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# Characterization of Efficacy and Flavonoid Levels in Sorghum Extracts of Suri 4 Variety with Different Levels of Drought Stress

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ABSTRACT

Bioherbicides that utilize the allelopathic properties of sorghum plants that experience drought stress are fundamental to sustainable agriculture. This study aimed to look at the efficacy characteristics and flavonoid levels of Suri 4 extracts that have experienced various levels of drought stress. Extract materials were prepared from May to July 2024 at Bengkulu University Greenhouse, Kandang Limun, Bengkulu City, and *bioassay* tests were conducted in August 2024. Flavonoid analysis was performed at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University in August 2024. This research used a completely randomized design with one factor. One sorghum variety, Suri 4, was planted with water stress treatment through watering patterns every day, every 2 days, every 3 days, every 4 days, and every 5. The *bioassay* experimental unit was a petri dish, and the experiment was repeated four times. Each petri dish was given 10 mL of water extract, and 25 mung bean seeds were planted and incubated for three days. The results showed that the highest flavonoid levels were achieved by the 5-day watering pattern (3841.65 µg/g), and the lowest flavonoid levels were completed by the daily watering pattern (2948.07 µg/g). The highest germination inhibition was due to the sorghum water extract from the 4-day and 5-day watering patterns and was the best bioherbicide pioneer


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## INTRODUCTION

Weeds are one of the significant constraints in crop production due to their ability to compete with main crops for essential resources such as water, nutrients, light, and growing space. This competition can significantly reduce crop productivity, with the degree of competition varying depending on the type of weed, its population density, and environmental conditions. If not appropriately managed, this competition can lead to yield reductions of up to 30-50% (Chauhan et al., 2020). Therefore, effective weed management strategies are needed to ensure resource access for main crops while supporting the sustainability of agricultural systems.

Environmentally friendly weed management is an important approach to maintaining agricultural productivity. This approach involves using methods such as natural compound-based bioherbicides, crop rotation, organic mulching, and mechanical techniques that minimize negative environmental impacts. Bioherbicides containing allelopathic compounds, such as flavonoids in sorghum, have proven effective in inhibiting weed growth without leaving harmful chemical residues. In addition, crop rotation helps to break the life cycle of weeds naturally, thereby reducing weed populations in agricultural fields.

Sorghum has excellent potential in weed management thanks to its content of allelopathic compounds such as sorgoleone, which effectively inhibits weed germination and growth. Research shows that sorghum extracts, such as sorgaab, can reduce weed density by up to 30% without damaging soil productivity (Susilo et al., 2021). In addition, sorghum can adapt to challenging environmental conditions, such as tolerance to drought and nutrient-poor soils, making it suitable to support sustainable agricultural systems.

Drought as a form of environmental stress can increase levels of bioactive compounds in sorghum, including flavonoids that have allelopathic properties. Kustiawan & Kusuma (2019) reported that sorghum flavonoid levels increased under drought conditions, increasing its allelopathic potential. This opens up opportunities to develop more effective and environmentally friendly sorghum-based bioherbicides. Further research is needed to understand the relationship between environmental stress and allelopathic properties of sorghum, especially on marginal lands such as Inceptisol, to support its broader application in sustainable agriculture. This study aims to evaluate seed germination characteristics (*bioassay* method) and flavonoid content of extracts (laboratory test) obtained through different levels of drought stress.

## LITERATURE REVIEW

Sorghum is a cereal crop that can withstand dry conditions and grow on marginal soils, making it ideal for dry climate regions. In drought situations, sorghum increases the production of secondary metabolites, such as phenolics, flavonoids, and terpenoids, which act as protective mechanisms against pathogens, herbivores, and competitors (Varela et al., 2022; Akula & Ravishankar, 2011).

Sorghum also contains allelopathic compounds, including sorgoleone, a phenolic compound produced by its roots. Sorgoleone is effective in inhibiting germination and growth of weeds around sorghum. The production of sorgoleone and other secondary metabolites increases significantly when sorghum is subjected to drought stress (Zuo et al., 2021).

