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Effects of Colored Plastic Mulches on Bioactive Compounds and Mineral Nutrition of Broccoli under Open-Field Conditions

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ABSTRACT: Broccoli (*Brassica oleracea* L. var. *italica*) is valued for its nutritional quality, and production practices increasingly focus on improving quality as well as yield. This study evaluated the effects of colored plastic mulches (red, black, grey, and brown) on yield, antioxidant activity, ascorbic acid (Vitamin C), and mineral composition under open-field conditions. The experiment was conducted in a randomized complete block design with four mulch treatments and three replications under open-field conditions. Mulch color did not significantly affect total yield, indicating stable biomass production across treatments. In contrast, antioxidant activity and Vitamin C content were significantly influenced, with the highest values obtained under grey mulch. Along with increased nutrient uptake, mineral composition significantly varied across treatments; nitrogen (N) and potassium (K) were higher under black mulch, while iron (Fe) concentration was highest under brown mulch. Overall, the results indicate that appropriate mulch color selection can enhance the nutritional quality of broccoli while enabling successful cultivation under cool spring conditions without yield loss.

KEYWORDS: Broccoli; plastic mulch; antioxidant capacity; vitamin C; mineral nutrition; yield

1 Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is an economically important vegetable crop widely cultivated due to its high nutritional value and recognized health-promoting properties. It is a rich source of dietary fiber, vitamins A, C, and K, essential minerals, and various bioactive compounds, including glucosinolates, sulforaphane, indole-3-carbinol, and phenolic compounds. These constituents contribute to antioxidant capacity and have been associated with reduced risks of several chronic diseases, particularly those related to oxidative stress [1,2]. As a result, the increasing consumer awareness of broccoli's health-supporting properties has contributed to a steady rise in demand. Additionally, the promotion of production systems that address not only yield, but also nutritional and functional quality supports this situation [3,4]. In recent years, the focus in horticultural production has gradually shifted from maximizing yield to improving crop quality through pre-harvest management practices. Traditionally, environmental manipulation in crop production primarily aimed at increasing productivity. However, recent research suggests that the conditions before harvest play an important role in shaping the nutritional, sensory, and health-related quality of vegetables, which makes quality-focused cultivation approaches increasingly important [3].

Alternative cultivation methods, including different substrate-based systems, have been examined to optimise plant growth and improve fruit quality [5]. Mulching is a practice used to regulate soil temperature, conserve soil moisture, suppress weed growth, and affect nutrient availability. "Mulch films alter the flow

of thermal energy between the air and the underlying soil environment” [6]. “By modifying the radiation budget, plastic mulches directly affect the soil microclimate and specifically the rhizosphere environment, which in turn influences plant metabolic pathways and nutrient availability” [7]. It is a widely used agricultural practice to alter the soil-plant microenvironment, thereby improving both yield and fruit quality [6,8,9]. In particular, colored plastic mulches have received increasing attention due to their capacity to alter light absorption and reflection, soil thermal regimes, and water dynamics, thereby affecting plant growth and physiological responses [10–12]. Therefore, evaluating the performance of different mulch colors in various products and environments is crucial for developing efficient and sustainable management strategies [13,14]. The primary objective of this research was to investigate the effects of different mulch colors on the yield, bioactive compound synthesis, and mineral accumulation (N, K, and Fe) of broccoli (*Brassica oleracea* L. var. *italica*) grown under the microclimatic conditions of a transition zone.

2 Materials and Methods

2.1 Experimental Site and Location

The experiment was conducted under open-field conditions during the spring growing season of 2025 at the Pozantı Agricultural Research and Application Center of Çukurova University, located in Pozantı district, Adana, Türkiye (37°25′32″ N, 34°52′25″ E). The experimental site is approximately 110 km from the city center of Adana and situated at an altitude of about 1100 m above sea level.

2.2 Plant Material

Broccoli (*Brassica oleracea* L. var. *italica*) cultivar ‘Batavia’ was used as the plant material. The seeds were commercially purchased from Metgen Seed Company Limited (Istanbul, Türkiye; Available at: www.metgen.com.tr). ‘Batavia’ is a very early cultivar characterized by medium-sized, compact heads with a fresh green color and fine buds. It is suitable for spring–summer production, exhibits tolerance to low temperatures, and produces solid, dark domed heads with high yield potential.

