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Inhibition of seed germination under water extracts of sorghum (*Sorghum bicolor* L.) and its ratoon cultivated in swamp land

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Abstract The results showed that the highest inhibition was found in the root of the main plant and the stem of the ratoon plant, where the normal growth was 6.00% and 22.00%, plumule length 3.29 cm and 3.57 cm, radicle length 0.84 cm and 2.58 cm, plumule wet weight 0.033 g and 0.030 g, radicle wet weight 0.003 g and 0.008 g), sprouts wet weight 0.075 g and 0.075 g, plumule dry weight 0.0033 g and 0.0030 g, radicle dry weight 0.003 g and 0.008 g, respectively. Likewise, those the same plant parts had cotyledon dry weight of 0.0185 g and 0.0168 g, ungrowth seeds 48.00% and 47, 00%, cotyledon wet weight 0.043 g and 0.038 g, as well as abnormal sprouts 46.00% and 31.00%, respectively. These findings indicate that the roots of sorghum main plant and its stems cultivated in swamp land become the best source of bioherbicides.

Keywords: Allelopathy, Bioherbicide, Organic agriculture, Organic farming, Autotoxicity

Introduction

The potential of allelopathy as a bioherbicide for agricultural cultivation continues to be developed through research and its application in the field. Although allelopathy cannot completely control weeds, it can suppress weed growth by inhibiting seed germination and the growth of other plants (Li *et al.*, 2019). Allelopathy, as a bioherbicide, produces allelochemical compounds that inhibit weed growth. Allelochemical inhibition mechanism is nearly identical to that of synthetic herbicides (Darmanti, 2018). Allelopaths derived from root exudation, stem and root residues are identified in sorghum (Sorghum halapense) (Alsaadawi *et al.*, 2013; Susilo *et al.*, 2020). Sorghum root residues affect plant growth (Amb and Ahluwalia, 2016). Allelopathic compounds produced by sorghum can inhibit the growth of other plants. Sorghum stem

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extract at a concentration of 10 g/L inhibited the germination of lettuce, tomato, and pigweed seeds higher than sorghum root extract (Marchi *et al.*, 2008). Application of water extract of sorghum suppressed the biomass of weeds *Trianthema portulacastrum* and *Cyperus rotundus* up to 40% (Iqbal *et al.*, 2020). Allelopathy of sorghum root exudation inhibited root and shoot development of wheat by 44.12% and 36.04%, respectively (Naby and Ali, 2021).

Allelopath can be produced from almost any part of the plant, including the roots, stems, leaves, flowers, and seeds. More research is needed to determine which part of the sorghum plant exhibits the greatest allelopathy. Allelopath is a byproduct of metabolism. Plants have particular tissues that synthesize and release secondary metabolites, as concluded by Latif *et al.* (2017). Sorghum stems, leaves, and roots contain secondary metabolites: ferulic, p-coumaric, syringic, vanillic, p-hydroxybenzoic, m-hydroxybenzoic, and protocatechuic acids, which can inhibit photosynthesis and seed germination, induce water stress, and cause stomatal closure stomata (Hussain *et al.,* 2021).

In addition to the source of allelopathy, environmental variables affect the allelopathic content of sorghum. Plants will produce more secondary metabolites if they are stressed and subjected to biotic and abiotic stress (Setyorini and Yusnawan, 2016). According to Susilo *et al.* (2021a), an aqueous extract of sorghum grown in swampy land with dry conditions at a concentration of 7.5% can inhibit the germination of sorghum seeds. In addition, Susilo *et al.* (2021b) stated that the best source of bioherbicides was from plants that had experienced abiotic stress in drought, particularly in Histosols. *In vitro* seed germination used to test the allelopathic potential of sorghum plant parts. The study was intended to determine the inhibition of seed germination treated with water extract of different parts of sorghum cultivated in swamp land.

