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STUDY OF SAMBILOTO *Andrographis paniculate* Ness. EXTRACT AS AN ECO-FRIENDLY BIOCONTROL ON WEED SEED GERMINATION AND GROWTH

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Abstract. *The use of synthetic herbicides causes many problems, including pollution, a decrease in soil organic content, and weed resistance to specific herbicides. Plants provide a source of novel phytotoxic compounds that can be evaluated in the search for efficient and ecologically safe herbicides. This research aimed to examine the allelochemical bioherbicidal activity of sambiloto (Andrographis paniculate* Ness*) leaf extract (SLE) to suppress weed seed germination and growth. The experiment was designed to investigate the effect of the SLE as a potent weed seed germination inhibitor and retard weed growth. In this experiment, a non-factorial randomized block design was used, with treatments* SLE_0 *(0% Extract),* SLE_1 *(Extract 0.1%),* SLE_2 *(Extract 0.5%),* SLE_3 *(Extract 1.0%), and* SLE_4 *(Extract 1.5%). The result showed that weed height and biomass were significantly reduced after the extract was applied. The plant-based extracts contained 0.1114% flavonoids and 0.1628% tannins. The findings suggest that this sambiloto herbaceous extract has a promising future in modern plant protection as bioherbicide to suppress germination and weed growth. Therefore, the application of these bio-products could be beneficial for sustainable agricultural practices.*
Keywords: *bioherbicide; sambiloto; weed extract*

1. Introduction

Plant-based extracts growth inhibitory activity has dramatically increased for potential weed management. Recently, the idea is to utilize substances derived from plants to address the issues brought on by synthetic herbicides. The use of synthetic herbicides causes many issues, namely the cost of providing expensive herbicides, environmental pollution, decreasing soil organic content, and weeds that develop resistance to specific herbicides (Sarjono & Zaman, 2017; Hendarto, 2017) and human health concerns (Dayan *et al*., 2009; Ishak *et al*., 2021; Isda *et al*., 2013; Singh *et al*., 2003). In addition, when weeds are not managed, the yield of crops can decrease by 20-80% in general (Radhakrishnan *et al*., 2018; Syahputra *et al*., 2011). Therefore, studies on plant-based herbicides (bioherbicides) are critical because there is a need to search for environmentally safer substances with weed-suppressing ability. The plant-based extracts are less toxic, safe for humans and easily decomposed in the soil, so they do not cause residue (Godlewska *et al*., 2021). According to (Riskitavani & Purwani, 2013), using bioherbicides does not directly affect cultivated plants and has a slight chance of causing environmental pollution.

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The term allelopathy is classified as a biochemical interaction that involves both suppression and activation or one of them, via the production of substances from one plant, including microbes (Rai *et al*., 2021; Harahap & Rahmat, 2015) on another plant (Rashid *et al*., 2010). Furthermore, the allelopathic action of several plant species was evaluated, and it was discovered that some families suppressed the root growth of lettuce by more than 80% (Ishak *et al*., 2021; Qasem & Foy, 2001). Metabolites produced by some traditional medicinal herbs may potentially be used as bioherbicides. Sambiloto (*Andrographis paniculata* Ness) is a medicinal plant in Indonesia under various names. Since ancient times, Javanese have used this multi-branched bush as an efficacious snake poison treatment. Sambiloto belongs to the Acanthaceae family that has been used for centuries to cure digestive and respiratory diseases, fever, herpes, throat infection, and various other chronic and infectious illnesses, such as well as malaria. Its active phytochemicals have been extensively researched for their antioxidant, anti-microbial, and anti-fungal properties (Rofida, 2020; Zein *et al*., 2013).

