International Journal of Agricultural and Natural Sciences
E-ISSN: 2651-3617 17(2): 165-180, 2024
doi: 10.5281/zenodo.11408221
Research Article

ALLELOPATHIC POTENTIAL OF SELECTED SHADE TREE SPECIES TO CONTROL WEEDS IN TEA PLANTATIONS

S.R.W.M.C.J.K. Ranawana*, P.W. Pathinayake, P.E. Kaliyadasa, K.G. Prematilake

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

Corresponding Author:
E-mail: chandima@uwu.ac.lk

(Received 12th March 2024; accepted 15th April 2024)

ABSTRACT. The detrimental effects of synthetic herbicides on the environment and human health have spurred increased interest in eco-friendly alternatives for weed control, including allelopathy. A bioassay and a pot study were conducted to assess the allelopathic potential of four common shade tree species (*Gliricidia sepium*, *Calliandra calothrysus*, *Erythrina lithosperma*, and *Grevillea robusta*) to control weeds in tea plantations in Sri Lanka. The study also examined the most effective concentration of the leaf extract and the extraction techniques. Aqueous extracts were prepared using dry powders of mature leaves at four concentrations (2, 4, 6, and 8% w/v) using hot and cold-distilled water. Bioassays were performed using lettuce as the indicator plant. The effect of different leaf extracts on seed-germination parameters depended on their concentration ($P<0.05$). There was no significant difference between hot and cold water extraction ($P>0.05$). *G. sepium* exhibited the highest allelopathic effect, evidenced by the lowest values for germination percentage (17.8%), germination index (0.4), seedling vigor index (0.1), and the highest inhibition in radical (83.3%), and hypocotyl (89%) elongation followed by *G. robusta* both at 8% concentration. Most phytotoxic extracts (*G. sepium*, *G. robusta*, *E. lithosperma* each at. 6, and 8% concentrations (w/v)) were further tested on three weed species (*Cleoma aspera*, *Bidens pilosa*, *Ageratum conyzoides*) planted in pots in three replicates. *G. sepium* recorded the highest weed growth suppression, followed by *G. robusta* at 8% concentration. *G. sepium* at 8% recorded the significantly highest dead weed count (*C. aspera*, 100%; *B. pilosa* and *A. conyzoides*, 66.6%), followed by *G. robusta* at 8%. In conclusion, leaf extracts of *G. sepium* and *G. robusta* at 8% can potentially be used to control broad-leaf weeds in tea lands. Further investigations under field conditions are warranted.

Keywords: *Bioassay, Germination, Hot and cold-water extraction, Phytotoxic, Seedling vigor*

INTRODUCTION

The Growth and yield of tea plants are significantly hampered by weeds, primarily because they compete for essential resources such as light, water, space, and nutrients. Furthermore, weeds also disrupt crucial field operations such as plucking, fertilizer application, and pruning. Therefore, effective weed management emerges as a pivotal aspect of field operations, necessitating regular attention to ensure the sustainable productivity of tea cultivations [1]. However, managing weeds in tea plantations has become increasingly challenging due to the high cost of labour and other inputs such as synthetic herbicides [2]. The increased reliance on synthetic herbicides has led to several serious issues, including the evolution of herbicide resistance in weeds, shifts in weed species populations, surface-water pollution, and negative effects on human and animal health [3, 4]. Meanwhile, global concerns regarding the detrimental impacts of synthetic herbicide use on the environment and human health have shifted the focus of plant scientists toward finding eco-friendly alternatives for weed control in agricultural production systems. In this context, harnessing allelopathic phenomenon for weed control may offer a sustainable, economical, and eco-friendly alternative to chemical weed control [5-7]. Allelopathy refers to the beneficial or harmful effects that one plant can have on another, encompassing both crop and weed species, by the release of chemicals (secondary metabolites) from plant parts by leaching (from aerial parts of plants during rain, dew, and fog), root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems [8]. Allelopathy has been utilized for weed control in various crops, including wheat [9], cotton [10], rice [11], maize [12], canola [13], and mungbean [14]. Several plant species are known to possess allelopathic potential, including *Senna occidentalis* L. [15], *Helianthus annus* L. [16-18], Eucalyptus spp. [19, 20], *Camellia sinensis* (L.) Kuntze [21, 22], *Lantana camara* [23], *Mangifera indica* [24], *Mechalia Champaca* [25], *Medicago sativa* L. [26], *Azadirachta indica* [27] and *Sorghum bicolor* L. [12, 28, 29]. Several phytotoxic compounds, known as allelochemicals, have been isolated from plant tissues and soils such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, *β-Resorcyclic acid*, vanillic acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, ferulic acid, sinapinic acid (trans and cis forms), myricetin, chlorogenic acid, isochlorogenic acid, scopolin, annuionones and α-naphathol [30-32]. These natural compounds offer excellent potential for formulating new bio-herbicides [33, 34] due to their unique mode of action [35]. This would help in addressing the issue of herbicide resistance. Furthermore, the high specificity of allelochemicals would enable the development of highly specific herbicides [36]. The primary obstacle facing sustainable modern crop protection lies in the restricted access to bioherbicides. Present-day scientists can explore allelopathic plants as a potential reservoir for discovering and extracting novel allelopathic compounds. By evaluating their effectiveness in both controlled laboratory settings and real-world agricultural conditions, promising substances can be identified and suggested for developing innovative natural herbicides, thus promoting sustainable farming practices [7, 37]. Only a limited number of natural herbicides derived from allelochemicals are commercially available, highlighting the necessity for additional options with enhanced crop selectivity. Modern biotechnological tools and refined extraction techniques hold promise for identifying, testing, and deploying more allelochemicals in weed management practices [38].

