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The inhibition of seed germination treated with water extract of sorghum (*Sorghum bicolor*, L.) cultivated in Histosols

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Abstract Allelopathy is a form of suppressing plant growth due to the release of toxins into the surrounding plants. Sorghum (*Sorghum bicolor*, L.) is a plant producing an allelopathic compound. Abiotic stress to sorghum planted in Histosols can determine the allelochemical release process. Application of an aqueous sorghum extract can control weeds nearby the main crop. The inhibition of seed germination treated with water extract of sorghum grown in marginal lands (Histosols) with different pattern of water application is determined. A watering pattern consisted of wet, alternate of a week wet and dry pattern, alternate of a week dry and wet pattern, dry and Ultisols (as a control). The concentration of root water extract of sorghum consisted of 0.0%, 2.5%, 5.0%, 7.5%, 10.0%, 15.0%, 20.0%, and 25.0%. The results showed that the highest inhibition of sorghum seed germination was under 7.5-10% concentration of water extract at dry Histosols as indicated by the lowest plumula and radicle fresh and dry weight. This finding indicated that drought stress sorghum in Histosols produces the highest allelopathic compound. Therefore, the plant has the potential as a source of bioherbicides.

Keywords: Abiotic, Allelochemical, Autotoxic, Stress, Marginal

Introduction

Allelopathy is a form of plant growth inhibition due to the release of toxins into surrounding plants. Allelopathy is a secondary metabolite compound that functions to reduce the impact of abiotic stress on plants. Secondary metabolites produced by plants are the main components in a strong defense mechanism in plants (Cluzet *et al.,* 2020). Allelopathic compounds control weeds and indirectly increase crop yields (Cheema and Khaliq, 2000). The application of allelopathy in agricultural cultivation can support environmental and ecosystem sustainability because it reduces chemical herbicides.

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Sorghum is one of the plants that secrete allelopathy, so it is widely used as a natural herbicide (Farooq *et al.,* 2013). Many kinds of researches on the potential of sorghum to produce allelopathy have been carried out, one of which is the test results of the potential of sorghum to produce allelopathy in different parts of the sorghum plant, such as roots. Based on the research results of Cheema and Khaliq (2000) aqueous extract of sorghum roots can suppress weed biomass by up to 50% and increase wheat yield by 14% on application of 5% aqueous extract of sorghum roots. The aqueous extract of sorghum roots also affected soybean sprouts resulting in shorter radicles than control (Correia *et al.,* 2005).

However, the allelopathic properties of plants when interacting with other plants or with compounds in the soil are different because of many influencing factors. There are differences in the mechanism of the allelopathic process in plants which are influenced by several factors, including environmental factors, soil, and growth effects (Sowiński *et al.,* 2020). Sorghum planted on Histosols may have a different growth response and allelopathic content from sorghum grown on other lands. In addition, the allelopathic content will be different in sorghum under abiotic stress drought conditions.

Residual sorghum biomass can also interact with other plants and weed seeds in the same land. Allelochemical toxicity released by sorghum can inhibit seed germination. As the test results on mung bean seeds, aqueous extracts of leaves, stems, and roots of sorghum significantly reduced the rate and time of germination (Moosavi *et al.,* 2011). Therefore, it is needed to find out the allelopathic inhibition potential of sorghum plants on seed germination. The main objective of this study was to determine the inhibition of germination of seeds treated with sorghum extract in Histosols at different irrigations.

Materials and methods

Plant material and experimental design

The main element in water extract is the Numbu sorghum variety. Four-week-old sorghum shoots and roots were picked and dried in the sun for five days before being dried in a 70°C oven for 72 hours. Dry bean curd is cut into 1-2 cm pieces and ground into a powder, which is then utilized as an aqueous extract. The experiment used Completely Randomized Design (CRD) with 2 factors and 5 replications The first factor was a watering pattern for four weeks, consisting of wet, alternate of a week wet and dry pattern, alternate of a week dry and wet pattern, dry and Ultisols (as a control). The second factor was the

concentration of root water extract of sorghum, which consisted of 0.0%, 2.5%, 5.0%, 7.5%, 10.0%, 15.0%, 20.0%, and 25.0%.

Water extract preparation

250 g of dry sorghum powder (25% treatment) was soaked with 1000 mL of distilled water and stirred for 24 hours using a screw at room temperature. The mixture of extracts and water is filtered through a cloth and then filter paper. The extract was put in a labeled container and stored in the refrigerator until the material was used for experiments. For concentrations of 2.5%, 5%, 7.5%, 10%, 15%, 20% and 25% of these extracts were also prepared by the same procedure.

Bioassay with water extract on filter paper

The purpose of this bioassay test was determined the growth inhibition of sorghum seed germination as a result of water-soluble allelochemical compounds. Two layers of filter paper were placed in a 9 cm diameter petri dish. 25 grains of sorghum seeds were planted in each petri dish and 10 mL of aqueous extract was added at different concentrations according to treatment (2.5%, 5%, 7.5%, 10%, 15%, 20% and 25%) each. petri dish. The Petri dishes were then incubated in the growth chamber for five days. All experimental series (each combination of concentration and irrigation pattern) were repeated five times.

Measurement of experiment variables

Measurement of the experimental variables was carried out on the percentage of normal germination (%), percentage of abnormal germination (%), length of plumula (cm), the total length of plumula (cm), length of radicles (cm), the total length of radicles (cm), dry weight of germination with cotyledons (g), dry weight of germination without cotyledons (g), plumula fresh weight (g), radicle fresh weight (g), fresh cotyledon weight (g), plumula dry weight (g), radicle dry weight (g), and cotyledon dry weight (g).