A. paniculata leaves have a distinct odor, and the plants are thought to contain essential compounds. This leaf has been widely reported to be an effective natural biocontrol (Prakoso *et al*., 2018). Flavonoids, saponin, alkaloids, and tannin are found in *A. paniculata* (Dwivedi *et al*., 2021; Hu *et al*., 2018; Utami, 2016; Ratnani *et al*., 2012). Sambiloto extract contains flavonoids, saponins, alkaloids, and tannins that can be applied as bioherbicides. These compounds may interfere with biochemical and physiological reactions during seed germination. Early in the germination process, the mechanisms of degradation of storage material accumulated in seeds usually proceed extremely quickly, resulting in lipid degradation (Rys *et al*., 2022). Sambiloto have many advantages and the potential to become a large-scale bioherbicide. Plant extracts have recently received much attention as a practical way to improve particular crop production sustainability and, specifically, to produce bioherbicides. In the current study in Indonesia, only certain plant species were found to have allelopathic potential in controlling weeds, and only limited studies on sambiloto potential as bioherbicides were conducted. As a result, more research will be needed to understand and confirm whether sambiloto can be used to treat weeds growth. Following that, the goal of this work is to identify the allelopathic substances in *A. paniculata* (Sambiloto) extract and investigate their capacity to inhibit weed seed germination and growth.

2. Methods

2.1. Experimental design

To test the effect of SLE on weed growth, a field study was performed. This study was arranged in Randomized Block Design (RBD) with five treatments. The factors used were Sambiloto leaf extract (SLE) consisting of SLE_0 (Without Sambiloto leaf extract (control), SLE_1:

Sambiloto leaf extract concentration 0.1%, SLE_2: Sambiloto leaf extract concentration 0.5%, SLE_3: Sambiloto leaf extract concentration 1.0%, SLE_4: Concentration of Sambiloto leaf extract 1.5%. Each treatment has three replications.

2.2. Extract preparation, application, and weed growth observation

The preparation of the SLE was conducted using 1 kg leaf powder, collected from Nabila herbs producer in Situbondo, East Java, which was then macerated with 10 liters of methanol for 6 hours with manual stirring using a stir bar. The solution was stored for 1x24 hours and then filtered. The macerate from the filtering obtained was evaporated using a vacuum rotary evaporator (Buchi Rotavapor R-100, Switzerland) until all the methanol had evaporated so that a thick extract was obtained. The flavonoid and tannin content of the sambiloto leaf extract was determined at the Institut Pertanian Stiper Laboratory in Yogyakarta.

The leaf extracts were made in five concentration levels, namely 0; 0.1; 0.5; 1.0; 1.5 g/ml. In environmentally friendly agriculture, using plants with high allelopathic potential as bioherbicide in the form of aqueous extracts is gaining popularity. Therefore, each extract solution was made by mixing and dissolving each with 1 ml of 10% dimethyl sulfoxide solvent and adding distilled water. Then, the solution was stirred until homogeneous. The application of the extract was carried out once at the beginning of the application.

The germination parameters observed included weed growth and identification of weed species. The determination of weeds was done by counting the number of weeds growing on each experimental field every week, then averaged at the end of the observation, and then analyzed using a variance test at the 5% level. Weed identification was made by observing the weed species that grow in each observation area, then taking samples of four dominant weeds that increase in size to compare, and then counting the number of weeds that grow in each observation area. Weed identification was conducted using the polytechnic library's internal weed collection guide.

The growth parameters included weed height (cm), and the number of leaves (strands) was observed every week by counting the number of fully opened weeds. The wet weight was measured at the end of the observation using an analytical balance; furthermore, the dry weight was measured after the samples dried in the oven at 80˚C for 24 hours.

2.3. Data Analysis

The observations' results were analyzed using the Analysis of Variance (ANOVA). If the analysis of variance results revealed a significant effect, it was further tested using the Least Significant Difference (LSD) at the 5% level.

