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Allelochemical effect of Ageratum conyzoides L. leaf extract on Soybean [Glycine max (L.) Merr. cv Grobogan] growth

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Abstract. Soybean [Glycine max (L.) Merr. cv Grobogan] is one of the most important commodities in Indonesia, but its production is often decreasing due to weed disturbance. One type of weed that has an allelopathic effect is A. conyzoides. Allelopathy is a phenomenon of plant releasing a chemical exudate into the environment that can suppress the growth of other plants. This study aims to determine the total phenolic content of A. conyzoides and examine the effect of the A. conyzoides allelochemical extract against the growth of soybean. The experiment was performed in completely randomized design (CRD) with one factor of concentration of allelochemical extract of A. conyzoides. Each treatment has five replications. Treatments with different concentrations, i.e., 0%, 5%, 10%, 15% and 20%. The parameters measured were the plant height, wet weight, dry weight, chlorophyll a, chlorophyll b and total chlorophyll were analyzed by Analys of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at the level of 95%. Total phenols contained in extract of A. conyzoides leaf is 16.121 µg GAE/g. The results showed that the higher the concentration of the allelochemical of A. conyzoides leaf extract reducing plant height, wet weight, dry weight, growth tolerance index (GTI), chlorophyll a, chlorophyll b and total chlorophyll.

Keywords: Allelophaty, Ageratum conyzoides, soybean seed

1. Introduction

Soybean [Glycine max (L.) Merr.] is one of the agriculture commodities that is the needed in Indonesia, because it can be consumed in various processed food products. According to Kilkoda [15], soy contains more than 40% protein and 10-15% fat, while soybean sprouts contain 94% water, 3% protein, and 0.1% fat. One type of local soybean that is widely cultivated in Indonesia is the Grobogan cultivars. According to Balitkabi [3], this cultivar has the advantage of short lifespan (76 days), big pod size, high productivity, and higher protein content that reaches 43.9%. These advantages make high demand for soybean production. According to BPS [4], soybean production in 2016 reached 963.18 thousand tons of dry beans, an increase of 8.19 thousand tons (0.86 percent) compared to 2015.

Increased public demand for soybeans is not balanced with soybean production in Indonesia. National soybean production has not been able to meet the needs of the community so that Indonesia must import 2.26 million tons of soybean by 2016 [4]. The low productivity of soybean in Indonesia caused by competition of resources with weeds as well as weed allelopathy competition [13]. Weeds are undesirable plants especially in the cultivated land because it can interfere with the growth and decrease the productivity of cultivated the plants [12]. One type of weed that often grows on the soybean cultivation field is babandotan (Ageratum conyzoides). Hafsah et al [10] state that the population of A. conyzoides is often more dominant than other weeds in a field, thus reducing the growth of cultivated plant. This weed has allelopathic capability that is a state in which plant releases...
chemical exudates into the environment that can suppress the growth of the other plants. Babandotan leaf capability as allelopathy was identified because of 3 phenolic acids, ie, gallic acid, coumaric acid and protocatechins acid which is a phenol compound and can inhibit the growth of some plants [18].

Allelochemicals can cause plants to experience oxidative stress. Oxidative stress is a condition of cell damage caused by Reactive Oxygen Species (ROS). According to Sharma et al. [21], ROS is an unpaired, unstable, highly reactive molecule and is often referred to as free radicals. ROS under normal circumstances plays an important role for biological function, regulation of cell growth, and as an intracellular signal to mediate some plant responses. However, when the ROS concentration exceeds normal, it can cause tissue damage and impaired biological function of the plant. According to Eberhardt [9], ROS causes cell damage in three ways: peroxidation of lipid components of cell membranes and cytosol. Causes a series of fatty acid reductions (otocatalysis) resulting in membrane and organelle cell damage; DNA damage that can lead to DNA mutations can even lead to cell death; Modified proteins are oxidized by the formation of cross-linking of proteins, through sulfhydryl mediators of some labile amino acids such as cysteine, methionine, lysine, and histidine.

