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Research Article

Application time of *Wedelia trilobata* leaf extract as a bioherbicide for controlling dominant weeds in soybeans

Faisal Abdillah [1], Dyah Weny Respatie [1,*], and Aziz Purwantoro [1]

[1] Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Sleman 55281, Yogyakarta, INDONESIA
* Corresponding author (✉ wenyrespatie@ugm.ac.id)

Abstract

Wedelia leaf extract at a 40% concentration has been reported as an effective bioherbicide for inhibiting the growth of Cyperus rotundus, a dominant weed in soybean cultivation; however, information regarding the most effective application timing remains unavailable. Consequently, this study aimed to determine the optimal application time of Wedelia (Wedelia trilobata L.) leaf extract as a bioherbicide to control dominant weeds, specifically broadleaf weed (Tridax procumbens L.) and purple nutsedge (Cyperus rotundus L.), in soybean crops. The research employed a split-plot design, where the main plots consisted of the plant species (Tridax procumbens, Cyperus rotundus, and soybean), while the subplots consists of three application timings: 7 days before planting (D-7), at the time of planting (D0), and 7 days after planting (D+7), which were then compared against a C0- (negative control=without treatment) and a C0+ (positive control=using the chemical herbicide Pendimethalin (application within 7 days before planting) with dose 3 L ha^{-1}). The results indicated that 7 days before planting (D-7) was the most effective time for applying the 40% Wedelia leaf extract in inhibiting the growth of Tridax procumbens and Cyperus rotundus in soybean cultivation.

Keywords: *Cyperus rotundus*; timing application; *Tridax procumbens*; weed control

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Introduction

Weed control in soybean cultivation areas is very crucial because the presence of weeds can inhibit the growth and development of soybean plants through competition for nutrients, sunlight, water, CO_2, and growing space. This competition can negatively impact soybean crop yield, potentially causing losses of up to 80% (Aisyah & Nugroho, 2019). Weeds have a very strong and aggressive ability to inhibit cultivated plants, even under unfavorable conditions (Aanchal & Menon, 2025). Two dominant weed species found in soybean areas are purple nutsedge (*Cyperus rotundus*) and broadleaf weed (*Tridax procumbens*). These two weeds can further affect soybean plant growth by releasing allelochemicals, which inhibit the physiological processes occurring in the crop. Therefore, the presence of these two dominant weed species must be controlled as part of the effort to increase soybean plant yield productivity (Puspita et al., 2017).

The control of purple nutsedge (*Cyperus rotundus*) and broadleaf weed (*Tridax procumbens*) can be achieved by applying herbicides to soybean cultivation areas. However, the continuous and prolonged use of chemical herbicides can significantly damage soil conditions. Negative impacts resulting from persistent chemical herbicide use include causing resistance in several weed species, degrading the soil's biological and physical condition, reducing the population of beneficial microorganisms, and leaving residues that affect air quality and human health (Aditiya, 2021). Consequently, current technological advancements have led to the widespread development of natural herbicides, often called bioherbicides, which are environmentally friendly alternatives designed to replace the use of chemical herbicides.

The *Wedelia* plant (*Wedelia trilobata* L.) is an ornamental plant that often grows wild in cultivated areas; thus, it is frequently considered a weed because its presence is thought to interfere with crops. The leaves of the *Wedelia* plant have a high phenol compound content, which makes them a potential source material for bioherbicides targeting dominant weeds in soybean cultivation (Li et al., 2013). Previous research has reported that the application of 40% concentration of the *Wedelia* leaf extract was able to affect plant height, inhibit leaf growth, and reduce the total length of leaves and roots of the weed *Cyperus rotundus* (Uyun et al., 2024).

Knowing the potential of the *Wedelia* plant as a bioherbicide, it is subsequently necessary to analyze the appropriate application time so that the *Wedelia* leaf extract can effectively inhibit the growth of dominant weeds in soybean cultivation areas. This analysis of application timing is essential because it is closely related to determining whether the *Wedelia* leaf extract should be categorized as a pre-emergence or a post-emergence herbicide.

