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Allelopathic effect of sorghum root extract and its potential use as a bioherbicide

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Abstract The bioherbicide of sorghum root extract had a significant effect on seed germination. It inhibited the seedling growth of test plants, i.e., rice (*Oryza sativa*) and mung bean (*Vigna radiata*). The test plant treatment significantly affected the radicle and plumule length, radicle and plumule fresh weight, and the radicle and plumule dry weight. Bioherbicide concentration, on the other hand, affected all observed variables i.e. normal and abnormal sprouts, radicle and plumule length, and radicle and plumule weight. The percentage of normal and abnormal sprouts, radicle and plumule length, and radicle fresh weight correlated with the test plant and bioherbicide concentration. The higher the concentration of sorghum root extract, the lower the normal sprouts, radicle length, plumula length, and the fresh weight of mung bean (representing broad-leaf weed) and rice (representing narrow-leaf weeds). The regression analysis showed that LC 50% sorghum root extract was 5.24% for rice and 4.93% for mung bean. In summary, sorghum root extract can be a bioherbicide to control both narrow and broadleaf weeds in marginal land of coastal environment.

Keywords: Biopesticide, Dhurrin, Organic agriculture, Sorgoleone, Weed control

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a potential cereal crop supporting Indonesia's food and energy diversification programs (Subagio and Aqil, 2013). Besides its function as a food source, sorghum also contains antioxidants, minerals, protein, and fiber. Sorghum can adapt to various growing environments, including marginal land (Syafruddin *et al*., 2015). Sorghum contains allelochemical compounds, including sorgoleone. Sorgoleone (2 hydroxy-5-methoxy-3 - [(8'Z, 11'Z) -8 ', 11', 14'-penta decatriene] -p-benzoquinone) is a hydrophobic molecule having activity as a

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bioherbicide. In addition, sorgoleone in root hair can inhibit weed growth without harming cultivated plants. Sorgoleone sorghum is produced from both the seeds and the roots (Zhu *et al*., 1018).

Franco *et al*. (2011) reported that the amount of sorgoleone produced by seeds was not different from that produced by roots, both fresh and stored. Broadleaf weeds and grass are reportedly susceptible to sorgoleone, which is applied pre-emergent and post-emergent both in greenhouses and in the field. Sorgoleone works by interfering with the absorption of solutes and water and inhibiting electron transport in chloroplasts and mitochondria to inhibit photosynthesis in plants. Jesudas *et al*. (2015) reported that the effectiveness of sorgoleone was comparable to that of synthetic herbicides.

Studying by Einhellig and Souza (1992) indicated that sorgoleone inhibited the radical elongation of *Eragrostis* tef, and seed growth of *Abutilon theophrasti*, *Datura stramonium*, *Amaranthus retroflexus*, *Setaria viridis*, *Digitaria sanguinalis*, and *Echinochloa crusgalli*. Sorghum shoot extract affected the seedling growth, plumule and radicle length, plumule and radicle weight, and total dry weight of *Triticum aesativum* L., *T. durum* L., *Hordeum spontaneum*, *Avena fatua* and *Phalaris minor* (Naby and Ali, 2020). These results indicate that sorgoleone at low concentrations has biological activity (allelopathic). The research results of Cheema and Khaliq (2000) show that sorgaab (sorghum water extract) control weeds between 35% - 49% and increase the yield of sorghum between 10% - 21% while the chopped stover at a dose of 2-6 tons/ha, immersed in the soil at planting date, controls weeds by 40 - 50% and increase sorghum yield by 15%. Sorgaab also suppresses the growth of *Trianthema portulacastrum* weeds (broadleaf weeds), *Echinochloa colona* and *E. crussgalli* (grass weeds), as well as *Cyperus rotundus* and *C. iria* (nut weeds) (Khaliq *et al*., 2013).

