Bozok Journal of Science
Volume 4, No 1, Page 22-33 (2026)
Research Article DOI: 10.70500/bjs.1924022

Integrated Assessment of Antioxidant-Related Traits in *B. rapa* ssp. *oleifera* Seeds under Different Sowing Densities

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Received: 06.04.2026 Accepted: 26.04.2026 Final Version: 04.05.2026

Abstract

This study investigated the effects of different sowing densities on the antioxidant profile of *B. rapa* ssp. *Oleifera* seeds using an integrated analytical approach. Field trials were conducted in a randomized complete block design with four replicates at sowing densities of 150, 200, 250, and 300 seeds m^{-2}. Total phenolic content, total flavonoid content, DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) were measured. Because these parameters were expressed in different units, the data were standardized using the z-score method to enable integrated evaluation. The results showed that antioxidant-related traits responded to sowing density in a parameter-dependent manner rather than following a single common trend. The second sowing density produced the most balanced antioxidant profile, whereas the first density had the highest FRAP value. Total flavonoid content was highest at the third sowing density, while total phenolic content reached its highest level at the fourth density. However, the increase in phenolic content at the highest density was not accompanied by a comparable increase in antioxidant activity. These findings indicate that the optimum sowing density should be evaluated not only in terms of yield, but also with respect to antioxidant-related biochemical quality traits.

Keywords: Oilseed turnip, Sowing density, Antioxidant activity, Phenolic compounds, Flavonoids

1. INTRODUCTION

B. rapa ssp. *oleifera* (oilseed turnip) belongs to one of the most economically important plant genera – the genus *Brassica*. Numerous characteristics made it an object of extensive research – high plasticity, versatility of application, and the ability to satisfy various agricultural needs. It can be grown for roots, leaves, and seeds (Kayaçetin, 2020; Kayaçetin, 2023; Cartea et al., 2021; Aydın et al., 2024). Moreover, nowadays, its cultivation is considered not only as a field crop but also as a source of bioactive compounds that can improve human health. Biochemical research related to *Brassica* species tends to be focused primarily on phenolics, flavonoids, and antioxidants since they are the main criteria of functional value and nutritive importance (Dias, 2022).

An efficient antioxidative potential is not determined by one compound, and its estimation implies the consideration of several mechanisms – accumulation of phenolics, formation of flavonoids, ability to scavenge radicals, and redox potential. Thus, it is quite usual to evaluate total phenolics, total flavonoids, DPPH radical scavenging capacity, and ferric reducing antioxidant power (FRAP) (Sajjadi, 2025). However, sometimes these parameters do not tend to behave similarly: while one sample shows excellent redox potential, another does not have increased scavenging activity, and an increase in flavonoid content does not necessarily correspond to increased phenolic content. Therefore, the overall pattern of these indicators should be considered as informative as the indicators themselves (Čalkaitė et al., 2022; Amarowicz et al., 2024; Thani et al., 2024). In such circumstances, cultivation plays an especially important role. Agronomic factors, sowing density among them, affect plant development and growth. They influence canopy architecture, light absorption, nutrient availability, and competitiveness. Consequently, apart from changing growth rate and yield, sowing density may change the metabolism through which bioactive compounds appear and accumulate. Intensified competition and stress caused by increased sowing density can result in redistribution of carbon flow, whereas other sowing densities may stimulate metabolic reactions that are necessary for secondary metabolite formation (Škerget et al., 2005; Celis et al., 2014). However, although the number of studies related to the effect of sowing density on yield is considerable, research devoted to the effect on biochemistry

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and metabolism is much smaller, particularly for antioxidants. There are numerous studies related to the effect of sowing density on *B. rapa* seeds' composition, but antioxidant properties are rarely examined as separate parameters. Therefore, it is difficult to say which sowing density is best for antioxidant properties, since different indicators are used for the analysis, and a single factor is considered to evaluate the effect of sowing density on antioxidant activity.

The problem is to determine not whether a given parameter increases or not but how all the parameters related to antioxidant activity interact with each other in response to sowing density. It can be resolved with the help of integrated approaches. Normalizing and transforming data obtained in different analyses on one scale allows the comparison of the results and the definition of which sowing density promotes the highest degree of antioxidant interaction between the analyzed properties. In the case when the indicators tend to contradict each other, z-score calculation serves as a better option than simple transformation. Therefore, the goal of this paper is to evaluate antioxidant-related properties in *B. rapa* seeds under various conditions of sowing density by means of the integrated evaluation of indicators, including total phenolic content, total flavonoid content, DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP).

2. MATERIAL AND METHODS

2.1 Chemicals

All chemicals and analytical-grade standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagents used to measure total phenolic content and antioxidant activity such as Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), gallic acid, rutin, and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid]. Ultrapure water with a resistivity of 18.2 MΩ·cm (Milli-Q, Millipore) was used throughout all experimental procedures.