Materials and methods

Collection site

Wedelia leaves were collected from the Muntilan area, Magelang District, Central Java Province, Indonesia (7°35'10.6"S and 110°17'59.9"E) from December 2024 to June 2025. Wedelia leaves were taken fresh and free from disease symptoms.

Making a solution of Wedelia leaf extract

The collected *Wedelia* leaves were first cleaned under running water and then dried, either by using an oven at 40 °C for 48 hours or by drying them under direct sunlight for approximately 5 days until completely dry. Once dried, the leaves were pulverized and refined. The resulting *Wedelia* leaf powder was then sieved using a 16-mesh screen. To achieve a 40% extract concentration, the maceration extraction method was employed by mixing 40 grams of the powder into 100 mL of distilled water (aquadest). The mixture was allowed to sit for 24 hours, after which the solution was filtered using a cloth to obtain the pure *Wedelia* leaf extract. The final solution was stored in a refrigerator before being ready for application to the soybean crops (Uyun et al., 2024).

Bioassays

The experiment was implemented using a split-plot design. The main plots consisted of three different plant species: the broadleaf weed Tridax procumbens, the sedge weed *Cyperus rotundus*, and the soybean crop. These three types of plants are

planted individually or separately in each block. Tridax procumbens is planted using seeds, *Cyperus rotundus* is planted using tubers with the same tuber weight, namely 1 g. Meanwhile, soybeans are planted using seeds. The subplots comprised five application timings for the *Wedelia* leaf extract: D-7 (seven days before planting), D0 (at planting), and D+7 (seven days after planting), C0+ (positive control=using the chemical herbicide pendimethalin (application within 7 days before planting) with a dose of 3 L ha^{-1}), and a C0- (negative control = no application). The treatment used with three types of application times is based on the classification of herbicide types based on their application time. They are pre-emergence, which is applied before weeds or plants grow, post-emergence (applied after weeds appear), and pre-planting (before seeds are planted). Every treatment combination within the main plots was replicated in 3 blocks, with each block containing 6 polybags per treatment. This research was conducted in a greenhouse.

The *Wedelia* leaf extract was applied by drenching the soil based on the polybag soil's field capacity. The calculated volume of the solution given was 300 mL per polybag. Planting was carried out simultaneously with the D0 application timing, or 7 days after the bioherbicide was applied for the D-7 treatment.

The experiment was observed for a total duration of 4 WAP (weeks after planting), which was chosen to align with the critical growth period of the soybean plant. Observations were specifically conducted at 2 WAP and 4 WAP to collect comparative growth data, which served as the basis for the subsequent plant growth analysis.

Observations of growth parameters

In this study, we evaluated several parameters to assess the extent of effectiveness of the *Wedelia* leaf extract application at various timings in inhibiting weed growth. The observations included analysis of the bioherbicide extract's pH and electrical conductivity. We calculate the weed or plant germination percentage until the end of the 4 WAP observation using the following formula.

$$GP = \frac{\sum n}{N} \; x \; 100\%$$

where GP is germination percentage; n is the number of seeds germinated; and N is the total number of seeds sown.

The fresh and dry weight of the roots and shoots, the number of tillers, the area of roots and leaves, stomatal opening width, stomatal density, chlorophyll content, greenish leaves (measure it using a chlorophyll meter type SPAD-502Plus), and the fresh and dry weight of *Cyperus rotundus* tubers. These variables were measured at 2 and 4 WAP. The collected dry weight and leaf area data were used to calculate the net assimilation rate (NAR) and the relative growth rate (RGR). Furthermore, periodic observations were conducted every 3 days starting one week after planting, covering plant height and the number of leaves.

$$\text{NAR} = \frac{W2 - W1}{t2 - t1} \; x \; \frac{\ln L2 - \ln L1}{L2 - L1}$$

where W1, W2 are the total plant dry weight at the first and second sampling; L1, L2 are the total leaf area at the first and second sampling; t1, t2 are the time intervals between samplings.

$$\text{RGR} = \frac{\ln W2 - \ln W1}{t2 - t1}$$

where ln is the natural logarithm; W1, W2 are the total dry weight at the beginning and end of the period; t2-t1 is the duration of the growth period.