Materials and methods

Sorghum plant preparation and experimental design

The research was conducted in the swamps located in Kandang Limun Village, Muara Bangkahulu District, Bengkulu City, Bengkulu Province, Indonesia, and in the laboratory between December 2020 and August 2021. The experiment used a completely randomized design (CRD) with five replications. The treatments were control, water extract, plant leaves, plant stems, roots, ratoon plant leaves, ratoon plant stems, and ratoon plant roots.

The extract was derived from the Numbu variety sorghum's main plant and the sorghum ratoon plant. The experiment used leaves, stems, and roots of main and ratoon of sorghum. The main crops and ratoons were cultivated in swamps to test for allelopathy. Sorghum seeds of the Numbu variety were used as a test plant. Parts of the plant shoot (leaves + stems) and roots of 7-week-old sorghum plants were harvested and dried for 14 days. After that, the dry shoot was cut into 1-2 cm pieces and dried at 70^{o}C for 72 hours. The material was then ground into powder using a grinder. The powder was used as a source of extract in this experiment.

Water extract preparation

Dried sorghum powder from the main plant and ratoon was weighed as much as 100 g (for a concentration of 10%) and soaked with 1000 mL of distilled water. The sorghum extract was stirred for 24 hours using a shaker at room temperature. After stirring for 24 hours, the mixture was filtered through a Whatman filter paper. The filtrate was then used to test sorghum seed germination.

Bioassay experiment

The bioassay test was designed to determine whether allelochemical sorghum extract inhibited the germination of Numbu sorghum seeds. Two layers of filter paper were placed in a 9 cm diameter Petri dish. Twenty-five grains of sorghum seeds were placed in a Petri dish and 10 mL of 10% sorghum extract was poured over the seeds. The Petri dishes were then incubated at room temperature for five days. The observed variables included the percentage of normal germination (%), percentage of abnormal germination (%), percentage of ungerminate seeds (%), seedling fresh weight (g), plumule length (cm), radicles length (cm), plumule fresh weight (g), radicle fresh weight (g), cotyledon fresh weight (g), plumule dry weight (g), radicle dry weight (g), and cotyledon dry weight (g).

Data analysis

Data were analyzed statistically by ANOVA at confidence level of 95%. If there is a significant difference, it is continued with the Duncan Multiple Range Test (DMRT) test with a significance level of $P < 0.05$.

Results

The results showed that the aqueous extract treatment significantly affected all observed variables except the fresh weight of the cotyledons (Table 1). Sorghum extract treatment had a significant effect on the percentage of normal seedlings. The main plant root extract treatment resulted in the lowest normal seedling (6.00%) and ratoon stem (22.00%). Seedlings can grow abnormally when exposed to leaf and main stem extracts, as well as ratoon leaves and roots. The control treatment generated the highest normal seedlings (89.00%) (Table 2). These results showed that sorghum extract could inhibit seed germination, and extracts from main plant roots and ratoon stems contained more allelopathy inhibitory compounds than other extract sources.

Table 1. Summarizes of ANOVA for variables obeserved in this experiment

No	Variables	Extract source	Coefficient variation (%)
1	Percentage of normal germination	23.40 **	24.20
2	Percentage of abnormal germination	22.06 **	30.99
3	Percentage of ungerminate seeds	7.98 **	41.19
4	Plumule length	47.78 **	10.38
5	Radicles length	47.66 **	18.87
6	Plumule fresh weight	46.14 **	10.24
7	Radicle fresh weight	14.47 **	33.81
8	Cotyledon fresh weight	2.47 tn	12.93
9	Seedling fresh weight	34.75 **	8.82
10	Plumule dry weight	46.14 **	10.24
11	Radicle dry weight	12.31 **	35.53
12	Cotyledon dry weight	2.98 *	10.61

Note : **: highly significant different, *: significantly different, ns: not significantly different

Table 2. The effect of the extract source on the percentage of normal seedlings, abnormal seedlings, ungerminate seeds and plumule length, radicle