3. Results and Discussion

3.1. Weed viability

The treatment of sambiloto leaf extract did not significantly affect the growth of weeds at 1 – 4 weeks after application (WAA). The effect of sambiloto leaf extract on weeds growth can be seen in Figure 1. The treatment showed that the extract's concentration was still unable to inhibit the germination of weed seeds in the experimental field. However, if we look at the growing weeds of 1-4 WAA, the sambiloto leaf extract 1.5% tends to inhibit the germination of weed seeds in the experimental field when compared to other treatments. Allelopathy can reduce seed germination and slow down germination time because allelochemical compounds cause inhibition of the enzyme's activity that degrade food reserves in seeds so that the growth energy produced is deficient and over a long time will reduce germination potential. In addition, soil preparation was also carried out before application, which decreased weed seed deposits in the soil. The field preparation issue is in line with the statement (Amin *et al*., 2016) that tillage carried out before weeds produce seeds can significantly reduce the number of total seed deposits in the soil.

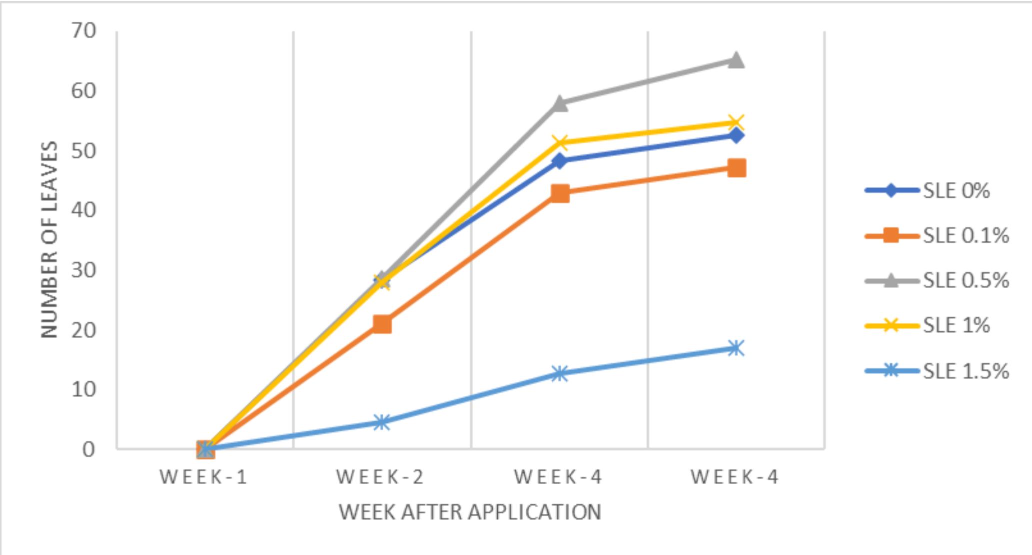

Figure 1. The effect of sambiloto leaf extract on weed growth

The application of extracts with concentrations of 0.1%, 0.5%, 1.0%, and 1.5% did not show a significant effect because the weed growth rate increased every week and the control treatment. This is owing to the somewhat varied concentrations of each extract, as well as the extract's low concentration. Low concentration extracts may take longer to have an effect. According to Shakkira *et al.* (2022), *A. paniculata* has an allelophatic action at concentrations of 25-30%. However, the 1.5% extract treatment was more able to inhibit the growth of weeds compared to the control treatment. Based on laboratory examination, the sambiloto leaf extract used in these experiments includes allelopathic chemicals in the form of flavonoids (0.1114%) and tannins (0.1628%), which potentially prevent weed development in the experimental field. Moreover, (Dwivedi *et al.*, 2021) profiled the secondary metabolite of sambiloto and confirmed that phenolics, flavonoids, and their derivatives were present in its methanol extract. According to (Yuliani *et al.*, 2009), allelochemicals produced by plants can have a destructive, inhibiting, and detrimental effect on plants in the surrounding environment.

The 1.5% bioherbicide extract treatment showed better results than the other treatments. At 4 MSA, 1.5% extract treatment gave the smallest weed growth inhibitory value of 17.00, and 0.5% extract treatment gave the most immense weed growth inhibitory value of 65.33. The concentration of sambiloto leaf extract 1.5% showed the best concentration that has the potential to control pre-growing bioherbicide.

