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Allelochemicals of Goatweed (*Ageratum conyzoides* L.): Potential as a Natural Herbicide for Weed Control

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ABSTRACT: Ageratum conyzoides L., commonly known as goatweed, is a weed with allelopathic properties and potential as a source of natural herbicides. This study aimed to evaluate the allelopathic potential of goatweed in inhibiting the germination of rice test plants, determine the optimum concentration of goatweed extract for inhibiting the growth of the test plants, and establish the Inhibitory Concentration 50% (IC50) of goatweed extract. The experiment was conducted using a Completely Randomized Design (CRD) with a single factor and five replications. The factor tested was the concentration of goatweed allelopathic extract consisting of 0%, 2.5%, 5%, 7.5%, and 10%. The data were analyzed using Analysis of Variance (ANOVA) at the 5% F-level. Significant results were further tested using Orthogonal Polynomial and Least Significant Difference (LSD) tests. The IC50 was determined using linear regression analysis. The control treatment (without extract) did not inhibit germination or seedling growth, while concentrations between 2.5% and 10% inhibited the germination and seedling growth of the test plants. The inhibitory effect increased with higher concentrations of the allelopathic extract. The highest inhibition of growth was observed at a 10% concentration. The IC50 for abnormal seedlings was 7.47%, which was lower than that for normal seedlings at 11.42%, while the IC50 for plumule length was 6.18%, radicle length 5.88%, plumule dry weight 6.78%, and radicle dry weight 5.31%. Therefore, the aqueous extract of goatweed has potential as a natural herbicide, as it effectively inhibits germination and the growth of the test plants.

Keywords - allelopathy, bioherbicide, organic farming, sustainable agriculture

I. INTRODUCTION

Weeds grow in unwanted places and compete with cultivated plants for nutrients, water, light, and space [1]. The presence of weeds in agricultural land can affect the quality of crop production [2], making weed control essential to reduce competition with the main crops. Weed control using synthetic herbicides has become common due to its ease and relatively low cost. However, its use negatively impacts the environment and can trigger weed resistance [3]. This negative impact has led to the need for more environmentally friendly alternatives for weed control. One effort is using allelopathic compounds from plants as natural herbicides, which are considered safer because they do not contain harmful substances.

Natural herbicides are considered safer because they do not leave residues that pollute the soil and are safe for humans and animals. The allelopathic ability of plants can be utilized as an effective natural herbicide, similar to chemical herbicides [4]. One weed with potential as a natural herbicide is goatweed (*Ageratum conyzoides* L.), which contains allelopathic compounds such as alkaloids, flavonoids, coumarins, benzofuran, and terpenoids [5].

Plants release allelopathic compounds through their roots, pollen, and vapors from plant parts, inhibiting the growth of surrounding plants [6] [7]. The potential of allelochemicals as natural herbicides is significant because their mechanism is similar to synthetic herbicides [3]. Research shows that goatweed leaf extract effectively inhibits the growth of weeds, such as *Paspalum conjugatum* [8] and *Imperata cylindrica* [9].

Herbicide testing on test plants, such as rice, is conducted to determine its level of effectiveness. The toxicity indicator of the plant extract is measured by IC50, which is the concentration that inhibits 50% of the growth of the test organism [10] [11]. Research on the allelopathic effects of goatweed on rice germination and vegetative growth is still limited, making further studies necessary to assess the potential of this natural herbicide.

The research aims to test the allelopathic potential of goatweed in inhibiting the germination of rice (*Oryza sative*, L.*)* as the test plant, determine the optimal concentration of goatweed aqueous extract that can inhibit the vegetative growth of the rice test plant, and determine the Inhibitory Concentration 50% (IC50) of goatweed extract in a laboratory test.