2. Method
The experiment was performed in completely randomized design (CRD) with one factor of concentration of a allelochemical extract of A. conyzoides. Each treatment has five replications. Treatments with different concentrations, i.e., 0%, 5%, 10%, 15% and 20%. The parameters measured were the plant height, wet weight, dry weight, chlorophyll a, chlorophyll b and total chlorophyll were analyzed by Analysis of Variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT) at the level of 95%.

The main materials used were seed of [Glycine max (L.) Merr. cv. Grobogan] obtained from Balitkabi Malang, Ageratum conyzoides leaf obtained in Tembalang, Semarang, methanol, Folin-Ciocalteau, Na-Carbonate 7.5%, gallic acid and acetone 80 %.

The main tools used are mortar and pestel, centrifugation, UV-Vis spectrophotometer, oven and 10 x 20 cm sized polybag.

2.1. Selection of Soybean Seed
Selection of soybean seeds based on size uniformity. The soybean seed was selected and sterilized by dipping it into 1% hypochlorite solution for 5 minutes and rinsing with aquadest 3 times.

2.2. Total Phenol Determination
Total phenol content measurement was done by spectrophotometric method using Darmanti et al. [7], leaf samples of A. conyzoides of 0.5 g were mashed. The sample was homogenized with 2.5 mL of methanol, filtered with paper weight and final volume adjusted to 5 mL by adding methanol. Then 20 μL mixed extract solution, 1.58 mL of aquadest and 100 μL of Folin-Ciocalteau reagent were incubated for 8 min. 300 mL 7.5% sodium carbonate was added then the mixed solution was incubated at 30 °C for 30 min. The absorbance value was measured using a spectrophotometer at 769 nm. The total phenol content was calculated using the standard curve of gallic acid.

2.3. Allelochemical A. conyzoides Extract Preparation
Leaf extraction of Ageratum conyzoides using Darmanti et al. [7] leaf extract of A. conyzoides concentration 100% was made by A. conyzoides leaves was dried in dark conditions for 24 hours. The leaves were smoothed with blender, then extracted with aquadest by a ratio of 1: 1 weight/volume for 24 hours. The extract was filtered three times using filter paper, then diluted with aquadest to reach the extract concentration of 20%, 15%, 10% and 5%, then stored in dark and low temperatures. Controls were made by using aquades without the addition of extracts.
2.4. Treatment

Planting media made by mixing the soil and husk with a ratio of 3:1. Planting media put in 10 x 20 cm polybags to ¾ part. Each polybag is given ten soybean seeds cv. Grobogan. Ten soybean seeds are placed into polybags that have been filled with planting medium for each experiment. Leaf extract of *A. conyzoides* was added to each polybag of the same volume using a spray pipette. Controls were made with water without the addition of extracts. During the treatment of extracts or water was kept in excessive amounts. Provision of *A. conyzoides* leaf extract was performed daily with the same volume and time for each treatment until the 3-week-old plant.

2.5. Growth Observation

Observation of soybean plant growth was done at 3rd-week. Fresh weight, dry weight, plant height measured. The Germination Tolerance Index (GTI) was calculated, and the total content of chlorophyll a, chlorophyll b and total chlorophyll were determined. The parameter calculation is performed according to the formula used by Akinci dan Sermin [1] as follows:

\[
\text{GTI} = \frac{1}{N} \sum_{i=1}^{n} \frac{PC_{ri}}{PC_{ci}} \times 100\%
\]

- \(N\) : Number of morphological parameters used
- \(PC_{ri}\) : The value of each of the i-th treatment parameters
- \(PC_{ci}\) : The value of each of the i-th control parameters

