Research Article

Allelopathic Potential of Aqueous Extracts of Fresh Leaves of *Mucuna bracteata* DC and *Cymbopogon citratus* DC on Weed Germination and Growth

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Abstract | *Mucuna bracteata* and *Cymbopogon citratus* are fast-growing plants, easy to find and contain various secondary metabolites. Therefore, these two types of plants can be used as an alternative bioherbicide for weed control. This study aims to determine the effect of aqueous extracts from fresh leaves of *C. citratus*, *M. bracteata* and a mixture of both on the germination and growth of *Paspalum conjugatum* and *Borreria alata weeds*.Treatments consisted of extracts of *M. bracteata, C. citratus* and a mixture of both extracts at various concentrations (0% or control, 20% and 60%). Spraying of the extracts started at the time of seed planting, then spraying was done every 3 days for 30 days. All aqueous extract treatments showed toxicity effects on weeds as indicated by the inhibition of germination and growth of *P. conjugatum* weed and *B. alata* weed. *Mucuna bracteata* extract was more effective than *C. citratus* extract. The mixed extract (*C. citratus* + *M. bracteata*) was more effective than the single extract. The highest percentage of germination and growth inhibition was found in the single extract of *M. bracteata* and mixed extract (*M. bracteata* + *C. citratus*) with concentrations of 40% and 60%. Based on the results of this study, 40% fresh extract of M. *bracteata* or 40% mixed extract of *C. citratus* + *M. bracteata* can be applied by spraying directly to the field to inhibit weed emergence and weed growth in agricultural land as an alternative weed control.

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Introduction

Weeds are a problem in crop cultivation practices as a result of over-intensive land use. Weeds compete with crops for growing space, light, water, and nutrients, resulting in reduced crop quality, reduced yields, and delays in harvest time (Monteiro and Santos, 2022; Kubiak *et al*., 2022). Weeds are also hosts for insect pests and pathogens that attack crops and disrupt water flow (Abouziena and Haggag, 2016). About 1800 weed species have caused yield reductions of up to 31.5% (Kubiak *et al*., 2022).

Common weed controls are physical, mechanical and chemical. Physical and mechanical control includes tillage and hand weeding. Tillage results in increased weed population and soil erosion. Hand weeding is labor intensive and expensive (Sharma and Rayamajhi, 2022). Chemical weed control using herbicides results in contamination of soil, water, air, crops and can kill non-target organisms (Kubiak *et al*., 2022), reduce farmland biodiversity and loss of natural enemies of crop pests or pollinators (Petit *et al*., 2011). Chemical herbicides also cause toxicity to animals and plants, which in turn affects human health (Marin-Morales *et al*., 2013). The various health and environmental problems arising from the use of synthetic herbicides have led many to start implementing organic farming. More sustainable solutions in weed management are being implemented to reduce the impact of herbicide use. One alternative to sustainable weed management is through the phenomenon of allelopathy. Allelopathy is a biological interaction between plants, both inhibiting and promoting growth through the role of chemical compounds produced by plants. Plant allelopathy can be used to control weeds in various ways, such as through ground cover crops, organic mulch, crop rotation, ground cover crops and plant extracts. Plant extracts containing allelochemicals can also be used as bioherbicides. Phytotoxins from plant extracts can be used as alternative bioherbicides to replace herbicides because they are easily decomposed and safe for the environment (Smith-Fiola and Gill, 2014; Abbas *et al*., 2018; Khamare and Marble, 2022; Scavo and Mauromicale, 2021; Kostina-Bednarz *et al*., 2023). The phytotoxicity activity of allelochemicals can inhibit weed growth. The use of allelochemicals can be an alternative to reduce herbicide use (Dahiya *et al*., 2017; Abbas *et al*., 2021; Kostina-Bednarz *et al*., 2023).

Various plant extracts containing allelochemicals can inhibit weed germination and growth. Treatment of aqueous extracts from fresh leaves inhibits weed germination and growth, and increases damage to weeds (Fatonah *et al*., 2014). The treatment of aqueous extracts from dry leaves inhibits weed germination and growth (Sihombing *et al*., 2012; Taupik *et al*., 2022; Ismail *et al*., 2016; Ishak and Sahid, 2014). These studies generally use dry extracts and in single form (without mixture). Dry extracts require large amounts of plant material. One alternative to reduce the need for plant material and increase the toxicity effect of plant extracts is to use plant material in fresh form and in the form of a mixture of extracts from two plants, for example *Mucuna bracteata* and *Cymbopogon citratus*. Extracts from fresh plant material (without drying) do not require more plant material and not many chemicals are lost. Mixed extracts allow allelochemicals to have a higher toxicity effect due to the synergistic effect of allelochemicals (Mushtaq *et al*., 2010; Abbas *et al*., 2018).