3.2. Weed identification

The weed identification results showed several types of weeds at the study site, as shown in Figure 2. The dominant weed was *Elephantopus carolinianus*, with the highest population in the 0.5% extract treatment and the lowest in the 1.5% extract treatment. The lowest weed population was *Oplismenus hirtellus*, found in the 1.0% extract treatment. Weed species and populations in the experimental field can be seen in Figure 2.

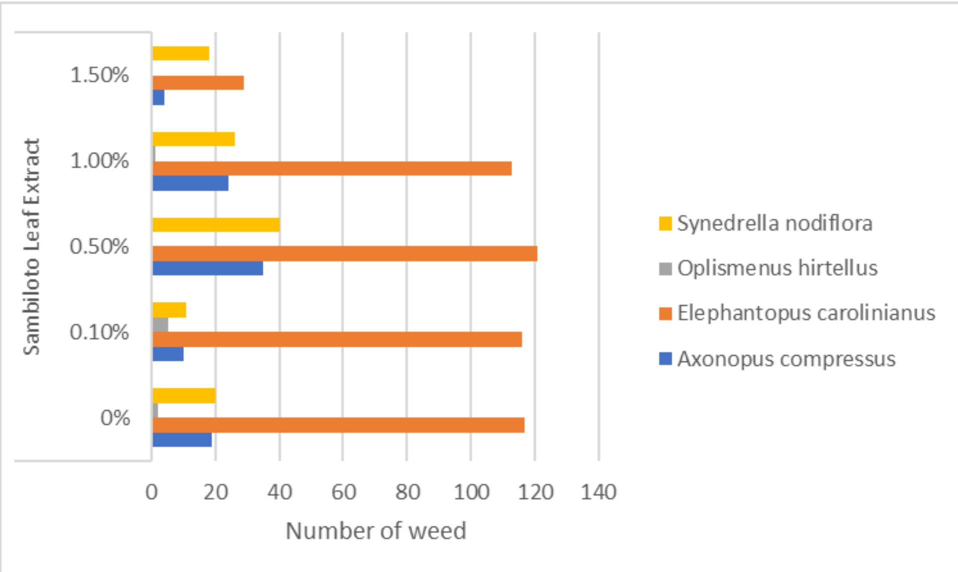

Figure 2. Types and number of weeds identified after application of sambiloto leaf extract (SLE)

The low weed population in each treatment was potentially caused by the influence of allelochemical compounds, flavonoids, and tannins in the sambiloto leaf extract. The same trend was detected in control, although for particular types of weeds, such as *E. carolinianus* and *O. hirtellus*, the SLE application had a greater affect. According to (Prokop & Veverka, 2003), spraying herbicide effectiveness is determined by several factors, including an ability of the leaves to intercept spray droplets, drying up of the droplet in air or on the surface of the leaves, leaking droplets through leaves, and being penetrated of the droplets into the plant's canopy. It can be challenging to identify which of these characteristics is the most relevant, and further investigation was required to determine whether aspect is slightly or substantially important, particularly for the field experiment. Besides that, the difference in the number of weeds in each treatment indicated the presence of allelochemical compounds inhibiting certain weeds' germination and growth. Broadleaf weeds such as *Elephantopus carolinianus* became the dominant weed in each treatment, and weeds such as *Oplismenus hirtellus* became the weeds with the lowest population. These results indicate that the extract of sambiloto leaf extract is more effective in controlling grass weeds than broad leaf weeds. The grass weed *Axonopus compressus* had a smaller population than the broadleaf weed *Synedrella nodiflora*. Cogon grass (*Imperata cylindrica*) extracts contained flavonoids and tannins. The extract inhibited plant growth by inhibiting the function of gibberellin, thereby inhibiting plant growth. Furthermore, it was more effective in controlling weeds from the carpet grass (*Axonopus compressus*) and bermudagrass (*Cynodon dactilon*) groups than broadleaf (*Boreria alata*) (Erida *et al*., 2019; Paiman *et al*., 2022). Based on the report, it can be concluded that the allelochemical compounds contained in the cogon grass extract have not been able to suppress the growth of broadleaf weeds.