2.6. Chlorophyll Content Determining

The measurement of chlorophyll content was done by using Kamble *et al* [12]. Leaves *A. conyzoides* are weighed as much as 0.1 g, then the pieces of leaves were destroyed using mortar and pestle. 10 ml of 80% acetone was added. Dissolve a few moments until the chlorophyll dissolves, then strain with filter paper. 3 ml of the solution was inserted into cuvette and analyzed using spectrophotometer with wavelength 645 and 663 nm. The concentration of chlorophyll was calculated using the following formula:

\[
\begin{align*}
\text{Chlorophyll a (mg/L)} &= 12.7 (A_{663}) - 2.69 (A_{645}) \text{ mg/l} \\
\text{Chlorophyll b (mg/L)} &= 22.9 A_{645} - 4.68 (A_{645}) \text{ mg/l} \\
\text{Total Chlorophyll (mg/L)} &= 8.02 A_{663} + 20.2 (A_{645}) \text{ mg/l}
\end{align*}
\]

2.7. Data Analysis

Data were analyzed using One Way-Analysis of Variance (ANOVA). If the results of the variance analysis show, any real effect tested further by using Duncan's Multi-Range Test (DMRT) of 5% level and data were analyzed using SPSS.

3. Results and Discussion

Total phenol analysis was performed to prove that *A. conyzoides* contained allelochemicall in the form of phenol. The results of total phenol *A. conyzoides* leaf extract with spectrophotometric method showed total phenol content of 16.121 μg GAE (Gallie Acid Equivalent)/g fresh weight. The ability of inhibition of phenol compounds on plant growth depends on the amount of concentration. Previous research has been conducted by Dores *et al.* [8] which revealed that the results of total phenol content of leaf extract of *A. conyzoides* using spectrophotometry method were 14.722 μg / g in fresh weight.
Table 1. Soy chlorophyll content \([\text{Glycine max} \ (\text{L.}) \ \text{Merr. cv. Grobogan}]\) with allelochemical treatment at the 54th hour

| Treatment | Parameter Means (mg/L) | chlorophyll a | chlorophyll b | Total chlorophyll |
|-----------|------------------------|---------------|---------------|------------------|
| P0        |                        | 7.0246\text{a} | 2.9072\text{a} | 9.9293\text{a}   |
| P1        | 5.9352\text{ab}       | 2.9855\text{a} | 8.9184\text{b} |
| P2        | 5.5867\text{b}        | 2.4769\text{a} | 8.0616\text{b}c|
| P3        | 5.5210\text{b}        | 2.5838\text{a} | 8.1027\text{b}c|
| P4        | 5.2421\text{b}        | 1.9872\text{b} | 7.2275\text{b}c|

\[P0 = \text{control, P1 = A. conyzoides 5\% leaf extract treatment, P2 = A. conyzoides 10\% leaf extract treatment, P3 = A. conyzoides 15\% leaf extract treatment, P4 = A. conyzoides 20\% leaf extract treatment. Figures on the same parameters followed by different letters show significant differences with the DMRT advanced test at sig. 5\%}\]

Table 2. Fresh weight, dry weight, and height of soybean \([\text{Glycine max} \ (\text{L.}) \ \text{Merr. cv. Grobogan}]\) with allelochemical treatment at the 54th hour

| Treatment | Parameter Means |
|-----------|-----------------|
|           | FW  | DK  | Height |
| P0        | 1.98\text{a}  | 0.23\text{a} | 32.22\text{a} |
| P1        | 1.58\text{b}  | 0.18\text{b} | 30.20\text{b} |
| P2        | 1.55\text{bc} | 0.15\text{c} | 24.67\text{b} |
| P3        | 1.53\text{bc} | 0.15\text{c} | 24.55\text{b} |
| P4        | 1.28\text{c}  | 0.13\text{d} | 22.57\text{b} |

\[P0 = \text{control, P1 = A. conyzoides 5\% leaf extract treatment, P2 = A. conyzoides 10\% leaf extract treatment, P3 = A. conyzoides 15\% leaf extract treatment, P4 = A. conyzoides 20\% leaf extract treatment, FW = fresh weight and DW = dry weight. Figures on the same parameters followed by different letters show significant differences with the DMRT advanced test at 5\%}\]

Figure 1. The growth of soybean cv. Grobogan with allelochemical treatment from \textit{A. conyzoides} extract for 54 hours
4. Discussion

The presence of phenolic allelochemical stress in *A. conyzoides* results in the formation of ROS (Reactive Oxygen Species) in excessive amounts of soybean crops. Increasing the formation of ROS in excessive amounts causes soybean to experience oxidative stress characterized by the presence of obstacles to germination and plant growth. Li *et al.* [10] the mechanism of inhibition of plant growth by phenol by altering the permeability of the membrane thus inhibiting the absorption of plant nutrients, influencing the synthesis of endogenous plant hormones, affecting the various functions and activities of enzymes, affecting the process of photosynthesis and protein synthesis, and inhibit plant cell division and elongation.

