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Full Length Article

Allelopathic Activity of Leaf and Root Extracts of *Clidemia hirta* to Morpho-Physiological Characters of Rice (*Oryza sativa*) and Some Weeds of Rice Field

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Abstract

The decline in worldwide rice production could be attributed to weed infestations. Weeds hinder the growth of rice plants, leading to reduced rice production. Conversely, the study and potency of extract of *Clidemia hirta* D.Don as bioherbicide in rice is limited. Based on these characteristics, *Cl. hirta* is suspected to have allelopathic potential as an herbicide to enhance rice production by inhibiting weed growth. Therefore, this research aimed to analyze the extract of the invasive alien species (IAS) plant *Cl. hirta* as an allelopathic agent in inhibiting weed growth in rice cultivation. The research methods include the identification and inventory of plant seeds, the preparation of leaf and root extracts of *Cl. hirta* in a water-based solvent, allelopathy extract tests on rice and weeds in the seedling and vegetative growth stages, morphological character analysis as a physiological response to the extract, and phytochemical tests. The tested weed species include *Ageratum conyzoides*, *Echinochloa crus-galli*, *Cyperus iria* and *Ludwigia octovalvis*, while the rice variety used is IR64 as a control. In summary, both leaf and root extracts of *Cl. hirta* inhibit the growth of *A. conyzoides*, *E. crusgalli*, *Cy. iria* and *L. octovalvis*. In contrast, rice growth was actually stimulated by the extract. Therefore, this study is expected to serve as a reference for the development of bioherbicides for rice cv. IR64.

Keywords: Allelopathy; *Clidemia hirta*; IAS; Rice cv. IR64; Weeds

Introduction

Rice (*Oryza sativa* L.) production in Indonesia in 2018 to 2019 has decreased; particularly there is a decline of 1.3 million tons (Fendiyanto *et al.* 2019). The decrease in rice production could be due to weed infestations. Weeds inhibit the growth of rice plants, leading to reduced rice production. Some of the weed species found in the fields include *Monochoria vaginalis, Marcilea crenata, Salvinia molesta, Paspalum distichum, Leersia hexandra, Cyperus difformis*, and *Cy. iria*. The abundance of weed species that can invade rice crops increases the likelihood of reduced rice plant growth, which can threaten national food security. Therefore, rice weeds need to be inhibited using other plants with allelopathic potential, such as *Clidemia hirta* D.Don.

Cl. hirta is one of the invasive plant species. Invasive alien species (IAS) plants often invade national parks, tourist destinations, agricultural lands, and existing vegetation in Indonesia. It has a biochemical activity to fight pathogens and acts as antibacterial agents. Likewise, *Cl. hirta* provides a high zone of inhibition for several types of bacteria, including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhii* (Pratami *et al*. 2021). The compounds contained in *Cl. hirta* are thought to have other effects on other organisms. In addition, *Cl. hirta* is able to grow well among several other types of plants such as Cyperaceae. It is suspected that *Cl. hirta* has active compounds that can inhibit the growth of grass weeds such as *Cy. iria* in the field. In preliminary research, administering *Cl. hirta* extract apparently had an inhibitory effect on *Cy. iria*. Therefore, it is important to carry out research on the effect of *Cl. hirta* extract on the growth of several types of weeds. The agricultural land in Indonesia covers 8.59 million hectares out of a total land area of 192 million hectares (Fendiyanto

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et al. 2019). Agricultural land is gradually unable to meet the local food supply needs, partly due to the disturbance caused by invasive plants attacking rice (Hossain 2009). Invasive plants can cause economic and environmental disruptions and have adverse effects on human health (Alpert *et al.* 2000). However, the IAS plant *Cl. hirta* has the potential to be used as allelopathy to enhance rice production by inhibiting weed growth.

