

Potential Waste Water Extract from Ratoon Plant Sorghum (*Sorghum bicolor* L.) Produced in Swamp Land as Bioherbicide

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ABSTRACT: Allelopathy is a phenomenon of inhibition of plant growth due to toxins. Sorghum is one of the allelopathy-producing plants that have the potential as a bioherbicide. Research on the source of extracts from ratoon sorghum waste extract as a bioherbicide has never been done. The purpose of this study was to determine the potential of ratoon sorghum waste extract as a bioherbicide. The study was conducted in MuaraBangkahulu, Bengkulu City, Indonesia, from January to April 2022. The study used a completely randomized design. The first factor was the source of extract from the ratoon sorghum organ, which consisted of leaves, stems, and roots. The second factor is the concentration which consists of 0%, 5%, 10%, and 15%. Experiments using the bioassay method. Each petri dish was poured with 10 ml of wastewater extract, and 20 mung bean seeds were sown and incubated for three days. The results showed that all extract sources with all concentration levels in germination growth were higher than the control, indicated by the percentage of normal germination and radicle length. This finding indicates that the source extracted from the first extract waste has a role, such as liquid organic fertilizer (LOF). Therefore, the source extracted in this experiment could not be used as a source of bioherbicides.

Keywords- Histosols, opportunities, ratoon sorghum, secondary extracts, vegetable herbicides

I. INTRODUCTION

Allelochemical compounds derived from sorghum, especially ratoon, have the potential to be developed into effective and environmentally friendly bioherbicides to control weeds. The ratoon of sorghum is one of the sources of secondary metabolites that act as plant allelopathy. According to [1], allelopathic compounds can control weeds and indirectly increase crop yields. Although the use of allelopathy is not entirely able to control weeds, the role of allelopathy can reduce weed populations by inhibiting seed germination and plant growth mechanisms [2].

The potential of allelopathy as a bioherbicide is to produce allelochemicals with an inhibitory system similar to synthetic herbicides [3]. It is known that sorghum has relatively high allelopathy derived from the exudation of roots, stem residues, and sorghum roots [4]. Root residue is one factor that affects plant growth competitively [5]. The application of allelopathy to staple crops can support a sustainable environment and ecosystem because it can function to reduce the use of synthetic herbicides. According to [6], allelochemical extracts derived from sorghum are environmentally friendly vegetable herbicides. Application of water extract of sorghum or sorghum plants can control weeds around cultivated plants.

Research on the potential of sorghum in producing allelopathy has been carried out. One of them is the potential for sorghum plant organs (roots, stems, and leaves) to produce different allelopathy from ratoon sorghum plants produced in swampland. Based on the results of research by [1], sorgaab from sorghum roots can suppress weeds up to 50% and increase wheat crop yields by 14% at a concentration of 5%. Sorgaab derived from the roots of the sorghum plant also affected soybean sprout radicles which were shorter than controls [7]. Water extracts from different organ sources will produce different responses by the test plant [8].

Allelopathic properties of plants will be different when interacting in the soil because there are several influencing factors. These factors include environmental factors, soil, and the influence of growth [9]. Sorghum plants grown on marginal soils (swamps) have different growth and development responses as well as allelopathic content when compared to sorghum grown on other soil types. According to [10], sorghum cultivated in swampy areas with a dry irrigation pattern produces a higher allelopathic content than the wet irrigation pattern. In addition, the allelopathic content was thought to be different in different sources (organs) of sorghum plants.

Allelochemical toxicity released by sorghum plants can inhibit seed germination. Based on the test results on mung bean seeds, the aqueous extract of the leaves, stems, and roots of sorghum reduced the rate and time of germination significantly differently [11]. The concentration of the extract has a significant role in the effectiveness of the inhibition of the test plant. According to [12], the concentration of sorghum extract cultivated in swampland

from 7.5 to 10% had the highest inhibition on germination of sorghum seeds in the bioassay test. The use of water extracts from sorghum plants, especially plant organs, has resulted in the inhibition of the test plant. However, research on utilizing the first extract waste as a bioherbicide has never been carried out. The purpose of this study was to determine the inhibition of germination treated with ratoon sorghum waste extract (secondary extract) cultivated in swamps with different concentrations.

II. METHODOLOGY

Plant material

The research was conducted in Muara Bangkahulu, Bengkulu City, Indonesia, from January to April 2022. The definition of the main crop is sorghum which has undergone vegetative and generative phases until the first harvest (for 3.5 months) and is cultivated in swampland. The water extract material in this experiment came from the ratoon sorghum plant. Ratoon plants are plants that grow back after the main (first) crop is harvested. The ratoon plants that had grown for the past 7 weeks were harvested in the form of a trunk consisting of a canopy (stems and leaves) and roots. The shoots and roots of the sorghum ratoons were harvested and dried in the sun for 10 days. Dry bean (roots, stems, and leaves) cut 1-2 cm. Then it was dried in an oven at 70°C for 72 hours. Berangkas are mashed using a grinder or blender. The resulting powder is an extract material in this experiment.