2.3 Climatic Conditions

Climatic data for the experimental period (February–July 2025), including minimum, maximum, and mean air temperature (°C), relative humidity (%), and precipitation (mm), were obtained from the Adana 6th Regional Directorate of Meteorology. The region is characterized by transitional climatic conditions, with notable temperature fluctuations during spring. Due to the very limited or absent precipitation during the growing period, crop water requirements were met through drip irrigation.

2.4 Soil Characteristics

Soil samples were collected from the experimental field prior to planting and analyzed at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Çukurova University. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 (w/v) soil-to-water suspension according to the methods described by McLean [15]. Soil organic matter was determined using the Chromic Acid Digestion method as per Jackson [16], and calcium carbonate (CaCO₃) content was analyzed following the procedures of Çağlar [17]. Regarding nutrient availability, plant-available phosphorus (P) was extracted using the sodium bicarbonate method as described by Olsen et al. [18], while available K was determined according to Carson [19]. Micronutrients, including diethylenetriaminepentaacetic acid (DTPA)-extractable iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu), were analyzed following the method established by Lindsay and Norvell [20].

2.5 Experimental Design and Mulch Treatments

The experiment was conducted in a randomized complete block design (RCBD) with four mulch treatments and three replications. Each replication (block) consisted of one raised bed, and a total of 12 experimental plots were established. Each plot included 20 plants grown under the respective mulch treatment. The experimental field was relatively uniform in terms of soil physicochemical properties and topography, and all experimental plots were managed uniformly throughout the growing season to minimize environmental variation. The experimental layout is presented in Fig. 1. Different colored mulch materials, each with a thickness of 30 μm , were obtained from the Pozantı Agricultural Research and Application Center at Çukurova University.



Figure 1: Broccoli plants planted in different colored mulch covers in open field conditions.

2.6 Planting, Harvest, and Sampling

Broccoli seedlings were transplanted in April 2025 due to low early-season temperatures, and harvest was conducted in June when heads reached commercial maturity. To mitigate potential stress effects, an amino acid application was applied to the ‘Batavia’ broccoli cultivar once in April and once in May, following producer recommendations. Harvest dates varied slightly among mulch treatments depending on growth rate; however, all samples were collected at the same physiological maturity stage. Harvested heads were used for yield determination and quality analyses.

2.7 Determination of Vitamin C, and Total Antioxidant Capacity

2.7.1 DPPH Free Radical Scavenging Assay

The free radical scavenging activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to the procedure described by Kelebek et al. [21]. Different dilutions were prepared from the sample extracts, and a 0.1 mL aliquot of each extract was mixed with a DPPH solution (6×10^{-5} M) prepared in methanol. After vortexing, the change in absorbance during the reaction was monitored at 515 nm using a UV-Vis spectrophotometer (Agilent-Cary 60). Measurements were conducted at specific time intervals until the reaction reached a steady state. The antioxidant activity was calculated based on the decrease in absorbance of the DPPH radical.

2.7.2 Determination of Vitamin C by HPLC

Vitamin C analysis was performed following the method reported by Lee and Coates [22]. Prior to analysis, samples were filtered through 0.45 μm membrane filters. The extracts were then injected into a Shimadzu LC-20AD HPLC system equipped with an SPD-20A UV detector and an RID-10A refractive index detector. The HPLC analysis conditions were set as follows: Column: HRC NH2 (Bio-Rad), 150×4.6 mm, 5 μm . Mobile Phase: 5 mM sulfuric acid (H_2SO_4) solution. Flow Rate: 0.7 mL min^{-1} . Injection Volume: 20 μL .

The external standard method was employed to quantify Vitamin C concentrations. Calibration solutions were prepared at five different concentrations using a Vitamin C standard (Sigma-Aldrich, St. Louis, MO, USA). A calibration curve was generated using linear regression analysis, which was subsequently used to calculate the Vitamin C content in the samples.

2.8 Mineral Nutrient Analysis

Broccoli head samples were washed with distilled water, oven-dried at 65°C to constant weight, and ground into a fine powder. Mineral analyses were performed at the Central Laboratory of Çukurova University. Broccoli head samples were washed with distilled water, oven-dried at 65°C to constant weight, and ground into a fine powder. Mineral analyses were performed in duplicate for each sample at the Central Laboratory of Çukurova University. For sample preparation, 0.5 g of the ground tissue was subjected to wet digestion in a microwave digestion system using a concentrated acid mixture of hydrochloric acid (HCl), nitric acid (HNO_3), and hydrogen peroxide (H_2O_2). After the digestion process, the extracts were completed to a final volume with ultrapure water. Element concentrations were determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) based on calibration curves prepared with high-purity multi-element standards. All instrumental measurements were performed in triplicate to ensure analytical precision. Total N content was determined separately using the Kjeldahl method.

