Allelochemical effects of Chromolaena odorata L. against photosynthetic pigments and stomata of Ageratum conyzoides l. leaves

by Endah Dwi Hastuti

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Allelochemical effects of *Chromolaena odorata* L. against photosynthetic pigments and stomata of *Ageratum conyzoides* l. leaves

E D Yuliyani[1], S Darmanti[2], and E D Hastuti[2]

[1]Student in Department Biology, Faculty of Science and Mathematics, Diponegoro University
[2]Department Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia
Email: darmantisri@yahoo.co.id

1. Introduction

Weeds are plants that grow wild on cultivated land that cause losses. Therefore it needs that they need to be controlled [17]. Siam weed (*Chromolaena odorata* L.) is a weed of woody shrubs with large habitus. As an invasive plant, it can grow in all places such as agricultural and plantation land, grasslands, roadside, riverbanks, home yards, and cultivated forest areas. Siam weed is very fast growing, and breeding forms a community so that it can hinder the growth of other plants through competition [24]. The existence of weeds in the area of cultivated plants can cause losses regarding both quantity and quality of products. It is due to competition in obtaining water, nutrients, light, CO_2 and living space between plants and weeds and allelopathy [19].

Allelopathy is a secondary metabolite produced by plants, algae, bacteria and fungi that can affect the growth and development of agricultural systems [15]. Allelopathy affects plants through the release of chemicals into the environment. These allelopathic chemicals are called allelochemical [10]. Allelochemical released into the environment through several ways, such as exudation or excretion by plant organs, volatility through the leaves in the form of gas via stomata, dissolution or leaching of

fresh leaves through rainwater or dew, dissolution of decomposed litter and transformation of microorganisms [11]. The siam weed has allelochemical compounds found in all plant organs such as leaves, stems and roots in the form of essential oils, alcohols, flavonoids, tannins, alkaloids, terpenes, kromen, chromon, benzofuran, coumarin, sterols, limonene, saponins and phenols which include protocatechuic -coumaric, ferulic, p-hydroxybenzoate and vanillic acid [9]. The flowers contain palmitic acid, linoleic acid and 2,6-dimethoxyphenol [1].

Allelochemical compounds can inhibit the growth of other plants through the inhibition of cell division [12]. Allelochemical mechanisms in inhibiting growth and development through complex processes. The inhibition process begins with the occurrence of chaos in the plasma membrane structure, modification of the membrane channel, or loss of the function of the ATPase enzyme. This process will affect the absorption of ions and water which then affects the opening of the stomatal and photosynthetic processes [16]. Allelochemical causes decreased root activity to absorb nutrients so that photosynthesis is disrupted. Leaf and root cells of the plant have a structure of cell membranes composed of phospholipid bilayers, proteins, and carbohydrates. Phenol will stick to the membrane lipids and cause fat solubility to decrease which results in damage to the cell membrane, thus affecting other organelles in the cell membrane, namely mitochondria, chloroplasts, and vacuoles. [5]. Damage to the membrane system causes the absorption of water and nutrients dissolved by epidermal cells and neighboring cells that lead into the closure cell to become inhibited, which will affect the opening and closing of the stomata. This results in a decrease in the number of stomata and inhibition of chloroplast activity in cells can affect the synthesis of chlorophyll and carotenoids.

The availability of high-yielding weeds in the wild and the large leafy plant habitus can be used as an alternative as herbicide material. It is supported by a lack of toxic allelochemical compounds found in all plant organs. An herbicide is a weed control by utilizing the content of allelochemical compounds produced by a plant, which can suppress the growth of other weeds. Bioherbicide is environmentally friendly because it does not contain harmful ingredients, does not leave residues or contaminate the soil, so it is safe for humans and animals [14].

One of the weeds that must be controlled by the population is *A. conyzoides* L. It is an annual weed that has high adaptability, so it can grow in all places and often grows on soybean cultivation. *A.conyzoides* weeds are categorized noxious weed (weeds are dangerous and very detrimental) and are difficult to control by herbicides or weeding [13]. This study aims to examine the allelochemical effects of different organs, that is leaf, stem, and root of *Chromolaena odorata* L. on different concentrations against photosynthetic pigments and the number of stomata *A.conyzoides* L. leaves

2. Method

The main materials used is *A.conyzoides* L. leaves and *C.odorata* L. obtained in Tembalang, Semarang Jawa Tengah, distilled water, transparent nail polish, filter paper, aluminum foil, and 80%acetone. The main tools used are UV-Vis spectrophotometer, optilab microscope, glass preparation, measuring cup, glass beaker, glass funnel, Erlenmeyer, test tube, test tube shelves, drop pipettes, mortar, and pestle, label paper, masking tape, and scissors.