The water extract material used in this experiment came from extract waste (solid substrate). The initial extract is extracting three ingredients, namely roots, stems, and leaves, from the ratoon plant. After extraction at a concentration of 20%, the waste is then fermented for one month. After the fermentation process is complete, the extraction follows the treatment in this experiment again.

Experimental design

Experimental Design Completely Randomized Design. This experiment was arranged in a two-factor factorial pattern. The first factor is the extracted source, consisting of leaves from the ratoon plant, stems from the ratoon plant, and roots from the ratoon plant. The second factor was the concentration of the extract, consisting of 0%, 5%, 10%, and 15%. The experiment was repeated 4 times, and the experimental unit was a petri dish.

Water extract preparation

The process of making the first extract was as follows: 200 g of dry powder of ratoon sorghum (leaves, roots, and stems) (20% concentration) was soaked with 1000 mL of distilled water and stirred for 24 hours using a drain at room temperature. The mixture of extract and water is filtered through cloth and then filter paper. The process of making the second extract (waste) was as follows: 200 g of dry solid waste (the result of making the first extract) (20% concentration) was soaked in 1000 mL of distilled water and stirred for 24 hours using a switch at room temperature. The mixture of extract and water is filtered through cloth and then filter paper. Then the extract was put into a labeled container and ready to be used in this experiment.

Bioassay with water extract

Bioassay test of aqueous extract on filter paper in a 9 cm diameter petri dish. The purpose of the bioassay test was to determine the growth inhibition of mung bean seed germination due to water-soluble allelochemical compounds. Two layers of filter paper were in a petri dish. 20 mung bean seeds were planted in each petri dish, and 10 mL of water extract was added at the concentration according to the treatment (0%, 5%, 10%, and 15%) added to each petri dish. Then, it was incubated in the growth chamber for 3 days. All treatments in the experiment were repeated 4 times.

Measurement of experiment variables

Observation variables consisted of normal germination percentage (%), abnormal germination percentage (%), hypocotyl length (cm), radicle length (cm), cotyledon wet weight (g), hypocotyl wet weight (g), sprout wet weight (g), and radicle wet weight (g).

Statistic analysis

The observational data obtained were statistically analyzed to produce ANOVA and continued with the LSD test if there was a significant difference between the mean and the significance level set at $P < 0.05$.

III. RESULTS AND DISCUSSION

The variables observed in this experiment were the percentage of normal germination, percentage of abnormal germination, hypocotyl length, radicle length, cotyledon wet weight, hypocotyl wet weight, sprout wet weight, and radicle wet weight. Based on the variance table, it was shown that the extracted source had a significant effect on the percentage of normal germination, abnormal germination percentage, radicle length, hypocotyl wet weight, and radicle wet weight, as shown in Table 1. This indicates that the extract source treatment applied in this experiment had a positive response on some of these germination variables. The treatment of extract concentration significantly affected the variables of normal germination percentage, abnormal germination percentage, hypocotyl length, radicle length, cotyledon wet weight, and radicle wet weight. This indicates that the aqueous extract concentration treatment applied in this experiment had a significant response to most germination variables. An interaction between the extracted source and the extract's concentration on the observed variables: percentage of

normal germination, percentage of abnormal germination, radicle length, and cotyledon wet weight.

Table 1. Recapitulation of the results of the F test of the potential waste water extract of sorghum ratoon extract as a bioherbicide

No	Variable	Extract source (S)	Extract concentration (C)	interaction (S x C)	Coefficient of diversity (%)
1	Normal germination	17.50 **	7.76 **	3.90 **	3.25
2	Abnormal germination	19.75 **	7.86 **	4.63 **	31.49
3	Length hypocotyl	1.51 ns	8.15 **	0.47 ns	10.69
4	Length radicle	29.14 **	73.31 **	3.35 *	14.19
5	Cotyledon wet weight	1.73 ns	2.90 *	2.39 *	12.35
6	hypocotyl wet weight	4.45 *	2.36 ns	0.54 ns	13.14
7	Wet weight of sprouts	0.80 ns	0.75 ns	0.75 ns	23.58
8	Radicle wet weight	3.83 *	5.27 **	2.16 ns	20.64

Note : * : significantly
 ** : very significantly
 ns : no significant

The effect of the extracted source on the length of the hypocotyl showed no significant effect. However, there was a tendency that extracts from stems from ratoon plants produced higher hypocotyl lengths. The lowest hypocotyl length was achieved by extracts derived from leaves. This indicates that extracts from the ratoon plant, mainly stem organs (secondary extract waste fermenting for one month), cannot produce inhibitory power. The effect of extract concentration on hypocotyl length showed a significant effect. The extract concentration of 10% resulted in the highest hypocotyl length, but it was not significantly different from the concentration of 5%. The lowest hypocotyl length was achieved with 0% concentration or control. The findings of this data indicate that the application of the extract resulted in a response to stimulating germination growth in this test plant (mung bean). The application of plant organ water extract did not inhibit germination growth.

