

Effect of Nutrient Management on Antioxidant Potential of Black Gram and Green Gram: A Comparative DPPH and ABTS Assessment

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Abstract

Vigna mungo (Black gram) and *Vigna radiata* (Green gram) are important pulse crops containing diverse bioactive compounds with antioxidant potential. The present study compared the radical-scavenging activity of both species grown under organic (Panchagavya) and inorganic (Diammonium phosphate) fertilizer management using DPPH and ABTS assays. Antioxidant activity was expressed as IC₅₀ values. DPPH IC₅₀ ranged from 301.24 to 757.64 µg mL⁻¹, while ABTS IC₅₀ ranged from 403.84 to 880.35 µg mL⁻¹. Lower IC₅₀ values were observed in Diammonium phosphate-treated samples, with *V. mungo* showing the lowest values in both assays. These results indicate differences in radical-scavenging potency associated with species and nutrient management.

Keywords: *Vigna mungo*, *Vigna radiata*, antioxidant activity, DPPH, ABTS, Panchagavya, Diammonium phosphate, IC₅₀.

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Introduction

Pulses are nutritionally important food legumes and constitute an essential component of diets in many developing and developed countries because of their high-quality plant protein, dietary fiber, minerals, vitamins and biologically active phytochemicals. In addition to their primary nutritional functions, pulses contain a diverse range of secondary metabolites, including phenolic acids, flavonoids, tannins, saponins and other antioxidant constituents that may contribute to their functional and health-promoting properties. Among the commonly consumed pulses, *Vigna mungo* (L.) Hepper (Black gram) and *Vigna radiata* (L.) R. Wilczek (Green gram or mung bean) are important food legumes in India and other Asian countries. Their seeds contain several bioactive constituents associated with antioxidant and other biological activities. Previous investigations have demonstrated considerable variation in the concentration and composition of phenolic compounds and antioxidant capacity among pulse species and cultivars, indicating that genotype, seed characteristics and growing conditions can influence their functional properties (Shahidi & Wanasundara, 1992; Xu & Chang, 2008; Venkidasamy *et al.*, 2019). Mung bean is particularly recognized for its bioactive polyphenols, including vitexin and isovitexin, which contribute to its nutraceutical potential (Hou *et al.*, 2019). More recently, Hadi *et al.*, (2025) reviewed *V. mungo* and reported the occurrence of flavonoids, tannins, phenolic compounds and other phytochemicals associated with diverse biological activities, including antioxidant activity.

The antioxidant characteristics of pulses are influenced not only by genetic background but also by agronomic practices, nutrient availability, environmental conditions, maturity and post-harvest processing. Nutrient-management practices can alter plant growth, physiological metabolism and the synthesis or accumulation of secondary metabolites, potentially resulting in differences in the antioxidant properties of harvested grains. Recent research has confirmed substantial variation in total phenolic content, flavonoid concentration and antioxidant capacity among different legume species. Multescu *et al.*, (2024), for example, reported marked differences in phenolic and flavonoid contents and antioxidant capacity among several legumes, with mung bean showing a comparatively high flavonoid concentration. Similarly, Zhang *et al.*, (2024) demonstrated significant differences in nutrient and metabolite profiles among soybean, pea, mung bean and common bean and highlighted the contribution of metabolite composition to differences in antioxidant properties. In *V. radiata*, Desai and Modi, (2024) also demonstrated that extraction technique and solvent influenced the recovery of phenolic and flavonoid compounds and the measured antioxidant activity, emphasizing the importance of sample preparation and chemical composition when evaluating antioxidant potential. Evaluation of antioxidant activity using more than one analytical approach is therefore important for obtaining a broader assessment of the radical-scavenging potential of pulse grains. The DPPH assay is a widely used spectrophotometric method based on the reduction of the stable 2,2-diphenyl-1-

picrylhydrazyl radical by antioxidant molecules (Brand-Williams *et al.*, 1995), whereas the ABTS assay measures the ability of antioxidant compounds to quench the ABTS radical cation and can respond to a broad range of hydrophilic and lipophilic antioxidant constituents (Re *et al.*, 1999). These assays provide complementary information because antioxidant activity can vary according to the chemical characteristics of the compounds present and the analytical system used. Recent evidence further indicates that antioxidant capacity among legumes may be associated with differences in phenolic and flavonoid composition, although the strength of these relationships can differ between assays and legume types (Multescu *et al.*, 2024; Zhang *et al.*, 2024). Therefore, the present study was undertaken to comparatively evaluate the antioxidant activity of *V. mungo* and *V. radiata* cultivated under organic (Panchagavya) and inorganic (Diammonium phosphate) nutrient-management conditions using DPPH and ABTS radical-scavenging assays. The study provides an assessment of whether nutrient-management practices and species differences are associated with variation in the antioxidant potential of these nutritionally important pulse crops.

