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Research article

# Effects of *Melaleuca cajuputi* leaf extract on inhibition of seed germination and seedling growth of 12 weed species

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## Abstract

**Importance of the work**: Plant natural products are sources of numerous compounds with antimicrobial, pesticide and allelopathic properties.

**Objectives**: To test *Melaleuca cajuputi* leaf and powder extracts for their inhibitory effect on seed germination and seedling growth of 12 weed species.

**Materials & Methods**: Petri dish and soil-based experiments were conducted to determine the efficacy of different percentages of the leaf powder and leaf extracts on the inhibition of weed seed germination.

**Results**: Weed seed germination and seedling growth were inhibited by *M. cajuputi* leaf extract better than by branch extract in a Petri dish experiment, with inhibition better on broadleaf weeds than narrowleaf weeds. There was complete inhibition (100%) of the seed germination of the broadleaf weeds, *Eclipta prostrata*, *Borreria alata*, *Tridax procumbens*, *Gomphrena celosioides*, *Amaranthus viridis* and the narrowleaf weed, *Chloris barbata* with *M. cajuputi* leaf extract at amounts of 0.1−1.0 g dry weight. The major essential oil found in the *M. cajuputi* leaf extract was eugenol, with 0.015−0.2 M of eugenol inhibiting the seed germination and seedling growth of *Achyranthes aspera*.

**Main finding**: In the soil-based experiment, there was 100% inhibition of weed seed germination using 6% leaf powder and 4−6% leaf extracts, which supported the potential use of *M. cajuputi* leaf extract as a natural herbicide for safe and sustainable agriculture.

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## Introduction

Allelopathy is the ability of a plant to inhibit or stimulate the growth of other organisms in its environment by releasing allelochemical compounds (Duke et al., 2000; Trezzi et al., 2016; Lim et al., 2019). These compounds, mainly secondary metabolites, are introduced into the environment via exudation, decomposition, leaching and volatilization and may be toxic to or stimulate the growth of other plant species or soil microorganisms (Duke et al., 2000; Hoagland, 2001; Mallik and Williams, 2009). Allelochemicals which can inhibit other plant species as natural herbicides have several benefits over synthetic compounds. For example, they have a short half-life and are considered safe from an environmental toxicology standpoint (Duke et al., 2007). Furthermore, such natural compounds have diversity in their molecular structures and hence may avoid the development of herbicide-resistant weeds to some extent. Nowadays, the number of herbicide-resistant weeds is increasing (Fischer et al., 2000; Duke et al., 2002) and may be related to the fact that synthetic herbicide design has been biased toward certain types of chemical structures (Bhowmik and Inderjit, 2003). It has been reported that the over-use of a chemical herbicide, glyphosate, resulted in weed resistance to the herbicide which in turn resulted in the world-wide use of Paraquat due to its ability to control glyphosate – resistant weeds, even though it is forbidden in Europe (Eubank et al., 2008; Beckie, 2011). Low levels of contamination of paraquat in surface water, ground water and soil extracts have been found, which have posed a risk to human health (Amondham et al., 2006; Botta et al., 2020; Guijarro et al., 2018).

*Melaleuca cajuputi*, the cajuput tree or paper bark tree ('samet khao' in Thai) is a member of the Myrtaceae family that occurs in hot and humid climates, naturally in swamp forests between the raised sea beaches and the coastal alluvial flats behind the sandy beaches or mangrove forest (Isah et al., 2023). It has been known for its beneficial phytochemical properties and has been used for ethnomedicine e.g. (to relieve coughs and colds, Brophy et al., 2013). Other studies have shown several potential applications for the phytochemical extract from this plant, as insecticide (Bakar et al., 2012), as a functional food due to its high antioxidant activities with low toxicity (Al-Abd et al., 2015; Noor et al., 2020) and as a control agent against aflatoxigenic strain of *Aspergillus flavus* (Chaudhari et al., 2022). Its allelopathy was observed based on its water extract that apparently suppressed the germination and growth of seedlings of the grass, *Chloris barbata* (Othman et al., 2019). Furthermore, Kueh et al. (2018, 2019) reported the presence of caryophyllene and humulene (which have allelopathic effects) in the extract of *M. cajuputi* leaves and further showed that the extract caused injuries on tissue-cultured weed. However, other terpenoid compounds, such as eugenol from *Eugenia* spp., inhibited the growth of two weed species (*Cassia occidentalis* and *Bidens pilosa*) and reduced the total chlorophyll level and cellular respiration. Ahuja et al. (2015) showed the effect of eugenol on germination, root and coleoptile growth and chlorophyll content in *Avena fatua*.

