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Phenolic and Flavonoid Responses of Bauji Shallots to Ammonium Sulfate under Floating Cultivation

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Abstract

Shallot is a high value horticultural crop valued for its flavor, aroma, and medicinal properties, and is rich in phenolics and flavonoids that contribute to antioxidant activity. Limited dryland and fluctuating demand in South Sumatra highlight the need for alternative systems such as floating cultivation. This study aimed to evaluate the effects of different ammonium sulfate (ZA) doses on the bulb yield, total phenolic, and flavonoid contents of Bauji shallots grown under floating cultivation. The experiment used a randomized complete block design with three replications and four ZA fertilizer treatments (0, 60, 180, and 360 kg ha^{-1}). Observed variables included total phenolic and flavonoid contents measured by spectrophotometry and dry bulb weight. The results indicated that the ZA application significantly increased dry bulb weight and total phenolic content but did not affect flavonoids. The highest bulb yield and flavonoid content were obtained with 60 kg ha^{-1}, whereas phenolics peaked under the control (0 kg ha^{-1}), suggesting a trade-off between yield and secondary metabolite accumulation. Correlation analysis revealed a positive association between bulb weight and flavonoid content, whereas phenolics were negatively related to fertilizer input. As the first report on Bauji shallots under floating cultivation, these findings indicate that moderate ZA fertilization (60 kg ha^{-1}) optimizes bulb yield while maintaining bioactive compound levels. However, further multi-season studies are needed for broader recommendations.

Keywords: fertilization; flavonoid content; medicinal plant; phenolic compounds; plant extracts

INTRODUCTION

Shallots are spice vegetables widely used as a primary ingredient in cooking and traditional medicine, giving them high economic value (Shahrajabian et al., 2020). Beyond their culinary and economic importance, shallots are also rich in secondary metabolites, particularly phenolic compounds and flavonoids. These bioactive molecules exhibit strong antioxidant properties and reduce oxidative stress (Rudrapal et al., 2022), as well as demonstrating anti-inflammatory (Al-Khayri et al., 2022), anticancer (Kopustinskiene et al., 2020), antibacterial (Rosyada et al., 2023), and minimizing the occurrence of diabetes complications (Jin et al., 2023). Such health promoting potentials make shallots an essential research subject for their role in preventing and managing chronic diseases. The distinctive taste and aroma of shallots originate from the compound isoalliin, an isomer of alliin and the dominant S-alk(en)yl-L-cysteine sulfoxide present in shallots, which is formed through processes influenced by the plant's sulfur content (Hasrianda and Setiarto, 2022).

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In Indonesia, the availability of shallots often becomes limited before major religious and national holidays (Yuniarti et al., 2023), primarily due to farmers' inability to meet market demand. In South Sumatra Province, the annual consumption requirement for shallots reaches 23,288 tons, while local production amounts to only 8,965 tons (Statistics of Indonesia, 2024; Statistics of South Sumatra Province, 2024a; 2024b). One of the main obstacles farmers face in increasing shallot production is the limited availability of dryland suitable for cultivation, as the province's landscape is predominantly composed of swampy lowlands and peatlands (Wildayana and Armanto, 2018).

To meet local demand, shallots are often transported from major producing provinces, such as Central Java, one of Indonesia's largest shallot production centers (Rosyid et al., 2021). However, this approach poses logistical challenges. Transporting shallots over long distances requires time, and because shallots are bulb vegetables with limited storability, this method does not provide a sustainable supply solution (Sarjani et al., 2018). Another alternative is cultivating shallots in shallow swamp areas with water levels less than 25 cm during the rainy season (Armanto et al., 2018). Previous studies by Susilawati et al. (2021) have shown that when the soil water table falls below 20 cm from the surface, shallot growth and yield are adversely affected, leading to suboptimal production levels. Consequently, this cultivation method remains insufficient to meet the demand for shallot consumption in South Sumatra.

An innovative approach to overcoming these challenges is using floating cultivation systems (Pyka et al., 2020; Sofian et al., 2023), which enable shallot production in wetland areas. This system utilizes floating rafts on flooded lands, where plants are grown in polybags supported on buoyant structures (Hasbi et al., 2017). Floating cultivation allows crop production on otherwise unsuitable land, though nutrient management remains critical for optimal growth (Wawan and Fikrawati, 2021).

