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## Effect of water stress and varieties on the bioherbicidal effectivity of sorghum aqueous extracts

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**Abstract** The 5% water extract from Super 1 sorghum variety irrigated every 5 days, Suri 4 irrigated every 4 and 5 days, and Mandau irrigated every five days was more toxic compared to other treatments, as indicated by the lowest percentage of seed germination, seedlings fresh weight, fresh weight of plumule and radicle, and plumule and radicle length, as well as the higher percentage of dead seedlings and fresh weight of dead seedlings. These findings suggested that the Super 1, Suri 4, and Mandau sorghum varieties, with irrigation frequencies of 4 or 5 days, can produce allelopathic compounds that can be used as bioherbicides to control broadleaf weeds.

**Keywords:** Bioherbicide, Natural weed control, Sorghum extract efficacy, Sustainable agriculture, Water stress

### Introduction

Weeds compete with crops for resources, and their presence can lead to reduced yields (Little *et al.*, 2021). Weeds decrease crop yields by utilizing available resources such as water, nutrients, and growing space (Farooq *et al.*, 2020). On the other hand, successful crop cultivation is determined by several factors, including superior seeds, land degradation, harvesting, and post-harvest practices, climate change, and the management of pests, diseases, and weeds (Farooq *et al.*, 2023; Musara *et al.*, 2021). Farmers control weeds manually (Tibugari *et al.*, 2020) or use synthetic herbicides due to labor shortages (Chikowo *et al.*, 2008).

Weeds that interfere with crops and reduce yields can be broadleaf, grass, or sedges (Tibugari *et al.*, 2020). Manual weed control costs more than synthetic herbicides. However, excessive and irresponsible use of synthetic pesticides frequently harms the environment and health (Scavo *et al.*, 2020). Therefore, appropriate weed control methods are needed, which are essential for sustainable

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agricultural systems. In implementing weed control methods, a comprehensive assessment is required to ensure a positive impact on farm sustainability while also being environmentally sustainable. Investment in environmentally friendly weed management is crucial for sustainable crop production.

Organic and environmentally friendly weed management is essential to address the issue of weed resistance to synthetic herbicides (Tubieleh and Souikane, 2020). According to Arif *et al.* (2015), using organic herbicides derived from allelopathic plants is one alternative for controlling weeds while being environmentally friendly. Allelopathy, therefore, is one solution for controlling weeds in crop production (Farooq *et al.*, 2020). Allelochemicals are often employed to control weeds and allelopathic plants as cover crops in weed management (Alsaadawi *et al.*, 2015). Allelopathy can be combined with other methods for sustainable weed control (Kundra *et al.*, 2023).

Applying bioherbicides will benefit all stakeholders in developing effective and environmentally friendly weed management strategies and reducing dependence on synthetic herbicides (Iqbal *et al.*, 2020). Implementing allelopathy as one of the weed control efforts in sustainable agricultural systems in developing countries is essential. However, the proposed control methods must be simple and economically viable to ensure the successful application of allelopathy. Environmentally friendly weed control strategies can be achieved by utilizing widely available, easily grown plants with allelopathic potential (Nornasuha and Ismail, 2017).

Sorghum is one of the plants that produces allelopathic compounds and is widely adaptable for growth. In addition to being able to produce and adapt to various environmental conditions, sorghum also has allelopathic compounds. Sorghum is tolerant to drought and can adapt to marginal lands with low nutrient content and rainfall (Ali *et al.*, 2023). According to Kandhro *et al.* (2016), sorghum is an allelopathic plant capable of inhibiting other plant species. Sorghum contains allelopathic compounds and can suppress weed growth in cotton plants. Sorghum water extract can reduce weeds' density, fresh weight, and dry weight by 29%, 31%, and 27%, respectively (Ashraf and Akhlaq, 2007).

Using sorghum from various varieties as raw material for bioherbicides is suspected to produce different allelopathic potentials in suppressing weeds. One strategy to enhance allelopathic properties is through genetic modification. The allelopathy produced should also be safe or non-toxic to both humans and ecosystems, capable of increasing crop productivity, and have affordable application costs (Amb and Ahluwalia, 2016). According to Susilo *et al.* (2021a), water extracts of sorghum produced from dry cultivation (water stress) on swamps and Ultisol have the potential to serve as bioherbicides. Susilo *et al.*

(2021b) state that applying sorghum water extracts at a concentration of 5% to 10% from dry swamps exhibits higher inhibition than lower concentrations.