II. METHODOLOGY

The research was conducted from February to April 2024 in the Agronomy laboratory and greenhouse of the Department of Agricultural Cultivation, Faculty of Agriculture, University of Bengkulu. This research was carried out in two stages: testing the effectiveness of goatweed bioherbicide on the test plant in the laboratory and testing the growth of the test plant in the greenhouse. The research employed a Completely Randomized Design (CRD) with a single factor. The concentrations of goatweed extract (P) consisted of P1 = 0%, P2 = 2.5%, P3 = 5%, P4 = 7.5%, and P5 = 10%. Each treatment was repeated five times.

Preparation of Goatweed Extract

Goatweed is oven-dried at 70℃ for three days. The extract from goatweed stems and roots was prepared using the extraction method with distilled water as the solvent. The dried goatweed was ground using a blender. Then, 100 g of goatweed powder is weighed, dissolved in 900 ml of distilled water, and left for 24 hours. Afterward, the extract is filtered using Whatman No. 1 filter paper into an erlenmeyer flask. The filtrate obtained is a liquid referred to as stock solution, with a concentration of 10%.

Effect of goatweed allelopathic concentration on seed germination

The petri dishes used as the germination medium are washed with 5% bleach, cleaned with 70% alcohol, and lined with Whatman No. 1 filter paper. Next, each petri dish was filled with 10 ml of goatweed extract. Rice seeds, serving as the test plant, are placed in each petri dish with 25 seeds per dish. The germination was observed over seven days.

Effect of goatweed allelopathic concentration on plant growth

A total of 150 kg of ultisol is used as the growing medium and placed into buckets with dimensions of 22 cm in length and 20 cm in width. The growing medium was then arranged in a greenhouse. Seven-day-old seedlings from the lab test were transplanted into the growing medium, with one seedling per planting hole. Fertilization was done once at the time of planting with a dosage of Urea at 300 kg/ha or 0.15 g/bucket, TSP at 100 kg/ha or 0.05 g/bucket, and KCl at 100 kg/ha or 0.05 g/bucket. Watering is carried out every three days with 1 liter of water per session. Pest control was done manually by removing pests by hand and spraying Decis insecticide once a week with a concentration of 1 ml/L of water. Harvesting is done 45 days after planting. by cleaning the plant from the planting medium with water and then separating the roots from the crown to observe

Variables observed

Germination test in the laboratory

The variables observed in the laboratory test using petri dishes were conducted on ten seedlings in each petri dish. The data obtained were then averaged to calculate the percentage of normal and abnormal seedlings, the length of the radicle and plumule, the dry weight of the radicle and plumule, and the total dry weight.

Plant growth evaluation in the greenhouse

The observed variables included plant height, number of leaves, leaf length and width, number of tillers, and dry weight of roots and shoots were measured once a week, six times. The dry weight was measured after the plant samples were oven-dried at 70°C for three days until the weight became constant. Then, the samples were weighed using a digital scale.

Data analysis

1. The data were statistically analyzed using Analysis of Variance (ANOVA) at a 5% F significance level. If significant differences were found, further analysis was conducted using Orthogonal Polynomial for the germination test and the Least Significant Difference (LSD) test for the plant growth test.
2. A linear regression equation determined the Inhibition Concentration (IC) 50% of goatweed extract on seed germination. IC50 = a (50) + b
3. Leaf Area (cm^2). The leaf area is calculated using the formula LA = L x W x C.
    a. Note: LA: Leaf Area, L: Leaf Length, W: Leaf Width, C: Correction Factor
    b. The correction factor for rice leaf area is 0.76 [12].
4. Relative Shoot Length (RSL).
   $RSL = \frac{\text{Shoot length in extract}}{\text{Shoot length ini control treatment}} \times 100\%$ [13].
5. Relative Root Shoot Weight (RRSW)
   $RRSW = \frac{\text{Root dry weight in extract} + \text{Shoot dry weight in extract}}{\text{Root dry weight ini control} + \text{Shoot dry weight in control}} \times 100\%$ [13].
6. Shoot Root Ratio
   $\text{Shoot Root Ratio} = \frac{\text{Shoot dry weight}}{\text{Root dry weight}}$ [13].