Extract source	Normal seedlings (%)	Abnormal seedlings (%)	Ungerminate seeds (%)	Plumule length (cm)	Radicle length (cm)
Control	89 a	7 d	3 d	6.78 b	6.63 b
Main plant shoot	64 bc	9 d	27 bc	7.12 b	6.24 b
Stem main plant	51cd	12 d	37 ab	5.69 c	4.07 c
Root main plant	6 e	46 a	48 a	3.29 e	0.84 e
Ratoon shoot	44 d	22 c	34 ab	4.63 d	4.65 c
Ratoon stem	22 e	31 b	47 a	3.57 e	2.58 d
Ratoon root	78 ab	11d	11cd	8.96 a	11.38 a

Note: 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 main plant root extract and ratoon stem generated more abnormal seedling growth, 46% and 31%, respectively, while the control treatment produced the least (7%). The main plant root extract treatment also inhibited seed germination (48.00%), while ratoon stem (47.00%) inhibited seed germination and was higher than other treatments. In the control treatment, only 3% of seeds did not germinate (Table 2).

Sorghum extract, alike to seed germination, significantly affected plumule and radicle length. The main plant root and ratoon stem extract generated the shortest plumules, 3.29 cm and 3.57 cm, respectively, while the control treatment produced the longest plumules, 6.63 cm. The main plant root and ratoon stem extract generated the shortest radicle, 0.84 cm, and 2.58 cm, respectively, while the ratoon root extract treatment produced the longest (11.38 cm) (Table 2). In addition to seed germination and plumule and radicle development, sorghum extract had a significant effect on seedling weight (Table 3). The main plant root and ratoon stem extract generated the lowest plumule fresh weight, while the ratoon root extract produced the highest plumule fresh weight.

Table 3. The effect of extract source on the fresh weight of plumules, radicles, cotyledons, and seedlings

Extract source	Plumula fresh weight (g)	Radicles fresh weight (g)	Cotyledons fresh weight (g)	Seedlings fresh weight (g)
Control	0.058 b	0.013 bc	0.038	0.108 b
Main plant shoot	0.060 b	0.018 b	0.035	0.108 b
Stem main plant	0.045 c	0.010 c	0.030	0.088 c
Root main plant	0.033 d	0.003 d	0.043	0.075 c
Ratoon shoot	0.045 c	0.010 c	0.038	0.088 c
Ratoon stem	0.030 d	0.008 cd	0.038	0.075 c
Ratoon root	0.080 a	0.028 a	0.038	0.148 a

Note: 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 main plant root and ratoon stem extract treatments had the lowest radicle fresh weight, 0.003 g, and 0.008 g, respectively, while the ratoon root extract treatment had the highest (0.028 g). Sorghum extract did not affect cotyledon's fresh weight. However, the main plant root extract produced greater cotyledon fresh weight than the other treatments (0.043 g). Table 3 further reveals that the main plant root and ratoon stems extract achieved the lowest fresh weight of seedlings which were 0.075 g and 0.075 g, respectively, whereas the control treatment's fresh weight of seedlings reached 0.108 g.

The sorghum extract treatment was significantly affected the dry weight of plumules, radicles and cotyledons (Table 4). The lowest plumula dry weight was obtained from the main plant root extract (0.0033 g) and the ratoon stem (0.0030 g), and the highest was achieved by the control treatment (0.0058 g) and the main plant leaf extract (0.0060 g). The main plant root and the ratoon stem extract had the lowest dry weight of the radicle. Meanwhile, the main plant stem extract treatment yielded the lowest cotyledon dry weight, though it was not significantly different from the control, main plant shoot, ratoon shoot, and ratoon root treatment (Table 4).