Several experiments have reported a relationship between environmental growth factors and plant varieties regarding allelopathic content. Research on the relationship between allelopathy and abiotic environments with abiotic stress has been conducted; however, studies on allelopathy in water-stressed environments on Inceptisols with various sorghum varieties have not been conducted. This research will evaluate water extracts from several sorghum varieties grown in Inceptisols with different irrigation frequencies. The study aimed to evaluate the inhibitory effects of water extracts from various sorghum varieties with varying irrigation frequencies on seed germination.

## **Materials and methods**

### ***Extract source***

Five sorghum varieties, namely Super 1, Super 2, Suri 4, Bioguma, and Mandau, were planted in Inceptisol. Each variety was subjected to water stress treatments with different irrigation frequencies: daily, and every 2, 3, 4, and 5 days. The extract material was obtained from sorghum harvested at 4 weeks after planting. After harvesting, the plant shoots and roots were dried under sunlight for 14 days and then dried in an oven at 70°C for 72 hours. Subsequently, the dried material was ground using a grinder, and the resulting powder was extracted with water.

### ***Water extract preparation***

To obtain a 5% concentration, 50 g of dried sorghum powder was soaked in 950 mL of distilled water and stirred for 24 hours using a shaker at room temperature. After stirring, the mixture was filtered using Whatman filter paper. This water extract was then used for bioassay test.

### ***Bioassay experiment***

The bioassay was conducted in the laboratory using mung bean (*Vigna radiata*) as the test plant. A total of 25 mung bean seeds were placed in a Petri dish (9 cm in diameter) that was previously lined with 2 sheets of filter paper. Then, 10 mL of sorghum water extract (5% concentration) from various varieties and water stress treatments was added to each Petri dish. The Petri dishes were incubated in a growth chamber for 3 days. The research design used in the

bioassay was a completely randomized design, one factor. Each treatment was replicated 4 times.

### *Variables*

The observed variables were percentage of seed germination, percentage of dead seedling, seedling fresh weight, dead seedling fresh weight, plumula and radicle fresh weight, and plumula and radicle length.

### *Statistic analysis*

Data were analyzed statistically using Anova at the 5% level. If there are statistically differences between treatments, they are tested further with Least Significant Different (LSD).

### **Results**

The treatment of water extracts from various sorghum varieties and irrigation frequency had highly significant affected on percentage of seed germination, percentage of dead seedling, seedling fresh weight, dead seedling fresh weight, plumula and radicle fresh weight, and plumula and radicle length (Table 1).

**Table 1.** Effect of water extract of various sorghum varieties and irrigation frequency on seedling germination and growth

Variable	Treatments	Coeff. variation (%)
Percentage of seed germination	10.70 **	22.102
Percentage of dead seedlings	10.70 **	16.14
Seedling fresh weight	6.53 **	31.97
Dead seedling fresh weight	16.00 **	16.02
Plumula and radicle fresh weight	49.89 **	23.13
Plumula and radicle length	79.52 **	18.40

\*\* = highly significant different

The highest germination inhibition and seedling fresh weight were produced by sorghum varieties Super 1, Suri 4, and Mandau with irrigation frequencies of every 4 or 5 days. Sorghum extract from the Super 1 variety, with an irrigation frequency of every 5 days, Suri 4, with an irrigation frequency of every 4 or 5 days, and Mandau, with an irrigation frequency of every 5 days, resulted in 10% or fewer live seedlings, meaning they were able to suppress germination by 90% to 100% (Table 2). Less frequent irrigation resulted in extracts that tended to be more toxic. Sorghum varieties Super 1, Suri 4, and

Mandau with irrigation frequencies of every 4 or 5 days produced biomass whose water extracts most inhibited seed germination and suppress weedling weight.

