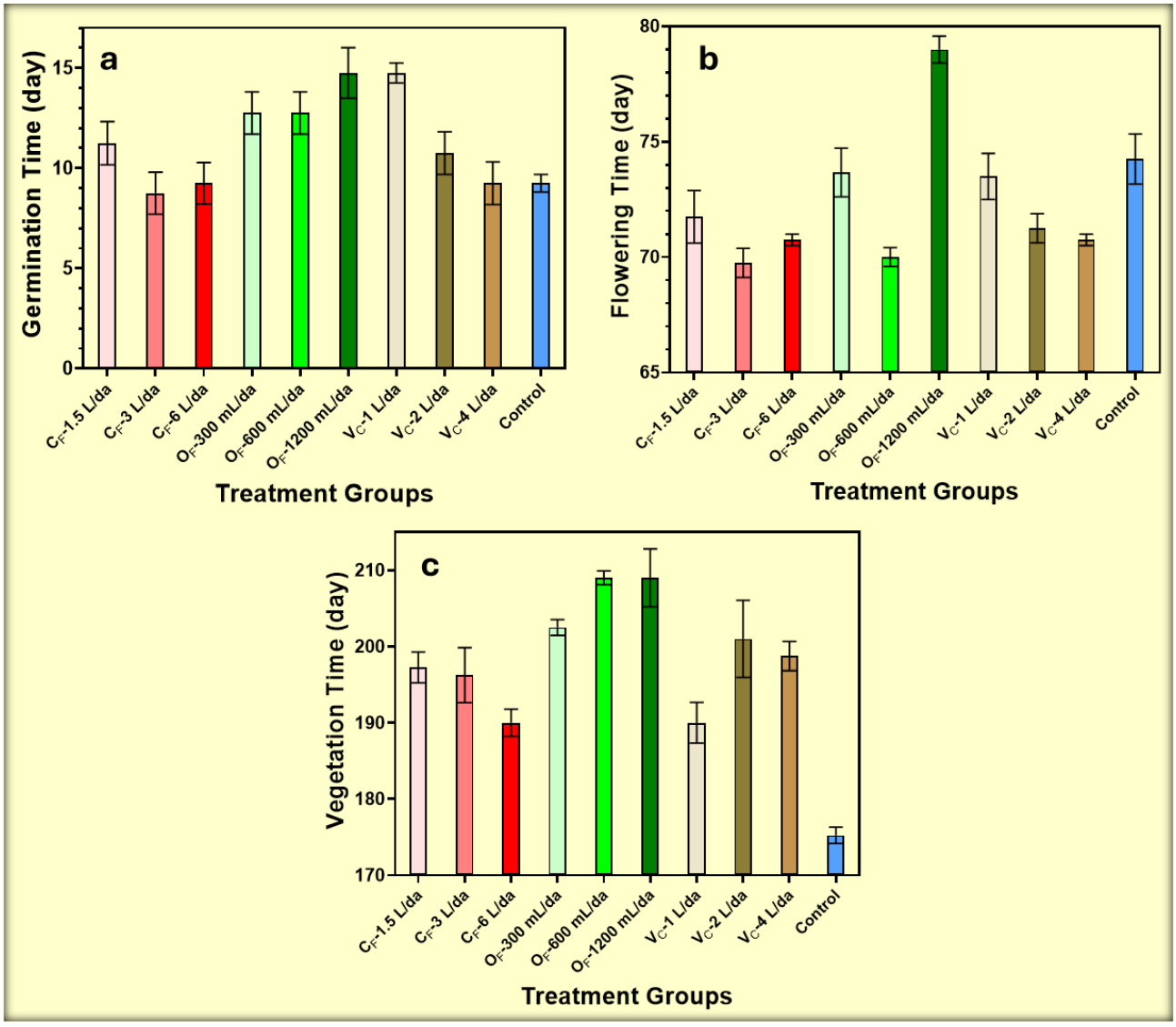

Figure 2. Average germination (a), Flowering (b), and Vegetation (c) times for treatment groups

3.2. Time of Flowering (day)

The flowering time is defined as the interval between the planting of the samples in the pots and the onset of flowering in more than half of the plants [34]. The study's flowering duration ranged from 68 to 79 days across various treatment groups. The earliest flowering was recorded in the C_F-3 L/da group at 68th days, followed closely by the O_F-600 mL/da group, where flowering occurred on the 70th day. The latest flowering event was observed in the group treated with O_F-1200 mL/da on the 79th day. Additionally, the control group displayed delayed flowering, starting on the 75th day. These flowering periods across different treatment groups are presented in Figure 2b, highlighting the differential impacts of fertilization regimes on flowering dynamics.