Mucuna bracteata and *C. citratus* can be a source of bioherbicide. *Mucuna bracteata* is a ground cover crop that is widely planted in various plantations because it has a high biomass. This plant is resistant to drought and shade, is not favored by livestock, and is able to compete with weeds. *Mucuna bracteata* contains compounds such as flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids, and saponins (Kumar *et al*., 2009; Natarajan *et al*., 2012; Abigail and Dashak, 2020). *Cymbopogon citratus* or lemongrass is widely found in various regions, has a strong lemon-like aroma, mostly used as spices in food or ingredients for the cosmetics industry. The plant is a producer of alkaloids, saponins, flavonoids, polyphenols, anthraquinones, steroids, and various essential oils (geranial, neral, and myrcene) (Gbenou *et al*., 2013; Pagare *et al*., 2015; Oladeji *et al*., 2019; Gupta *et al*., 2019). Lemongrass is widely cultivated in Indonesia with a production of about 30-50 tons/ha and can be harvested 3-6 times a year for 4-6 years (Sofiah, 2008).

The effectiveness of the extract in controlling weeds needs to be tested against dominant weeds such as *B. alata* and *P. conjugatum*. *Borreria alata* is a broadleaf weed that easily germinates. *Borreria alata* and *P. conjugatum* are dominant weeds in oil palm plantations (Fatonah and Herman, 2011; Dahliani and Elban, 2019; Kurniadie and Umiyati, 2019). *Borreria alata* is the dominant weed in tomato and chili fields (Abdullah *et al*., 2020). This study aims to test the phytotoxic effects of aqueous extracts of *C. citratus, M. bracteata* and a mixture of the two extracts on the germination and growth of *P. conjugatum* and *B. alata* weeds.

Materials and Methods

Experimental design

Research in the form of single factor experiment, with a randomized block design. Treatment in the form of extract concentration of *M. bracteata* and *C. Citratus*, ie single extract (7 treatments) compared to mixture of extract (three treatments) which can be seen in Table 1. Each treatment was repeated five times. All treatments were tested on both weeds (*B. alata* and *P. conjugatum*).

Preparation of extracts, planting seeds of weeds and treatment of extracts

Preparation of extract was carried out by means of fresh leaves of *C. citratus* and *M. bracteata* weighed according to treatment (20%, 40%, and 60%) plus water until it reached one liter, then blended. For example, an extract with a concentration of 20% is made by weighing 200 g of plant material plus water until it reaches one liter, then blended and filtered. Seeds of *B. alata* and *P. conjugatum* were poured on the soil surface evenly in each polybag. Each polybag contained 20 seeds. The application of *M. bracteata* and *C. citratus* extracts were done after seed planting in same day. Extracts were sprayed on soil and weed surfaces every three days for 30 days.

Observation of weed germination and growth

Observations were made daily for 30 days after planting. The variables observed were germination (seedling emergence time, germination percentage, germination inhibition) and growth (fresh weight, number of leaves, shoot length, number of roots, root length, growth inhibition), weed seedling damage, and weed seedling mortality. Inhibition of extract germination shows the effectiveness of the extract in inhibiting weed seed germination with the formula:

$$\text{Germination inhibition} = \frac{\%\text{ germination of control treatment} - \%\text{ treatment germination}}{\%\text{ germination of control treatment}} \times 100\%$$

Growth inhibition showed the extract effectiveness in inhibiting the growth of weed seedlings with formula:

$$\text{Growth inhibition} = \frac{\text{Fresh weight of control treatment} - \text{fresh weight of extract treatment}}{\text{Fresh weight of control treatment}} \times 100\%$$

The obtained data were analyzed by using Analysis of Variance (ANOVA). If analysis of variance showed the significant effect, the further test done with Duncan's Multi Range Test (DMRT) at significance of 5 % by using SPSS 17.