Statistic analysis

Data were subjected to ANOVA at level of 5%. If there was a significant difference between the averages, the means were analized using Duncan's test at 5%.

Results

The variables observed in this experiment were the percentage of normal germination 3 DAP and 4 DAP, the percentage of abnormal germination 3 DAP and 4 DAP, plumule length, total plumule length, radicle length, total radicle length, dry weight of complete sprouts (plumule, root, and cotyledons), dry weight of incomplete sprouts (without cotyledons), plumule fresh weight, radicle fresh weight, fresh cotyledon weight, plumule dry weight, radicle dry weight, and cotyledon dry weight.

Table 1. Recapitulation of sorghum water extract inhibition with the treatment of different irrigation patterns and concentrations

No	Variable	Watering Pattern (A)	Extract concentration (B)	Interaction (A x B)	CV (%)
1	Normal germination 3 DAP	3.13 *	2030.45 **	10.93 **	13.81
2	Normal germination 4 DAP	2.66 *	1986.65 **	5.50 **	13.93
3	Abnormal germination 3 DAP	27.99 **	101.19 **	9.42 **	31.49
4	Abnormal germination 4 DAP	35.09 **	109.91 **	7.95 **	32.84
5	Plumula length	22.60 **	2908.91 **	9.45 **	10.11
6	Total Plumula length	22.57 **	2911.07 **	9.46 **	10.63
7	Radicular length	8.79 **	2622.19 **	4.09 **	14.16
8	Total Radicular length	8.74 **	2624.46 **	4.10 **	15.32
9	DW germination + cotyledons	4.49 **	20.82 **	1.07 ns	5.40
10	DW germination without cotyledons	21.61 **	2054.59 **	9.07 **	11.44
11	Plumula fresh weight	19.35 **	1643.57 **	5.75 **	13.50
12	Radicular fresh weight	10.85 **	380.78 **	5.60 **	37.43
13	Cotyledon fresh weight	3.21 *	152.67 **	6.39 **	4.65
14	Plumula dry weight	20.29 **	1545.17 **	7.45 **	11.73
15	Radicular dry weight	6.90 **	829.02 **	6.68 **	18.95
16	Cotyledon dry weight	4.32 **	162.61 **	1.58 *	5.81

* : significantly
** : very significantly
ns : no significant
DW : dry weight
DAP : days after planting
CV : Coefficient Variation

The variance showed that the treatment pattern of water application had a significant effect on all experimental variables (sorghum seed germination variables). The concentration treatment of sorghum water extracts significantly affected all germination observation variables. The interaction between the pattern of water administration and the concentration of water extract significantly affected all observed variables, except the dry weight of complete sprouts, as shown in Table 1.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the percentage of

normal sprouts at 3 DAP and 4 DAP, as shown in Table 2. All treatments of water application (wet swamp soil, wet-dry swamp soil, dry-wet swamp soil, dry swamp, and dry Ultisol) with 0% aqueous extract concentration produced the highest percentage of normal sprouts which were 88.00%, 88.50%, 88.50%, 90.50%, and 88.00% at 3 DAP and 90.50 %, 89.50 %, 89.50 %, 91.00 %, and 90.00 % at 4 DAT, repectively. This shows that all water application patterns did not inhibit germination when combined with 0% aqueous extract concentration because there was no inhibitory power of toxic compounds in this treatment.

Table 2. Effect of interaction of irrigation patterns and concentration of water extracts on normal germination, abnormal germination, plumula length, and total plumula length

Treatment	Normal germination 3 DAP (%)	Normal germination 4 DAP (%)	Abnormal germination 3 DAP (%)	Abnormal germination 4 DAP (%)	Length plumula (cm)	Total length plumula (cm)
Watering pattern (A)						
W	24.50 b	28.63 ab	22.75 c	18.13 c	1.93 c	18.29 c
WD	27.44 a	29.00 a	32.19 b	30.56 b	2.21 a	21.09 a
DW	27.06 a	27.94 abc	20.69 c	19.81 c	2.13 ab	20.24 ab
D	26.13 ab	26.44 c	27.69 b	27.31 b	1.78 d	16.77 d
UD	26.38 ab	26.44 c	42.19 a	41.69 a	2.09 b	19.88 b
Prob. F > 5 %	0.0175	0.0363	0.0001	0.0001	0.0001	0.0001
Concentration (B)						
0% (0)	88.70 a	90.10 a	6.50 e	5.60 e	6.97 a	68.70 a
2.5 %	73.20 b	81.80 b	18.60 d	9.60 e	3.94 b	38.40 b
5 %	40.10 c	40.80 c	42.90 b	41.30 b	3.10 c	29.99 c
7.5 %	8.40 d	9.40 d	64.40 a	63.20 a	1.14 d	10.41 d
10 %	0.00 e	0.00 e	47.00 b	46.90 b	0.70 f	5.92 e
15 %	0.00 e	0.00 e	27.60 c	27.60 c	0.16 f	0.63 f
20 %	0.00 e	0.00 e	18.80 d	18.80 d	0.10 f	0.00 f
25 %	0.00 e	0.00 e	7.00 e	7.00 e	0.10 f	0.00 f
Prob. F > 5 %	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction A x B						
W x 0 %	88.00 a	90.50 a	5.50 lmn	3.50 mn	7.06 ab	69.59 ab
WD x 0 %	88.50 a	89.50 ab	6.00 lmn	5.50 lmn	7.05 ab	69.44 ab
DW x 0 %	88.50 a	89.50 ab	5.50 lmn	5.50 lmn	7.26 a	71.58 a
D x 0 %	90.50 a	91.00 a	8.50 lmn	8.00 lmn	6.87 bc	67.66 bc

Table 2. (Cont.)