III. RESULTS AND DISCUSSION

Research Overview

No seeds had germinated on the first day of the germination test. On the fifth day, the seeds germinated significantly, which was set as the period for calculating the germination percentage of rice. The plants grown in the greenhouse were attacked by grasshoppers at 2 WAT (week after transplanting) (Figure a), causing leaf breakage (Figure b) and the pest attack, with an intensity of approximately 4%, did not affect plant growth.

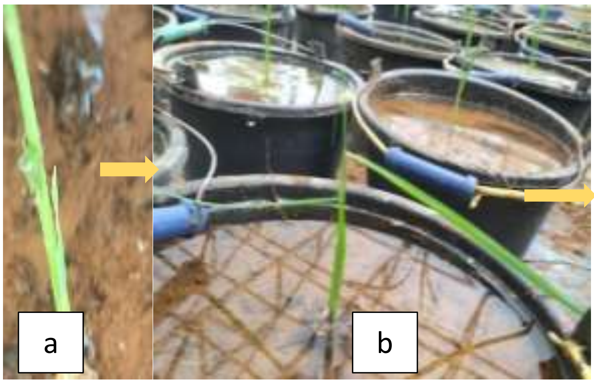

Figure 1. Rice test plant attacked by pest (a) grasshopper (b) broken leaf

The goatweed extract had a negative impact on the germination and growth of the test plant (Figures 2a and 2b). The higher the extract dose, the more germination and plant growth are suppressed. Allelopathic compounds are secondary metabolites that inhibit the germination and growth processes [14].

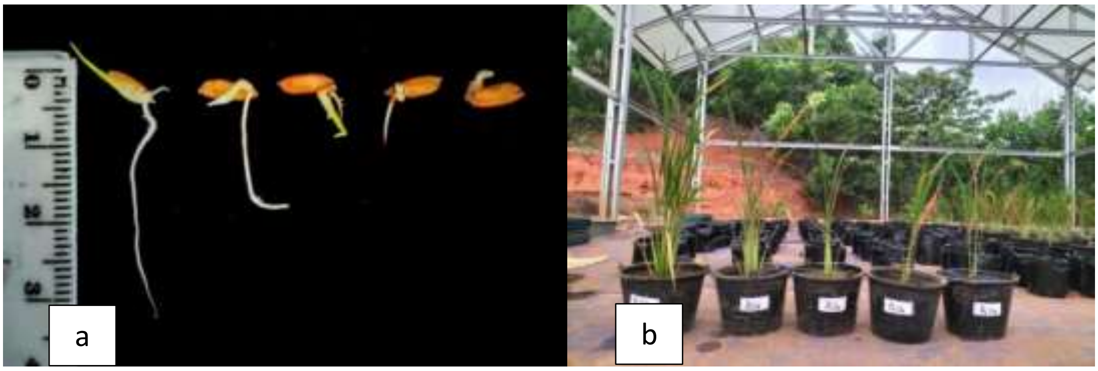

Figure 2. The allelopathic effect of goatweed on the germination and vegetative growth of the test plant: (a) germination test in the laboratory, (b) plant growth in the greenhouse

The effect of goatweed extract on seed germination

Goatweed extract significantly affected seed germination. The treatment resulted in normal and abnormal germination data with a non-normal distribution, necessitating data transformation using the formula √(x+0.5). The variance analysis results showed that the extract concentration significantly affected all observed variables (Table 1).

Table 1. Summary of Variance Analysis for Rice Germination Variables.

Variable	F-Calculated	Coeff Var (%)	F-table (5%)
Normal seedling percentage ^{t}	172.95*	2.71	
Abnormal seedling percentage ^{t}	92.76*	2.96	
Radicle length	361.14*	4.37	
Plumule length	299.35*	4.39	5.19
Radicle dry weight	607.90*	4.06	
Plumule dry weight	264.18*	4.05	
Total dry weight	920.81*	2.58	

Note : *= significantly different, ^{t}= data transformed $\sqrt{x + 0,5}$

Goatweed extract significantly affected the percentage of normal and abnormal seedlings, the length of the radicle and plumule, the dry weight of the radicle and plumule, and the total dry weight.