Results and Discussion

Phytotoxic effects of aqueous extracts of M. bracteata DC And C. citratus DC on weed germination

The results of analysis of variance (ANOVA) showed that the treatment of *M. bracteata* and *C. citratus* extracts significantly affected the germination of *B. alata* and *P. conjugatum* seeds (Table 2). The mean germination time and germination percentage of weeds at various concentrations of *M. bracteata* and *C. citratus* extracts are presented in Table 3.

Table 1: *Treatment of single and mixture extracts of M. bracteata and C. citratus at various concentrations.*

Aqueous extracts	Extract concentration (%)	Details
Control	0	Air
M. bracteata	20	200 g/l M. *bracteata extract*
M. bracteata	40	400 g/l M. *bracteata extract*
M. bracteata	60	600 g/l M. *bracteata extract*
C. citratus	20	200 g/l of *C. citratus extract*
C. citratus	40	400 g/l of *C. citratus extract*
C. citrates	60	600 g/l of *C. citratus extract*
M. bracteata + *C. citratus*	20	100 g/l *C. citratus* extract + 100 g/l *M. bracteata* extract
M. bracteata + *C. citratus*	40	200 g/l of *C. citratus* extract + 200 g/l of *M. bracteata* extract
M. bracteata + *C. citrates*	60	300 g/l *C. citratus* extract+300 g/l *M. bracteata* extract

Table 2: *ANOVA results of the effect of water extracts of M. bracteata and C. citratus on germination of B. alata and P. conjugatum weeds.*

Weeds	Germination parameters	Sum of squares	Degrees of freedom	Mean square	F-ratio	Significant (p)
Borreria alata	Seedling emergence time	340.171	9	56.695	6.538	0.000**
	Germination percentage	24741.520	9	2749.058	141.485	0.000**
Paspalum conjugatum	Seedling emergence time	1128.900	9	125.433	5.295	0.000**
	Germination percentage	4208.000	9	467,556	24,289	0.000**

*Notes: **: significant at p<0.01*

Table 3: *Phytotoxic effects of aqueous extracts of M. bracteata and C. citratus on germination of B. alata and P. conjugatum weeds.*

Aqueous extracts	Extract concentration (%)	Germination time (days)		Germination persentage (%)	
		B. alata	***P. conjugatum***	***B. alata***	***P. conjugatum***
Control	0	6.4[a]	7.8[a]	77[c]	32[c]
M. bracteata	20	11.6[b]	9.2[a]	5.8[ab]	5[ab]
M. bracteata	40	11[b]	30[b]	1[a]	NG[a]
M. bracteata	60	30[d]	30[b]	NG	NG[a]
C. citratus	20	10.4[b]	8.6[a]	8.4[b]	10[b]
C. citratus	40	12.6[b]	7.2[a]	4.6[ab]	6[ab]
C. citrates	60	13.2[b]	8.8[a]	5.2[ab]	5[ab]
M. bracteata + C. citratus	20	17.6[c]	13.4[a]	6.4[ab]	4[ab]
M. bracteata + C. citratus	40	NG	NG	NG	NG
M. bracteata + C. Citrates	60	NG	NG	NG	NG

Note: The mean number followed by different letters shows a significant difference at the 5% test level based on the DMRT test. NG : seeds do not germinate.

The treatment of *M. bracteata* and *C. citratus* extracts slowed down the germination time and decreased the germination percentage. All treatments of *C. citratus* and *M. Bracteata* extracts, both single extracts and mixed extracts slowed down the germination time and reduced the germination of *B. alata* weeds. The 40% treatment of *M. Bracteata* extract resulted in a very low percentage of *Borreria alata* weed germination (1%), while the 60% treatment of *M. Bracteata* extract resulted in *B. alata* not germinating. The treatment of 20 to 60% *C. citratus* extract did not slow down the germination time of *P. conjugatum* weed seeds, but decreased the germination percentage. The treatment of *M. bracteata* extract, both single extract and mixed extract of *M. bracteata + C. citratus* at the lowest concentration (20%) did not slow down the germination time, but at higher concentrations (40% and 60%) resulted in *P. conjugatum* seeds not germinating (0% germination percentage). The mixture of *M. bracteata + C. citratus* extracts at 40% and 60% concentration resulted in *B. alata* and *P. conjugatum* seeds not germinating.