The effect of the extracted source on the wet weight of the hypocotyl showed a significant effect. The lowest hypocotyl wet weight was achieved by extracts derived from leaves. This indicates that extracts from the ratoon plant, especially stem and root organs, did not produce inhibitory power. Leaf organs have the potential to produce higher inhibitory power than stems and roots. The effect of extract concentration on the wet weight of the hypocotyl showed no significant effect. There is a tendency that the higher the concentration, the higher the inhibitory power produced (up to a concentration limit of 10%).

The effect of the extracted source on the wet weight of the radicle showed a significant effect. The lowest radicle wet weight was achieved by extracts derived from roots. This indicated that extracts from the ratoon plant, especially the roots, were more capable of producing inhibition of germination. Leaf and stem organs produce the same inhibitory power. The effect of extract concentration on radicle wet weight showed a significant effect. Extract concentrations of 10% and 15% resulted in the highest wet weight of the radicle. The lowest radicle wet weight was achieved by 0% and 5% concentrations. The findings of these data indicate that the application of the extract resulted in a response to stimulating germination growth. Application of aqueous extracts from fermented plant organs showed no inhibition.

Table 2. Average hypocotyl length, hypocotyl wet weight, radicle wet weight, and wet weight of sprouts

Treatment	Length hypocotyl (cm)	Hypocotyl wet weight (g)	Radicle wet weight (g)	Wet weight of sprouts (g)
Extract source :				
The leaves of the ratoon plant	5.35	0.119 b	0.054 ab	0.338
The steam of the ratoon plant	5.72	0.134 a	0.058 a	0.314
The root of the ratoon plant	5.57	0.133 a	0.048 b	0.305
Extract concentration :				
0 %	4.91 c	0.129	0.044 c	0.298
5 %	5.80 ab	0.129	0.051 bc	0.343
10 %	6.04 a	0.138	0.060 a	0.322
15 %	5.44 b	0.125	0.058 ab	0.313

Note : the numbers followed by the same letter in the same column were not significantly different in the Least Significance Different (LSD) test level of 5%.

The effect of the extracted source on the wet weight of sprouts showed no significant effect. The effect of extract concentration on the wet weight of sprouts showed no significant effect. There was a tendency that no

treatment (control) produced the lowest wet weight of sprouts. Application of aqueous extracts derived from fermented ratoon sorghum plant organs showed no inhibition. The application of aqueous extract tends to have the property of stimulating the growth of test plant germination.

The interaction between the extracted source and the extract concentration showed a significant effect on the percentage of normal germination, percentage of abnormal germination, radicle length, and cotyledon wet weight, as shown in Table 3. All treatments of extract sources (leaves, stems, and roots) with 0% aqueous extract concentration resulted in the highest percentage of normal sprouts is 100%. However, this was not significantly different from the interaction of stems with a concentration of 5%, leaves with a concentration of 10%, and leaves with a concentration of 15%. Have indicated that all the interactions mentioned above did not provide inhibition to germination. It is suspected that there are no toxic compounds in this treatment. Root interactions with a concentration of 15% resulted in the lowest percentage of normal germination. Have indicated that the interaction produces the highest inhibitory power. Although the definition of the highest inhibition here is that there is no inhibition in this experiment.

Table 3. Effect of interaction of extract source and concentration of water extracts on normal germination, abnormal germination, radicle length, and cotyledon wet weight

