
Impact of water stress and sorghum varieties on flavonoid content and the bioherbicidal potential of water extracts for weed control

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Abstract The results showed that the Super 1 sorghum variety under drought stress with watering every 4 days produced the highest total flavonoid content (4120.61 µg/g), while the Bioguma variety with watering every 2 days had the lowest total flavonoid content (2859.83 µg/g). A longer drought stress period led to the higher the flavonoid content. The allelopathic extract sources showed varying levels of effectiveness in inhibiting weed growth. Sorghum aqueous extracts (based on variety and drought stress level) were able to control grass weeds, except *Eleusine indica*, which showed higher tolerance. Sedge was effectively suppressed by extracts from the Super 1 variety under 4- or 5-day stress intervals and the Suri 4 variety with 5-day stress. Broadleaf weeds were effectively controlled by extracts from the Super 1 variety under 4-day drought stress. Sorghum aqueous extracts effectively controlled weeds, with weed control efficiency ranging from 69.19% to 77.52%. The highest weed control efficiency (77.52%) was achieved using extracts from Super 1 under 5-day drought stress and Suri 4 under 4-day stress. Consequently, sorghum aqueous extract can be considered a possible bioherbicide for efficient weed management. To identify the most effective allelopathic constituents for weed management, additional experiments with extracts from different sorghum varieties subjected to greater drought stress (beyond 5 days) will be needed.

Keywords: Bioherbicide, Flavonoid, Weed control, Sorghum water extract, Sustainable agriculture

Introduction

Weeds compete for space, light, water, and nutrients, which affect production systems and integrated agriculture. This has been attributed to significant crop yield losses and even crop extinction (Colbach *et al.*, 2020; Horvath *et al.*, 2023; Da Silva *et al.*, 2022; Little *et al.*, 2021).

In contemporary farming systems, the use of industrial agricultural herbicides continues to dominate weed control practises. Although these

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industrial pesticides have a negative impact, especially in extrapolated and repeated applications, they provide an immediate short-term solution. Negative impacts include contamination of soil and water, nontarget organism mortality, residual toxicity, and the potential of creating a new generation of herbicide-resistant weeds (Parven *et al.*, 2025; Baćmaga *et al.*, 2024; Ofosu *et al.*, 2023; Gao *et al.*, 2024). These issues justify the search for new methods of weed control that are more ecologically balanced and sustainable.

Allelopathy can be defined as the biochemical process involving plants' release of secondary metabolites such as phenolics, tannins, and flavonoids. Increasing recognition supports this concept as a potential base for developing bioherbicides to sustain agriculture (Khamare *et al.*, 2022; Kostina-Bednarz *et al.*, 2023). Sorghum is among the most studied allelopathic crops because of its production of sorgoleone besides different phenolic acids through root exudates and residues left after the crop (Weston *et al.*, 2013). Flavonoids found in aqueous extracts from sorghum show very high biological activity hence making them top candidates considered for natural bioherbicidal agents.

Sorghum thus has potential as a natural bioherbicide or weed suppressant under different genetic and environmental conditions of the crop, particularly drought. The profile of allelochemicals in sorghum is highly determined by its genetic diversity together with expression levels invoked through environmental factors such as drought. Flavonoids are activated by drought; several genes have been mapped very close to loci controlling increased accumulation of flavonoids (Yue *et al.*, 2025). The metabolites function both ways: antioxidants and allelochemicals against neighboring plants including weeds (Patil *et al.*, 2024). Large genotypic differences found between phenolic acids and flavonoid content under dry conditions that result from interaction effects between genotype and environment on forming total allelopathic potential in sorghum (Tibugari *et al.*, 2019).

Genotype by environment interaction decisively determines the allelopathic profile of sorghum. However, barely little is known about their interaction on flavonoid biosynthesis and associated bioherbicidal activity. The present study therefore investigates sorghum under abiotic stressed coastal agroecosystems both for its potential use as a sustainable bioherbicide and also to identify varieties with higher secondary metabolite content.

Materials and methods

Experimental site and design

There were two sequential experiments conducted in 2024 at the Faculty of Agriculture, University of Bengkulu, Indonesia. The first experiment

determined total flavonoid content in sorghum under different irrigation intervals and regimes while the second experiment assessed phytotoxic effects of aqueous extracts of sorghum on weed growth. Both experiments were set up as a factorial arrangement between variety and irrigation interval within a Randomized Complete Block Design (RCBD).