Allelopathy is a phenomenon where plants release chemicals into the environment that have harmful effects on other plants (Zhang *et al*. 2021). Putnam (1988) and Ismaini (2015) explained that the negative effects of allelopathy depend on the concentration of the chemicals it contains. Waller (1987) stated that the results of secondary metabolites such as terpenoids, phenols, alkaloids, fatty acids, steroids, and polyacetylenes can function as allelochemicals. Furthermore, Inderjit (1996) stated that the release of allelochemicals is facilitated by various processes such as dissolution from the plant's surrounding parts, exudates, stems, microbial activity, plowing plant residues into the soil, and the decomposition of dry matter residues. Allelopathy activity can act as bioherbicide purposes. Bioherbicides are herbicidal substances derived from natural sources including plants (Zhang *et al*. 2021). Thus, compounds contained in *Cl. hirta* can be used to inhibit weed growth. Therefore, this research aimed to analyze the extract of the invasive alien species (IAS) plant *Cl. hirta* as an allelopathy agent in inhibiting weed growth in rice cultivation as an effort to enhance rice production.

Materials and Methods

Materials

Weed plants used, namely *Ageratum conyzoides*, *Echinochloa crus-galli*, *Cy. iria*, and *Ludwigia octovalva*, were obtained from the SEAMEO BIOROP Weed Control Laboratory. We used rice IR64 variety, developed by PT Petrokimia Gresik. The research was conducted at the Ecology Laboratory, Plant Physiology and Biomolecular Laboratory, and Greenhouse of the Department of Biology, IPB University, Bogor, Indonesia.

Determination of the best media for testing the vegetative growth of plants

Several media with different material compositions were used to determine the best medium for weed growth. The tested media compositions were soil: sand (4:1), soil: compost (4:1), and soil. The four weed species were sown in each of these media, resulting in 12 experimental units. The plant was watered daily. In addition, the height was also measured. The measurement of height was determined from the tallest plant, and these parameters were used to determine the best medium for plant growth.

Preparation of allelopathy extracts

The allelopathy extracts were made using the leaves and roots of *Cl. hirta*. These extracts were used for the germination and vegetative growth treatments. To make the allelopathy extracts, 25 mg of plant material was ground into 100 mL of distilled water. The mixture was macerated and then filtered. The filtrate was used as the mother solution. Before being used for germination and vegetative growth experiments, the mother solution was diluted with distilled water to achieve concentrations of 10, 20 and 40%.

Allelopathy extract test on germination and vegetative stages

The germination test was performed by placing 10 seeds of each plant in separate petri dishes with filter paper. Allelopathy extracts at concentrations of 10, 20 and 40% were added to each petri dish with seeds. Distilled water was used for the control. Observations of germination were made every day for 12 days, measuring the percentage of germination and the length of seedling roots. The vegetative growth test was conducted on individual plants. Several seeds of each plant were planted in soil-filled polybags for germination. After two weeks, two plants were left in each pot. We observed the morpho-physiological characters of the plants after two months especially in vegetative stages.

Allelopathy treatments were applied to five plant types using two *Cl. hirta* extracts (root and leaf) at three concentrations (10, 20 and 40%). The experiment to test allelopathy extracts during the germination phase was designed and conducted using a completely randomized design (CRD) with a factorial treatment design consisting of two factors.

The first factor involved the types of plant seeds, comprising five levels:
V1: Rice, IR64 variety
V2: *A. conyzoides*
V3: *E. crus-galli*
V4: *Cy. iria*
V5: *L. octovalvis*

The second factor included allelopathy extract treatments with eight levels. They are C0: No allelopathy extract treatment (negative control), C1: Treatment with 10% leaf *Cl. hirta* extract, C2: Treatment with 20% leaf *Cl. hirta* extract, C3: Treatment with 40% leaf *Cl. hirta* extract, C4: Treatment with 10% root *Cl. hirta* extract, C5: Treatment with 20% root *Cl. hirta* extract, C6: Treatment with 40% root *Cl. hirta* extract, and C7: Treatment with herbicide (positive control). The combination of these two factors resulted in 40 treatment combinations, each replicated three times, totaling 120 experimental units.

