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# Optimal Concentration and Application Timing of Teak Leaf Extract (*Tectona grandis*) as a Bioherbicide Against *Tridax procumbens* in Soybean

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## ABSTRACT

Weed competition during soybean cultivation induces stress, leading to reduced yields. One of the dominant weeds in soybean cultivation is *Tridax procumbens*, and weed control is generally performed using herbicides, but can cause environmental pollution. Therefore, natural herbicides that are environmentally friendly and sustainable are required. Teak leaves, which contain allelopathy and are abundantly available in Indonesia, have the potential to be used as a bioherbicide. This study aimed to determine the optimal concentration and application time of teak leaf extract as a bioherbicidal agent against *T. procumbens* during soybean cultivation. The study was performed at the laboratory and greenhouse of the Faculty of Agriculture, Universitas Gadjah Mada, Sleman, Special Region of Yogyakarta. The research was conducted in two stages. The first stage was the germination stage, which used a complete randomised design with 10%, 20%, and 40% teak leaf extract concentrations. Meanwhile, the second stage was the initial growth stage using a complete randomised block design with treatments consisting of application 7 days pre-planting, planting time, and application 7 days post-planting. The 10% concentration of teak leaves was able to inhibit the germination force and vigour index of *T. procumbens* and did not inhibit soybean germination. Therefore, a 10% teak leaf extract concentration was applied in the second stage, and optimal results at the time of application at planting were obtained because it was able to inhibit up to 100% of *T. procumbens* growth. However, further field-scale studies are needed to evaluate its practical application.

*Keywords:* Allelopathy, bioherbicide, germination, seedling growth, soybeans, teak, *Tridax procumbens*

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## INTRODUCTION

Soybeans are one of Indonesia's preferred crops. In 2021, Indonesia's domestic soybean production was 613,300 tons, a 3.01% decrease from that of 2020. Furthermore, in 2023, soybean production declined to 576,300 tons or a decrease of 3.09% (Hulu, 2023). These declines in soybean production in Indonesia can be caused by reduced production area or constraints on soybean cultivation, such as biotic and abiotic stressors. One of these biotic stressors that inhibits soybean cultivation is weed competition.

Crop-weed competition alters how crops interact with light, space, water, and nutrients (Kaur et al., 2018). Weed competition can reduce soybean crop yield by up to 80% (Silva et al., 2015). Every gram of weed dry matter reduces crop yields because the weeds absorb one-third of the fertiliser nutrients during their early growth, including nitrogen, phosphorus, potassium, calcium, and magnesium (Kaur et al., 2018). One of the main weed species found in soybean production is *Tridax procumbens* (Rodrigues & Vivian, 2011). The impact of weeds on soybean crops necessitates weed control efforts to maintain high yields. Weed control can be performed mechanically or chemically. However, when used excessively, chemical control with herbicides can harm the biosphere by decreasing soil fertility, increasing pest populations, polluting the environment, and leading to resistant weed populations (Rajak et al., 2023).

Teak leaves contain allelopathic compounds. When teak leaves fall to the ground due to defoliation, these compounds can affect the growth of surrounding plants. Previous studies have shown that teak leaf extracts suppress the germination and growth of several plant species through allelopathic activity (Kato-Noguchi, 2021). The teak plant is widely planted in Yogyakarta, especially in the Gunung Kidul Regency. Teak plant leaves can be used as a bioherbicide because of their allelopathic properties and their abundant presence in the surrounding area. Using methanol-extracted teak leaf extract can reduce weed populations by 56% and inhibit the growth of *Echinochloa colona* and *Echinochloa crus-galli* (Kole et al., 2016).

Innovations in alternative weed control that are practical and environmentally friendly, such as bioherbicides, have been identified and attracted much interest. Moreover, the application of the leaf extract of cardoon (*Cynara cardunculus* L. var. altilis DC.) can inhibit the germination of four weeds, namely *Amaranthus retroflexus* L., *Portulaca oleracea* L., and *Stellaria media* (L.) Vill., and *Anagallis arvensis* L. (Scavo et al., 2020). Previous studies have also demonstrated the bioherbicidal potential of several plant extracts. Furthermore, *Cleome rutidosperma* contains toxic allelopathic compounds that can interfere with the germination of other weeds in rice cultivation, such as *Echinochloa crus-galli* (L.) P. Beauv., *Fimbristylis miliacea* (L.) Vahl, *Oryza sativa* f. *spontanea* Roshev, *Leptochloa chinensis* (L.) Nees, and *Cyperus iria* L. (Motmainna et al., 2024).