Among nitrogen fertilizers, urea is the most commonly used source but suffers from high volatilization losses under flooded and floating conditions due to rapid hydrolysis and ammonia emission (He et al., 2025). In contrast, ammonium sulfate fertilizer supplies nitrogen directly in the ammonium form, which is less prone to volatilization, while also providing sulfur essential for secondary metabolite synthesis (Powlson and Dawson, 2022). As $(NH_4)_2SO_4$, ammonium sulfate enhances nitrogen use efficiency, promotes vegetative growth, and stimulates sulfur dependent compounds that contribute to the distinctive flavor and aroma of shallots (Hasanah et al., 2021). This study aims to evaluate the effects of varying ammonium sulfate fertilizer doses on the bulb yield, total phenolic, and flavonoid contents of shallots cultivated in a floating cultivation system. According to current evidence, this study was the first to investigate Bauji shallots under floating cultivation with varying ammonium sulfate doses, providing new insights into nutrient level responses in phenolic and flavonoid contents.

MATERIALS AND METHOD

Study site

The experiment was conducted in 5 Ulu Darat Village, Seberang Ulu I Sub-district, Palembang, Indonesia. The site is geographically located at 104°45'45.1" E and 3°00'12.7" S, with an elevation of approximately 6 m above sea level (Figure 1). The study was conducted from December 2024 to February 2025 in a constructed pond measuring 2.5 m × 3.5 m in a residential area. During the experimental period, the average air temperature was 26.6 °C, relative humidity averaged 85.5%, the average daily rainfall was 9.6 mm, daily sunshine duration was 3.1 hours, and the mean wind speed was 2.1 m s^{-1}. Climatic data were obtained from the nearest meteorological station (Meteorology, Climatology, and Geophysics Agency of Palembang).

Procedures

Cultivation pond and floating rafts

The cultivation pond was constructed using *gelam* wood (local hardwood) and bamboo slats, and lined with a plastic tarp to retain water. The pond measured 3.4 m × 2.4 m and was filled with rainwater. After settling, the water exhibited a pH range of 4.5 to 5.0, while the temperature was maintained at 25 to 26 °C throughout the experiment. Floating rafts were fabricated using 1.5 l plastic water bottles as flotation devices, following the method described by Siaga et al. (2019), combined with bamboo slats as the supporting frame (Figure 2). Each raft measured 1 m × 2 m, and the experiment used three units.

