

Research Article

Allelopathic potential of *Zea mays*, *Senna spectabilis*, and *Muntingia calabura* for suppression of tea-associated weeds

Chandima Ranawana*, Kasun Jayalath, Ewon Kaliyadasa, Wasana Jeewanthi and Bhagya Samarasinghe

Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, 90000, Sri Lanka.

Abstract: Allelopathic potential of three plant species, namely, *Zea mays*, *Senna spectabilis*, and *Muntingia calabura* to control weeds was investigated via bioassays and field studies. The specific objectives were to identify the most phytotoxic plant extract, its effective concentration, the phytochemical extraction method, the allelochemical release mode, and the field efficacy in controlling weeds. Plant extracts were prepared with dry powders of leaves/husks in four concentrations (4, 6, 8, and 10% w/v) using hot and cold-distilled water. The modes of releasing allelochemicals (decomposition, volatilization, and leaching) were identified using pot bioassay, dish-pack, and sandwich methods, respectively. Lettuce *Lactuca sativa* was used as an indicator in bioassays. Meanwhile, the three most allelopathic extracts/materials were tested in the field by spraying and mulching. Results revealed no significant difference among hot and cold-water extractions ($P > 0.05$). The 10% concentration showed the highest phytotoxicity. *M. calabura* and *S. spectabilis* showed the highest phytotoxicity, evidenced by the lowest germination (22-23%), followed by *Z. mays* (44%). Leaching was prominent in *S. spectabilis*, as evidenced by the lowest germination (61%) and the highest inhibitory effects on radical (77%) and hypocotyl (71%) elongation. Volatilization was prominent in *S. spectabilis* and *M. calabura*, while decomposition was notable in *Z. mays* (leaves) and *S. spectabilis*. Mulching was more effective than spraying (10%, 450 ml m⁻²), with *Z. mays* mulching recording the lowest weed emergence, followed by *M. calabura* (77-84% weed dry weight reduction). In conclusion, *S. spectabilis* and *M. calabura* demonstrate high allelopathic potential, followed by *Z. mays*, highlighting their potential for eco-friendly weed control.

Keywords: Bioassay, Decomposition, Eco-Friendly, Leaching, Phytotoxicity, Volatilization

Introduction

Weed management is one of the most important field operations in tea cultivation. Improper

weed management causes a considerable reduction in the productivity of both vegetatively propagated (VP) teas (5-9%) and seedling teas (5-15%) (Premathilake, 2003). Weeds disturb

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*Corresponding authors: chandima@uwu.ac.lk

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the growth of tea plants and field operations such as plucking, fertilizer application, pruning, and so on. Successfully managing weeds is critical, especially during the early stages of tea establishment.

Weed management in the field includes prevention, cultural, mechanical, biological, and chemical methods. Continued application of synthetic herbicides causes adverse effects on the soil environment and living beings. The application of herbicides left residuals in the made tea, affecting the quality standards. Moreover, weeds may become resistant to herbicides due to repeated use of the same herbicide for several years in the same field (Jhala and Knezevic, 2017). This necessitates the current need for eco-friendly herbicides as a sustainable alternative to synthetic herbicides. Suppressing weeds by harnessing allelopathy might be an innovative alternative (Jabran and Farooq, 2013). Allelopathy is characterized as the harmful or beneficial direct or indirect effects of one plant on another through the development and release of secondary metabolites into the environment (Premathilake, 2003; Cheng and Cheng, 2015). Allelopathy is categorized into two types, true allelopathy and functional allelopathy (Duke, 2015). True allelopathy is the release of toxic substances from their origin in plants (Duke, 2015). Functional allelopathy is the release of toxic substances resulting from chemical transformations by microorganisms (Inderjit *et al.*, 2002; Jabran and Farooq, 2013). These chemicals accrue and persist for a substantial time in the plant, thereby causing significant interference with the growth and development of neighbouring plants (Einhelling, 2008), which can be either a crop or a weed.

