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Allelopathy Potential of Jamaican Cherry (Muntingia calabura L.) Leaf Extract in Inhibiting the Germination and Growth of Amaranthus spinosus L. Weed

Rita Wahyuningtiyas, Sri Darmanti*, Endah Dwi Hastuti

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ABSTRACT

This study examined the effects of an aqueous extract of *Muntingia calabura* L. leaves on the inhibition of germination and growth of *Amaranthus spinosus*. The study used a single-factor Completely Randomized Design, namely, the concentration of extract with treatment levels of 0, 2, 4, and 6% for germination and 0, 10, 20, and 30% for growth. Data were analyzed using Analysis of Variance and Duncan's Multiple Range Test at a confidence level of 95%. The results showed that treatment with the aqueous of leaf extracts decreased the percentage of germination, germination index, radicle, and stem length of the sprouts and slowed the germination time. During growth, it decreased the plant height, number of leaves, fresh weight, dry weight, and extended flowering time. The higher the concentration of the extract, the greater the decrease in the germination and growth of the weed. At concentrations of 10 and 20%, the contents of chlorophyll a, chlorophyll b, and carotenoids increased, but at a concentration of 30%, the contents began to decrease. At a concentration of 6%, germination decreased by 75%, germination index decreased by 87.47%, sprout length decreased by 27.51%, radicle length decreased by 26.20%, and germination time increased by 14.74%. At a concentration of 30%, the plant height was 6.48%, the number of leaves was 38.13%, the fresh weight was 20.64%, and the dry weight was 18.36%; however, the flowering time increased by 40%.

Keywords: Allelochemicals, germination, growth, inhibition, *Muntingia calabura* L

INTRODUCTION

Plant cultivation is often constrained during the growth period owing to the presence of weeds. According to Winarsih (2019), the weeds in agricultural land decreases the growth, production, and quality of agricultural products. Spinach is a weed that grows on agricultural lands. According to Iamonico (2015), spinach (*Amaranthus spinosus*) is a weed belonging to the genus Amaranthus, which usually grows on agricultural land and causes a decrease in agricultural production. Additionally, Fatimah *et al.* (2013) spinach weed is one of the seven types of weeds that grow widely in Indonesia, especially on the Java Island. Weeds can be controlled using various methods and synthetic herbicides are often used. However, chemical control has negative effects on the surrounding environment. Chemical herbicides have been directly associated with environmental pollution (Singh *et al.* 2020).

An alternative method for overcoming the presence of weeds is the use of natural herbicides (bioherbicides). Bioherbicides are organic compounds that can be used to control or kill weeds. Their ingredients come from natural materials in the form of plants, so they do not pollute the environment and are relatively safe for humans or other living organisms (Budiyanto 2017). The natural material that can be used is kersen (Jamaican cherry, *Muntingia calabura* L.) leaves, which are easily found in the surrounding environment and contain secondary metabolites, which are allelochemical compounds. Plants can release secondary metabolites that have positive or negative effects on the functioning of plants or other surrounding organisms (Ashraf *et al.* 2017). The compounds were identified as allelochemicals that included flavonoids, alkaloids, phenolics, momilactone, jasmonate, glucosinolic, hydroxamic acids, brassinosteroids, amino acids, terpenoids, and carbohydrates. Kersen leaves qualitatively contain flavonoid components, triterpenoids, tannins, saponins, and steroids (Pujaningsih *et al.* 2019), alkaloids (Fariestha *et al.* 2018), sterols, glycosides (Buhian *et al.* 2016), quercetin (Pelaez *et al.* 2018), triterpenoids, and polyphenol compounds that exhibit antioxidant activity (Aligita *et al.* 2018).

