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Growth suppression of *Echinochloa crus-galli* by leaf extracts of *Malachra capitata*

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Abstract This study investigated the distribution of allelopathic activity among different plant parts (roots, stems, and leaves) of *Malachra capitata* and evaluated its potential as a natural herbicide against barnyardgrass (*Echinochloa crus-galli*). Among the three plant parts examined, leaf extracts exhibited the strongest phytotoxic activity. The 75% ethanolic leaf extract had slightly effect on seed germination but markedly inhibited root elongation, reducing root length by 85.41% at 0.50% a.i. (w/v) and nearly completely suppressing root development at 0.75% a.i. (w/v). In post-emergence pot experiments, the *M. capitata*-derived natural herbicide significantly suppressed barnyardgrass growth in a concentration-dependent manner. At 6.00% a.i. (w/v), plant height was reduced by 50.6% compared with the control at 21 days after treatment, while shoot dry weight decreased by 45.21%. Treated plants also exhibited phytotoxic symptoms, including chlorosis, necrosis, and, most notably, severe stunting, indicating prolonged suppression of vegetative growth rather than rapid plant mortality. Physicochemical characterization showed that pH remained stable across all treatments, suggesting that the herbicidal activity was primarily attributable to the allelochemical constituents of the extract. These findings demonstrate that leaf-derived *M. capitata* extracts effectively inhibit the growth of barnyardgrass and represent a promising source of plant-based natural herbicides.

Keywords: Allelopathy, Barnyardgrass, Leaf extract, Plant-derived herbicide, Weed control

Introduction

Chemical herbicides remain the primary strategy for weed management because of their rapid and effective control. However, their continuous and intensive use has accelerated the evolution of herbicide-resistant weed

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populations, substantially reducing the efficacy of many commercial herbicides. Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.), one of the most troublesome weeds in rice production systems worldwide, has evolved resistance to multiple herbicide modes of action. To date, 85 confirmed cases of herbicide-resistant barnyardgrass have been reported across 16 countries, with resistance occurring most frequently to penoxsulam, bispyribac-sodium, cyhalofop-butyl, propanil, and quinclorac (Damalas and Koutroubas, 2023; Wu *et al.*, 2019). Furthermore, excessive herbicide use has raised concerns regarding environmental contamination, adverse effects on non-target organisms, and potential risks to human health. These challenges have stimulated the search for alternative weed management strategies. Among them, plant-derived natural products have attracted considerable attention because they contain diverse allelochemicals with herbicidal activity and are generally more biodegradable than synthetic herbicides (Charoenying *et al.*, 2022). Crude plant extracts are particularly attractive because they retain a complex mixture of bioactive compounds, including phenolics, flavonoids, alkaloids, tannins, and terpenoids. These compounds may act individually or synergistically to inhibit seed germination and suppress plant growth through disruption of essential physiological and biochemical processes.

Malachra capitata L., a member of the family Malvaceae, is an invasive weed widely distributed throughout tropical and subtropical regions, including Thailand (Vaishali and Alka, 2019; Wichittrakarn *et al.*, 2026). Several factors influence the allelopathic activity of plants, such as developmental stage or the plant part from which the extract is obtained (Inderjit and Duke, 2003; Laosinwattana *et al.*, 2007). Previous studies have demonstrated its allelopathic potential. Kamsan *et al.* (2015) reported that aqueous extracts prepared from dried leaves exhibited stronger phytotoxic activity than those from fresh leaves, indicating that extraction conditions and plant materials can markedly influence allelochemical activity. More recently, Wichittrakarn *et al.* (2026) showed that incorporating *M. capitata* residues into soil effectively suppressed weed seed emergence, further supporting its potential as a source of natural herbicidal compounds. Nevertheless, little information is available regarding the distribution of herbicidal activity among different plant parts or the post-emergence efficacy of *M. capitata*-derived extracts. Therefore, this study aimed to compare the herbicidal activities of extracts prepared from different plant parts of *M. capitata*, identify the most active plant material, and evaluate the post-emergence efficacy of a formulated *M. capitata*-derived natural herbicide against barnyardgrass. The findings provide a basis for developing plant-based herbicides as an alternative approach for sustainable weed management.