Total phenolic content was evaluated to estimate the potential allelopathic activity of teak leaf extract, as phenolic compounds are known to contribute to phytotoxic effects (Saludes-Zanfaño et al., 2024). Allelopathic effects can be evaluated through seed germination parameters (Li et al., 2024). Therefore, germination rate, germination index, and the number of abnormal sprouts were measured in this study. The root-stem ratio in soybean was evaluated to determine the relative sensitivity of root and stem growth to allelopathy (Xiao et al., 2024). Dry weight was measured as an indicator of the overall growth response of *T. procumbens* to teak leaf extract treatment (Respatie et al., 2024).

However, the effective concentration and optimal application timing of teak leaf extract remain unclear. Due to its allelopathic properties, teak leaf extract has potential as an environmentally friendly bioherbicide for weed management. Therefore, this study aimed to determine the effective concentration and application timing of teak leaf extract against weeds.

## MATERIALS AND METHODS

### Experimental Site

Fallen teak leaves were gathered from the experimental field of the Agricultural Faculty of Universitas Gadjah Mada, Banguntapan, Bantul District, Special Region of Yogyakarta Province, Indonesia (07º48'17" S, 110º24'45" E). Naturally fallen teak leaves were used in this research to support the utilisation of unused plant waste. The leaves were stored in dry conditions at room temperature before further processing. The germination experiment was conducted in November 2023 at the Plant Management Laboratory, and the field experiment was conducted from January to February 2024 at the greenhouse of the Agricultural Faculty of Universitas Gadjah Mada.

### Experimental Design

The experimental design in the concentration and germination experiments was conducted using a single-factor, completely randomised design with four different concentrations (0, 10, 20, and 40%) and three replications. The extract concentrations were selected based on the study by Budianto et al. (2023), who reported that teak leaves contain approximately 4.3 ± 0.15% (w/w). The 10% concentration was assumed to represent the minimum inhibitory level because it contained the lowest proportion of phenolic compounds. Furthermore, the concentrations were increased to ensure a clear and significant difference between treatments and to evaluate the dose-dependent inhibitory effects of the extract.

Thereafter, a field experiment was conducted using a randomised complete block design with three treatments consisting of application 7 days pre-planting (D-7), planting time (D-0), and application 7 days post-planting (D+7), with four replications.

## Extract and Solution Preparations

The teak leaves used were leaves that had fallen from the tree; therefore, they were already in a dry state. The teak leaves were ground into powder using a grinder. The powder was weighed according to the desired concentration: 10 g, 20 g, and 40 g of teak leaf powder were mixed with 100 mL of distilled water to prepare 10%, 20%, and 40% extracts, respectively. Therefore, the extraction ratio was maintained consistently at *n* g:100 mL for all treatments.

The mixtures were kept at room temperature (20-25 °C) for 24 h. After extraction, the mixtures were filtered through muslin cloth (<0.1 mm diameter) and then through Whatman No. 1 filter paper to obtain the final extracts. The extraction process was repeated for each experimental replicate to ensure reproducibility. All extracts were maintained at room temperature prior to application.

## Determination of the Phenolic Content Equivalent to Gallic Acid

To determine the total phenol content equivalent to gallic acid, 50 mg of the sample was combined with 0.5 mL of Folin-Ciocalteu phenol reagent and 7.5 mL of distilled water. The mixture was shaken and allowed to stand for 10 min. Following this, 1.5 mL of 20% sodium carbonate solution was added. The mixture was shaken again and left for an additional 10 min. Finally, distilled water was added to make up a total volume of 10 mL, and the absorbance was measured at 760 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

## Biochemical Analysis of the Teak Leaf Extract

Biochemical analysis of teak leaf extracts was performed by measuring the value of acidity (pH) and electrical conductivity (EC) of the teak leaf extracts with concentrations of 10%, 20%, and 40%, compared with the control (distilled water). The pH was measured by pH Tester HI189107 pHep, and EC are measured with an EC meter model number HI8731.

