




## Allelopathic Effect of Methanol Extract of Parthenium Plant Parts on Seed Germination and Seedling Growth of *Digitaria sanguinalis* L. and *Eleusine indica* L.

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### ABSTRACT

For sustainable weed management, herbicides produced from natural ingredients could be an excellent environmentally acceptable alternative to synthetic chemical herbicides. This study looked at the allelopathic effects of methanol extract of *Parthenium hysterophorus* stem, leaf, and flower on seed germination and seedling growth of two weed species, crabgrass (*Digitaria sanguinalis* L.) and goosegrass (*Eleusine indica* L.). Five different concentrations of the extract (0, 25, 50, 75, 100, and 150 g L<sup>-1</sup>) were compared. Percent inhibition in seed germination, root and shoot growth were calculated and EC<sub>50</sub> (concentration required to cause 50% growth inhibition) of test weeds were measured. The extracts affected the seed sprouting and seedling growth of test weed species significantly. Leaf and flower extracts were found more phytotoxic than the stem extract. An amount of 50 g L<sup>-1</sup> of plant mass was enough to suppress effectively all parameters of the test weed species (designated as EC<sub>50</sub>). The leaf and flower of *Parthenium hysterophorus* might contain a number of plant inhibitors or phytotoxins, which could be exploited in future to create a natural herbicide.

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## 1. Introduction

Weed causes significant loss in crop production by competing with crop plant for limiting resources. Weed management by chemical herbicides leads to development of herbicide resistance in weeds and are the reasons for environmental pollution. Therefore, eco-friendly method of weed control is recommended to sustain our agriculture. Goosegrass (*Eleusine indica*), and crabgrass (*Digitaria sanguinalis*) are the two important weeds particularly in upland crops including rice causing significant yield losses (Jalaludin et al. 2010, Turner et al. 2012).

*Parthenium hysterophorus* L. is an allergenic and calcitragant which affect human health, livestock production and crop yields. Its effective control is a vital issue in the country of infestation. Utilization of this colonial plant for developing value-added product is novel research. It has been reported by a number of scientists that this plant is an allelopathic plant (Singh 2005; Batish et al. 2009; Pati & Chowdhury, 2016). It is an annual herbaceous plant that reproduces mostly through seeds. After sprouting, the young plant produces a basal rosette of finely lobed, bright green leaves that are 8–20 cm long and 4–8 cm wide. The rosette stage can continue to grow under unfavorable circumstances and reach a maximum length of 2.5 m (Tiku

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et al. 2014). The flower heads are terminal and somewhat hairy; they consist of several small white capitula-shaped florets. Usually, each head has five productive ray florets, although occasionally six or eight. Thousands of branches, which develop in separate clusters, produce compressed black seeds about 2 mm in size (Bashar et al., 2021).

Allelopathy is described as chemical's positive or negative impacts substances formed primarily by plant, microbe, and fungal secondary metabolism on the growth and establishment of neighboring plants or microorganisms, as well as the dynamical processes of agricultural and natural eco-systems (Latif et al. 2017). It's a complicated phenomenon that's influenced by a variety of internal and external circumstances. Due to its intricacy, the explanation is a difficult endeavor that necessitates knowledge from a variety of professions (Scognamiglio et al. 2015). Allelochemicals are plants that release secondary metabolites into the environment. They are anti-inflammatory substances that belong to a variety of chemical classes, primarily phenolic compounds and terpenoids (Scavo et al. 2019). The exploitation of allelopathic activity of this weed plant is important for knowing the possibility of utilizing the plants to generate bioherbicide to sustain our agroecosystem and for organic agriculture. Sufficient studies on the allelopathic effects of methanol extracts on aforesaid weeds are lacking. This strategy will aid in reducing reliance on chemical herbicides, reducing the likelihood of weed resistance to herbicides, reducing health risks and environmental damage and strengthening the national economy. Some previous reports reveal the allelopathic potential of *Parthenium hysterophorus* extracts in different plant species. However, inadequate evidence is available on the influence of methanol extract of *Parthenium* on the sprouting and seedlings development of crabgrass and goosegrass, which are the major weeds of many of the field crops. Not only that many reports have in *parthenium* individual parts but also lackings of cooperative study on *parthenium* different parts. Therefore, the main objectives of this study is to see the allelopathic effect of methanol extract made from the aerial portions of *Parthenium hysterophorus* on target species to develop bioherbicides based on natural products.