**Table 2.** The effect of water extracts from various sorghum varieties and irrigation frequency on seed germination and fresh weight

Sorghum varieties and irrigation frequency	Seed germination (%)	Dead seedling (%)	Seedling fresh weight (g)
Control treatment	90.00 <sup>a</sup>	10.00 <sup>i</sup>	2.460 <sup>a</sup>
<b>Super 1</b>			
Every day	20.00 <sup>fgh</sup>	80.00 <sup>bcd</sup>	0.321 <sup>efgh</sup>
Every 2 day	30.00 <sup>def</sup>	70.00 <sup>def</sup>	0.450 <sup>cdefgh</sup>
Every 3 day	25.00 <sup>efg</sup>	75.00 <sup>cde</sup>	0.340 <sup>defgh</sup>
Every 4 day	40.00 <sup>cde</sup>	60.00 <sup>efg</sup>	0.562 <sup>cdefg</sup>
Every 5 day	10.00 <sup>ghi</sup>	90.00 <sup>abc</sup>	0.127 <sup>fgh</sup>
<b>Super 2</b>			
Every day	40.00 <sup>cde</sup>	60.00 <sup>efg</sup>	0.656 <sup>cdef</sup>
Every 2 day	45.00 <sup>bcd</sup>	55.00 <sup>fgh</sup>	0.815 <sup>bcde</sup>
Every 3 day	40.00 <sup>cde</sup>	60.00 <sup>efg</sup>	0.611 <sup>cdefg</sup>
Every 4 day	55.00 <sup>bc</sup>	45.00 <sup>gh</sup>	0.795 <sup>cde</sup>
Every 5 day	60.00 <sup>b</sup>	40.00 <sup>h</sup>	0.864 <sup>bcd</sup>
<b>Suri 4</b>			
Every day	25.00 <sup>efg</sup>	75.00 <sup>cde</sup>	0.517 <sup>cdefgh</sup>
Every 2 day	25.00 <sup>efg</sup>	75.00 <sup>cde</sup>	0.488 <sup>cdefgh</sup>
Every 3 day	30.00 <sup>def</sup>	70.00 <sup>def</sup>	0.693 <sup>cde</sup>
Every 4 day	5.00 <sup>hi</sup>	95.00 <sup>ab</sup>	0.079 <sup>fgh</sup>
Every 5 day	0.00 <sup>i</sup>	100.00 <sup>a</sup>	0.000 <sup>h</sup>
<b>Bioguma</b>			
Every day	10.00 <sup>ghi</sup>	90.00 <sup>abc</sup>	1.340 <sup>b</sup>
Every 2 day	20.00 <sup>fgh</sup>	80.00 <sup>bcd</sup>	0.402 <sup>cdefgh</sup>
Every 3 day	25.00 <sup>efg</sup>	75.00 <sup>cde</sup>	0.329 <sup>efgh</sup>
Every 4 day	35.00 <sup>def</sup>	65.00 <sup>def</sup>	0.693 <sup>cde</sup>
Every 5 day	30.00 <sup>def</sup>	70.00 <sup>def</sup>	0.551 <sup>cdefg</sup>
<b>Mandau</b>			
Every day	55.00 <sup>bc</sup>	45.99 <sup>gh</sup>	0.908 <sup>bc</sup>
Every 2 day	55.00 <sup>bc</sup>	45.00 <sup>gh</sup>	0.826 <sup>bcde</sup>
Every 3 day	55.00 <sup>bc</sup>	45.00 <sup>gh</sup>	0.824 <sup>bcde</sup>
Every 4 day	35.00 <sup>def</sup>	65.00 <sup>def</sup>	0.503 <sup>cdefgh</sup>
Every 5 day	10.00 <sup>ghi</sup>	90.00 <sup>abc</sup>	0.130 <sup>fgh</sup>

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

The highest dead seedling fresh weight (1.468 g) was produced by sorghum extract from the Suri 4 variety with an irrigation frequency of every 5 days, while the control treatment at 0.165 g produced the lowest. Sorghum extracts from the Super 1, Suri 4, and Mandau varieties with an irrigation frequency of every 5 days resulted in the lowest fresh weight of plumules + radicles, while the shortest

plumule + radicle length was produced by the Suri variety with an irrigation frequency of every 5 or 4 days (Table 3). In general, the Suri 4 variety was more sensitive to sorghum allelopathy than other varieties, as indicated by the shortest plumule + radicle length.