Applying nitrogen, phosphorus, and potassium (NPK) fertilizer to *A. caudatus* at three levels resulted in observed flowering times between 75 and 78 days. The earliest flowering was observed at the dose of 120-100-80, while the latest flowering was observed at 18-46-30 [35]. Similar to the current study, these findings suggest that higher doses of nitrogen, phosphorus, and potassium may promote earlier flowering, likely due to enhanced nutrient availability that accelerates the plant's physiological development [36]. This variation highlights the importance of optimizing fertilization levels to achieve desired growth and phenological outcomes in *A. caudatus*. Adjusting nutrient input based on the plant's developmental needs can significantly impact the timing of flowering, overall growth, and yield. The differential responses observed at various fertilizer doses emphasize the need for precise nutrient management strategies to enhance agricultural productivity while maintaining plant health and resource efficiency [37].

3.3. The Vegetation Period (day)

The vegetation period is defined as the time from the date of emergence in pots to the date of harvest [34]. In the present study, plants sown on April 18, 2022, experienced early maturation due to the summer growing season and the application of fertilizers. Among the treatment groups, the longest vegetative period was observed in the O_F-1200 mL/da group at 215 days, followed by the O_F-600 mL/da group at 211 days. In contrast, the closest vegetation period to the control group was noted in the C_F-6 L/da group, ranging from 187 to 190 days. The vegetative periods for the respective groups are illustrated in Figure 2c, highlighting the influence of different fertilization regimes on the time of plant development.

The combined application of poultry manure at 15 tonnes/ha and urea-N at 30 kg/ha resulted in a significantly enhanced growth response in *A. cruentus*, compared to treatments with urea-N or poultry manure alone. This treatment led to the longest vegetative lifespan, recorded at 70 days, highlighting the synergistic effect of organic and inorganic nutrient sources on plant growth [38]. This suggests that integrating organic and synthetic fertilizers provides a more balanced nutrient availability, fostering optimal plant development and effectively extending vegetative phases. The duration of vegetative growth in plants can be significantly influenced by the type and combination of fertilizers applied, as shown in multiple studies. For instance, a study by Agegnehu et al. [39] demonstrated that combining organic manure with inorganic fertilizers extended the vegetative growth period of crops by improving nutrient availability and uptake efficiency. Similarly, Rahimi et al. [40] found that organic fertilizers, such as vermicompost, can prolong the vegetative phase by enhancing soil structure and nutrient content, allowing plants to access nutrients gradually. Integrating organic and inorganic fertilizers optimizes nutrient release, facilitating sustained plant growth and extended vegetation periods, critical for optimizing yield and plant health.

3.4. Total Phenolic and Flavonoid Content

The Soxhlet method's methanol extract yields% of *A. caudatus* seeds varied between 7.30% (O_F-300 mL/da) and 13.38% (C_F-1.5 L/da). The results demonstrate that the treatment of fertilizers considerably impacts crop yield (Table 2.). The treatment of organic fertilizer (O_F) demonstrated varying responses, with the highest yield observed at the 600 mL/da dose (9.13%) and the lowest at 300 mL/da (7.30%), suggesting that higher doses of O_F are generally more effective. The treatment of vermicompost (V_C) showed a clear positive correlation between dosage and yield, with the highest yield recorded at 4 L/da (13.14%), surpassing the control and other treatments. Chemical fertilizer (C_F) treatments also resulted in high yields, particularly at 1.5 L/da (13.38%), indicating that C_F, like V_C, significantly improves yield at appropriate dosages. However, the yield slightly decreased at the higher C_F dose of 6 L/da (13.31%) compared to 1.5 L/da, suggesting that excessive application may not proportionally increase yield. Overall, the V_C and C_F treatments yielded the highest increases, with the optimal results observed at moderate to high application rates.