Due to their practicality and cost-effectiveness, it is crucial to explore locally available plant species with higher allelopathic potential for controlling weeds in tea plantations. Several shade tree species are commonly cultivated in tea plantations, and some of them may contain allelochemicals that can effectively suppress weed growth. Therefore, exploiting potential allelopathic shade tree species is a timely necessity, where shade tree lopping after pruning and leaf litter can be utilized to suppress weeds. *Calliandra calothrysus*, *Erythrina lithosperma*, *Acacia pruinosa, Gliricidia sepium*, *Grevillea robusta*, *Albizzia moluccana* and *Albizzia chinesis* are some of shade tree species found in tea plantations in Sri Lanka [39]. While some studies have explored the allelopathic potential of certain shade tree species such as *Gliricidia sepium* [40, 41] and *Calliandra calothrysus* [36] on maize, there is a noticeable gap in the scientific evidence pertaining to the allelopathic capabilities of other shade tree species. Allelopathy is a complex biological phenomenon, as the allelopathic activity may be inhibitory, neutral or stimulatory, even within the same species and under different ecological conditions [41]. Production and activity of allelochemicals depend on ecological conditions. Therefore, investigating the allelopathic potential of these plant species under different ecological conditions is necessary if allelopathic phenomenon is to be effectively utilized in weed management programmes. Additionally, the allelochemical extraction technique and its concentration may also determine the effectiveness of employing allelopathy in weed management. At low concentration, allelochemicals sometimes stimulate growth, while at high concentrations, they suppress the growth [42, 43]. Consequently, there remains a lack of comprehensive understanding regarding the allelopathic properties of numerous other shade tree species as well as in a technique for extracting allelochemicals, determining their optimal concentration and identifying target weed species. Considering the timely need to apply allelopathic phenomenon for eco-friendly weed control, a series of bioassays and a pot study were conducted to investigate the allelopathic potential of some selected shade tree species commonly grown in tea plantations. This study also aimed to identify the most effective extraction technique (hot and cold distilled water extraction), the concentration that produces the most phytotoxic effect, and the target weed species susceptible to allelopathic effects.

MATERIALS AND METHODS

Lettuce seed bioassays and a pot experiment were conducted at the Uva Wellassa University, Badulla, Sri Lanka (6.9819° N, 81.0763° E). The experiment lasted four months.

Lettuce Seed Bioassay

Lettuce seed bioassays were arranged using a three-factor factorial, completely randomized design (CRD) with three replicates under laboratory conditions. The factors included leaf extracts of shade tree species (*Calliandra calothrysus, Erythrina lithosperma, Gliricidia sepium,* and *Grevillea robusta*), extraction method (hot and cold distilled water extraction), and concentration (2, 4, 6, and 8% w/v).

Mature leaves of the aforementioned four shade tree species were cleaned and oven-dried separately in perforated paper bags at 45°C for 48 hours [44]. The plant materials were then ground into fine powder and sieved through a 1 mm sieve. Stock solutions were prepared by dissolving 10 g of each powder in 100 ml of hot and cold distilled water separately. All 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 [22]. The stock solutions were diluted to obtain 2, 4, 6, and 8% (w/v) concentrations.