During the past decades, the weed-suppressive ability of allelochemicals has drawn significant attention. Several phytotoxic compounds known as “allelochemicals” have been isolated from plant tissues and soils. These natural compounds offer excellent potential for formulating new herbicidal solutions or key compounds for new herbicides because of their unique mode of action (Duke *et al.*, 2000; Vyvyan, 2002; Haig *et al.*, 2005). This would

help overcome herbicide resistance. Further, the great specificity of allelopathic chemicals would enable the development of selective herbicides. Allelochemicals may be more biodegradable than traditional herbicides. Several plants express allelopathic effects, such as *Gliricidia sepium* (Oyun, 2006; Kaboneka *et al.*, 2020), *Senna occidentalis* L. (Asad and Bajwa, 2005), *Calliandra calothyrsus* (Kaboneka *et al.*, 2020), *Helianthus annuus* L. (Tehmina and Bajwa, 2005; Ashrafi *et al.*, 2008), *Eucalyptus* spp (Ejaz *et al.*, 2004), *Camellia sinensis* (L.) Kuntze (Rezaeinodehi *et al.*, 2006; Waris *et al.*, 2016), *Lantana camara* (Kong *et al.*, 2006), *Ageratum conyzoides* (Kaliyadasa and Jayasinghe, 2018), *Mangifera indica* (El-Rokiek *et al.*, 2010), *Azadirachta indica* (Khanam *et al.*, 2020) and *Sorghum bicolor* L. (Cheema *et al.*, 2004; Weston *et al.*, 2013; Kremer and Reinbott, 2021). Prematilake and Liyanage (2011) reported that an aqueous solution from *Mechalia champaca* seeds can be used as a natural weed killer, particularly against broadleaf weeds. Although allelopathy was used for weed control in several crops, including wheat (Cheema *et al.*, 2000a), cotton (Cheema *et al.*, 2000b), rice (Irshad and Cheema, 2004), maize (Cheema *et al.*, 2004), canola (Jabran *et al.*, 2008), and mungbean (Cheema *et al.*, 2001), its effectiveness in controlling weeds in tea plantations has not been evaluated yet. A comprehensive examination of the allelopathic potential of locally available plant species and their response patterns is key to designing a cost-effective, eco-friendly approach to weed management in tea lands. Further, it is essential to identify the effective concentration at which each specific response occurs if allelopathic interaction is to be used in weed management. Also, the plant extraction technique and the method of application may determine the effectiveness of employing the allelopathy phenomenon in weed management. Allelochemicals are released into the environment by several mechanisms, including foliar leaching, root exudation, volatilization, and decomposition or leaching from plant litter (Birkett *et al.* 2001). However, there is

insufficient information on the mechanisms of allelochemical release across different plant species.

Therefore, the current study investigates the allelopathic potential of locally available plant species, namely, Maize *Zea mays*, Kaha-kona *Senna spectabilis*, and Jam tree *Muntingia calabura*. Previous studies on the allelopathic effects of these plants are limited, particularly for *Muntingia calabura* L. Antesa and Antesa (2012) reported the allelopathic potential of *Muntingia calabura* L. and suggested that it releases allelochemicals primarily through leaching. The allelopathic potential of aqueous extracts from both fresh and oven-dried maize leaves, as well as their root exudates, has been documented (Al-Tawaha and Odat, 2010; Ahmad and Bano, 2013; Ma et al., 2022), although studies specifically addressing tea-associated weeds are lacking. Prajitha and Bai (2024) observed allelopathic effects of *Senna spectabilis* and highlighted the need for further investigation into the mechanisms of allelochemical release and their modes of action. Additionally, Subi et al. (2024) identified several allelochemicals in *Senna spectabilis*. Actually, these materials are easily available at no cost. For example, maize plant residues left in the field after harvesting can be used for weed control. Similarly, the other two plant species are fast-growing and naturally found in the tea fields. The high biomass production of these plant species makes them potential candidates for weed control in tea plantations. The present study aimed to identify the most phytotoxic plant extract and its concentration, the most effective phytochemical extraction method, the mode of releasing allelochemicals, and the effectiveness of spray application and mulching as field applications.

Materials and Methods

The research was conducted as a series of bioassays (indicator plant: lettuce) and field studies at the Uva Wellassa University, Badulla, Sri Lanka (6.9819° N, 81.0763° E) as described below.

Preparation of plant extracts

Z. mays leaves, *Z. mays* husks, *S. spectabilis* leaves, and *M. calabura* leaves were cleaned and oven-dried separately in perforated paper bags at 45 °C for 48 hours (Al-Samarai et al., 2018). Plant materials were milled into fine powder and sieved through a 1 mm sieve. Then, the stock solutions were prepared by dissolving 10 g of each powder in 100 ml of hot distilled water and in 100 ml of cold distilled water, respectively. All the samples were kept at room temperature for 24 hours. Each solution was filtered through four layers of cheesecloth to remove debris and then centrifuged at 3000 rpm for 10 min. The supernatant was filtered through one layer of Whatman no.1 filter paper (Waris et al., 2016). The stock solution (10 g in 100 ml; w/v) was diluted to get 4, 6, and 8% (w/v) concentrations. A stock solution was used at 10% (w/v) as the treatment.