Materials and methods

Plant materials

Whole plants of *M. capitata* were collected from an agricultural field at King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The plants were separated into roots, stems, and leaves, washed thoroughly with tap water to remove adhering soil and debris, and dried in a hot-air oven at 45 °C for 72 h. The dried materials were cut into small pieces and stored at room temperature until extraction.

Bioassays of aqueous extracts from different plant parts

Ten g of dried plant were soaked in 90 mL of distilled water at 4 °C for 72 h. The extracts were filtered through Whatman No. 1 filter paper and used immediately for bioassays. Extracts from roots, stems, and leaves were evaluated at concentrations of 1.25, 2.5, 5.0, and 10.0% (w/v) for their effects on barnyardgrass seed germination and seedling growth. Twenty seeds were placed in 9-cm glass Petri dishes lined with germination paper moistened with 5 mL of each treatment solution. Distilled water served as the control. Dishes were incubated in a growth chamber at 25/31°C (night/day) under a 12 h light/12 h dark photoperiod. Germination was recorded after 7 days, and root and shoot lengths were measured. Germination inhibition and seedling growth inhibition were calculated relative to the control.

Bioassays of ethanolic extracts

The plant part exhibiting the strongest inhibitory activity in the aqueous extract screening was selected for ethanol extraction. Dried plant material was extracted three times with 75% (v/v) ethanol, and the combined filtrates were concentrated under reduced pressure at 45 °C using a rotary evaporator. The crude extract was formulated with dimethylformamide (DMF) and Tween 80 at a ratio of 2:3:5 (crude extract: DMF: Tween 80) to improve its dispersion in aqueous solution before biological evaluation. The resulting stock solution was diluted with distilled water to final concentrations of 0.50, 0.75, and 1.00% a.i. (w/v) and evaluated using the same Petri dish bioassay described above.

To verify that the observed phytotoxicity was not caused by physicochemical properties of the formulations, pH and osmotic potential were determined. Osmotic potential was estimated from electrical conductivity (EC) using the equation: Osmotic potential (mbar) = 0.36 × EC (µS/cm).

Post-emergence herbicidal activity

Barnyardgrass seedlings were grown in plastic pots (15 cm diameter × 15 cm depth) filled with sandy loam prepared by mixing soil and sand at a ratio of 3:1 (v/v). Two-week-old seedlings were sprayed with the formulated *M. capitata* extract at concentrations of 2, 4, and 6% active ingredient (a.i.) using a hand sprayer at an application volume equivalent to 120 L/rai. Distilled water was applied as the control. Treated plants were maintained in a glasshouse (28-30 °C, natural light, 64-69% relative humidity), and irrigation was withheld for 24 h after application. Plant height was recorded at 7, 14, and 21 days after treatment (DAT), and shoot dry weight was determined at 21 DAT.

Statistical analysis

All experiments were conducted using a completely randomized design with four replicates. Data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Tukey's HSD test at $p < 0.05$.

Results

Effects of aqueous extracts from different parts of M. capitata

The aqueous extracts obtained from different parts of *M. capitata* exhibited varying inhibitory effects on the germination, and growth of barnyardgrass depending on the plant part and extract concentration (Figure 1). Among all plant parts, leaf extracts showed the strongest herbicidal activity compared with stem and root extracts. Leaf extracts at 10% w/v concentration completely inhibited barnyardgrass germination, while shoot and root growth were also totally suppressed. At 5% w/v concentration, leaf extracts did not significantly affect germination or shoot; however, root growth was strongly inhibited by 80.20%, indicating that root development was more sensitive to the extract than shoot growth. Lower concentrations of leaf extracts (1.25-2.5% w/v) produced only slight inhibitory effects on shoot and root elongation. Stem extracts exhibited moderate inhibitory activity. At 10% w/v concentration, stem extracts inhibited germination by 18.75%, while shoot and root length were reduced by 36.28% and 90.52%, respectively. In contrast, root extracts showed the weakest inhibitory effects among all plant parts. Even at the highest concentration, root extracts inhibited germination by 13.75%, whereas root and shoot growth inhibition reached 36.61% and 18.25%, respectively.