The phytochemical components of *M. cajuputi* vary with locality and age (Othman et al., 2019) and potentially with genetics; thus further studies are needed to investigate the geographical range of this plant. More importantly, in addition to its effects on narrowleaf weeds, further investigation is warranted on its allelopathy against broadleaf weeds, as well as the allelopathic potential of different *M. cajuputi* plant parts. Therefore, the present study was conducted to evaluate the inhibitory effects of *M. cajuputi* leaf and branch extracts and powder, using the pure compound on the seed germination of 12 species of weed and their subsequent growth in Petri dish and soil-based experiments.

## Materials and Methods

### *Plant materials and extraction*

Mature leaves and branches of *M. cajuputi* were separately collected in April (dry season) from Chonburi province, Thailand. The voucher specimen number BKF191396 was obtained from the office of the forest herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand. The specimens were dried in an oven at 65–70°C for 48 hr. After drying, each type of plant material was ground into small pieces (to pass through a 50–100 mesh) and macerated with methanol (Carlo Erba; Rodano MI, Italy) for 72 hr. Later, a rotary evaporator was used to remove any methanol from the extract until completely dry. The crude extracts were stored at 4°C before use.

### *Inhibition of seed germination and seedlings growth*

Seeds of 12 different broadleaf (*Eclipta prostrata*, *Borreria alata*, *Tridax procumbens*, *Ruellia tuberosa*, *Gomphrena celosioides*, *Amaranthus viridis*, *Portulaca oleracea*, *Euphorbia heterophylla*, *Achyranthes aspera*) and narrowleaf weeds (*Rottboellia cochinchinensis*, *Chloris barbata*, *Cenchrus echinatus*) were collected from a corn field at the Corn and Sorghum Research Station, Nakhon Ratchasima, Thailand. Crude extracts of *M. cajuputi* leaf and branch were used at concentrations per 10 mL of dry ethanol of 0.1 g dry weight, 0.25 g dry weight, 0.5 g dry weight and 1.0 g dry weight in separate Petri dishes. A sterile filter paper (Whatman No.1) of 100 mm diameter was placed in each Petri dish (90 mm diameter) before adding the crude extract dissolved in 10 mL methanol. The Petri dish was allowed to dry. Then, sterile distilled water was added to final concentrations per 10 mL water of 0.1 g dry weight, 0.25 g dry weight, 0.5 g dry weight and 1.0 g dry weight. Then, 25 seeds of each weed species were evenly placed onto the previously treated filter paper in each Petri dish. The control treatment was 10 mL water without *M. cajuputi* leaf or branch extract. The Petri dishes were incubated at room temperature (28–32°C) for 5 d. The experiment was repeated with four replicates. The length of each seedling was measured in centimeters and the percentage of inhibition of seed germination and seedling growth were evaluated using Equation 1:

$$\% \text{ Inhibition of seed germination, short growth or root growth} = [100 - (100 \times G^i)] / G^c$$

where $G^i$ = % seed germination, shoot growth or root growth of each treatment and $G^c$ = % seed germination, shoot growth or root growth of the control.

### *Phytochemical constituents*

The crude methanolic extract of the *M. cajuputi* leaves was dried under vacuum, after which liquid-liquid extraction was performed using hexane, dichloromethane, ethyl acetate and 30% methanol. The phytochemical constituents of each fraction were analyzed using gas chromatography-mass spectrometry (GC-MS) and a HP-INNOWAX column (30 cm × 0.25 mm diameter) at 50–250 °C (increased at 4°C/ min) and a mass selective detector was used with an EI of 70 eV.

### *Inhibition of seed germination and seedling growth from pure compound*

Eugenol was a major monoterpene found in *M. cajuputi* leaf methanolic extract in the present study and it is well-known that eugenol is one of the allelochemical active monoterpenes in this plant (Ahuja et al., 2015). Therefore, it was hypothesized that eugenol may be the important active ingredient responsible for the inhibitory effect. To prove this hypothesis, the experiment was carried out using a medium-tolerance, narrow-leaf weed, *A. aspera*, according to the results of the present study. Commercial eugenol was purchased from Sigma-Aldrich and prepared to concentrations per 10 mL methanol of 0.05 g, 0.1 g, 0.2 g and 0.4 g per Petri dish. Then, *A. aspera* seeds were placed in each Petri dish following the same protocol described above. The data collection was also the same as for the previous experiment.