Phytochemical analysis

Phytochemical analysis is a qualitative test to determine the presence of various active compound groups contained in *Cl. hirta* extracts. This analysis was conducted based on Harbone (1987) and Kancherla *et al*. (2019). The identification involved testing for flavonoids, saponins, tannins, quinones, coumarins, steroids, triterpenoids and alkaloids. The leaf and root extracts of *Cl. hirta* were analyzed to identify differences in their compound content. Regarding the Flavonoid, Saponin, Tannin and Quinone Test, a total of 10 mL of the extract was heated in a water bath, and the solution was divided into four tubes. The first tube received approximately 100 mg of magnesium powder, followed by 1 mL of concentrated hydrochloric acid and 3 mL of amyl alcohol. The solution was shaken vigorously and allowed to stand. The appearance of red, yellow, or orange colors in the amyl alcohol layer indicated the presence of flavonoids. The second tube was shaken vertically for 10 s and the formation of stable foam, which persisted for 10 min, indicated the presence of saponins. The third tube received several drops of 1 N sodium hydroxide, and the development of a red solution indicated the presence of quinones. The fourth tube received several drops of 1% iron (III) chloride solution, and the formation of a dark blue or greenish-black solution indicated the presence of tannins. Regarding the steroid and triterpenoid test, two mL of the extract were mixed with 18 mL of ether and macerated for 2 h. The mixture was then filtered, and the filtrate was collected. The filtrate was evaporated in an evaporating dish until residue was obtained. To the residue, Liebermann-Burchard reagent (2 drops of acetic anhydride and 1 drop of concentrated sulfuric acid) was added. The formation of a red color indicated the presence of triterpenoids, while the appearance of green indicated the presence of steroids. For alkaloid test, two mL of the extract were moistened with ammonia and then mixed with chloroform. Organic layer was transferred to a new reaction tube and treated with 10% hydrochloric acid. The acid layer was then transferred to a new reaction tube and a few drops of Dragendorff reagent were added. The formation of a brick-red precipitate indicated the presence of alkaloids. The Harbone's methods were verified using phyto-constituents evaluation based on Kancherla *et al*. (2019).

Statistical analysis

Statistical analysis was carried out with the R version 4.4.0 program with the Agricolae package. The analysis used in this research includes analysis of variance (ANOVA), Duncan Multiple Range Test (DMRT) and *t*-student test (Fendiyanto *et al.* 2019).

Results

The determination of the best medium for testing the vegetative growth of plants

The best planting media composition for testing vegetative growth on the four weeds is soil mixed with compost in a 4:1 ratio (Fig. 1). *E. crus-galli* and *Cy. iria* grew taller on soil: compost media compared to soil and soil: sand. Soil:sand media also showed significant differences with soil media in *E. crus-galli* and *Cy. iria*. However, *A. conyzoides* and *L. octovalvis* did not show significant differences in growth due to the media (Fig. 1). Because the composition of soil media mixed with compost showed significant differences in several plants, we used this composition for further experiments.

The preparation of *Cl. hirta* extracts

Cl. hirta was obtained from the Experimental Garden of IPB Dramaga in four trash bags, and then its roots, stems, and leaves were separated. Leaves and roots were extracted using a water-based solvent. A total of 1.775 g of leaves and 300 g of roots were obtained. From these materials, a leaf extract solution of 8 L and a root extract solution of 2 L were prepared. The ratio of the amount of leaves and roots that could be used for extraction was disproportionate, and the roots of this plant were relatively hard, making them difficult to grind. The presence of *Cl. hirta* in the IPB Dramaga campus area has also become scarce and relatively hard to find. Based on this comparison, it is concluded that root extraction is relatively challenging, so there is a need for an alternative to increase the amount of material for extraction.

Effect of *Cl. hirta* extract at various doses on the growth of *E. crus-galli*

Cl. hirta extract showed that *E. crus-galli* inhibited growth at concentrations of 10, 20 and 40%. The highest inhibition occurred at a concentration of 40%, while the lowest inhibition occurred in the control. The negative control is a control that uses distilled water without *Cl. hirta* extract. *E. crus-galli* roots showed significant growth inhibition ($P < 0.05$) after being treated with *Cl. hirta* extract when compared with controls (Fig. 2).