Treatment	Normal germination 3 DAP (%)	Normal germination 4 DAP (%)	Abnormal germination 3 DAP (%)	Abnormal germination 4 DAP (%)	Length plumula (cm)	Total length plumula (cm)
UD x 0 %	88.00 a	90.00 ab	7.00 lmn	5.00 lmn	6.63 c	65.22 c
W x 2.5 %	51.00 d	74.00 d	43.00 defgh	20.00 ijkl	3.70 e	36.00 e
WD x 2.5 %	75.00 c	86.00 abc	16.00 klm	4.00 mn	4.61 d	45.08 d
DW x 2.5 %	77.00 bc	83.00 c	12.00 klmn	6.00 lmn	4.57 d	44.65 d
D x 2.5 %	82.00 b	84.00 bc	11.00 klmn	8.00 lmn	3.20 g	31.03 g
UD x 2.5 %	81.00 b	82.00 c	11.00 klmn	10.00 klmn	3.62 ef	35.23 fg
W x 5 %	38.00 ef	40.50 f	46.00 defg	39.50 efgh	2.75 h	26.44 h
WD x 5 %	50.00 d	50.50 e	34.50 fghi	34.00 ghi	3.38 fg	32.70 fg
DW x 5 %	38.00 ef	38.00 fg	47.00 cdef	46.00 defg	3.22 g	31.10 g
D x 5 %	32.00 fg	32.50 g	48.50 cdef	48.50 cdef	2.44 i	23.35 i
UD x 5 %	42.00 e	42.50 f	38.50 efghi	38.50 fgh	3.74 e	36.40 e
W x 7.5 %	19.00 g	24.00 h	54.00 bcd	49.00 cdef	1.26 j	11.58 j
WD x 7.5 %	6.00 i	6.00 i	55.00 bcd	55.00 bcd	1.06 j	9.63 j
DW x 7.5 %	13.00 h	13.00 h	68.00 ab	68.00 ab	1.28 j	11.78 j
D x 7.5 %	4.00 i	4.00 i	70.00 a	70.00 ab	1.03 j	9.3 j
UD x 7.5 %	0.00 i	0.00 i	75.00 a	74.00 a	1.06 j	9.78 j
W x 10 %	0.00 i	0.00 i	30.50 hij	30.00 hij	0.38 k	2.78 k
WD x 10 %	0.00 i	0.00 i	52.00 cde	52.00 cdef	1.29 j	11.89 j
DW x 10 %	0.00 i	0.00 i	32.00 ghij	32.00 ghij	0.39 k	2.83 k
D x 10 %	0.00 i	0.00 i	49.50 cde	49.50 cdef	0.39 k	2.84 k
UD x 10 %	0.00 i	0.00 i	71.00 a	71.00 a	0.03 j	9.28 j
W x 15 %	0.00 i	0.00 i	3.00 lmn	3.00 mn	0.41 k	0.00 k
WD x 15 %	0.00 i	0.00 i	54.00 bcd	54.00 cde	0.10 k	0.00 k
DW x 15 %	0.00 i	0.00 i	1.00 mn	1.00 n	0.10 k	0.00 k
D x 15 %	0.00 i	0.00 i	18.oo jkl	18.00 klmn	0.10 k	0.00 k
UD x 15 %	0.00 i	0.00 i	62.00 abc	62.00 abc	0.10 k	3.13 k
W x 20 %	0.00 i	0.00 i	0.00 n	0.00 n	0.10 k	0.00 k
WD x 20 %	0.00 i	0.00 i	32.00 ghij	32.00 ghij	0.10 k	0.00 k

Table 2. (Cont.)

Treatment	Normal germination 3 DAP (%)	Normal germination 4 DAP (%)	Abnormal germination 3 DAP (%)	Abnormal germination 4 DAP (%)	Length plumula (cm)	Total length plumula (cm)
DW x 20 %	0.00 i	0.00 i	0.00 n	0.00 n	0.10 k	0.00 k
D x 20 %	0.00 i	0.00 i	13.00 klmn	13.00 klmn	0.10 k	0.00 k
UD x 20 %	0.00 i	0.00 i	49.00 cdef	49.00 cdef	0.10 k	0.00 k
W x 25 %	0.00 i	0.00 i	0.00 n	0.00 n	0.10 k	0.00 k
WD x 25 %	0.00 i	0.00 i	8.00 lmn	8.00 lmn	0.10 k	0.00 k
DW x 25 %	0.00 i	0.00 i	0.00 n	0.00 n	0.10 k	0.00 k
D x 25 %	0.00 i	0.00 i	3.00 mn	3.00 mn	0.10 k	0.00 k
UD x 25 %	0.00 i	0.00 i	24.00 jkl	24.00 ijk	0.10 k	0.00 k
Prob. F > 5 %	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

W = wet swampland, WD = wet-dry swampland, DW = wet-dry swampland, D = dry swampland, UD = dry Ultisol; the numbers followed by the same letters in the same column are not significantly different in the Duncan's Multiple Range Test (DMRT) level of 5%

All treatment patterns of water application (wet swamp soil, wet-dry swamp soil, dry-wet swamp soil, dry swamp soil, and dry Ultisol) with a water extract concentration of 7.5% resulted in a low normal germination percentage of 19.00 each. %, 6.00 %, 13.00 %, 4.00 %, and 0.00 % at 3 DAT and 24.00 %, 6.00 %, 13.00 %, 4.00 %, and 0.00 % at 4 DAT. This shows that the interaction between dry Ultisols with a water extract concentration of 7.5% exhibited the lowest normal germination. Still, it was not significantly different from that at dry swamps and the pattern of wet-dry swamps at a concentration of water extract 7.5%. Furthermore, the lowest percentage of normal germination occurred at a concentration of 10% to 25% aqueous extract in all water application patterns.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the percentage of abnormal sprouts at 3 DAP, and 4 DAP is shown in Table 2. In general, all treatments of water patterns with 0% aqueous extract concentration resulted in the lowest percentage of abnormal sprouts, which were 5,50 %, 6,00 %, 5,50 %, 8,50 %, and 7,00 % at 3 DAP and 3,50 %, 5,50 %, 5,50 %, 8,00 %, and 5.00 % at 4 DAP, respectively. This indicates that all patterns of water applications combined with 0% aqueous extract concentration did not inhibit germination, so the resulting abnormal sprouts were small.