### *Soil-based experiments*

Despite the inhibitory effect of the leaf extract on weed germination and growth in Petri dishes, it necessary to further investigate such effects when the extracts are applied in soil. Five weed species were used (*R. tuberosa*, *E. heterophylla*, *A. aspera, R. cochinchinensis* and *C. echinatus*) whose responses to *M. cajuputi* leaf extract were identified to follow a concentration-dependent manner in the previous experiment. Preparation of the *M. cajuputi* leaf and branch extracts was the same as described in the previous experiment. In the present experiment, the crude extract was weighed and dissolved in water to concentrations of 2% dry weight, 4% dry weight and 6% dry weight, while the *M. cajuputi* leaf and branch powder was separately mixed with soil at concentrations on a weight to weight basis of 2% dry weight, 4% dry weight and 6% dry weight. Paraquat dichloride (Syngenta Nantong Crop Protection Co., Ltd., Indonesia) was used as a positive control at a concentration of 0.2% (volume per volume). Slightly acidic sandy clay soils and red-brown in color were collected from the Corn and Sorghum Research Station, Nakhon Ratchasima, Thailand. The soils were fumigated using 10% dazomet to avoid weed contamination. The mixtures of soil and *M. cajuputi* extract and powder were added to clay pots (15 cm diameter). Control pots were grown without *M. cajuputi*. In separate pots, 20 seeds of each weed species were embedded in the soil at a depth of 3 cm. The pots were placed in the greenhouse and the plants were watered with

200 mL each pot once a day in the morning using a plastic container. The percentage of seed germination and the seedling growth were measured at 30 d after planting.

### *Statistical analyses*

The data on the percentage germination and growth of the 12 weed species were subjected to analysis of variance followed by Tukey's post hoc tests and the results were considered significant at $p < 0.05$. Two-way analysis of variance was performed to verify any effects of the extract concentrations on each weed group (broadleaf and narrowleaf). The analyses was carried out using the SAS software (SAS 9.4, 2023, NC, USA).

## Results

### *Inhibition of seed germination by M. cajuputi extract*

The percentage yields of the *M. cajuputi* leaf and branch extracts were 6.2% and 5.3%, respectively. Both the *M. cajuputi* leaf and branch methanol extracts significantly inhibited seed germination and seedling growth of all 12 weed species (Table S1), with 100% seed germination inhibition of broadleaf and narrowleaf weeds with the *M. cajuputi* leaf extract at amounts of 0.1 g/10 mL, 0.25 g/10 mL, 0.5 g/10 mL and 1.0 g/10 mL. However, the *M. cajuputi* branch extract was less potent than the leaf extract because higher concentrations were required to achieve 100% inhibition of each weed species (Tables 1 and 2; Fig. 1).

Further analysis using 2-way analysis of variance revealed that the extract was more potent in suppressing germination and shoot growth of broadleaf weeds than narrowleaf weeds (Fig. 2). However, the difference in potency was only at low concentrations (0.1 g/10 mL and 0.25 g/10 mL), whereas at higher concentrations (0.5 g/10 mL and 1 g/10 mL) there was no significant difference in the % suppression and reached 100% at a concentration of 1 g/10 mL.

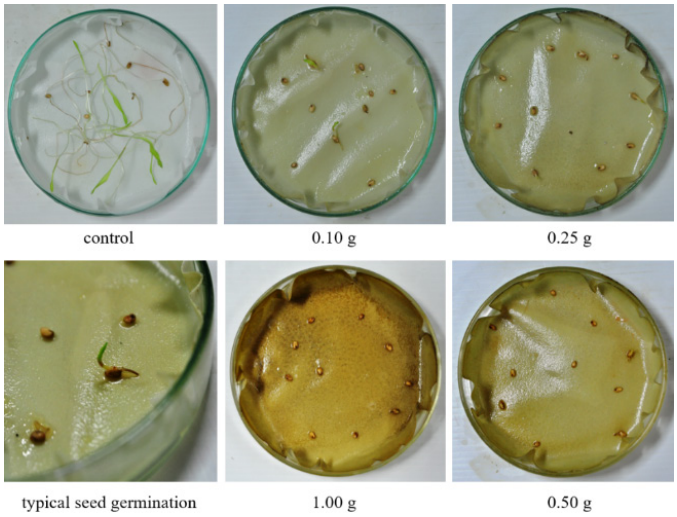


**Fig. 1** Inhibition of *Cenchrus echinatus* seed germination by various amounts of *Melaleuca cajuputi* leaf extract dissolved in 10 mL water

**Table 1** Means of percentage inhibition of weed seed germination and shoot growth from *Melaleuca cajuputi* leaf extract

| Inhibition of | Concentration (g) | | | |
|---|---|---|---|---|
| | 0.1 | 0.25 | 0.5 | 1 |
| Seed germination | | | | |
| Broadleaf | 84.32±1.1$^{a}$ | 92.85±0.9$^{b}$ | 96.24±0.6$^{c}$ | 100±0.0$^{d}$ |
| Narrowleaf | 70.21±5.9$^{a}$ | 86.29±3.1$^{b}$ | 95.87±2.1$^{c}$ | 100±0.0$^{c}$ |
| Shoot growth | | | | |
| Broadleaf | 86.58±2.2$^{a}$ | 93.65±0.8$^{b}$ | 96.74±1.6$^{c}$ | 100±0.0$^{d}$ |
| Narrowleaf | 65.81±2.6$^{a}$ | 87.06±5.8$^{b}$ | 97.76±1.8$^{c}$ | 100±0.0$^{c}$ |

Mean values ($n = 4$) followed by (SD) with different lowercase superscripts in same row are significantly ($p \leq 0.05$) different.