The experiment was laid out in a randomized complete block design having two factors of treatment: Factor A comprising five varieties of sorghum, namely Super 1, Super 2, Suri 4, Mandau and Bioguma; and Factor B comprising levels of irrigation interval treatment imposed at one day (daily), two days, three days, four days and five days. Each treatment combination was replicated five times with two polybags (30 × 40 cm; 10 kg soil capacity) each planted with one plant per experimental unit giving a total number of 250 experimental units for Experiment 1 as well as seventy (70) for Experiment 2 which is sufficient replication to allow proper statistical analysis.

Day temperature inside the greenhouse was maintained between 28 and 32 °C, while night temperature was maintained between 22 and 25 °C, with a relative humidity of about 70-80 %. Irrigation was standardized at 1 L of water per polybag per irrigation event.

Preparation of Sorghum aqueous extracts

Sorghum plants were harvested four weeks after sowing. Shoots and roots were cleaned, sun-dried for 14 days, oven-dried at 70 °C for 72 h, and ground into fine powder. Extracts were prepared at a 1:10 (w/v) ratio by soaking 100 g of sorghum powder in 1 L of distilled water for 24 h, followed by filtration through Whatman No. 1 filter paper. Extracts were applied immediately in the bioassays to minimize degradation of active compounds.

Flavonoid quantification

The concentration of flavonoids was determined by the colorimetric method described by Chang *et al.* (2002) with slight modification. About 100 mg sample powder was hydrolyzed with 4 N HCl at 110°C for 2 h, extracted with diethyl ether and dried. The residue was reacted sequentially with 5% sodium nitrite, 10% aluminum chloride and 1M sodium hydroxide. Samples were brought to a final volume of 5 mL and the absorbance measured at 510 nm using a UV-Vis spectrophotometer. Flavonoid concentration was calculated from a standard curve prepared using quercetin and expressed as mg quercetin equivalents (QE)/g dry weight (DW). Each treatment replicate was analyzed in triplicate.

Weed suppression assay

Weed emergence came from the natural seed bank which was present in the soil used for experiment. Parameters measured included summed dominance ratio (relative abundance of weed species within the study area), total weed dry weight (biomass of all weed species per unit area), dominant weed dry weight (biomass of dominant species *Echinochloa indica* and *Phyllanthus zeylanica*) and Weed Control Efficiency, calculated using the formula. All weeds were oven dried at 70 °C for 72 h and expressed as g DW per polybag.

Statistical analysis

Flavonoid content and weed suppression data were subjected to two-way ANOVA (variety × irrigation interval) using SPSS v.27. Normality and homogeneity of variance were checked before analysis. Means were separated by least significant difference (LSD) test at $p \leq 0.05$. Results are presented as mean ± standard error (SE). Interaction plots have been provided to show any significant effects.

Results

Experiment 1: Flavonoid profiling of Sorghum under drought stress

Both variety ($p < 0.01$) and irrigation interval ($p < 0.01$) had highly significant effects on total flavonoid content with a highly significant effect of variety × irrigation interaction ($p < 0.01$) (Table 1).

Table 1. Interaction of sorghum varieties and drought stress on flavonoid content

Variables	Treatment			Coefficient Variation (%)
	Variety (V)	Drought stress (S)	Interaction (V x S)	
Total Flavonoid content	5.91 **	2.04 **	4.25 **	0.001

** = highly significant

The concentration of flavonoids increased under moderate drought (irrigation at 4-5day intervals) while levels were lower with daily or 2-day interval irrigation. The highest content was recorded in Super 1, with a 4-day interval of irrigation (4120.61 mg QE/g DW), and the lowest was recorded in Bioguma, with a 2-day interval of irrigation (2859.83 mg QE/g DW).

Table 2. Interaction between sorghum varieties and drought stress on the total flavonoid content of sorghum aqueous extracts

Irrigation time	Variety					Average
	Super 1	Super 2	Suri 4	Mandau	Bioguma	
	-----µg/g-----					
every day	3342.25 s	3165.26 w	2948.07 x	3241.81 v	3467.93 n	3233.06 e
every 2 days	3684.02 i	3387.33 p	3331.21 t	3351.49 r	2859.83 y	3322.78 d
every 3 days	3604.91 l	3366.96 q	3758.00 h	3991.34 c	3845.01 f	3713.25 b
every 4 days	4120.61 a	3536.46 m	3423.46 o	3901.77 d	3861.07 e	3769.07 a
every 5 days	4067.01 b	3625.00 k	3841.65 g	3285.51 u	3655.11 j	3694.86 c
Average	3763.76 a	3416.20 e	3713.25 b	3554.38 b	3538.19 c	

Note: numbers followed by the same letter are not significantly different based on the 5% LSD test.