## 2. Materials and Methods

### 2.1. Site description

The experiment was conducted at the Weed Science Laboratory, Department of Crop Science, Faculty of Agriculture at Universiti Putra Malaysia (3°02' N, 101°42' E, elevation 31 m), Selangor, Malaysia.

### 2.2. Experimental design and treatments

Completely randomized designs (CRD) with four replications were used to set up factorial studies (plant parts and concentrations) in a growth chamber at a temperature of 25°C. Three different plant parts of *Parthenium*: leaf, stem, and flower were extracted in methanol solvent. Treatments comprised of leaf methanol extract, stem methanol extract, flower methanol extract at six different concentrations of 25, 50, 75, 100 and 150 g L<sup>-1</sup> with distilled water as a control (Aslani et al. 2015).

### 2.3. Preparation of plant materials

*Parthenium hysterophorus* plants (excluding root) were obtained from Ladang Infoternak Farm, Sungai Siput, Perak, Malaysia. Collected plants were washed with tap water for removing the dust particles, and then air-dried at room temperature (24-26°C) for three weeks. The leaves, stems, and flowers were separated from each other. The dried plant components were mashed into a fine powder using a laboratory blender and sieved through a 40-mesh sieve.

The seeds of two weed species namely crabgrass and goosegrass were collected from an agricultural field at the University Putra Malaysia. The damaged seed and other inert materials was discarded and maintained uniformity of the seeds. Weed seeds were air-dried and stored at -18°C temperature before use.

### 2.4. Extract preparation

The methanol extracts were made according to the procedure published by Ahn & Chung (2000) and Aslani et al. (2014). In a conical flask, 100 g powder of *parthenium* leaves, stems or flowers powder was immersed in 1000 ml of 80% (80:20, v/v) methanol separately. The conical flask was then covered in paraffin and shaken in an orbital shaker at 150 rpm agitation speed (Orbit shaker 719, Lab-Tech, Malaysia) for 48 hours at 24-26°C room temperatures. To remove debris, the solutions were filtered through four layers of cheesecloth and centrifuged for one hour at 3000 rpm in a 5804/5804 R centrifuge (Eppendorf, Germany). One layer of Whatman No. 42 filter paper was used to filter the supernatant. The solutions were re-filtered via a 0.2-mm Nalgene filter to avoid microbial development (Lincoln Park, NJ-based Becton Dickinson percent Labware). Using a rotary evaporator (R 124, Buchi Rotary Evaporator, Germany), the supernatants were evaporated from the extract to dryness (a thick mass of coagulated liquid) under vacuum at 40°C and then collected the sample. The mean extraction yield was 16.66 g from a 100 g powdered sample of *parthenium* as determined by the following formula-

Extract weight (g)/powder weight (g) × 100= Extraction percentage

Each stock extracts from *parthenium* leaves, stems and flowers were diluted using appropriate amount of sterile purified water to generate extract concentrations of 25, 50, 75, 100, and 150 g L<sup>-1</sup> and water (distilled) was used as a control. Before usage, all the extracts were stored at 40°C temperature in the dark.

### 2.5. Germination and growth bioassays

Before starting bioassays, the seeds of test weed species were steeped in 0.2 percent potassium nitrate (KNO<sub>3</sub>) for 24 hours and then rinsed with distilled water. Thirty crabgrass and goosegrass seeds were put on Whatman No. 1 filter paper in a sterilized 90 × 15 mm Petri dish. Ten ml of methanol extract was administered in Petri dishes at six concentrations (25, 50, 75, 100 and 150 g L<sup>-1</sup>) and distilled water as a control. All of the Petri dishes were placed in a growth chamber under fluorescent light and incubated at 30°C (day) and 20°C (night) on a 12 h photoperiod (8500 lux). The relative humidity was maintained between 30 to 50%. The covers of the Petri dishes were not sealed to facilitate gas exchange and

prevent anaerobic conditions. The germination of the seeds was 99%.