Controlling weeds is crucial since they has lower crop production. Weeds compete with cultivated plants for nutrients, water, sunlight, and space (Rao, 2000). In addition, weeds are also hosted for several pests and pathogens. In large-scale crop cultivation, weed control is carried out using synthetic herbicides, considered effective and efficient. However, the excessive use of synthetic herbicides over a long period harms the environment, yields; weeds become resistant and the loss of natural enemies of weeds (Zimdahl, 2007). To overcome the adverse effects of synthetic herbicides, environmentally friendly herbicides are needed. The advantage of using natural compounds as herbicides is that the degradation of natural compounds in the environment is faster than synthetic chemical compounds, thereby reducing environmental pollution and groundwater contamination. Most of the natural compounds produced by plants are not harmful to human health (Kruse *et al*., 2000).

Research on environmentally friendly weed control by utilizing several weeds containing chemical compounds (allelochemicals) has been widely carried out. Several weed species have potential as organic herbicides (Setyowati and Suprijono, 2000). Apart from weeds, several plant species have been reported to contain allelochemicals sucs as *Medicago sativa* (Yang *et al*., 2020), *Hordeum vulgare* (Modhej *et al*., 2021), *Pennisetum glaucum* (Malik *et al.*, 2019), *Helianthus annuus* (Fuentes-Gandara, 2019), *Triticum aesativum* (Jabran, 2017), *Archidendron jiringa* (Nurjanah *et al*., 2020) and *Sorghum bicolor* (Susilo *et al*., 2020; Harsono and Setyowati, 2020; Susilo *et al*., 2021).

Allelopathy has potential as a bioherbicide to replace chemical herbicides (Fujii, 2001). Thus, the potential for allelopathy benefits the environment and humans and saves farmers' money on chemical herbicides. Several studies have shown that the use of allelopathy can replace synthetic herbicides. The combination of sorghum and sunflower extracts can reduce the use of chemical herbicide Dial Gold by up to 50% (Kandhro *et al*., 2015), the use of sorgaab or the combination of sorgaab + chemical herbicide can improve weed control and increase crop yields (Jesudas *et al*., 2015).

The allelopathic activity of *S. bicolor* varies depending on the cultivar, climatic conditions, and plant growth stage. Moreover, the allelochemical composition of sorghum tissues varies (Jabran *et al*., 2015). According to Santos *et al*. (2014), even though *S. bicolor* is considered an allelopathic species, its toxic effect varies depending on numerous aspects such as genotype, concentration, plant density, fertility, and soil moisture. The bioherbicide tested in this study was the Keller variety cultivated on marginal land in the coastal area of Bengkulu, Indonesia. The study aimed to determine the Lethal Concentration (LC) 50% of sorghum root extract in the test plants, namely rice (*Oryza sativa*) and mung beans (*Vigna radiata*).

Materials and methods

Preparation of extract

Sorghum var. Keller roots were harvested from the field, stored, and dried at room temperature. Dry sorghum roots are chopped into 3-5 cm lengths and powdered in a grinder. Sorghum root powder was weighed at 100 g and extracted with 1000 mL of water. After 24 hours of incubation, the extract was filtered using Wathman filter paper. The result of this extraction was sorghum root extrac for the treatments. The dilution was carried out according to the treatment, namely concentration 0% = 0 mL sorghum root extract + 100 mL aquadest; concentration 2.5%, = 25 mL of sorghum root extract + 75 mL of aquadest; concentration 5% = 50 mL sorghum root extract + 50 mL aquadest; concentration 7.5% = 75 mL sorghum root extract + 25 mL aquadest; and 10% concentration = 100 mL sorghum root extract + 0 mL aquadest.

Bioassay

The bioassay was carried out in the laboratory at room temperature (25 ± 5^0C) using a petri dish as a planting medium. Petri dish rinsed with bay clean and disinfected with 70% alcohol, then the bottom of petri dish covered with Whatman No. 1 filter paper for seed germination. Each petri dish received 10 mL of sorghum root extract bioherbicide. Twenty-five (25) rice and mung bean seeds were germinated into each petri dish and grown for one week.

The variables observed were the percentage of normal sprouts (%), percentage of abnormal sprouts (%), radicle length (cm), plumule length (cm), radicle and plumule fresh weight (mg), and dry weight of radicle and plumule (mg). The percentage of normal and abnormal sprouts was calculated at four to seven weeks after planting. The length and fresh weight of the radicle and plumule were measured seven days after planting, while the dry weight of the radicle and plumule was determined after oven drying at 60^0C or until the weight was constant.