## Bioassays

The experiment was arranged in a completely randomised design consisting of control treatment (A0) and teak leaf extract concentrations of 10% (A1), 20% (A2), and 40% (A3). Soybean and *T. procumbens* seeds were germinated separately in a petri dish lined with filter paper treated with 10 mL of teak leaf extract. Each treatment consisted of 10 seeds with three replications for each plant species. Seed germination was observed for 7 days.

Several germination parameters, including germination rate, germination index, and abnormal sprout formation, were evaluated to assess the effects of teak leaf extract on seed growth.

Sprouts were classified as abnormal if they had spiral hypocotyls and small primary roots. Below are the formulas used during this study, as shown in Equations 1 - 3:

$$\text{Germination Rate (\%)} = \left(\frac{Number\ \ of\ Total\ Germinated\ \ Seeds}{Total\ \ of\ Number\ \ Seeds\ Tested}\right) \times 100 \qquad [1]$$

$$\text{Germination Index} = \Sigma \frac{G_t}{D_t} \qquad [2]$$

where $G_t$ denotes number of seeds germinated on day-$t$, and $D_t$ corresponds to the Day of the count

$$\text{Abnormal Sprouts (\%)} = \left(\frac{Number\ \ of\ Total\ Abnormal\ \ Sprouts}{Total\ \ of\ Number\ \ Germinated\ \ Seeds}\right) \times 100 \qquad [3]$$

### Greenhouse experiment

The experiment was conducted under greenhouse conditions with temperatures ranging from 25-35 °C and relative humidity ranging from 50-90%. Soybean and *T. procumbens* were planted in 15 × 15 cm polybags containing 1L of sterilised soil media. Five soybean cultivars, *Anjasmoro* and 20 *T. procumbens* seeds, were planted in separate polybags. The 250 mL teak leaf extract solution was applied to each polybag at the specified treatment concentration. Three application treatment times were used in this study: before planting (D-7), at planting (D-0), and after planting (D+7). 250 mL of teak leaf extract was applied to the polybags. Watering was performed once per application time treatment. The variables measured at this stage were germination rate and dry weight. The following formula was used for the germination rate:

$$\text{Germination Rate (\%)} = \left(\frac{Number\ \ of\ Total\ Germinated\ \ Seeds}{Total\ \ of\ Number\ \ Seeds\ Tested}\right) \times 100 \qquad [4]$$

Dry weights were measured by placing the plant parts into envelopes and oven-drying at 65 °C-85 °C for 48 h until a constant dry weight was obtained, then weighing.

The root/shoot ratio was calculated using the following formula:

$$\text{Root/Shoot Ratio} = \frac{Root\ dry\ weight}{Shoot\ dry\ weight} \qquad [5]$$

### Statistical Analysis

Data analysis was performed using analysis of variance with a significance level of 5% (α = 0.05). If the data were significant, a post hoc test was used. Further tests for germination and growth were analysed using the Tukey test at the 5% significance level. All data analysis in this study was performed using R version 4.2.1.

## RESULT AND DISCUSSION

### Chemical Properties of the Teak Leaf Extract

The total phenol content of the dried teak leaf extract was 4.36% gallic acid equivalent (w/b), which was similar to the value reported by Budianto et al. (2023) for teak leaves (4.3 ± 0.15% w/b). The phenol content in the teak leaf extract was also higher than that reported for *Thymus capitatus*, a plant known to inhibit seed germination. Algandaby and El-Darier (2018) reported that T. capitatus contained 3842 mg/100 g phenolic compounds, equivalent to 3.8% (w/b). These results indicate that teak leaf extract contains phenolic compounds with potential bioherbicidal activity.

The pH and EC values of the teak leaf extract at various concentrations are shown in Figure 1. The pH of the teak leaf extracts increased with increasing concentration, indicating alkaline pH levels, with values in the range of 8-8.3. The EC value of the teak leaf extract followed a similar trend to the pH value, with higher EC values observed with higher teak leaf extract concentrations, with the highest value at 40% concentration, with 1506 µS. The pH value affects the availability of nutrients in the media. The salt and ion content of the solution influences the pH concentration and EC, which in turn affect plant growth by modifying the osmotic pressure and enzyme activity in the cells (Santos et al., 2021).