## 2.6. Data collection

The number of seeds germinated was counted and radicle & hypocotyl lengths were measured with a ruler at seven days after seeding. The radicle and hypocotyl lengths were assessed using Image J software (Mirmostafaei et al.; 2020) and the inhibitory effect of parthenium extracts on germination, radicle length and hypocotyl length was calculated using the following equation

$$I = 100 (C - A)/C \text{ (Kordali et al. 2009)}$$

Where, "C" is the control's mean radicle and hypocotyl length, and "A" is the aqueous extracts' mean radicle and hypocotyl length and "I" is the percentage of inhibition.

## 2.7. Statistical analysis

Two-way Analysis of Variance (ANOVA) was done using SAS (Statistical Analysis System) procedures, version 9.4. (Cary, NC, USA) to determine whether there was a significant difference between each treatment and control. Separation of treatment means from control was ascertained at 0.05 probability levels as per the LSD test.

Effective dosages capable of suppressing 50% of germination, radicle development, and hypocotyl growth were calculated using  $EC_{g50}$ ,  $EC_{r50}$ , and  $EC_{h50}$ , respectively. The  $EC_{g50}$ ,  $EC_{r50}$  and  $EC_{h50}$  values were calculated using Probit analysis based on the percent of radicle and hypocotyl growth inhibition, respectively. The following equation was used to create an inhibitory index (Re) for each of the most active extract and most sensitive plants for each plant tested:

$$EC_{g50n} \text{ (germination)} + EC_{r50n} \text{ (radicle)} + EC_{h50n} \text{ (hypocotyl)} = \text{Rank (R}_e\text{)}$$

Where  $R_e$  is the plant's rank n, and  $EC_{g50n}$ ,  $EC_{r50n}$ , and  $EC_{h50n}$  are the amounts of plant extract n that inhibit 50% of germination, radicle growth, and hypocotyl growth, respectively. The lowest  $R_e$  value indicates the most inhibitory effect and the most sensitive plants, while the highest  $R_e$  value indicates the least allelopathic effect of the extract.

## 3. Results and Discussion

### 3.1. Allelopathic effect of the areal parts of parthenium extracts

The germination and initial growth of crabgrass (*Digitaria sanguinalis*) and goosegrass (*Elesine indica*) were used to test the possible allelopathy of methanol extract of the aerial portions of parthenium at different doses. When compared to the control, parthenium leaf, stem and flower concentrations had significant effect on seed germination, radicle and hypocotyl length (Table 1). We observed a modestly stimulatory effect on seed germination, radicle length and hypocotyl length at lower doses, 25 g L<sup>-1</sup> especially on crabgrass but an inhibitory response was observed at

higher dosages. Plant extracts are hypothesized to impede germination by having osmotic influences on imbibition rate, which therefore prevented germination and, in particular, cell elongation (El-Mergawi & Al-Humaid 2019). The extraction of several active phyto-chemicals (e.g. flavonoid, phenolics, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH)) with methanol as an extraction solvent have been reported by Dhawan & Gupta (2017). The germination kinetics of crude extracts were studied and it was shown that there is a significant variation in germination kinetics between the doses of methanol crudes of parthenium plants (Pati & Chowdhury 2016). Batish et al. (2009) and Singh et al. (2005) reported that the growth and development of numerous field crops were inhibited by parthenium methanol extract and residues.

Goosegrass failed to sprout entirely when given a high concentration of methanol extract of parthenium, on the other hand, it grew well at low concentrations up to 25 g L<sup>-1</sup>. In case of both the weeds, the concentration of 50 g L<sup>-1</sup> resulted in a 100% reduction in germination. The extracts of stem were less effective in growth inhibition of crabgrass and goosegrass compared to the leaf and flower extracts (Table 1). There was a gradual decrease in seed germination with the increase in concentration of extracts obtained from different parts of parthenium. These results showed that leaf aqueous extract of parthenium exhibited significant inhibitory effect on seed germination and had adverse effect than the stem as reported by (Shafiq et al. 2020).