Phytotoxic bioassay of plant extracts

A three-factor factorial, completely randomized design was used, with three replicates. Factors were plant type, extraction method (hot or cold distilled water), and concentration (4, 6, 8, and 10% w/v). Bioassays were conducted using lettuce seeds. Lettuce seeds were sterilized using water: sodium hypochlorite solution @ 10:1. Ten lettuce seeds were placed on each sterilized petri dish (9 cm diameter) lined with Whatman No.1 filter paper, and 5 ml of solution from each was added to each petri dish (Gariglio, 2002). Distilled water was used as the control. The %germination (Waris et al. 2016) was evaluated after incubating at 25 °C for 5 days using the following equation.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds sown}}$$

Mode of releasing allelochemicals

The modes of releasing allelochemicals, viz., volatilization, leaching, and decomposition, were identified using the dish-pack method (Fujii et al., 2005), the sandwich method (Fujii et al., 2003; Fujii et al., 2004), and the pot bioassay (Ranagalage et al., 2014), respectively. In each experiment, a completely randomized

design was used with three replicates. Four types of plant materials, including *Z. mays* leaves, *Z. mays* husks, *S. spectabilis* leaves, and *M. calabura* leaves, were tested as treatments in each mode.

For the dish-pack method, a dish with “six wells” was used. The leaves/husks of the plant species were cut into 2 × 2 mm pieces and placed into one of the wells of a 6-well multi-dish. Whatman No. 1 filter paper and 0.7 ml of distilled water were added to the other 5 wells, along with 6 lettuce seeds in each well. The dishes were covered with aluminum foil and sealed with tape. Four separate plates were used for each of the four plant materials. All the dishes were incubated in the dark for 3 days at 25 °C, and data were recorded after 4 days (Fujii, 2005).

For the sandwich method, 0.75% autoclaved agar medium (5 ml per well) was poured into 6-well multi-dishes as a basal layer. After the base agar had solidified entirely, cut leaf/husk pieces from four plant materials were placed equidistantly on the base agar (10 pieces per well). Then, the leaf/husk pieces were covered by pouring another 0.75% agar medium (5 ml per well). To prepare the control set-up, liquefied 0.75% agar was poured into one of the wells without leaves. When the agar had fully solidified, surface-sterilized lettuce seeds (5 per well) were sown on the agar surface. The multi-dish was covered with plastic tape, labelled, wrapped in aluminum foil, and incubated in the dark at 25 °C for 3 days. The data were recorded after 3 days (Fujii *et al.*, 2003, 2004).

For the pot bioassay, glass beakers were used. Each beaker was filled with 500 g of soil mixed with 6 g of each plant material separately. Water was added to each beaker to maintain adequate moisture. Surface-sterilized lettuce seeds were uniformly placed at a depth of about 1 cm in each beaker after 2 weeks of residue incorporation. Seedling emergence rate at the soil surface was measured daily for 20 days after seeding (Ranagalage *et al.*, 2014).

% germination (eq. 01 above) and %inhibition of radical and hypocotyl elongation (Hong *et al.* 2003) of lettuce were evaluated in each experiment. %inhibition of radical and hypocotyl elongation was calculated using the following equation.

$$\% \text{ inhibition} = [1 - (\text{RL or HL}_{\text{treatment}} / \text{RL or HL}_{\text{control}})] \times 100 \quad (\text{eq. 02})$$

RL: Radical length HL: Hypocotyl length

Field evaluation of plant materials and their extracts

Mulching: Mulching was applied with finely chopped materials from the three plant species in a weed-free area (1 m² each). It was replicated thrice. Treatments were compared with a control plot maintained without mulch (Campiglia, 2010). Dry weights of emerged weeds in each plot were measured at 1, 2, and 3 months after mulching.

Spraying: The most phytotoxic plant extracts (*S. spectabilis* leaves, *M. calabura* leaves, and a cocktail mixture of both at 10% concentration) were tested under field conditions using quadrats (using a 1 m² quadrat divided into four parts). Spray application was performed at a 10% (w/v) concentration (450 ml m⁻²) on a randomly selected field (Chhokar, 2015). Plant extracts were sprayed onto the foliage of weeds that emerged in the field three weeks after land clearing. The prominent weeds in the tested field were *Hedyotis auricularia* (25%) and *Ageratum conyzoides* (33%), while *Paspalum conjugatum* (15%), *Desmodium triflorum* (13%), *Emilia sonchifolia* (8%), *Sonchus wightianus* (3%) and *Euphorbia heterophylla* (3%) were also present. The dry weights of weeds in each plot were measured 3 weeks after spray application; plots sprayed with water served as the control.

