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Evaluation of the Aqueous Extracts of *Celosia argentea* L. (Plumosa Group) as a Bioherbicide for Dominant Weeds in Soybean Cultivation

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ABSTRACT

Weed infestation is a major constraint on soybean (*Glycine max* (L.) Merrill) production, necessitating alternative weed management strategies. Plant-derived bioherbicides may offer a viable alternative. This study aims to evaluate the potential of a bioherbicide derived from *Celosia argentea* L. (Plumosa Group) against soybeans (*Glycine max* L. (Merrill)), purple nutsedge (*Cyperus rotundus* L.), and blackjack (*Bidens pilosa* L.). Two experiments were conducted: a laboratory bioassay using a completely randomised design to test extracts from the roots, stems, leaves, and flowers at concentrations of 0, 10, 20, and 40 g L^{-1}, followed by an early growth study in a greenhouse using a completely randomised block design. For the second experiment, the flower extract at a concentration of 40 g L^{-1} was selected due to its inhibitory effect on germination. The flower extract recorded the highest total phenolic content (11.77% w/w). The 40 g L^{-1} treatment reduced germination rates in soybeans (from 73.33% to 38.67%), purple nutsedge (from 84.00% to 48.00%), and blackjack (from 85.33% to 52.00%) in bioassay tests. In early growth studies, the extract affected the plants' morphological, physiological, and anatomical characteristics, such as root development, chlorophyll content, and stomata. These weed species exhibited lower stomatal aperture width and density. The results indicate that the flower extract of *C. argentea* has bioherbicidal potential, and that 40 g L^{-1} was the most effective concentration for inhibiting germination among those tested. Further research is needed to optimise application conditions and enhance weed suppression while minimising negative impacts on soybeans.

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INTRODUCTION

Soybeans (*Glycine max* (L.) Merrill) are one of the most important food crops and

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sources of plant-based protein in Indonesia. In 2020, per capita soybean consumption reached 11-12 kg per year, with domestic production at 0.63 million metric tons, lower than the demand of 3.29 million metric tons (Harsono et al., 2022). Soybean imports reached 2.670.086 metric tons in 2021 (Sibuea et al., 2024). Competition with weeds for water, nutrients, light, and growing space is a limiting factor for soybeans (Bidira et al., 2021). This competition can reduce crop yields by 18-76% (Setyowati et al., 2025). *Bidens pilosa* L. is a fast-growing weed that produces more than 30.000 seeds per plant (Chauhan et al., 2019). This weed has been reported to dominate soybean fields in several regions, such as Zimbabwe and the United States (Chivinge & Schweppenhauser, 1995). The leaves of C4 weeds in soybean cultivation, such as *Cyperus rotundus* L., are adapted to high light intensity (Peerzada, 2017). This weed is difficult to control because it can regrow from tubers and rhizomes (Peerzada, 2017). The allelopathic effects of purple nutsedge on the growth of other plants have been reported (Benvenuti, 2025).

Chemical herbicides are the most common way of controlling weeds. However, the overuse of herbicides can pollute air, water, and soil and affect the balance of soil microorganisms and ecosystems (Baćmaga et al., 2024). Therefore, sustainable alternatives such as plant-based bioherbicides can be developed. *Celosia argentea* L. contains bioactive compounds such as cyclic peptides, saponins, phenols, fatty acids, amino acids, and minerals (Zhang et al., 2016). Various phenolic compounds have been studied because of their different structures, from simple phenols to complex polymers (Alara et al., 2021).

Previous studies have shown that plant extracts can inhibit weed germination and growth. An extract of *Cosmos sulphureus* significantly reduced the size of the stems, roots, and leaves of purple nutsedge (Respatie et al., 2019). *Ricinus communis* extract was the most effective among extracts of *Piper tuberculatum*, *Dipteryx lacunifera,* and *Jatropha gossypiifolia* in inhibiting the germination of *Bidens bipinnata* (Lopes et al., 2022). In a study by Uyun et al. (2024), a 40% concentration of wedelia leaf extract was able to inhibit the germination and growth of purple nutsedge. Research on Wedelia extract using the same ingredients and concentration showed that it could inhibit the growth of purple nutsedge and soybeans when applied at planting (Abdillah et al., 2026). Kusumawati et al. (2026) reported that at a 10% concentration, teak leaf extract inhibited the germination of *Tridax procumbens*. Research on bioherbicides also reports that chrysanthemum stem and leaf extracts at a concentration of 40 g L^{-1} can inhibit the growth of coat button weeds (Yumna et al., 2026).

Physicochemical characteristics and biological activity of *C. argentea* extract as a potential bioherbicide. Total phenolic content was determined using the Folin-Ciocalteu method and expressed in gallic acid equivalents (Pérez et al., 2023). Measurements of physicochemical properties related to water availability and plant physiological responses,

such as pH, electrical conductivity, and osmotic potential (Mashreghi et al., 2024). Extract efficiency was assessed through germination rates and vigour indices of soybeans, purple nutsedge, and blackjack (Li et al., 2025). Early growth can be evaluated based on plant height, number of leaves, root length, root area, leaf area, fresh weight, dry root weight, and dry shoot weight (Wang et al., 2025). Leaf greenness serves as an indirect proxy for chlorophyll content, which is measured using a SPAD meter (Vishwakarma et al., 2023). Spectrophotometric estimates of chlorophyll a, chlorophyll b, and total chlorophyll were conducted to assess potential disruptions to photosynthesis (Hussain et al., 2024). Studies examining stomatal anatomy indicate that stomatal density and stomatal aperture width influence transpiration characteristics and gas regulation (Liu et al., 2024).