Allelochemicals are non-nutrient chemicals produced by plants as secondary metabolites that can inhibit the germination or growth of the surrounding plants (Cotrut 2018). These compounds inhibit weed growth and stomatal closure, interfere with mineral absorption, affect photosynthesis and respiration, cause water pressure, and interfere with enzymatic activity (Ashraf *et al.* 2017). The mechanism of allelochemical inhibition begins after the target plant is

Faculty of Science and Mathematics, Universitas Diponegoro, Semarang 50275, Indonesia
* Corresponding Author:
Email: sridarmanti@lecturer.undip.ac.id

exposed to allelochemicals, causing the plant to quickly produce reactive oxygen species (ROS) in the contact area and alter the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and ascorbic acid peroxidase (APX) to combat oxidative stress. This leads to an imbalance in the antioxidant systems of the plants (Cheng and Zhihui 2015). ROS play two important roles in plants depending on their concentration. Low ROS concentrations are beneficial because they play a role in molecular signaling when plants are under stress. By contrast, high ROS concentrations can damage various cellular components (Kapoor *et al.* 2019).

Jayalath *et al.* (2021) found that kersen leaf dry powder extract at a concentration of 10% showed the highest inhibiting effect on lettuce germination with a germination power of 22–23%. Therefore, this study aimed to determine the potential of kersen leaf aqueous extract at various concentrations as a bioherbicide for inhibiting germination and growth, and to determine the optimal concentration that can inhibit germination and growth.

METHODS

A Shimadzu 1240 UV-VIS spectrophotometer was used as an analytical tool. Kersen leaves (*Muntingia calabura* L.) and spinach seeds (*Amaranthus spinosus* L.) were obtained from the campus area of Diponegoro University.

The leaves were extracted according to the procedure described by Wardhani *et al*. (2018). A 100% extract concentration was prepared by smoothing the leaf cuttings and distilled water at a ratio of 1:1 (v/v). The fine leaves were placed in an Erlenmeyer flask, stored in a refrigerator for 24 h, and filtered using cloth and filter paper. Furthermore, according to the requirements of the study, it was diluted with distilled water to obtain extracts with concentrations of 2, 4, and 6% for germination observation, and concentrations of 10, 20, and 30% for growth observation.

The seeds were germinated in a 90 mm-diameter Petri dish with a 3-layer filter paper base. Each dish was filled with 10 spiny spinach seeds that were selected based on their uniform size and color. Spinach seeds in each dish were dripped with 10 drops of aqueous extract according to the label (0, 2, 4, and 6%). The extract was administered every 6 h, and the treatment was discontinued after seven days of experimentation.

Germination was monitored every six hours. The observed parameters were germination percentage (%G), average germination time (MGT), germination index (GI), root length, and stem germination. Germination parameters were measured using the formulas of Zhang *et al.* (2020) and Thongtip *et al.* (2022), as follows:

$$\%\mathrm{G} = \frac{\text{Number of germinated seed}}{\text{Total number of seed}} \quad \text{.....(1)}$$

$$\mathrm{MGT} = \frac{\sum \mathrm{Gt.Tt}}{\sum \mathrm{Gt}} \quad \text{................................(2)}$$

$$\mathrm{GI} = \sum \frac{\mathrm{Gt}}{\mathrm{Tt}} \quad \text{......................................(3)}$$

where:
Gt = Number of seeds germinated on the *t*-day
Tt = *t*-day

The growth treatment began with the selection of old and dried spinach seeds that were soaked for 24 h and then sown. Sowing was stopped after three weeks, and the weeds reached a height of ± 8 cm. Seedlings were selected based on the same height and number of leaves. Twenty homogeneous weed seedlings were collected, planted in polybags, and acclimatized for 1 week.

Spinach weeds were sprayed periodically using the aqueous extract at different concentrations (0, 10, 20, and 30 %). Ten sprays were made on the upper and lower surfaces of the leaves every three days in the morning. The treatment was administered 14 times during the 6 weeks of the trial and discontinued 42 DAP (day after planting. Each experiment was repeated five times.

The observed growth parameters included plant height, number of leaves, fresh weight, dry weight, chlorophyll a, chlorophyll b, and carotenoid content. Observations were made from the 1st to 6th week of the experiment.