Fresh leaf extracts of *M. bracteata* and *C. citratus* showed high effectiveness in inhibiting the germination of weed seeds of *B. alata* and *P. conjugatum* with a germination inhibition percentage of 84.37% to 100% (Table 6). The treatment of *M. bracteata* extract resulted in 100% germination inhibition (no germination) in the 60% concentration treatment for *B. alata* weed and 40% and 60% concentration treatments for *P. conjugatum* weed. Germination inhibition of *C. Citrates* extract on *B. alata* weeds did not reach 100%, but germination inhibition reached 100% on *P. conjugatum* weeds (40% and 60% concentration treatments). Germination inhibition from a mixture of *M. bracteata + C. citratus* extracts reached 100% at 40% and 60% extract concentrations, both on *B. alata* and *P. conjugatum*.

The results showed that fresh leaf extracts of *M. bracteata* and *C. citratus* inhibited the germination of *B. alata* and *P. conjugatum* weed seeds with a high percentage of germination inhibition (84.37% - 100%). Extracts of *M. bracteata* and *C. Citratus* inhibit weed germination due to the content of allelochemicals in the extract which results in weeds not germinating because the seeds die or delay germination. Seeds die because allelochemicals cause increased cell permeability and lipid peroxidation, so that the seed embryo is damaged and dies. Another possibility is that the seeds are still alive but delayed in germinating because allelochemicals inhibit the germination process including hydrolysis of food reserves, respiration, and embryo growth. This is related to the effect of allelochemicals in inhibiting water absorption, cell elongation, and cell division, respiration, enzyme activity and function, phytohormone metabolism, and gene expression (Li *et al*., 2010; Soltys *et al*., 2013; Khalaj *et al*., 2013; Feng *et al*., 2017; Tanase *et al*., 2019; Bachheti *et al*., 2020).

The mixed extract of *M. bracteata + C. Citratus* was more effective in inhibiting germination than the single extract, where *B. alata* or *P. conjugatum* weed seeds did not germinate at concentrations of

40 and 60%. The mixed extract of *M. bracteata* + *C. Citratus* contains various secondary metabolites, namely a mixture of compounds contained in *M. bracteata* (flavonoids, tannins, alkaloids, glycosides, terpenoids, steroids, and saponins) (Natarajan *et al*., 2012; Rane *et al*., 2019; Abigail and Dashak, 2020) and compounds in *C. citrates* (alkaloids, saponins, flavonoids, polyphenols, anthraquinones, steroids, and various essential oils) (Gbenou *et al*., 2013; Oladeji *et al*., 2019; Gupta *et al*., 2019). The interaction of these various compounds leads to higher toxicity that inhibits seed germination.

This study used aqueous extracts of fresh leaves of *M. bracteata* and *C. citratus*. Fresh leaf extracts require less plant material when compared to dried leaf extracts. Other studies that used aqueous extracts from dried leaves of one plant species showed lower effectiveness in inhibiting weed germination. Water extract from dried leaves of *Pueraria javanica* with a concentration of 54% showed 53% germination inhibition on *Asystasia gangetica* weed (Fatonah *et al*., 2013). Other studies using aqueous extracts from dried leaf leaves generally use lower concentrations, because a little dry matter requires a lot of plant material. A low concentration (6.67%) of aqueous extract from the dried leaves of the legume *Leucaena leucocephala* inhibited the germination of *Emilia sonchifolia* weed seeds with a 32% inhibition percentage (Ishak and Sahid, 2014). The aqueous extract of the dried leaves of *Jatropha curcas* with a concentration of 15% inhibited the germination of *Parthenium hyesterophorus* seeds with 46% inhibition effectiveness (Khan *et al*., 2017). However, there are other studies that use aqueous extracts from dried leaves with low concentrations showing high effectiveness in inhibiting weed germination. Water extract from dried leaves of *P. javanica* at a concentration of 6.67% inhibited the germination of *E. indica* weed seeds with an inhibitory effectiveness of 95% (Ismail *et al*., 2016). A 10% aqueous extract of the dried leaves of *Chromolaena odorata* inhibited the germination of field grass weeds with a 100% inhibition rate (seeds did not germinate) (Suwal *et al*., 2010). The aqueous extract of the dried leaves of *Crotalaria juncea* inhibited the germination of *Amaranthus hybridus* weed with 100% inhibition (no seeds germinated) (Skinner *et al*., 2012). A 20% aqueous extract of *Rhanterium eapposum* inhibited the germination of *Chenopodiastrum murale* weed with up to 100% inhibition effectiveness (Al-Harbi, 2018). A 20% aqueous extract of *Datura metel* leaves inhibited *Parthenium hysterophorus* weed germination with 100% inhibitory effectiveness (Ramachandran and Venkataraman, 2016).