**Table 3.** The effect of water extracts from various sorghum varieties and irrigation frequency of seedling growth

Sorghum varieties and irrigation frequency	Dead seedling fresh weight (g)	Plumula and radicle fresh weight (g)	Plumule + radicle length (cm)
Control treatment	0.165 <sup>k</sup>	0.148 <sup>a</sup>	7.311 <sup>a</sup>
<b>Super 1</b>			
Every day	0.839 <sup>efg</sup>	0.024 <sup>efgh</sup>	1.183 <sup>b</sup>
Every 2 day	0.794 <sup>fgh</sup>	0.025 <sup>efgh</sup>	1.163 <sup>b</sup>
Every 3 day	0.770 <sup>fgh</sup>	0.015 <sup>hijk</sup>	1.200 <sup>b</sup>
Every 4 day	0.627 <sup>ghi</sup>	0.022 <sup>efghi</sup>	1.227 <sup>b</sup>
Every 5 day	0.949 <sup>def</sup>	0.008 <sup>kl</sup>	0.577 <sup>c</sup>
<b>Super 2</b>			
Every day	0.686 <sup>ghi</sup>	0.029 <sup>defg</sup>	1.207 <sup>b</sup>
Every 2 day	0.614 <sup>hij</sup>	0.025 <sup>efgh</sup>	1.243 <sup>b</sup>
Every 3 day	0.631 <sup>ghi</sup>	0.019 <sup>gh</sup>	1.283 <sup>b</sup>
Every 4 day	0.504 <sup>ij</sup>	0.022 <sup>efghi</sup>	1.233 <sup>b</sup>
Every 5 day	0.399 <sup>j</sup>	0.011 <sup>ijk</sup>	1.223 <sup>b</sup>
<b>Suri 4</b>			
Every day	1.030 <sup>cde</sup>	0.028 <sup>defg</sup>	1.160 <sup>b</sup>
Every 2 day	0.979 <sup>def</sup>	0.031 <sup>cdef</sup>	0.127 <sup>c</sup>
Every 3 day	1.068 <sup>cd</sup>	0.047 <sup>b</sup>	1.200 <sup>b</sup>
Every 4 day	1.246 <sup>bc</sup>	0.000 <sup>l</sup>	0.600 <sup>c</sup>
Every 5 day	1.468 <sup>a</sup>	0.000 <sup>l</sup>	0.000 <sup>d</sup>
<b>Bioguma</b>			
Every day	1.448 <sup>ab</sup>	0.040 <sup>bc</sup>	1.150 <sup>b</sup>
Every 2 day	1.126 <sup>cd</sup>	0.032 <sup>cde</sup>	1.227 <sup>b</sup>
Every 3 day	1.019 <sup>de</sup>	0.027 <sup>defg</sup>	1.160 <sup>b</sup>
Every 4 day	0.949 <sup>def</sup>	0.043 <sup>b</sup>	1.217 <sup>b</sup>
Every 5 day	0.909 <sup>def</sup>	0.028 <sup>defg</sup>	1.263 <sup>b</sup>
<b>Mandau</b>			
Every day	0.653 <sup>ghi</sup>	0.030 <sup>cdefg</sup>	1.257 <sup>b</sup>
Every 2 day	0.546 <sup>ij</sup>	0.036 <sup>bcd</sup>	1.250 <sup>b</sup>
Every 3 day	0.590 <sup>hij</sup>	0.024 <sup>efgh</sup>	1.250 <sup>b</sup>
Every 4 day	0.642 <sup>ghi</sup>	0.020 <sup>fghij</sup>	1.227 <sup>b</sup>
Every 5 day	1.063 <sup>cd</sup>	0.010 <sup>jkl</sup>	0.577 <sup>c</sup>

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

## Discussion

The highest inhibition of germination was produced by the Super 1, Suri 4, and Mandau varieties' water extracts with an irrigation frequency of once every 4 or 5 days. The longer the interval between irrigation showed the higher the inhibition effect produced by the Super 1, Suri 4, Bioguma, and Mandau varieties. Thus, the greater the water stress on the sorghum plants revealed the higher the inhibition effect suppressing germination and seedling growth.

The differences in water stress levels resulted in varying allelopathic content in sorghum. The variation in sorghum growth under different water stress conditions affected the plant's physiological processes. According to Asadi and Eshghizadeh (2021) who stated that water-stressed sorghum plants experienced to decrease in the activity of antioxidant enzymes, namely catalase, ascorbate peroxidase, and peroxidase. Additionally, the proline content in the leaves and the membrane stability index decrease. Furthermore, water stress in sorghum plants also reduces starch synthesis enzyme activities (Bing *et al.*, 2014), disrupting sorghum metabolism and affecting the production of secondary metabolites involved in forming allelopathic compounds.