Experimental design and statistical analysis

The design used Completely Randomized Design (CRD), replicated five times, with two factors: the concentration of the extract and the type of test plant. The first factor was the bioherbicide concentrations consists of 5 levels, namely, 0%, 2.5%, 5%, 7.5%, and 10%. The second factor consisted of 2 (two) test plants, namely mung bean (representing broad-leaf weeds) and rice (representing narrow-leaf weeds). The data were analyzed statistically using analysis of variance (ANOVA) at the F-test level of 5%. Data that showed a significant difference were subsequently evaluated using Polynomials Orthogonal and regression analysis to determine the Lethal Concentration (LC) 50%.

Results

The bioherbicide of sorghum root extract had a significant effect on germination and inhibited the seedling growth and development of rice and mung beans. The test plant treatment significantly affected the radicle and plumule length, radicle and plumule fresh weight, and the radicle and plumule dry weight (Table 1). The treatment of bioherbicide concentration, on the other hand, affected all observed variables except plumule dry weight. There was an interaction between the test plant and the concentration of bioherbicides on the percentage of normal and abnormal sprouts, radicle and plumule length, and radicle fresh weight. A summary of the analysis of variance is presented in Table 1.

Table 1. Summary of analysis of variance

Variable	T	C	TxC	CV (%)
Normal sprout (%)	2.77 ns	293.23**	50.73*	12.61
Abnormal sprout (%)	2.77 ns	293.23**	50.73*	13.19
Radicle length	116.24 **	13.12*	3.07*	13.78
Plumule length	521.83 **	45.91*	3.34*	12.82
Radicle fresh weight	32.06*	3.55*	12.02*	14.53
Plumule fresh weight	528.65**	4.41*	0.87ns	14.83
Radicle dry weight	24.63*	5.55*	0.25ns	20.86
Plumule dry weight	341.21**	2.21ns	0.65ns	18.95

Note: ns= no significant effect; *= significant effect at 5% level; *** = significant effect at the level of 1%; T=test plant; C= bioherbicide concentration; T x C= interaction of T x C; CV= coefficient variation

The coefficient of variance (CV) ranges from 12% to 21%. The CV value was low (≤ 30%), which indicates a high degree of accuracy. The higher the accuracy, the higher the validity of the conclusions obtained (Hanafiah, 2008).

Bioassay

There was an interaction between the concentration of bioherbicide and the test plant on the percentage of normal and abnormal sprouts, radicle length, plumula length, and radicle fresh weight (Table 1). The interaction between bioherbicides concentration and the mung bean test plant showed a negative linear relationship. Increasing the concentration of bioherbicides lowered the normal sprouts of mung beans. In the control treatment (0%), the normal number of sprouts was 81%, while at the 5% and 10% concentrations, it decreased to 49.6% and 18.08%, respectively. The interaction of bioherbicide concentration and rice test plants showed a negative linear relationship. All rice seeds germinated normally (100%); however, bioherbicide treatments at 5% and 10% concentrations reduced normal rice sprouts to 52.64% and 0%, respectively (Figure 1).

The concentration of sorghum root bioherbicide with the test plant showed a positive linear interaction with the percentage of abnormal sprouts (Table 1). Abnormal sprouts are sprouts that do not have the potential to become normal plants under optimum conditions. The interaction between bioherbicides concentration and the mung bean test plant showed a positive linear relationship. In the control treatment, the abnormal number of sprouts was 18.88%, while at the 5% and 10% concentrations, it increased to 50.40% and 81.92%, respectively. The interaction of bioherbicide concentration and rice test plant showed a negative linear relationship with the y = -10.88x + 107.04 and R^2 = 0.925. At control treatment, all rice sprouts grew normally, while at 5% concentrations of bioherbicides, the abnormal sprouts increased to 47.36%, and at 10%, all sprouts grew abnormally (Figure 2).