Lettuce seeds were surface sterilized with water: sodium hypochlorite solution (10:1 v/v) for 10 minutes and rinsed thoroughly several times with sterile water to prevent contamination. Surface-sterilized lettuce seeds were placed at equal distances on a Whatman no.1 filter paper in sterilized 9 cm Petri dishes (15 seeds per petri dish). 5 ml of leaf extracts was added separately to each petri dish. Distilled water was used as the control.

Lettuce seed germination percentage (Eqn. 1), radicle, and hypocotyl lengths were recorded after incubation at 25°C for 7 days [21, 22]. Seeds were considered germinated when the seed coat ruptured and the emergent radicle reached a length of 2 mm. Percentage inhibition of radicle and hypocotyl elongation of lettuce seedlings (Eqn. 2) [45], germination index (GI; Eqn. 3) [46, 47], and seedling vigor index (SVI; Eqn. 4) [48] were also calculated after 7 days using the following equations.

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

Eqn. 1

$$\text{Percentage inhibition (\%)} = [1 - \frac{(RL\ or\ HL_{treatment})}{(RL\ or\ HL_{control})}] \times 100$$

Eqn. 2

$$\text{Germination index (GI)} = \sum \frac{(Gt)}{(Tt)}$$

Eqn. 3

Where, Gt = Number of seeds germinated on t^{th} day; Tt = Number of days up to t^{th} day.

$$\text{Seedling Vigor Index (SVI)} = \text{Germination percentage (\%)} \times \text{seedling total length (cm)}$$

Eqn. 4

Pot experiment

Based on the results of the bioassays, six most phytotoxic extracts were selected for further investigation through a pot study. Accordingly, leaf extracts (cold distilled water extraction) of *Erythrina lithosperma*, *Gliricidia sepium*, and *Grevillea robusta* each at two different concentrations, *namely* 6% and 8% (w/v) were tested on three selected weed species commonly found in tea lands (*Ageratum conyzoides*, *Bidens pilosa*, and *Cleoma aspera*). Selected weeds with uniform growth were planted individually on polythene bags (one plant per bag) filled with soil collected from a tea land. The experiment was laid out in three-factor factorial CRD with three replicates under partially-controlled environment conditions in a plant house of the Uva Wellassa University of Sri Lanka. The three factors included leaf extract (shade tree species), weed species, and concentration levels. Leaf extracts were applied to the weeds planted in polythene bags once a week using a hand sprayer as drench application for three consecutive weeks. Distilled water was sprayed as the control. Weed responses were regularly monitored for 3 weeks. Visual injury symptoms, such as wilting and chlorosis, were evaluated on a scale from 1 to 4, with 1 indicating no injury and 4 indicating severe injury, at weekly intervals (Table 1). Dead weed count was recorded weekly, and plant height (HT) and weeds dry weight (DW) were measured at three weeks after weeds establishment. The plants were oven-dried at 60°C for 48 h for dry weight measurements. Growth suppression in weeds treated with different leaf extracts relative to the control (distilled water) was assessed using the following equation (Eqn. 5).

$$\text{Percentage reduction in HT of DW (\%)} = [1 - \frac{(HT\ or\ DW_{treatment})}{(HT\ or\ DW_{control})}] \times 100$$

Eqn. 5

Where, HT = Plant height; DW = Dry Weight

Table 1. *Description of the categories used in the visual assessments of wilting [49] and chlorosis (modified from Sudahono et al. [50]) of weed plants.*

Category	Stage	Visual Characters
	Wilting Stage	
1	Normal (not wilted)	No signs of wilting
2	Slightly wilted	Slight leaf angel changes but no folding, rolling or changes in leaf surface structure
3	Wilted	Strong change of leaf angle or protrusion of veins on the leaf surface
4	Severely wilted	Very strong change of leaf angle or protrusion of veins on the leaf surface with beginning necrosis
	Chlorosis Stage	
1	Normal (not chlorosis)	Healthy green leaves
2	Slightly chlorosis	Yellowish-green interveinal areas, green veins
3	Chlorosis	greenish–yellow interveinal areas or yellow interveinal areas, green veins
4	Severely Chlorosis	Yellow–white interveinal areas, pale green veins

Statistical analysis

The germination parameters of the bioassays were initially analyzed using a three-way Analysis of Variance (ANOVA). Subsequently, the six most phytotoxic extracts were selected and further analyzed along with the control (distilled water) using one-way ANOVA. Treatment means were compared using Tukey's mean comparison test. Data from the pot experiment (reduction in plant height and dry weight relative to the control and dead weed count) were analyzed using three-way ANOVA. Visual injury symptoms were analyzed using the 1-Sample Sign Test. All statistical analyses were conducted using the Minitab 16 software package.