The preparation of the extract for the analysis of pigment content was based on a study by Bukhari *et al.* (2019). Fresh leaf samples (0.5 g) were weighed, ground in an acetone solution using a mortar & pestle, and filtered. The filtrate volume was 10 mL. The pigment content of the extracts was analyzed using spectrophotometer at wavelengths (λ) of 646 nm, 663 nm, and 470 nm. Pigment content was calculated according to Wellburn (1994):

$$Ca = 12.21\,A663 - 2.81\,A636 \quad \text{.......... (1)}$$
$$Cb = 20.13\,A646 - 5.03\,A663 \quad \text{.......... (2)}$$
$$C_{X+C} = \frac{1000\,A470 - 3.27\,Ca - 104\,Cb}{198} \quad \text{........... (3)}$$

where:
A = Absorbance
Ca = Chlorophyll a
Cb = Chlorophyll b
C x+c = Carotenoid

The data obtained from the study were analyzed using Analysis of Variance (ANOVA), followed by

Duncan's Multiple Range Test (DMRT). Statistical analysis was performed using IBM SPSS Statistics 27.

RESULTS AND DISCUSSION

The results showed that kersen (*Muntingia calabura* L.) leaf aqueous treatment at different concentrations affected the germination and growth of spiny spinach weed (*Amaranthus spinosus* L.). The higher the concentration of the extract, the lower the decrease in germination percentage, germination index, germination length, and weed radicular length. Conversely, the higher the concentration of the extract, the longer the germination time of the spinach seeds (Table.1).

Phenolic compounds in allelochemicals, such as in kersen leaf aqueous, disrupt the activity of the hormone gibberellin, which inhibits the activities of peptidase, α-amylase, and lipase. As a result, hydrolysis of food reserves, such as proteins, starches, and fats, in the endosperm or cotyledons are inhibited. This results in low energy available for germination; therefore, the ability of seeds to germinate will also decrease. Thus, there is a decrease in the germination percentage and germination index of spinach seeds, as well as an increase in the time required for seeds to germinate (Dordevic *et al.* 2022, Aisah *et al.* 2018). This is in accordance with the findings of Ghimire *et al*. (2020) that *Mischantus scchariflorus* leaf extract can suppress weed seed germination in a concentration-dependent manner. Application of M. *sacchariflorus* leaf extract at a concentration of 10,000 ppm reduced the germination of *Bidens frondosa*, *Echinochloa crus-galli*, and *Erigeron canadensis* L seeds by up to 100%.

A decrease in the length of the sprouts and spinach radicula was observed at a concentration of 6% (Table 1). This may be because the overhaul of food reserves from the cotyledons that are transferred to the point of growth of the plumula and radicula is not optimal. In addition, allelochemical exposure to phenol compounds causes damage to the spindle thread during cell nuclear division. Damage to the spindle thread interferes with the movement of the centriole towards the cell pole and results in the non-formation of the cell dividing wall, so cell division does not occur. If cell division is inhibited, the formation of other plant organs is also inhibited. Disturbances in germination due to allelochemicals can also occur because of disturbances in phytohormones. Allelochemicals, in the form of phenols and terpenoids, can interfere with the activity of gibberellin and cytokinin during germination, thus affecting the division process. Cytokinin play important roles in mitosis (Aristya *et al.* Nov. 2015; Aisah *et al.* 2018).

Kersen leaf aqueous extract inhibited germination at a concentration of 6%; however, at lower concentrations, there was no noticeable decrease in germination. This is in line with the findings of Zohaib *et al.* (2014), that increased germination inhibition in *Vigna radiata* and *V. mungo seeds* occurred in tandem with an increase in the concentration of *Vicia sativa* extract. The extracts at concentrations of 3% and 4% significantly inhibited seed germination. However, El-Mergawi and Abdulrohman (2019) argued that allelochemical extracts at low concentrations could stimulate root and stem elongation during the early growth period of plants. The results of the analysis showed that treatment with 10 and 20% kersen leaf aqueous extract increased the content of chlorophyll a, chlorophyll b, and carotenoids, while a concentration of 30% decreased the three parameters (Table 2).