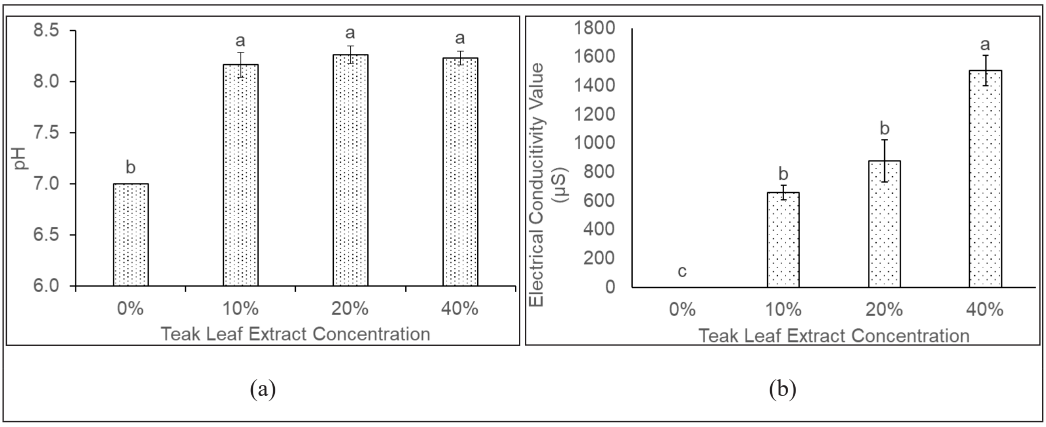



*Figure 1.* (a) pH value and (b) electrical conductivity value of teak leaf extract at various concentrations

## Bioassays

In Figure 2a, the germination rate of seeds in the application of dried teak leaf extract concentration did not cause a significant decrease from the control treatment. Soybean resistance to teak leaf extract application at high pH and EC values is due to the soybean cultivar *Anjasmoro*.

Figure 2b shows no significant difference in the application of 10% and 20% teak leaf extract concentrations compared to the control for the soybean vigour index. Meanwhile, there was a significant difference in the vigour index of soybeans after application of the 40% concentration compared to the control, but no significant difference between the 40% and the 10% or 20% teak leaf extract concentrations. The decrease in soybean vigour index at higher extract concentrations suggests that elevated allelochemical exposure may interfere with early seedling development. Increasing extract concentration led to greater inhibition, resulting in a decrease in vigour index (Respatie et al., 2024).

This study found the highest abnormal germination rate in the 40% teak leaf extract treatment, at 16.67% (Figure 2c). The germination process requires amylase, which breaks down complex carbohydrates into simpler molecules (Gunathunga et al., 2024). Using a teak leaf extract containing phenol may inhibit amylase activity, resulting in reduced germination and abnormal sprouting. Allelochemicals inhibit microtubule formation, leading to abnormal growth (Reigosa et al., 2006).