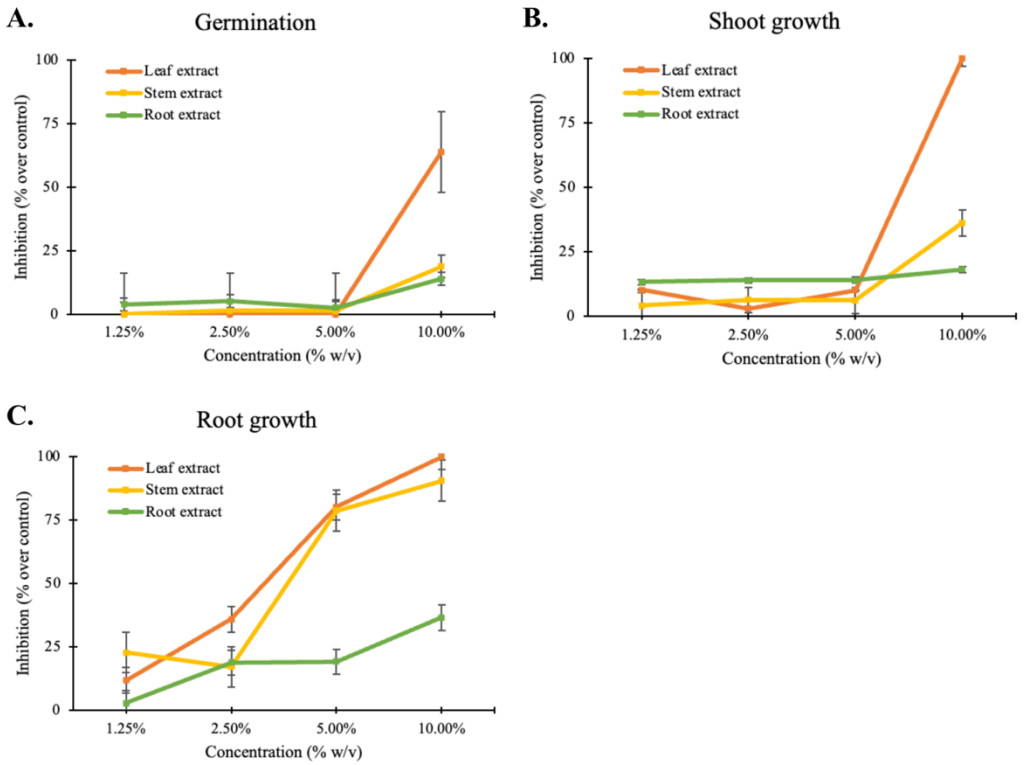


Figure 1. Effect of aqueous extracts from different parts of *M. capitata* on seed germination (A) and shoot (B) and root(C) growth of barnyardgrass

Physicochemical characterization of M. capitata crude extracts

The physicochemical properties of the *M. capitata*-derived natural herbicide are summarized in Table 1. The pH of all treatment solutions remained relatively constant, ranging from 5.24 to 5.29, with no significant differences among concentrations ($p > 0.05$). In contrast, EC increased significantly with increasing extract concentration, from 991.73 µS/cm at 0.50% to 10,878.67 µS/cm at 6.00% ($p < 0.05$). Accordingly, the estimated osmotic potential became progressively more negative as extract concentration increased. These results indicate that the extract formulations exhibited similar acidity across all treatments, whereas EC and osmotic potential varied proportionally with extract concentration.

Table 1. pH value, EC, and osmotic potential of treatment solutions according to concentration of *M. capitata*-derived natural herbicide

Treatment	pH	EC (μS/cm)	Osmotic potential (mbar)
Petri dish assay (% w/v)			
0.50	5.26 ± 0.03a	991.73 ± 24.47f	357.02 ± 8.81f
0.75	5.24 ± 0.01a	1,424.67 ± 11.72e	512.88 ± 4.22e
1.00	5.24 ± 0.03a	1,788.33 ± 37.82d	643.80 ±13.62d
Pot experiment (% w/v)			
2.00	5.29 ± 0.06a	2,945.67 ± 134.81c	1,060.44 ± 48.53c
4.00	5.26 ± 0.03a	7,144.00 ± 45.18b	2,571.84 ± 16.26b
6.00	5.29 ± 0.03a	10,878.67 ± 69.37a	3,916.32 ± 24.97a

The data are presented as means ± S.D. In each column, Data followed by the same letter do not differ by the Tukey's HSD test ($p < 0.05$).