**Table 2** Percentage inhibition of weed seed germination from *Melaleuca cajuputi* leaf extract

| Treatment | Concentration (g) | Inhibition of weed seed germination (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. prostrata* | *B. alata* | *T. procumbens* | *R. tuberosa* | *G. celosioide* | *A. viridis* | *P. oleracea* | *E. heterophylla* | *A. aspera* | *C. barbata* | *R. cochinchinensis* | *C. echinatus* |
| Control | 0.00 | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{d}$ | 0.00$^{c}$ | 0.00$^{c}$ | 0.00$^{f}$ | 0.00$^{e}$ | 0.00$^{c}$ | 0.00$^{b}$ | 0.00$^{e}$ | 0.00$^{d}$ |
| Leaves | 0.10 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 80.00±6.1$^{b}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 63.48±1.8$^{d}$ | 21.59±4.9$^{d}$ | 39.05±12.5$^{b}$ | 100.0±0.0$^{a}$ | 93.93±5.3$^{a}$ | 71.55±6.5$^{b}$ |
| | 0.25 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 76.73±7.3$^{c}$ | 58.9±6.9$^{c}$ | 71.39±8.9$^{b}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 87.45±5.5$^{b}$ |
| | 0.50 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 89.97±4.7$^{b}$ | 76.16±3.6$^{b}$ | 87.57±6.7$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.00±0.0$^{a}$ |
| | 1.00 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.00±0.0$^{a}$ |

Mean values ($n = 4$) ± SD with different lowercase superscripts in same row are significantly ($p \leq 0.05$) different.

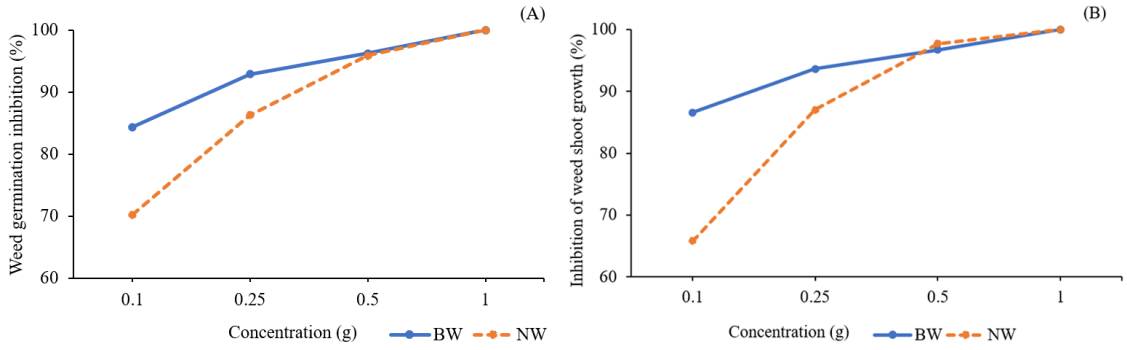


**Fig. 2** Interaction plots of concentration and inhibition of broadleaf (BW) and narrowleaf (NW) weeds using leaf extract of *M. cajuputi*: (A) inhibition of weed seed germination; (B) inhibition of weed shoot growth

### *Inhibition of shoot growth by M. cajuputi extract*

After treating of seedlings with *M. cajuputi* leaf and branch extracts for 5 d, the shoot growth of all 12 species of weed seedlings was strongly inhibited (Table 3 and S2). The inhibition potency was clearly higher for the leaf extract than the branch extract at concentrations of 0.1–0.25 g/10 mL. However, the potency was almost equal at higher concentrations. The overall results suggested that there were higher levels of active constituents in the leaf than the branch. Thus, the chemical constituents of *M. cajuputi* leaf extract were further investigated.

### *Gas chromatography-mass spectrometry analysis*

In total, 27 compounds were identified in the *M. cajuputi* leaf methanolic extract using GC-MS analysis, with the major compounds in descending order being eugenol, trans-caryophllene, dehydro-p-cymene, α-selinene, eudesma-4 (14),11-diene, caryophyllene oxide, α-humulene and *β*-elemene, respectively. The highest percentage was for eugenol (34.38%) which might be the active compound in *M. cajuputi* leaf extract (Table 4).