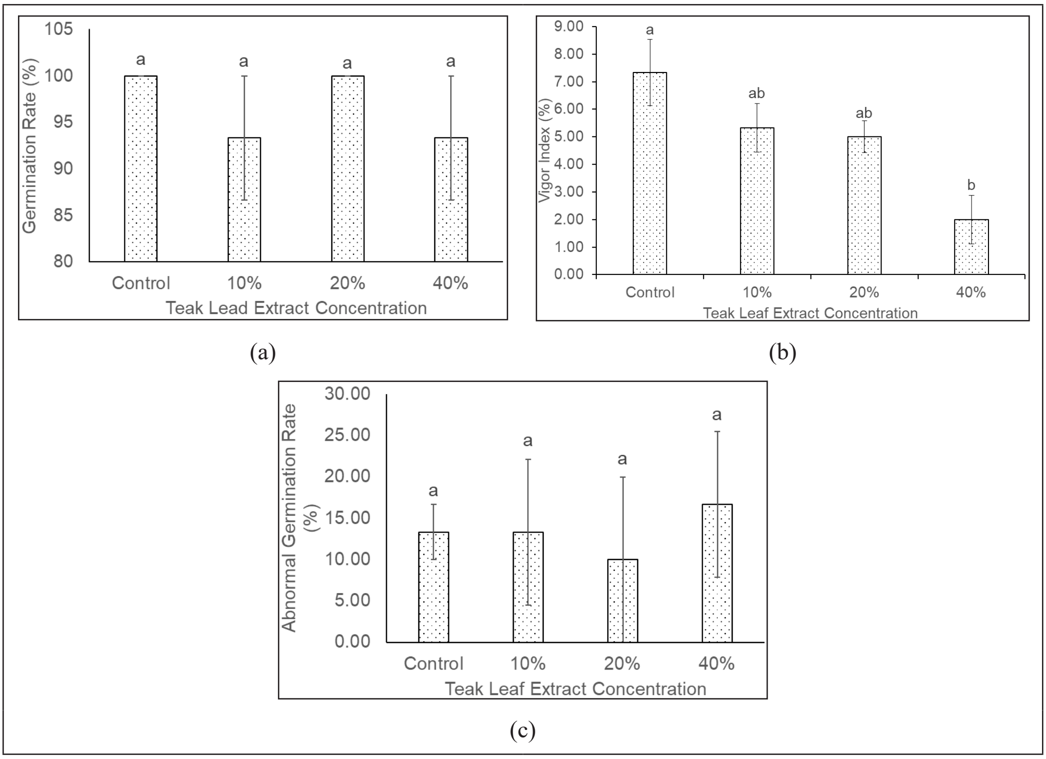
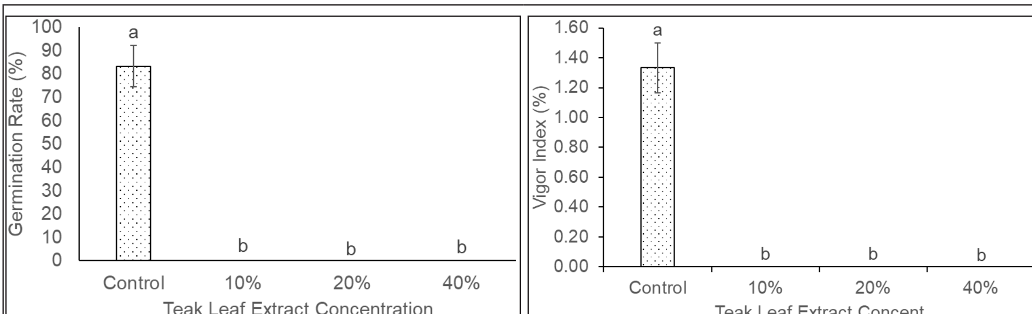



*Figure 2.* (a) Germination rate; (b) vigour index; and (c) incidence of abnormal soybean sprouts at different concentrations of teak leaf extract

The germination of *T. procumbens* weed showed significant differences (Figure 3a). In the control treatment, the germination rate of *T. procumbens* reached 80%, whereas applications of teak leaf extract at 10%, 20%, and 40% resulted in a 0% germination rate. The results of this study indicate that inhibition increases with increasing extract concentration, a finding similar to that reported by Respatie et al. (2024) for the Wedelia plant extract. Similar inhibitory effects of plant-based bioherbicides on weed germination have also been reported in several allelopathic plant species. Aqueous extracts of *Bidens bipinnata* L., *Ricinus communis* L., and *Piper tuberculatum* Jacq. significantly inhibited the germination and seedling growth of *B. bipinnata* (Lopes et al., 2022).

Teak leaves contain several phytotoxic compounds, including phenolics and quinones, which may contribute to germination inhibition (Kato-Noguchi, 2021). Therefore, a 10% concentration can completely inhibit the growth of *Tridax procumbens* and is considered effective when applied in subsequent stages of this study. The inhibition of seed germination may be attributed to allelochemicals in the bioherbicidal extract, which penetrate the seeds, reduce gibberellin content and α-amylase activity, and simultaneously increase antioxidant enzyme activity (Li et al., 2024). Furthermore, Somala et al. (2024) report that allelochemicals penetrating seed tissues induce the accumulation of reactive oxygen species (ROS) and elevate malondialdehyde (MDA) levels. This process results in oxidative damage to cellular membranes and may inhibit seed germination.