In order to quantify the TPC of *A. caudatus*, a calibration curve was generated using gallic acid as the standard at varying concentrations (0.1–1.0 mg/mL). Once the regression equation had been determined, TPC in both the control and treatment groups was calculated based on this equation. The TPC of the treatment groups ranged from 0.29 to 2.46 mg GAE/g (gallic acid equivalent). The fertilizer treatments enhanced the TPC in seeds compared to the control group (Table 2.). The highest TPC was observed in the O_F-300 mL/da group, with a value of 2.46 mg GAE/g, while the lowest phenolic content was recorded in the control group, at 0.29 mg GAE/g. The differences between groups were statistically significant ($p< 0.05$). Although applying vermicompost and chemical fertilizers at different doses resulted in only minor variations in TPC, an approximately 200% increase in TPC observed between the O_F-300 mL/da and the other organic fertilizer treatments is noteworthy. In a study investigating the TPC of the aerial parts of *A. caudatus* at different times, TPC ranged from 18.3 to 33.7 mg GAE/g. The highest TPC levels were observed at the early vegetative and grain-filling stages as well as at the beginning of the flowering stage.

In contrast, the lowest TPC was determined in extracts from the shoot and budding stages [17]. The average value of TPC determined as a result of combined animal manure applied to *A. viridis* at different doses (0, 20, 40, 60, and 80 kg urea N/ha) was 463.15 mg GAE/g and minor variations were detected among the groups [41]. The application of nitrogen fertilizer to *A. hypochondriacus* at doses of 120, 180, and 240 kg/ha resulted in TPC values of 13.4, 14.6, and 13.7 mg GAE/g, respectively [42]. In the present study, the TPC of *A. caudatus* was lower than the values reported in the above studies. These findings clearly show that different fertilizers and their doses cause various effects on polyphenol accumulation.

The TFC of the treatment groups ranged from 0.50 to 1.26 mg QE/g (quercetin equivalent). Fertilizer treatments generally significantly enhanced the TFC in seeds compared to the control group (Table 2). The differences between groups were statistically significant ($p< 0.05$). The highest TFC was recorded in the V_C-2 L/da group, measuring 1.26 mg QE/g, whereas the O_F-600 mL/da group exhibited the lowest phenolic content at 0.50 mg GAE/g. In addition, the O_F-300 mL/da and the O_F-600 mL/da groups exhibited flavonoid levels similar to those of the control group. In contrast, the O_F-1200 mL/da (0.97 mg QE/g) and the V_C-1 L/da (0.92 mg QE/g) groups show a significant increase compared to the control group. C_F-1.5 L/da (1.13 mg QE/g) exhibited the second-highest TFC in chemical fertilizer treatments. Vermicompost and chemical fertilizer treatments significantly increased the TFC, while organic fertilizer treatments remained at lower levels. These findings indicate that the effects of different fertilizer types and doses on the TFC are significant.

Many studies have systematically investigated the phytochemical composition of various Amaranthus species, focusing on their bioactive compounds, such as polyphenols, flavonoids, and antioxidants. These studies have demonstrated significant variation in phytochemical content across different species and under varying environmental and agricultural conditions, highlighting the importance of genetic and external factors in influencing the phytochemical profiles of Amaranthus plants. For instance, in their study, Li et al. [43] reported that the TFC of the leaf extract from *A. caudatus* was 6.5 mg CAE/g, while Akin-Idowu et al. [44] reported a TFC of 8.9 mg CE/100 g. In the study by Ali et al. [45], it was stated that Farm Manure (FYM) and humic acid application to *A. viridis* grown in different regions significantly affected TPC and TFC, especially 15 t/ha FYM application resulted in the highest TPC (46.24 µg/GAE g FW) and TFC (182.16 µg/RE g DW). Furthermore, the application of humic acid exhibited a more irregular trend than FYM. The highest total phenolic content (43.30 µg/GAE g FW) and total flavonoid content (169.97 µg/RE g DW) data were obtained at 30 kg/ha. In line with the current and recent studies, applying fertilizers to plants at optimal doses can enhance polyphenol accumulation. However, the observed responses varied between regions and treatments, indicating that environmental and agricultural factors may influence these effects.