Data analysis

Data were analyzed using Analysis of variance (ANOVA) in Minitab 17. Mean comparisons were performed using Tukey's Pairwise Comparisons.

Results

Identification of the most phytotoxic plant extract

According to the results of the lettuce bioassay, two-way and three-way interactions were not significant ($P > 0.05$) for %germination. Moreover, bioassay results revealed no significant difference between hot and cold distilled water extractions ($P > 0.05$), while only the main effects of plant extraction method and concentration were significant for germination percentage (Table 1). % germination decreased with increasing extract concentration. A concentration of 10% recorded the lowest germination percentage (Table 1).

Table 1 Percentage of lettuce seed germination as affected by the type of plant extract and its concentration.

Treatment	%germination ¹
Type of Plant Extract (PE)	
<i>Z. mays</i> husk	35.0 ^{bc}
<i>Z. mays</i> leaves	53.0 ^{ab}
<i>S. spectabilis</i> leaves	23.0 ^c
<i>M. calabura</i> leaves	22.0 ^c
Control (distilled water)	90.0 ^a
Concentration (C; w/v)	
4%	52.1 ^a
6%	41.7 ^{ab}
8%	25.0 ^{bc}
10%	14.6 ^c
Control (distilled water)	90.0 ^a
P values	
PE	<0.001
C	<0.001
PE × C	0.188

¹ Values presented are means and respective p-values of interaction and main effects. Mean values followed by the same letters are not significantly different according to Tukey's pairwise comparison test at $P < 0.05$.

M. calabura and *S. spectabilis* demonstrated the highest allelopathic effect as evidenced by

the lowest lettuce germination percentage (22%), followed by *Z. mays* (average of leaves and husks; 44%).

Identification of the mode of releasing allelochemicals

The results of the study identifying the modes of allelochemical release are presented below.

Volatilization (Dish-pack method): According to the results, there was no significant effect of different plant extracts on % germination at the 0.05 level (Table 2). However, plant extracts had a significant effect ($P < 0.05$) on radicle and hypocotyl elongation. Volatilization was prominent in both *S. spectabilis* (%inhibition of RL and HL, 47.9% and 53.2%, respectively) and *M. calabura* (%inhibition of RL and HL, 45.8% and 53.2%, respectively) as evidenced by the highest inhibitory effect on radicle and hypocotyl growth.

Leaching (Sandwich method): None of the plant extracts had a significant effect on %germination ($P > 0.05$; Table 2). However, hypocotyl and radicle elongation were significantly inhibited under different plant extracts compared to the control ($P < 0.05$). *S. spectabilis* recorded the highest inhibition of radicle and hypocotyl elongation leaves, followed by *Z. mays* leaves. That means leaching was prominent in *S. spectabilis* as evidenced by the highest inhibitory effect on radical (77%) and hypocotyl (71%) elongation.

Decomposition (Pot bioassay): There was a significant inhibitory effect on lettuce seed germination ($P < 0.05$; Table 2). The lowest germination rate of 26.7% was recorded in *Z. mays* leaves, followed by *S. spectabilis* leaves (30%). Therefore, among the four plant materials tested, decomposition as a mode of releasing allelochemicals was notable in *Z. mays* and *S. spectabilis* leaves. The effect of plant extracts was not significant for hypocotyl and radical growth.

Table 2 Percentage of lettuce seed germination, radical length (RL), inhibition of RL, hypocotyl length (HL), and inhibition of HL under four different plant extracts compared to control (distilled water) as recorded in the dish-pack method, sandwich method, and pot bioassay.