This study was designed to evaluate the physicochemical properties of extracts from various parts of *C. argentea* as well as the effect of flower extracts on the germination and early growth of weeds and soybeans. The hypothesis proposed is that the response of weeds and soybeans to the extract treatment will differ. The main concerns are the ability to inhibit weed growth and the potential for phytotoxicity in cultivated plants. The results of this study are expected to provide a scientific basis for the development of plant-based bioherbicides. The uniqueness of this study lies in the combination of physicochemical characterisation of the extracts with a comparative evaluation of their inhibitory effects on weeds and soybeans as bioherbicide candidates. However, the physicochemical properties of *C. argentea* require further investigation. To develop bioherbicides, studies are needed on the effects of *C. argentea* on target weeds and soybeans. This study was designed to evaluate the physicochemical properties of extracts from various parts of *C. argentea*, as well as the effects of these extracts on the germination and early growth of weeds and soybeans. The hypothesis of this study is that weeds and soybeans will respond differently to the extract treatments. The main challenges faced are estimating weed growth inhibition and the potential for phytotoxicity toward cultivated plants. It is hoped that this study will provide a scientific basis for the development of plant-based bioherbicides. The uniqueness of this study lies in the combination of physicochemical characterisation of the extracts with a comparative evaluation of their effects on the growth of weeds and soybeans.

Literature Review

The present study is in agreement with earlier reports on plants as potential bioherbicides. *Cosmos sulphureus* greatly reduced purple nutsedge growth, especially in stem and root size (Respatie et al., 2019). In addition, Lopes et al. (2022) tested several plants as bioherbicide extracts, and the Ricinus communis extract showed the highest efficiency in inhibiting the germination of Bidens bipinnata. Also, Uyun et al. (2024) show that wedelia leaf extract at 40% concentration can inhibit the germination and growth of purple nutsedge. It was reported that the extract inhibited the growth of purple nutsedge and soybean at

planting using the same material and concentration (Abdillah et al., 2026). Moreover, a 10% concentration of teak leaf extract can inhibit the germination of *Tridax procumbens* (Kusumawati et al., 2026). The buttonweed growth can be inhibited by chrysanthemum stem and leaf extracts at 40 g L-1 (Yumna et al., 2026). This study presents information on the potential of *C. argentea* extract as a bioherbicide. The objective of the present study was to determine physicochemical characteristics of red *C. argentea* and its effects on germination, early growth, and physiological characteristics of soybean, purple nutsedge, and blackjack plants. The differential sensitivities of the three species to the extract indicate the importance of evaluating weed-inhibition effectiveness and selectivity. Growth of roots and shoots, leaf area, chlorophyll content, and stomatal characteristics were also measured to elucidate the plants' response to the extract treatment and potential disturbances in physiological processes.

MATERIALS AND METHODS

Plant Cultivation

The research was conducted in September 2023 in Ngaditirto Village, Selopampang Subdistrict, Temanggung Regency, Central Java, Indonesia (7.374889° LS, 110.142852° BT) using *Celosia argentea* L. (Plumosa Group). The place is situated at an altitude of 960 m above sea level with an average temperature of 23°C. Seeds of *C. argentea* were sown in 10 × 10 cm polybags. Manure was used as a base fertiliser. *C. argentea* seedlings were transplanted to the field at 2 weeks of age with a planting spacing of 20 cm × 20 cm. NPK fertiliser was applied 30 days after planting. Pesticides were sprayed whenever pests and diseases attacked the *C. argentea* plants. The *C. argentea* plants were harvested by pulling up the roots after 120 days. Then the plant parts were separated (roots, stems, leaves, flowers). These parts were dried in an oven at 48 °C until completely dry. The dried material was ground in a grinder into a fine powder. The test plant was the Anjasmoro soybean variety, selected by mass selection from the Mansuria inbred line population. This variety is resistant to leaf rust and has pods that do not split easily. It has a flowering period of 35.7-39.4 days and a pod maturity period of 82.5-92.5 days, with a plant height of 64-68 cm. Meanwhile, purple nutsedge and blackjack weeds were collected from Tri Dharma University Experimental Garden, Banguntapan, Bantul, Special Region of Yogyakarta, which is an area with high soybean cultivation intensity.

Treatment and Experimental Design

Bioassay

The bioassay was conducted at the Crop Production Management Laboratory, Faculty of Agriculture, Gadjah Mada University. In this study, the red flowers of *C. argentea* were used

based on the results of physicochemical extract testing. This study employed a completely randomised design (CRD) with two factors: test plant species, namely soybeans and weeds (purple nutgrass and blackjack). The second factor was concentration, namely 0, 10, 20, and 40 g L^{-1}. In this study, the red flower parts of *C. argentea* were used. The results of the physicochemical analysis indicated that these parts had the greatest potential as a bioherbicide. The experiments were conducted in Petri dishes with a diameter of 6 cm. Each Petri dish contained 10 seeds. Each treatment combination was repeated three times. Each seed in every Petri dish was treated with 2 mL of extract using a measuring pipette. To maintain moisture, 5 mL of additional liquid was added per Petri dish until day 14. Germination was allowed to proceed for 14 days, after which the seeds... The bioassay was conducted at the Plant Production Management Laboratory, Faculty of Agriculture, Gadjah Mada University. In this study, the red flowers of *C. argentea* were used based on the results of physicochemical extract testing. This study employed a completely randomised design (CRD) with two factors: test plant type, namely soybeans and weeds (purple nutsedge and blackjack). The second factor was concentration: 0, 10, 20, and 40 g L^{-1}. In this study, the red flower parts of *C. argentea* were used. The results of physicochemical analysis showed that these parts had the greatest potential as a bioherbicide. The experiments were conducted in Petri dishes with a diameter of 6 cm. Each Petri dish contained 10 seeds. Each treatment combination was repeated three times. Each seed in each Petri dish was treated with 2 mL of extract using a measuring pipette. To maintain moisture, 5 mL of water was added to each Petri dish until day 14. Germination was allowed to proceed for 14 days, after which the seeds were harvested.

Early Growth Study

This early growth study was conducted in the greenhouse of the Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia. The greenhouse environmental conditions included an average temperature of 31 °C, relative humidity of 60%, and light intensity of 25,000 lux. This early-growth study was conducted to determine the effects of bioherbicides on soybeans and weeds (purple nutsedge and blackjack). The experimental design used was a randomised complete block design (RCBD) with two factors. The first factor was the type of test plant, namely soybeans and weeds (purple nutsedge and blackjack). The second factor was the bioherbicide treatment with red-flowered *C. argentea* at concentrations of 0 and 40 g L^{-1}. These concentrations were selected based on recommendations from a previous bioassay indicating that 40 g L^{-1} could inhibit weed growth. Each treatment combination was replicated three times. Thus, there were 18 germination trays, each containing 25 holes. Consequently, the total number of experimental plants was 450.