**Table 4** Chemical constituents found in *Melaleuca cajuputi* methanolic leaf extract based on gas chromatography-mass spectrometry analysis

| Retention time (min) | Compound | Peak area (%) |
|---|---|---|
| 7.70 | Sabinene | tr |
| 7.80 | α-Terpinene | tr |
| 8.02 | p-Cymene | 0.74 |
| 8.10 | α-Terpinolene | - |
| 12.70 | Dehydro-p-cymene | 4.16 |
| 13.58 | Furfural | 0.63 |
| 14.11 | α-Copaene | tr |
| 15.98 | Linalool | 0.10 |
| 17.08 | *β*-Elemene | 2.67 |
| 17.20 | Trans-caryophllene | 6.14 |
| 17.42 | Terpinen-4-ol | 0.69 |
| 19.19 | α-Humulene | 2.68 |
| 19.37 | *β*-Selinene | 0.56 |
| 19.73 | α-Amorphene | 0.61 |
| 19.86 | 1, 8-Menthadiene-4-ol | 0.11 |
| 20.12 | α-Terpineol | 0.35 |
| 20.27 | Germacrene D | 0.53 |
| 20.56 | Eudesma-4(14),11-diene | 3.77 |
| 20.71 | α-Selinene | 3.99 |
| 21.67 | δ-Cadinene | 1.48 |
| 23.66 | 1S,cis-calamenene | 0.36 |
| 24.26 | p-Cymene-8-ol | 1.18 |
| 27.35 | Caryophyllene oxide | 3.50 |
| 30.86 | Spathulenol | 1.34 |
| 41.43 | Phytol | 0.55 |
| 47.10 | Hexadecanoic acid | 10.06 |
| 48.77 | Eugenol | 34.38 |

tr = trace amount

**Table 3** Percentage inhibition of weed shoot growth from *Melaleuca cajuputi* leaf extract

| Treatment | Concentration (g) | Inhibition of weed shoot growth (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. prostrata* | *B. alata* | *T. procumbens* | *R. tuberosa* | *G. celosioide* | *A. viridis* | *P. oleracea* | *E. heterophylla* | *A. aspera* | *C. barbata* | *R. cochinchinensis* | *C. echinatus* |
| Control | 0.00 | 0.0$^{c}$ | 0.0$^{c}$ | 0.0$^{c}$ | 0.0$^{d}$ | 0.0$^{c}$ | 0.0$^{c}$ | 0.0$^{c}$ | 0.0$^{f}$ | 0.0$^{f}$ | 0.0$^{b}$ | 0.0$^{f}$ | 0.0$^{c}$ |
| Leaves | 0.10 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 63.5±4.7$^{b}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 47.8±4.9$^{d}$ | 76.9±5.5$^{c}$ | 46.2±3.1$^{d}$ | 100.0±0.0$^{a}$ | 90.93±4.5$^{ab}$ | 51.2±3.8$^{c}$ |
| | 0.25 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 61.7±3.5$^{c}$ | 81.1±6.5$^{d}$ | 82.1±4.9$^{b}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 79.1±5.2$^{b}$ |
| | 0.50 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 78.9±3.7$^{b}$ | 91.8±5.0$^{b}$ | 93.3±2.6$^{ab}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ |
| | 1.00 | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ | 100.0±0.0$^{a}$ |

Mean values ($n$ = 4) ± SD with different lowercase superscripts in same row are significantly ($p \leq 0.05$) different.

### *Inhibition by eugenol of seed germination and seedling growth of weeds*

As expected, eugenol clearly inhibited the germination of the weed seeds and seeding growth, as shown by the reductions in the shoot and root growth. The magnitude of the inhibitory effects increased with concentration, with the highest concentration (0.12 M) inhibiting 77.0±14.3% of seed germination, 87.5±12.7% of shoot growth and 80.6±16.6% of root growth, averaged across weed species (Table 5).

### *Inhibition by leaf powder and extract in soil-based experiment*

The soil-based experiment produced similar results to the previous experiments in Petri dishes, with the leaf powder and leaf extract being more potent than the branch powder and extract (Table 6) and the potency of all product types seemed to increase with increasing concentration (Fig. 3). Notably, the relative potency between the powder and the extract products of leaves varied with the weed species. For examples, the extract was more potent than the powder in inhibiting seed germination of *E. heterophy* and *A. aspera*, whereas the powder produced a higher percentage inhibition with *R. tuberosa*, *R. cochinchin* and *C. echinatus*. Furthermore, the *M. cajuputi* leaf extract had greater inhibition of broadleaf weeds than narrowleaf weeds.