According to Eberhardt [9], ROS causes cell damage in three ways: peroxidation of the lipid component of the cell membrane and cytosol. Causes a series of fatty acid reductions (otocatalysis) resulting in membrane and organelle cell damage. DNA damage that can lead to DNA mutations can even cause cell death. Modified proteins are oxidized by the formation of cross-linking proteins, through sulfhydryl mediators over some labile amino acids such as cysteine, methionine, lysine, and histidine.

Khang [14] stated that chlorophyll synthesis is influenced by various factors such as light, sugar or carbohydrate, water, temperature, genetic factors, and nutrients (N, Mg, Fe, Mn, Cu, Zn, S, and O). The disturbance of nutrient and water uptake causes the decrease of chlorophyll content of soybean plants, especially the nitrogen element which will be converted by glutamine acid synthetase enzyme into glutamic acid. Glutamic acid is important for chlorophyll biosynthesis. In line with the statement of Cheng & Zhihui [5] 250mM ferulic acid inhibits the absorption of ammonium (NO3) in corn seeds.

The low content of soybean chlorophyll result from phenol then affects the ability of photosynthesis. The declining photosynthesis capability will be followed by a decrease in the rate of growth. Accordings to Elisante et al. (2013) chlorophyll is a complex molecule that serves as the absorption of light, the transfer of energy and electrons in the process of photosynthesis. Leaf chlorophyll content is used as one of the elemental parameters in understanding the response of plants to environmental stresses caused by allelochemistry. The results showed that all treatment of allelochemical extract of *A. conyzoides* decreased the content of chlorophyll a, chlorophyll b and total chlorophyll of soybean crops when compared with controls indicating that soybean plants were stressful. The result is in line with the research of *Khang (2016)* who stated allelochemistry in the form of phenolic acid can affect the activity of photosynthesis and respiration plants. Administration of 10 - 30 μM of caffeine, cumarin, ferulat, cinnamate, and vanillic acid can reduce the content of chlorophyll and soy photosynthesis products (*Glycine max*).

The decrease in chlorophyll due to phenolic allelochemistry is also expressed by Li *et al.* [16] the impact of phenolic allelochemistry on the most important photosynthesis is to reduce chlorophyll

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**Table 3. Mean of fresh weight tolerance index, dry weight, and height of soybean plant [*Glycine max* (L.) Merr. cv. Grobogan] with allelochemical treatment for 54 hours**

| Treatment | Average Parameter | FW  | DW  | Height |
|-----------|-------------------|-----|-----|--------|
| P0        |                   |     |     |        |
| P1        |                   | 0.27| 0.26^a| 0.31^a |
| P2        |                   | 0.26| 0.22^b| 0.26^b |
| P3        |                   | 0.26| 0.21^b| 0.13^c |
| P4        |                   | 0.22| 0.18^c| 0.14^c |

P0 = control, P1 = *A. conyzoides* 5% leaf extract treatment, P2 = *A. conyzoides* 10% leaf extract treatment, P3 = *A. conyzoides* 15% leaf extract treatment, P4 = *A. conyzoides* 20% leaf extract treatment. **FW** = fresh weight and **DW** = dry weight. Figures on the same parameters followed by different letters show significant differences with the DMRT advanced test at 5%
content. According to Macias et al. [17], the process of photosynthesis of plants is directly proportional to the amount of chlorophyll present in leaf tissue that plays an important role in photochemical reactions. Normal plants can grow well and have a greater amount of chlorophyll than plants that experience stress conditions. So based on research results, decrease in soy chlorophyll indicates that soybean plants experience stress conditions caused by the presence of allelochemistry.