Table 4. The effect of extract source on the dry weight of the plumule, radicle, and cotyledon

Extract source	Plumula dry weight (g)	Radicle dry weight (g)	Cotyledon dry weight (g)
Control	0.0058 b	0.0010 b	0.0160 abc
Main plant shoot	0.0060 b	0.0013 b	0.0150 bc
Stem main plant	0.0045 c	0.0010 b	0.0138 c
Root main plant	0.0033 c	0.0003 c	0.0185 a
Ratoon shoot	0.0045 c	0.0010 b	0.0160 abc
Ratoon stem	0.0030 d	0.0008 bc	0.0168 ab
Ratoon root	0.0080 a	0.0025 a	0.0160 abc

Note: 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%

Discussion

Except for the fresh weight of the cotyledons, the aqueous extract treatment of sorghum had a significant effect on all observed variables. The extract treatment from the main plant's roots and ratoon stems produced the lowest normal seedlings, while the control treatment generated the highest normal seedlings. These findings revealed that the main plant root and ratoon stem extract treatments suppressed the most test plant seed germination and that the extracts from the main plant roots and ratoon stem contained more allelopathic compounds than other treatments. The control treatment (no extract treatment) had no effect on seed germination. According to the findings of the same study, Marchi *et al.* (2008), the seed germination percentage was significantly decreased by the application of a water extract of sorghum stem at a dose of 10 g/l. Seed germination is the growth and development of an essential part of an embryo in a seed that demonstrates its ability to grow normally in an appropriate environment. Normal germination occurs when seedlings exhibit good and normal growth abilities (Ance, 2003).

Compared to normal seedlings, the main plant root and ratoon stems extract treatment produced the highest percentage of abnormal seedlings and the lowest percentage of ungerminated seeds. In contrast, the control treatment generated the fewest abnormal seedlings. The more abnormal seedlings produced, the increased the inhibition process, and vice versa. Thus, sorghum extract produces allelopathy that affects the germination of the test plant's seeds. According to Amb and Ahluwalia (2016), allelopathy released by plants inhibits seed germination. The presence of phytotoxic polyphenols could be a factor causing the high inhibition of seed germination (Tubeileh and Souikane, 2020).

The main plant root and ratoon stem extract treatments resulted in the shortest plumule. Thus, the main plant's roots and the ratoon's stem have higher allelopathic than other plant parts. According to the findings of Naby and Ali (2021), allelopathic compounds from sorghum can reduce the length of shoots and roots on grassy weeds. Sorghum's allelopathic reduces plant growth due to the presence of sorgoleon. Sorgoleon is a hydrophobic phytotoxic compound that inhibits photosynthesis and electron transport.

The application of main plant roots and ratoon stem extracts inhibited the growth of the test plant more than other treatments, as indicated by the findings of this study. The inhibition of the seed growth process will result in abnormal seedlings, and the seeds will not germinate. Plants cannot grow normally into new individuals with complete root, stem, and leaf organs as a result of the allelopathic release of sorghum extract. Plants respond in different ways when exposed to extracts from various organ sources, as concluded by Susilo *et al.* (2021a). Inhibition of water absorption causes low water content. Eventually, stomata closure occurs so that photosynthesis is inhibited and will have an impact on inhibiting the growth of the target plant. Inhibition of this physiological process leads to inhibition of shoot elongation. On the other hand, in normal growth, plant organs absorb more water and store the products of photosynthesis, thereby increasing the fresh weight.

Germination is an important stage in the plant's life cycle, which is marked by the appearance of new roots and shoots. According to Elisa (2006), germination is the process of reactivating the resting embryonic axis in the seed to develop seedlings. The embryonic axis develops during the process of seed growth and maturation. Germinating seeds are distinguished by the presence of a radicle or plumule that emerges from the seed. Allelopathic compounds in the extract can inhibit seedling growth by inhibiting cell division and elongation activities. Fitter and Hay (1991) confirmed that terpenoids, flavonoids, and phenols are compounds that inhibit cell division. Wattimena (1987) also suggested that phenolic compounds inhibit the metaphase phase of mitosis. Disruption of the metabolic phase leads to inhibition of mitosis, bringing about the inhibition of cell division and elongation. This inhibition does not increase cell number and size, so seedling growth is not impeded. The number and size of cells do not increase when plant metabolic processes are inhibited, hampering seedling growth.