Effects of leaf-derived crude extract on barnyardgrass growth

Extraction of 50 g of dried *M. capitata* leaves with 75% ethanol yielded 3.12, 2.04, and 1.31 g of crude extract from the first, second, and third extraction cycles, respectively, giving a total crude extract yield of 6.47 g (12.94%, w/w dried leaves). The effects of the formulated leaf-derived crude extract on barnyardgrass seed germination and early seedling growth are shown in Figure 2. Seed germination was slightly affected by the treatments (Figure 2A). Seed germination was defined as radicle protrusion exceeding 2 mm beyond the seed coat (Steinbrecher and Leubner-Metzger, 2017).

Interestingly, the extract markedly inhibited early post-germination root development (Figure 2B). The mean root length of the control seedlings was 3.70 cm, whereas treatment with 0.50% (w/v) extract reduced root length to 0.54 cm. Increasing the concentration almost completely suppressed root elongation. Consequently, although the tested seeds satisfied the criterion for germination, the absence of root development indicates that the seedlings were unable to establish after germination. These findings indicate that the *M. capitata* leaf-derived crude extract exerts its primary phytotoxic effect after germination by severely restricting root development rather than inhibiting the germination process itself.

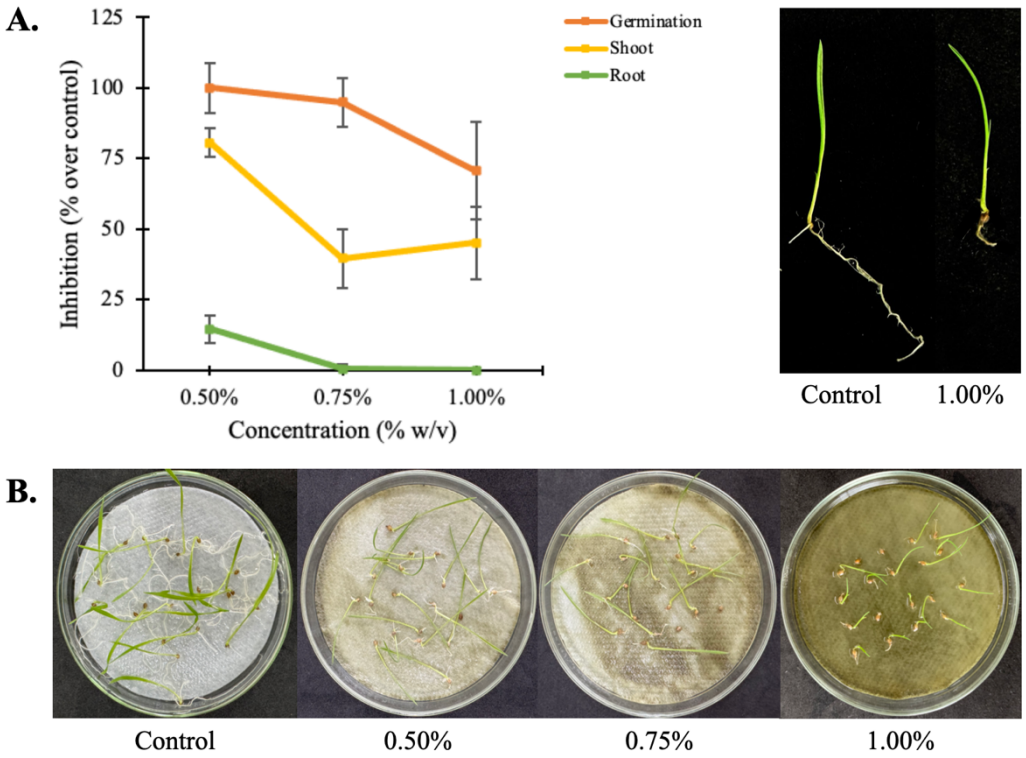


Figure 2. Effects of leaf-derived ethanolic extracts of *M. capitata* on seed germination and seedling growth of barnyardgrass. (A) Seed germination, root length, and shoot length of seedlings treated with different concentrations of the extract. (B) Representative photographs of barnyardgrass seedlings 7 days after treatment