Statistical analysis

The data obtained from the observations were analyzed using analysis of variance (ANOVA) at a significant level of $\alpha = 5\%$. If significant differences were found among the results, a Tukey's Honestly Significant Difference (HSD) post-hoc test was performed at a 95% confidence level to determine the effective application timing of the *Wedelia* leaf extract. All statistical tests were conducted using the R-Studio software version 4.5.1.

Results and discussion

The test results for the *Wedelia* leaf extract showed that the extract is acidic and has a relatively low electrical conductivity. The observed pH value of the solution was 5.8, and the electrical conductivity value was 1999 $\mu S\ cm^{-1}$. Under these conditions, the acidic pH of a solution applied to a plant can potentially affect the plant's condition, such as inhibiting growth by disrupting the process of nutrient and mineral absorption by the plant (Pratami et al., 2020).

The performance of Tridax procumbens under different treatments

The results (Table 1) showed that the weed was only able to grow in two treatments. The negative control (C0-), or no bioherbicide application, and the bioherbicide application at time D+7 (seven days after planting), with respective percentages of 11.1% and 10.0%. This condition occurred because, at the D+7 application timing, the weeds had already emerged, thus there was no significant inhibitory effect on the germinating power of the Tridax procumbens weed. Therefore, it can be interpreted that the application of the Wedelia leaf extract at both the D0 (at planting) and D+7 timings effectively inhibited the growth of *Tridax procumbens* until the end of the observation period. This effectiveness may be due to the *Wedelia* leaf extract containing gallic acid, a compound that inhibits cell division during the germination process of *Tridax procumbens* seeds (Respatie et al., 2024).

Table 1. The means of the germination percentage of Tridax procumbens weeds that grew up to 4 weeks after planting in various treatments of *Wedelia* leaf extract application times.

Treatment	Germination percentage of *Tridax procumbens* weed (%)
C0-	11.10a
C0+	0.00b
D-7	0.00b
D0	0.00b
D+7	10.00a
CV (%)	23.57

Note: Numbers in the column followed by the same letter indicate non-significant differences based on the HSD test ($\alpha = 5\%$), and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

Additionally, the extract of *Wedelia* leaf was also found to contain compounds such as phenolics, which can certainly have an impact, such as inhibiting weed growth (Anh et al., 2021). It has been proposed that tannin compounds can inhibit plant growth, disrupt respiratory control in mitochondrial organelles, and interfere with the transport of Ca^{2+} and PO_4^{3-} ions. This mechanism allows tannin to inhibit the

growth of the Tridax procumbens weed (Nugroho et al., 2023). The massive growth of Tridax procumbens weeds in soybean plantations can certainly affect soybean growth by releasing allelochemical compounds that can attack soybean roots (Andriana et al., 2019). Therefore, the use of Wedelia leaf extract can be considered appropriate in inhibiting the growth of *Tridax procumbens*.

Observations on growth parameters of the weed Tridax procumbens (Table 2) showed that the C0+ treatment did not differ significantly from the D-7 and D0 application time treatments for these parameters. This condition indicates that the results of the D-7 and D0 application time treatments did not allow the weed to grow until the end of the observation period (4 WAP). Consequently, this can be interpreted as the D-7 and D0 application time treatments inhibiting the growth of *Tridax procumbens* by suppressing the germination of *Tridax procumbens* seeds. Based on these findings, *Tridax procumbens* only grew in the C0- and D+7 treatments. In the D+7 application time treatment, the weed could grow because the *Wedelia* leaf extract was applied after the weed had already grown.

Table 2. Several growth parameters of *Tridax procumbens* weeds at 2 WAP and 4 WAP.