**Table 5** Percentage inhibition of eugenol on seed germination, shoot growth and root growth of *Achyranthes aspera*

| Eugenol (M) | Inhibition of *A. aspera* (%)[1] | | |
|---|---|---|---|
| | Seed germination | Shoot growth | Root growth |
| 0 (control) | 0.0±0.0$^{h}$ | 0.0±0.0$^{h}$ | 0.0±0.0$^{h}$ |
| 0.015 | 36.2±7.1$^{f}$ | 25.5±5.4$^{g}$ | 24.2±4.5$^{g}$ |
| 0.03 | 45.7±12.8$^{e}$ | 65.5±6.5$^{c}$ | 54.7±7.9$^{d}$ |
| 0.06 | 62.5±10.5$^{c}$ | 74.2±11.3$^{b}$ | 61.0±8.4$^{cd}$ |
| 0.12 | 77.0±14.3$^{ab}$ | 87.5±12.7$^{a}$ | 80.6±16.6$^{a}$ |
| LSD 95% | 6.0 | 6.5 | 7.5 |
| CV (%) | 8.4 | 7.6 | 10.3 |
| F test | ** | ** | ** |

LSD = least significant difference; CV = coefficient of variation.
Mean values ($n$ = 4) ± SD with different lowercase superscripts in same row are significantly ($p \leq 0.05$) different.
** = significant at $p \leq 0.01$.

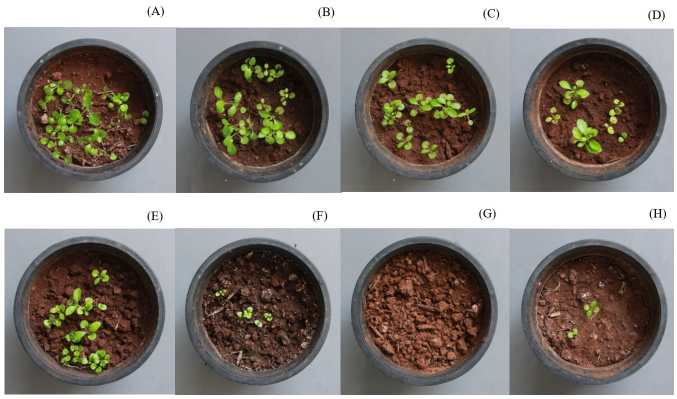


**Fig. 3** Inhibition of *Ruellia tuberosa* seed germination by *Melaleuca cajuputi* branch powder at concentrations of (A) 0; (B) 2%; (C) 4%; (D) 6% and leaf powder at concentrations of (E) 2%; (F) 4%; (G) 6%, compared to (H) 0.2% paraquat dichloride

**Table 6** Allelopathy effect of *Melaleuca cajuputi* leaf and branch on percentage inhibition of weed seed germination in soil-based experiment