Treatment	Normal germination (%)	Abnormal germination (%)	Cotyledon wet weight (g)	Radicle length (cm)
Extract source (S)				
Leaf	100.00 a	0.00 c	0.123	8.66 a
Steam	96.56 b	3.13 b	0.129	8.81 a
Root	93.44 c	6.13 a	0.119	6.13 b
Prob. F > 5 %	<.0001	<.0001	0.1936	<.0001
Concentration (C)				
0 %	100.00 a	0.00 c	0.134 a	3.90 c
5 %	97.08 b	2.92 b	0.119 b	8.38 b
10 %	95.42 bc	4.58 ab	0.123 ab	10.26 a
15 %	94.17 c	5.42 a	0.118 b	8.91 b
Prob. F > 5 %	<.0001	0.0004	0.0498	<.0001
Interaction S x C				
Leaf x 0 %	100.00 a	0.00 c	0.140 a	4.09 f
Steam x 0 %	100.00 a	0.00 c	0.123 abc	3.88 f
Root x 0 %	100.00 a	0.00 c	0.140 a	3.73 f
Leaf x 5 %	100.00 a	0.00 c	0.113 c	8.87 bc
Steam x 5 %	98.75 ab	1.25 c	0.135 ab	9.70 abc
Root x 5 %	92.50 c	7.50 b	0.110 c	6.58 de
Leaf x 10 %	100.00 a	0.00 c	0.115 bc	11.50 a
Steam x 10 %	92.50 c	7.50 b	0.135 ab	11.14 a
Root x 10 %	93.75 c	6.25 b	0.120 abc	8.15 cd
Leaf x 15 %	100.00 a	0.00 c	0.125 abc	10.18 ab
Steam x 15 %	95.00 bc	3.75 bc	0.123 abc	10.50 ab
Root x 15 %	87.50 d	12.50 a	0.105 c	6.05 e
Prob. F > 5 %	0.0047	0.0016	0.0496	0.0109

Note :The numbers followed by the same letters in the same column are not significantly different in the Least Significance Different (LSD) test level of 5%.

All treatments of extract sources (leaves, stems, and roots) with 0% aqueous extract concentration resulted in the lowest percentage of abnormal sprouts, namely 0.00%. However, this was not significantly different from the interaction between leaves with a concentration of 10% and leaf interactions with a concentration of 15%. Have indicated that all the interactions mentioned above did not provide inhibition of germination resulting in abnormally low germination. The interaction of roots with extract concentration of 15% resulted in the highest percentage of abnormal germination. Have indicated that the interaction produces the highest inhibitory power. Although the highest inhibition is defined here, there is no inhibition in this experiment because the numbers obtained are still in the low category.

All treatments of extract sources (leaves, stems, and roots) with all concentrations of the extract resulted in higher cotyledon wet weight, except for leaves with a concentration of 5%, roots with a concentration of 5%, leaves with a concentration of 10%, and roots with a concentration of 15%. Based on these data, this data shows that the

combination of the extracted source and the concentration of the extract resulted in high cotyledon wet weight due to high germination growth. The water extract applied did not produce inhibition but instead stimulated the growth of the germination itself.

All treatments of extract sources (leaves, stems, and roots) with 0% aqueous extract concentration resulted in the lowest radicle lengths of 4.09 cm, 3.88 cm, and 3.73 cm, respectively. The interaction between the extracted source and the extract concentration resulted in the highest radicle length being achieved by the interaction between stems with a concentration of 5% (9.70 cm), leaves with a concentration of 10% (11.50 cm), stems with a concentration of 10% (11.14 cm). Leaves with a concentration of 15% (10.18 cm) and stem with a concentration of 15% (10.50 cm). Based on this data, it is shown that, in general, extract sources (mainly stems and leaves) combine extract concentrations of 5%, 10%, or 15% to produce low inhibitory power. This indicates that these interactions tend to produce plant growth-promoting hormones. The extracted source used in this experiment tends to work as liquid organic fertilizer (POC).

The source of the extract and the concentration of the extract with the essential ingredients coming from waste extract (substrate), which has been fermented for one month, is not possible as a bioherbicide. The extract produced in this experiment disappeared as POC, which was thought to have become its secondary metabolite compound. The application of organic fertilizer is an alternative to reduce the negative impact of using inorganic fertilizers. Organic fertilizers are fertilizers that come from plants or livestock. Organic fertilizers provide macro and micronutrients needed for plant growth and development. Organic fertilizers also help increase the soil's physical, chemical, and biological activity [13] [14].

Bioherbicides are part of vegetable biopesticides. According to [15], vegetable biopesticides result from the extraction of certain parts of plants that contain secondary metabolites and are toxic to certain plants. From the results of this experiment, it was found that the extract that was applied did not have inhibition but had properties to stimulate germination growth. It is necessary to conduct further research on this extracted material in the growth test of other types of plants. Extracts derived from waste extracts that have been fermented for one month are findings that may be good POC ingredients in the future. LOF provides nutrients that plants readily absorb. LOF can be applied by spraying onto plants. Organic fertilizers can be made from various sources of organic matter [16], one of which is extract waste made from fermented ratoon sorghum.

IV. CONCLUSION

It was concluded that generally, the interaction between all extract sources and all concentration levels resulted in higher germination growth than control, which was indicated by normal germination percentage and radicle length. This finding indicates that the extracted source from the first extract waste has a role, such as liquid organic fertilizer as a growth promoter. Therefore, the extracted source cannot be used as a source of bioherbicides.

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