2.2 Plant Material and Experimental Plot

Seeds of *Brassica rapa* ssp. *oleifera* were used as the plant material in this study. Seeds used for analysis purposes were taken from a field experiment carried out in the Keçiören area in Ankara, Türkiye and harvested in 2025. Field experiments were set up in the Keçiören experimental farm in the province of Ankara. The most prominent environmental and soil conditions of the experimental site are listed in Table 1.

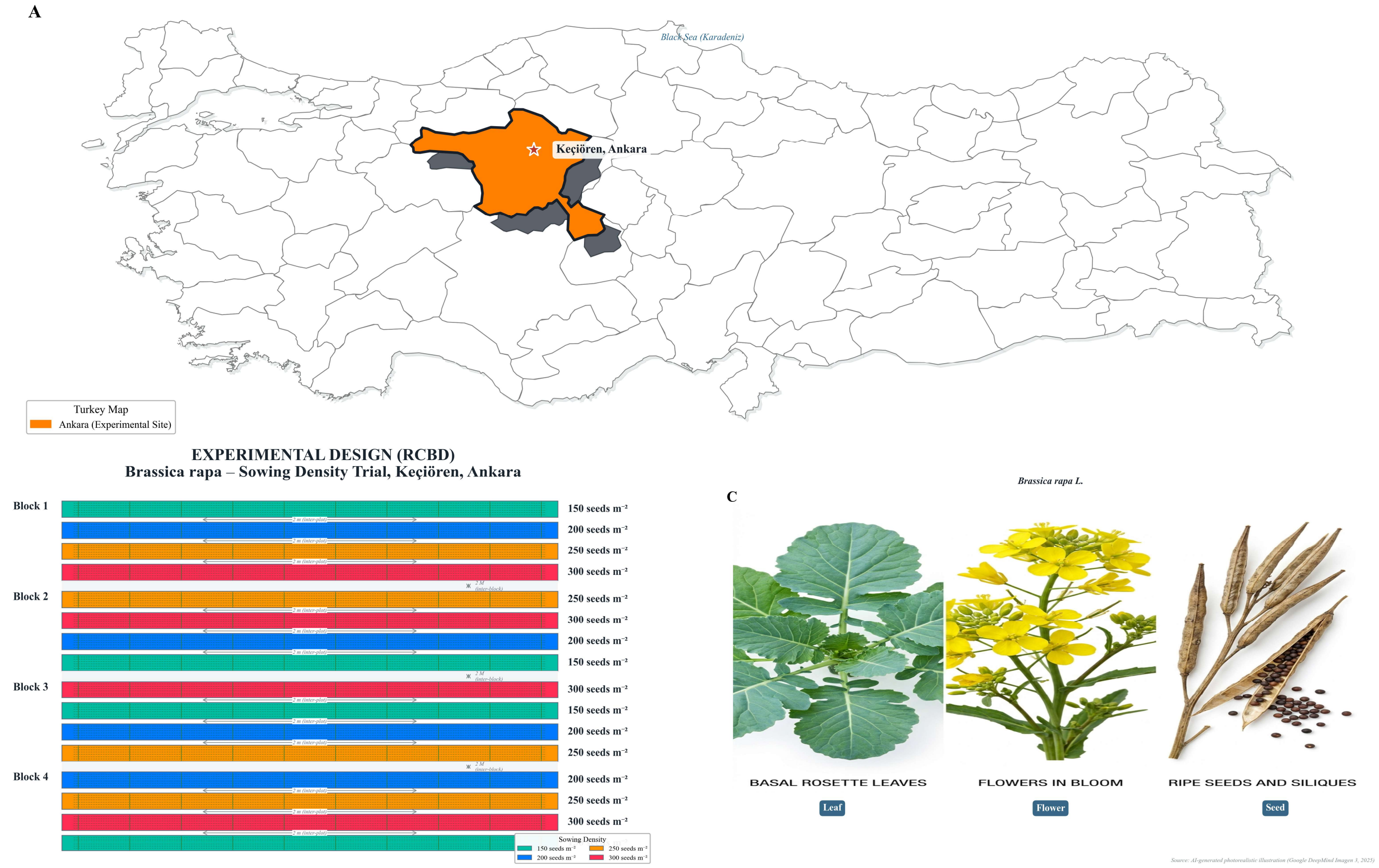
Table 1. Main environmental and soil characteristics of the Keçiören experimental plot

Property	**Value**
Location	Keçiören, Ankara, Türkiye
Altitude	860 m
Climate type (Köppen-Geiger)	Dsa
Soil texture	Clay
pH	7,70
EC (dS m^{-1})	0,50
Organic matter (%)	1,40
$CaCO_3$ (%)	17,30
Total N (%)	0,08
P_2O_5 (mg kg^{-1})	22,70
K_2O (mg kg^{-1})	963,60
Long-term mean maximum temperature	36.8 °C
Long-term mean minimum temperature	-9.5 °C
Relative humidity range	43–76%

Note: EC = electrical conductivity; $CaCO_3$ = calcium carbonate; P_2O_5 = available phosphorus; K_2O = available potassium. Temperature and relative humidity values are based on long-term climatic records for the study area2.3. Experimental Design and Sowing Densities

The field experiment was established in a randomized complete block design with four replications. Four different sowing densities were applied in the study: 150, 200, 250, and 300 seeds m^{-2}. These densities corresponded approximately to seed rates of 300, 400, 500, and 600 g da^{-1}, respectively. The sowing densities were selected based on previous studies conducted under similar agroecological conditions.