| Concentration | Treatment | Inhibition of weed seed germination (%) [1] | | | | |
|---|---|---|---|---|---|---|
| | | E.heterophylla | R. tuberosa | A. aspera | R. cochinchinensis | C. echinatus |
| | Control | 0.00±0.0 | 0.00±0.0 | 0.00±0.0 | 0.00±0.0 | 0.00±0.0 |
| 2% | Leaf powder | 69.48±6.8$^{cd}$ | 85.71±4.1$^{abc}$ | 74.16±8.6$^{bc}$ | 72.73±9.3$^{abc}$ | 61.19±7.9$^{cde}$ |
| | Leaf extract | 95.12±5.2$^{ab}$ | 71.43±8.3$^{cd}$ | 30.33±6.3$^{ef}$ | 59.11±11.1$^{bcd}$ | 79.10±5.0$^{bc}$ |
| | Branch powder | 29.41±9.1$^{g}$ | 56.18±6.8$^{de}$ | 26.83±5.3$^{f}$ | 49.31±8.1$^{cde}$ | 14.10±6.0$^{g}$ |
| | Branch extract | 39.70±7.0$^{fg}$ | 47.19±5.9$^{e}$ | 14.63±6.3$^{g}$ | 22.94±7.4$^{e}$ | 32.12±4.2$^{f}$ |
| 4% | Leaf powder | 76.86±4.6$^{cd}$ | 94.36±3.6$^{ab}$ | 85.27±7.7$^{ab}$ | 81.82±7.2$^{ab}$ | 73.14±3.3$^{dc}$ |
| | Leaf extract | 100.00±0.0$^{a}$ | 81.43±6.3$^{bc}$ | 44.94±5.4$^{d}$ | 65.89±6.9$^{a–d}$ | 89.55±4.4$^{ab}$ |
| | Branch powder | 51.47±3.7$^{ef}$ | 80.90±5.0$^{bc}$ | 37.80±3.0$^{def}$ | 57.53±5.3$^{bcd}$ | 44.87±3.5$^{ef}$ |
| | Branch extract | 63.23±5.3$^{de}$ | 64.05±3.5$^{d}$ | 30.46±4.6$^{ef}$ | 34.25±3.5$^{de}$ | 47.44±5.7$^{def}$ |
| 6% | Leaf powder | 82.93±3.3$^{bc}$ | 100.00±0.0$^{a}$ | 91.01±4.0$^{a}$ | 92.04±3.4$^{a}$ | 88.06±2.4$^{ab}$ |
| | Leaf extract | 100.00±0.0$^{a}$ | 91.43±2.3$^{ab}$ | 66.29±2.9$^{c}$ | 86.37±4.7$^{ab}$ | 100.00±0.0$^{a}$ |
| | Branch powder | 70.58±2.8$^{cd}$ | 82.83±4.3$^{bc}$ | 70.73±2.3$^{c}$ | 64.38±4.8$^{bcd}$ | 74.36±3.6$^{bc}$ |
| | Branch extract | 72.06±4.6$^{cd}$ | 82.06±2.6$^{bc}$ | 40.27±3.7$^{de}$ | 56.17±2.7$^{bcd}$ | 75.32±3.2$^{bc}$ |
| 0.2% | Paraquat dichloride | 86.2±7.9 | 92.3±3.8 | 88.7±9.4 | 86.4±5.4 | 82.0±2.8 |
| | Total average | 70.9 | 78.13 | 51.06 | 61.88 | 64.94 |
| | CV (%) | 10.5 | 9.70 | 13.30 | 23.90 | 15.40 |
| Treatment (T) | LSD (99%) | 18.56 | 12.96 | 20.95 | 19.62 | 19.39 |
| | F test | ** | ** | ** | ** | ** |
| Concentration | LSD (99%) | 10.66 | 10.82 | 9.71 | 21.19 | 14.33 |
| (C) | F-test | ** | ** | ** | ** | ** |
| (M)×(C) | F test | ns | ns | ns | ns | ns |

LSD = least significant difference; CV = coefficient of variation
Mean values ($n$ = 4) ± SD with different lowercase superscripts in same row are significantly ($p \leq 0.05$) different.
** = significant at $p \leq 0.01$

In this experiment, a chemical herbicide, paraquat dichloride (0.2% weight per weight) was used as a positive control and produced a percentage of inhibition in the range 82.0±2.8–92.3±3.8% across five weed species. The inhibitory effect of *M. cajuputi* leaf extract tended to be lower than that of paraquat at low concentrations (2−4%) but the potency was almost equal at the 6% concentration of the leaf extract (*versus* 0.2% paraquat).

## Discussion

Chemical herbicides have caused environmental contamination as well as human health problems worldwide (Rolando et al., 2017; Islam et al., 2018; Zimdahl, 2018). This necessitates investigating natural herbicides and plant extracts that show high potential (Li et al., 2010; Dayan et al., 2012; Ladhari et al., 2013; Jabran et al., 2015; Motmainna et al., 2021). The series of experiments conducted herein revealed that the leaf and branch extracts of *M. cajuputi* contained chemical compounds that inhibited the germination and growth of weed seedlings. Although it is known that the leaves and stems of several *Melaleuca* species, including *M. cajuputi*, produce essential oils that play important roles in traditional medicines in South-East Asia (Brophy et al., 2013), the present study explored the potential use of *M. cajuputi* extract as herbicides. The relative potency of leaf and branch extracts (leaf extract > branch extract) corroborated the fact that plant essential oils in the glands or trichomes of plants are more abundance in leaves than in other plant parts (Brophy et al., 2013). In addition, Laosinwattana et al. (2009) found that the aqueous leaf extract of *Aglaia odorata* had a greater level of inhibition of the germination and seedling growth of barnyard grass and wild pea than branch extract.

The extract was more potent against broadleaf than narrowleaf weeds. This finding was well supported by the anatomy of the plant, because the shoot apexes of broadleaf weeds are directly exposed to herbicide on the horizontally extended leaves. In contrast, the shoot apexes of narrowleaf species are encased by coleoptiles that protect against herbicide. However, at high concentration, both narrowleaf and broadleaf weeds were equally inhibited, perhaps because the concentration was the optimum for the inhibition of gibberellins, which are a growth hormone required for seeding growth (Amaral da Silva et al., 2005).