Parameter	Plant age	Treatment				
		C0-	C0+	D-7	D0	D+7
Fresh weight of shoot (g per plant)	2 WAP	0.032a	-	-	-	0.039a
	4 WAP	0.153a	-	-	-	0.180a
Leaf area (cm^2 per plant)	2 WAP	1.819a	-	-	-	1.326a
	4 WAP	4.837b	-	-	-	11.004a
Fresh weight of roots (g per plant)	2 WAP	0.003a	-	-	-	0.004a
	4 WAP	0.006a	-	-	-	0.007a
Root area (cm^2 per plant)	2 WAP	1.096a	-	-	-	0.997a
	4 WAP	1.096a	-	-	-	1.162a
Root length (cm per plant)	2 WAP	4.533a	-	-	-	3.400a
	4 WAP	3.667a	-	-	-	2.000a
Root number per plant	2 WAP	2.667a	-	-	-	2.667a
	4 WAP	3.000a	-	-	-	3.000a
Plant height (cm per plant)	2 WAP	2.300b	-	-	-	3.300a
	4 WAP	3.200a	-	-	-	4.100a
Leaf number per plant	2 WAP	2.667a	-	-	-	2.333a
	4 WAP	4.000a	-	-	-	4.667a
Shoot dry weight (g per plant)	2 WAP	0.008a	-	-	-	0.005b
	4 WAP	0.027b	-	-	-	0.054a
Root dry weight (g per plant)	2 WAP	0.001a	-	-	-	0.001a
	4 WAP	0.003a	-	-	-	0.003a
Root/shoot ratio	2 WAP	0.166a	-	-	-	0.302a
	4 WAP	0.105a	-	-	-	0.053a

Note: Numbers in the row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), the (-) This sign indicates no data, that the seeds did not germinate, and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The weed growth between the C0- and D+7 treatments was not significantly different. For example, considering the parameters of plant height and fresh shoot weight, the D+7 application time treatment produced a higher result compared to the C0- treatment. This indicates that the application of the *Wedelia* leaf extract affected the weeds that had already grown. The application of *Wedelia* leaf extract caused the soil to become acidic, which prompted a response in the plants to continuously adapt, such as growing taller. This condition is certainly because the plants, feeling

threatened, attempted to adapt by reaching for nutrients from sunlight so they could continue to grow (Sukma, 2015).

The 40% concentration of *Wedelia* leaf extract can affect the weed's germination process, thereby inhibiting its growth. From the results (Figure 1), it can be observed that the morphology of the *Tridax procumbens* weed in the negative control treatment showed no significant difference compared to the D+7 application time treatment. Conversely, no *Tridax procumbens* weed growth was found in the positive control, D-7, and D0 application time treatments, thus making morphological observation impossible.

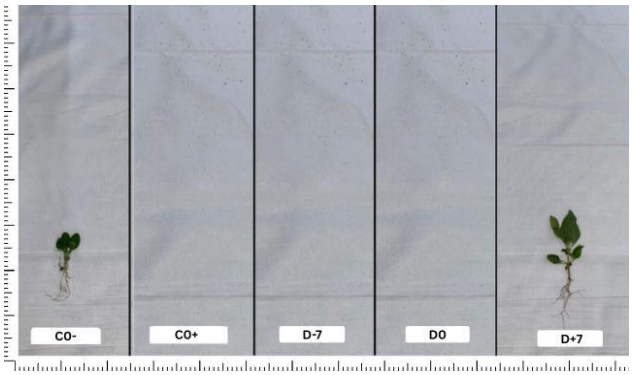

Figure 1. The appearance of the height or performance of *Tridax procumbens* weed at the end of the observation period (4 WAP). C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The leaf greenness, stomatal aperture width, and stomatal density of T. procumbens did not differ significantly between the weed growth treatments (Table 3), namely C0- and D+7. In contrast, all parameters for the D-7 and D0 application time treatments could not be observed because weeds did not grow. The net assimilation rate (NAR) and relative growth rate (RGR) for the D+7 application time treatment were higher than for C0- because the D+7 treatment produced a larger leaf area, which likely allowed for a higher net assimilation rate. The significant differences based on the letter notation (a or b) actually only differentiate the range of observed data results. However, the observed data indicate that the differences in results are not significantly different. In this context, a high net assimilation rate can increase the relative growth rate, and this condition is certainly supported by other factors, such as environmental factors that promote growth.