Sowing was carried out manually at the Keçiören location. The plot size was arranged as 6 m × 1.8 m (10.8 m^2), and each plot consisted of six rows with a row spacing of 30 cm. 1.5 m was left between plots and 2 m between blocks. To minimize border effects, the two outer rows of each plot and 0.5 m from both the beginning and the end of each row were excluded from evaluation. Based on this arrangement, the effective sampling area was accepted as 6 m^2. The location of the experimental site and the general layout of the field plots are presented in Figure 1.



Source: AI-generated photorealistic illustration (Google DeepMind Imagen 3, 2025)

Figure 1. Location of the Keçiören experimental site where different sowing density treatments were applied in *Brassica rapa*, together with general views of the experimental plots.
(A) Geographic location of the Keçiören experimental site in Ankara, Türkiye. **(B)** Schematic representation of the randomized complete block design (RCBD) used in the sowing density trial, showing the four blocks and the distribution of the sowing density treatments. **(C)** Representative images of Brassica rapa L. at different developmental stages, including basal rosette leaves, flowering plants, and mature siliques with seeds.

All cultivation practices were carried out under the same field conditions so that the observed differences could be attributed mainly to sowing density. Seed samples were obtained from the effective sampling area of each plot, and all analyses were performed using these samples.

2.4 Sample Seed Preparation and Extracts Preparation

Initial preparation of seed samples of *Brassica rapa* consisted of cleaning, homogenization, and grinding the seeds into powder in the Foss mill for future analysis. The prepared powder was kept in a sealed container at 4 °C until further use. The extraction process involved mixing 0.5 g of seed powder with 10 ml of 80% methanol followed by shaking at 150 rpm for 60 minutes and centrifugation at 5000 rpm for 5 minutes. Supernatants underwent two repeated extractions using the same procedure. All collected extracts were mixed and diluted to 30 ml with 80% methanol. Analysis was done within 24 hours after the extraction.

2.5 Total Phenolic and Flavonoid Content

2.5.1 Total Phenolic Content (TPC)

The total phenolic content determination was performed according to the Folin–Ciocalteu method. Initially, 0.5 ml of the sample extract was added to 2.5 ml of the 10% (v/v) Folin–Ciocalteu reagent solution. After that, 2.0 ml of 7.5% sodium carbonate solution was added. The whole solution was kept in darkness at room temperature for 30 minutes. Further measurement of absorbance was performed at a wavelength of 765 nm. The total phenolic content was calculated in milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw) according to the calibration curve made on a gallic acid standard (Ozcan et al., 2009).

2.5.2 Total Flavonoid Content (TFC)

To determine the total flavonoid content in a sample, aluminum chloride colorimetric method was used. It implied adding 0.5 ml of the diluted sample to 0.15 ml of 5% $NaNO_2$. Six minutes later, 0.15 ml of 10% $AlCl_3$ solution was added. After another 5 minutes, 1.0 ml of 1 M NaOH was added and diluted to 5 ml with distilled water. Measurement of absorbance was carried out at a wavelength of 510 nm. Results were reported as milligrams of rutin equivalents per gram of dry weight (mg RE g^{-1} dw) using rutin calibration curve (Shraim et al., 2021).

2.6. Determination of Total Antioxidant Capacity

2.6.1. DPPH Radical Scavenging Activity

A volume of 0.1 ml of extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm (Gulcin & Alwasel, 2023).

2.6.2. FRAP Assay

The FRAP assay was performed according to the ferric reducing method. The FRAP reagent was prepared from a mixture of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ, and 20 mM $FeCl_3 \cdot 6H_2O$. A volume of 3.0 ml of FRAP reagent was added to 100 µl of extract, and the mixture was incubated at 37 °C for 30 min. Absorbance was measured at 593 nm. Antioxidant capacity was expressed as µmol Trolox equivalents g^{-1} dry matter using a Trolox standard curve (Avelino, 2024).

2.7. Integrated Evaluation Based on z-Score Analysis and Statistical Analysis

A z-score standardization approach was used to evaluate the relative effects of different sowing densities on antioxidant-related parameters in an integrated way. Values for DPPH, FRAP, total phenolic content, and total flavonoid content were standardized within each parameter and converted into z-scores. Since lower IC_{50} values in the DPPH assay indicate higher antioxidant activity, a direction adjustment was applied before standardization. Thus, higher z-score values consistently represented better performance across all parameters. In addition, antioxidant-related parameters were also evaluated separately within each sowing density based on their z-scores to identify relatively stronger and weaker traits under each treatment. The standardized data were interpreted using a heat map and a ranking approach.