Table 2. % yields, TPC, and TFC of *A. caudatus* seed methanol extracts

Treatments	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Control	7.87	0.29 ± 0.00[a]	0.62 ± 0.09[a]
O_F-300 mL/da	7.30	2.46 ± 0.02[g]	0.59 ± 0.16[a]
O_F-600 mL/da	9.13	0.46 ± 0.00[b]	0.50 ± 0.06[a]
O_F-1200 mL/da	8.81	0.48 ± 0.00[b]	0.97 ± 0.01[bc]
V_C-1 L/da	8.46	1.10 ± 0.01[d]	0.92 ± 0.03[bc]
V_C-2 L/da	10.70	1.46 ± 0.01[f]	1.26 ± 0.07[d]
V_C-4 L/da	13.14	1.47 ± 0.03[f]	0.98 ± 0.03[bc]
C_F-1.5 L/da	13.38	1.24 ± 0.00[e]	1.13 ± 0.01[cd]
C_F-3 L/da	11.56	1.13 ± 0.01[d]	0.92 ± 0.05[bc]
C_F-6 L/da	13.31	1.01 ± 0.04[c]	0.74 ± 0.09[ab]

Control (No fertilizer), O_F: Organic fertilizer, V_C: Vermicompost, C_F: Chemical fertilizer. The letters in each column did not differ significantly at $p < 0.05$.

3.5. Antioxidant DPPH Radical-Scavenging Activity

Antioxidants function as the primary defense against oxidative damage induced by free radicals. DPPH is a stable synthetic molecule commonly employed to evaluate various antioxidant compounds' free radical scavenging capabilities [46]. The antioxidant properties of the seed methanol extract were evaluated using the DPPH assay across a concentration range of 0.5 to 50 mg/mL. The inhibition percentages were determined by comparing the results with ascorbic acid, used as the reference standard. All tested treatment groups exhibited significant DPPH activity at different concentration-dependent ratios (Figure 3), and the IC_{50} values of the *A. caudatus* seed methanol extracts ranged from 3.34 to 6.80 mg/mL. DPPH radical scavenging activity of seed extracts was affected by fertilizer type and fertilizer doses. The O_F-300 mL/da demonstrated the highest free radical scavenging activity at the maximum 50 mg/mL concentration among the tested samples. It achieved an inhibition percentage of approximately 89.95% (IC_{50}: 3.90±0.02 µg/mL) ($p<0.05$), exhibiting its superior antioxidant potential compared to other materials tested (Figure 4).