Table 3. Stomatal aperture width, stomatal density, net assimilation rate, and relative growth rate of Tridax procumbens

Parameter	Treatment				
	C0-	C0+	D-7	D0	D+7
Greenish leaves	27.650a	-	-	-	30.030a
Stomatal aperture width (μm)	6.340a	-	-	-	5.440a
Stomatal density (mm^2)	106.170a	-	-	-	104.940a
NAR (g cm^{-2} per week)	0.003b	-	-	-	0.006a
RGR (g g per week)	0.561b	-	-	-	1.174a

Note: Numbers in the row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), the (-) This sign indicates no data, that the seeds did not germinate, and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The performance of Cyperus rotundus under different treatments

The percentage of tuber regrowth capacity of *C. rotundus* between the C0+ treatment and the D-7 treatment did not differ significantly and yielded results smaller than the other application time treatments (Table 4). This implies that the application of the *Wedelia* leaf extract 7 days before planting can be considered more effective, compared to other application times, in inhibiting the growth of the *Cyperus rotundus*.

Table 4. The average tuber regrowth percentage of *Cyperus rotundus* that grew up to 4 weeks after planting in various treatments of *Wedelia* leaf extract application times

Treatment	Tuber regrowth percentage of *Cyperus rotundus* weed (%)
C0-	75a
C0+	22b
D-7	36b
D0	67a
D+7	69a
CV (%)	19.15

Note: Numbers in the column followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

Table 5. Several growth parameters of *Cyperus rotundus* weeds at 2 WAP and 4 WAP.

Parameter	Plant age	Treatment				
		C0-	C0+	D-7	D0	D+7
Fresh weight of shoot (g per plant)	2 WAP	0.93a	0.31a	0.43a	0.66a	0.71a
	4 WAP	6.43ab	8.71a	4.22b	8.14ab	7.96ab
Leaf area (cm^2 per plant)	2 WAP	30.87a	8.32a	12.52a	23.74a	25.08a
	4 WAP	224.75a	243.87a	152.28a	265.92a	287.43a
Fresh weight of roots (g per plant)	2 WAP	0.11a	0.02a	0.02a	0.08a	0.09a
	4 WAP	1.86a	0.82a	1.10a	1.50a	2.11a
Root area (cm^2 per plant)	2 WAP	9.22a	2.20a	4.02a	4.98a	4.35a
	4 WAP	62.68a	31.94a	36.65a	55.60a	55.49a
Root length (cm per plant)	2 WAP	7.70a	4.20a	4.00a	5.10a	5.10a
	4 WAP	28.00a	11.70b	17.00ab	17.70ab	25.70ab
Root number per plant	2 WAP	14.00a	3.33b	9.33ab	10.33ab	11.67a
	4 WAP	30.33a	20.67a	19.67a	21.67a	29.00a
Plant height (cm per plant)	2 WAP	30.90a	38.57a	25.33a	37.30a	34.07a
	4 WAP	56.00a	55.67a	38.00b	52.67ab	51.33ab
Leaf number per plant	2 WAP	9.67a	11.33a	9.67a	9.67a	11.67a
	4 WAP	24.33a	23.67	26.00a	30.33a	26.67a
Shoot dry weight (g per plant)	2 WAP	0.15a	0.04b	0.05b	0.10ab	0.10ab
	4 WAP	1.23a	1.45a	0.77a	1.45a	1.37a
Root dry weight (g per plant)	2 WAP	0.03a	0.01a	0.01a	0.02a	0.03a
	4 WAP	0.52a	0.19a	0.30a	0.40a	0.54a
Root/shoot ratio	2 WAP	0.20a	0.16a	0.22a	0.23a	0.24a
	4 WAP	0.46a	0.13a	0.37a	0.29a	0.43a
Number of shoots/tubers	2 WAP	1.30a	1.00a	1.00a	1.00a	1.00a
	4 WAP	6.00a	4.33a	4.33a	5.67a	6.67a

Note: Numbers in the row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

This condition is further reinforced by the results of several other observational parameters, such as the root dry weight parameter of 0.30 g at 4 WAP, indicating that the D-7 application time treatment produced smaller yields compared to other application time treatments (Table 5). This indicates that when the root dry weight is lower and the root length is shorter, the plant absorbs less water and nutrients, thereby inhibiting its growth (Nugroho et al., 2023).