Pigments are secondary metabolites that provide color to plant organs. Photosynthetic pigments, namely chlorophyll a, chlorophyll b, and carotenoids, capture energy from the sun and convert H_20 and CO_2 into energy and organic compounds (Upadhyay 2018). ALA (5-amino-levulinate acid) is an important component of the chlorophyll process. ALA is converted into

Table 1 Percent germination (%), germination time (h), germination index, germination length, and radicular length *of A. spinosus* L. upon *M. calabura* L. *leaf extract treatment* at different concentrations

Variable	Concentration of extract (%)			
	0	2	4	6
Germination percentage (%)	53.33 ± 20.55 ^{a}	36.67 ± 17.00 ^{ab}	20.00 ± 8.16 ^{bc}	13.33 ± 4.71 ^{c}
Germination time (h)	21.74 ± 1.80 ^{b}	22.72 ± 1.62 ^{b}	23.66 ± 1.12 ^{ab}	25.50 ± 0.71 ^{a}
Germination index	4.63 ± 2.13 ^{a}	2.32 ± 1.20 ^{b}	0.88 ± 0.27 ^{b}	0.58 ± 0.43 ^{b}
Sprout Length (cm)	2.98 ± 0.13 ^{a}	3.13 ± 0.35 ^{a}	3.05 ± 1.73 ^{a}	2.16 ± 0.21 ^{b}
Length of radicula (cm)	1.87 ± 0.11 ^{a}	1.87 ± 0.11 ^{a}	1.64 ± 0.21 ^{a}	1.38 ± 0.19 ^{b}

Remarks: The numbers on the same line and followed by the same letter showed no significant difference between the treatments based on the DMRT follow-up test with a 95% confidence level.

Table 2 The pigment content of chlorophyll a (µg/g), chlorophyll b (µg/g), and carotenoids (µg/g) *of A. Spinosus* L. upon aqueous treatments of *M. calabura* L. leaf extract at different concentrations

Parameter	Concentration extract (%)			
	0	10	20	30
Chlorophyl a (µg/g)	443.93 ± 15.85 ^{b}	474.20 ± 20.81 ^{a}	489.85 ± 3.93 ^{a}	432.70 ± 9.36 ^{b}
Chlorophyl b (µg/g)	228.95 ± 13.95 ^{b}	239.62 ± 17.73 ^{ab}	269.86 ± 15.37 ^{a}	223.88 ± 13.79 ^{b}
Carotenoid (µg/g)	91.61 ± 7.02 ^{b}	110.24 ± 9.14 ^{a}	118.72 ± 3.18 ^{a}	85.26 ± 3.59 ^{b}

Remarks: The numbers on the same line and followed by the same letter showed no significant difference between the treatments based on the DMRT follow-up test with a 95% confidence level.

protoporphyrin IX through a series of reactions. Furthermore, magnesium kelatase facilitates the insertion of divalent magnesium ions into the protoporphyrin IX ring to form magnesium protoporphyrin IX. Furthermore, magnesium prochlorophillide IX undergoes methyl esterification, cyclisation, and reduction to form protochlorophillyl, which is catalyzed by protochlorophillyl reductase (POR) under light conditions to produce chlorophyll a. Catalytic chlorophyll synthase (CS) combines phytisol pyrophosphate (PhyPP) molecules to produce chlorophyll a. Chlorophyll b is formed by two oxidation reactions with chlorophyll a molecule with the help of chlorophyll a oxygenase (Lu *et al.* 2023).

The photosynthetic pigment content of plants is related to their primary metabolite content (Sonebe *et al.* 2020). However, the increased chlorophyll a, chlorophyll b, and carotenoid content upon the 10% and 20% extract treatments were inversely proportional to the decreased growth of spinach weeds. This is not in accordance with the opinion of Hariandi *et al*. (2019), that the rate of photosynthesis is positively correlated with the plant chlorophyll content. A high chlorophyll content increases the rate of photosynthesis. Based on Ding *et al*. (2020), the main changes caused by oxidative stress due to allelochemicals are the disruption of the flow of assimilates from the source (leaves) to the sink. Inhibition of photosynthesis results to the zinc tissue results in the accumulation of dissolved sugars and sucrose in the leaf organs thereby inhibiting growth.