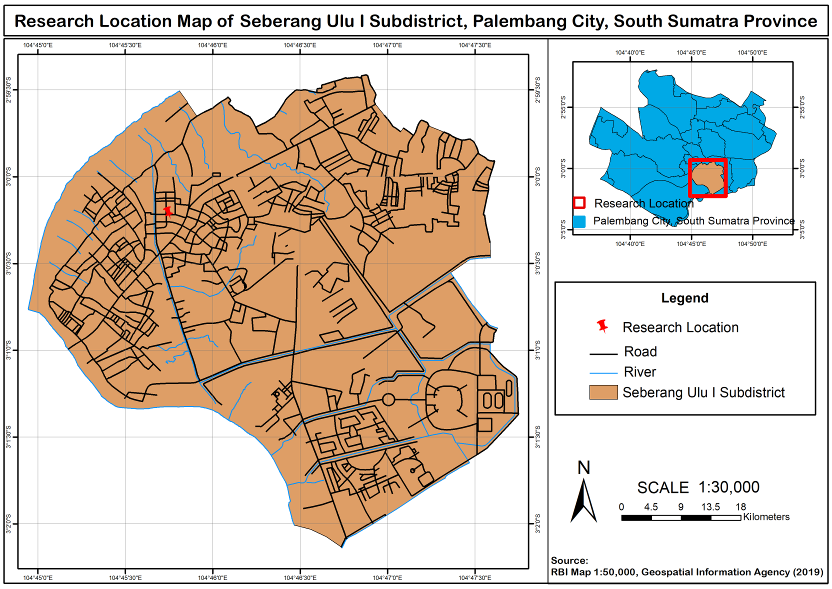


Figure 1. Research location map

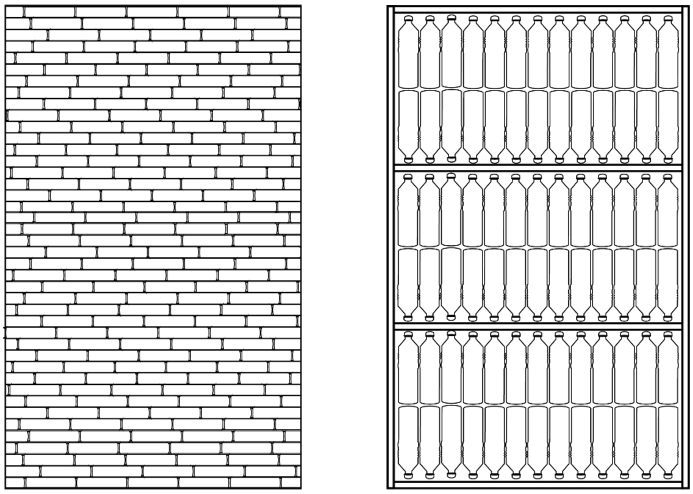


Figure 2. Floating raft top and bottom view

Planting media

The planting medium consisted of a mixture of topsoil, burned rice husk, and cow manure in a 2:1:1 ratio. The pH of the soil ranged from 4.5 to 6.0. The mixture was placed into 25 cm × 25 cm polybags before transplanting. Basal fertilization was applied once, one week before planting (Solagracya et al., 2025), with NPK 16:16:16 at 200 kg ha^{-1} (Susilawati et al., 2022), SP-36 at 150 kg ha^{-1} (Hadiawati et al., 2023), and KCl at 105 kg ha^{-1} (Suminartika et al., 2022) to ensure nutrient availability during early growth.

Seed preparation

Shallot seedlings of the Bauji variety were obtained from mature bulbs (≥ 3 months old) with firm outer skin, small size (2 to 3 g), uniform shape, and free from pests, diseases, or mechanical damage to ensure optimal growth. This cultivar was selected for its moderate adaptability to peatland conditions (Lestari and Maftu'Ah, 2021), making it a promising candidate for floating cultivation.

Planting procedure

Before planting, the shallot bulbs were trimmed by approximately one-third of their length to promote sprout emergence and stimulate lateral bulb formation (Tome et al., 2022). The bulbs were then soaked in a fungicide solution at a concentration of 1 ml l^{-1} for 5 minutes. Planting was conducted by inserting the bulbs into the soil medium and gently twisting them to ensure firm contact between the bulb base and the planting substrate.

Crop maintenance

Weed management was performed manually by hand pulling. Pest and disease control was carried out using integrated methods. Mild disease symptoms were treated with biological agents, primarily *Trichoderma* sp. In cases of severe infestation, infected plants were removed to prevent further spread.

Harvesting

Shallots were considered physiologically mature and ready for harvest when approximately 60% of the foliage had wilted and turned yellow at approximately 60 days after planting.

Bioactive compound analysis

After harvest, only the bulb portion of the shallot samples was used. The bulbs were thinly sliced and air-dried, followed by oven-drying at 35 °C for 7 days. The dried samples were then ground into a fine powder using a blender and passed through a medium-sized mesh (approximately 2 mm) to obtain a homogeneous shallot powder extract.

Total flavonoid

This content was determined using UV-Vis spectrophotometry at 415 nm. The extract was reacted with aluminum chloride ($AlCl_3$) in 50% methanol to form a detectable flavonoid–$AlCl_3$ complex, following the method described by Aminah et al. (2017) and Ramadhani et al. (2020). Results were expressed as milligrams of quercetin equivalent per gram of sample (mg QE g^{-1}).

Total phenolic

This content was analyzed using the Folin–Ciocalteu colorimetric method. The extract was mixed with the Folin-Ciocalteu reagent and incubated under controlled conditions to allow the formation of a blue-green chromophore. Absorbance was measured at 725 nm using a UV-Vis spectrophotometer, as described by Muthia et al. (2023). Results were expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE g^{-1}).

Methods

The experiment was arranged using a randomized complete block design (RCBD) with four levels of ammonium sulfate (ZA) fertilizer and three replications. Fertilizer applications were carried out 1 and 3 weeks after planting (WAP), following the procedure described by Idly et al. (2024). Each replication consisted of 25 plants, resulting in 300 experimental units. The ZA fertilizer treatments were as follows: S0 = control (no fertilizer), S1 = 60 kg ha^{-1}, S2 = 180 kg ha^{-1}, S3 = 360 kg ha^{-1}.

Data analysis

Data were analyzed using analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test at a 5% significance level, polynomial regression, and Pearson's correlation analysis. The observed variables were dry bulb weight, total phenolic, and flavonoid content.