All treatment patterns of water application with an aqueous extract concentration of 7.5% resulted in high abnormal germination percentages, especially in dry Ultisol, dry swampland, and dry-wet swamp soil, which were

75.00%, 70,00 %, and 68.00 % at 3 DAT and 74.00 %, 70,00 %, and 68.00 %, at 4 DAP, respectively. Furthermore, the percentage of abnormal germination also occurred between dry Ultisols and a concentration of 10% water extract, which is 71.00%, as shown in Table 2. This shows that the interaction between dry Ultisols and the concentration of water extract is 7.5% and 10% resulted in the highest abnormal germination, but not significantly different from the pattern of water application in dry-swamp soil and the pattern of water application in wet-dry swamp soil at a water extract concentration of 7.5%.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the variable length of the plumules, as shown in Table 2. In general, all treatments of the pattern of water application with a concentration of 0% water extract produced the highest sprouted plumule length because there was no inhibitory power in the form of toxic compounds in the concentration of water extract 0%. Germination growth showed normal conditions. The treatment of watering patterns (especially in wet-swamp soil, dry-wet marshland, and dry-swamp soil) with a 10% water extract concentration resulted in the lowest total plumule length.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the variable length of the radicle, as shown in Table 3. All treatments of the pattern of water application with a concentration of 7.5% water extract resulted in low radicle length, then at a concentration of 10% water extract resulted in a higher radicle length. This indicates that the interaction between all types of water application patterns with 7.5% and 10% water extract concentrations resulted in the lowest radicle length. This result is associated with the inhibitory power of the aqueous extract applied to the bioassay media. In general, the higher concentration resukted in the shorter the radicle length. The concentration of aqueous extract to produce the shortest radicle length was achieved by 10%.

The interaction between the pattern of water aapplication and the concentration of the aqueous extract showed a significant effect on the total radicular length variable. All treatment patterns of water application with 7.5% water extract concentration resulted in low total radicular length, then at 10% aqueous extract concentration resulted in the lowest total radicular length. This indicates that the interaction between all types of water application patterns with 7.5% and 10% concentrations of water extract resulted in the lowest total radicular length. The concentration of aqueous extract to produce the shortest total radicular length achieved by 10% is shown in Table 3.

Table 3. Effect of interaction of irrigation patterns and concentration of water extracts on radicle length, total radicle length, dry weight germination with cotyledon, and dry weight germination without cotyledon

Treatment	Length radicles (cm)	Total length radicles (cm)	Weight dry germination with cotyledon (g)	Weight dry germination without cotyledon (g)
Watering pattern (A)				
W	2.36 a	22.55 a	0.0270 ab	0.0021 c
WD	2.25 ab	21.42 ab	0.0270 ab	0.0025 a
DW	2.22 ab	21.21 ab	0.0276 a	0.0025 a
D	1.92 c	18.18 c	0.0268 bc	0.0020 c
UD	2.11 b	20.07 b	0.0261 c	0.0023 b
Prob. F > 5 %	0.0001	0.0001	0.0021	0.0001
Concentration (B)				
0 %	10.33 a	102.32 a	0.0242 c	0.0071 a
2.5 %	4.50 b	44.01 b	0.0256 b	0.0047 b
5 %	1.62 c	15.10 c	0.0261 b	0.0037 c
7.5 %	0.46 d	3.57 d	0.0276 a	0.0016 d
10 %	0.15 e	0.48 e	0.0276 a	0.0009 e
15 %	0.10 e	0.00 e	0.0276 a	0.0001 f
20 %	0.10 e	0.00 e	0.0285 a	0.0000 f
25 %	0.10 e	0.00 e	0.0289 a	0.0000 f
Prob. F > 5 %	0.0001	0.0001	0.0001	0.0001
Interaction A x B				
W x 0 %	10.84 a	107.40 a	0.0241	0.0070 b
WD x 0 %	10.06 cd	99.56 cd	0.0244	0.0069 b
DW x 0 %	10.34 bc	102.40 bc	0.0244	0.0078 a
D x 0 %	9.69 d	95.89 d	0.0239	0.0070 b
UD x 0 %	10.74 ab	106.34 bc	0.0240	0.0068 b
W x 2.5 %	4.91 ef	48.10 ef	0.0254	0.0045 d
WD x 2.5 %	5.28 e	51.83 e	0.0250	0.0055 c
DW x 2.5 %	4.75 f	46.48 f	0.0263	0.0054 c
D x 2.5 %	3.99 g	38.85 g	0.0263	0.0044 de
UD x 2.5 %	3.58 g	34.78 g	0.0249	0.0040 e
W x 5 %	1.71 h	16.10 h	0.0264	0.0030 f
WD x 5 %	1.71 h	15.71 h	0.0259	0.0043 e
DW x 5 %	1.65 h	15.51 h	0.0266	0.0040 e
D x 5 %	1.06 i	9.59 i	0.0259	0.0031 f
UD x 5 %	1.96 h	18.60 h	0.0259	0.0044 de
W x 7.5 %	0.94 i	8.38 i	0.0274	0.0017 gh
WD x 7.5 %	0.38 j	2.83 j	0.0272	0.0017 gh
DW x 7.5 %	0.63 ij	5.28 ij	0.0282	0.0021 g

Table 3. (Con.)

