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The Effect of Drought Stress on Flavonoid and Phenolic Content in Super 1 Sorghum Extract

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ABSTRACT

Allelopathic-based bioherbicides sourced from drought-stressed sorghum plants help advance organic farming. This study aimed to assess the levels of flavonoids and phenols in extracts from Super 1 sorghum varieties exposed to drought stress. Preparation and analysis of the extracts occurred at the Integrated Laboratory of the Faculty of Mathematics and Natural Sciences, Bengkulu University, from July to August 2025. For extraction, a randomized block design with a single factor was used in a greenhouse. In this experiment, one Super 1 sorghum variety was subjected to water stress through varying irrigation schedules: daily, every four days, every five days, and every six days. Plants were harvested at 45 days, sun-dried, then oven-dried at 70°C for 48 hours. Dried cobs were ground into a fine powder and analyzed for flavonoid and phenol levels. Results showed that irrigation every 5 days yielded extracts with the highest flavonoid level (1045 µg/g). Meanwhile, the lowest flavonoid levels were recorded with irrigation every 6 days and daily, giving 320 µg/g and 330 µg/g, respectively. Regarding phenol content, the highest amount was found in extracts watered every 6 days (3310 µg/g), and the lowest amount was observed with daily irrigation, at 1150 µg/g

INTRODUCTION

Global climate change has increased the frequency of droughts, challenging agriculture, especially for food crops that require water. Crops adaptive to abiotic stresses, such as sorghum (Sorghum bicolor L. Moench), are thus increasingly important (Hossain et al, 2022; Khalifa & Eltahir, 2023).

Given these climate challenges, sorghum stands out as a cereal crop with high tolerance to marginal land and drought, making it a potential alternative food source, animal feed, and bioenergy raw material (Nghiem et al., 2016; Rooney et al., 2007; Hossain et al., 2022; Susilo et al.,2020). One of the superior varieties widely developed in Indonesia is Sorghum Super 1, which has high productivity and is adaptive to various environmental conditions. However, the utilization of sorghum has so far been more focused on seed production, while the potential of its secondary metabolite content has not been widely studied (Yustianisa & Diana 2023).

Drought stress increases Reactive Oxygen Species (ROS) in plants. In response, plants produce more secondary metabolites, particularly flavonoids and phenols, which act as antioxidants and exhibit potential as pharmaceuticals, functional foods, and bioherbicides (Rao & Zheng, 2025; Patil et al., 2024; Mohagheghian et al., 2025).

Many studies explore sorghum's drought responses, but little is known about the effect on flavonoid and phenolic content in the Super 1 sorghum variety. This is key for understanding adaptation and leveraging sorghum's bioactive compounds (Yue et al., 2025; Aru et al., 2023).

Based on the above, this study uniquely examined how drought stress alters flavonoid and phenolic content in Super 1 sorghum extracts. These results provide specific insights that can aid the development of sorghum not only as a food source but also as a distinctive provider of secondary metabolites, supporting targeted innovation in sustainable agriculture and bio-based industries (Cunha et al., 2024; Yue et al., 2025).The purpose of this study was to examine the flavonoid and phenol content of Super 1 sorghum extracts subjected to drought stress

LITERATURE REVIEW

Sorghum is a cereal crop that can thrive in marginal soils and withstand dry climates. The production of secondary metabolites such as phenolics, flavonoids, and terpenoids increases under drought conditions. These metabolites function as defenses against pathogens, herbivores, and enemies (Akula & Ravishankar, 2011).

Sorgoleone is a phenolic compound produced by sorghum roots that acts as an allelopathic agent, inhibiting the growth and germination of nearby weeds. Under drought stress, sorghum increases the production of sorgoleone and other secondary metabolites, which strengthen its defenses against competing plants. Drought promotes these biochemical changes by increasing oxidative stress and activating enzymes related to phenylpropanoid biosynthesis, resulting in higher concentrations of phenolic compounds, such as sorgoleone. As a result, sorghum becomes more effective as a natural bioherbicide (Khan et al., 2020).

Sorghum has strong natural weed-fighting abilities, particularly in dry areas prone to drought. This makes sorghum useful as a natural weed killer (Weston & Duke, 2003). In these areas, sorghum helps control weeds and reduce the need for chemical herbicides, which can harm the environment (Dayan, 2019).

Building on this, it is essential to note that drought stress in sorghum enhances the production of allelopathic secondary metabolites, which can be utilized as bioherbicides. Further research is needed to identify the specific active compounds responsible for these allelopathic effects and to explore their potential use in broader, sustainable, and environmentally friendly agricultural systems (Bhowmik & Inderjit, 2003 ; Susilo et al., 2023 ; Susilo et al., 2024 ; Susilo et al., 2021). Akula and Ravishankar (2011) also reported that abiotic stress, such as drought, can increase the production of secondary metabolites in plants, including flavonoids.As natural antioxidants, flavonoids protect plants from environmental oxidative stress. Khan et al. (2020) stated that drought activates enzymes in the phenylpropanoid biosynthesis pathway, which is responsible for the production of flavonoids. According to Ferdinando et al. (2014), flavonoids play an important role in protecting plant cells from damage caused by drought stress. Their production increases in response to environmental stress, making them important secondary metabolites in plant adaptation. Phenolic compounds (phenolic acids) have been internationally recognized as allelopathic agents with great potential for development into environmentally friendly bioherbicides (Hoang et al., 2021).