RESULTS AND DISCUSSION

An increase in the dosage of ZA fertilizer resulted in a significant decrease in shallot dry bulb weight and total phenolic content (Table 1). In contrast, total flavonoid content showed no statistically significant differences among treatments. These results suggest that dry bulb weight and phenolic compounds were more responsive to changes in nitrogen and sulfur availability than flavonoids.

The application of fertilizer significantly influenced shallot dry bulb weight at 8 WAP. The 60 kg ha^{-1} fertilizer dose resulted in the highest dry weight, at 4.0 g (Figure 3a). This indicates moderate nitrogen input (60 kg ha^{-1}) supports optimal bulb development. The polynomial regression explained only 34.15% of the variation (R^2 = 0.3415) (Figure 3b), suggesting that dry bulb weight was weakly associated with ZA dosage and strongly influenced by other environmental or physiological factors. The final yield of bulbs is a direct function of the balanced relationship between soil nutrient uptake, favorable environmental conditions, and efficient metabolic activity within the plant.

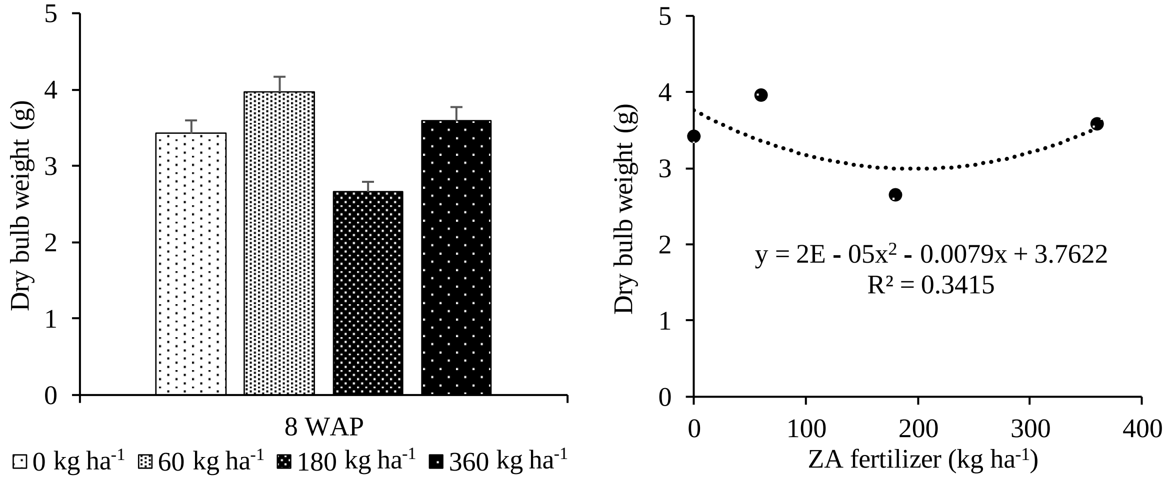
Table 1. Effects of ZA fertilizer dosage on shallot physiology and bioactive traits

Variable	F-value	CV (%)
Dry bulb weight	8.31^{*}	10
Total phenol content	6.21^{*}	6
Total flavonoid content	3.09^{ns}	12
F-table 5%	4.76	
F-table 1%	9.78	