Normal and abnormal seedling percentage

Normal seedlings show the potential to develop into perfect plants when grown under optimal conditions, while abnormal seedlings do not. Observing the percentage of normal seedlings aims to determine the number of seedlings that can grow and develop well.

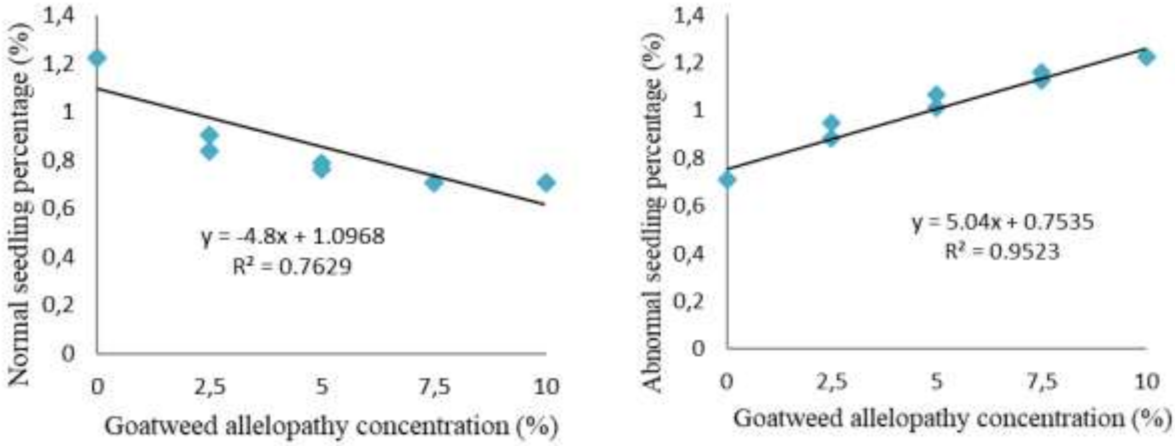

Figure 3. Effect of goatweed allelopathy concentration on the percentage of normal and abnormal seedling

The polynomial orthogonal test on the variable percentage of normal seedlings formed a negative linear regression (Figure 3). The coefficient of determination (R^2) value of 0.7629 indicates a strong relationship between the goatweed allelopathy and the emergence of normal seedlings, meaning that the concentration of goatweed allelopathic influences 76.29% of normal germination. In comparison, other variables influence the remaining 23.71%. The R^2 value = 0.9535 for abnormal seedlings indicates a very strong relationship between abnormal seedlings and the concentration of goatweed allelopathy [15]. This value shows that 95.35% of abnormal seedlings are influenced by goatweed allelopathy extract, while only 4.65% are affected by variables other than goatweed allelopathy. [15] stated that an R^2 value of 0.90 and above falls into the very strong category, R^2 = 0.75-0.90 into the strong category, R^2 = 0.50 is categorized as moderately strong, and an R^2 value of 0.25 is categorized as weak.

Lower concentrations of goatweed allelopathy result in a higher percentage of normal seedlings, indicating that an increase in goatweed extract concentration has a negative effect on seed germination. This negative effect of the goatweed extract is due to the disruption of the metabolism of food reserves as phenolic compounds enter the seeds. [16] reported that the allelopathic extract of goatweed significantly reduced wheat seed germination compared to the control treatment.

The seeds treated with 0% allelopathy concentration (control) exhibited good root growth, especially in the primary root, well-developed hypocotyls, and healthy plumule growth with green leaves. In contrast, applying goatweed allelopathy extract at concentrations of 2.5% to 10% resulted in seedlings with short, yellowing primary roots, twisted plumules, and stunted growth. [17] reported that goatweed allelopathy inhibits cell division, elongation, and enlargement, which are related to cell and organ growth, thus hindering seedling development. [18] stated that the entry of phenolic compounds and their derivatives, such as coumarin, cinnamic acid, and benzoic acid, can affect several vital processes such as cell division, mineral absorption, ion uptake, respiration, water balance, photosynthesis, protein synthesis, chlorophyll, and phytohormone production.