Treatment	Length radicles (cm)	Total length radicles (cm)	Weight dry germination with cotyledon (g)	Weight dry germination without cotyledon (g)
D x 7.5 %	0.19 j	0.95 j	0.0277	0.0013 i
UD x 7.5 %	0.14 j	0.40 j	0.0276	0.0000 k
W x 10 %	0.14 j	0.40 j	0.0277	0.0004 jk
WD x 10 %	0.25 j	1.45 j	0.0273	0.0014 hi
DW x 10 %	0.10 j	0.00 j	0.0281	0.0007 j
D x 10 %	0.11 j	0.13 j	0.0278	0.0006 j
UD x 10 %	0.15 j	0.43 j	0.0271	0.0014 hi
W x 15 %	0.00 j	0.00 j	0.0286	0.0000 k
WD x 15 %	0.00 j	0.00 j	0.0278	0.0000 k
DW x 15 %	0.00 j	0.00 j	0.0283	0.0000 k
D x 15 %	0.00 j	0.00 j	0.0262	0.0000 k
UD x 15 %	0.00 j	0.00 j	0.0272	0.0006 j
W x 20 %	0.00 j	0.00 j	0.0286	0.0000 k
WD x 20 %	0.00 j	0.00 j	0.0291	0.0000 k
DW x 20 %	0.00 j	0.00 j	0.0295	0.0000 k
D x 20 %	0.00 j	0.00 j	0.0278	0.0000 k
UD x 20 %	0.00 j	0.00 j	0.0273	0.0000 k
W x 25 %	0.00 j	0.00 j	0.0285	0.0000 k
WD x 25 %	0.00 j	0.00 j	0.0294	0.0000 k
DW x 25 %	0.00 j	0.00 j	0.0295	0.0000 k
D x 25 %	0.00 j	0.00 j	0.0285	0.0000 k
UD x 25 %	0.00 j	0.00 j	0.0249	0.0000 k
Prob. F > 5 %	0.0001	0.0001	0.3921	0.0001

W = wet swampland, WD = wet-dry swampland, DW = wet-dry swampland, D = dry swampland, UD = dry Ultisol; the numbers followed by the same letters in the same column are not significantly different in the Duncan's Multiple Range Test (DMRT) level of 5%.

The pattern of water application and the concentration of water extract showed a significant effect on the dry weight of complete sprouts, as shown in Table 3. The treatment with water extract concentration of 7.5%, 10%, 15%, 20% and 25% resulted in the higher complete sprout weight compared to the aqueous extract concentration of 0%, 2.5%, and 5%. This indicates that the starting concentration of 7.5% aqueous extract has affected germination activity so that the impact of cotyledons is less developed during the germination process.

The interaction between the pattern of water application and the concentration of the aqueous extract showed a significant effect on the weight of sprouts without cotyledons. All treatment patterns of water application with a water extract concentration of 7.5% resulted in a low weight of sprouts without cotyledons. Furthermore, a concentration of 10% aqueous extract produced the

lowest weight of sprouts without cotyledons. This indicated that the interaction between all types of water aapplication patterns with 7.5% and 10% aqueous extract concentration resulted in the lowest seedling weight without cotyledons. This is related to the inhibitory power of the aqueous extract applied to the bioassay media. The pattern of water application in dry-marsh, dry-wet, and wet soil resulted in the lowest seedling weight without cotyledons. In general, higher concentration of water extract, produced lower weight of sprouts without cotyledons as seen in Table 3.

Table 4. Effect of interaction of irrigation patterns and sorghum extract concentration on fresh weight of plumula, fresh weight of radicles, fresh weight of cotyledons, dry weight of plumula, dry weight of radicles, and dry weight of cotyledon