Research shows that drought triggers the accumulation of secondary metabolites in sorghum in response to environmental stress (Vogler et al., 2019). Drought triggers oxidative stress, activating enzymes in the phenylpropanoid biosynthetic pathway and producing phenolic compounds such as sorgoleone (Khan et al., 2020). This increase in sorgoleone concentration amplifies the allelopathic effect of sorghum against competing plants, making it more effective as a natural bioherbicide.

The utilization of sorghum as a natural bioherbicide is based on its high allelopathic potential, especially in dry or marginal lands that often experience drought (Weston & Duke, 2003). Under these conditions, sorghum can be used as a natural strategy for weed control while reducing dependence on synthetic herbicides that have the potential to pollute the environment (Dayan, 2019).

Drought stress in sorghum increases the production of allelopathic secondary metabolites that can potentially be used as bioherbicides. Further research is needed to identify the specific active compounds responsible for these allelopathic effects and explore their application in a broader, sustainable, environmentally friendly agricultural system (Bhowmik & Inderjit, 2003). Abiotic stress in the form of drought also spurs secondary metabolites, especially flavonoids. According to Akula & Ravishankar (2011), abiotic stresses such as drought can increase the production of secondary metabolites in plants, including flavonoids. Flavonoids function as natural antioxidants that protect plants from oxidative stress caused by environmental stress. Khan et al. (2020) argue that drought increases enzymatic activity in the phenylpropanoid biosynthetic pathway, which plays a role in flavonoid production. These flavonoids help plants deal with oxidative stress and serve as a defense mechanism against external stress. Ferdinando et al., (2014) suggested that flavonoids are essential in protecting plant cells from damage caused by drought stress. Flavonoid production increases significantly in response to environmental stress, making it one of the primary secondary metabolites in plant adaptation.

## METHODS

### Plant material

This study utilized water extract obtained through a series of treatment stages. Sorghum variety Suri 4 was planted on Inceptisol soil media with five variations of water stress treatment: watering every day, every 2 days, every 3 days, every 4 days, and every 5. Plants were harvested after 4 weeks of age, then the crown and roots were separated, dried in the sun for 14 days, and continued with oven drying at 70°C for 72 hours. The dried plants were chopped into small pieces measuring 2-3 cm, then ground using a grinder or blender until they became powder. It was used as raw material for preparing aqueous extracts in flavonoid analysis and *bioassay* tests.

**Water Extract Preparation**

One hundred grams of sorghum powder (5% concentration) was soaked in 1900 mL of distilled water and stirred continuously for 24 hours at room temperature using a stirrer. The solution was then filtered with a cloth and filter paper to produce a clear extract. This extract was labeled and stored for use in *bioassay* tests.

***Bioassay* ith Water Extract on Filter Paper**

*bioassay* tests were conducted to test the ability of sorghum water extracts containing allelopathic compounds, such as flavonoids, to inhibit mung bean seed germination. A total of 25 mung bean seeds were placed in a 9 cm diameter petri dish coated with two layers of filter paper, then 10 mL of 5% concentration of water extract was added. Petri dishes were incubated in a growth chamber for 3 days. Each treatment combination, involving sorghum variety and water stress level, was repeated four times to increase the accuracy of the results.

**Measurement of Experiment Variables**

Variables observed included total flavonoid content, germination percentage, percentage of non-germinated seeds, wet weight of live and dead sprouts, and wet weight and length of plumula and radicle.

**Statistic Analysis**

The study used a completely randomized design with one factor and four replications. Data analysis was performed statistically using ANOVA, and differences between treatments were tested using the LSD test at a significance level of $P < 0.05$.

**RESULT**

The observation variables in this study were flavonoid levels, test plant germination with variables of percentage of live sprouts, percentage of non-growing sprouts, wet weight of live sprouts, damp weight of non-living sprouts, wet weight of plumula + radicle, and length of plumula + radicle. Based on the table of variance, it shows that the treatment of sorghum extract variety Suri 4 with different levels of drought stress shows a very significant effect on flavonoid content, percentage of live sprouts, percentage of non-living sprouts, wet weight of live sprouts, wet weight of non-living sprouts, wet weight of plumula + radicle, and length of plumula + radicle shown in Table 1.