Statistical analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC, USA), and graphs were prepared using OriginPro (OriginLab Corporation, Northampton, MA, USA). The experiment was conducted as a randomized complete block design (RCBD) with four sowing densities (150, 200, 250, and 300 seeds m^{-2}) and four blocks. Each parameter was analyzed in three independent experiments, with two parallel replicates in each experiment. Statistical significance was accepted at $p < 0.05$.

For multivariate analysis, the biochemical variables were z-score standardized to remove scale differences among parameters. Principal component analysis (PCA) was then performed to evaluate treatment distribution and associations among antioxidant-related traits. To examine the response of antioxidant-related parameters to sowing density, regression analyses were performed, and linear or quadratic models were selected according to the observed data pattern and goodness of fit. The fitted models were presented with 95% confidence intervals. Pearson correlation coefficients were also calculated to assess relationships among the measured variables, and the results were visualized using a correlation heatmap. Sowing density was expressed as seeds m^{-2} throughout the statistical analysis and presentation of results.

3. RESULTS AND DISCUSSION

3.1 Integrated evaluation of antioxidant activity in *Brassica rapa* seeds under different sowing densities

In the examined dataset, no linear correlation between DPPH antioxidant activity in *Brassica rapa* seeds and sowing density was identified (Table 2). Optimum DPPH activity was recorded at the second level of sowing density; the third level had moderately good DPPH activity, while the first one showed the lowest performance. For the fourth level of sowing density, DPPH activity was not fully absent but appeared to be relatively poor compared to the second and third ones. This observation may indicate that moderate rather than extremely low or high plant density favors free radical scavenging capacity.

The impact of plant density on photosynthetic pigments, competition for light or resources, and environmental stress, in general, can potentially influence phytochemical production. In Brassica crops, environmental and agronomic factors are likely to have a considerable effect on phytochemical content, which depends on the intensity of the effect from the environment (Biondi et al., 2021).

Table 2. Standardized z-score performance of DPPH, total phenolic content, total flavonoid content, and FRAP in *Brassica rapa* seeds under different sowing densities

Sowing density	DPPH	Phenolic	Flavonoid	FRAP
1	-0.50	-0.03	0.07	0.67
2	0.35	0.10	-0.55	0.63
3	0.18	-0.38	0.67	-0.11
4	-0.04	0.31	-0.19	-1.19

However, when analyzed separately, the FRAP data provided a different pattern of correlation (Table 2). As can be seen from Table 2, the most prominent relative effectiveness of samples was reached at the first and second sowing densities. From that point on, a sharp decrease began, and the reducing power declined drastically by the fourth sampling density. Thus, it can be stated that the ferric reducing capacity is more sensitive to plant density increase compared to the DPPH activity.

Under higher density, increased competition for available light, nutrients, and space might limit the biosynthesis or efficacy of plant substances responsible for electron transfer-based reducing capacity (Demmig-Adams et al., 2022). It is known that cultivation conditions, like density and irrigation, have an impact on the phenolic composition and antioxidant activity in Brassica products; moreover, at some levels of density, the phytochemical compounds responsible for reduction potential seem to be especially susceptible (Biondi et al., 2021).

Table 3. Standardized z-score distribution of DPPH, total phenolic content, total flavonoid content, and FRAP across parameters within each sowing density in *Brassica rapa* seeds

Sowing density	FRAP	Flavonoid	Phenolic	DPPH
1	1.29	0.04	-0.18	-1.15
2	0.99	-1.36	-0.06	0.43
3	-0.45	1.29	-1.04	0.21
4	-1.42	0.14	0.91	0.37

If comparing the DPPH and FRAP together, one can conclude that these tests have reacted to the changes in sowing density differently. It is methodically sound since, as it is mentioned above, the first test measures free radical scavenging capacity, while the second one quantifies ferric reducing power via the mechanism of single electron transfer (Lin & Zhao, 2010; Avelino, 2024). From the available literature, it can be concluded that usually the FRAP value correlates well with the Folin-Ciocalteu, while the DPPH does not correlate very well with any other test used for antioxidant activity measurement. This means that the antioxidant behavior measured with the DPPH assay cannot be considered similar. On the contrary, the opposing dynamics in the two tests within one substance should be expected since antioxidants consist of multiple components. Therefore, in *Brassica* products, the antioxidant activity is dependent not only on the concentration of phenolics but also their composition.

When assessing the effects of sowing densities using assays of antioxidant activity, there was a noticeable shift in the dominant antioxidant response (Table 3). With the first and second densities, FRAP exceeded DPPH, meaning that antioxidant activity with those densities was mostly determined by reducing capacity. At the third and fourth densities, however, DPPH outstripped FRAP, indicating that the dominant antioxidant response appeared to shift with increasing plant density. At low and moderate densities, where environmental conditions, especially light, are relatively favorable and intraspecific competition is lower, electron transfer might be more prevalent in terms of the defense capacity. With high density, the system seems to be rebalancing towards the radical scavenging mechanism. The literature suggests that plant density may impact on the antioxidant activity and phytochemical composition of *Brassica* (Li et al., 2022; Sakinah et al., 2022; Cowden et al., 2024). Taken together, the combined use of DPPH and FRAP provided a broader view of antioxidant activity under different sowing densities.