Treatment	Plumula fresh weight (g)	Radicular Fresh weight (g)	Fresh weight of cotyledon (g)	Plumula dry weight (g)	Radicular dry weight (g)	Cotyledon dry weight (g)
Watering pattern (A)						
W	0.0145 c	0.0022 b	0.0399 ab	0.0017 b	0.0006 bc	0.0251 a
WD	0.0162 b	0.0022 b	0.0394 b	0.0020 a	0.0006 ab	0.0247 a
DW	0.0165 b	0.0022 b	0.0394 b	0.0020 a	0.0007 a	0.0252 a
D	0.0147 c	0.0021 b	0.0408 a	0.0017 b	0.0005 c	0.0248 a
UD	0.0187 c	0.0033 a	0.0399 ab	0.0020 a	0.0005 c	0.0239 b
Prob. F > 5%	0.0001	0.0001	0.0155	0.0001	0.0001	0.0027
Concentration (B)						
0%	0.0543 a	0.0114 a	0.0332 g	0.0053 a	0.0020 a	0.0017 g
2.5 %	0.0311 b	0.0034 b	0.0334 g	0.0035 b	0.0014 b	0.0210 f
5 %	0.0278 c	0.0034 b	0.0398 e	0.0033 c	0.0007 c	0.0225 e
7.5 %	0.0092 d	0.0003 c	0.0369 f	0.0006 d	0.0002 d	0.0261 d
10 %	0.0056 e	0.0004 c	0.0421 d	0.0009 f	0.0001 e	0.0269 d
15 %	0.0008 f	0.0001 c	0.0466 a	0.0002 f	0.0001 e	0.0276 bc
20 %	0.0001 f	0.0001 c	0.0449 b	0.0001 f	0.0001 e	0.0286 a
25 %	0.0001 f	0.0001 c	0.0433 c	0.0001 f	0.0001 e	0.0282 ab
Prob. F > 5 %	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction A x B						
W x 0 %	0.055 a	0.0108 bc	0.0343 mno	0.0053 b	0.0019 b	0.0172 m
WD x 0 %	0.048 b	0.0098 c	0.0329 op	0.0052 bc	0.0019 b	0.0177 lm
DW x 0 %	0.057 a	0.0112 b	0.0325 op	0.0058 a	0.0021 a	0.0168 m
D x 0 %	0.055 a	0.0121 b	0.0324 op	0.0053 b	0.0019 b	0.0170 m
UD x 0 %	0.056 a	0.0134 a	0.0338 nop	0.0049 c	0.0020 ab	0.0173 m
W x 2.5 %	0.029 efg	0.0019 hij	0.0331 op	0.0034 fgh	0.0013 d	0.0210 jk
WD x 2.5 %	0.0320 de	0.0020 hij	0.0311 p	0.0041 d	0.0016 c	0.0196 kl
DW x 2.5 %	0.0316 de	0.0026 ghi	0.0327 op	0.0039 de	0.0017 c	0.0210 jk
D x 2.5 %	0.0261 gh	0.0028 gh	0.0337 nop	0.0032 hi	0.0014 d	0.0221 ij
UD x 2.5 %	0.0368 c	0.0076 d	0.0368 klm	0.0033 ghi	0.0009 e	0.0210 jk
W x 5 %	0.0225 i	0.0035 fg	0.0396 hijk	0.0025 j	0.0007 g	0.0235 ghi

Table 4. (Con.)

Treatment	Plumula fresh weight (g)	Radicular Fresh weight (g)	Fresh weight of cotyledon (g)	Plumula dry weight (g)	Radicular dry weight (g)	Cotyledon dry weight (g)
WD x 5 %	0.0301 ef	0.0050 e	0.0393 ijkl	0.0036 ef	0.0009 ef	0.0217 ijk
DW x 5 %	0.0281 fg	0.0025 ghi	0.0364 nml	0.0035 fg	0.0008 fg	0.0227 ij
D x 5 %	0.0237 hi	0.0013 ijk	0.0391 jkl	0.0025 j	0.0004 hi	0.0229 hij
UD x 5 %	0.0345 cd	0.0050 e	0.0396 hijk	0.0037 ef	0.0008 ef	0.0217 ijk
W x 7.5 %	0.0064 kl	0.0006 jk	0.0349 mno	0.0015 m	0.0005 h	0.0258 ef
WD x 7.5 %	0.0097 jk	0.0002 k	0.0345 mno	0.0018 kl	0.0001 j	0.0256 efg
DW x 7.5 %	0.0116 j	0.0007 jk	0.0369 klm	0.0020 k	0.0003 ij	0.026 cdef
D x 7.5 %	0.0088 jk	0.0001 k	0.0365 lmn	0.0014 m	0.0001 j	0.027 bcdef
UD x 7.5 %	0.0097 jk	0.0001 k	0.0416 efghij	0.0015 lm	0.0001 j	0.0263 bcdef
W x 10 %	0.0027 m	0.0009 jk	0.0406 ghij	0.0005 no	0.0001 j	0.0275 abcde
WD x 10 %	0.0094 jk	0.0006 jk	0.0398 ghij	0.0014 m	0.0002 j	0.0261 def
DW x 10 %	0.0030 m	0.0001 k	0.0417 efghij	0.0008 n	0.0001 j	0.0276 abcde
D x 10 %	0.0035 lm	0.0002 k	0.0467 b	0.0007 n	0.0001 j	0.0274 abcde
UD x 10 %	0.0093 jk	0.0003 k	0.0419 efghij	0.0015 lm	0.0002 j	0.0258 ef
W x 15 %	0.0001 m	0.0001 k	0.0463 b	0.0001 o	0.0001 j	0.0287 ab
WD x 15 %	0.0001 m	0.0001 k	0.0496 b	0.0001 o	0.0001 j	0.0279 abcde
DW x 15 %	0.0001 m	0.0001 k	0.0450 bcd	0.0001 o	0.0001 j	0.0284 abcd
D x 15 %	0.0001 m	0.0001 k	0.0509 a	0.0001 o	0.0001 j	0.0263 cdef
UD x 15 %	0.0037 lm	0.0001 k	0.0412 fghij	0.0007 n	0.0001 j	0.0267 bcdef
W x 20 %	0.0001 m	0.0001 k	0.0463 b	0.0001 o	0.0001 j	0.0287 ab
WD x 20 %	0.0001 m	0.0001 k	0.0457 bc	0.0001 o	0.0001 j	0.0292 a
DW x 20 %	0.0001 m	0.0001 k	0.0458 b	0.0001 o	0.0001 j	0.0296 a
D x 20 %	0.0001 m	0.0001 k	0.0445 bcde	0.0001 o	0.0001 j	0.0279 abcde
UD x 20 %	0.0001 m	0.0001 k	0.0424 defgh	0.0001 o	0.0001 j	0.0274 abcde
W x 25 %	0.0001 m	0.0001 k	0.0452 bcd	0.0001 o	0.0001 j	0.0286 abc
WD x 25 %	0.0001 m	0.0001 k	0.0422 defghi	0.0001 o	0.0001 j	0.0295 a
DW x 25 %	0.0001 m	0.0001 k	0.0442 bcdef	0.0001 o	0.0001 j	0.0296 a
D x 25 %	0.0001 m	0.0001 k	0.0428 cdefg	0.0001 o	0.0001 j	0.0286 abc
UD x 25 %	0.0001 m	0.0001 k	0.0422 defghi	0.0001 o	0.0001 j	0.0250 fgh
Prob. F > 5 %	0.0001	0.0001	0.0001	0.0001	0.0001	0.0472