The effect of extract treatment from sorghum variety Suri 4 showed a significant impact. The highest flavonoid levels were achieved by watering every 5 days (3841.65 µg/g) and were significantly different from other watering treatments. The lowest flavonoid levels were achieved by watering daily (2948.07 µg/g) and were substantially different from other varieties shown in Table 2. Research, in general, shows that the longer the watering interval of sorghum plants of the Suri 4 variety, the more flavonoid levels produced increase. Kustiawan & Kusuma (2019) revealed that drought conditions can trigger increased plant flavonoid compounds. Their research shows that plants that experience stress from lack of water produce higher amounts of flavonoids in response to environmental stress. Zhang et al. (2021) added that abiotic stress, especially drought, encourages plants to produce secondary metabolites such as flavonoids to protect against unfavorable environmental conditions. In addition, Ali et al., (2023) found that drought-stressed sorghum plants showed increased flavonoid levels, which play a role in strengthening plant resistance to stress due to water deficit.

Table 1. Recapitulation of Flavonoid Levels and Test Plant Germination Due to Treatment with Water Extract of Sorghum Variety Suri 4 with Different Levels of Water Stress

| Variables observation | Treatment | Coefficient of variation (%) |
|---|---|---|
| Flavonoid | 2353,00 ** | 0,12 |
| Percentage of live sprouts | 27,58 ** | 36,36 |
| Percentage of dead sprouts | 27,58 ** | 14,97 |
| Wet weight of live sprouts | 24,77 ** | 44,64 |
| Wet weight of dead sprouts | 49,82 ** | 12,10 |
| Plumula and radicle wet weight | 75,70 ** | 25,96 |
| Length of plumula and radicle | 153,23 ** | 19,82 |

** = very significantly different

The effect of water extract treatment from various levels of drought stress on the percentage of live sprouts showed a significant impact. The control achieved the highest rate of live sprouts and was significantly different from other watering patterns. The lowest percentage of live sprouts was completed by the watering pattern every 5 days and every 4 days, as shown in Table 3. The data shows that the control achieved the most extended watering interval and the lowest percentage of live sprouts. This indicates that the test plants were affected by the extract obtained from the drought-stressed plants, where different watering patterns produced the highest inhibition. The data shows that the provision of extracts can inhibit the germination process. Based on Susilo et al., (2024), a water extract from the ratoon variety Suri 4 leaves can inhibit the germination of sorghum seeds, produce the highest level of abnormal sprouts, and make the lowest length of plumula and radicle.

Table 2. Average Flavonoid Content of Sorghum Extracts of Suri 4 Variety with Different Levels of Water Stress

| Watering treatment | Flavonoid (µg/g) |
|---|---|
| Every day | 2948,07 e |
| Every 2 days | 3331,21 d |
| Every 3 days | 3758,00 b |
| Every 4 days | 3423,46 c |
| Every 4 days | 3841,65 a |

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

The effect of water extract treatment of various levels of drought stress on the percentage of sprouts that did not grow showed a significant impact. The watering pattern achieved the highest rate of unsprouted sprouts every 5 days and 4 days. The control achieved the lowest percentage of non-sprouts and significantly differed from the other treatments shown in Table 3. The data shows that extracts from drought influence the test plant-stressed plants, where variations in watering patterns produce the highest inhibition. Susilo & Pujiwati (2023) stated that water extracts from the ratoon stem of the Suri 4 variety, in particular, as well as water extracts from the stem organs of sorghum ratoon in general, have the potential to become bioherbicides with the ability to inhibit the growth of test plants.