Plant Extract	%germination	RL (mm)	% Inhibition RL	HL (mm)	% Inhibition HL
Mode of release: Volatilization (Dish-pack method) ¹					
<i>Z. mays</i> husk	79.1 ^a	21.5 ^a	9.7 ^c	17.9 ^a	3.8 ^c
<i>Z. mays</i> leaves	84.5 ^a	17.9 ^b	24.8 ^b	13.1 ^b	29.6 ^b
<i>S. spectabilis</i> leaves	75.8 ^a	12.4 ^c	47.9 ^a	8.7 ^c	53.2 ^a
<i>M. calabura</i> leaves	76.1 ^a	12.9 ^c	45.8 ^a	8.7 ^c	53.2 ^a
Control	87.1 ^a	23.8 ^a		18.6 ^a	
P value	0.073	< 0.001	< 0.001	< 0.001	< 0.001
Mode of release: Leaching (Sandwich method) ¹					
<i>Z. mays</i> husk	100 ^a	15.7 ^{ab}	34.8 ^b	10.9 ^a	13.5 ^b
<i>Z. mays</i> leaves	94.4 ^a	14.7 ^b	39.0 ^b	10.2 ^{ab}	19.0 ^{ab}
<i>S. spectabilis</i> leaves	61.1 ^a	5.4 ^c	77.6 ^a	3.6 ^b	71.4 ^a
<i>M. calabura</i> leaves	83.3 ^a	18.7 ^{ab}	22.4 ^b	11.7 ^a	7.1 ^b
Control	94.4 ^a	24.1 ^a		12.6 ^a	
P value	0.938	<0.001	0.009	0.009	0.025
Mode of release: Decomposition (Pot bioassay) ¹					
<i>Z. mays</i> husk	66.7 ^a	22.8 ^a	1.8 ^a	63.2 ^a	0.1 ^a
<i>Z. mays</i> leaves	26.7 ^b	21.5 ^a	7.4 ^a	59.2 ^a	4.9 ^a
<i>S. spectabilis</i> leaves	30.0 ^b	22.5 ^a	2.9 ^a	56.3 ^a	9.6 ^a
<i>M. calabura</i> leaves	46.7 ^{ab}	21.3 ^a	8.0 ^a	58.9 ^a	5.4 ^a
Control	80.0 ^a	23.2 ^a		62.3 ^a	
P value	0.002	0.057	0.123	0.086	0.141

¹ Detailed methodology is provided in the Materials and Methods section.

Values presented are means and respective p-values of interaction and main effects. Mean values followed by the same letters are not significantly different according to Tukey's pairwise comparison test at $P < 0.05$.

Field evaluation of plant extracts

Spray application

There was no significant difference ($P > 0.05$) in weed dry weight at 4 weeks after spraying among treatments, although the cocktail mixture recorded the lowest weed dry weight (Fig. 1).

Mulch application

Mulching was more effective than spray application. At four weeks after mulching, the lowest weed dry weight was recorded in the plots mulched with *Z. mays* husks, followed by *Z. mays* and *M. calabura* leaves (Fig. 2). Reduction in weed dry weights compared to the control was 85%, 82% and 77% in *Z. mays* husks, *Z. mays* leaves, and *M. calabura* leaves, respectively.

There was a significant reduction in weed dry weight compared to the control, even after eight weeks of mulching with all four planting materials (averaging 80% reduction). Mulching with *Z. mays* husks and *M. calabura* was found to be effective in suppressing weed growth even after 12 weeks, which is evidenced by the lowest weed emergence recorded by the plots mulched with *Z. mays* husks and *M. calabura* leaves. It was, on average, a 76% reduction (on a weed-dry-weight basis) compared to the control. *Z. mays* husks take much time to decompose, and for the ground exposure, it reduces weed emergence. The release of allelochemicals *M. calabura* leaves may affect the weed emergence.