Efficacy of M. capitata-derived natural herbicide in pot experiments

The post-emergence herbicidal activity of the *M. capitata*-derived natural herbicide against barnyardgrass is presented in Table 2 and Figure 3. No significant differences in plant height were observed among treatments at 7 DAT ($p > 0.05$). However, the inhibitory effect became more pronounced over time. This trend persisted until 21 DAT, when plants treated with 6.00% herbicide reached only 18.24 cm, representing approximately 50.6% lower plant height than the control (36.92 cm). A similar trend was observed for shoot dry weight at 21 DAT. The highest concentration significantly reduced dry biomass to 7.32 g, corresponding to a reduction of approximately 45.21% relative to the untreated control (13.36 g).

Visual observations were consistent with the quantitative measurements (Figure 3). Following post-emergence application, treated barnyardgrass plants exhibited concentration-dependent toxic symptoms, including leaf chlorosis and necrosis. The most prominent symptom, however, was severe growth suppression, characterized by pronounced stunting and limited elongation of newly emerging leaves. At the highest concentration, plants remained markedly shorter than the untreated control throughout the experimental period, indicating that the *M. capitata*-derived natural herbicide primarily inhibited subsequent vegetative growth rather than causing rapid plant mortality.

Table 2. Plant length and dry weight of barnyardgrass after post-emergence application of the *M. capitata*-derived natural herbicide

Treatment	Plant length (cm)			Dry weight (g)
	7 DAT	14 DAT	21 DAT	
0% (Control)	12.26 ± 1.18a	26.21 ± 2.66a	36.92 ± 3.20a	13.358 ± 4.24a
2.00%	10.6 ± 2.80a	21.11 ± 4.23ab	31.86 ± 4.24ab	14.656 ± 4.16ab
4.00%	10.28 ± 1.51a	19.28 ± 2.04bc	28.28 ± 3.02b	9.658 ± 2.52bc
6.00%	9.12 ± 1.48a	14.77 ± 1.73c	18.24 ± 1.19c	7.318 ± 1.27c

Data are presented as means. Different letters in the same column indicate significant difference according to Tukey's HSD test at the $p<0.05$ level.

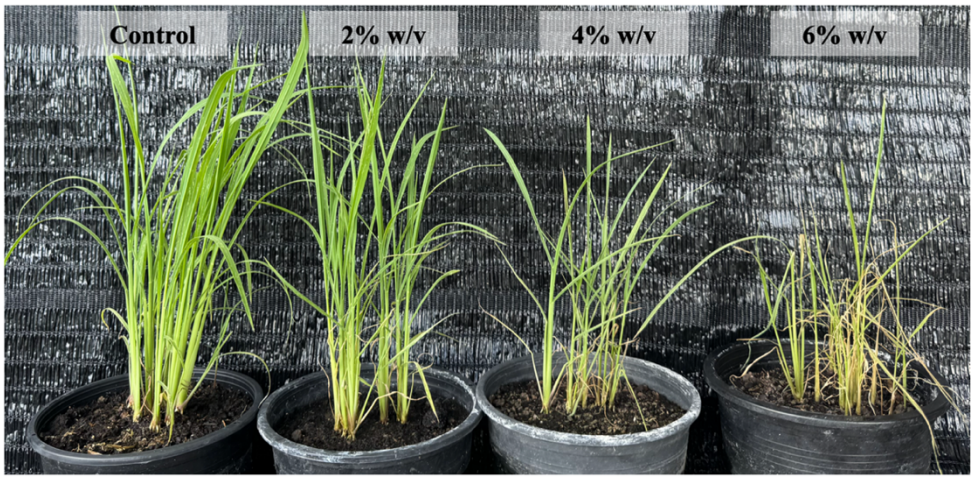


Figure 3. Efficacy of post-emergence application of the *M. capitata*-derived natural herbicide at 0, 2, 4, and 6% on barnyardgrass. Photographs were taken 7 days after treatment