2.1. Preparation of Siam Weed Extract

The obtained siam weed roots are washed clean. The leaves, stems, and roots were dried in dark conditions. Siam weed was mashed, then extracted with water at a ratio of 1: 1 weight/volume. The extract was filtered two times using a cloth and filter paper to obtain a concentration of 100%; the extract was diluted with water according to the concentration of the treatment, that was 10%, 20%, 30%, and 40%. Control was made using tap water without the addition of extract [2].

2.2. Treatment

The treatment starts after the plant is 21 days old. The extract of leaves stems and siam weed roots were carried out by spraying the extract on the entire surface of *A.conyzoides* L. leaves every two days in the morning, according to the treatment concentration of 0%, 10%, 20%, 30% and 40% with the same volume of 10 ml. The treatment was carried out for 28 days.

2.3. Photosynthetic Pigment Detection

A.conyzoides L. leaves are weighed as much as 0,1 g, then the pieces of leaves were crushed using mortar and pestle. 10 ml of 80% acetone was added. Until the chlorophyll was dissolved strain with filter paper. 3 ml of the solution was filled into the cuvette and analyzed using spectrophotometer with a wavelength of 645 nm and 663 nm [8]. The concentration of chlorophyll was calculated using the following equation:

$$\text{Chlorophyll b (mg/L)} = 22{,}9\ (A_{663}) - 4{,}68\ (A_{645})\ \text{mg/l}$$
$$\text{Chlorophyll a (mg/L)} = 12{,}7\ (A_{663}) - 2{,}69\ (A_{645})\ \text{mg/l}$$
$$\text{Total Chlorophyll (mg/L)} = 8{,}02\ (A_{663}) + 20{,}2\ (A_{645})\ \text{mg/l}$$
$$\text{Carotenoids} = \frac{\{(A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645}) \times V \times 10^3)\}}{112{,}5 \times 0{,}1 \times 10}$$

2.4. Number of Stomata

Stoma numbers of *A.conyzoides* L. leaves were calculated by printing leaf surfaces using nail polish, which applied on the bottom surface of the leaves. After drying the nail polish is removed and observed with a microscope. Even the stoma is calculated in the area of view 4,794 μm^2 [6].

2.5. Data Analysis

The study used a Completely Randomized Design (RAL) and factorial pattern with two factors (3X5). The first factor is the type of organ siam, i.e., leaf, stem, and root and the second factor is the concentration of the extract, i.e., 0%, 10%, 20%, 30%, and 40%. Each treatment, replicated five times. The parameters measured were the concentration of photosynthetic pigments and number of stomata. Results data were analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at the test level of 95%.

3. Results and Discussion

All treatments showed that siam weed extract with different concentrations reduced photosynthetic pigments compared to controls. Root extract was more influential in reducing chlorophyll b, total chlorophyll and carotenoids compared to leaf and stem extracts (Table 1).

Allelochemical extracts reduce the levels of photosynthetic pigments. It because allelochemicals interfere with the permeability of cell membranes, thereby inhibiting the absorption of water and dissolved nutrients. Allelochemical causes decreased root activity to absorb nutrients so that photosynthesis is disrupted. Root cells have a cell membrane structure composed of phospholipid bilayers, proteins, and carbohydrates. Phenol will stick to the membrane lipids and cause fat solubility to decrease which results in damage to the cell membrane, thus affecting other organelles in the cell membrane, namely mitochondria, chloroplasts, and vacuoles. The inhibition of chloroplast activity in cells can affect the synthesis of chlorophyll and carotenoids [5]. In accordance with Wardani's study, which stated that *A. conyzoides* leaf extract causes a decrease the levels photosynthetic pigments of soybean plants cv Grobogan) [20]. Another study conducted by Susilowati showed that the levels of chlorophyll and carotenoids of thorn spinach weeds were decreasing along with the addition of concentrations of old leaf extracts and leaf roots[18].

Table 1. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of *A. conyzoides* L. leaves due to the treatment of different *C. odorata* L. organs of with a different concentration.