Methanol extracts had significant phytotoxic effects on the weeds' radicle and hypocotyl lengths as well. Leaf extract at doses equal to or greater than 50 g L<sup>-1</sup> significantly reduced the radicle length of target weed species ( $P < 0.05$ ). Even in case of goosegrass, the radicle length reduced at 25 g L<sup>-1</sup>. On the other hand, both the weed species showed no germination as well as no radicle and hypocotyl development with higher concentrations (50 to 150 g L<sup>-1</sup>) except with stem extract. Among the aerial parts of the parthenium plant, the leaf displayed the most inhibition in weed parameters than the other parts. From the concentration level applied from 25 to 150 g L<sup>-1</sup> of methanol extract of parthenium, these reduced the radicle length at the ranges of 56-100%, 66-100% and 47-100% by leaf, stem and flower, respectively. The weed species, crabgrass and goosegrass were severely affected by the leaf and flower methanol extracts in the concentration of 50 to 150 g L<sup>-1</sup>. However, the crabgrass was inhibited by the stem extract at concentrations of 100 to 150 g L<sup>-1</sup> only. The aerial parts of parthenium extract had a substantial effect on seed germination, radicle and hypocotyl length reduction as noticed in this investigation. These effects grew stronger as the concentration level increased. These findings are reliable with those of Wakjira et al. (2005) and Mersie & Singh (1987) who reported a robust link between increased extract concentrations and greater weed plant toxicity. Similarly, Motmainna et al. (2021) found that the extracts from parthenium had a significant impact on the germination, hypocotyl and radicle length of a few weed species. As the concentration was raised, the magnitude of suppression was increased as well. The extract obtained from 100 mg *Couroupita guianensis* leaves with methanol completely inhibited germination of lettuce or significantly delayed germination of barnyard grass (Khan et al. 2016).

**Table 1.** Effect of leaf, stem and flower methanol extracts of *Parthenium hysterophorus* on germination, radicle and hypocotyl length of two weed species

Weed species	Conc.(g L <sup>-1</sup> )	Extract of leaf			Extract of stem			Extract of flower		
		Germination (%)	Radicle length (cm)	Hypocotyl length (cm)	Germination (%)	Radicle length (cm)	Hypocotyl length (cm)	Germination (%)	Radicle length (cm)	Hypocotyl length (cm)
Crabgrass	0	97.77±2.22a (0)	0.23±0.02a (0)	0.85±0.03a (0)	97.77±2.22a (0)	0.23±0.02a (0)	0.85±0.03a (0)	97.77±2.22a (0)	0.23±0.02a (0)	0.85±0.03a (0)
	25	36.66±1.92b (62.5)	0.10±0b (56.52)	0.22±0.01b (74.12)	55.55±2.22b (40.48)	0.20±0b (66.67)	0.60±0.04b (17.81)	45.55±2.93b (51.19)	0.10±0b (47.37)	0.26±0b (54.39)
	50	0c (100)	0c (100)	0c (100)	31.10±2.22c (66.68)	0.19±0.01bc (68.33)	0.49±0.01c (32.88)	0c (100)	0c (100)	0c (100)
	75	0c (100)	0c (100)	0c (100)	5.55±1.11d (94.05)	0.17±0.01c (71.67)	0d (100)	0c (100)	0c (100)	0c (100)
	100	0c (100)	0c (100)	0c (100)	0e (100)	0d (100)	0d (100)	0c (100)	0c (100)	0c (100)
	150	0c (100)	0c (100)	0c (100)	0e (100)	0d (100)	0d (100)	0c (100)	0c (100)	0c (100)
Goosegrass	0	83.33±1.92a (0)	0.20±0.02a (0)	0.80±0.01a (0)	83.33±1.92a (0)	0.20±0.02a (0)	0.80±0.01a (0)	83.33±1.92a (0)	0.20±0.02a (0)	0.80±0.01a (0)
	25	0b (100)	0b (100)	0b (100)	12.22±1.11b (83.34)	0.14±0.01b (22.22)	0.30±0.01b (46.43)	26.66±3.84b (66.67)	0.12±0b (62.5)	0.53±0.01b (46.46)
	50	0b (100)	0b (100)	0b (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)
	75	0b (100)	0b (100)	0b (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)
	100	0b (100)	0b (100)	0b (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)
	150	0b (100)	0b (100)	0b (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)	0c (100)