Interaction plots showed distinctly different responses of genotypes to water stress. Flavonoid accumulation in the drought-tolerant varieties (Super 1, Suri 4) under 4–5-day irrigation was much higher than that of the sensitive varieties (Bioguma, Super 2) which registered a low response (Table 2).

Experiment 2: Bioherbicidal effects of Sorghum aqueous extract on weed

There were no statistically significant changes in the dominant weed species among the different treatments of sorghum extract or under regimes of drought stress, but an interesting trend was observed. In the control treatment, grass-dominated weed communities transformed into broadleaf-dominated ones after applying the extract, as indicated by changes in Summed Dominance Ratio (SDR) values. Total weed richness was reduced from nine species in the control to between five and eight species across different sorghum varieties and stress conditions (Table 3). This adds more evidence to what has been documented before about allelopathy of sorghum on suppression of weed germination and growth; hence it can still be considered a potential sustainable tool for integrated weed management.

At the species level, *Eleusine indica* was the most dominant grass across treatments with SDR values ranging from 29.3% (control) to 50.0% (SP14). *Phyllanthus zeylanica* was the leading broadleaf species and contributed from 17.5% (SU44) to 32.6% (SU45). Grasses, sedges, and broadleaf weeds were consistently represented across all treatments (Table 3). Species richness, however, varied among treatments. The control plots supported the highest number of dominant weed species (nine), whereas sorghum extract treatments reduced species richness to between five and eight species, depending on variety and irrigation regime. The application of sorghum extracts combined with drought stress significantly to highly significantly affected total weed dry weight,

dry weight of dominant weeds (*E. indica* and *P. zeylanica*), and weed control efficiency.

Table 3. Composition of dominant weeds under the combination of variety and drought stress treatments

Weed species	Summed Dominance Ratio (SDR) (%)						
	C	SP14	SP15	SU44	SU45	M4	M5
Grasses:							
<i>Eleusine indica</i>	29.3	50.0	38.2	34.6	41.0	29.3	39.8
<i>Eleutherine palmifolia</i>	5.4			8.8			
<i>Panicum colonum</i>	5.4						
<i>Leptochloa chinensis</i>	3.3						
<i>Dactyloctenium aegyptium</i>	17.3	7.9				7.3	
Broadleaf weeds:							
<i>Mimosa pudica</i>					5.8		
<i>Portulaca oleracea</i>					3.8	5.8	6.9
<i>Pouzolzia zeylanica</i>	30.0	24.5	17.6	17.5	32.6	22.6	24.6
<i>Phyllanthus urinaria</i>			3.9	9.4		11.2	3.2
<i>Spenochlea zeylanica</i>		4.9	21.7	4.1			12.8
<i>Melochia corchorifolia</i>	2.0		10.8	17.5	5.9	6.4	
<i>Ageratum conyzoides</i>			7.6				
<i>Acalypha indica</i>	2.7	12.6		3.9	10.8	12.6	4.7
<i>Ludwigia octovalvis</i>							3.5
<i>Amaranthus spinosus</i>	3.7						
Sedge:							
<i>Cyperus rotundus</i>				4.2		4.9	4.5

Note: C: control; SP14: Super 1, irrigation every 4 days, SP15: Super 1, irrigation every 5 days, SU4: Suri 4, irrigation every 4 days, SU5: Suri 4, irrigation every 5 days, M4: Mandau, irrigation every 4 days, M5: Mandau, irrigation every 5 days.

Table 4. Effects of sorghum aqueous extracts on weed biomass and weed control efficiency

Sorghum variety and irrigation time combination treatments	Total weed dry weight (g)	Dry weight <i>Eleusine indica</i> (g)	Dry weight <i>Pouzolzia zeylanica</i> (g)	Weed control efficiency (%)
Control	22.18 a	9.05 a	5.52 a	0.00 b
Super 1, every 4 days	8.23 b	5.85 ab	1.43 b	69.19 a
Super 1, every 5 days	6.01 b	2.55 b	2.12 ab	77.52 a
Suri 4, every 4 days	7.86 b	3.73 b	1.40 b	77.52 a
Suri 4, every 5 days	8.75 b	2.48 b	1.71 b	70.59 a
Mandau, every 4 days	7.44 b	2.65 b	3.54 ab	72.14 a
Mandau, every 5 days	8.07 b	5.04 ab	2.64 ab	69.79 a

Different letters indicate significant differences at LSD $p \leq 0.05$.