On the other hand, the allelochemical compounds from the sambiloto leaf extract were more effective in suppressing the growth of weeds from the grass group. The 0.5% extract treatment became the treatment with the highest total weed population of 196 weeds. The 1.5% extract treatment became the treatment with the lowest total weed population of 51, compared to the control treatment with a total weed population of 158. The concentration of the sambiloto leaf extract, 1.5%, is the best in inhibiting the growth of weeds from the grass group and has the potential to become a pre-emergent bioherbicide.

3.3. Weed height

The application of sambiloto leaf extract significantly affected weed height at 2 to 4 weeks after application (WAA). The highest plant height at 4 WAA was found in the 1.0% extract treatment and significantly different from the 1.5% extract treatment. The effect of extract treatment on weed height growth can be seen in Table 1. The highest weed height at 4 WAA was found in Extract 1.0% treatment of 6.23 cm. The increase in growth is because the experimental land was located on an edge shaded from direct sunlight, so groundwater availability is higher than in other treatments. Sunlight affects the evaporation of groundwater (evapotranspiration). As stated by (Runtunuwu *et al*., 2008), the evapotranspiration rate is influenced by several factors, such as sunlight, air temperature, humidity, wind, and water quality. The availability of more water than other treatment areas made weeds that grow in the 1.0% extract treatment area have better resistance to allelochemical compounds of sambiloto leaf extract.

The lowest weed height at 4 WAA was found in the 1.5% extract treatment of 3.00 cm, and this was not statistically different from the control treatment. The growth weed's inhibition is potential because the sambiloto leaf extract contains allelochemical compounds. The chemical content of sambiloto, include andrographolide, neo-andrographolide, paniculin, minerals (potassium, calcium, sodium), flavonoids, gritty acid, and tannins (Dwivedi *et al.*, 2021). The flavonoid and tannin compounds play a role in inhibiting the growth of weed height.

Table 1. Effect of extract treatment on weed height growth

Treatment	Week after application (cm)			
	1	2	3	4
SLE 0%	0.00	1.33 ± 0.09 b	2.50 ± 0.11 b	3.83 ± 0.11 bc
SLE 0.1%	0.00	1.83 ± 0.03 b	2.57 ± 0.07 b	4.20 ± 0.14 bc
SLE 0.5%	0.00	1.27 ± 0.13 b	3.37 ± 0.06 b	4.70 ± 0.07 bc
SLE 1.0%	0.00	4.07 ± 0.71 a	5.43 ± 0.57 a	6.23 ± 0.71 a
SLE 1.5%	0.00	0.67 ± 0.06 b	1.70 ± 0.04 b	3.00 ± 0.07 c

Note: the numbers followed by the same letter show no significant difference according to the 5% LSD Test.

Furthermore, (Mirza *et al.*, 2020) described that tannin could inhibit growth, eliminate respiratory control in mitochondria and interfere with Ca^{+2} and PO_4^{3-} ion transport. Tannin compounds could also inactivate the amylase, proteinase, lipase, and urease. Moreover, it can further inhibit the hormone gibberellins. Other compounds in the form of flavonoids have a role in the growth process, which acts as a potent inhibitor of IAA-oxidase. This inhibition includes a series of complex processes that go through several metabolic activities, which include growth regulation through disturbances in growth regulators, nutrient uptake, photosynthesis, respiration, stomata opening, protein synthesis, carbon accumulation, and pigment synthesis (Astuti *et al.*, 2017). An extract concentration of 1.5% was the best concentration in slowing weed height growth. With this, it can be concluded that the higher the concentration of the extract of bitter leaf bioherbicide, the greater its ability to inhibit the growth of weed height.