The treatment of extracts of the main plant roots and ratoon stems also produced the shortest radicle length. Radicles are more sensitive to the presence of sorghum extract due to direct contact with the growing medium. According to Netsere (2015), the radicle was more retarded than the plumule against *Parthenium hysterophorus* L. extract allelopathy. The presence of allelopathic also causes gibberellins and IAA hormones to malfunction, inhibiting radicular growth.

Ratoon root extract had the most extended radicle length. This result demonstrates that sorghum extract does not permanently inhibit seedling growth but instead stimulates growth. This phenomenon occurs due to the characteristics of the sorghum extract source. The ratoon root extract contained mostly inactive mature roots (rotten and compost-like), with only a few active or lived roots. The inactive roots are due to lack of allelopathy. Allelopathy can stimulate plant growth at low doses and concentrations (Maqbool *et al.,* 2013; Farooq *et al.,* 2013).

Treatment with aqueous extracts from main plant roots and ratoon stems resulted in the lowest fresh and dry weights of plumules, radicles, and cotyledons. The extract derived from the ratoon root produced a much more plumula fresh weight. This result reveals that the extracted material derived from the main plant's root and ratoon stem consistently has the most significant inhibitory influence. Before allelopathic interaction, the first, physiological processes during germination are the absorption of water by imbibition or osmosis, the second, breakdown of more minor, more uncomplicated, water-soluble, and transportable molecular compounds, the third, distribution of the breakdown products, and the fourth, rearrangement, the fifth process is respiration, which involves the reorganization of food reserves, and the sixth is growth at the growth point. Gardner *et al.* (1991) suggested that stem elongation was influenced by gibberellin hormone activity. Gibberellins play a role in stimulating cell division, cell enlargement, and stem elongation. According to Einhellig (1995), allelochemical mechanisms inhibit plant growth by inhibiting phytohormone activity. Allelopathic compounds in sorghum extract are thought to inhibit gibberellin activity. This disrupts cell division in the interlayer meristem, hampering seedling elongation.

Extract materials derived from sorghum roots and stems may thus have bioherbicide potential. According to Tetelay (2003), Allelopathy can inhibit plant growth by interfering with root growth. The presence of phenolic compounds interferes with auxin transport from shoots to roots and with cytokinin synthesis in roots. Cytokinins are known to play a role in root cell division and differentiation, while auxin is a compound that promotes root elongation (Gardner *et al.,* 1991). Allelopathic compounds absorbed by the roots will thus inhibit growth, particularly on the root surface in direct contact with the extract.

The treatment of main plant roots and ratoon stems resulted in seedlings' lowest fresh and dry weight. Plumule, radicle, and cotyledon fresh weight was all reduced by main plant root and ratoon stem extract. These three variables are components of the seedling fresh weight variable. Finally, the presence of sorghum extract allelopathy can reduce seedling fresh weight.

According to Javaid *et al.* (2006), sorghum stem extract suppressed germination more than sorghum root extract. At a concentration of 25%, extracts from sorghum roots inhibited seed germination. The phenolic compounds found in sorghum extract can inhibit seedling growth. The decrease in fresh weight indicates that the growth process is being slowed. Plant growth is inhibited due to the disruption of the water absorption process and the inhibition of the photosynthesis process. The total water content and the yield of photosynthesis (photosynthate) in the plant are referred to as fresh weight. Inhibiting water absorption by plant cells and photosynthesis reduces water content and photosynthesis outcomes.

The lowest normal seedlings, length of plumule, length of the radicle, fresh and dry weight of plumule and radicle resulted from extract treatment of main plant root and ratoon stem. Both treatments produced a higher percentage of ungerminated seeds and abnormal seedlings than extracts from the main plant's leaves and stems, as well as ratoon leaves and roots. These findings indicated that sorghum plant organs derived from the roots of the main plant and ratoon stems cultivated in swamps can be a better source of bioherbicides than other plant parts.

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