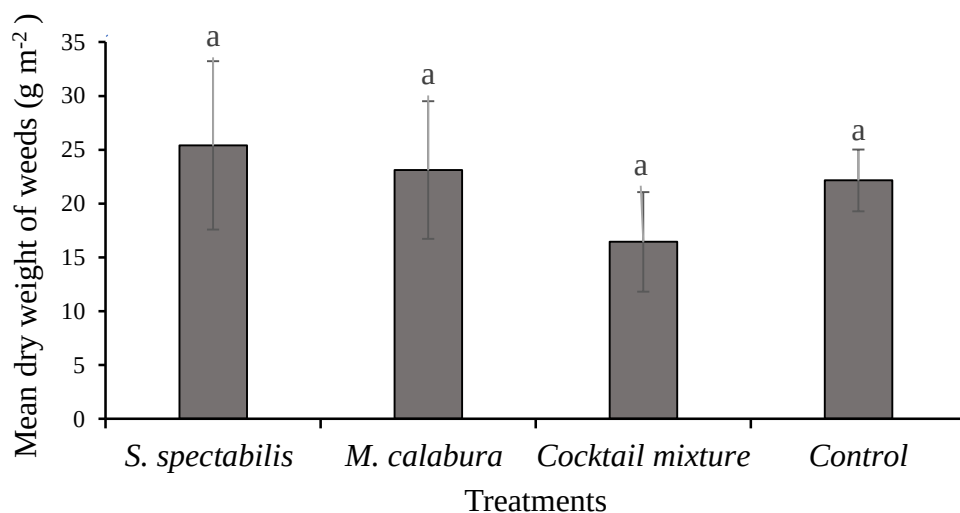


Figure 1 Weed dry weights at 4 weeks after spraying of selected plant extracts and their cocktail (1:1) formulations compared to the control. Concentration and the application rate of the plant extracts are 10% w/v and 450 ml m⁻², respectively. Plots sprayed with water served as the control.

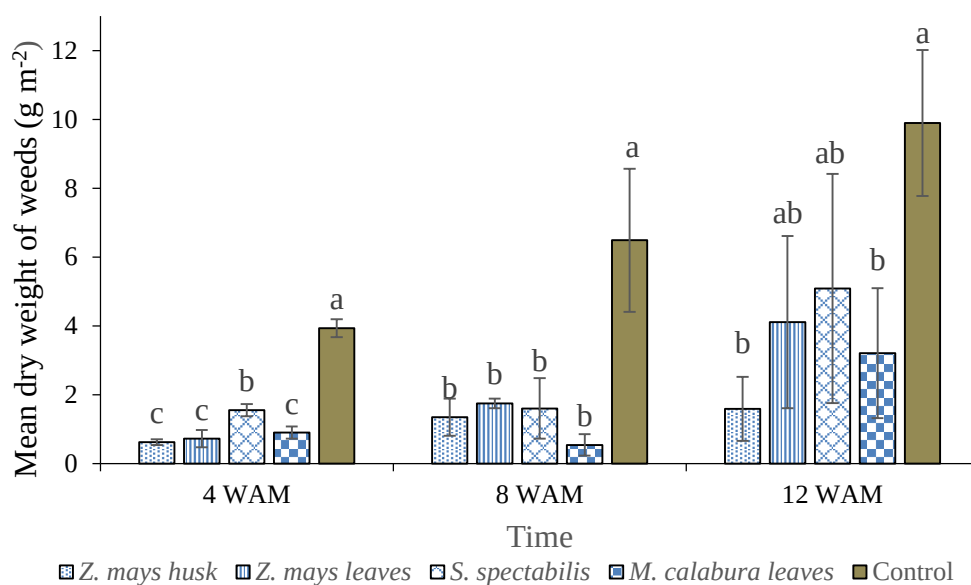


Figure 2 Dry weights (g) of weeds emerged in plots at 4, 8, 12 weeks after mulching (WAM) with four different planting materials compared to the control (without mulch).

Discussion

The present study demonstrated that *S. spectabilis* and *M. calabura* exhibited the

highest allelopathic potential, followed by *Z. mays*. Bioassay results revealed no significant difference between hot and cold distilled water extractions ($P > 0.05$), which is consistent with

the findings of Waris *et al.* (2016), who reported that both hot and cold-water extracts of tea produced statistically similar effects on wheat and maize seed germination. The allelopathic effect observed in this study was concentration-dependent, in agreement with previous studies showing that higher extract concentrations result in greater phytotoxicity (Wu *et al.*, 2003; Koodkaew *et al.*, 2018).

M. calabura is a fast-growing tree with multiple medicinal uses, and it is well-known around the world as “Jamaican cherry” (Mahmood *et al.*, 2014). *M. calabura* is native to southern Mexico, Central America, the Greater Antilles, Trinidad, and St. Vincent. It is also found in India, Sri Lanka, and Southeast Asia, such as Malaysia, Indonesia, and the Philippines (Mahmood *et al.*, 2014). Although the antibacterial and insecticidal activities of *M. calabura* (Bandeira *et al.*, 2013; Nasution *et al.*, 2020) have been reported previously, its allelopathic potential has not yet been extensively studied. Flavonoid compounds like flavones, flavanones, flavans, and biflavans identified in *M. calabura* leaves (Nshimo *et al.*, 1993) may be ascribed to the allelopathic effect reported in this study.

S. spectabilis is widely distributed in tropical and subtropical areas. It is used in folk medicine due to its good therapeutic value (Jothy *et al.*, 2012). Distribution across widespread geographic regions has led to a diverse array of bioactive secondary metabolites in this plant, including alkaloids, steroids, and flavonoids (Selegato *et al.*, 2017). Therefore, we can assume that the observed allelopathic effect of *S. spectabilis* may be due to the activity of those secondary metabolites.