Materials and Methods

Seeds of *Vigna mungo* (Black gram) and *Vigna radiata* (Green gram) cultivated under two nutrient-management practices were used for the present antioxidant evaluation. The organic treatment consisted of Panchagavya, whereas Diammonium phosphate was used as the inorganic fertilizer treatment. Samples representing both treatments were collected for biochemical analysis and subjected to methanolic extraction.

Preparation of methanolic extracts

The collected pulse samples were processed and extracted using methanol. The resulting extracts were used for the evaluation of free-radical-scavenging activity by DPPH and ABTS assays. The antioxidant activity was assessed at different concentrations of the extracts, and the corresponding IC₅₀ values were determined.

DPPH Radical-Scavenging Assay

DPPH radical-scavenging activity was evaluated using a spectrophotometric method based on the reduction of the DPPH radical. Different concentrations of the methanolic extracts were made up to 100 µL with methanol, followed by the addition of 3 mL of 0.004% methanolic DPPH solution. Methanol (100 µL) with 3 mL of DPPH solution served as the control, while rutin was used as the reference standard. The reaction mixtures were thoroughly mixed and incubated for 30 min at 27 °C. Absorbance was measured at 517 nm against a methanol blank. The percentage of radical-scavenging activity was used to determine the IC₅₀ value, representing the concentration of extract

required to produce 50% inhibition, following Brand-Williams *et al.* (1995).

ABTS Radical-Cation Scavenging Assay

ABTS radical-cation scavenging activity was determined according to the method of Re *et al.* (1999). ABTS (7 mM) was reacted with potassium persulfate (2.4 mM) and kept in the dark for 12–16 h at room temperature to generate the ABTS^{•+} radical cation. The resulting solution was diluted with ethanol and adjusted to an absorbance of 0.700 ± 0.02 at 734 nm at 25 °C. Approximately 1 mL of the diluted ABTS^{•+} solution was mixed with the sample extracts and Trolox standard, vortexed and incubated for 30 min at room temperature. Absorbance was then recorded at 734 nm. The antioxidant response was used to determine the IC₅₀ values in comparison with the Trolox reference standard.

All antioxidant measurements were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD). The DPPH and ABTS results were compared between the Panchagavya and Diammonium phosphate treatments in *V. mungo* and *V. radiata*. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple-range test, with significance considered at P < 0.05.

Results and Discussion

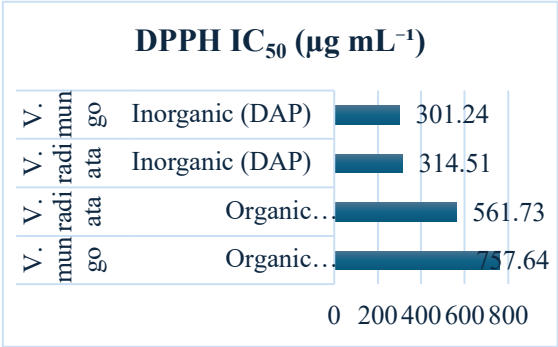
DPPH Radical-Scavenging Activity

The DPPH assay revealed clear differences in the reported IC₅₀ values between organic(Panchagavya) and inorganic fertilizer(DAP) treatments in both pulse species. The highest IC₅₀ was recorded in organically treated *V. mungo* (757.64 ± 3.53 µg mL⁻¹), followed by organically treated *V. radiata* (561.73 ± 2.98 µg mL⁻¹). In contrast, inorganic-treated *V. radiata* and *V. mungo* showed lower IC₅₀ values of 314.51 ± 3.11 and 301.24 ± 4.27 µg mL⁻¹, respectively. The numerical difference between treatments was therefore pronounced in both species.