3.2 Integrated evaluation of antioxidant content in *Brassica rapa* seeds under different sowing densities

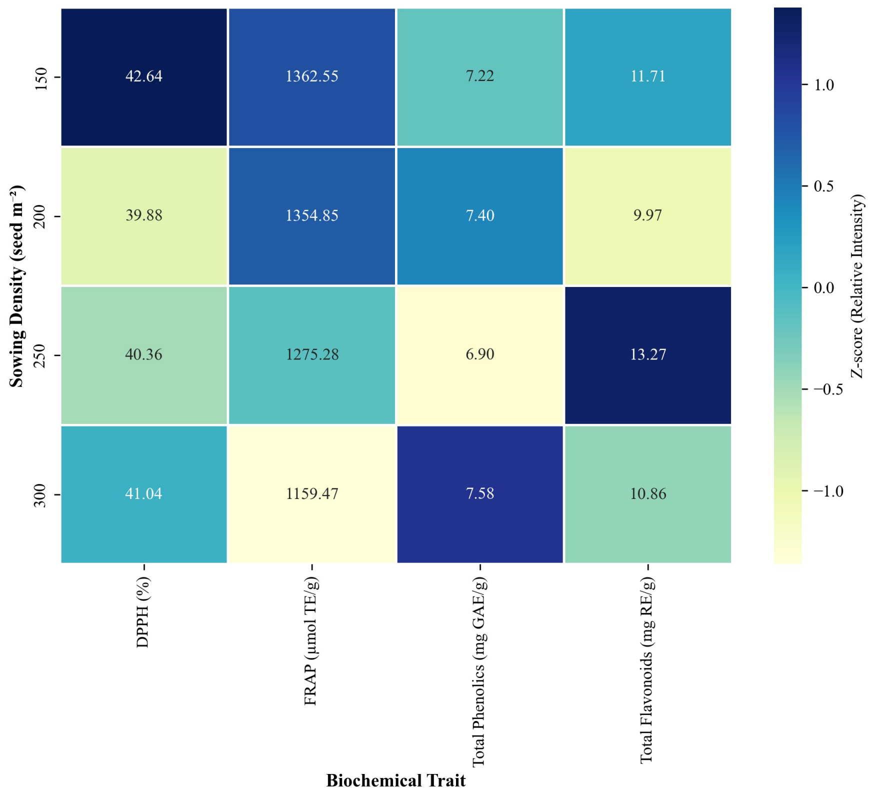


Figure 2. Z-score heatmap of standardized biochemical traits across sowing densities. Each cell represents the relative performance (Z-score) of individual biochemical traits (DPPH, FRAP, Total Phenolics, and Total Flavonoids) across four distinct sowing densities (150, 200, 250, and 300 seed m^{-2}). Numerical values denoted within cells represent the original measured mean values for each trait-density combination.

The response to changes in sowing density in terms of total phenolic content within the dataset in which this trait appeared to be more prominent did not follow a strictly positive or negative trend either (see Table 2). Namely, the fourth sowing density provided the best relative value in this regard, the second one – the second-best, the first one was close to a neutral state, and the worst performance corresponded to the third sowing density. The presented results point towards the likelihood that phenolic content can rise in low-density environments, as well as when a certain degree of competition takes place. This overall distribution of standardized biochemical responses across sowing densities is more clearly illustrated in the heatmap shown in Figure 2. As shown in Figure 2, the biochemical response pattern was not uniform across sowing densities. The 150 seed m^{-2} treatment was relatively more favorable for DPPH and FRAP, whereas total phenolics reached their strongest relative position at 300 seed m^{-2} and total flavonoids were more prominent at 250 seed m^{-2}. This pattern indicates that sowing density affected antioxidant-related traits in a trait-specific manner rather than producing a single common optimum for all biochemical parameters.