The radicle is the embryonic root of the plant and grows downward in the soil. It is the first thing to emerge from the seed and down into the ground to allow the seed to suck up water and send out its leaves so that it starts photosynthesizing. The interaction of sorghum root bioherbicide concentrations significantly affected radicle length (Figure 3). The interaction between bioherbicides concentration and the mung bean test plant showed a negative linear relationship with the equation of y = -0.1234x + 7.1988 and R^2 = 0.9476. Increasing the concentration of bioherbicides (2.5%) lowered the radicle length of mung beans by 0.3 cm. At concentration of 5%, the mung bean radicle length was 6.58 cm, while at 10% decreased to 5.96 cm. The interaction of bioherbicide concentration and rice test plant showed a negative linear relationship with the y = -0.3084x + 5.8372 and R^2 = 0.9464. In line with mung bean, increasing the concentration of bioherbicides by 2.5% lowered rice radicle length. At 5%, the rice radicle length was 4.29 cm, and 2.75 cm at 10%.

The plumule is the part of the embryo that develops into the shoot bearing the plant's leaves. The plumule gives rise to aerial shoots. There was an interaction between the concentration of sorghum root bioherbicide and the test plants on the plumule length (Table 1). The interaction between bioherbicides concentration and the mung bean test plant showed a negative linear relationship with the equation of y = -0.1615x + 3.6313 and R^2 = 0.8998. Increasing the concentration of bioherbicides (2.5%) lowered the plumule length of mung beans by 0.4 cm. At a concentration of 5%, the mung bean plumule length was 1.17 cm, while at 10% decreased to 0.62 cm. The interaction of bioherbicide concentration and rice test plant showed a negative linear relationship with the y = -0.109x + 1.714 and R^2 = 0.959. The equation shows that increasing the concentration of bioherbicides by 2.5% lowered radicle length by 0.2 cm. In line with mung bean, increasing the concentration of bioherbicides by 2.5% decreased rice plumule length. At 5%, the rice radicle length was 2.82 cm, and 2.02 cm at 10% (Figure 4).

The interaction of sorghum root bioherbicide concentrations with test plants on radicle fresh weight is shown in Table 1. The interaction between bioherbicides concentration and the mung bean testing plant showed a negative linear relationship with the equation of y = -2.7013x + 65.295 and R^2 = 0.8939. Increasing the concentration of bioherbicides (2.5%) lowered the radicle fresh weight of mung bean by 6.753 mg. At control treatment, the mung bean radicle fresh weight was 65.295 mg, while at 5% and 10%, bioherbicides concentrations decreased radicle fresh weight to 51.788 mg and 38.282 mg, respectively. The interaction of bioherbicide concentration and rice test plant showed a positive linear relationship with the equation of y = -0.8991x + 36.494 and R^2 = 0.8931. Increasing the concentration of bioherbicides (2.5%) increased rice radicle fresh weight up to 2.247 mg. Rice radicle fresh wight at control treatment, 5% dan 10% of bioherbicides treatments was 36,494 mg; 31.998 mg and 27.503 mg respectively (Figure 5).

The differences in allelopathy concentration did not affect the fresh weight of plumules, dry weight of radicles, and fresh plumules in mung bean and rice is shown in Table 2. Sorghum root bioherbicide at various concentrations had no significant effect on the fresh weight of plumules, dry weight of radicles, and dry weight of plumules. Thus, there was no interaction between allelopathy concentration and the test plant. This study showed that plumule fresh weight, radicle dry weight, and plumule dry weight decreased with the increased concentration of bioherbicides.

LC 50% of the percentage of normal germination, percentage of abnormal germination, radicle length, plumule length, radicle fresh and dry weight, plumule fresh and dry weight are shown in Table 3.

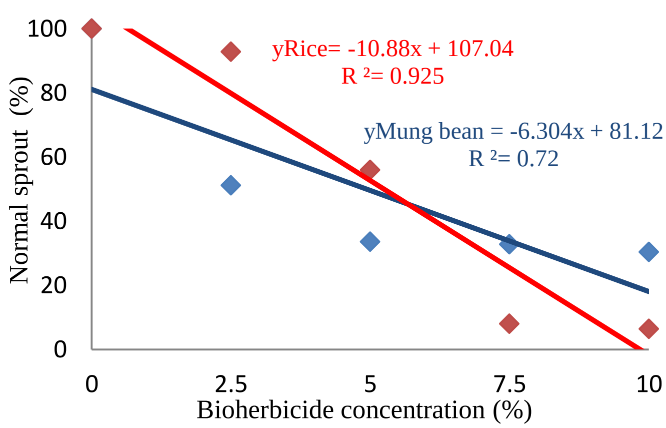

Figure 1. Interaction of bioherbicide concentration on normal sprouts of rice and mung bean