Preliminary test of the effect of various concentrations of *Cl. hirta* extracts on the morphology of crowns and roots of *E. crus-galli* in the germination phase

Cl. hirta extract at various concentrations given to *E. crus-galli* can inhibit germination, reduce crown growth, and damage the root system. The highest root and shoot damage to *E. crus-galli* occurred after administration of 40% extract. Morphological damage to *E. crus-galli* given 10 and 20% extract showed relatively similar damage, where the roots were stunted and the crown was damaged when compared to the control (Fig. 3).

Effect of various concentrations of *Cl. hirta* extracts on shoot and root morphology of *A. conyzoides, E. crus-galli, Cy. iria* and *L. octovalvis* in the germination and vegetative phases

In general, *Cl. hirta* extract can inhibit the growth of *A.*

conyzoides, E. crus-galli, Cy. iria, and *L. octovalvis* both in the germination and vegetative phases (Table 1). Leaf and root extracts from *Cl. hirta* inhibited the growth of *A. conyzoides* significantly ($P < 0.05$) at concentrations of 10, 20 and 40% in both the Germination phase and vegetative stage when compared with the negative control (C-, without extract). In *E. crus-galli*, the highest inhibition occurred in the root extract when compared to the leaf extract. The highest inhibition in *E. crus-galli* occurred at a concentration of 20% in both the germination and vegetative phases. In *Cy. iria*, in general inhibition occurred in both the germination phase and the vegetative phase at concentrations of 10, 20 and 40% in both root and leaf extracts. In *L. octovalvis*, effective inhibition occurred in leaf extract at concentrations of 20 and 40% of leaf extract (Table 1). Rice was used as a comparison for the application of *Cl. hirta* extract against weed growth. Rice (*Oryza sativa* cv. IR64) showed no growth inhibition, and specifically root and leaf extracts in the germination phase actually increased rice growth significantly ($P < 0.05$). However, in the vegetative phase, *Cl. hirta* extract also inhibited rice growth (Table 1).

Phytochemical investigation

Cl. hirta leaf extract shows the presence of Flavonoid, Alkaloid, Tannin, Quinone, Saponin and Triterpenoid compounds. In addition, *Cl. hirta* root extract only showed the presence of three compounds detected particularly Flavonoids, Quinone, and Triterpenoids (Table 2).

Discussion

Cl. hirta is a plant that is an invasive alien species (IAS) and some vegetation in rice fields and dry land on rice farms shows dominance (Tjitrosoedirdjo 2005; Steenis 2006). *Cl. hirta* is thought to contain certain chemical compounds that can inhibit the growth of other plants in an environment (Pejchar and Mooney 2009). *Cl. hirta* as an IAS is often considered detrimental to the environment, but its biological use is still relatively little researched (Lowe *et al.* 2000; Purwono *et al*. 2002). *Cl. hirta* extract is known to inhibit the growth of other organisms such as several *E. coli, S. aureus,* and *S. typii* (Pratami *et al.* 2021). Studies on the use of *Cl. hirta* as a bioherbicide on rice growth have never been carried out even though *Cl. hirta* growth tends to dominate on rice agricultural land and it can be seen that some weeds have lower dominance values (Ismaini 2015).

Cl. hirta extract made is an extract derived from the leaves and roots. The use of these two parts of the organ is to test which extract has the most effect as a natural bioherbicide on several weed species. Using plant parts as an extract source has been reported to increase the biological activity of the extract used particularly as bioherbicides against *Impatiens platypetala* (Dorning and Cipollini 2006). The bark of *Cl. hirta* will be used as additional material, mixed with the roots for extraction. This choice is based on the fact that the bark of some plants has the potential to yield bioactive compounds. Bark extracts from several plants, such as matoa, have the potential as antibacterials against *S. aureus*, while langsat bark has

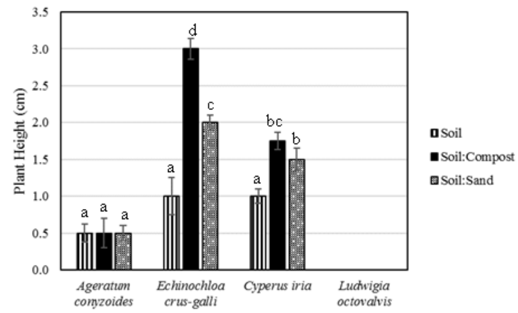

Fig. 1: The best media composition for the growth of the four weeds is a mixture of soil and compost in a 4:1 ratio. Different letters indicate significant differences in plant height based on the Duncan Multiple Range Test (DMRT) ($P < 0.05$)