Discussion

The present study is demonstrated that *M. capitata* possesses considerable potential as a source of natural herbicidal compounds. Among the three plant parts examined, leaf extracts exhibited the strongest phytotoxic activity against barnyardgrass, indicating that allelochemicals are unevenly distributed within the plant. This variation is consistent with previous studies showing that allelopathic activity is influenced by plant organ, developmental stage, and environmental conditions, all of which affect the biosynthesis and accumulation of secondary metabolites (Boonmee *et al.*, 2018; Uddin *et al.*, 2012). Previous studies have demonstrated that allelopathic activity differs markedly among plant organs. In several species, leaf extracts consistently exhibit stronger phytotoxic effects than extracts from stems or roots. For instance, leaf and flower extracts of *Parthenium hysterophorus* inhibited *Eragrostis tef* seed germination, whereas stem and root extracts had little effect (Tefera, 2002). Similarly, among different organs of *Drimys brasiliensis*, leaf extracts showed the greatest inhibitory activity against wheat and radish germination (Anese *et al.*, 2015). These observations are consistent with the present study, where leaf extracts of *M. capitata* exhibited the strongest herbicidal activity against barnyardgrass. The superior activity of the leaf extract is likely attributable to the fact that leaves are the primary site of photosynthesis and secondary metabolite biosynthesis. Consequently, leaf tissues often accumulate higher concentrations of bioactive compounds involved in plant defense, resulting in greater allelopathic activity than other plant organs. The physicochemical characterization further supports the conclusion that the observed herbicidal activity was primarily attributable to the bioactive constituents of the extract rather than to indirect effects of solution acidity. Although EC and osmotic potential increased with extract concentration, the variation in these physicochemical properties within the tested range did not affect seed germination, consistent with previous report (Mushtaq *et al.*, 2019; Wu *et al.,* 2019). Therefore, the progressive inhibition of barnyardgrass growth observed in this study is found to be more likely attributable to the phytotoxic constituents of the *M. capitata* extract than to physicochemical stress.

Interestingly, the ethanolic leaf extract had slightly affected on seed germination but strongly inhibited root elongation after germination. Even at the highest concentration, many seeds satisfied the criterion for germination, whereas root development was suppressed. Similar responses have been reported for numerous allelopathic plant extracts, in which inhibition of seedling growth is often more pronounced than inhibition of germination because young root tissues are particularly sensitive to allelochemicals (Amâncio *et al.*, 2021). Root

growth relies on continuous cell division and elongation and is therefore readily disrupted by phytotoxic compounds that interfere with cell metabolism, membrane integrity, hormone homeostasis, or nutrient uptake (Yan *et al.*, 2020). Consequently, although germination occurred, the complete inhibition of root development would likely prevent successful seedling establishment under natural conditions. The greater activity of the ethanolic extract compared with the aqueous extract may be attributed to the intermediate polarity of 75% ethanol, which efficiently extracts a broader range of phytochemicals, including both moderately polar phenolics and flavonoids, than water alone (Kajdžanoska *et al.*, 2011).

The pot experiment further demonstrated that the formulated *M. capitata*-derived natural herbicide suppressed the post-emergence growth of barnyardgrass. Visual symptoms, including leaf necrosis, and severe stunting, were accompanied by significant reductions in plant height and shoot dry weight. These observations are suggested that the extract did not induce rapid plant death but instead restricted subsequent vegetative growth by suppressing shoot elongation and biomass accumulation.

Overall, the results are demonstrated that leaf-derived extracts of *M. capitata* effectively suppress the growth of barnyardgrass by inhibiting root development during the early seedling stage and restricting vegetative growth after post-emergence application. Given the increasing prevalence of herbicide-resistant barnyardgrass populations worldwide, *M. capitata*-derived herbicides may represent a promising complementary tool for sustainable weed management. Nevertheless, further studies are required to identify the active compounds responsible for the observed phytotoxicity, elucidate their modes of action, and evaluate their efficacy, crop selectivity, and field performance under practical agricultural conditions.

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Conflicts of interest

The authors declare no conflict of interest.

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