Allelochemicals are released from plants or plant parts by a variety of processes, such as leaching from above-ground plant parts, volatilization, root exudation, stem flow, microbial activity, plowing of plant residues in the soil, and dry residue decomposition (Ambika, 2013). According to our study, leaching was most pronounced in *S. spectabilis*, as indicated by the lowest germination rate and the strongest inhibition of radical and hypocotyl

elongation. Volatilization was significant in *S. spectabilis* and *M. calabura*, whereas decomposition notable in *Z. mays* (leaves) and *S. spectabilis*. There is little scientific evidence on the allelopathic effects of *Z. mays* residues on germination and seedling growth (Garcia and Anderson, 1984; Martin *et al.*, 1990), possibly due to the release of allelochemicals during decomposition.

Information on different modes of releasing allelochemicals across various plant species would be necessary for selecting the best extraction procedures (E.g., volatiles may require specialized procedures) and field application methods (E.g., Certain allelochemicals are released during decomposition). Allelochemicals can be released over time from all plant tissues, including leaves, stems, roots, flowers, seeds, rhizomes, pollen, bark, and buds (Weston and Duke, 2003). In the present study, only some specific plant parts have been selected. Therefore, it is important to investigate other parts of the chosen plants too.

The nature and concentration of allelochemicals released by the plant into the environment depend on the plant itself and some biotic and abiotic factors. Plant factors include plant species, cultivar, age, and the type of tissue under consideration for allelochemical production. Environmental factors regulating allelochemical production and release include pathogen infestation, physical injuries, or abiotic factors such as drought, temperature, soil characteristics, rainfall, nutrient deficiency, irradiation, competitors, and exposure to ultraviolet radiation (Mahmood *et al.*, 2013). The release of allelochemicals into the external environment is influenced by their chemical properties, including molecular weight, polarity, and concentration within the plant. Allelochemicals are most often released in mixtures in conjunction with other closely related metabolites (Macías *et al.*, 2007).

The presence of metabolites in complex mixtures may significantly affect allelopathic activity. Therefore, activity might be associated with complex molecular

interactions, including synergy, antagonism, and enhanced effects in the presence of other metabolites (Albuquerque *et al.*, 2011). To estimate the bioactivity of allelochemicals, a dose-response study is important to establish their potential effects on the environment. When studies utilize a dose significantly higher than the concentration(s) naturally present in the soil, the results are generally difficult to interpret from an allelopathic perspective. Moreover, the mode of action of allelochemicals can differ when applied at doses well beyond those encountered in nature (Fujii and Hiradate, 2007).

It is well known that even a substance showing strong phytotoxic activity on target plants in laboratory experiments may not perform satisfactorily in field conditions due to the influence of several soil factors like soil pH, organic carbon, organic matter, and available nitrogen (Khanh *et al.*, 2005; Islam *et al.*, 2018). Therefore, more emphasis should be placed on evaluating the bioactivity of allelopathic substances or allelopathic plant extracts under both laboratory and field conditions, as well as across different field application methods. If the extracts or the isolated compound show strong activity under both laboratory and field conditions, they could be recommended for new natural herbicide development.

In field trials, mulching and spray applications were evaluated, and mulching proved more effective than spraying (10%, 450 ml m⁻²). *Zea mays* mulching resulted in the lowest weed emergence, followed by *M. calabura*, which reduced weed dry weight by 77–84%. Poor spray performance might be due to the degradation of allelochemicals under high light and temperature. Additionally, some microbial activities may contribute to poor spray performance. Moreover, the volume applied for the spraying may be insufficient for better performance. Further investigation is suggested with higher concentrations or application rates.

Although selecting or identifying allelopathic plants is much easier, isolating and

identifying strong allelopathic substances is difficult, time-consuming, and requires very sophisticated equipment. Hence, very few studies have been conducted to isolate and identify the allelopathic substances from allelopathic plants. Researchers have reported that many substances exhibit strong phytotoxicity against various target plant species under laboratory conditions. However, their phytotoxic potential under field conditions has not yet been reported.

In conclusion, *M. calabura* and *S. spectabilis* showed the highest allelopathic effect, followed by *Z. mays*. Extracts can be prepared with either hot or cold water as there is no significant difference in allelopathic effect between those two methods. As a mode of releasing allelochemicals to the environment, volatilization was prominent in both *S. spectabilis* and *M. calabura*. Leaching was prominent in *S. spectabilis*. Decomposition mode was notable in *Z. mays* (leaves) and *S. spectabilis*. *Z. mays*, especially husks, and *M. calabura* leaves are effective as mulch in controlling weeds. However, the tested spray application rate (10%, 450 ml m⁻²) is insufficient for significant weed control and requires further investigation into the effective concentration and application frequency.