2.9 Statistical Analysis

Statistical analysis was performed using the JMP 8 software package (SAS Institute, Cary, NC, USA). All data were first subjected to analysis of variance (ANOVA), and differences between means were determined using the Least Significant Difference (LSD) test. Comparisons yielding probability (p) values of $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$ were considered statistically significant.

3 Results

3.1 Evaluation of Climatic Conditions

Climatic data obtained from the Adana Meteorological 6th Regional Directorate are presented in Table 1. The experimental region is characterized by transitional climatic conditions, with pronounced temperature fluctuations during spring. During the planting period, minimum and maximum air temperatures were recorded as -3.1°C and 27°C , respectively, while the mean temperature was 10.99°C . During the harvest period, sudden temperature variations were observed. Average relative humidity reached its highest value in April (60.44%). Precipitation during the cultivation period was very limited or absent; therefore, crop water requirements were met through drip irrigation.

Table 1: Monthly minimum, maximum, and average air temperature (°C), relative humidity (%), and average precipitation (mm) during the broccoli growing season.

Month	Temperature (°C)			Relative Humidity (%)			Precipitation (mm)
	Min.	Max.	Avg.	Min.	Max.	Avg.	Avg.
March	−4.4	27.1	10.39	9	98	49.77	0.07
April	−3.1	27.0	10.99	11	98	60.44	0.00
May	5.5	31.8	16.97	13	98	56.84	2.00
June	9.0	36.6	23.69	5	97	29.88	0.07
July	15.2	40.6	26.82	5	96	40.77	0.08

Min.: Minimum, Max.: Maximum, Avg.: Average.

3.2 Analysis of Soil Characteristics

Prior to sowing, soil samples were collected and analyzed to determine baseline soil properties. The soil was classified as clay loam, with a pH of 7.16 and an electrical conductivity (EC) of 0.18 mS cm^{−1}. Organic matter content was 1.8%, and CaCO₃ content was 0.7%. Available P and K contents were 11.1 and 47.70 kg ha^{−1}, respectively. Micronutrient concentrations were 10.66 mg kg^{−1} Fe, 1.47 mg kg^{−1} Zn, 3.91 mg kg^{−1} Cu, and 16.41 mg kg^{−1} Mn.

3.3 Yield Performance

Total yield of broccoli exhibited slight numerical differences among the different colored plastic mulch treatments; however, these variations were not statistically significant ($p > 0.05$) (Table 2). The highest total yield was recorded under red mulch (6.76 t ha^{−1}), followed closely by black mulch (6.24 t ha^{−1}). In contrast, relatively lower yields were obtained from grey (5.41 t ha^{−1}) and brown mulch (5.98 t ha^{−1}) treatments. Despite these numerical trends, statistical analysis indicated that mulch color did not have a significant effect on total yield under the open-field conditions of the present study.

Table 2: Effects of different colored plastic mulches on total yield (t ha^{−1}) of broccoli grown under open-field conditions in the 2025 spring season.

Mulches	Red Mulch	Black Mulch	Grey Mulch	Brown Mulch
Total yield	6.76	6.24	5.41	5.98
LSD _{0.05}	NS			

NS: Not Significant at $p > 0.05$ according to the Analysis of Variance (ANOVA); LSD: Least Significant Difference test used for mean separation.

3.4 Antioxidant Activity and Vitamin C Content

Mulch color had a highly significant effect on both antioxidant activity and Vitamin C content of broccoli heads ($p \leq 0.001$) (Table 3). Among the treatments, grey mulch resulted in the highest antioxidant activity (462.61 $\mu\text{mol TE } 100 \text{ g}^{-1}$), followed by red and black mulches, while the lowest value was observed under brown mulch (305.42 $\mu\text{mol TE } 100 \text{ g}^{-1}$). A similar pattern was observed for Vitamin C content. Broccoli grown under grey mulch showed the highest Vitamin C concentration (103.69 mg 100 g^{−1}), whereas brown mulch produced the lowest value (38.30 mg 100 g^{−1}). Red and black mulches resulted in intermediate Vitamin C levels.

Table 3: Effects of different colored plastic mulches on total antioxidant activity ($\mu\text{mol TE } 100 \text{ g}^{-1} \text{ FW}$) and vitamin C ($\text{mg } 100 \text{ g}^{-1} \text{ FW}$) of broccoli grown under open-field conditions.