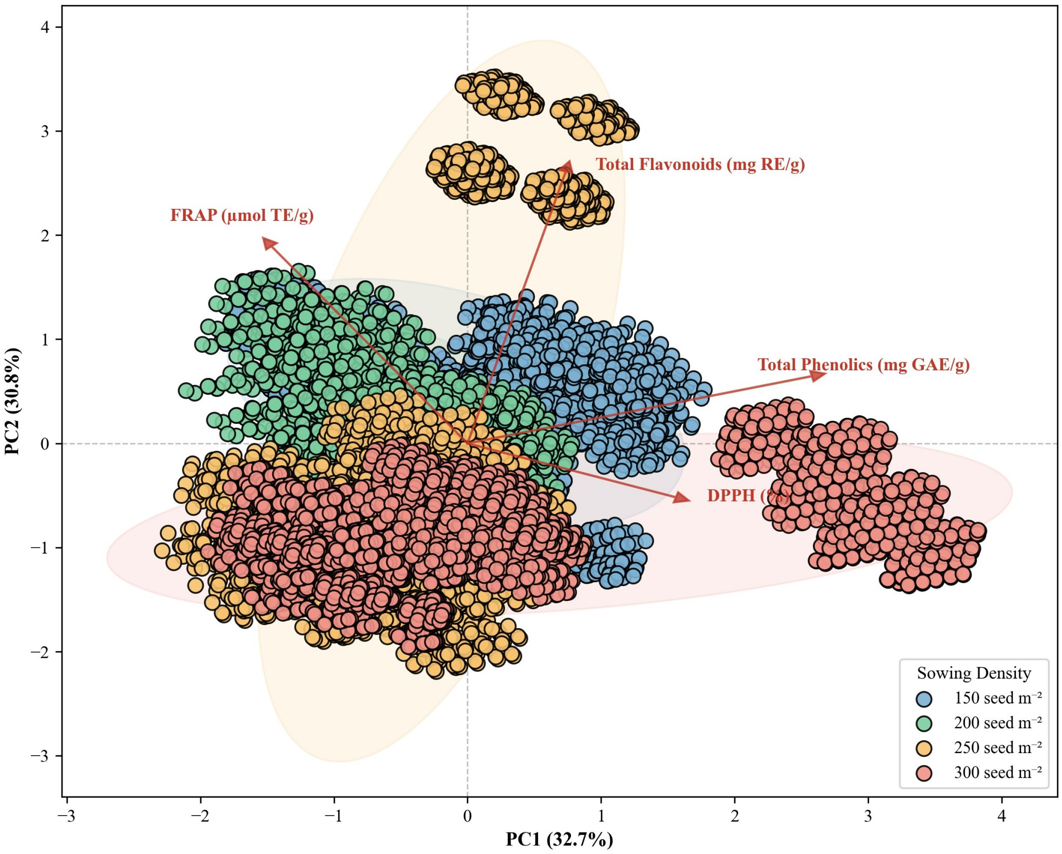


Figure 3. Principal Component Analysis (PCA) biplot of biochemical parameters. The biplot illustrates the distribution of treatments across the first two principal components (PC1 and PC2), which explain the majority of the total variance. Data points represent individual replicates color-coded by sowing density (150, 200, 250, and 300 seed m^{-2}), accompanied by 95% confidence ellipses. Red vectors demonstrate the loadings of the measured biochemical traits onto the principal components.

The PCA biplot revealed that the biochemical traits did not vary in a fully uniform manner across sowing densities but instead formed partially distinct response patterns (Figure 3). PC1 and PC2 together explained a substantial proportion of the total variance, indicating that the first two components captured the main structure of the dataset. DPPH and total phenolics were positioned more strongly along the positive side of PC1, whereas FRAP was associated more with the negative PC1 and positive PC2 direction. Total flavonoids showed a stronger contribution along the positive PC2 axis. In terms of treatment distribution, the 300 seed m^{-2} group tended to align more closely with DPPH-related variation, while the 200 seed m^{-2} treatment was positioned more in the FRAP-associated region. The 250 seed m^{-2} treatment showed a closer association with the flavonoid direction. Overall, the PCA supports the view that sowing density altered the biochemical profile in a trait-dependent rather than uniform manner.

Phenolics are known major components in plant defense metabolism (Kostidis & Karabourniotis, 2024), meaning that the observed superiority of the fourth sowing density could reflect a broader defense response caused by dense planting of seeds. When assessing total flavonoid content, it became clear that the third sowing density provided the best relative performance, followed by the first one, with flavonoid content declining at the fourth sowing density while reaching its lowest levels at the second one. In other words, flavonoids might not respond universally to changing environmental cues as total phenolic content. Flavonoids are secondary metabolites whose production is directly affected by environmental conditions such as light quality and oxidative stress. The pronounced superiority of the third sowing density in terms of flavonoid content appears to indicate that the competition was sufficient to stimulate a metabolic response, but not too intense to cause excess stress (Liu et al., 2021; Patil et al., 2024; Nayak et al., 2025). Combining results for phenols and flavonoids makes it clear that these two metabolite classes responded differently to changes in sowing density. Although both originated from the phenylpropanoid pathway, these metabolites do not react uniformly to environmental stimuli and metabolic processes (Zhang et al., 2022). This means that increased total phenolic content does not guarantee increased total flavonoid content. In other words, antioxidant content is not a single-dimensional trait but reflects the uneven distribution of metabolites across different branches of secondary metabolism. Therefore, a conclusion about the quality of *B. rapa* seeds based exclusively on any of these antioxidants is not fully appropriate. To further visualize the multivariate relationship among these biochemical traits and sowing density treatments, a PCA biplot was generated (Figure 3).