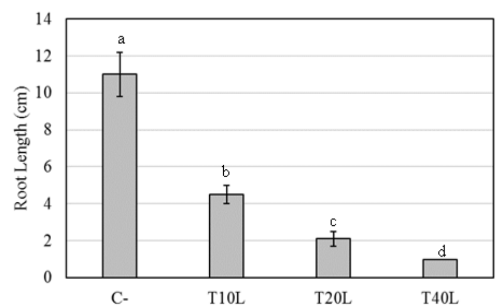

Fig. 2: An increase in the concentration of *Cl. hirta* leaf extract leads to an increase in the inhibition of *E. crus-galli* seedling root growth ($\alpha = 0.05$). C-: Control aquadest without *Cl. hirta* extract, 10 L: 10% of leaves extract, 20 L: 20% of leaves extract and 40 L: 40% of leaves extract. Different letters indicate significant differences in plant height based on the Duncan Multiple Range Test (DMRT) ($P < 0.05$)

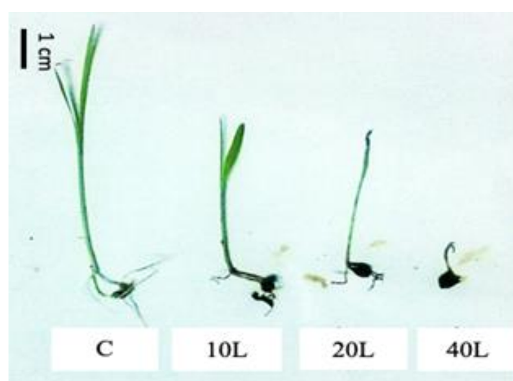

Fig. 3: The morphology of *E. crus-galli* seedlings after 12 days of treatment with *Cl. hirta* leaf extract shows inhibited growth that corresponds with an increase in concentration

Table 1: Effect of *Cl. hirta* extract from leaf and root on plant height of *A. conyzoides, E. crus-galli, Cy. iria, L. octovalvis* and *Oryza sativa* cv. IR64