Differences in the effectiveness of various extracts in inhibiting the germination of various weeds are related to the content of allelochemicals (plant species), treatment techniques and the level of weed sensitivity to allelochemicals. This study used a mixed treatment of fresh extracts of *M. bracteata* + *C. Citrates* on *B. alata* and *P. conjugatum* weed seeds grown in soil in polybags. The water extract treatment was carried out by spraying seeds, weeds that have grown and the soil surface evenly at three-day intervals for 30 days. Other studies are generally conducted in the laboratory by planting weed seeds on filter paper in a petri dish, and water extracts are moistened on the seeds and filter paper (Suwal *et al*., 2010; Sisodia and Siddiqui, 2010; Patil *et al*., 2013; Woranoot *et al*., 2015; Al-Harbi, 2018; Taupik *et al*., 2022). Another study was conducted on soil in polybags, but the extract was done by soaking the seeds in an aqueous extract (Khan *et al*., 2017). Another study was conducted by extracting various extracts using organic solvents with a low extract concentration (50 mg/l) that could inhibit germination with a high degree of inhibition (Caser *et al*., 2020). We planted the tested weeds in soil in polybags to illustrate conditions that are almost the same as in the field.

Phytotoxic effects of aqueous extracts of M. bracteata and C. citratus on weed growth

ANOVA results showed that the treatment of *M. bracteata* and C. citratus extracts significantly affected the growth of *B. alata* and *P. conjugatum* weeds (Table 4). The mean fresh weight, number of leaves, plant height, and number of roots in various treatments of *M. bracteata* and *C. citratus* extracts are presented in (Table 5).

The results showed that the treatment of *C. citratus* and *M. bracteata* extracts reduced the growth of *B. alata* and *P. conjugatum* weeds. Growth reduction began at the lowest extract concentration treatment (20%). The 20% treatment of *C. citratus* extract inhibited fresh weight, plant height, and number of root. The decrease in the number of leaves of *P. conjugatum* began to occur in the treatment of 20% *C. citratus* extract, but the decrease in the number of leaves of *B. alata* began to occur in the treatment of 40% *C. citratus* extract. The number of leaves of *B. alata* and *P. conjugatum*

Table 4: *ANOVA results of the effect of water extracts of M. bracteata and C. citratus on growth of B. alata and P. conjugatum weeds.*

Weeds	Growth parameters	Sum of squares	Degrees of freedom	Mean square	F-ratio	Significant (p)
Borreria alata	Fresh weight	4339.171	9	482.130	391.386	0.000**
	Number of leaves	866.030	9	96.226	191.770	0.000**
	Shoot length	368.576	9	40.953	651.660	0.000**
	Number of root	390.559	9	43.395	128.900	
Paspalum conjugatum	Fresh weight	2655.383	9	295.043	1780.443	0.000**
	Number of leaves	172.583	9	19.176	7.752	0.000**
	Shoot length	273.120	9	30.347	106.857	0.000**
	Number of root	337.372	9	37.486	10.167	0.000**

*Notes: **: significant at p<0.01*

Table 5: *Phytotoxic effects of aqueous extracts of M. bracteata and C. citratus on growth of B. alata and P. conjugatum weeds.*