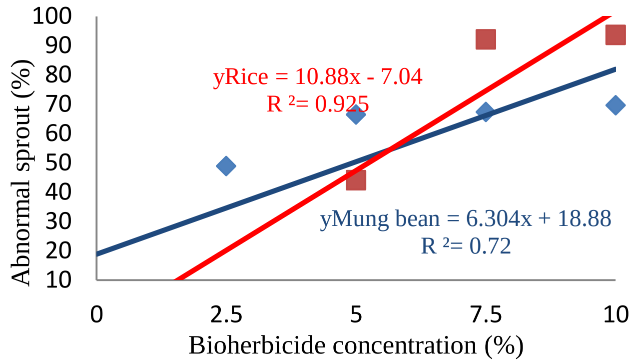

Figure 2. Interaction of bioherbicide concentration on abnormal sprouts of rice and mung bean

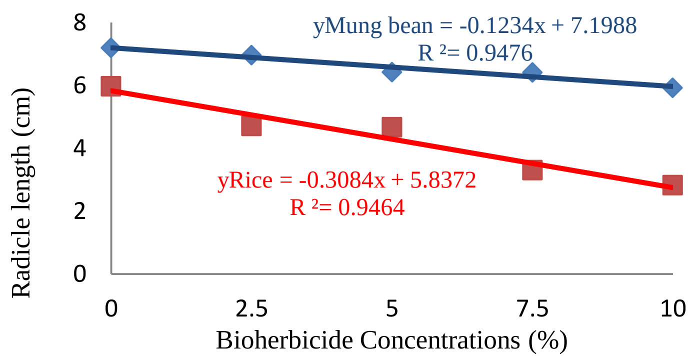

Figure 3. Interaction of bioherbicide concentration on radicle length of rice and mung bean

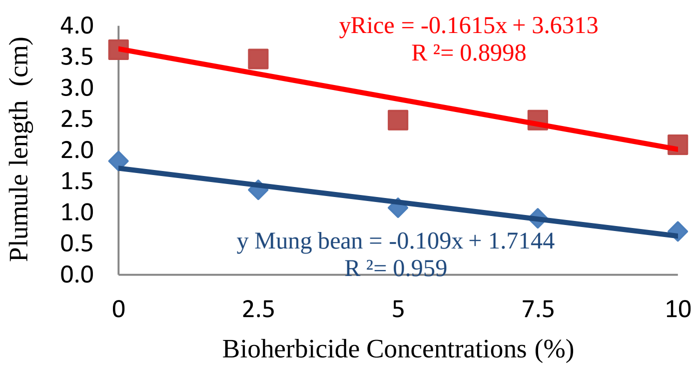

Figure 4. Interaction of bioherbicide concentration on plumule length of rice and mung bean

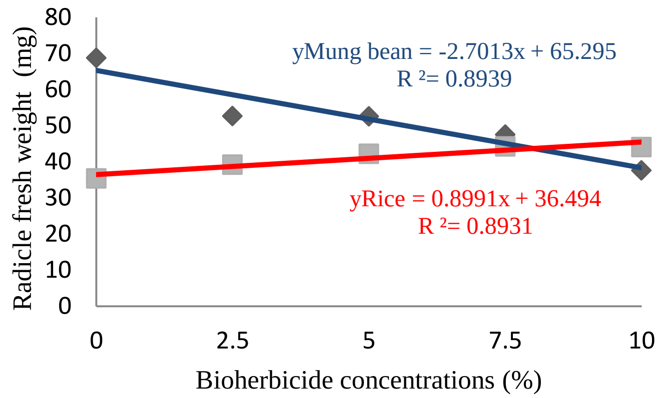

Figure 5. Interaction of bioherbicide concentration on radicle fresh weight of rice and mung bean

Table 2. Average of fresh weight of plumule, dry weight of radicle, and dry weight of plumule on mung bean and rice sprouts