In fact, a redistribution of content along antioxidant metabolites becomes even more apparent when evaluating these variables internally in accordance with their role in the antioxidative defense. First, phenolic compounds prevailed at the second and fourth sowing densities, whereas at the first and third densities, the prevalence went towards flavonoids. The presented results suggest that at certain densities, it suggests that the metabolic response may have shifted towards flavonoid accumulation, whereas at others, it is phenolics that are synthesized more actively. If the third and fourth densities are considered together, then it becomes clear that sowing density affects not only the amount of substances produced, but also the distribution ratio between them. It seems reasonable to assume that such phenomena occur due to competition for light and resources, leading to different forms of defense (Murchie & Burgess, 2022). Indeed, as the density of planting increases, there is not only competition for growing space, but also a shift in metabolic pathways in terms of light quality and oxidative reactions. Namely, phenolic compounds appear to respond more widely to the environmental situation and can be associated with defense, whereas flavonoids respond more closely to light conditions and oxidative stress (Ferreyra et al., 2021). The fact that flavonoids prevail at low density levels and phenolic compounds are synthesized more abundantly in dense environments means that may reflect a redistribution in the relative contribution of phenolic and flavonoid pools in response to changing environmental stimuli.

Thus, the impact of sowing density on antioxidant content was revealed on the quantitative and compositional levels. The partial overlap of the response of phenolic and flavonoid content means that assessment of seed quality cannot be performed relying only on the amounts of antioxidants. It is necessary to pay attention to the ratios between the substances themselves and the activity of these compounds. Therefore, total phenolic and flavonoid contents, as well as activity measured with DPPH and FRAP, should be considered when selecting the optimal sowing density.

3.3. Evaluation of the relationship between antioxidant content and antioxidant activity in *Brassica rapa* seeds

When antioxidant content results were considered together with antioxidant activity data, a more informative pattern emerged (Tables 2 and 3). At the third sowing density, the prominence of flavonoid content alongside the relatively favorable position of DPPH suggests that flavonoids may have contributed more strongly to free radical scavenging capacity. The direction and shape of these trait-specific responses across sowing density are further summarized by the fitted regression models presented in Figure 4. Among the evaluated traits, FRAP showed the clearest density-dependent pattern, with a significant quadratic decline as sowing density increased, whereas DPPH displayed only a weak curvilinear response. In contrast, total phenolic and total flavonoid contents did not show statistically meaningful linear trends, suggesting that sowing density influenced antioxidant activity more strongly than total antioxidant content.

The DPPH assay mainly reflects radical scavenging ability, and the response in this assay depends on the capacity of antioxidant compounds to donate hydrogen or electrons. For this reason, a flavonoid-enriched profile would be expected to exert a more favorable effect on DPPH from a biochemical point of view (Yeo & Shahidi, 2019; Gulcin and Alwasel, 2023).

By contrast, at the fourth sowing density, phenolic content was relatively strong, whereas FRAP remained clearly weak. This indicates that an increase in total phenolics was not reflected to the same extent in reducing power. One likely reason is that the FRAP assay depends not only on the total amount of phenolics, but also on the subgroup distribution and redox characteristics of those compounds (Avelino, 2024; Zhang et al., 2025). Indeed, although FRAP is often more closely related to Folin-Ciocalteu values, DPPH does not always behave in the same direction as other assays. This shows that different antioxidant assays reflect different functional aspects of the antioxidant system (Abramovič et al., 2017, 2018).

For this reason, the relationship between content and activity is not linear, but compositional and functional. An increase in a particular group of compounds does not necessarily produce a simultaneous increase in all antioxidant assays (Granato et al., 2018). What matters is not only the total level of phenolics or flavonoids, but also which phenolic subgroups dominate and which reaction mechanisms those compounds preferentially support (Platzer et al., 2022). Plant phenolics are structurally diverse, and the same total content level may therefore produce different responses across antioxidant assays (Vuolo et al., 2019).

The pattern observed in *Brassica rapa* seeds supports this interpretation. At sowing densities where flavonoid content was more prominent, DPPH also showed a more favorable profile, suggesting that radical scavenging capacity was influenced more strongly by certain compound groups. In contrast, at a density where phenolic content was relatively high, FRAP did not increase to the same extent, showing that antioxidant activity cannot be explained by total content alone. The effect of sowing density was therefore not limited to altering total antioxidant content; it also reshaped the functional nature of that content.