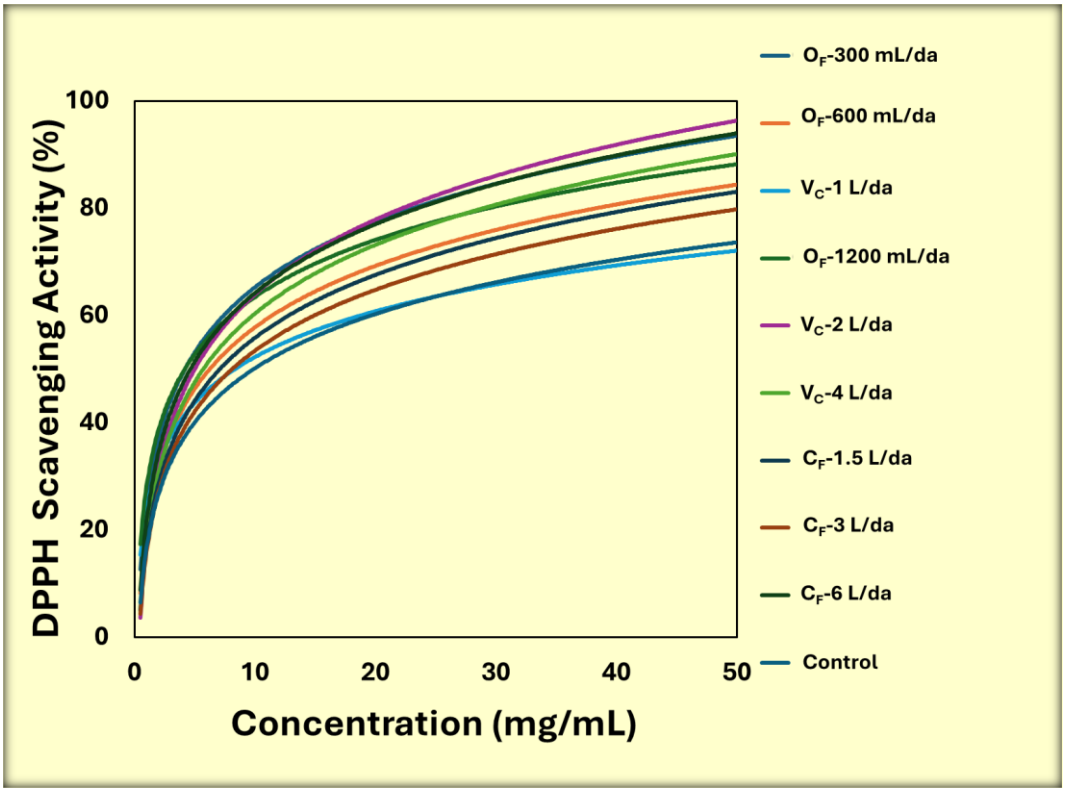

Figure 3. DPPH scavenging activity treatment groups

In the vermicompost and chemical fertilizer treatment groups, especially V_C-1 L/da and O_F-6 L/da showed concentration-dependent DPPH radical scavenging activity and were the most effective treatments in neutralizing DPPH free radicals at the highest concentrations in their groups and 88.22% (IC_{50}: 4.83±0.02 µg/mL) and 82.35% (IC_{50}: 3.82±0.01 µg/mL) a remarkable inhibition percentages were obtained, respectively. These findings reveal that fertilizers applied at appropriate doses increase the plant's antioxidant activity, which can potentially strengthen the stress resistance of the *A. caudatus* by increasing the free radical scavenging capacity. Munene et al. [47] reported that two different *Amaranthus* varieties (AB6 and AB7) fed with 3 N forms (NO_3, NH_4^+, and NH_4NO_3) exhibited superior scavenging capacity compared to the control group. In this study, ammonium exhibited superior DPPH scavenging activity with lower IC_{50} values (0.06 mg/mL for AB7 and 0.3 mg/mL for AB6) than nitrate treatments. This suggests that plants treated with ammonium have greater antioxidant potential, requiring less volume to neutralize 50% of DPPH radicals than those treated with nitrate or ammonium-nitrate mixtures.