In this study, *soybean* seeds of the Anjasmoro variety, uniformly sized purple nutsedge tubers (0.5 g), and blackjack seeds were used. The soil was sterilised by drying in an oven at 100 °C before being placed in the germination trays. Before use, the germination trays were tightly sealed with black tape to minimise loss of the bioherbicide solution. Next, the seedlings were transferred to the germination trays, with one seedling per hole. The bioherbicide solution was applied once at 0 DAS (days after planting) by adding 5 mL to each hole in the tray. Watering was performed every three days using a sprayer to maintain adequate soil moisture.

Extraction

The roots, stems, leaves, and flowers of red *C. argentea* were dried to a constant weight in an electric oven at 40 °C for 48 hours. After the dried plant parts were collected, they were ground into a powder and stored in airtight plastic bags. The plant material powder was extracted with 1000 mL of distilled water adjusted to predetermined concentrations: 0 grams for the control treatment, 10 grams for the 10 g L^{-1} treatment, 20 grams for the 20 g L^{-1} treatment, and 40 grams for the 40 g L^{-1} treatment. After 24 hours, the solutions were filtered using Whatman No. 1 filter paper (Uyun et al., 2024).

Analytical Procedures

Total Phenolic Content

The Folin-Ciocalteu reagent was used to measure the total phenolic content. To do this, 50 mg of the sample was mixed with 0.5 mL of the reagent and 7.5 mL of distilled water. After mixing the concoction, it was incubated for ten minutes. Next, 1.5 millilitres of 20% sodium carbonate was added. After shaking the mixture once again, the solution was allowed to stand for ten minutes. The ultimate volume was 10 mL when distilled water was added. The OD_{760} of the solution was measured after it had been diluted 25 times (Kupina et al., 2019).

pH Measurement

The pH of all extracts was measured with a calibrated pH meter to assess the acidity level.

Electrical Conductivity (EC) and Osmotic Potential (Ψs)

The EC of the extracts was measured utilising an EC meter to determine the total dissolved salt content. The Ψs were calculated by multiplying the EC value by -0.036 and expressed in megapascals (MPa) (McDonald & Wilson, 1979).

Observed and Data Collection

Germination Parameters

Germination was observed at 14 DAS during the germination and early growth studies.

The germination percentage (%) was calculated as in Equation 1 (International Seed Testing Association [ISTA], 1999):

$$Germiation\ Percentage\ (\%) = \frac{Number\ of\ total\ germinated\ seeds}{Total\ number\ of\ seeds} \times 100\% \quad [1]$$

Vigour was observed daily, and the index was ascertained using the following formula (ISTA, 1999) as in Equation 2:

$$Vigour\ Index = \sum \frac{\text{Number of sprouts on day n}}{n} \times 100\% \quad [2]$$

Growth Parameters

The plants were harvested 14 days after planting, and the following growth parameters were measured.

Plant Height

The measurement is taken from the soil surface to the highest point of the plant for soybean and the blackjack weed. The height of the purple nutsedge weed is measured from the base of the crown to the highest leaf tip.

Root Length

The length of the root is measured using a ruler with the unit in cm. The measurement is taken from the base of the root, which is the boundary between the root and the crown, to the tip of the longest root.

Number of Leaves

The quantity of leaves is monitored every day by tallying the leaves produced on each plant. The number of leaves on a soybean plant is determined by counting the trifoliate leaves, but the number of leaves on a blackjack and purple nutsedge plant is determined by counting the leaves that are created on each plant.

Number of Roots

On Soybean and Blackjack plants, observations were made by counting the roots that grow from the main root. On Purple nutsedge, the number of roots was counted based on the roots that grow from the tuber.

Leaf Area

The leaf area is measured using a leaf area meter. The leaves are arranged on white paper, then placed on the leaf area meter with sufficient lighting. Then, click on measurements to show numbers; the units of measurement on the leaf area meter are mm2.

Root Area

The root area is measured using a leaf area meter. The roots were cleaned of any remaining growing medium and then arranged on the device according to the operating procedure. The roots were placed on white paper and then positioned on the leaf area meter under adequate lighting.

Fresh Shoot Weight

The shoot parts of the plants Soybean, Blackjack, and Purple nutsedge were separated from the root parts, then weighed using an analytical balance. The measurement results are expressed in grams.

Dry Shoot Weight

The shoot part is separated from the root, then dried using an oven at 80 °C until a constant weight is achieved. After that, the samples were weighed using an analytical balance.

Fresh Root Weight

The roots of soybean, blackjack, and purple nutsedge were separated from the canopy, then weighed using an analytical balance. The measurement results are expressed in grams.

Dry Root Weight

The roots are separated from the shoot, then dried in an oven at 80 °C until a constant weight is achieved. The next sample is weighed using an analytical scale. The measurement results are expressed in mg per plant.

Physiological Parameters

Physiological parameters were observed at the final harvest (14 days after planting). Observations included leaf greenness, chlorophyll a, chlorophyll b, and total chlorophyll. Greenness of the lower, middle, and upper leaves was measured with a SPAD meter. The chlorophyll content was determined by grinding 1 g of leaf tissue with a mortar and pestle and mixing it with 20 mL of 80% acetone. The solution was filtered through a Whatman No. 1 filter paper, transferred to a test tube, and the OD_{663} and OD_{645} were measured utilising a spectrophotometer (Lichtenthaler, 1987) as in Equations 3 to 5:

$$\text{Chlorophyll a (mg g}^{-1}) = (0.0127 \times A663 - 0.00269 \times A645) \times 20 \text{ mL} \quad [3]$$

$$\text{Chlorophyll b (mg g}^{-1}) = (0.00229 \times A645 - 0.00468 \times A663) \times 20 \text{ mL} \quad [4]$$

$$\text{Total chlorophyll} = ([20.2 \times A645]) + (20 \text{ mL}/[1000 \times 1 \text{ g}]) \text{ mg g}^{-1} \text{ of leaf fresh weight.} \quad [5]$$

Leaf Anatomy

Stomata were observed at 14 DAS utilising the leaf imprinting method. The lower leaf surface was coated with nail polish and left to dry for 10 min. The dried polish layer was carefully removed using adhesive tape and placed on a glass slide. Stomatal density and opening width were observed with a microscope at 10× and 40× magnification (Paul et al., 2017).