Means and Standard Error are used to express data. At p<0.05, the mean for each plant is not much different with the same letters in the column. The percentage reduction in comparison to the control is indicated by the values enclosed in parenthesis

**Table 2.** The half-inhibitory effect of *Parthenium hysterophorus* methanol extracts to the test weed species as well as the sensitivity of the weed species

Weed species	Leaf methanol extract				Stem methanol extract				Flower methanol extract			
	ECg50	Ecr50	Ech50	Rank	Ecg50	Ecr50	Ech50	Rank	Ecg50	Ecr50	Ech50	Rank
Values in g L <sup>-1</sup> (lower-upper)												
Crabgrass	0	0	0	0	31.62 (16.36-42.85)	20.79 (0)	44.41 (0)	96.82	0	0	0	0
Goosegrass	0	0	0	0	0	0	0	0	0	0	0	0
Rank	0	0	0	0	31.62	20.79	44.41	96.82	0	0	0	0

The quantities of extracts that inhibit 50% of germination, root growth, and hypocotyl enlargement, respectively, are designated as EC<sub>g50</sub>, EC<sub>r50</sub>, and EC<sub>h50</sub>.

**Table 3.** Mean inhibitory indices of different plant parts extracts of *Parthenium hysterophorus* on crabgrass and goosegrass

Weed species	Concentrations (g L <sup>-1</sup> )	Parthenium plant parts			Mean of weed species
		Leaf	Stem	Flower	
Crabgrass	0	0	0	0	0
	25	64.38	41.65	50.98	52.33
	50	100	55.96	100	85.32
	75	100	88.57	100	96.19
	100	100	100	100	100
	150	100	100	100	100
Average		77.39	64.36	75.16	72.30
Goosgrass	0	0	0	0	0
	25	100	50.66	58.54	69.73
	50	100	100	100	100
	75	100	100	100	100
	100	100	100	100	100
	150	100	100	100	100
Average		83.33	75.11	76.42	78.28
Mean of plant parts		80.36	69.73	75.79	

All parts of *Parthenium hysterophorus* plant influenced the germination, radicle and hypocotyl length of weed species that were examined in a dose-dependent manner. Because of their superior strength, efficacy and consistency in suppressing seed germination and seedling growth, extracts of parthenium leaf and flower was the most promising candidate for bioherbicide isolation.

### 3.2. Effects of methanol extracts on initial growth parameters of test weed species

Table 2 shows the half inhibitory effects of methanol extracts of parthenium plant parts as well as the sensitivity of the weed species. It is obvious that the stem extract has a lesser efficacy than the leaf and flower extract with the stem extract's rank value of 96.82 and the leaf and flower rank value of 0. The leaf extracts of *Justicia adhatoda* with methanol showed that the foxtail millet and barnyard grass are germinating below 50 % both in the laboratory condition and in the pot experiment at their maximum concentration (Khan et al. 2021). Islam et al. (2013) reported that the aqueous methanol extract of *Litchi chinensis* leaves were significantly inhibited the hypocotyls of cress, lettuce and alfalfa, and the root growth of all test plant species and complete inhibition of lettuce seed germination and a significant delay of germination on barnyard grass.

### 3.3. Mean inhibitory indices of different plant parts

Table 3 represents the mean inhibitory indices of different plant parts extracts on crabgrass and goosegrass. It was observed that mean plant parts showed highest inhibition in leaf (80.36) than flower (75.79) and lowest in stem extract from both weed species. Another, mean of weed species, it was found that goosegrass (78.28) was more affected than crabgrass (72.30).

## 4. Conclusion

The aerial parts, especially leaf of *Parthenium hysterophorus* plant might contain phytotoxins, which affected the seed germination and seedling growth of two selected weed species like crab grass and goose grass. Methanol extracts of leaf and flower may be used for isolation and identification of the phytochemicals present in it with the hope of generating a natural herbicide which could be used to integrate weed control in sustainable farming systems.

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### Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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