Table 1: IC₅₀ Values of DPPH Radical Scavenging Activity

Species	Treatment	DPPH IC ₅₀ (µg mL ⁻¹)	SD
<i>V. mungo</i>	Organic (Panchagavya)	757.64	3.53
<i>V. radiata</i>	Organic (Panchagavya)	561.73	2.98
<i>V. radiata</i>	Inorganic (DAP)	314.51	3.11
<i>V. mungo</i>	Inorganic (DAP)	301.24	4.27

Figure 1. DPPH Radical-Scavenging Activity Expressed as IC₅₀ Values



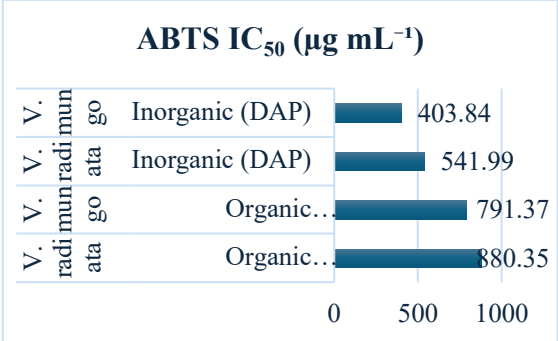
ABTS Radical-Cation Scavenging Activity

The ABTS assay showed a similar numerical pattern. The highest reported IC₅₀ was observed in organically treated *V. radiata* (880.35 ± 15.03 µg mL⁻¹), followed by organically treated *V. mungo* (791.37 ± 5.41 µg mL⁻¹). The inorganic treatments recorded lower values of 541.99 ± 8.89 µg mL⁻¹ in *V. radiata* and 403.84 ± 16.61 µg mL⁻¹ in *V. mungo*. Thus, the reported IC₅₀ values were consistently higher under Panchagavya management than under inorganic fertilizer treatment.

Table 2: IC₅₀ Values of ABTS Radical Scavenging Activity

Species	Treatment	ABTS IC ₅₀ (µg mL ⁻¹)	SD
V. radiata	Organic (Panchagavya)	880.35	15.03
V. mungo	Organic (Panchagavya)	791.37	5.41
V. radiata	Inorganic (DAP)	541.99	8.89
V. mungo	Inorganic (DAP)	403.84	16.61

Figure 2. ABTS Radical-Cation Scavenging Activity Expressed as IC₅₀ Values.



Interpretive note: Because IC₅₀ conventionally represents the concentration required to produce 50% inhibition, lower IC₅₀ generally indicates greater radical-scavenging potency. Accordingly, the direction of antioxidant superiority should be confirmed from the original inhibition curves/calculations before making a biological claim.

The present study demonstrated clear differences in the radical-scavenging responses of *Vigna mungo*

and *Vigna radiata* cultivated under organic (Panchagavya) and inorganic (DAP) nutrient-management conditions. Based on the conventional interpretation of IC₅₀ values, the Diammonium phosphate -treated samples exhibited greater radical-scavenging potency than the Panchagavya-treated samples in both DPPH and ABTS assays, as indicated by their substantially lower IC₅₀ values. Among the treatments evaluated, Diammonium phosphate -treated *V. mungo* exhibited the lowest IC₅₀ values for both DPPH (301.24 ± 4.27 µg mL⁻¹) and ABTS (403.84 ± 16.61 µg mL⁻¹), indicating the strongest radical-scavenging potency under the conditions of the present experiment. The consistent trend observed across the two independent antioxidant assays strengthens the evidence that fertilizer management was associated with differences in antioxidant response.

The results further indicate that antioxidant potential cannot be attributed solely to the fertilizer source, because the response may reflect interactions among genotype, nutrient availability, environmental conditions and the accumulation of different classes of bioactive compounds. Recent investigations have demonstrated substantial variation in phenolic, flavonoid and antioxidant characteristics among legumes and mung bean genotypes, supporting the importance of species and biochemical composition when interpreting antioxidant responses (Multescu et al., 2024; Zhang et al., 2024). Recent work specifically examining *V. mungo* has also documented diverse phytochemicals, including phenolic compounds, flavonoids and tannins, and their association with antioxidant properties (Hadi et al., 2025). Therefore, the present findings provide evidence for differences in antioxidant potency but do not establish a direct biochemical mechanism by which Diammonium phosphate produced the observed response. In particular, higher or lower IC₅₀ values should not be interpreted as direct evidence of increased or decreased concentrations of individual antioxidant compounds without corresponding phytochemical measurements.