METHODOLOGY

This study used water extracts obtained through several treatment stages. Super 1 sorghum varieties were planted in coastal soil under four water stress treatments: daily irrigation, every 4 days, every 5 days, and every 6 days. Plants were harvested at 40 days of age; tops and roots were separated immediately after harvesting. The separated plant parts were first sun-dried for 14 days, then transferred to an oven at 70°C for an additional 72 hours. Once dried, the plant material was chopped into uniform pieces (1–2 cm) and then ground into a powder using a blender. This powder served as the raw material for water extraction during the analysis of flavonoids and phenols.

For flavonoid analysis, weigh 10 mg of extract and dissolve it in 10 ml of solvent. Take 0.5 mL of this solution and add 1.5 mL of 96% alcohol, 0.1 mL of 10% AlCl3, 0.1 mL of 1 M CH3COONa, and 2.8 mL of distilled water. Homogenize the mixture and let it stand for 30 minutes. Measure the mixture at a wavelength of 435 nm. Apply the same procedure for the standard series.

The phenol analysis method involves weighing 10 mg of extract dissolved in a 10 mL measuring flask. Take 0.2 ml of the solution and add 15.8 ml of distilled water. Next, add 1 ml of R. Folin, let it sit for 8 minutes. After that, add 3 mL of 20% Na2CO3 and let it stand for 30 minutes. Measure at a wavelength of 765 nm. Do the same with the standard series.

The research variables included flavonoid and phenol content. This study used a completely randomized design with one factor and four replicates for the preparation of extract materials. Data were analyzed statistically using ANOVA, and differences between treatments were tested using the LSD test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

The observation variables in this study were the flavonoid and phenol levels contained in the extract. The analysis of variance table shows that the treatment of Super 1 sorghum water extract with different levels of drought stress had a significant effect on the flavonoid and phenol levels, as shown in Table 1.

Table 1. Recapitulation of the Analysis of Variance of Flavonoid and Phenol Levels

Observation variables	**Treatment**
Flavonoids	5.95 *
Phenols	3.55 *
* = significant effect	

The effect of treatment with an extract from the Super 1 sorghum variety showed a significant impact on flavonoid content. The highest flavonoid content was achieved by watering every 5 days (1045.0 μg/g) and was significantly different from watering every day or every 6 days; however, it was not significantly different from watering every 4 days, as shown in Table 2. This study shows that watering every day to every 5 days produces flavonoid content from low to high. Furthermore, the treatment with irrigation every 6 days resulted in a decrease in flavonoid levels. This indicates that as the level of drought stress increases, flavonoid levels also increase, but this effect reaches its limit at the drought stress level corresponding to the treatment with irrigation every 5 days.

This pattern is common: flavonoids, a class of plant secondary metabolites with antioxidant properties, increase during moderate stress (5 days of watering), then decrease when stress becomes too severe (6 days). Mild to moderate drought activates the phenylpropanoid pathway, which involves key enzymes such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and chalcone isomerase (CHI), thereby increasing flavonoid synthesis to counteract reactive oxygen species (ROS)—highly reactive molecules that can damage cells. At the 5-day interval, stress signals, such as abscisic acid (ABA, a plant hormone) and hydrogen peroxide (H_2O_2, a type of reactive oxygen species, or ROS), are strong enough to trigger flavonoid production but have not yet damaged the metabolic machinery. More severe stress causes a sharp decline in photosynthesis (as stomata, the pores on leaf surfaces, close, resulting in low carbon dioxide (CO_2) availability and depletion of energy molecules like ATP and NADPH). Carbon substrates, such as phenylalanine and malonyl-CoA, as well as energy, are required for flavonoid biosynthesis, leading to a decrease in production. Under severe drought, excess ROS begin to damage membranes and enzymes, including those vital for the flavonoid pathway.

Table 2. Average Flavonoid and Phenol Content of Super 1 Sorghum Extracts With Different Levels of Drought Stress

Irrigation treatment	Flavonoid (μg/g)	Phenols (μg/g)
Every day	330.0 b	1150.0 b
Every 4 days	655.0 ab	2565.0 ab
Every 5 days	1045.0 a	2050.0 ab
Every 6 days	320.0 b	3310.0 a

Note: Numbers followed by the same letter in the same column are not significantly different in the LSD 5% test.