Plant extract	Extract concentration (%)	Fresh weight (mg)		Number of leaves		Shoot length (mm)		Number of root	
		Ba	Pc	Ba	Pc	Ba	Pc	Ba	Pc
Control	0	3110[c]	2452[e]	9.36[d]	4.88[c]	95.3[c]	81.5[e]	8.02[c]	5.46[d]
M. bracteata	20	40[b]	140[bc]	7.53[b]	2.70[b]	12.4[b]	9.7[cd]	1.14[b]	1.05[ab]
M. bracteata	40	NG	NG	NG	NG	NG	NG	NG	NG
M. bracteata	60	NG	NG	NG	NG	NG	NG	NG	NG
C. citratus	20	110[b]	910[d]	8.87[cd]	4.87[c]	15.3[b]	16.3[d]	2.13[c]	2.43[c]
C. citratus	40	100[b]	460[bcd]	7.47[b]	3.00[b]	14.2[b]	10.1[cd]	1.31[b]	1.29[bc]
C. citrates	60	110[b]	660[cd]	9.07[cd]	2.70[b]	15.4[b]	10.2[cd]	1.36[b]	1.87[bc]
M. bracteata + C. citratus	20	50[b]	50[b]	8.20[bc]	1.80[a]	0.90[a]	5.1[bc]	1.48[b]	1.00[ab]
M. bracteata + C. citratus	40	NG	NG	NG	NG	NG	NG	NG	NG
M. bracteata + C. Citrates	60	NG	NG	NG	NG	NG	NG	NG	NG

Note: The mean number followed by different letters shows a significant difference at the 5% test level based on the DMRT test. Ba: B. alata, Pc: P. conjugatum. NG: Seeds do not germinate (weeds do not grow).

weeds in the treatment *of M bracteata* and *C. citratus* extracts showed almost the same results as the control treatment and only a slight decrease, but the difference in fresh weight between the extract treatment and the control was higher. This is related to the very small size of leaves and stems in weeds treated with *M. bracteata* and *C. citratus* extracts which can be seen in Figures 1 and 2. The treatment of a single extract of *M. bracteata* and a mixture of extracts of *M. bracteata* + *C. citratus* reduced growth starting at a concentration of 20%. The growth appearance of *P. conjugatum* and *B. alata* weeds is presented in Figures 1 and 2. Fresh leaf extracts of *M. bracteata* and *C. citratus* showed high effectiveness in inhibiting the growth of *B. alata* and *P. conjugatum* weeds with a growth inhibition percentage of 96% to 100% (Table 6). This can be seen from the very small size of the weeds (Figures 1 and 2).

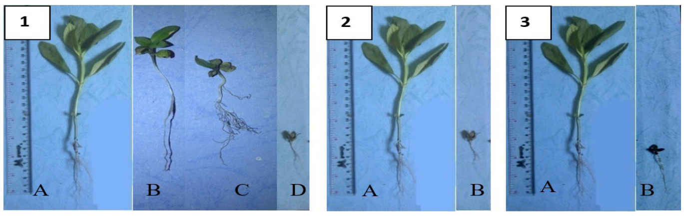

Figure 1: *Weed growth of B. alata at various types and concentration of extract : (1) C. citratus extract (A. 0%, B. 20%, C. 40%, d. 60%) (2) M. bracteata extract (A. 0%, B. 20%) and (3) mixed extract (C. citratus + M. bracteata) (A.0%, B. 20%).*

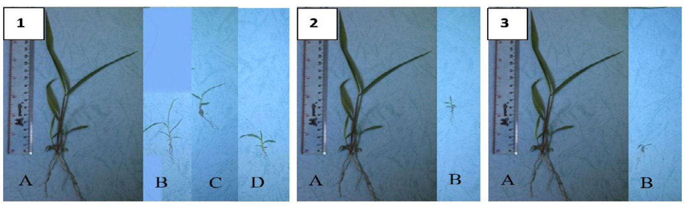

Figure 2: *Weed growth of P. conjugatum at various type and concentration of extract : (1) C. citratus extract (A. 0%, B. 20%, C. 40%, D. 60%) (2) M. bracteata extract (A. 0%, B. 20%) and (3) mixed extract (C. citratus + M. bracteata) (A.0%, B. 20%).*

Research on the use of plant extracts to inhibit weed growth generally uses leaf extracts or other parts that have been dried. Dry extract treatment with low concentrations generally inhibits weed growth. Treatment of 18% dry extract of *Calopogonium mucunoides* inhibited the growth of *Asystasia gangetica* in polybags with a decrease in growth (fresh weight) of 93% (Sihombing *et al*., 2012). Treatment of 6.67% dry leaf extract of *Leucaena leucocephala* on *Ageratum conyzoides* in petridish reduced growth with a percentage reduction of 50% (Ishak and Sahid, 2014). Biological test results of 6.67% dry extract of *Pueraria javanica* leaves on *Chromolaena odorata* weeds reduced growth by 97% (Ismail *et al*., 2016). Treatment of 2.5% dry extract of *Parthenium hysterophorus* leaves reduced the hypocotyl length of weeds *Digitaria sanguinalis* and *Eleusine indica* with an inhibition percentage of 100% (Bashar *et al*., 2022). Treatment of 10% dry extract of *Chromolaena odorata* leaves reduced the shoot height of barnyard grass with a decrease of 91.64% (Suwal *et al*., 2010). The treatment of dried leaf extracts from various plant species inhibited weed growth at lower extract concentrations than fresh leaf extracts of *M. bracteata* and *C. citratus*. However, the dry extract in its application requires more plant material than the fresh extract.