Overall, the findings demonstrate that nutrient-management practices and pulse species can influence the measured antioxidant characteristics of *V. mungo* and *V. radiata*. The agreement between DPPH and ABTS results provides useful evidence for comparative assessment of antioxidant radical-scavenging potency. However, further studies incorporating total phenolic content, total flavonoid content, individual phenolic and flavonoid profiling, and additional antioxidant assays such as FRAP or ORAC would provide a more comprehensive understanding of the biochemical basis of the observed differences. Measurement of individual compounds such as vitexin, isovitexin and phenolic acids using HPLC or LC-MS/MS would be particularly valuable for identifying compounds associated with the response. Future experiments

should also include adequate biological replication and statistical comparison of treatment means to establish the significance of species $\times$ nutrient-management interactions. Thus, the present study provides useful preliminary evidence that nutrient-management practices are associated with variation in the antioxidant potential of Black gram and Green gram and highlights the importance of integrating agronomic management with biochemical characterization when evaluating the functional quality of pulse grains.

Conclusion

The present investigation demonstrated distinct differences in the antioxidant responses of *Vigna mungo* and *Vigna radiata* under Panchagavya and DAP fertilizer management. In both DPPH and ABTS assays, the Diammonium phosphate -treated samples showed lower IC₅₀ values than the corresponding Panchagavya-treated samples, indicating greater radical-scavenging potency according to conventional IC₅₀ interpretation. Among the samples evaluated, Diammonium phosphate -treated *V. mungo* exhibited the lowest DPPH IC₅₀ ($301.24 \pm 4.27 \mu\text{g mL}^{-1}$) and ABTS IC₅₀ ($403.84 \pm 16.61 \mu\text{g mL}^{-1}$), suggesting comparatively stronger antioxidant potency in the two assays. Panchagavya-treated samples showed higher IC₅₀ values in both assays. These findings indicate that nutrient-management practices may influence the antioxidant characteristics of pulse grains. However, the present antioxidant assays alone cannot identify the specific compounds responsible for the observed differences. Further determination of phenolic compounds, flavonoids and individual antioxidant constituents, together with validated IC₅₀ calculations and appropriate statistical comparisons, would provide stronger evidence for explaining the biochemical basis of the treatment effects.

References

- Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), 25-30.
- Desai, K., & Modi, N. R. (2024). Evaluation of extraction techniques and solvents for phytochemical screening, antioxidant and antimicrobial activity of mungbean (*Vigna radiata* (L.) R. Wilczek) seeds. *Indian Journal of Natural Products and Resources (IJNPR)*[Formerly Natural Product Radiance (NPR)], 15(4), 565-577.
- Hadi, F., Sardar, H., Alam, W., Aschner, M., Alzahrani, F. M., Halawani, I. F., ... & Khan, H. (2025). *Vigna mungo* (Linn.) Hepper: ethnobotanical, pharmacological, phytochemical, and nutritious profile. *Phytochemistry Reviews*, 24(2), 1119-1142.
- Hou, D., Yousaf, L., Xue, Y., Hu, J., Wu, J., Hu, X., ... & Shen, Q. (2019). Mung bean (*Vigna radiata* L.): Bioactive polyphenols, polysaccharides, peptides, and health benefits. *Nutrients*, 11(6), 1238.
- Multescu, M., Culetu, A., & Susman, I. E. (2024). Screening of the nutritional properties, bioactive components, and antioxidant properties in legumes. *Foods*, 13(22), 3528.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9-10), 1231-1237.
- Shahidi, F., Janitha, P. K., & Wanasundara, P. D. (1992). Phenolic antioxidants. *Critical reviews in food science & nutrition*, 32(1), 67-103.
- Xu, B., & Chang, S. K. (2008). Antioxidant capacity of seed coat, dehulled bean, and whole black soybeans in relation to their distributions of total phenolics, phenolic acids, anthocyanins, and isoflavones. *Journal of agricultural and food chemistry*, 56(18), 8365-8373.
- Zhang, W., Zhao, Y., Yang, H., Liu, Y., Zhang, Y., Zhang, Z., Li, Y., Wang, X., Xu, Z., & Deng, J. (2024). Comparison analysis of bioactive metabolites in soybean, pea, mung bean, and common beans: Reveal the potential variations of their antioxidant property. *Food Chemistry*, 457, 140137.