Concen-tration	**Mung bean**			**Rice**		
	PFW (mg)	RDW (mg)	PDW (mg)	PFW (mg)	RDW (mg)	PDW (mg)
0%	62.00	24.00	36.00	27.43	33.93	11.874
2.5%	61.35	23.27	31.56	19.09	31.64	10.89
5%	57.82	23.15	30.84	18.33	29.60	10.86
7.5%	53.97	18.98	30.64	18.22	24.18	10.10
10%	52.41	16.66	28.17	17.17	23.75	9.48

Note: PFW: plumule fresh weight, RDW: radicle dry weight, and PDW: plumule dry weight

Lethal Concentration 50% (LC 50%) sorghum root bioherbicide

Table 3. Lethal Concentration (LC 50%) of bioherbicides on mung bean and rice

Variabel	**LC 50% mung bean**	**LC 50% rice**
Percentage of normal sprouts	4.93	5.24
Percentage of abnormal sprouts	4.93	5.24
Radicle length	-344.01	-143.21
Plumula length	-443.82	-286.94
Radicle fresh weight	5.66	15.04
Plumula fresh weight	12.22	-30.00
Radicle dry weight	-40.59	-14.21
Plumula dry weight	-22.65	-173.57

Discussion

Bioassay

The higher the concentration of bioherbicides, the growth of sprouts of rice, and mung bean, was increasingly inhibited. The inhibition of germination growth was due to the decrease in root permeability because the phenolic compounds released by the sorghum root extract entered the seeds. These phenolic compounds interfered with germination enzymes, especially those related to the breakdown of carbohydrates. Seed germination inhibition is related to disruption of the mitotic process in the embryo. Meanwhile, sprout growth inhibition is related to nutrient mobilization from the endosperm to the seed embryo. The high protein content indicates the inhibited mobilization of nutrients into the seed embryo. Narwal (2000) stated that the decomposition of previous crop residues and the release of allelochemicals coincides with the germination and early seedling growth of subsequent crops. As a result, allelochemicals affect the establishment and development of the next crop.

This study showed that increasing the concentration of sorghum root bioherbicides increased the percentage of abnormal sprouts and decreased the percentage of normal germination of the test plants. At 0% concentration of bioherbicide (control treatment) resulted in normal root development, especially primary roots. The absence of tissue damage indicates normal developing hypocotyl. Plumula grows normally, and the leaves are green. Epicotyl also grows normally without tissue damage. The sprouts had shorter primary roots at bioherbicide concentrations of 2.5%, 5%, 7.5%, and 10%. The sprouts do not grow and develop properly. The abnormal sprouts were indicated by twisted plumules, swollen cotyledons, and short roots.

Allelopathy inhibits the process of cell division, elongation, and enlargement. This process is related to the growth and size of plant cells and organs, so that plant height or length is inhibited. Bhadoria (2011) reported that allelochemicals' effect could be seen on plant growth and development by inhibiting plant growth. Seed darkening and swelling, short roots or radicles, necrosis of root tips, discoloration, and lack of root hairs. Research on the percentage of seedling mortality conducted by Mubarak and Sayed (2009) reported that *Acacia nilotica* leaf extract on *Zea mays* and *Sorghum bicolor* increased plant mortality rate by 50% and 28.6%, respectively, compared to the control treatment.

Increasing the concentration of sorghum root bioherbicide decreased the percentage of normal germination and inhibited root elongation of the tested plant. Sorghum root extract contains active phenolic compounds and their derivatives, inhibiting the growth of radicle length in mung bean and rice. In addition, phenolic compounds can also affect the function of specific enzymes in synthesizing proteins around the elongation of the radicle, reducing cell permeability and impairing the role of the hormones IAA and gibberellins (Einhellig, 1995). Skinner (2006) reported that the allelopathy of *Gliricidia sepium* and *Acacia auriculiformis* at a concentration of 3% could inhibit root length, root weight, plant height, and plant weight by up to 50%. In addition, Randhawa *et al*. (2012) stated that sorghum extract also inhibited the growth of *Trianthema portulacadtrum* radicle.