The results of this study showed that the treatment of water extracts from fresh leaves of *M. bracteata* and *C. citratus*, both single extracts and mixed extracts showed high growth inhibition effectiveness on *B. alata* and *P. conjugatum* weeds. The effectiveness of growth inhibition reached 96.28 to 100% ranging from the lowest concentration (20%) to the highest concentration (60%). Research on the use of plant extracts to inhibit weed growth generally uses leaf extracts or other parts that have been dried. Treatment of dried leaf extracts from various plant species inhibited weed growth at lower extract concentrations than fresh leaf extracts of *M. bracteata* and *C. citratus*. However, dry extracts in their application required more plant material than fresh extracts. The treatment of 18% dry extract of *C. mucunoides* inhibited the growth of *A. gangetica* in polybags with a 93% reduction in growth (fresh weight) (Sihombing *et al*., 2012). Treatment of 6.67% dry leaf extract of *Leucaena leucocephala* on *Ageratum conyzoides* in petridish reduced growth with a percentage decrease of 50% (Ishak and Sahid, 2014). Biological test results of 6.67% dry extract of *P. javanica* leaves on *C. odorata* weeds reduce growth by 97% (Ismail *et al*., 2016). The treatment of 2.5% dry extract of *Parthenium hysterophorus* leaves reduces the hypocotyl length of *Digitaria sanguinalis* and *Eleusine indica* weeds with a percentage inhibition of 100% (Bashar *et al*., 2022). The treatment of 10% dry extract of *C. odorata* leaves reduced the shoot height of barnyard grass with a decrease of 91.64% (Suwal *et al*., 2010).

The results showed that mixed extracts from two plants (*M. bracteata* + *C. citratus*) were more effective than single extracts (from one type of plant). This was indicated by lower growth parameter values and higher percentage of growth inhibition in weeds treated with mixed extracts (Tables 5 and 6). This is because the mixed extracts from two types of plants contain more types of secondary metabolites and synergize in inhibiting growth. These results are supported by other studies which also show that mixed extracts of several plants are more effective in inhibiting weed germination and growth. Water extracts from a mixture of two types of plants (Sorghum + sunflower) or more (Sorghum + sunflower + Brassica; Sorghum + sunflower + Brassica + mulberry) were more effective than extracts from one type of plant in inhibiting germination and growth of *Trianthema portulacastrum* weeds (Mushtaq *et al*., 2010).

Table 6: *Inhibition of aqueous extracts of M. bracteata and C. citratus on germination and growth of weeds.*

Extracts	Extract concentration (%)	Germination inhibition (%)		Growth inhibition (%)	
		B. alata	***P. conjugatum***	***B. alata***	***P. conjugatum***
Control	0	0	0	0	0
M. bracteata	20	92.46	84.37	99.85	99.41
M. bracteata	40	98.7	100	100	100
M. bracteata	60	100	100	100	100
C. citratus	20	89,09	84.37	99.64	96.28
C. citratus	40	94.02	100	99.72	98.10
C. citrates	60	93.24	100	99.65	97.29
M. bracteata + C. citratus	20	91.68	87.5	99.84	99.79
M. bracteata + C. citratus	40	100	100	100	100
M. bracteata + C. Citrates	60	100	100	100	100

The growth of *B. alata* and *P. conjugatum* weeds is inhibited due to the entry of allelochemicals from *C. citratus* and *M. bracteata* extracts into plant tissues, starting from ungerminated seeds to weed seedlings. The effect of allelochemicals on weed germination then affects weed growth. The germination time in the extract treatment was slower than the control, which was 5 to 10 days for *B. alata* weed and 1 to 6 days for *P. conjugatum* weed. The slow germination resulted in lower weed growth. Another possibility is that the extracts of *C. citratus* and *M. bracteata* applied directly on the weeds have a direct effect on various growth processes. Various allelochemicals inhibit photosynthesis, respiration, and ATP synthesis (Hussain and Reigosa, 2011; Cheng and Cheng, 2015; Soltys *et al*., 2013; Latif *et al*., 2017), inhibit water and nutrient uptake, oxidative stress (Ogunsusi *et al*., 2018; Novakoski *et al*., 2020; Staszek *et al*., 2021) and alter cell permeability and membrane function (Omezzine *et al*., 2014; Latif *et al*., 2017; Staszek *et al*., 2021).