3.4. Number of weed leaves

The treatment of sambiloto leaf extract on the number of weed leaves 2-4 weeks after application (WAA) was not significant. The impact of giving sambiloto leaf extract on the number of weed leaves can be seen in Figure 3. Application of the sambiloto leaf extract did not significantly reduce the number of weed leaves. However, at 4 WAA, the 1.5% extract treatment reduced the number of weed leaves by 4.00 leaves compared to the control treatment, which was 5.33 leaves. The allelochemical compounds in sambiloto leaf extract could reduce the number of weed leaves that can suppress cell division in weeds. Allelochemical compounds also inhibit the process of photosynthesis (the entry of CO_2 into the stomata). Leaf extracts could cause damage to the weed tissue, resulting in slow leaf formation, and leaves that are already formed will fall off due to necrosis and chlorosis (Baroroh, 2018). Allelochemical compounds also interfere with the activity of the gibberellin hormone, which plays an essential role in the process of cell elongation in weed stems. Weed stem elongation is related to leaf formation; if stem elongation is disturbed, the leaf formation process will also be disrupted (Saleem *et al.*, 2013).

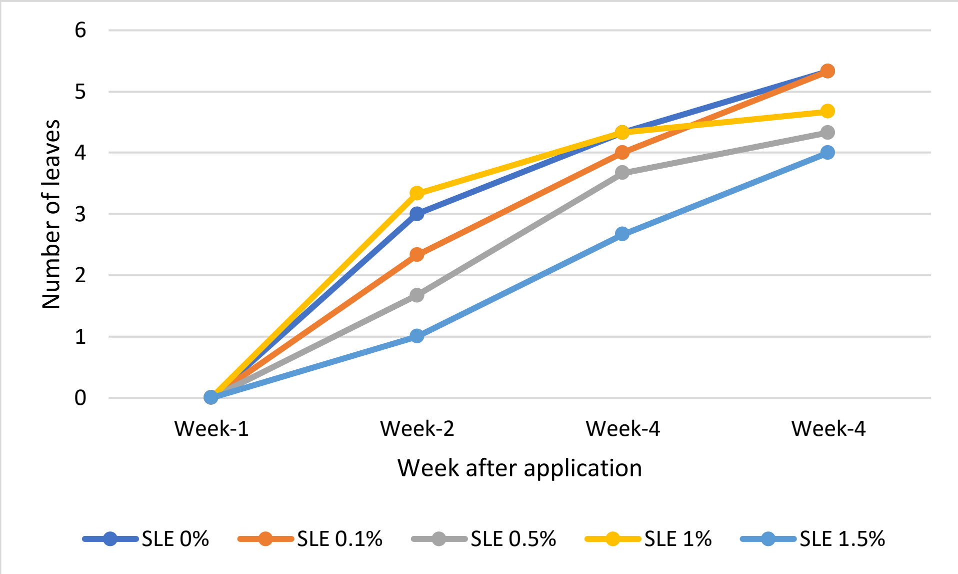

Figure 3. The effect of sambiloto leaf extract on the number of weed leaves

Furthermore, (Pebriani *et al.*, 2013) explained that the allelochemical mechanism inhibits the activity of phytohormones. The results of the study by (El-Rokiek *et al.*, 2010) showed that mango leaf powder at different levels showed phytotoxicity that affected the number of shoots/tubers and the number of leaves. This toxicity phenomenon explains that allelochemical compounds in bitter leaf extract have the potential to inhibit hormone activity in the process of cell elongation. Sambiloto leaf extract treatment of 1.5% was the best concentration in inhibiting the addition of the number of leaves on weeds compared to the control treatment. Sambiloto leaf extract can be used as a pre-growing bioherbicide to inhibit cell elongation in weeds, impacting the number of weed leaves.