Plants also divert resources to vital maintenance (osmoregulation, damage repair) rather than secondary metabolite synthesis. Extreme drought triggers leaf senescence. During senescence, chlorophyll and secondary metabolites can be degraded or oxidized. Some flavonoids are relocalized or used as "ROS sinks," resulting in lower measurable levels. Differences in leaf phase (older/withered), very low tissue water content (which causes less efficient extraction), or data normalization (e.g., fresh vs. dry weight) can also make values appear lower at 6 days. However, Kustiawan and Kusuma (2019) revealed that drought conditions can trigger an increase in flavonoid compounds in plants. Their research indicates that plants experiencing water stress produce higher amounts of flavonoids in response to environmental pressure. Similarly, Zhang et al. (2021) found that abiotic stress, particularly drought, can stimulate the production of secondary metabolites, including flavonoids, as a plant protection mechanism against unfavorable conditions. In addition, Patil et al. (2024) found that flavonoids play a crucial role in plant resistance to environmental stress, serving as antioxidants, UV protectors, and signal regulators, and their production increases during stressful conditions.

The effect of treatment with extract from Super 1 sorghum variety showed a significant effect on phenol content. The highest phenolic content was achieved by watering every 6 days (3310.0 μg/g) and was significantly different from daily watering; however, it was not significantly different from watering every 4 days or every 5 days, as shown in Table 2. This study shows that watering every day to every 6 days produces phenolic content from low to high. This indicates that as

Phenols are adaptive secondary metabolites that are closely related to oxidative stress responses. When plants experience increasingly severe drought stress, phenol levels typically increase in proportion to the stress intensity. The process can be explained as follows: 1) Watering every day (control, no stress). With optimal water conditions, plants do not produce many ROS. The activity of the phenylpropanoid pathway (PAL, a key enzyme in phenol synthesis) is relatively low, so phenol levels are also low. 2) Watering every 2–4 days (mild to moderate stress). Water deficiency begins to be felt, so the stomata close partially and photosynthesis slows down, resulting in the formation of moderate amounts of ROS. Plants respond by increasing phenol synthesis to protect cells from oxidative damage. 3) Watering once every 5 days (moderate to severe stress). Dry conditions are more pronounced, causing a significant increase in ROS. Phenylalanine ammonia-lyase (PAL) enzyme activity increases, accelerating phenol accumulation. At this stage, phenols function as powerful antioxidants and also as secondary osmoregulators to maintain cell integrity. 4) Watering every 6 days (severe stress). Plants are under the most stress, resulting in very high ROS levels. Under these conditions, plants “maximize” phenol production as a last-resort defense mechanism.Although the biosynthesis of other metabolites may decrease due to energy limitations, phenols often remain elevated because the phenylpropanoid pathway is closely linked to the plant's basic defense response (stress-induced compounds).

Thus, phenol levels follow a linear pattern, increasing with drought levels. This increase occurs because continuously rising ROS strongly signals the activation of the phenylpropanoid pathway, which stimulates the production of phenols. Because phenols are more "adaptive constitutive" than flavonoids, their production continues even as stress becomes more severe. Plants, therefore, prioritize phenols as main defense compounds under extreme conditions. Phenol levels increase from daily watering to once every 6 days because plants respond to increasing oxidative stress by boosting phenol synthesis for antioxidant and protective defense.

Research on barley shows that drought stress increases total phenolic content and antioxidant enzyme activity. The phenylpropanoid pathway is activated, and phenolics act as antioxidants and ROS scavengers (Mohagheghian et al., 2025). Drought stress triggers the accumulation of phenolic compounds through the upregulation of the phenylpropanoid pathway, including enzymes such as PAL, CHS, and others, to counteract the negative effects of stress (Sharma et al., 2019). The accumulation of phenolic acids and flavonoids increases as an adaptive response to drought. This is explained by the modulation of the phenylpropanoid pathway, which plays a role in stress mitigation (Park et al., 2023). Phenylpropanoid metabolic activity increases under abiotic stress, aiding in the detoxification of ROS and protecting cells from oxidative damage. Key genes such as PAL, C4H, 4CL, CHI, and F3H are activated (Aluko et al., 2025). The phenylpropanoid pathway is closely integrated with stress adaptation mechanisms, producing polyphenols—such as phenols and flavonoids—that possess antioxidant properties, membrane-stabilizing functions, and roles in osmotic adjustment (Rao & Zheng, 2025).

CONCLUSION AND RECOMMENDATION

Extracts from drought-stressed plants irrigated every 5 days had the highest flavonoid content (1045.0 μg/g), whereas the lowest flavonoid levels were found with both daily irrigation (330.0 μg/g) and irrigation every 6 days (320.0 μg/g). In contrast, irrigation every 6 days resulted in the highest phenol content (3310.0 μg/g), while daily irrigation yielded the lowest phenol content (1150.0 μg/g). Overall, higher drought stress generally resulted in elevated flavonoid and phenol contents. Thus, extracts from sorghum subjected to specific drought stress levels appear to be optimal candidates for the development of bioherbicides.

FUTHER STUDY

This research still has delays, so it is necessary to conduct further research related to the topic The Effect of Drought Stress on Flavonoid and Phenolic Content in Super 1 Sorghum Extract in order to improve this research and add insight for readers.

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