The result indicated that a monoterpene was the major component in *M. cajuputi* followed by an oxygen-containing monoterpene. This corresponded with the result of Noor et al. (2020), even though the extraction method (steam distillation) was different. There are several techniques to obtain the chemical components in plant material, with the major components obtained depending on the method. For example, Jajaei et al. (2010) extracted *M. cajuputi* leaf collected from Malaysia using supercritical fluid extraction and hexane extraction. They found the maximum yields were obtained using solvent extraction, with the major components from GC-MS analysis consisting of sesquiterpenes, followed by their oxygenated derivatives and polyphenolic ketones. In addition, Kueh et al. (2019) showed that caryophyllene and humulene were two major sesquiterpenes among nine compounds found in *M. cajuputi* leaves from Malaysia, which were extracted based on supercritical $CO_2$ extraction and analyzed using GC-MS. However, the extract induced weed bud injuries ranging from chlorosis to necrosis. It might be possible that this plant contained various groups of active compounds and the different extraction methods isolated the different major components. In addition, their study indicated that the variation in the major constituents in *M. cajuputi* depended on the geographical distribution of plants which was associated with their environmental and genetic background (Kadu et al., 2012; Sikuten et al., 2021). Thus, further study is recommended to compare different plant samples and extraction methods to get the best natural herbicide compounds from *M. cajuputi*.

In this experiment pure eugenol inhibited narrowleaf weed seed germination and seedling growth. Similar inhibitory effect was observed in the eugenol from the essential oil of cinnamon and clove (Tworkoski, 2002; Oliveira et al., 2016). Several studies have attempted to understand the mechanisms of such inhibition; however, the results are still inconclusive. Vaid et al. (2010) reported the reduction of total chlorophyll and decreased cellular respiration in two broadleaf weed species (*Cassia occidentalis* and *Bidens pilosa*) along with growth inhibition caused by eugenol. Furthermore, Ahuja et al. (2015) found that eugenol induced the generation of reactive oxygen species, leading to oxidative stress and membrane damage in root tissue. At present, the mechanisms of action of natural product herbicides have received much interest and the proposed mechanisms include inhibition of cell division, impacting nutrient uptake, inhibition of extension growth, inhibition of photosynthesis, the adverse effect of respiration, the effect of protein synthesis, changes in membrane permeability, and the inhibition of enzyme activity (Duke, 2017).

However, Kueh et al. (2019) reported that caryophyllene is the active allelopathic compound found in *M. cajuputi* leaf *via* supercritical $CO_2$ extract. It might be the synergistic effect of more than one compound in each plant species that plays a role in allelopathy (Moon et al., 2011; Vasilakoglou et al., 2012; Richards et al., 2016).

*M. cajuputi* extract and powder were compared in a soil-based experiment because plant powder is much easier for farmers to prepare than plant extract and the amounts of active compounds might be different. The soil-based experiment showed that *M. cajuputi* leaf extract and powder inhibited both broadleaf and narrowleaf weed species. In addition, high concentration of *M. cajuputi* leaf extract and powder showed 100% inhibition of both broadleaf and narrowleaf weed species, which was better than using paraquat dichloride at low concentration. The evidence that leaf extract and leaf powder performed differently in different weed species suggested that the chemical constituents of these products might be different, with different weed species responding differently to various allelopathic agents. It is possible that the leaf powder may contain extra allelopathic compounds which could not be extracted (Ploliński et al., 2022). Furthermore, the synergistic effect among compounds may trigger different responses by weeds (Hummelbrunner and Isman, 2001; Richards et al., 2016). Such evidence has been provided by Laosinwattana et al. (2010) who found that the dried leaf powder of *Suregada multiflorum* had stronger inhibition effects than the extract. However, plant extracts are frequently more active than plant powder because the bioactive compounds are more concentrated in the extract (Vongsak et al., 2013).

The present study provided evidence that *M. cajuputi* leaf extract and powder have high potential for use as a natural herbicide which could reduce negative environmental impacts and avoid the development of herbicide-resistant weeds.

## Conclusion

Leaf and branch methanol extracts of *M. cajuputi* inhibited the seed germination and seedling growth of 12 weed species, with higher inhibition potency on broadleaf weeds than narrowleaf weeds and the leaf extract was more potent than the branch extract. Among the 27 compounds identified in the *M. cajuputi* leaves, eugenol was the major compound (34.38%); its inhibitory effects on the seed germination and growth of weeds have been confirmed using pure eugenol. Furthermore, the soil-based experiment using powder and extract of *M. cajuputi* leaves and branches revealed that leaf products had better inhibitory effects on weeds and that both types of products (powder and extract) inhibited the seed germination and seedling growth of weeds. Furthermore, the experiments showed that the inhibitory effects increased with increasing extract or powder concentrations; however, the relative potency of the powder and the extract varied with the species of weed.

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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