Note: CV = Coefficient of variation; * = Significant; ^{ns} = Not significant

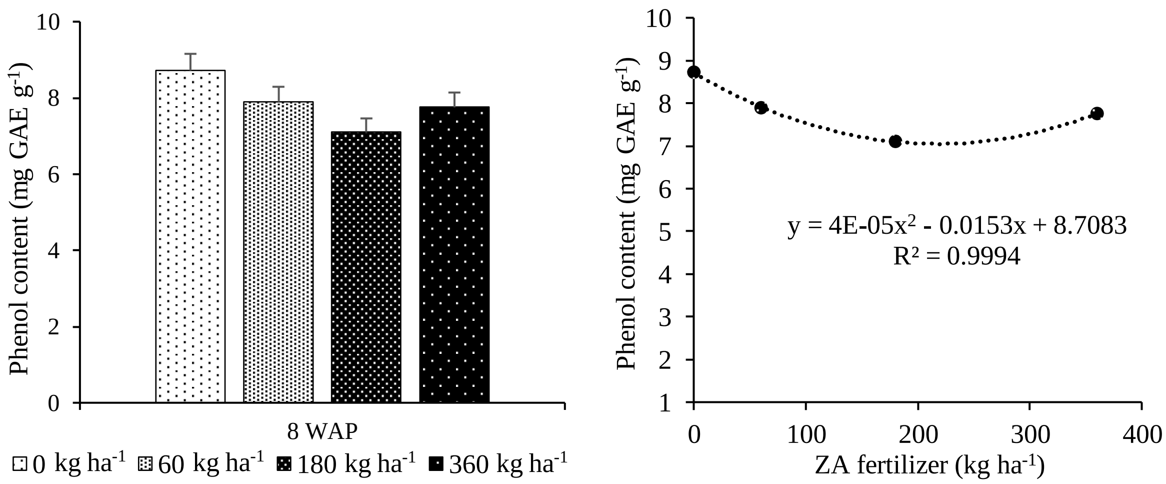
The control treatment (0 kg ha^{-1}) produced the highest phenolic content at 8.72 mg GAE g^{-1}, whereas higher ZA doses (180 to 360 kg ha^{-1}) reduced the phenolic content to 7.11 to 7.75 mg GAE g^{-1} (Figure 4a). The polynomial regression analysis revealed that the tested dose range of ZA explained 99.94% of the variation in phenol content ($R^2 = 0.9994$) (Figure 4b). This decrease is associated with the plant's stress response (Buchory et al., 2020), as phenolic production tends to increase under nutrient-deficient conditions. In the absence of fertilizer, nitrogen and sulfur deficiencies likely induced metabolic stress, thereby triggering the biosynthesis of phenolic compounds as a protective mechanism and part of their defense strategy. Phenols are potent antioxidants that help plants cope with various environmental stresses (Sharma et al., 2019).

These findings are consistent with previous studies by Narvekar and Tharayil (2021) and Ma et al. (2023), which reported increased phenolic content under low nitrogen or unfertilized conditions (Figure 4a). Under stress conditions, the plant's defense system is activated, particularly the phenylpropanoid pathway (Yadav et al., 2020), synthesizing protective phenolic compounds. The observed pattern, a decrease in phenol content from the control to a medium dose, followed by a slight increase at higher doses, is consistent with the carbon-nitrogen (C–N) allocation theory (Chen et al., 2024). This theory



a. b.

Figure 3. Shallot dry bulb weight (a) and polynomial regression analysis of shallot dry bulb weight (b)



a. b.

Figure 4. Total phenolic contents (a) and polynomial regression analysis of total phenolic contents (b)

explains that nitrogen deficiency significantly reduces the plant's ability to absorb CO_2, which is essential for photosynthesis and the subsequent production of assimilates. This triggers the accumulation of secondary metabolites. As nitrogen levels increase to a medium range, carbon is reallocated toward growth, reducing phenolic compounds.

No statistically significant variation in total flavonoid content was observed among the ZA fertilizer treatments. However, descriptive analysis indicated that the application of 60 kg ha^{-1} ZA fertilizer yielded the highest flavonoid content (6.64 mg QE g^{-1}) (Figure 5). This suggests that at this dosage, the availability of nitrogen and sulfur was within the optimal range to support flavonoid biosynthesis. Sufficient nutrient levels likely promoted the maximal activity of key enzymes in the flavonoid biosynthetic pathway. This observation is supported by previous studies conducted by Shafira et al. (2024) and Marlin et al. (2022), which reported that moderate nitrogen application (approximately 60 to 90 kg ha^{-1}) significantly increased flavonoid content. In contrast, higher fertilizer doses (e.g., 180 to 360 kg ha^{-1}, as applied in this study) tended to reduce flavonoid levels.

The contrasting patterns between flavonoid and phenol accumulation in response to ZA fertilizer doses suggest that, although both compounds originate from the same biosynthetic pathway, their production is regulated through distinct mechanisms. This highlights the flexibility of plant secondary metabolism in adapting to environmental and nutritional variations. These findings are consistent with previous studies, which indicate that different molecular processes control the biosynthesis of flavonoids and non-flavonoid phenols (Davies et al., 2020).

The Pearson correlation analysis among variables revealed distinct relationships between yield formation and secondary metabolite accumulation in shallots (Table 2). Phenol content declined with increasing ZA doses but showed a weak association with dry bulb weight, while flavonoid content strongly and positively correlated with yield. This suggests that flavonoids contribute more directly to bulb development than phenols. Therefore, moderate nitrogen application optimizes the balance between growth and secondary metabolite synthesis, leading to improved yield performance.