Allelochemical compounds in *A. conyzoides* leaf extract were able to give a significant effect in reducing fresh weight and dry weight of soybean crops with increasing concentrations of 5%, 10%, 15%, and 20% (Table 2). The decrease in both parameters indicates that the growth process is inhibited both directly such as inhibition of IAA synthesis, gibberellin, and disruption of cell membranes and indirect obstacles through photosynthesis. According to Alfandi & Dukat [2], fresh weight is the total content of water and photosynthesis results in the plant body. Barriers to water absorption and photosynthesis process cause the total water content and photosynthesis results to decrease in plants. These indirect barriers will also affect the decrease of dry weight. The higher concentration of allelochemical *A. conyzoides* leaves extracts given decreased mean fresh weight and dried soybean crop. This trend is by the opinion of Yulifrianti et al. [22] who stated that water absorption barrier by phenol compounds was causing the lack of water content, in which resulting in stomata closure, so the process of photosynthesis is inhibited and will affect in inhibiting the growth of target plants. In line with research in Hafsah et al. [10] that the extract of *Ageratum conyzoides* decreases the fresh and dried weight of mustard plants.

Allelochemistry affects photosynthesis and plant growth by lowering chlorophyll content. Decrease in plant capacity to accumulate chlorophyll which is an important component in the process of photosynthesis can affect the decrease of fresh weight and dry weight of plants. This is in accordance with the statement of Yulifrianti et al. [22] allelochemical compounds capable of inhibiting the process of photosynthesis so that plant growth becomes inhibited and the dry weight of the plant becomes reduced. In line with research Elisante et al. (2013) giving *Datura stramonium* extract gave a significant effect on the decrease of fresh weight and dry weight of legume *Wightii neonotonia*.

The decrease of soybean plant height is caused by the disruption of growth hormone activation. One of the hormones that play an important role in elongating the stem is auxin and cytokines. The existence of stress in the form of allelochemistry can disrupt cell division and enlargement so that high growth of soybean plants become obstructed. In line with research by Kilkoda [15], the presence of weeds *A. conyzoides* and *Borreria alata* can inhibit the growth of soybean crops from 3 different varieties (Grobogan, Gepak, and Gema).

The results (Table 2) showed that all treatments inhibited the height of soybean crops compared with controls. This is in accordance with the opinion of Isda et al. [11] that the presence of allelochemical compounds in the form of phenol will inhibit cytokinin activity. This barrier causes cell division in the shoot meristem part is disrupted so that it can inhibit high growth in plants.

GTI (Growth Tolerance Index) according to Akinci & Sermin [1] is an index showing the tolerance ability of a plant to environmental stress. The growth tolerance index is set by 1 divided by the number of morphological parameters used. Giving allelochemical extract of *A. conyzoides* affected decreasing growth tolerance index (dry weight and plant height) but did not affect growth tolerance index (fresh weight). The higher the concentration of allelochemical leaf extract of *A. conyzoides* the lower the average germination tolerance index produced (Table 3). GTI is based on three values of morphological parameters, i.e. fresh weight, dry weight and soybean plant height. This is in accordance with the opinion of Darmanti et al [7] GTI (Growth Tolerance Index) shows the effect of stress factors on plant growth and development. The higher allelochemical concentration of *A. conyzoides* leaf extracts given the lower the value of GTI obtained, in proportion to the decreased mean fresh weight, dry weight and soybean plant height.

5. Conclusion
Ageratum conyzoides is a soybean weed containing total phenol in leaves as much as 16.121 μg GAE / g fresh weight with spectrophotometric method. Allelochemical extract of Ageratum conyzoides significantly decreased fresh weight, dry weight, plant height, chlorophyll a, chlorophyll b, total chlorophyll, and growth tolerance index (dry weight, and height) but did not affect the tolerance index of fresh weight of soybean cv. Grobogan. Expected in the future can be further research based on other factors, different variables, different plant species, different weed species and the number of samples more.

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