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Statement of Conflicting Interests

The Authors state that there is no conflict of interest.

Authors' Contributions

CR and EK conceived of the presented idea. CR and EK developed the methodology. KJ carried out the experiment. CR, KJ and WJ performed the formal Analysis. CR and BS prepared the original draft, and CR and EK reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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پتانسیل آلوپاتیک ذرت *Zea mays*، سنا *Senna spectabilis* و گیلاس جامائیکا *Muntingia calabura* برای کنترل علف‌های هرز چای

چاندیما رانوانا*، کاسون جایالات، ایوون کالیاداسا، واسانا جیوانتی و باگیا ساماراسینگه

گروه کشاورزی صادراتی، دانشکده علوم دامی و کشاورزی صادراتی، دانشگاه اووا و لاسا، بادولا، ۹۰۰۰۰، سریلانکا.

پست الکترونیکی نویسنده مسئول مکاتبه: chandima@uwu.ac.lk

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چکیده: پتانسیل آلوپاتیک سه گونه گیاهی *Zea mays*، *Senna spectabilis* و *Muntingia calabura* برای کنترل علف‌های هرز از طریق زیست‌سنجی و مطالعات مزرعه‌ای مورد بررسی قرار گرفت. اهداف خاص، شناسایی عصاره گیاهی با بیش‌ترین سمیت گیاهی، غلظت مؤثر آن، روش استخراج فیتوشیمیایی، نحوه آزادسازی آلووشیمیایی و اثربخشی آن در مزرعه برای کنترل علف‌های هرز بود. عصاره‌های گیاهی با پودر خشک برگ‌ها/پوسته‌ها در چهار غلظت (۴، ۶، ۸ و ۱۰ درصد وزنی/حجمی) با استفاده از آب مقطر گرم و سرد تهیه شدند. نحوه آزادسازی آلووشیمیایی‌ها (تجزیه، تبخیر و آبشویی) به ترتیب از طریق روش‌های زیست‌سنجی گلدانی، بسته‌بندی در ظرف و ساندویچ شناسایی شد. کاهو *Lactuca sativa* به عنوان شاخص برای زیست‌سنجی‌ها استفاده شد. در همین حال، سه عصاره/ماده با بیش‌ترین سمیت گیاهی در مزرعه با اسپری کردن و مالچ‌پاشی آزمایش شدند. نتایج نشان داد که تفاوت معنی‌داری بین عصاره‌های آب گرم و سرد وجود ندارد ($p > 0.05$). غلظت ۱۰ درصد بالاترین سمیت گیاهی را نشان داد *M. calabura* و *S. spectabilis* بالاترین سمیت گیاهی را نشان دادند که با کمترین جوانه‌زنی (۲۲-۲۳ درصد) و پس از آن *Z. mays* ۴۴ درصد (مشاهده شد. آبشویی در *S. spectabilis* برجسته بود که با کمترین جوانه‌زنی (۶۱ درصد) و بیش‌ترین اثر بازدارندگی بر طول شدن ریشه‌چه (۷۷ درصد) و هیپوکوتیل (۷۱ درصد) نشان داده شد. تبخیر در *S. spectabilis* و *M. calabura* برجسته بود، در حالی که تجزیه در *Z. mays* (برگ‌ها) و *S. spectabilis* قابل توجه بود. مالچ‌پاشی در مقایسه با اسپری (۱۰ درصد، ۴۵۰ میلی‌لیتر در مترمربع) مؤثر بود، به طوری که مالچ‌پاشی *Z. mays* کمترین میزان ظهور علف‌های هرز را ثبت کرد و پس از آن *M. calabura* (۷۷-۸۴ درصد کاهش وزن خشک علف‌های هرز) قرار گرفت. در نتیجه، *S. spectabilis* و *M. calabura* پتانسیل آلوپاتی بالایی را نشان می‌دهند و پس از آن *Z. mays* قرار دارد که پتانسیل آن را برای کنترل علف‌های هرز سازگار با محیط زیست برجسته می‌کند.

واژگان کلیدی: زیست‌سنجی، تجزیه، سازگار با محیط زیست، آبشویی، سمیت گیاهی، تبخیر