The water extracts of sorghum from the Super 1, Suri 4, and Mandau varieties with an irrigation frequency of once every 5 days resulted in a very high percentage of dead seedlings, ranging from 90% to 100%, as well as low seedling weight. The high inhibition of seed germination indicated that water-stressed sorghum is produced more toxic allelopathy than sorghum with lower water-stress levels. The lowest germination inhibition was observed in the control treatment or without allelopathic treatment. According to Ibrahim *et al.* (2022), drought stress negatively affects wheat seedling growth, including its biochemical attributes, such as reduced antioxidant enzyme activity. To Glab *et al.* (2017) stated that sorgoleone, one of the allelopathic components, can inhibit H<sup>+</sup>ATP-ase membrane activity. This resulted to reduce the water uptake and inhibited seedling growth. After ten days, seedlings showed the growth reduction due to the effects of sorgoleone (Weston and Czarnota, 2001). Phytotoxicity is caused by phenolic compounds from allelopathy in seedlings that inhibited plant development due to changes in water status, increased ABA content, and osmotic and oxidative stress (Araniti *et al.*, 2020).

Sorghum extracts of the Suri 4 sorghum variety, with an irrigation frequency of once every 5 days, showed the highest fresh weight of dead seeds, amounting to 1.468 g, and the lowest was the control, at 0.165 g. Mung bean seeds was used as the tested plant that treated with sorghum extract and experienced water stress which resulted in a high weight of dead seedlings or endosperm. This occurs because seeds are subjected to stress from sorghum

extract which exhibited a low metabolic activity, their weight remained high. On the other hand, the control treatment was the lowest fresh weight of dead seeds. This indicated that irrigation water without allelopathic treatment had a little to no affect on cotyledon breakdown during germination. Seeds with normal metabolic activity produce low dead seed weight and low fresh weight of dead seeds. This study showed that allelochemicals affect the physiological processes of target plants, including increased production of ROS (reactive oxygen species), which disrupts cell membranes, reduces mitochondrial function, and interferes with the activity of the phytohormone IAA. The increase in ROS content hinders seedling development. The higher the level of stress, the greater the endosperm weight produced. These findings aligned with Weston and Czarnota (2001), who stated that allelochemicals enhance plant growth at low concentrations but inhibit growth under high-stress conditions.

The germination ability showed that the sorghum extracts from the Super 1, Suri 4, and Mandau varieties with the most infrequent irrigation every 5 days revealing the most effectively suppressed seed germination, as indicated by the lowest plumule and radicle length and weight (Table 3). The study results showed that the more stressed the growing medium, the more toxic the allelopathy produced by the sorghum. The allelopathy can inhibit the germination and growth of mung bean seeds used as the test plant. Susilo *et al.* (2020) reported that sorghum extracts inhibit shoot growth due to the high production of allelopathic compounds. According to Inderjit and Keating (1999), allelopathy in plants under abiotic stress conditions, such as drought, tends to increase secondary metabolite production. The cyanogenic glycoside levels in sorghum plants significantly increase under drought conditions (Maqbool *et al.*, 2013). Weston and Czarnota (2001) stated that sorgoleone, a compound in allelopathy, constitutes 85–90% of the root exudates of sorghum. The phytotoxicity of allelopathy halts plant growth, photosynthesis, respiration, and seedling chlorosis, inhibiting growth. Although sorghum releases various compounds, such as phenolic compounds, vanillic acid, and benzoic acid, each cultivar releases different amounts of these compounds (Glab *et al.*, 2017).

The highest inhibition of test plant germination was produced by the Super 1 sorghum variety with an irrigation frequency of once every 5 days, the Suri 4 variety with an irrigation every 5 days, and the Mandau variety with irrigation every 5 days. The highest inhibition is indicated by the lowest percentage of live seedlings, lowest fresh weight of live seedlings, plumule and radicle fresh weight, and plumule and radicle length, as well as the highest rate of dead seedlings and the highest fresh weight of dead seedlings. These findings suggested that the Super 1, Suri 4, and Mandau sorghum varieties are shown to



be the potential bioherbicides with the highest and most toxic allelopathic compounds.

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