Data Analysis

All data were analysed using analysis of variance at a significance level of $\alpha = 0.05$. We applied Tukey's honestly significant difference (HSD) test at a 95% confidence level when significant differences were detected. A contrast test was employed to compare the bioactivities of *C. argentea* flower extract with those of standard compounds. Regression analysis assessed relationships between variables and treatments. Statistical analyses were performed using the agricolae package in R software version 3.2.2 (https://cran.r-project.org/web/packages/agricolae/).

RESULTS

C. argentea, known as the cockscomb plant, is an ornamental species that is popular due to its beauty. It has a short life cycle and has potential bioherbicidal properties as it

approaches senescence. The study revealed that the flower extract of *C. argentea* had the highest phenolic content. It can be applied at planting at a concentration of 40 g L^{-1} to inhibit germination of both soybeans and weeds. The results indicate that flower extract of *C. argentea* could be a bioherbicide. However, from this study, the selectivity to soybean plants should be considered, as it can inhibit germination at planting time.

Organ Analysis

The increased bioherbicide concentrations increased the osmotic pressure, leading to hyperosmotic conditions that favored Na^+ and Cl^- accumulation, leading to physiological disturbances. Na^+ accumulation inhibits enzymes involved in photosynthetic pigment biosynthesis, leading to chloroplast membrane degradation and oxidative stress, thus reducing chlorophyll content (Laamari et al., 2023). The results of this research showed the potential of bioherbicides on the flower of *C. argentea*.

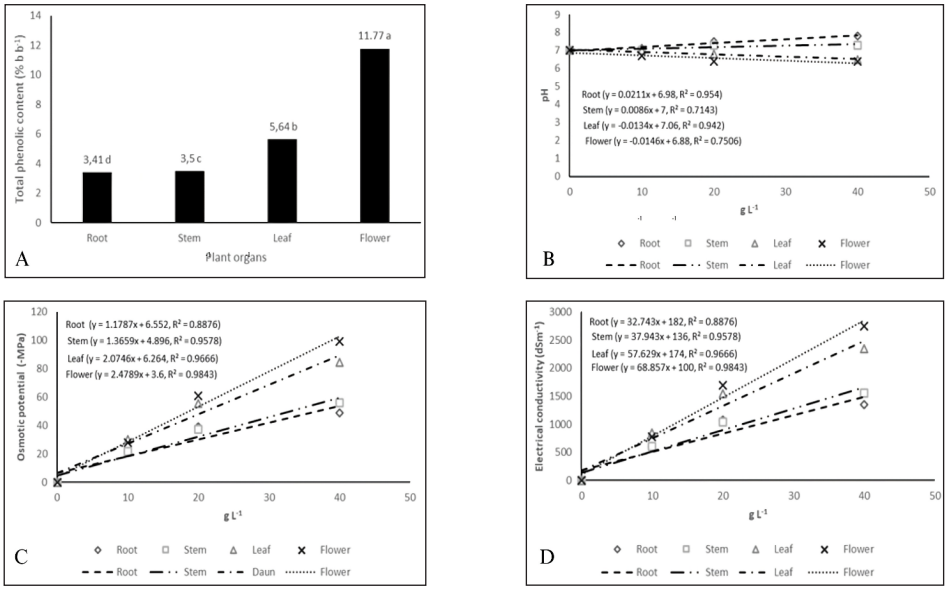


Figure 1. (A) Total phenolic content of the different plant organs (Tukey's test, $p \leq 0.05$); (B) pH; (C) osmotic potential; and (D) electrical conductivity as a function of the concentration of each *Celosia argentea* L. organ extract

Analysis of phenolic content showed that phenolic compounds were most highly concentrated in the generative parts, namely the flowers. The results of the analysis show that the red flower parts have the highest phenolic content (Figure 1A), at 11.77% w/w. In contrast, the root parts exhibit the lowest phenolic content at 3.41% w/w. The phenolic content in the stems is 3.5% w/w, while that in the leaves reaches 5.64% w/w. Differences in

phenolic content among plant parts are caused by variations in the distribution of secondary metabolites within plant tissues, indicating that each organ plays a distinct physiological role in the synthesis and accumulation of these compounds. Phenolic levels in leaves and flowers exhibited a concentration-dependent reduction with pH (Figure 1B), suggesting substantial phenolic acid levels. The flowers also demonstrated the highest EC, likely due to elevated concentrations of dissolved salts. According to Aslam et al. (2016), increased EC can induce cell leakage in plant tissues. This is attributable to the positive correlation between osmotic potential (Figure 1C) and electrical conductivity (Figure 1D).

Bioassay

The flower extracts showed the highest phenol content, EC, Ψs and the most acidic pH among all the plant organ extracts tested, indicating a strong bioherbicidal potential. In the first phase of testing, seeds of soybean, purple nutsedge, and blackjack were treated with flower extracts at 0, 10, 20 and 40 g L^{-1} (Figure 2). The extracts greatly reduced germination percentage (Figure 2A). The highest inhibition was observed at 40 g L^{-1}, corresponding to the highest phenol content. The seeds need water for imbibition during germination. Excess phenol inhibits this process and inhibits metabolic activity.

The results of the study show that increasing the concentration of the flower extract of *C. argentea* can reduce the germination percentage of soybeans, purple nutsedge, and blackjack. Based on the results, regression equations were found for the variables germination percentage (Figure 2A) and vigour index (Figure 2B). This indicates that an increase in the concentration of the flower bioherbicide *C. argentea* can reduce the germination percentage and vigour index. Li et al. (2010) showed that allelochemicals such as phenols lower cell membrane permeability, inhibit the transfer of metabolites from the endosperm to the growth points, and inhibit cell division and germination. The results show that the vigour index of the soybean is higher even with an increase in the concentration of the bioherbicide. This is because soybean seeds have a high rate of imbibition, which allows them to break dormancy quickly.