Transcriptomic and metabolomic analyses in species such as *Leymus chinensis*, *Foxtail millet*, and *Litsea coreana* have demonstrated that flavonoid accumulation is regulated by the expression of specific genes such as *phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), flavanone 3-hydroxylase (F3H), as well as transcription factors including myeloblastosis (MYB) and basic helix-loophelix (bHLH)*, which are not always involved in the regulation of total phenol content (Wu et al., 2024; Xie et al., 2024; Ye et al., 2025).

This differentiation is further supported by fertilization studies in various plant species such as *Elaeis guineensis*, *Justicia gendarussa*, *Amaranthus caudatus*, and *Adenostemma lavenia*, where identical nitrogen doses resulted in varying levels of flavonoid and phenol accumulation. These differences were influenced by environmental factors such as light intensity, shading, and the type of fertilizer applied (Marlin et al., 2022; Maulana et al., 2024; Shafira et al., 2024). Such findings reflect metabolic flux competition and the differential regulation of biosynthetic enzymes, whereby flavonoids may reach optimal accumulation at specific fertilizer doses. At the same time, other phenolic compounds tend to accumulate more under nutrient deficient conditions. Moreover, a molecular review by Shi et al. (2024) revealed that flavonoid biosynthesis is strongly influenced by environmental factors such as light, temperature, and hormonal signals, which activate the flavonoid pathway through specific regulatory cues.

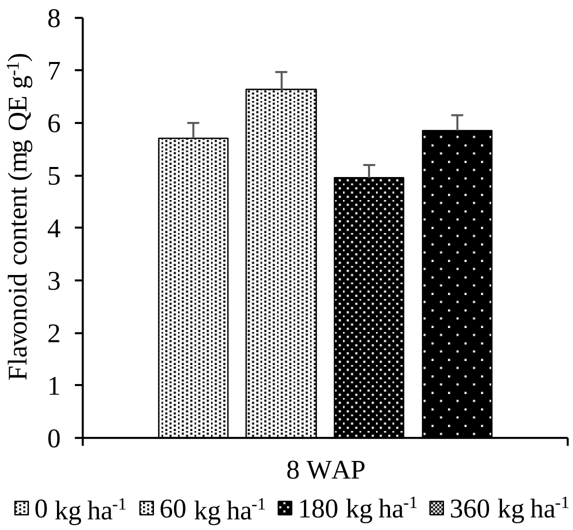


Figure 5. Total flavonoid content

Table 2. Pearson correlation

Variable		Total phenol content	Total flavonoid content	Dry bulb weight
Total phenol content	Pearson correlation	1		
	Sig. (2-tailed)			
	N	12		
Total flavonoid content	Pearson correlation	0.31	1	
	Sig. (2-tailed)	0.32		
	N	12	12	
Dry bulb weight	Pearson correlation	0.28	0.67*	1
	Sig. (2-tailed)	0.37	0.016	
	N	12	12	12

Note: *Significant at $p < 0.05$

Variations in phenolic and flavonoid accumulation under different ZA fertilizer treatments indicate that, although both originate from the phenylpropanoid pathway, the biosynthesis of flavonoids and non-flavonoid phenols is regulated by distinct enzymes and genetic mechanisms, resulting in different responses to nutrient availability. The highest dry bulb weight and flavonoid content observed at 60 kg ha^{-1} of ZA fertilizer, along with the highest phenol content recorded under the control (no fertilizer) condition, likely reflects the complex regulatory mechanisms of secondary metabolite biosynthesis, which are highly specific to each compound type, their associated genes, and the prevailing environmental conditions.

CONCLUSIONS

Varying ZA fertilizer doses in floating cultivation of Bauji shallots affected total phenolic, with the highest content at 0 kg ha^{-1} (8.72 mg GAE g^{-1}) under nutrient deficiency stress and dry bulb weight at 60 kg ha^{-1} (4.00 g). Flavonoid content showed no significant differences, although a tendency toward higher accumulation was observed at 60 kg ha^{-1} ZA (6.64 mg QE g^{-1}). These findings provide the first evidence of contrasting nutrient-level responses of phenolic and flavonoid pathways in Bauji shallots under floating cultivation. Future studies should be conducted across multiple seasons and shallot varieties, integrating soil property analysis and antioxidant assays to validate the consistency of ZA fertilization effects under floating cultivation systems.

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