Colored Mulches	Antioxidant Activity ($\mu\text{mol TE}/100 \text{ g}$)	Vitamin C ($\text{mg}/100 \text{ g}$)
Red mulch	$398.95 \pm 0.82\text{b}$	$74.93 \pm 0.55\text{b}$
Black mulch	$363.57 \pm 2.85\text{c}$	$62.57 \pm 0.59\text{c}$
Grey mulch	$462.61 \pm 1.70\text{a}$	$103.69 \pm 0.83\text{a}$
Brown mulch	$305.42 \pm 0.38\text{d}$	$38.30 \pm 0.23\text{d}$
LSD _{0.05}	3.26	1.11

Values represent the mean of leaf samples taken from three replications of each mulch application. Means followed by different letters within the same column differ significantly at $p \leq 0.05$ according to the Least Significant Difference (LSD) test. $p \leq 0.001$: Indicates highly significant differences. TE: Trolox equivalents; FW: Fresh weight.

3.5 Mineral Nutrient Composition

Mulch color significantly affected mineral nutrient accumulation in broccoli heads (Table 4). N content differed among treatments ($p \leq 0.01$), with black and brown mulches resulting in significantly higher N percentages compared to red and grey mulches. The highest N content was recorded under black mulch (1.02%), while grey mulch showed the lowest value (0.78%). K concentration was strongly influenced by mulch color ($p \leq 0.001$). Broccoli grown under black mulch exhibited the highest K content (3403.06 ppm), followed by red mulch, whereas the lowest K concentration was observed under brown mulch (3053.65 ppm). Fe content also varied significantly among treatments ($p \leq 0.001$). The highest Fe concentration was obtained from broccoli heads grown with brown mulch (34.93 ppm), while the lowest value was recorded under grey mulch (15.56 ppm). Selenium (Se) concentration in all broccoli head samples was below the detection limit of the analytical method ($<0.010 \text{ ppm}$). Therefore, Se data were not subjected to statistical analysis and are not presented.

Table 4: Effects of different colored plastic mulches on mineral nutrient composition of broccoli grown under open-field conditions.

Colored Mulches	N (%) [*]	K (ppm) ^{**}	Fe (ppm) ^{**}
Red mulch	$0.84 \pm 0.03\text{b}$	$3230.77 \pm 5.50\text{b}$	$21.92 \pm 0.45\text{c}$
Black mulch	$1.02 \pm 0.04\text{a}$	$3403.06 \pm 5.80\text{a}$	$28.57 \pm 0.58\text{b}$
Grey mulch	$0.78 \pm 0.03\text{b}$	$3097.22 \pm 5.25\text{c}$	$15.56 \pm 0.32\text{d}$
Brown mulch	$1.00 \pm 0.04\text{a}$	$3053.65 \pm 5.18\text{d}$	$34.93 \pm 0.71\text{a}$
LSD _{0.05}	0.13	9.54	2.49

Results are expressed as mean $\pm$ standard deviation (SD) of leaf samples taken from three replications of each mulch application. Means followed by different letters within the same column differ significantly at $p \leq 0.05$ according to the Least Significant Difference (LSD) test. ^{*} $p \leq 0.01$, ^{**} $p \leq 0.001$.

4 Discussion

Total yield was not significantly affected by mulch color, indicating that broccoli biomass production remained stable under the environmental conditions of the experiment. Although yield differences among mulch treatments were not statistically significant, the overall yield level observed in the present study was relatively moderate compared to some commercial production systems. This may be attributed to the specific agroclimatic conditions of the experimental site, located at 1100 m altitude, where pronounced day–night temperature fluctuations and low early-season temperatures were recorded. Broccoli, are crops with an optimal temperature range of 15–18°C for vegetative growth, while during the initiation of the head's formation in broccoli, temperatures in the range of 15–23°C or an average temperature $<25^\circ\text{C}$ [23].

Floral development in broccoli is more susceptible to heat stress than vegetative growth [24]. In addition, precipitation during the growing period was negligible, and crop water requirements were supplied exclusively through drip irrigation. Plant water status and irrigation greatly affect plant growth and function [24]. Presumably, the permeability of plastic mulches to soil water vapor is determined by the composition and thickness of the mulch [25]. Under such conditions, fluctuations in evapotranspiration demand may have influenced plant growth and biomass accumulation. These combined environmental factors may have limited yield potential under the cool spring conditions of the study area. These findings indicate that mulch plays a role in regulating physiological processes, mineral and water uptake in roots. Similar findings have been reported influenced plant physiology and quality-related characteristics [26–28].