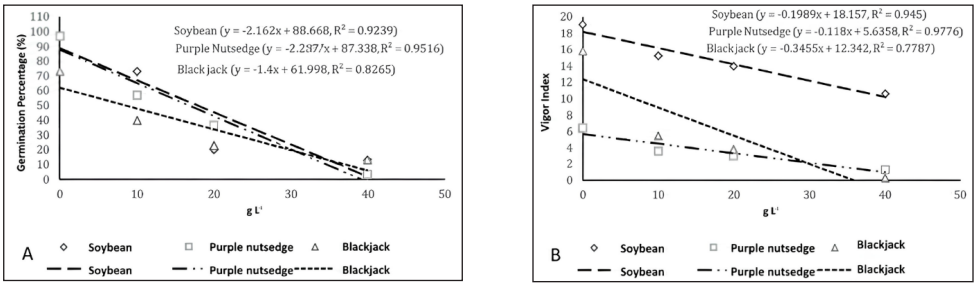


Figure 2. (A) Germination percentage (%) and (B) vigour index of soybean, purple nutsedge, and blackjack seeds under *Celosia argentea* L. extracts of different organs and concentrations. Values with varying letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

Early Growth

Based on the germination test results, 40 g L^{-1} flower extract was identified and used as the most effective inhibitory concentration for subsequent experiments.

Germination of Soybeans, Purple Nutsedge, and Blackjack

Treatment with flower extracts significantly reduced germination rates and vigour indices in purple nutsedge and blackjack, but less and insignificantly in soybeans. This variation is attributed to higher food reserves in the latter, mitigating bioherbicide-induced stress. In contrast, germination in purple nutsedge and blackjack declined markedly, most likely due to seed coat distortions and suppressed embryonic root elongation (Liu et al., 2021).

The germination rate for soybeans decreased from 73.33% to 38.67%, for purple nutsedge from 84% to 48%, and for blackjack from 85.33% (control) to 52% (Figure 3A). Similarly, the vigour index (Figure 3B) decreased significantly in purple nutsedge (8.82) and blackjack (8.67), while soybean vigour was unaffected, remaining at 14 following application of *C. argentea* at a concentration of 40 g L^{-1} (Figure 3B). Treatment with the flower extract significantly reduced the vigour index in barnyard grass and crabgrass, but the reduction was smaller and not significant in soybeans. This variation is due to the higher protein content in soybeans, which increases the randomness of germination over a relatively short period. The high imbibition rate in soybeans causes germination to occur more rapidly, as the water absorbed by the soybean seeds breaks the dormancy present in the seeds. Water absorption in the presence of high phenolic content results in a decrease in germination percentage (Figure 3A). However, the vigour index of purple nutsedge and blackjack weeds was significantly reduced, probably because of the seed coat distortion and inhibition of embryonic root elongation (Liu et al., 2021).

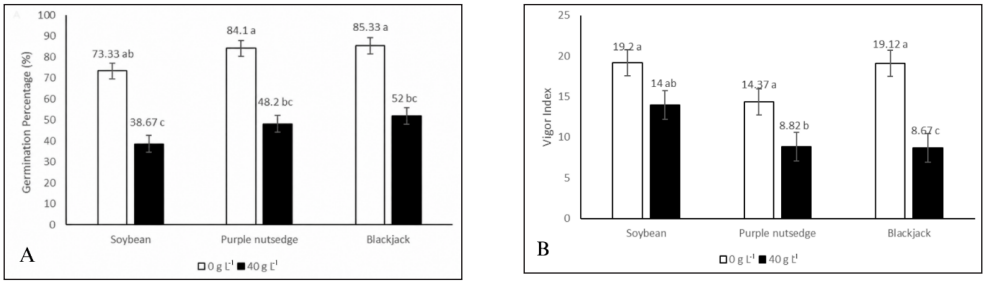
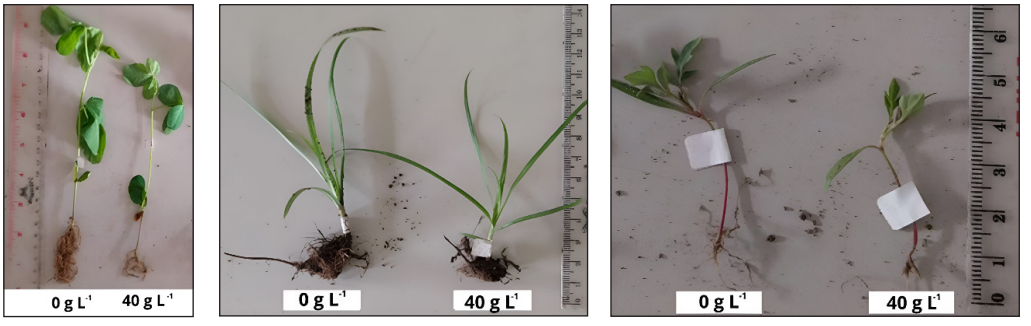


Figure 3. (A) Germination percentage (%) and (B) initial growth vigour index under treatment with 0 and 40 g L^{-1} *Celosia argentea* L. flower extracts. Values with different letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

Effect of Bioherbicide Application on Plant Morphology

The application of 40 g L^{-1} flower extract resulted in a reduction of plant height, leaf number, leaf area, and root area in all species (Figure 4). The reduction in height is ascribed to the suppression of photosynthesis and limited biosynthesis of carbohydrates (Powles & Yu, 2010). Leaf expansion was inhibited by allelopathic compounds (Rahmawati, 2024). The extract of the red flower highly suppressed root growth, with blackjack weeds having the least root area. Oxidative stress.