The vigour index of *T. procumbens* weed sprouts showed significantly different results between the control treatment and the teak leaf extract at 10%, 20%, and 40% concentrations (Figure 3b). In this case, applying a 10% concentration of dried teak leaf extract can inhibit the germination of *T. procumbens* weed seeds. Phenolic allelochemicals may interfere with seed imbibition and reduce α-amylase activity, thereby limiting carbohydrate mobilisation during germination (Madane & Patil, 2017). This disruption may subsequently affect mitotic activity and inhibit seed germination (Reigosa et al., 2006).




(a) (b)

*Figure 3.* (a) Germination rate and (b) vigour index of Tridax procumbens at different concentrations of teak leaf extract

The 10% concentration was considered the most effective treatment because it achieved the same inhibitory effect on *T. procumbens* as the 20% and 40% concentrations, while requiring less raw material. In addition, 10% concentration did not negatively affect soybean germination. Therefore, 10% concentration was chosen for the greenhouse experiment.

## Effect of Teak Leaf Extract Application on Soybean Plant Growth

The effect of the teak leaf extract application on soybean was indicated by the germination rate and root/shoot ratio at various teak leaf extract application times at 5 weeks after planting, as shown in Table 1. The germination percentage of soybean was similar between the control and teak leaf extract treatments, indicating that the application of 10% teak leaf extract did not affect soybean germination. At 5 weeks after planting, the control treatment showed the lowest root dry weight and the highest shoot dry weight, whereas the teak leaf extract treatments increased root dry weight and reduced shoot dry weight. These changes resulted in a higher root/shoot ratio, suggesting that soybean growth was more directed toward root development than shoot growth following teak leaf extract application. The high root/shoot ratio indicates that plant growth was more dominant in the roots compared to the shoots (Bláha, 2019). Similar growth responses have been reported in other allelopathic studies, where applying the allelopathic extract altered biomass allocation and root/shoot development (Xiao et al., 2024). One factor influencing this is the salinity resulting from the application of the teak leaf extract. The high EC values reflect the high salt concentrations in the teak leaf extracts (Corwin & Yemoto, 2017). High salt content can inhibit the initial growth of soybeans (Umida Toshtemirovna et al., 2023), which can lead to oxidative stress in soybeans, triggering lipid peroxidation and causing soybean growth inhibition, as shown by the root/shoot ratio (Uyun et al., 2024).

Table 1
*The germination rate of soybean growth and the root/shoot ratio of soybean five weeks after planting at various teak leaf extract application times*

| Treatments | Germination Rate (%) | Root/Shoot Ratio |
|---|---|---|
| Control | 81.67±20.64 a | 0.30±0.05 c |
| D-7 | 83.33±12.77 a | 0.37±0.10 bc |
| D-0 | 83.33±11.55 a | 0.52±0.07 a |
| D+7 | 85.00±17.53 a | 0.43±0.07 ab |
| CV (%) | 20.65 | 17.87 |

*Note.* Numbers in the same column followed by the same character were not significantly different based on the Tukey Test (α = 5%)

The effect of teak leaf extract treatment at various application times on the germination rate and dry weight of *T. procumbens* weed at 5 weeks after planting is shown in Table 2. There were no weeds growing in the D-0 treatment. Allelopathic compounds, especially phenolic compounds, can affect plant germination and growth by inhibiting respiration and enzyme activity during germination and growth (Zohaib et al., 2016). Therefore, food reserves, water imbibition, and transportation were effectively inhibited during the D-0 application or planting time. In the D-7 application treatment, or 7 days before planting, there was also inhibition, although this was not complete inhibition. Dried teak leaf extract contains allelochemical compounds in the form of phenols, which can inhibit plant germination (Li et al., 2021). In addition, these compounds may inhibit the vigour or strength of plant growth. Allelochemicals such as phenols can inhibit enzyme activity by degrading the seed's food reserves for germination. In particular, alpha-amylase activity decreased, thereby affecting the seeds' viability (Padilha et al., 2022).