Parameter	Plant Organ	Extract Concentration 0%	10%	20%	30%	40%	Average
Chlorophyll a (mg/l)	Leaf	7,11	6,28	5,39	4,87	3,82	5,49
	Stem	7,04	6,00	5,22	4,95	3,83	5,41
	Root	7,11	6,02	5,04	4,27	3,72	5,23

	Average	7,09[a]	6,10[b]	5,21[c]	4,70[d]	3,79[e]	(-)
Chlorophyll b (mg/l)	Leaf	12,36	10,46	9,71	8,82	7,24	9,74[b]
	Stem	12,44	11,12	10,32	9,35	7,98	10,24[a]
	Root	11,23	9,13	8,02	7,49	6,09	8,39[c]
	Average	12,01[a]	10,23[b]	9,36[c]	8,55[d]	7,14[e]	(-)
Total Chlorophyll (mg/l)	Leaf	9,87	8,69	7,80	7,16	6,87	8,08[a]
	Stem	9,63	8,86	8,31	7,96	6,95	8,34[a]
	Root	9,08	8,08	7,00	6,29	5,29	7,15[b]
	Average	9,52[a]	8,54[b]	7,70[c]	7,14[d]	6,37[e]	(-)
Carotenoids (mg/l)	Leaf	0,30[a]	0,25[b]	0,22[bc]	0,21[bc]	0,18[d]	0,23
	Stem	0,30[a]	0,27[ab]	0,25[b]	0,23[bc]	0,19[d]	0,25
	Root	0,30[a]	0,24[b]	0,22[bc]	0,20[c]	0,15[d]	0,22
	Average	0,30	0,25	0,23	0,21	0,17	(+)

- Numbers followed by different letters in the same column and row show significant differences with DMRT advanced tests at the 95% test level.

Allelochemical has many phytotoxic effects which cause the growth of target plants. The decrease in the activity of the H^+ ATPase enzyme in the cell membrane is the first disorder that causes non-specific anions and cations. It correlates with the inhibition of absorption of certain ions such as phosphates, nitrates, and magnesium [3]. Barriers to chlorophyll synthesis and trigger an increase in chlorophyll degradation [22] [23]. Inhibition of carotenoid synthesis [4] disrupts of electron transport in photosystem II (FS II). All of these disorders directly affect the decreased photosynthesis rate and target plant growth.

Table2. The number stomata of *A. conyzoides* L. leaves due to the treatment of different *C. odorata* L. organ with different concentrations

Parameter	Plant Organ	Extract Concentration 0%	10%	20%	30%	40%	Average
The number of stomata	Leaf	2,48[a]	2,05[b]	1,73[c]	1,47[d]	1,26[d]	1,79
	Stem	2,63[a]	1,94[b]	1,67[c]	1,45[d]	1,13[e]	1,76
	Root	2,32[a]	1,88[bc]	1,67[c]	1,49[d]	1,12[e]	1,69
	Average	2,47	1,95	1,68	1,47	1,16	(+)

- Numbers followed by different letters in the same column and row show significant differences with DMRT advanced tests at the 95% test level.