Table 3. Effect of Aqueous Extract Derived From Sorghum Variety Suri 4 with Different Levels of Water Stress on the Percentage of Live Sprouts, Percentage of Unsprouted Seeds, and Live Sprout Weight

| Watering treatment | Live sprouts (%) | Seeds do not grow (%) | Live sprout weight (g) |
|---|---|---|---|
| Control | 90.00 a | 10.00 c | 2.450 a |
| Every day | 25.00 c | 75.00 b | 0.517 bc |
| Every 2 days | 25.00 c | 75.00 b | 0.488 bc |
| Every 3 days | 30.00 b | 70.00 b | 0.668 b |
| Every 4 days | 5.00 c | 95.00 a | 0.079 c |
| Every 4 days | 0.00 c | 100.00 a | 0.000 c |

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

The effect of water extract treatment from various levels of drought stress on the weight of live sprouts showed a significant impact. The control achieved the highest live sprout weight and significantly differed from other watering patterns. Inhibition of sprouts, especially the highest live sprout weight variable, by watering patterns every five and every 4 days is shown in Table 3. This indicates that extracts from drought influence the test plant stressed plants, where variations in watering patterns produce maximum inhibition. Kumar et al., (2020) mentioned that plants exposed to environmental stress produce extracts containing compounds that can inhibit seed germination, as seen from the decrease in the percentage of seeds that successfully grow in the *bioassay* test.

The effect of water extract treatment from various levels of drought stress on dead seed weight showed a significant impact. The watering pattern achieved the highest dead seed weight every 5 days. The control achieved the lowest dead seed weight and significantly differed from the other treatments shown in Table 4. This indicates that the test plants exposed to extracts from plants with drought stress and variations in watering patterns experienced more significant inhibition than the control. Seeds as test plants affected by extracts tend not to undergo physiological germination, so the seed weight remains high.

In contrast, the control seeds experienced normal germination, which decreased their weight. Macías et al., (2020) explain that allelochemical compounds in plant extracts can interfere with the physiological process of germination by inhibiting the necessary metabolism. As a result, seed growth activity decreases, so seed weight does not decrease.

Table 4. Effect of Water Extract Derived from Sorghum Variety Suri 4 with Different Levels of Water Stress on Dead Seed Weight, Plumula + Radicle Weight, and Plumula + Radicle Length

| Watering treatment | Weight of dead seeds (g) | Weight of plumula + radicle (g) | Length of plumula + radicle (cm) |
|---|---|---|---|
| Control | 0.165 d | 0.148 a | 7.311 a |
| Every day | 1.030 c | 0.028 b | 1.160 b |
| Every 2 days | 0.979 c | 0.031 b | 1.127 b |
| Every 3 days | 1.068 bc | 0.047 b | 1.200 b |
| Every 4 days | 1.246 b | 0.000 c | 0.600 bc |
| Every 4 days | 1.468 a | 0.000 c | 0.000 c |

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

The effect of water extract treatment from various levels of drought stress on the weight of plumula + radicle showed a significant impact. The control achieved the highest weight of plumula + radicle and was significantly different from other watering patterns. The lowest plumula + radicle weight was achieved by the 5-day and every 4-day watering patterns shown in Table 4. This indicates that the test plants exposed to extracts from drought-stressed plants with varying watering patterns had the highest inhibition, which caused the low weight of plumules and radicles. Zhang et al., (2021) explained that drought stress triggers increased production of allelopathic compounds in donor plants, affecting test seed organs' growth, such as plumules and radicles, through interference with physiological and metabolic processes.

The effect of water extract treatment from various levels of drought stress on plumula + radicle length showed a significant impact. The control achieved the highest plumula + radicle length and significantly differed from the other watering patterns. The lowest plumula + radicle length was achieved by watering 5 days and every 4 days, as shown in Table 4. This indicates that the test plants affected by extracts from plants with drought stress and variations in watering patterns have the highest inhibition, so the length of the plumula and radicle is low. Cheng & Cheng (2021) explained that drought in donor plants increases the concentration of allelopathic compounds, which can inhibit the process of cell division and elongation in the plumula and radicle of test plants. Susilo & Pujiwati (2023) also found that water extracts from sorghum ratoon stems have the potential as bioherbicides with the ability to inhibit the growth of test plants.

## CONCLUSIONS

The 5-day watering pattern achieved the highest flavonoid content (3841.65 μg/g), and the daily watering pattern achieved the lowest (2948.07 μg/g). The sorghum water extract from the 4-day and 5-day watering patterns was the best bioherbicide pioneer, causing the highest germination inhibition.

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