3.5. Weed biomass

The application of sambiloto leaf extract significantly affected wet and dry weight four weeks after application (WAA). The highest wet and dry weights were found in the control treatment and significantly differed from the 1% and 1.5% SLE extract treatments. The effect of sambiloto leaf extract treatment on wet and dry weight can be seen in Table 2. The highest wet weight was found in the control treatment and significantly different from the 0.1%, 1.0%, and 1.5% extract treatments and not significantly different from the 0.5% extract treatment. The lowest wet weight was found in the 1.5% extract treatment and it was significantly different from the control treatment. The allelochemicals could cause weight loss. In addition, allelopathic extract inhibited fresh weight by more than 90%, pigment content by more than 80%, and plant height by more than 70%, according to the data by (Saric-krsmanovic *et al*., 2021). The compounds in the sambiloto leaf extract interfere with weeds' water absorption process to carry out photosynthesis. Allelochemical compounds are the main factors in reducing wet weight in weeds. The results of research by (Yulifrianti *et al*., 2015) showed that allelochemical compounds in mango leaf litter extract could reduce the wet weight of grinting grass weeds. Plant growth was inhibited at high concentrations by inhibiting the activity of enzymes needed in photosynthesis, and wet weight was reduced. Wet weight is the total water content and the results of photosynthesis in the plant body. Phenol compounds contained in sambiloto leaf extract can inhibit the growth of weeds. The inhibition occurs due to the disruption of the water absorption process. Lack of water absorption can inhibit the photosynthesis process, which causes the total water content to decrease in weeds.

Table 2. Effect of giving sambiloto leaf extract on wet and dry weight of weeds

Treatment	Wet weight	Dry weight
	--------------- (g) ---------------	
SLE 0%	0.4300 a	0.1233 a
SLE 0.1%	0.3133 b	0.1200 a
SLE 0.5%	0.4000 a	0.1100 a
SLE 1.0%	0.2700 c	0.0733 b
SLE 1.5%	0.2633 c	0.0767 b

Note: the numbers followed by the same letter show no significant difference according to the 5% LSD Test.

The sambiloto leaf extract treatment also significantly affected the dry weight of weeds. The effect of giving 0.5% extract was not significantly different from the control treatment, but at 1.0% and 1.5% extract concentrations, it was significantly different from the control treatment. Sambiloto leaf extract could inhibit the activity of enzymes (Dwivedi *et al*., 2021) needed in photosynthesis, reducing plant dry weight. Furthermore, the results research showed that the secondary metabolite of mango leaf extract could suppress the dry weight of walnut grass weed *(Phalaris minor*) (El-Rokiek *et al*., 2010; Saleem *et al*., 2013). Moreover, (Al-Shatti *et al*., 2014) added that less dry weight is probably due to chlorophyll damage, water absorption inhibition, and stomata closure. Their research described that growth inhibition of seedlings and crops was significantly related to chlorophyll content by reducing photochemical effectiveness and electron transfer rate of PS II, and nutrient uptake inhibition, which resulted in nitrogen and phosphate inadequacies. Furthermore, the decreased photosynthetic ability would decrease the rate of formation of plant organic matter; as a result, the dry weight value of the plant decreased. The 1.0% extract treatment was the best dose in reducing weeds' wet and dry weight, which was not significantly different from the 1.5% extract treatment. The result of this experiment indicates that the flavonoid and tannin compounds in the extract of the sambiloto leaf extract have the potential as pre-growing bioherbicides to reduce the wet and dry weight of weeds.

4. Conclusions

Sambiloto leaf extract was able to suppress weed height growth at a concentration of 1.5% and inhibit weed growth, which impacts reducing wet and dry weight at a concentration of 1.0%. Furthermore, the allelophatic impact of sambiloto leaf extract at 1% and 1.5% concentrations was comparable, with the former SLE concentration being more suggested. In this experiment, the sambiloto leaf extract, extracted using the methanol maceration method, contained 0.1114% flavonoids and 0.1628% tannins. The results of this experiment may support the idea that using plant extract as an alternative to synthetic plant protection products improves agricultural sustainability. Following that, more research into the identification and effects of allelochemicals secreted by sambiloto leaves should be conducted. This effort is critical for synthesizing bioactive compounds from herb species as a natural product for weed control.

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