Radicle and plumule length

The radicle is the part of the plant that develops from the embryo and becomes the first root during seed germination. The radicle supports and transports nutrients to be processed in other plant parts. The plumule is the embryonic axis that grows upward to form shoots and eventually develops into leaves. The research results show that the treatment with goatweed allelopathy concentrations significantly reduced the length of the radicle and plumule (Figure 4).

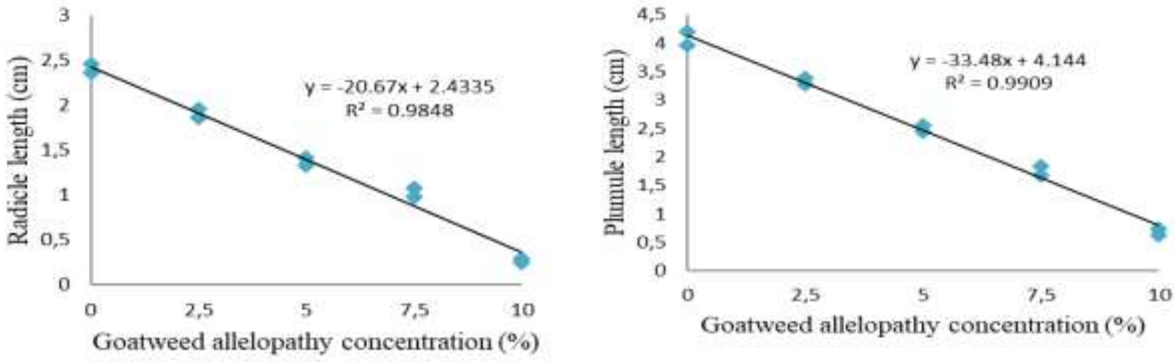

Figure 4. Effect of goatweed allelopathy concentration on radicle and plumule length

The coefficient of determination (R^2) values of 0.9848 and 0.9909 indicate that the application of goatweed extract has a very strong relationship with the length of the radicle and plumule, respectively [15]. The influence of goatweed concentration accounts for 98.48% of the radicle length and 99.09% of the plumule length.

The radicle length at a 0% concentration was 2.43 cm, while at a 2.5% concentration, it decreased to 1.92 cm, a reduction of 0.52 cm or 26.6%. The length of the radicle and plumule decreased as the allelopathy concentration increased. The plumule length at 0% concentration was 4.14 cm, and at 2.5% concentration, it dropped to 3.30 cm, a reduction of 0.84 cm or 25.5%.

The inhibition of radicle length may occur due to the obstruction of the diffusion process that transports the breakdown products of food reserves from the endosperm to the growth points of the plumule and radicle. [19] reported that goatweed extract has a strong allelopathic effect on the growth of radicles in rice test plants through mechanisms involving growth hormone inhibition, oxidative stress, and inhibiting enzyme activity. [20] stated that the toxicity of allelopathic compounds can be observed in seed growth, which becomes dark and swollen, with short radicles and a lack of root hairs. These are the visible morphological effects caused by allelopathy on plants.

[8] reported that increasing the concentration of goatweed leaf extract inhibits the plumule length during weed germination. [21] stated allelopathic compounds can inhibit cell elongation and proliferation. This study shows that the length of the radicle and plumule at the 0% treatment was longer compared to the treatments at 2.5%, 5%, 7.5%, and 10% concentrations.

Radicle and plumule dry weight

The radicle is a part of the plant embryo that develops into the root system, while the plumule is part of the plant embryo that develops into the upper part, including the stem and the first leaves. Observations of the dry weight of the radicle and plumule are often conducted in research to analyze the effects of treatments and evaluate plant growth. A higher dry weight generally indicates good and healthy development. The research results show that goatweed allelopathy significantly affects the dry weight of the radicle and plumule (Table 1).