Table 2 shows that the weed dry weight decreased after teak leaf extract applications at D-0 and D+7. This is due to the application of teak leaf extract, which contains allelopathy and triggers oxidative stress, causing a decrease in the weed's dry weight (Uyun et al., 2024). Meanwhile, there was an increase in the weed dry weight because the inhibition of teak leaf extract only lasted for a few days at the beginning of the application at D-7. Therefore, the allelopathic effect of the teak leaf extract did not affect the weed dry weight in the D-7 application.

Treatments of various application times showed different germination rates, as shown in Figure 4. The control treatment and D+7 application exhibited the same results, where the seeds began to germinate on the 3rd day. Meanwhile, the D-7 application treatment showed that the seeds began germinating on the 5th day. In the D-0 application, no *T. procumbens* seeds germinated. Germination inhibition was found in the D-7 treatments, and the inhibitory effect occurred until the 11th day after application. One weed seed germinated on the 4th or 11th day after applying the teak extract. Therefore, the teak leaf extract can inhibit up to the 11th day after application. However, further observations are required to evaluate the bioherbicidal activity of teak leaf extract.

Table 2
*The germination rate and dry weight of* Tridax procumbens *weeds that grew up to five weeks after planting in various treatments of teak leaf extract application time*

| **Treatments** | **Germination Rate (%)** | **Weed Dry Weight (g)** |
|---|---|---|
| Control | 28.75±8.54 a | 0.16±0.07 ab |
| D-7 | 6.25±2.50 b | 0.19±0.05 a |
| D-0 | 0.00±0.00 b | 0.00±0.00 c |
| D+7 | 22.50±6.45 a | 0.09±0.03 b |
| CV (%) | 4.42 | 3.76 |

*Note.* Numbers in the same column followed by the same character were not significantly different based on the Tukey Test (α = 5%)

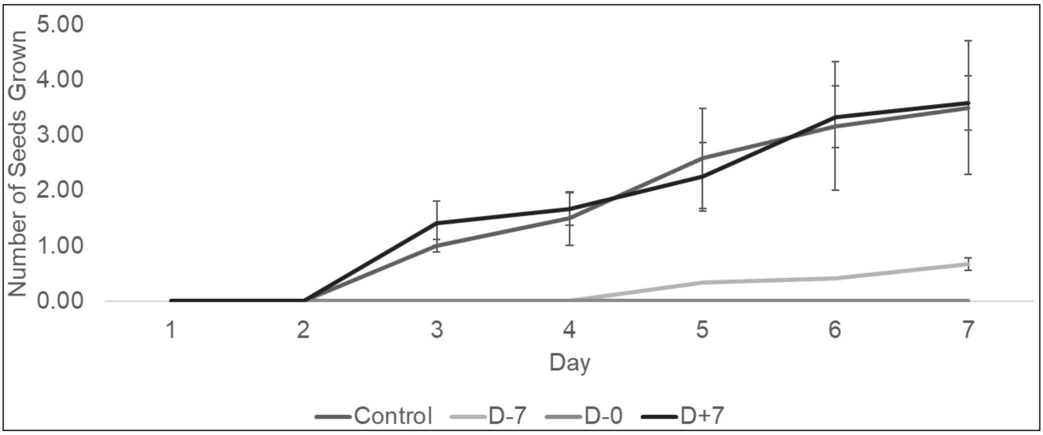



*Figure 4.* Germination rate of *Tridax procumbens* weed seeds at various teak leaf extract application times

The results of this study indicate that teak leaf extract at a concentration of 10% applied at planting time has the potential to be an environmentally friendly bioherbicide, as it inhibits the germination and growth of *T. procumbens* seedlings without disrupting soybean germination and growth. Furthermore, several regions in Indonesia have an abundance of underutilised fallen teak leaves. However, because this study was conducted in controlled environmental conditions (laboratory and greenhouse), further field-scale studies are needed to evaluate the effectiveness and stability of teak leaf extract under natural environmental conditions.

## CONCLUSION

The 10% teak leaf extract treatment showed potential for effective inhibition of *T. procumbens* germination in soybean production under controlled conditions. In addition, application at planting time appeared to be the most effective timing for suppressing *T. procumbens* growth using teak leaf extract as a potential bioherbicide. However, as this study was conducted under controlled environmental conditions in the laboratory and greenhouse, additional field-scale research is required to assess the effectiveness and stability of teak leaf extract in natural environments.

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