Sorghum extract treatments combined with drought stress significantly reduced total weed dry weight, including the dry weight of *E. indica* and *P. zeylanica*, compared with the control. Total weed dry weight declined from 22.18 g in the control to 6.01–8.75 g under treatments, with the lowest in Super 1 + irrigation every 5 days. The dry weight of *E. indica* decreased from 9.05 g (control) to 2.48–5.85 g, and *P. zeylanica* from 5.52 g to 1.40–3.54 g, with the lowest values in Suri 4 + irrigation every 5 days and Suri 4 + irrigation every 4 days, respectively. Weed control efficiency under sorghum treatments ranged 69.19-77.52%, compared to 0% in the control, with best performance observed in Super 1 + every 5 days, Suri 4 + every 4 days and Mandau + every 4 days.

Discussion

Flavonoid profiling of Sorghum under drought stress

Four weeks after treatment, significant differences appeared among the genotypes in total flavonoid content of sorghum due to aqueous extracts. This confirms a strong genetic control on biosynthesis pathway for flavonoids. The water deficit levels were also highly significant thus confirming that secondary metabolites are regulated under drought as an environmental stress factor and there was very high interaction between genotype by intensity of drought stressed which implies/flavonoid response is both genetically background dependent as well situational (environmental) condition dependent. These results imply that accumulation management (irrigation practice plus variety selection) of irrigation can be achieved through best practices involving varietal choice & irrigation management. Taleon *et al.* (2014) reported finding varieties with different contents depending on their growing environment/genotype while extreme weathers reduce flavanones but increase 3-deoxyanthocyanidin content.

Moderate drought condition (watering once in four days) hugely increased the flavonoid content, Super 1 variety recording a content of 4120.61 g/g while Bioguma watered once every two days recorded the lowest content at 2859.83 g/g. Similar results were obtained by Susilo *et al.* (2025), controlled drought enhances secondary metabolite accumulation in sorghum and Li *et al.* (2025), identified FLS (*flavone synthase*) as a key regulator under water stress. Flavonoid biosynthetic genes are upregulated by drought thereby increasing levels of myricetin, orientin among other compounds (Santos *et al.*, 2024; Ren *et al.*, 2022; Yue *et al.*, 2025). Therefore, as much as an accumulation is strongly dependent on genotype metabolic trigger for flavonoid biosynthesis in sorghum drought stress Moderate drought (4–5-day irrigation) consistently enhanced flavonoid production, aligning with previous findings in sorghum (Pinheiro *et al.*, 2021)

and other crops such as maize (Li *et al.*, 2021) and wheat (Guo *et al.*, 2025). The result is also similar to that reported by Pinheiro *et al.* (2021) in sorghum, Li *et al.* (2021), in maize and Guo *et al.* (2025) in wheat among others that a moderate drought enhances the production of flavonoids.

Flavonoids function as antioxidants, protecting plants from reactive oxygen species (ROS) generated under drought. The stress-induced ROS comprising superoxide, hydrogen peroxide and hydroxyl radicals activate secondary metabolic pathways among which flavonoid synthesis occupies a prime position mostly mediated by PAL (*phenylalanine ammonia-lyase*) enzyme (Sposito, 2008). A moderate stress promotes an increased synthesis of flavonoids as an adaptive response but under a severe drought condition biosynthesis is likely to be suppressed due to inhibition of metabolism. Similar mechanism has been reported in *Populus* spp., *Camellia sinensis* and medicinal plants (Ahmed *et al.*, 2021; Gu *et al.*, 2020; Selmar and Kleinwachter, 2013).

Flavonoids can thus be considered markers of drought tolerance since their content is usually higher in tolerant genotypes (Scavo *et al.*, 2020; Tubeileh and Souikane, 2020). Outside sorghum, a drought-enhanced accumulation of flavonoids has been associated with improved resistance to stresses in apple, maize, and wheat (Rao *et al.*, 2024; Li *et al.*, 2021; Guo *et al.*, 2025). Mechanistically secondary metabolism pathways including the pathway of flavonoid biosynthesis are strongly activated by ROS under an upsurge of ROS that drought imposes. *phenylalanine ammonia-lyase* (PAL) and *flavone synthase* (FLS) shall be expressed so that eventually myricetin-orientin-like compounds are accumulated for adaptation to stress (Li *et al.*, 2025).