This study showed that increasing the concentration of sorghum root bioherbicide could inhibit germination, shorten radicle and plumule and reduce the fresh weight of the mung bean radicle. The results also showed that sorghum root bioherbicides at high concentrations (10%) reduced the fresh weight of mung bean radicles. On the other hand, at the same extract concentration, the fresh weight of rice increased. This increase in weight is due to the rice seeds being observed and weighed. Salisbury and Ross (1995) stated that the increase in plant fresh weight occurred due to the absorption of large amounts of water by plant cells, followed by an increase in the rate of plant photosynthesis. The increased rate of photosynthesis further increases carbohydrates' formation rate, which is a source of energy for other metabolic activities.

The inhibition of sprouts showed allelopathic activity in the process of cell elongation and multiplication. Allelochemicals released from plants can inhibit germination, growth of shoots, and roots of other plants, affecting nutrient uptake and causing the plant to die. Allelopathy also inhibits plant root development, radicle and coleoptile elongation, necrosis at root tips, leaf discoloration, and reduces plant dry weight (Arowosegbe *et al*., 2012). According to Lim *et al*. (2019), the presence of phenols compounds of allelochemical inhibits cytokinin activity. Inhibition of cytokinin enzyme activity interferes with shoot meristem cell division. The experiment by Sitanggang (2018) showed that a 5% concentration of sorghum extract produced the lowest plumule length of 4.15 cm.

This study also showed that as allelopathy concentrations rise, so does the target plant's allelopathic inhibition. The negative effect of allelopathy occurred in almost all observed variables except the percentage of abnormal germination and fresh weight of the radicle on the test plant, rice. In line with Pabinru (1979); Dayan (2006); and Cheema *et al*. (2002), the factors that affect the inhibition of allelopathic compounds are concentration, type of target plant, and duration of inhibition. Shafer and Gorisson (1986) reported that there was a relationship between allelopathic activity and concentration. The greater the concentration of allelopathy, the greater the inhibition. The higher the concentration, the more poisoned the plant is. The inhibition process occurs at each phase of the seed germination process, starting from water absorption, activation of enzymes, respiration, and germination.

Lethal Concentration 50% (LC 50%) sorghum root bioherbicide

Research investigation by Cheema *et al*. (2000) showed that the application of sorghum mulch could reduce the dry weight of plants by 40-53% compared to the control, while sorgaab inhibited the dry weight of weeds by 32-40%. Another study showed that sorghum intercropped with plants had a positive effect on cotton plant growth (dry weight) and a negative impact on weeds (Santos *et al*., 2014). Intercropping one or two rows of sorghum in cotton can reduce nutsedge weed's density and dry biomass (Iqbal *et al*., 2007). Skinner (2006) found that *Clotalaria juncea* allelopathy significantly reduced the dry weight of *Amaranthus hybridus* and *Lolium multiflorum* weeds up to 100% and 65.38% compared to controls, respectively. The efficacy of allelopathy produced from plants varies depending on the type of plant tissue or plant organ. The content of allelopathic compounds is not evenly distributing in plants.

The purpose of calculating the LC value is to determine the toxicity of the tested compound caused 50% of death of the test plant. In this study, the value of LC 50% percentage of normal sprout in rice was 5.24% while mung bean 4.93%. Thus, sorghum root extract at a concentration of 2.5 to 10% inhibited the germination of the test plants. Einhellig (1995) reported that secondary metabolites of sorghum root extract inhibit the function of plant growth hormones, such as gibberellic acid (GA) and indole acetic acid (IAA). Inhibition of growth hormone synthesis cause decreased α-amylase enzyme activity and the hydrolysis of starch into glucose.

The LC-50% of rice is higher than that of mung bean. The difference in the root system of the two test plants resulted in different LC-50%. The mung bean test plant has a tap root system. As a result, when the radicle emerges from the seeds and comes into contact with bioherbicides, it is poisoned, stunting its growth and eventually dying. The rice plant has a fibrous root system. When the radicle is emerged and came into contact with the bioherbicide, the radicle was poisoned, and some sprouts died. Secondary roots, on the other hand, is emerged at the radicle germination site. As a result, rice had a higher LC 50% than mung bean. In conclusion, the higher the concentration of sorghum root extract, the lower the percentage of normal sprout, radicle length, plumule length, and radicle fresh weight of rice and mung bean. The lethal concentration (LC50%) of sorghum root as bioherbicide was 5.24% for rice and 4.93% for mung bean.

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