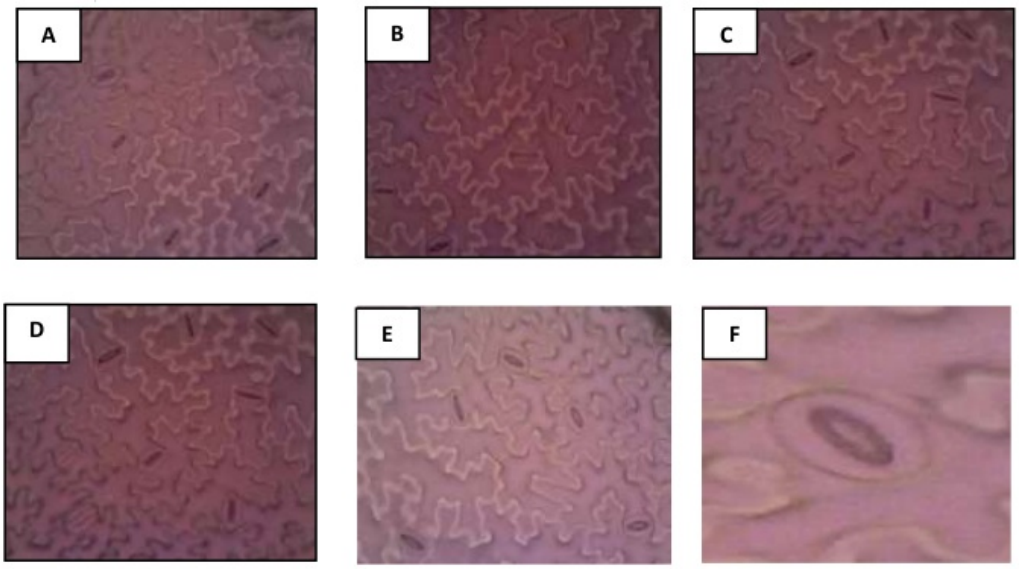

Figure1. Stomata of the lower surface of *A.conyzoides* L. leaves at concentration of (a) 0%, (b) 10%, (c) 20%, (d) 30% dan (e) 40% extrac of *C. odorata* (f) anatomy of *A. conyzoides* L. leaf stomata: 1. porous, 2. guard cell and 3. neighboring cells

The results showed that all treatment of turmeric extract with different concentrations reduced the number of stomata of *A.conyzoides* L. leaves compared with 0% (control) treatment. Weed siam root extract is more influential in reducing the number of stomata of *A.conyzoides* L. leaves compared to leaf and stem extracts. In accordance with Weston's research, which states that allelochemical compounds in the form of many flavonoids accumulate at the root tip. Roots usually produce many flavonoids which are stored as glycosides and will be released through root exudate and tissue decomposition [21]. The higher the Siam weed extract is given, the lower the number of stomata (Table 2).

Allelochemical affects the absorption of ion and water concentrations which then affect the opening of the stomata and photosynthesis process [16]. Allelochemical causes decreased leaf activity in absorbing water and dissolved nutrients so that photosynthesis is disrupted. Leaf cells have a cell membrane structure composed of phospholipid bilayers, proteins, and carbohydrates. Phenol will stick to membrane constituent lipids and cause fat solubility to decrease which results in damage to cell membranes, thus affecting other organelles within the cell membrane namely mitochondria, chloroplasts and vacuoles [5].

Cover cells take water through osmosis from surrounding cells, namely epidermal cells and neighboring cells, the closing cells will swell and become more turgid. Increased turgor pressure in the cover cell due to the surrounding water, the neighboring cells and epidermal cells entering the closing cell. The entry of water into the closing cell is due to the sugar concentration in the fluid of the closing cell increases and the plasma permeability of the water increases. It is caused by the activity of the amylase enzyme, phosphorylase and the increase in pH of the closing cell fluid. The photosynthesis process causes the levels of CO_2 in the chloroplast, the palisade tissue, and the parenchymal sponge to decrease; this is due to a portion of CO_2 being reduced to CH_2O. This reduction event causes H ions to decrease so that the pH of the environment becomes alkaline. The increase in osmosis of the contents of the cover cell causes the entry of water from neighboring cells, thereby increasing turgor and thin neighboring cell walls to expand as a result of stomata opening [6].

Damage to the membrane system in the leaves causes the absorption of water and nutrients dissolved by epidermal cells, and neighboring cells that lead into the closure cells become obstructed, which will affect the opening and closing of the stomata. It results in decreasing of stomata number and photosynthesis. Stomata begin to develop before meristematic activity in the epidermis is complete, and continue to develop for some time, as the leaves elongate and expand due to cell enlargement. With the influence of allelochemical, the cell enlargement process will be inhibited, and consequently, the resulting stomata will be small in number [7] (Figure 1). The function of stomata is influenced by many factors such as water status, K^+ ion concentration, and ABA signal. Allelochemical affects the opening of the stomata indirectly by modifying water status, hormonal balance, and ion absorption. The root is the first organ that is directly related to allelochemical. As a result, the absorption of water and ions will be disrupted, and the accumulation of ABA leaves will increase. Next, ABA will be channeled into guard cells, K^+ ions and water move out of the guard cells and result in stomatal closure. Stomata closure is directly related to its ability to inhibit the entry of K^+ ions into guard cells [12].

4. Conclusion

Extract of different organs of the Siam weed is leaves, stems and roots showed inhibition of photosynthetic pigments and stomata number of *A. conyzoides* L., but that root extract more inhibited. The higher concentration of the *C. odorata* L. weed extract given the greater inhibition of photosynthetic pigments and the number of stomata.

References

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