Treatment with 40 g L^{-1} of cockscomb flower extract affected the height of soybean plants (Figure 4A). Soybean plants treated with a concentration of 40 g L^{-1} were shorter and had smaller roots compared to those treated with 0 g L^{-1}. The same was observed in purple nutsedge (Figure 4B), where the *C. argentea* flower extract had a significant effect on plant height. In blackjack, the canopy and root sizes were smaller compared to the 0 g L^{-1} treatment (Figure 4C). Visually, it is evident that the response to the application of *C. argentea* extract at 40 g L^{-1} was consistent across all experimental plant species. This reduction in plant height was caused by the plants' response to the herbicide, which reduced the rate of photosynthesis and, consequently, carbohydrate biosynthesis (Powles & Yu, 2010). On day 14, the number of leaves, leaf area, and root area were reduced compared to the control group.



(A) (B) (C)

Figure 4. Effect of bioherbicide application on plant morphology in (A) soybean (B) purple nutsedge, and (C) blackjack. *Celosia argentea* L. flower extracts were used at 0 and 40 g L^{-1}

The application of bioherbicides affects the surface of the root hypocotyl. The thin cuticle allows phytotoxic compounds to penetrate root cells. This absorption causes changes in the cell cycle, membrane damage, and alterations in cell structure. Membrane damage results in reduced root elongation capacity (Figure 5B). In addition, the size of the metaxylem cells decreases due to these phytotoxic compounds (Hasan et al., 2021), which also disrupts cell elongation. Figure 5A shows a decrease in plant height when the bioherbicide *C. argentea* was applied at a concentration of 40 g L^{-1}. Inhibition of root

formation was caused by root damage, as evidenced by a reduction in root area (Figure 5D) and root number (Figure 5F) following treatment with flower extract at a concentration of 40 g L^{-1}. The reduction in root area resulted in decreased nutrient uptake and inhibited new leaf growth, as well as a decrease in leaf area (Figure 5C) and the number of leaves (Figure 5E).

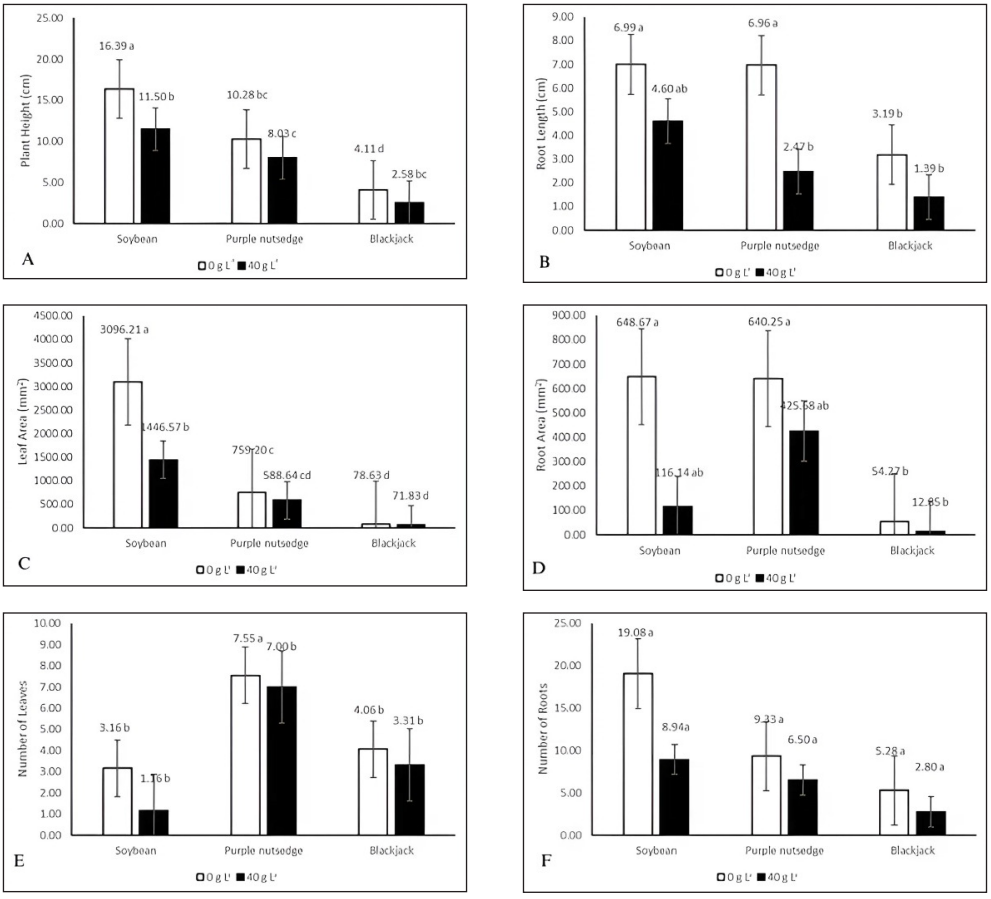


Figure 5. (A) Plant height, (B) root length (C) leaf area (D) root area (E) number of leaves, and (F) number of roots under treatment with 0 and 40 g L^{-1} *Celosia argentea* L. flower extracts. Values with varied letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

The study results showed a reduction in fresh shoot weight, fresh root weight, dry shoot weight, and dry root weight (Figure 6). For the fresh shoot weight variable (Figure 6A), it was observed that the application of the bioherbicide derived from the flower of *C. argentea* at 40 g L^{-1} reduced the shoot weight of soybean, purple nutsedge, and blackjack, with the lowest weight observed in the blackjack weed. The decrease in fresh shoot weight

was accompanied by a decrease in fresh root weight (Figure 6B); in soybeans, fresh root weight decreased from 0.098 to 0.77 g and from 0.585 to 0.116 g when treated with 40 g L^{-1} of bioherbicide. The decrease in fresh weight of both shoots and roots reduced shoot dry weight (Figure 6C) and root dry weight (Figure 6D). This decrease in dry weight is due to the plants' inability to produce assimilates under bioherbicide stress. This decrease occurs because the compound interacts with cytoplasmic membrane components, including proteins and lipids. As a result, the apical meristem browns, which can subsequently damage the root system (Kostina-Bednarz et al., 2023).