The treatment of *C. citratus* and *M. bracteata* extracts singly or in mixture inhibited the germination and growth of *P. conjugatum* and *B. alata*. The extracts also caused the death of seedlings, but death only occurred at 40% concentration for *M. bracteata*, with 100% mortality rate for *B. alata*. The death of *B. alata* weed occurred in the third week, with all the damage that appeared since the second week such as leaves turning red, yellowing leaves, brown spots, wilting, stems turning brown and gradually dying. The results showed that *B. alata* weed was more sensitive than *P. conjugatum*. This shows that dicotyledonous weeds (broadleaf weeds) are more sensitive than monocotyledonous weeds (narrow-leaved weeds). The death of *B. alata* is caused by allelochemicals contained in *M. bracteata* extract which are toxic to plants, so they can cause death. Allelochemicals inhibit photosynthesis, respiration, and are able to bind proteins (Soltys *et al*., 2013). *M. bracteata* is classified as Leguminosae which generally contains flavonoids which are included in the phenol group. Allelochemicals increase cell membrane permeability, result in solute leakage, and increase lipid peroxidation (Li *et al*., 2018; Ni *et al*., 2018; M'barek *et al*., 2019; Zhang *et al*., 2020). As a result, growth is inhibited or plant tissues die. Allelochemicals also inhibit nutrient uptake resulting in abnormal growth. Allelochemicals inhibit root elongation, cell division, and the formation of cell ultrastructure, thereby disrupting plant growth and development (Zeng *et al*., 2001; Li, 2010; Cheng and Cheng, 2015; Zhang *et al*., 2020).

A single 40% extract of *M. bracteata* or a mixed extract of *C. citratus* + *M. bracteata* was more effective in inhibiting weed germination and growth than a single extract of *C. citratus*. Important information from this study is that a single extract of 40% *M. bracteata* or a mixed extract of *C. citratus* + *M. bracteata* can be applied by spraying the extract directly on the soil, stem surface and leaves of weed seedlings. Weed germination inhibition is important in weed management as it reduces weed infestation in agricultural fields. This is important for controlling annual weeds or weeds that reproduce by using seeds, generally dicotyledonous weeds or broadleaf weeds (Sharma *et al*., 2020; Travlos *et al*., 2020; Bekuzarova *et al*., 2020; Farooq *et al*., 2020; Kubiak *et al*., 2022). Weed growth inhibition is important in weed management through its effect in increasing crop-weed competition, reducing weed regeneration and decreasing weed seed bank (Rao and Matsumoto, 2017; Jha *et al*., 2017; Farooq *et al*., 2020).

Conclusions and Recommendations

The treatment of mixed extracts (*C. citratus* + *M. bracteata*) was more effective than single extracts in inhibiting the germination and growth of *P. conjugatum* and *B. alata* weeds. The highest percentage of germination and growth inhibition was found in the single extract of *M. bracteata* and mixed extract (*C. citratus* + *M. bracteata*) at 40% and 60% concentrations. Based on the results of this study, 40% extract of fresh *M. bracteata* or a mixture of *C. citratus* + *M. bracteata* extracts can be applied by spraying directly on the soil to inhibit weed emergence in the field. Further testing is needed through direct treatment of the extract on weeds at various growth stages to determine the effect of the extract on weed growth and mortality.

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Novelty Statement

Mixed extracts (fresh leaves of *C. citratus* + *M. bracteata*) were more effective than single extracts in inhibiting weed germination and growth.

Author's Contribution

Siti Fatonah: planned the research, supervised the implementation of the research and wrote the publication.
Herman: assisted the research and proofread the writing for publication.
Dewi Yuni Safitri: conducted the research and compiled the research report.

Conflict of interest

The authors have declared no conflict of interest.

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