Flavonoids have allelopathic activities on the growth of neighboring plants. Most commonly from the root exudates or released by damaged tissues in which they inhibit cell division and root elongation photosynthesis of a competing plant and even its seed germination (Patil *et al.*, 2024; Leoni *et al.*, 2021; Kalinova *et al.*, 2007). Flavonoids act as natural bioherbicides that can induce ROS accumulation selectively in weeds with resultant oxidative stress and inhibition of growth. More importantly, their biodegradability together with target specificity makes them more sustainable than synthetic herbicides (Alsaadawi *et al.*, 2015; Jabran *et al.*, 2015). Phenolic and flavonoid-based extract bioherbicides have already been proven effective regarding weed suppression (Anwar *et al.*, 2021; Hasan *et al.*, 2021) proposed to reduce the use of herbicide for attaining sustainable agriculture (Kostina *et al.*, 2023; Becerra-Alvarez *et al.*, 2025).

Bioherbicidal effects of Sorghum aqueous extract on weed

Weed species, sorghum genotype and irrigation regime significantly influenced the allelopathic effects of sorghum extracts. Super 1 plus 4-day irrigation treatment (SP14) recorded a high suppression SDR=50% on grass *Echinochloa indica* while Mandau plus 4-day irrigation (M4) recorded minimal effect similar to control, 29.3%. On broadleaf weed (*Phyllanthus zeylanica*) selective response was observed with Super 1+5-day irrigation (SP15) and Suri 4+4-day irrigation (SU44) reducing growth to~17% while unexpectedly Suri 4+5-day irrigation (SU45) enhanced growth to 32.6%. Therefore, results show interaction between sorghum allelochemicals and irrigation pattern in weed response.

Flavonoids act as allelopathic agents besides antioxidants. The study found that the varieties with high content of flavonoids had also shown greater weed suppression, thus indicating directly the role played by flavonoids in allelopathy (Super 1, Suri 4). Flavonoids inhibit seed germination and disturb chloroplast function in weeds while making them accumulate ROS which results in oxidative damage to them (Patil *et al.*, 2024; Alsaadawi *et al.*, 2015).

Dominant weed flora varied between treatments but grasses, sedges *Cyperus rotundus* and broadleaves were present under all regimes. There were nine dominant species in the control plots which reduced to 6-8 species under sorghum-extract treatments SU44, SU45, M4 and M5. This shows that weed community structure is changed by sorghum extracts and irrigation management, even though all functional groups are not eliminated. The results obtained are supported by earlier findings that residues as well as extracts of sorghum suppress weeds improve soil quality and enhance crop yield in legumes (Ullah *et al.*, 2022; Susilo *et al.*, 2021; Patra *et al.*, 2025).

At four weeks, significant reductions were observed in total weed dry biomass (TWDB), dominant weed dry biomass (DWDB: *E. indica* and *P. zeylanica*), and weed control efficiency (WCE). Extract treatments reduced DWDB of *E. indica* to 2.48–5.85 g compared to 9.05 g in the control, with the lowest values under Suri 4 + 5-day, Super 1 + 5-day, and Mandau + 4-day irrigation. Similarly, DWDB of *P. zeylanica* declined to 1.40–3.54 g relative to the control. Weed control efficiency ranged from 69.19% to 77.52%, with Super 1 + 5-day and Suri 4 + 4-day irrigation achieving the highest suppression. These results demonstrate that sorghum water extracts combined with moderate drought stress are effective in reducing weed biomass.

This may be explained by species-specific sensitivity to flavonoid compounds. Similar species-dependent response has been reported in

allelopathic interaction of sorghum and sunflower extracts (Javaid *et al.*, 2006; Hussain *et al.*, 2021).

Sorghum is a highly allelopathic crop. Compounds such as sorgoleone are largely responsible for its inhibition of seed germination, root elongation and biomass accumulation in several weed species (Javaid *et al.*, 2006; Hussain *et al.*, 2021). Sorghum extract is biodegradable, environmentally safe, and inexpensive compared to synthetic herbicides. The efficiency range reported in this study (69-77%) is very close to previous findings of 60-80% suppression (Ullah *et al.*, 2022; Glab *et al.*, 2017). Tolerance by some other species, such as *E. indica*, highlights the need to implement integrated management strategies such as crop rotation or mulching, in conjunction with this organic extract, and at lower herbicide doses.

In conclusion, the Super 1 variety under 4–5-day irrigation intervals produced the highest flavonoid levels (up to 4120.61 µg/g) and exhibited superior weed suppression, particularly against grasses and broadleaf species. These findings confirm the potential of sorghum extracts as eco-friendly bioherbicides and provide a basis for developing sustainable weed management strategies.

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Conflicts of interest

The authors declare no conflict of interest.

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