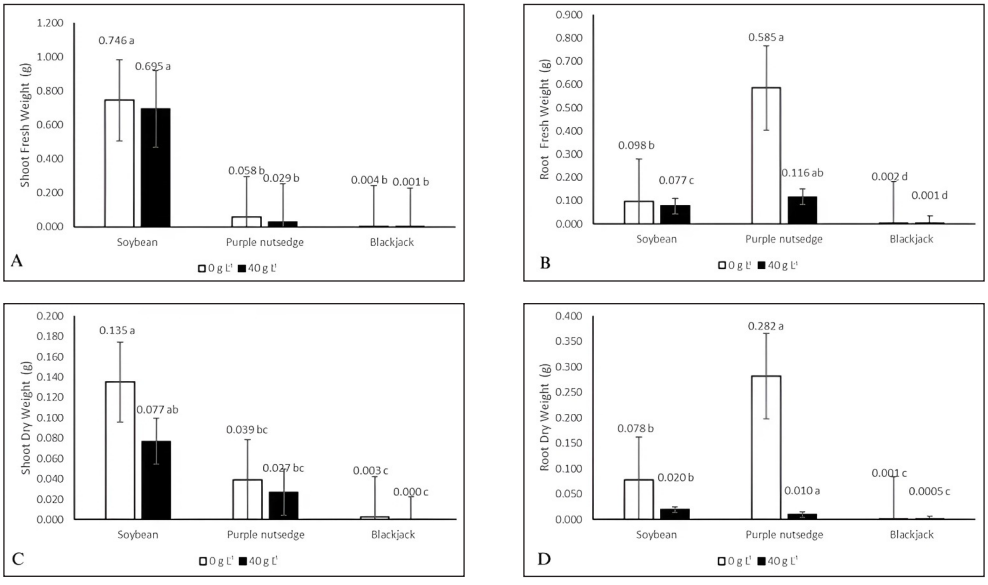


Figure 6. (A) Shoot Fresh Weight (B) root fresh weight (C) shoot dry weight and (D) root dry weights during the early growth phases of soybeans, purple nutsedge, and blackjack under treatment with *Celosia argentea* L. flower extracts at 0 and 40 g L^{-1}. Values with varied letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

Effect of Bioherbicide Application on Plant Physiology

The application of *C. argentea* extract significantly decreased the leaf chlorophyll a, chlorophyll b, and total chlorophyll levels (Figure 7). The reduction may be due to damage to the chloroplast pigments caused by oxidative stress and hence reduced photosynthesis. Reactive oxygen species (ROS) damage the integrity of the cell membrane, leading to leakage and desiccation, ultimately causing cell death (Hasan et al., 2021).

A significant decrease in leaf greenness scores was found in blackjack, with leaf greenness at 0 g L^{-1} showing 31.34 (Figure 7A), while at 40 g L^{-1}, the greenness decreased to 30.15. The chlorophyll a variable (Figure 7B) showed insignificant results in the

combination of *C. argentea* flower extract concentration and plant type, possibly because chlorophyll a levels were less sensitive to bioherbicide stress. Variable chlorophyll b levels (Figure 7C) showed significant results that influenced the decrease in total chlorophyll levels (Figure 7D). The suppression of chlorophyll may be caused by the inhibition of the biosynthesis process or the degradation of chlorophyll components, which can be affected by allelochemicals from bioherbicides (Uyun et al., 2024).

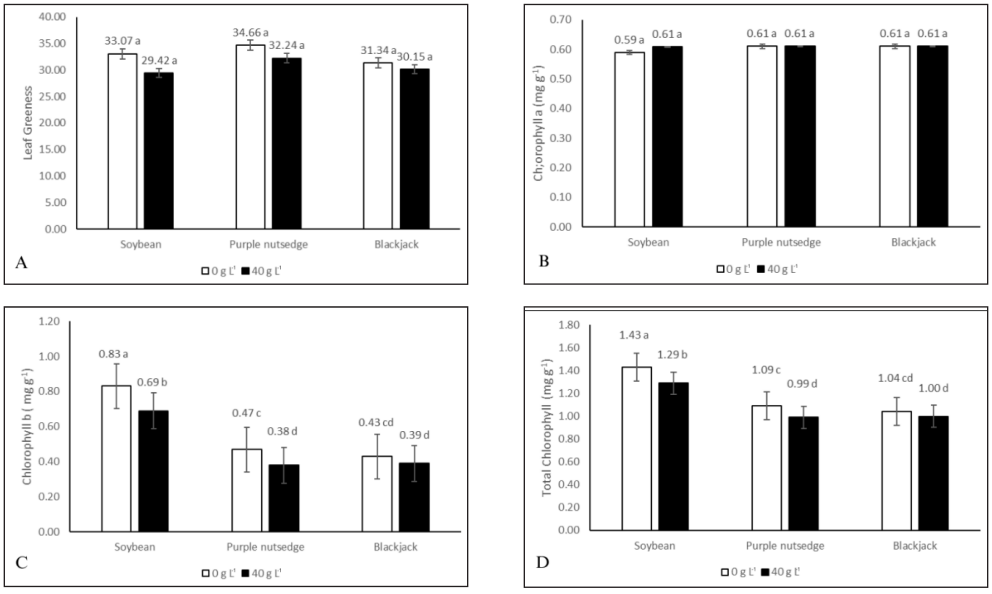


*Figure 7. (*A) Leaf greenness, (B) chlorophyll a, (C) chlorophyll b, and (D) total chlorophyll contents during the early growth phases of soybean, purple nutsedge, and blackjack treated with *Celosia argentea* L. flower extracts at 0 and 40 g L^{-1}. Values with varied letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

Impacts of Bioherbicide Application on Leaf Anatomy

The smallest stomatal aperture width was observed in blackjack weeds treated with flower extracts, while the highest aperture width was recorded in control soybean plants (Figure 8). The higher concentrations of bioherbicide increased the stomatal resistance but reduced the stomatal density, presumably due to the reduced Ψs. Genetic factors influence the variation in stomatal density, and it is important for the regulation of photosynthetic efficiency. Lower stomatal density leads to lower uptake of photosynthetic precursors and therefore lower overall photosynthetic capacity (Yahya et al., 2025).