From the morphological appearance (Figure 2), it can be seen that the diversity of the *C. rotundus* weed in the D-7 treatment was smaller compared to the results from the other application time treatments. This proves that the application of *Wedelia* leaf extract can affect the performance of *C. rotundus* weeds. Extensive proliferation of *C. rotundus* adversely affects soil quality by diminishing nutrient availability and suppressing enzymatic functions, which ultimately results in the decline of crop productivity (Liu et al., 2022). Therefore, the application of *Wedelia* leaf extract is needed as an alternative to suppress the growth and spread of *C. rotundus*.

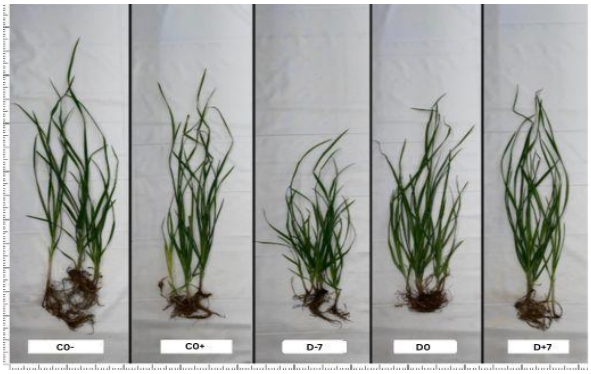

Figure 2. The appearance of the height or performance of *Cyperus rotundus* weed at the end of the observation period (4 WAP). C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The use of *Wedelia* leaf extract can increase abiotic stress on plants. The presence of abiotic stress will certainly affect the photosynthesis process, consequently leading to a decrease in plant biomass production (Riskitavani & Indah, 2013).

Based on several observation parameters (Table 6), the D-7 treatment yielded a smaller stomatal aperture compared to the other treatments, although the difference was not statistically significant. This condition, however, greatly impacts a plant's photosynthesis process. When the stomatal aperture in a plant is smaller, the CO_2 diffusion process into the leaf tissue is lower compared to plants with a larger stomatal aperture (Sakiroh & Aunillah, 2020). This consequence will also affect a plant's performance in its physiological processes and inhibit its growth. Other results showed that the NAR value of the C0+ treatment was higher than that of D-7. A lower NAR value indicates decreased photosynthetic efficiency. This suggests that the application time of the *Wedelia* leaf extract at D-7 was lower, thus better suppressing net photosynthetic efficiency and biomass production in *C. rotundus*, thus further inhibiting its growth.

Table 6. Stomatal aperture width, stomatal density, net assimilation rate, and relative growth rate *Cyperus rotundus*

Parameter	Treatment				
	C0-	C0+	D-7	D0	D+7
Greenish leaves	43.700a	39.330a	40.470a	41.170a	39.630a
Chlorophyll a (mg g^{-1})	0.280a	0.260a	0.280a	0.300a	0.240a
Chlorophyll b (mg g^{-1})	0.140a	0.130a	0.130a	0.180a	0.130a
Total chlorophyll (mg g^{-1})	0.850a	0.770a	0.820a	0.970a	0.740a
Stomatal aperture width (µm)	3.330a	3.450a	3.010a	3.220a	3.450a
Stomatal density (mm^2)	152.880a	116.260a	115.840a	131.690a	134.360a
NAR (g cm^{-2} per week)	0.010b	0.016a	0.012ab	0.011ab	0.011ab
RGR (g g per week)	0.895a	1.006a	0.982a	1.064a	1.030a