W = wet swampland, WD = wet-dry swampland, DW = wet-dry swampland, D = dry-swamp land, UD = dry Ultisol; the numbers followed by the same letters in the same column are not significantly different in the Duncan's Multiple Range Test (DMRT) level of 5%

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the fresh weight of plumules, as shown in Table 4. In general, all treatment patterns of water application with aqueous extract concentrations ranging from 7.5% to 10% resulted in low fresh weight of plumules, Morever, the concentration of 10% aqueous extract with the pattern of water application in wet-marsh, dry-wet,

and dry soils resulted in low fresh weight of plumules. This result confirms germination response on environmental stress.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the fresh weight of the radicle, as shown in Table 4. In general, all treatments of the pattern of water application with aqueous extract concentrations ranging from 7.5% to 10% resulted in low radicle fresh weight.

The interaction between the water application pattern and the aqueous extract concentration showed a significant effect on the fresh weight of the cotyledons, as shown in Table 4. In general, all treatments of the water application with the aqueous extract concentration ranging from 7.5% to 10% resulted in a low fresh weight of cotyledons. However, the dry-swamp water application resulted in a higher fresh weight of cotyledons when compared to other water application patterns, both at a water extract concentration of 7.5% and 10%. This indicates that the pattern of water supply for dry-swamp soil is less changing the cotyledons during the germination process so that the weight of fresh cotyledons is still relatively high.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the dry weight of plumules, as shown in Table 4. The dry weight of plumules in all 0% water application patterns (control) was higher than the other water treatment patterns. This is because 0% extract concentration treatment did not produce any toxic inhibitors. In general, all water treatment patterns with aqueous extract concentrations ranging from 7.5% to 10% resulted in lower plumule dry weights. Especially at the 10% aqueous extract concentration, the pattern of water aaplication in swamp soil, dry-wet in swamp soil, and dry swamp soil resulted in lower dry weight of plumules.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the dry weight of the radicle, as shown in Table 4. The dry weight of the radicle in all 0% water application patterns (control) had a relatively higher value than the other water treatment patterns. This is because the treatment with 0% extract concentration did not produce any inhibitory power. In general, all treatment patterns of water administration with aqueous extract concentrations ranging from 7.5% to 10% resulted in low radicle dry weight. This shows that all patterns of water application when combined with a water extract concentration of 7.5% or 10%, will produce the most effective inhibition of germination.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on the dry weight of the cotyledons, as shown in Table 4. The dry weight of the cotyledons in all 0%

water application patterns (control) had a lower value than the other water treatment patterns. This is because the treatment with 0% extract concentration did not produce any inhibitory power. The germination process took place normally, which resulted in the breaking of the cotyledons, which resulted in lighter cotyledon weights. In general, all water treatment patterns with aqueous extract concentrations ranging from 7.5% to 10% resulted in low cotyledon dry weight. However, the pattern of water supplying dry-swamp, wet-swamp, dry-wet swamp resulted in a higher dry weight of cotyledons compared to other watering patterns, especially at a concentration of 10% water extract.

Discussion

The analysis results showed an interaction between the pattern of water application and the concentration of the water extract, which had a significant effect on all observation variables, except the dry weight of complete sprouts. The treatment pattern of water application had a significant effect on all variables of sorghum seed germination. This is presumably due to differences in allelopathy levels in sorghum roots which are quite large at each level of the water supply pattern. Differences in the growth of sorghum plants under conditions of field water content and drought affect plant physiology. Based on the research results by Asadi and Eshghizadeh (2021), dry sorghum plants decreased the activity of antioxidant enzymes such as catalase, ascorbate peroxidase, and peroxidase enzymes, decreased proline content in leaves, and decreased membrane stability index. Drought in sorghum also reduces starch synthesis enzyme activities (Bing *et al.,* 2014), so that disturbances in the abiotic conditions of drought in sorghum plants indirectly affect the production of secondary metabolites in forming allelopathic compounds.

The treatment pattern of water application with an aqueous extract concentration of 7.5% resulted in a low percentage of normal germination at 3 DAP and 4 DAP, respectively. Sorghum root extract water 10-25% resulted in the lowest normal germination. This is different from the results obtained by Moosavi *et al.* (2011) that the germination of mung bean seeds was not significantly different from the control. However, the lowest germination was in the treatment of 20 g/l sorghum root water extract. In line with the results of the percentage of normal germination, the highest percentage of abnormal germination was at a water extract concentration of 7.5% - 10%, which was not significantly different from the water application pattern in dry-swamp soil and the water application pattern in wet-dry swamp soil at the water extract concentration. 7.5%. According to Weston and Czarnota (2001), the allelopathic effect was stronger when cultivating species with small seeds. This

confirms that each plant has a different response to allelopathy produced by sorghum plants.

The interaction between the pattern of water application and the concentration of water extract showed a significant effect on germination variables, such as plumule length, total plumule length, radicle length, total radicle length, dry weight of complete sprouts (plumule, root, and cotyledons), dry weight of incomplete sprouts (without cotyledons), plumule fresh weight, radicle fresh weight, fresh cotyledon weight, plumule dry weight, radicle dry weight, and cotyledon dry weight. The treatment pattern of water application with 10% aqueous extract concentration resulted in the lowest total plumule length and total radicle length. This indicates that the interaction between the pattern of water application and the concentration of 10% aqueous extract resulted in the lowest total plumule length and the lowest radicle length due to the effect of inhibitory power on the aqueous extract applied to the bioassay media. This is due to the inhibitory power on the aqueous extract given to the bioassay media. In general, the higher the concentration of aqueous extract given to the growing media, the lower the total radicle length.