Such results indicated a significant reduction in the stomatal aperture width (Figure 8A) and density (Figure 8B) in all weeds treated with 40 g L^{-1} of the flower extract in comparison to the control. The higher phenolic content of the extract probably increased the diffusion resistance of the leaf and favored stomatal closure. Also, from the figure, it

can be seen that the density is affected by the width of the aperture, with lower widths corresponding to lower density.

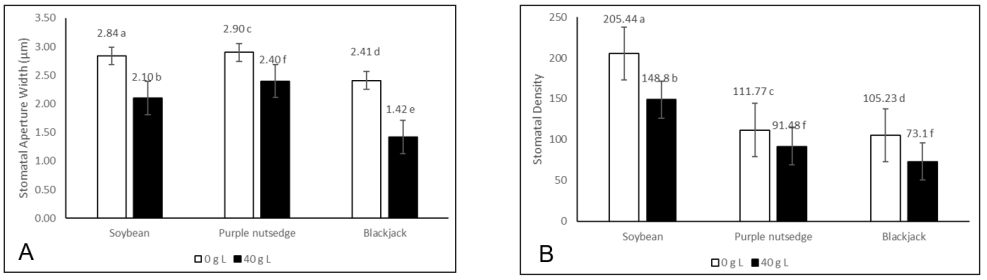


Figure 8. (A) Stomatal aperture width and (B) stomatal density during the early growth phase of soybean, purple nutsedge, and blackjack treated with *Celosia argentea* L. flower extracts at 0 and 40 g L^{-1}. Values with varied letters indicate statistically significant differences (Tukey's test, $p \leq 0.05$)

DISCUSSIONS

This finding is important in evaluating the bioherbicidal potential and possible phytotoxic effects of Celosia argentea flower extract on soybean and weeds. The bioassay and early growth responses of the test species differed, and this may give insight into their susceptibility to the extract. While many plant extracts have been reported as possible bioherbicides, the link between the physicochemical properties of *C. argentea* flower extracts and their inhibitory efficiency on weed germination and growth has not been studied.

The flower extract at 40 g L^{-1} strongly inhibited germination and early growth in soybean, purple nutsedge and blackjack weeds. The phytotoxic compounds inhibited cell division, reduced root area, and disturbed root growth, and consequently restricted nutrient absorption and leaf development. As a consequence of this, the leaf area was decreased, the chlorophyll content was decreased, and the overall productivity of the plant was decreased.

Bioherbicides can affect germination by interrupting imbibition, hydrolysing food reserves, and disturbing cell division and elongation (Wang et al., 2025). Root damage reduces water and nutrient uptake and their translocation in the vascular system (Hoang Anh et al., 2021). The inhibition of cellular respiration and ATP synthesis inhibits plant growth (Kostina-Bednarz et al., 2023). Drought stress is initiated by impaired water uptake and damage to roots and reduces stomatal conductance. Thus the chlorophyll content limits the growth of plants and accumulation of biomass.

The study results showed inhibition of germination processes in soybeans as well as weeds. Kusumawati et al. (2026) reported that the use of teak leaf bioherbicide extract at planting did not suppress soybean germination. However, it did prevent the germination of the weed T. *procumbens*. This is because of the different allelochemical content and

properties of *C. argentea* flowers and teak leaves. Since *C. argentea* produces a non-selective herbicide, it still inhibits germination of soybean when the bioherbicide is applied. Meanwhile, Abdillah et al. (2026) said that the 40% Wedelia bioherbicide is most effective when applied seven days before planting. The bioherbicide under this treatment was selective for soybean germination and inhibited the germination and growth of T. *procumbens* and Purple nutsedge. These studies indicate that soybeans and weeds have varied responses to different bioherbicide formulas and hence more research is required.

CONCLUSION

The physicochemical properties of *Celosia argentea* extracts varied among plant parts, with flower extract showing bioherbicidal potential. The extract inhibited germination and early growth of soybean, purple nutsedge, and blackjack. The early development of the plant was affected, as evidenced by the changes in root growth, leaf area, chlorophyll content, and stomatal characteristics. The results show the potential of flower extract as a weed controller and the need for crop selectivity. Application of *C. argentea* flower extract at 40 g L^{-1} can be used as a bioherbicide and may also affect the development of soybean. Further evaluation is needed to determine conditions of application under which weed suppression is achieved with minimal crop phytotoxicity. The study looked at germination and early growth in controlled conditions, and these may not completely reflect responses in the field. The roles of individual bioactive compounds and the underlying mechanisms were not fully established.

IMPLICATION OF THE STUDY

The results of the study show that *C. argentea* extract has the potential to be a bioherbicide that can inhibit weed growth in soybean crops. The most effective flower extract in suppressing germination and weed growth is C. *argentea* red flower organ extract with a concentration of 40 g L^{-1}, which shows an inhibiting effect compared to other organs and concentrations. Flower extract at this concentration can inhibit germination through a decrease in germination percentage and vigor index. In the early growth stage, it can reduce the height growth of plants, roots, and leaves. In addition, the application of these extracts can reduce the chlorophyll content and characteristics of the stomata. However, the effect of this extract still affects soybeans, so further research is needed on optimising the phytotoxic effect on weeds by minimising phytotoxic effects on soybeans. These findings are the basis for the development of weed control strategies that support environmentally friendly agriculture.

LIMITATION AND RECOMMENDATION FOR FUTURE RESEARCH

The current study has limitations that should be considered in future research. In this study, soybeans have not yet been selected for bioherbicides; therefore, research is needed to determine the right timing for applying red *C. argentea* extract. This research is still experimental in laboratories and greenhouses; therefore, field research is needed to validate the effects of *C. argentea* bioherbicides under field conditions. In addition, *C. argentea* toxicity needs to be increased through genetics, environmental conditions, and management practices that increase the secondary metabolites present in *C. argentea*. The follow-up research I recommend focuses on using nanotechnology to improve the effectiveness and efficiency of bioherbicide absorption by weeds. It is hoped that with this research, bioherbicides can be accepted by the community as environmentally friendly weed control. This follow-up research is expected to provide a comprehensive understanding of agricultural practices that use local resources for sustainable weed management strategies.

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