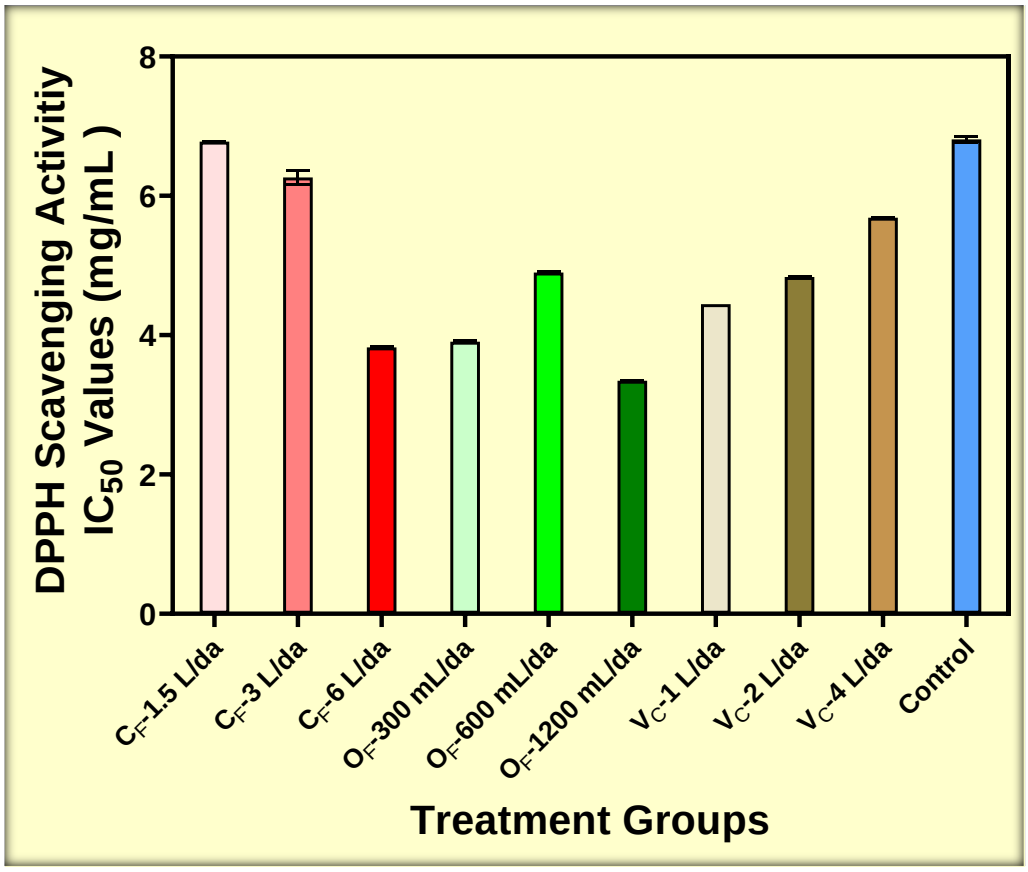

Figure 4. DPPH scavenging activity IC_{50} (mg/mL) values of treatment groups

Additionally, the ammonium-based extract demonstrated considerable antioxidant efficacy, with maximum inhibitory effects of 72.8% for AB6 and 86.1% for AB7 at a lower concentration of 2 mg/mL [47]. Muscolo et al. [48] discriminated that antioxidant activities expressed as DPPH and total antioxidant capacity (TAC) in lettuce grown with sulfur-based organic fertilizer (different concentrations) increased significantly compared to control and other fertilizer (chemical fertilizer and horse manure) applications. Antioxidant activities are closely associated with the composition and diversity of phytochemicals present in plants. These compounds play a critical role in protecting against oxidative stress, which is thought to be a key factor in the therapeutic potential of plants by reducing cellular damage caused by free radicals [49]. Among the four Amaranthus species examined (*A. dubius*, *A. spinosus*, *A. tricolor*, *A. viridis*), *A. spinosus* possessed the highest level of polyphenolic compounds known for their bioactive properties. This phytochemical richness corresponded to superior antioxidant and anti-inflammatory potential with lower IC_{50} values for both antioxidant (IC_{50}: 63.94±3.72 µg/mL) and anti-inflammatory (IC_{50}: 58.17±3.49 µg/mL) activity [19].

4. Conclusion

The current study's findings demonstrate that both organic and synthetic fertilizers play important roles in increasing bioactive compounds biological activities in Amaranthus and could potentially meet the global demand for healthier foods. Organic fertilizers, derived from natural sources, not only improve nutrient supply but also significantly increase the accumulation of phenolic compounds and enhance plant antioxidant activity. Due to their antioxidative properties, these phytochemicals are essential for improving plant resilience and human health. Organic fertilizers also support soil health by recycling waste and lowering greenhouse gas emissions, promoting more sustainable agricultural practices. In contrast, while efficiently delivering key nutrients like nitrogen, synthetic fertilizers must be carefully managed to avoid negative environmental impacts. Optimizing fertilizer treatment, especially organic types that boost phenolic content and antioxidant capacity according to specific crop demands and soil conditions, is essential for maintaining nutrient balance, promoting sustainability, and improving food production with minimal ecological impact. Future studies could explore the optimal ratios of organic and synthetic fertilizers tailored to different Amaranthus cultivars and varying environmental conditions to maximize bioactive compound production and antioxidant activity. In addition, research on the long-term effects of organic fertilizers on soil microbiome health and nutrient cycling could provide valuable insights for sustainable agriculture practices. Evaluating the combined effects of organic and synthetic fertilizers on a wider range of phytochemicals in Amaranthus may further reveal synergistic benefits, paving the way for more effective fertilization strategies in functional food production.

Author Contributions

All the authors equally contributed to this work. They all read and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Ethical Review and Approval

No approval from the Board of Ethics is required.

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