Based on the coefficient of determination (R^2) values of 0.9844 and 0.9741, the decrease in the dry weight of the radicle and plumule is strongly associated with the application of goatweed extract [15]. The reduction in the dry weight of the radicle and plumule is influenced by goatweed concentration, accounting for 98.44% and 97.41%, respectively. The dry weight of the radicle at a 0% concentration was 2.17 mg, while at a 2.5% concentration, it decreased to 1.66 mg, a reduction of 0.51 mg or 30.7%. Meanwhile, at the same concentration, the dry weight of the plumule decreased by 22.6%.

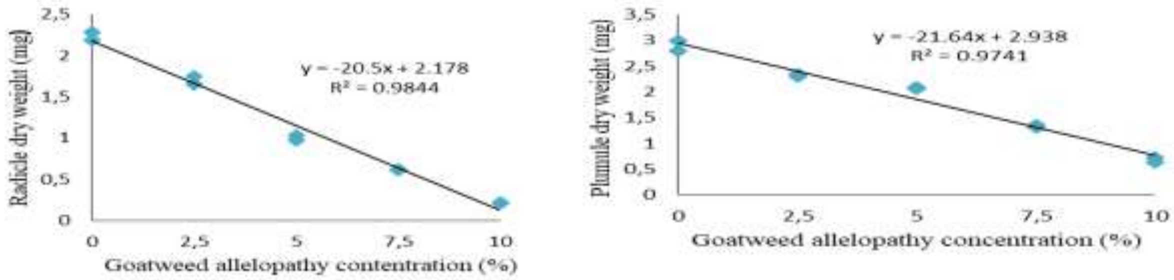

Figure 5. Effect of goatweed allelopathy concentration on the radicle and plumule dry weight

[22] stated that the allelopathic compounds in goatweed can affect the length and dry weight of the radicle in test plants. [17] reported that the application of goatweed bioherbicide significantly influences the dry weight of the plumule. The allelochemicals released by goatweed can inhibit other plants' germination, shoot growth, and root development, affecting nutrient absorption, impacting dry weight accumulation, and causing plant mortality [23].

Inhibition Concentration (IC50)

The IC50 concentration of goatweed allelopathy for radicle length and dry weight is lower, at 5.88% and 5.31%, respectively, compared to the IC50 for plumule length and dry weight, which are 6.18% and 6.78%. The lower IC50 concentration for the radicle is due to the direct contact of the rice seed radicle with the goatweed allelopathy extract during germination. On the other hand, the plumule does not come into direct contact with the goatweed allelopathy, necessitating a higher IC50 value compared to the radicle IC50.

Table 2. The IC50 of seedling growth, radicle and plumule length and dry weight

Variables	Regression correlation	IC50 (%)
Normal seedling percentage	y = -4.8x + 1.0968	11.42
Abnormal seedling percentage	y = 5.04x + 0.7535	7.47
Radicle length	y = -20.67x + 2.4335	5.88
Plumule length	y = -33.48x + 4.144	6.18
Radicle dry weight	y = -20.5x + 2.178	5.31
Plumule dry weight	y = -21.64x + 2.938	6.78
Total dry weight	y = -42.14x + 5.116	6.07

[24] stated that the entry of secondary metabolite compounds used as bioherbicides along with water into the seeds inhibits the induction of growth hormones such as gibberellic acid (GA) and indole-3-acetic acid (IAA). The inhibition of gibberellic hormone induction resulted in the inhibition of cell division and elongation, which hinders seedlings' germination and growth. Although seeds may still germinate, their germination is abnormal.

The Effect of Goatweed Allelopathy on Test Plant Growth

The treatment of goatweed allelopathy concentrations significantly affects plant height, leaf number, length, width, and area (Table 3).