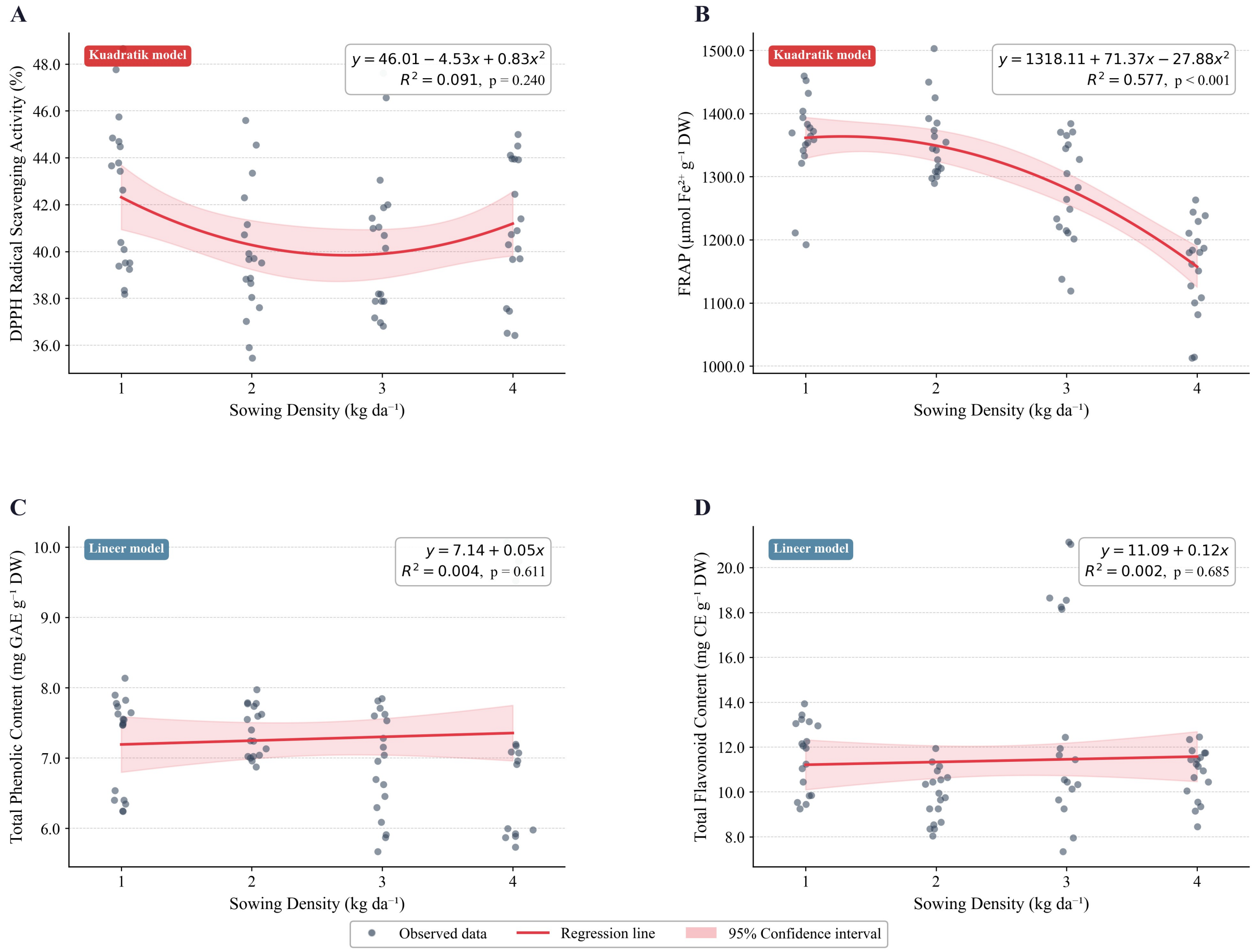


Figure 4. Regression models describing the relationship between sowing density and antioxidant-related traits in *Brassica rapa* seeds. **(A)** DPPH radical scavenging activity, **(B)** FRAP, **(C)** total phenolic content, and **(D)** total flavonoid content as affected by sowing density. Dots represent observed values, red lines indicate the fitted regression models, and shaded areas show the 95% confidence intervals. Quadratic models were fitted for DPPH and FRAP, whereas linear models were fitted for total phenolic and total flavonoid contents.

Overall, the relationship between antioxidant content and antioxidant activity was not one-dimensional, but multi-component in nature. Therefore, in evaluating seed quality in *B. rapa*, it is more reliable to interpret total phenolic and total flavonoid data together with activity assays such as DPPH and FRAP. This makes it possible to identify not only how sowing density affects compound levels, but also how those compounds are functionally expressed.

4. CONCLUSION

Sowing density significantly influenced the antioxidant profile of *Brassica rapa* seeds, and the response differed among parameters rather than following a single common pattern. While antioxidant activity assays and antioxidant content did not vary in the same way across densities, the integrated z-score approach showed that each sowing density produced a distinct biochemical profile. Intermediate densities provided the most balanced antioxidant performance, whereas higher density negatively affected some activity-related traits. These results indicate that the optimum sowing density should be evaluated not only in terms of yield, but also in relation to biochemical quality attributes linked to antioxidant potential.

AUTHOR'S CONTRIBUTIONS

Author 1: Conducted the antioxidant activity analyses, performed the statistical analysis, and contributed to manuscript writing. Author 2 and Author 3 contributed to field establishment, crop maintenance, harvesting, and manuscript writing.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

RESEARCH AND PUBLICATION ETHICS

The authors declare that this study complies with Research and Publication Ethics.

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