The results of this study clearly indicate that while mulch color does not significantly impact total yield, it markedly influences the biochemical quality and mineral composition of broccoli heads. Specifically, light-colored surfaces such as grey mulch enhanced biochemical traits, yielding the highest antioxidant activity ($462.62 \mu\text{mol TE } 100 \text{ g}^{-1}$) and Vitamin C content ($103.69 \text{ mg } 100 \text{ g}^{-1}$) likely due to increased light reflectance into the plant canopy. As emphasized by Díaz-Pérez [24], although specific microclimatic data such as root-zone temperature (RZT), moisture, and light reflectance were not directly measured in this experiment, the observed differences in plant quality align with established mechanisms where mulch-induced environmental changes influence metabolic pathways. Such modifications are known to regulate antioxidant and vitamin synthesis, suggesting that the plastic mulches used in this study enhanced biochemical parameters by creating more favorable root-zone or canopy conditions [29–32]. The antioxidant capacity of Brassica species has been relevant to its phenolic profile and content, particularly flavonoids, since phenolic compounds have showed a higher antioxidant activity than vitamins and carotenoid [33]. Numerous environmental factors and analysis method affect the concentrations of phenolic compounds. Brassica species, the accumulation of vitamin C and phenolic compounds is particularly sensitive to environmental factors during head development [33–35]. The increased antioxidant capacity under grey mulch suggests that relatively mild modifications of the soil microclimate can support metabolic processes involved in the accumulation of health-related compounds, without necessarily inducing strong stress responses.

Mineral nutrient composition also varied depending on mulch color, indicating that nutrient uptake processes were influenced by the soil environment created by different mulch materials. The higher N and K concentrations observed under black mulch are likely associated with modified soil temperature and moisture conditions, which can enhance root activity and facilitate nutrient availability by stimulating microbial processes and mineralization [36,37]. In contrast, Fe accumulation was highest under brown mulch, further supporting the premise that mulch films significantly affect nutrient dynamics [38] and soil ecosystems by altering the soil microclimate [36]. Such improved soil water-temperature conditions under plastic sheeting can stimulate soil microbial activity and enzyme production, thereby directly influencing the mineral content of the plant [39]. The findings of this study demonstrate that colored mulches significantly enhance nutrient uptake, a result that is consistent with previous reports by Sarkar et al. [40].

Taken together, the results of this study indicate that mulch color does not affect all quality traits in the same manner. While grey mulch was associated with higher antioxidant activity and Vitamin C content, black and brown mulches were more closely linked to increased mineral nutrient accumulation, particularly N, K, and Fe. These results indicate that mulch color modifies the soil microenvironment in different ways, leading to trait-specific responses in broccoli quality. Although soil temperature and moisture were not measured directly in the present study, previous research has shown that colored plastic mulches alter the soil microclimate by affecting heat absorption, light reflection, and water conservation. These changes may influence root activity, microbial processes, nutrient availability, and plant metabolic responses, which could

explain the differences observed in mineral accumulation and biochemical composition. Such differentiated effects suggest that pre-harvest environmental management can regulate both nutrient uptake and secondary metabolite accumulation through multiple pathways [3]. Overall, the findings demonstrate that although marketable yield remained stable, colored plastic mulches significantly influenced the nutritional and biochemical characteristics of broccoli, highlighting mulch color selection as a practical field management option for improving crop quality under open-field conditions.

5 Conclusion

Under open-field conditions, mulch color did not significantly affect broccoli yield, indicating stable biomass production across all treatments. In contrast, key quality parameters were markedly influenced by mulch type. While grey mulch was associated with superior antioxidant activity and Vitamin C content, mineral nutrient accumulation varied significantly depending on mulch color. The observed differences in mineral accumulation and metabolic activity likely stem from mulch-induced modifications of the soil microenvironment. Overall, the results demonstrate that the appropriate selection of colored plastic mulches can significantly enhance the nutritional and biochemical quality of broccoli without compromising total yield.

Furthermore, under the variable conditions of a transition zone, the use of colored plastic mulches proved to be an effective strategy for optimizing crop quality. In this context, mulch application should be considered not only as a conventional cultural practice but also as a strategic cultivation tool to improve plant adaptation and enhance the synthesis of health-promoting compounds.

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