Table 3. Analysis of Variance of growth components of rice

Variable	F-calculated	Coeff Var (%)	F-Table
Plant height	462.44*	4.11	
Leaves number	576.58*	7.83	
Leaf length	234.37*	2.46	
Leaf width	75.86*	4.74	
Laef area	459.61*	3.13	2.87
Tiller number	1388.69*	4.77	
Shoot Root Ratio	63.97*	10.86	
Relative Shoot length	91.21*	3.17	3.24
Relative Root Shoot Weight	237.30*	2.85	

Note: *= significantly different

Table 3 shows the effect of goatweed allelopathy on plant growth components. plant height, leaf number, leaf length, leaf width, leaf area, and the number of tillers decreased due to the application of goatweed allelopathy (Table 4).

Table 4. Effect of goatweed allelopathy concentration on test plant growth

Allelopathy concentration (%)	Variables						
	Plant height (cm)	Leaves number	Leaf area (cm^2)	Tiller number	Shoot Root Ratio	Relative shoot length	Relative root-to shoot weight.
0.0	75.55a	32.10a	48.01a	11.60a	1.18c		
2.5	66.32b	26.20b	38.32b	7.70b	1.28c	88.00a	9.89a
5.0	60.62c	8.20c	32.74c	3.70c	1.37bc	80.20b	9.00b
7.5	52.39d	6.20d	29.13d	2.30d	1.58b	69.60c	7.74c
10.0	48.69e	3.90e	20.93e	1.40e	2.72a	65.20d	6.19d

Note: Numbers followed by different letters in the same column indicate significant differences based on the LSD test at the 5% level.

Plant height, leaf number, leaf area, and the number of tillers were highest in the control treatment. At concentrations ranging from 2.5% to 10%, goatweed allelopathy reduced all observed plant growth components. At a concentration of 10%, the allelopathy most severely affected plant height, leaf number, leaf area, and the number of tillers. The research results indicate that as the concentration of allelopathy increases, the vegetative growth of the test plants is more suppressed. This finding suggests that higher concentrations of goatweed extract lead to increased allelopathic content, enhancing its toxicity. [25] stated that the allelopathic compounds in goatweed disrupt ATP function, impacting protein synthesis, stomatal opening, and several phytohormonal activities. [26] reported that allelopathic compounds, including flavonoids and phenolic compounds, inhibit cell division, thereby reducing hypocotyl length. Additionally, the inhibition of cell division and enlargement affects the physiological processes of plants, ultimately hindering plant growth.

The 10% concentration of goatweed extract resulted in a significantly higher shoot-to-root ratio than treatments with lower concentrations. The research findings indicate that root development is more sensitive to allelopathy than shoots. This disturbance is caused by the allocation of photosynthesis products directed more toward the shoots.[19] stated that allelopathic compounds from goatweed can inhibit photosynthesis, thereby stunting rice plants' growth and reducing rice roots' dry weight. The results of the subsequent tests indicate that the treatment with goatweed extract at a concentration of 10% produced lower and significantly different PTR and BATR than the lower concentrations. [27] reported that the allelopathic extract of Babandotan at a concentration of 10% also inhibited the growth of rice roots and shoots. [28] stated that phenols interacting with leaf chlorophyll inhibit various essential processes in photosynthesis, such as electron transport, energy transfer, and electron reception, thereby leading to the inhibition of photosynthesis. The inhibition of the photosynthetic process results in a decrease in the relative growth rate of the plants, as reflected in the reduction of dry matter accumulation. Consequently, there is a decrease in the dry weight of the roots and shoots of the plants.

IV. CONCLUSIONS

1. Goatweed (*Ageratum conyzoides*) can be a source of bioherbicide because the allelopathic compounds in goatweed inhibit the germination and vegetative growth of the test plant, rice (*Oryza sativa*).
2. A ten percent concentration of goatweed aqueous extract inhibits the vegetative growth of the rice more than lower concentrations.
3. A higher IC50% is required to inhibit normal rice seedlings compared to abnormal seedlings, and a lower IC50% is needed for radicle length and dry weight compared to that for plumula length and dry weight.

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