7.5% -10% of the aqueous extract concentration resulted in low seedling weight without cotyledons. Fresh weight of plumules at concentrations of aqueous extract ranging from 7.5% - 10% resulted in low fresh weight of plumules and fresh weight of radicles. According to Glab *et al.* (2017), sorgoleone, which is one of the components in allelopathy, can reduce H + ATPase membrane activity, affecting water absorption. The development of sprouts is disrupted. The study by Weston and Czarnota (2001) obtained similar result where the application of sorgoleone after ten days of seedlings experienced a significant decrease in growth. The impact of phytotoxicity on sprouts by phenolic compounds from allelopathy will decrease plant development through changes in water status, increased ABA content, osmotic stress, and oxidative stress (Araniti *et al.,* 2020).

Based on the results of the dry weight of the sprouts (Table 3), it showed that the treatment of water application in the swamp soil (wet, wet-dry, dry-wet, or dry) resulted in a higher seedling weight as a result of cotyledon compression due to environmental stress, so that the cotyledons were less dense. Treatment of aqueous extract concentrations ranging from 7.5% to 25% affected germination activity so that the cotyledons were less developed during the germination process. Darmanti (2018) explained that one of the effects of allelochemicals on the physiology of target plants is an increase in the production of ROS (reactive oxygen species), which disrupt cell membranes, a decrease in mitochondrial function, and impaired activity of IAA phytohormones. This study showed the development of sprouts was disturbed

because physiologically, phytohormones that function to support the growth and development of the plumule and radicle is inhibited.

The concentration of aqueous extract from 7.5% - 10% resulted in a low fresh weight of cotyledons. However, the dry-swamp groundwater application resulted in a higher fresh weight of cotyledons than other watering patterns. The higher the stress on the seed germination process, the higher the weight of the fresh cotyledons produced. Furthermore, the higher the concentration of aqueous extract, the higher the weight of the fresh cotyledons produced. This is in line with the study by Weston and Czarnota (2001) that allelochemicals increase plant growth at low concentrations and suppress its growth at high concentrations.

The analysis results showed an interaction between the pattern of water application and the concentration of water extract on the dry weight of the plumules and radicles . The 0% water treatment has a large weight because there is no inhibition effect. Meanwhile, at 10% water extract concentration resulted in low plumule dry weight, and the pattern of water application in swamp soil, dry-wet in swamp soil, and dry in swamp soil resulted in lower plumule dry weight. This shows the germination response to the inhibitory power of the aqueous extract concentration resulting from the higher stress water supply pattern. The pattern of water application with a stressed environment will result in higher allelopathy produced in sorghum plants as an ingredient in the water extract. The results of this study showed that the highest inhibitory power was dry-Ultisol, dry-swamp soil, and dry-wet-swamp soil at a water extract concentration of 7.5%. This shows that all patterns of water application when combined with a water extract concentration of 7.5% or 10%, will produce the most effective inhibition of germination. These results align with the research results by Susilo *et al.* (2020) that extra sorghum in dry swamp cultivation produces high allelopathic compounds and can inhibit shoots. According to Inderjit and Keating, (1999), the allelopathic potential of plants under abiotic stress conditions such as drought tends to increase the production of secondary metabolites. This is following Maqbool *et al.* (2013) that the levels of cyanogenic glycoside in sorghum plants will significantly increase in drought conditions.

The results showed that the cotyledon weight with 0% water treatment (control) had the lowest value. This is because the 0% extract concentration treatment did not produce any inhibitory power. The germination process took place normally, which resulted in the breaking of the cotyledons, leading to lighter cotyledon weights. In contrast, the concentration of aqueous extract from 7.5% to 10% resulted in a low dry weight of the cotyledons. However, the soil water supply pattern of dry-swamp, wet-swamp, and dry-wet swamp

resulted in a higher dry weight of cotyledons compared to other watering patterns, especially at a concentration of 10% water extract. This indicates that the pattern of water application has small effect on the seed cotyledons during the germination process so that the dry cotyledon weight produced is still relatively high. The higher the stress on the germination process, the higher the weight of the cotyledons produced.

Furthermore, the higher the concentration of aqueous extract, the higher the dry cotyledon weight produced. The breakdown of cotyledons may be caused by allelopathic compounds from the aqueous extract of sorghum roots. According to Weston and Czarnota (2001), sorgoleone (a compound in allelopathy) is 85-90% of sorghum root exudate, which has phytotoxicity to inhibit plant growth, inhibits photosynthesis and respiration, and seedlings will also experience chlorosis and stunted growth due to phytotoxicity from allelopathy. Sorghum produces many acidic primary phenolic compounds that are phytotoxins, but each sorghum cultivar has different levels of released compounds such as vanillic acid, benzoic acid, and phenolic compounds (Glab *et al.,* 2017). According Iannuci *et al.* (2012), wheat produces allelochemical compounds in 6 types of acids, namely 4-hydroxybenzoic acid, vanillic acid, citric acid, p-coumaric acid, pyruvic acid, and citric acid behind.

It was concluded that the highest inhibition of sorghum seed germination was at a concentration of 7.5-10% aqueous extract on dry Histosols indicated by the fresh weight of plumules and the lowest dry weight of radicles. This finding indicated that drought stress sorghum in Histosols produced the highest allelopathic compounds. Therefore, plants under these conditions have the potential as a source of bioherbicides.

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