Treatment with 10 and 20% kersen leaf aqueous extracts stimulated the formation of photosynthetic pigments in the form of chlorophyll a, chlorophyll b, and carotenoids in the target plants. This is in line with the findings of Siyar *et al.* (2019), which showed that the application of 15 g/L allelochemicals of whole weed extracts of *Melilotus officinalis*, *Avena fatua*, and *Polypogon hissaricus* significantly increased the chlorophyll content of wheat varieties Pirsabaq and Serin, but decreased the chlorophyll content of wheat varieties of Ata Habib when compared to controls. Treatment with both allelochemical extracts significantly increased the chlorophyll b content and total carotenoid content. Like the results of M'barek *et al.* (2019), allelochemicals from *Cupressus sempervirens* L., *Juniperus phoenicea* L., and *Tetraclinis articulata* (Vahl) leaf extracts increased carotenoid content in lettuce plants.

Treatment with 30% kersen leaf aqueous extract reduced the pigment content of chlorophyll a, chlorophyll b, and carotenoids. According to M'barek *et al*. (2019), allelochemicals trigger the activity of chlorophyllase responsible for chlorophyll damage and decrease chlorophyll synthesis, owing to changes in the structure of the thylakoid membrane. Zhang *et al.* (2016) stated that reduced pigment content affects the processes of photon reception, electron transport, and energy conversion. Chlorophyll plays an important role in light absorption, transfer, and transformation. Carotenoids are chromoprotein components that are useful in photosynthesis to protect the main components of the photosynthetic process from injury, owing to the presence of oxygen singlets. Decreased carotenoid content inhibits photon transfer in photosystem II. The treatment of 30% kersen leaf extract reduced the height of the plant, the number of leaves, fresh weight, dry weight, and the flowering time of spinach weeds. The highest number of weeds was observed in the 0% treatment (Table 3).

Treatment with different concentrations of kersen leaf aqueous lowered the height of the spinach weeds (Figure 1). High inhibition of spinach weeds is affected by the inhibition of growth hormone activities, such as auxin, gibberellin, and cytokinin. Additionally, Syahri *et al.* (2017) proposed that the decrease in weed height is caused by allelochemical compounds that interfere with IAA activity and formation. The allelochemicals in the kersen leaf extract also inhibited the activities of gibberellin and cytokinin. The phenols content caused by the administration of kersen leaf extract damaged the spindle thread during the metaphase phase of mitosis. This results in an increase in the number of cells and the inhibition of cell enlargement. The inhibition of the proliferation process obstructs plant organ formation, thereby decreasing plant growth.

Syahri *et al.* (2017) suggested that phenolic compounds can interfere with the transport of auxins from shoots to roots and interfere with cytokinin synthesis at the roots. In physiological processes, cytokinin stimulates cell division in meristematic tissues and root cell differentiation, and affects bud dominance, terminal bud growth, and aging. Allelochemicals inhibit growth-regulating hormone activity, resulting in the inhibition of the elongation and enlargement of spinach weed stem cells. This is in accordance with the findings of Alqarawi *et al.* (2018) that the allelochemical extract

Table 3 Plant height (cm), number of leaves, fresh weight (g), dry weight (g), and flowering time (DAP) *of A. spinosus* L. upon treatment of *M. calabura* L. leaf aqueous extract at different concentrations

Parameter	Concentration extract (%)			
	0	10	20	30
Plant height (cm)	128.32 ± 1.86 [a]	125.55 ± 2.83 [a]	125.10 ± 3.15 [a]	120.26 ± 1.35 [b]
Number of leaves	55.60 ± 4.39 [a]	49.20 ± 3.56 [b]	44.80 ± 2.49 [b]	34.40 ± 4.10 [c]
Fresh weight (g)	211.20 ± 8.67 [a]	173.80 ± 12.52 [b]	173.60 ± 25.01 [b]	167.60 ± 10.50 [b]
Dry weight (g)	29.40 ± 3.51 [a]	27.40 ± 1.67 [ab]	25.20 ± 2.78 [b]	24.00 ± 1.58 [b]
Flowering time (day)	21.00 ± 1.58 [b]	22.40 ± 1.14 [b]	22.40 ± 1.14 [b]	29.40 ± 2.41 [a]

Remarks: The numbers on the same line and followed by the same letter showed no significant difference between the treatments based on the DMRT follow-up test with a 95% confidence level.