Species	*C. hirta* Extract type	Treatment	Germination Stage (cm, Mean ± SD)	Vegetative Stage (cm, Mean ± SD)
Ageratum conyzoides	Leaf	C+	3.5 ± 0.1	0.0 ± 0.0
		C-	5.0 ± 0.1	7.0 ± 0.2
		T10	**2.0 ± 0.1**	**4.0 ± 0.1**
		T20	**1.8 ± 0.2**	**3.1 ± 0.1**
		T40	**1.8 ± 0.1**	**2.3 ± 0.1**
	Root	C±	3.5 ± 0.1	0.0 ± 0.0
		C-	5.0 ± 0.1	7.0 ± 0.2
		T10	**2.0 ± 0.2**	**3.5 ± 0.2**
		T20	**1.9 ± 0.1**	**3.0 ± 0.1**
		T40	**2.0 ± 0.0**	**3.1 ± 0.1**
Echinochloa crus-galli	Leaf	C±	27.0 ± 0.2	0.0 ± 0.0
		C-	14.0 ± 0.1	26.1 ± 0.2
		T10	20.0 ± 0.1	30.0 ± 0.1
		T20	19.2 ± 0.2	27.0 ± 0.3
		T40	24.0 ± 0.1	32.1 ± 0.1
	Root	C±	27.0 ± 0.2	0.0 ± 0.0
		C-	14.0 ± 0.1	26.1 ± 0.2
		T10	25.0 ± 0.2	36.1 ± 0.1
		T20	**6.5 ± 0.1**	**15.2 ± 0.1**
		T40	**11.5 ± 0.1**	**17.1 ± 0.1**
Cyperus iria	Leaf	C±	6.5 ± 0.2	0.0 ± 0.0
		C-	9.0 ± 0.2	19.9 ± 0.1
		T10	**7.1 ± 0.1**	**15.8 ± 0.1**
		T20	9.8 ± 0.2	**16.2 ± 0.2**
		T40	**5.2 ± 0.1**	**11.1 ± 0.1**
	Root	C±	27.0 ± 0.2	0.0 ± 0.0
		C-	14.0 ± 0.1	26.1 ± 0.2
		T10	**10.6 ± 0.3**	**21.5 ± 0.1**
		T20	**6.3 ± 0.2**	**13.2 ± 0.1**
		T40	**6.2 ± 0.1**	**14.1 ± 0.2**
Ludwigia octovalvis	Leaf	C±	2.4 ± 0.2	0.0 ± 0.0
		C-	1.5 ± 0.1	2.6 ± 0.1
		T10	1.5 ± 0.0	2.6 ± 0.1
		T20	**1.3 ± 0.2**	**2.0 ± 0.1**
		T40	**1.3 ± 0.0**	**1.4 ± 0.1**
	Root	C±	2.4 ± 0.2	0.0 ± 0.0
		C-	1.5 ± 0.1	2.6 ± 0.1
		T10	1.5 ± 0.1	2.6 ± 0.1
		T20	1.5 ± 0.1	**2.3 ± 0.0**
		T40	1.7 ± 0.0	2.6 ± 0.1
Oryza sativa cv. IR64	Leaf	C±	15.1 ± 0.2	0.0 ± 0.0
		C-	18.0 ± 0.2	32.0 ± 0.3
		T10	***20.0 ± 0.2***	**21.2 ± 0.1**
		T20	***23.0 ± 0.2***	**23.2 ± 0.1**
		T40	***28.0 ± 0.2***	30.0 ± 0.2
	Root	C±	15.1 ± 0.2	0.0 ± 0.0
		C-	18.0 ± 0.2	32.0 ± 0.3
		T10	***27.2 ± 0.2***	28.0 ± 0.2
		T20	***27.5 ± 0.1***	28.0 ± 0.1
		T40	***20.0 ± 0.0***	**20.1 ± 0.1**

Notes: **Bold numbers** indicate growth inhibition when compared with C- (control aquadest without extract) in the Student's T-test ($P < 0.05$). ***Numbers in italics and bold print*** indicate an increase in growth when compared with C- (control aquadest without extract) in the Student's T-test ($P < 0.05$). C+ is herbicides. T10, T20 and T40 are *Clidemia hirta* extract with concentrations 10, 20 and 40%, respectively.

Table 2: Phytochemical screening test of leaf and root extracts of *Cl. hirta*

Groups of compounds	Phytochemical Test	
	Leaves Extract	Root Extract
Flavonoid	+	+
Alkaloid	+	-
Tannin	+	-
Quinone	+	+
Saponin	+	-
Triterpenoid	+	+
Steroid	-	-

potential as an anticancer agent (Semuel 2008) and soursop bark has potential as an antioxidant (Suhandono *et al.* 2013). In this study, we used extract types derived from the leaves and roots of *Cl. hirta*.

Apart from making appropriate extracts, natural bioherbicide experiments will also be effective if growth observations are carried out using the right media. We conducted experiments using soil, compost and sand compositions to test what media were effective in growing weeds, especially *A. conyzoides, E. crus-galli, Cy. iria* and *L. octovalvis*. In other study, All four test weeds are wetland plants commonly found in rice fields. Soil: compost media (4:1) is believed to provide conditions suitable for paddy fields. This underpins the selection of soil: compost (4:1) as the medium for allelopathy testing on vegetative growth. The best composition obtained from this research was soil: compost (Fig. 1) so this media was used further in subsequent research.

Allelopathy is a biological process where one plant species releases chemicals into the environment that affect the growth, germination, or development of other nearby plants in their habitat (Scepanovic *et al.* 2007). These chemicals, known as allelochemicals, can have both positive and negative effects on neighboring plants. Allelopathy plays a role in plant competition, defense against herbivores and pathogens, and shaping plant community structure (Sastroutomo 1990). Some plants release allelochemicals from their roots, leaves, or seeds, while others may volatilize them into the air. Examples of allelopathic substances include phenolics, terpenoids, alkaloids, and cyanogenic compounds (Kancherla *et al*. 2019).