Note: Numbers in the row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), the (-) sign indicates that the seeds that are not germinating, and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The performance of soybean plant under different treatments

The soybeans in the C0+ treatment could not grow until the end of the observation period (Table 7 and Figure 3). Consequently, the morphological appearance could not be observed, and the percentage of germination capacity could not be determined. This was likely due to the active ingredient contained in the herbicide pendimethalin, which can inhibit the process of cell division (mitosis) in the soybean plant organs. The inhibitory mechanism involves inhibiting microtubule polymerization, thus affecting cell formation (Nehila et al., 2025). As a result, the cells in the soybean plants could not divide and elongate, potentially leading to inhibited soybean growth or even death of the soybean plants. The level (high or low) of the pendimethalin herbicide dosage also affects soybean plants. The safe limit for pendimethalin dosage is reported to range between 0.5 L ha^{-1} and 3 L ha^{-1} for soybean plants (Ayuningtyas et al., 2024). Therefore, the application of *Wedelia* leaf extract with a 40% concentration is considered safer for weed control in soybean cultivation.

Table 7. The means of the germination percentage of soybean seeds that grew up to 4 weeks after planting in various treatments of *Wedelia* leaf extract application times

Treatment	Germination percentage of soybean seeds (%)
C0-	82a
C0+	0b
D-7	52a
D0	67a
D+7	80a
CV (%)	19.15

Note: Numbers in the same column followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

Figure 3. The appearance of the height or performance of soybean at the end of the observation period (4 WAP). C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

Table 8 shows that in the C0+ treatment, the soybean plants managed to grow at 2 WAP, but their growth was stunted, and they eventually died because they could not sustain their growth. Furthermore, the average application time treatments at 4 WAP did not show a significant difference in results. Therefore, it can be concluded that the application of *Wedelia* leaf extract did not have a negative impact on soybean plant growth.

Table 8. Several growth parameters of soybean plants at 2 WAP and 4 WAP

Parameter	Plant age	Treatment				
		C0-	C0+	D-7	D0	D+7
Fresh weight of shoot (g per plant)	2 WAP	2.05a	0.81c	1.28bc	1.59ab	1.86ab
	4 WAP	7.61a	-	5.28a	6.05a	5.34a
Leaf area (cm^2 per plant)	2 WAP	72.26a	1.88d	19.11cd	38.04bc	59.20ab
	4 WAP	352.88a	-	233.15b	243.97ab	255.09ab
Fresh weight of roots (g per plant)	2 WAP	0.09a	0.01c	0.03bc	0.05ab	0.05ab
	4 WAP	0.33a	-	0.26a	0.32a	0.26a
Root area (cm^2 per plant)	2 WAP	5.80a	0.52c	2.54bc	3.89ab	3.86ab
	4 WAP	7.36a	-	5.05b	6.00ab	5.53ab
Root length (cm per plant)	2 WAP	9.17a	1.53b	5.60ab	7.43a	5.83ab
	4 WAP	13.43a	-	9.53a	10.67a	11.57a
Root number per plant	2 WAP	15.67a	0.67b	9.33a	13.67a	12.33a
	4 WAP	21.00a	-	16.67a	20.33a	27.33a
Plant height (cm per plant)	2 WAP	38.50a	2.33c	22.83bc	21.77ab	29.00ab
	4 WAP	87.83a	-	62.00a	67.33a	68.33a
Leaf number per plant	2 WAP	5.00a	2.00a	4.33a	4.00a	5.00a
	4 WAP	8.00a	-	7.33a	7.33a	7.67a
Shoot dry weight (g per plant)	2 WAP	0.32a	0.14b	0.14b	0.19b	0.27a
	4 WAP	2.01a	-	1.21a	1.30a	1.32a
Root dry weight (g per plant)	2 WAP	0.04a	0.01d	0.01cd	0.02bc	0.03ab
	4 WAP	0.13a	-	0.10a	0.11a	0.11a
Root/shoot ratio	2 WAP	0.13a	0.02b	0.10a	0.12a	0.11a
	4 WAP	0.07a	-	0.07a	0.08a	0.08a