Figure 1 *Amaranth spinosus* weed at 10 weeks after planting upon treatment of *M. calabura L.* leaf aqueous extract at different concentrations.

of *Rhazya stricta* leaves causes decreased growth of *Salsola villosa.*

The number of leaves began to decrease significantly in the 10% treatment, and the higher the concentration of the decline, the greater the decrease. The inhibition of spinach leaf formation by allelochemical compounds is due to the impaired activity of cytokinin and auxin hormones. Cytokinin functions in maintaining the size and structure of the apical meristem (SAM), whereas auxins are required for leaf formation and organogenesis. The low content of cytokinin hormones due to the administration of allelochemical compounds to the apical meristem of the shoot (SAM) inhibits the expression of genes that play a role in maintaining a high rate of cell division in the organizing center (OC) region of the apical meristem of the shoot. As a result, the function of the receptors responsible for blocking the effects of cytokinin and increasing auxins for leaf formation is inhibited. Low auxin accumulation leads to the inhibition of leaf primordium formation. The early stages of leaf development are aided by the activation of cytokinin hormones by regulators located in the marginal region of the blast ozone (MB) of the apical meristem of the shoot. However, exposure to allelochemicals disrupts this process, inhibiting the formation of compound leaves (Siregar *et al*. 2017. Wu *et al.* 2021).

The fresh weight of the spinach weeds decreased by 10%. The dry weight decreased starting with 20% extract treatment. The higher the concentration of the extract, the greater the decrease in fresh and dry weights of spinach weed. Susilo *et al.* (2023) reported that the fresh weight of plants is the content of photosynthesis and the total water. A decrease in fresh weight indicates that plant growth slows down. Plant growth was stunted owing to disruption of the photosynthesis process, which causes a decrease in the water content and photosynthate produced by plants. Dry weight is a measure of the material left in the plant after removing all water content has been removed. According to Krisman *et al*. (2016), the dry weight of plants indicates the size of plant growth, which reflects the number of organic compounds that have been synthesized by plants during the growth period. In addition, Namkeleja *et al.* (2014) mentioned that allelochemicals reduces plant water potential and inhibit the absorption of ions and minerals, causing a decrease in plant growth. Consequently, the fresh and dry weights of plants decreased. In line with Motmainna *et al.* (2021), the application of allelochemical extracts of *Parthenium hysterophorus* (20, 40, and 80 g/L) significantly decreased the fresh and dry weights of *Cyperus iria*, *Ageratum conyzoides*, and *Oryza sativa*.

The flowering time of spinach weeds increased with 30% extract treatment. This demonstrates that the allelochemicals of kersen leaf extract inhibits the flowering of spinach weeds. This was due to the inhibition of the phytohormonal activity. According to Chen *et al.* (2018), allelochemicals inhibit the production of phytohormones such as auxin, gibberellin, cytokinin, and abscisic acid. The inhibition of these phytohormones inhibits the activation of several genes that play a role in the induction of flowering, initiation of flower emergence, and differentiation. This results in the process of flower formation in plants being hampered (Siregar *et al*. 2017, Chen *et al.* 2018, Virral *et al.* 2020).

According to Chen *et al.* (2018), allelochemicals from *Cinnamomum japonicum* leaf litter doses of 20 g pot^{-1}, 40 g pot^{-1}, and 80 g pot^{-1} showed a decrease in the time of first flowering in *Impatiens balsamina.* Although there was no significant difference with the control, the higher the amount of litter used, the higher the inhibition effect. The treatment caused a change in the normal flowering patterns in the target plant, where there was a significant decrease in the number of *I. balsamina* flowers.

CONCLUSION

Allelochemicals in kersen leaf extracts inhibit the germination and growth of spinach. However, the sensitivity of spinach to the allelochemicals differs during different growth phases. Kersen leaf extract at a concentration of 6% caused a decrease in germination; the germination percentage decreased by 75%, the germination index decreased by 87.47%, the germination length decreased by 27.51%, and root length decreased by 26.20%, but the germination time increased by 14.74%. Kersen leaf extract at a concentration of 30% caused a decrease in spinach growth; plant height decreased by 6.48%, number of leaves decreased by 38.13%, fresh weight decreased by 20.64%, dry weight decreased by 18.36%, and flowering time increased by 40%.

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