Allelopathy of *Cl. hirta* leaf extract was tested on *E. crus-galli* seeds, resulting in the inhibition of seedling root length. Increasing the extract concentration led to reduced root length growth (Fig. 2). When the overall morphology was observed, there was a noticeable difference in the physiological status among different extract treatment levels. The control plant appeared healthy while increasing the extract concentration caused the plants to wilt (Fig. 3). This change is believed to be related to the inhibition of root growth. Plants obtain water and nutrients for their metabolism and growth through their roots. If the roots are damaged, the plant's growth will be disrupted.

Allelopathy tests on the vegetative growth of the four weed species and IR64 rice have been conducted, and morphological characteristics were observed during the first to third weeks after the application of the extracts (Table 1). The application of *Cl. hirta* leaf extract can inhibit the height growth of the four weed species, but it does not significantly affect IR64 rice. The application of root extract at low concentrations does not show inhibition in the height growth of the test plants, but at higher concentrations, it can inhibit growth (Table 1).

Allelopathy activity can indeed be harnessed for bioherbicide purposes. Bioherbicides are herbicidal substances derived from natural sources such as plants, fungi, bacteria or other organisms (Zhang *et al.* 2021). Instead of synthetic chemicals, they utilize natural compounds to control weeds and unwanted vegetation (Rice 1984; Bangun and Wiroatmodjo 1986; Gurning and Fagi 1986).

Allelopathic plants produce compounds that inhibit the growth of neighboring plants (Yuan *et al.* 2007), and these compounds can potentially be isolated and formulated into bioherbicides (McCoy *et al.* 2022). By leveraging the allelopathic activity of certain plant species (Soerjani *et al.* 1987), bioherbicides can offer an environmentally friendly alternative to synthetic herbicides (Qu *et al.* 2021).

Phytochemical screening conducted on leaf and root extracts of *Cl. hirta* has revealed differences in the composition of compound groups between these two plant parts. Compound groups that were found exclusively in leaf extracts are alkaloids, tannins, and saponins (Table 2). This difference in chemical composition between leaf and root extracts may contribute to the varying allelopathic effects they have on the test plants (Ruckli *et al.* 2014; Zhu *et al.* 2016; Kancherla *et al*. 2019). However, both leaf and root extracts of *Cl. hirta* contain compound groups such as flavonoids, quinones, and triterpenoids. Phenolic compounds, alkaloids, fatty acids, steroids, and polyacetylenes can act as allelochemicals (Waller 1987; Widhalm and Rhodes 2016; Yu *et al.* 2019). The release of allelochemicals in nature is facilitated by various processes, including leaching from plant parts, root exudates, stem activities, microbial activity, and decomposition of dry matter residues (Inderjit 1996; Stinson *et al.* 2006; Yu *et al.* 2019).

Research on the allelopathic potential of *Cl. hirta* is expected to serve as a reference for the development of bioherbicides. The widespread use of bioherbicides is one of the efforts to promote sustainable and environmentally friendly agriculture. In addition to its use as an herbicide, the mass utilization of *Cl. hirta* can also help reduce the population of this plant species. This is one way to control the population of invasive alien species, which tend to have negative economic and ecological impacts.

Conclusion

In summary, both leaf and root extracts of *Cl. hirta* D.Don inhibit the growth of *A. conyzoides*, *E. crus-galli, Cy. iria*, and *L. octovalvis*. In contrast, rice growth is significantly stimulated by the extract. Therefore, this study is expected to serve as a reference for the development of bioherbicides for rice cv. IR64. However, further studies related to the metabolic profiling of *Cl. hirta* extract as bioherbicides are still needed.

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Author Contributions

MHF, MPP and IRK wrote the manuscript, designed the experiment, and conducted morphophysiological tests. RDS edited the manuscript and conducted a phytochemical analysis. All authors edited the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data Availability

We hereby declare that all data reported in this paper are available and will be produced on demand.

Ethics Approval

Not applicable

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