Note: Numbers in the same row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), the (-) This sign indicates no data, that the seeds did not germinate, and treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The dry weights of both soybean shoots and roots across all *Wedelia* leaf extract application time treatments were also not significantly different (Table 8). Consequently, since the dry weight of a plant can reflect its nutritional status and can be a good indicator of plant growth and development (Sitorus et al., 2014), it can be definitively confirmed that the application of *Wedelia* leaf extract in soybean cultivation does not disrupt the soybean plants.

Soybean plants are very susceptible to weed attacks, especially during their vegetative stage. These plants require favorable environmental conditions, such as proper temperature, humidity, and sufficient light (Scott et al., 2021). Consequently, managing weeds during the soybean's critical period is necessary to ensure maximum growth.

Leaf greenness level, chlorophyll a, chlorophyll b, total chlorophyll, stomatal aperture, and stomatal density across the various application time treatments did not show a significant difference (Table 9). This means that the application of the *Wedelia* leaf extract did not interfere with the physiological processes in the plants. This condition indicates that the photosynthesis process in the soybean plants proceeded efficiently without any disturbance to the leaf stomatal system. The stomatal aperture and stomatal density can be influenced by cell division patterns, differentiation, and cell expansion during leaf organ development in a plant (Sultana et al., 2024).

Table 9. Stomatal aperture width, stomatal density, net assimilation rate, and relative growth rate of soybean

Parameter	Treatment				
	C0-	C0+	D-7	D0	D+7
Greenish leaves	38.500a	-	36.930a	37.270a	36.900a
Chlorophyll a (mg g^{-1})	0.300a	-	0.290a	0.300a	0.300a
Chlorophyll b (mg g^{-1})	0.330a	-	0.340a	0.320a	0.380a
Total chlorophyll (mg g^{-1})	1.250a	-	1.260a	1.230a	1.360a
Stomatal aperture width (µm)	2.620a	-	2.601a	2.231a	2.660a
Stomatal density (mm^2)	155.560a	-	158.850a	141.560a	182.100a
NAR (g cm^{-2} per week)	0.005a	-	0.007a	0.005a	0.004a
RGR (g g per week)	0.886ab	-	1.054a	0.920ab	0.775b

Note: Numbers in the same row followed by the same letter indicate non-significant differences based on the HSD test (α = 5%), the (-) sign indicates no data (the seeds did not germinate), treatment C0- (negative control = without treatment); C0+ (positive control = herbicide Pendimethalin); D-7 (seven days before planting); D0 (at planting); D+7 (seven days after planting).

The growth analysis results show that the relative growth rate for the D-7 treatment yielded a higher result compared to the D0 and D+7 (Table 9) application time treatments. This means that the soybean plants in the D-7 application time treatment had a very efficient and fast growth rate in converting biomass. Furthermore, the application of Wedelia leaf extract in the D-7 treatment is considered to have provided sufficient time for the bioherbicide to work effectively and partially degrade in the soil, allowing it to suppress weed growth without affecting the growth of the soybean plants. Therefore, in this context, the application of Wedelia leaf extract in the D-7 treatment inhibits weed growth but positively benefits soybean growth. The application of herbicides, or in this case bioherbicides, before planting is considered good because it targets weeds without affecting the initial growth process of soybean plants (Krähmer et al., 2021).

Conclusions

The application of *Wedelia* leaf extract as a bioherbicide with a concentration of 40% is able to inhibit weed growth. However, in determining the optimal application time, this study indicates that the application of *Wedelia* leaf extract 7 days before planting is considered more effective in inhibiting the dominant weeds, *Tridax procumbens* and *Cyperus rotundus*, without having a negative impact on soybean plant growth. This condition is based on the results of the dry weight parameters of *Tridax procumbens* and *Cyperus rotundus* shoots at D-7, which are small, and these variables are used as indicators to measure total biomass and photosynthesis rate.

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