RESULTS

Lettuce seed bioassay

Effects of leaf extracts of shade tree spp., allelochemical extraction method, leaf extract concentration and their interactions on lettuce seed germination parameters is shown in the Table 2. Three-way interaction between leaf extract, concentration, and extraction method was not significant for lettuce seed germination percentage (P = 0.712), germination index (P = 0.751), hypocotyl length (P = 1.000), radicle length (P = 0.958), percentage inhibition of radicle elongation (P = 0.864), percentage inhibition of hypocotyl elongation (P = 1.000), and seedling vigor index (P = 0.065; Table 2). Interaction effect of leaf extract and concentration was significant for all above parameters ($P < 0.05$; Table 2). There was no any significant difference between hot distilled and cold distilled water extraction methods ($P > 0.05$) on any germination parameters tested.

Table 2. *Main and interaction effects (P values) on different germination parameters of lettuce seeds treated with leaf extracts (LE) of four shade tree species (Calliandra calothrysus, Erythrina lithosperma, Gliricidia sepium, and Grevillea robusta) extracted using hot and cold distilled water extraction methods (EM) at two different concentration (C) levels*

Main/Interaction effect	Probability level (*P* value)						
	GP	GI	HL	IHE	RL	IRE	SVI
LE	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
C	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
EM	0.597	0.642	0.988	0.991	0.201	0.064	0.260
LE x C	0.001*	0.001*	0.001*	0.001*	0.008*	0.001*	0.001*
LE x EM	0.929	0.961	0.988	0.975	0.852	0.071	0.111
C x EM	0.858	0.961	0.993	0.998	0.377	0.424	0.354
LE x C x EM	0.712	0.751	1.000	1.000	0.958	0.864	0.065

*Significant at 0.05 probability level, GP: Germination percentage; GI: Germination index; HL: Hypocotyl length; RL: Radicle length; IRE: Percentage inhibition of radicle elongation; IHE: Percentage inhibition of hypocotyl elongation; SVI: Seedling vigor index.

Erythrina lithosperma, *Gliricidia sepium*, and *Grevillea robusta* each at 6% and 8% concentrations showed promising results for all germination parameters tested (highest phytotoxicity) and hence compared with the control as shown in the Table 3.

Table 3. *Effect of leaf extracts of three selected shade tree species (Erythrina lithosperma, Gliricidia sepium, and Grevillea robusta) at two concentration levels on different germination parameters of lettuce seeds.*

Leaf extract	Concentration (%) (w/v)	Seed germination parameters						
		GP (%)	GI	HL (mm)	IHE (%)	RL (mm)	IRE (%)	SVI
E. lithosperma	8	29.5^{cd} (69)	0.7^{bc}	5.2^{c}	80.8^{c}	2.4^{c}	77.7^{ab}	0.2^{cd}
	6	51.0^{b} (47)	1.1^{ab}	7.9^{b}	70.9^{d}	4.3^{bc}	60.2^{ab}	0.6^{b}
G. sepium	8	17.8^{e} (82)	0.4^{c}	3.0^{d}	89.0^{a}	1.8^{c}	83.3^{a}	0.1^{d}
	6	31.8^{cd} (67)	0.8^{bc}	3.8^{cd}	86.0^{ab}	6.1^{b}	43.5^{b}	0.3^{bcd}
G. robusta	8	21.9^{de} (77)	0.5^{c}	4.6^{c}	83.0^{dc}	4.2^{bc}	61.1^{ab}	0.2^{cd}
	6	39.8^{c} (59)	0.9^{bc}	7.3^{b}	73.2^{d}	6.0^{b}	44.4^{b}	0.5^{bc}
Control		96.7^{a}	1.7^{a}	27.2^{a}		10.8^{a}		3.4^{a}

Means in each column followed by the same letter are not significantly different at *P* = 0.05.
GP: Germination percentage; GI: Germination index; HL: Hypocotyl length; RL: Radicle length; IRE: Percentage inhibition of radicle elongation; IHE: Percentage inhibition of hypocotyl elongation; SVI: Seedling vigor index.
Data are expressed as means. n = 3. Values within parenthesis in GP column are inhibition percentages (%) relative to the control (distilled water).

Germination percentage

All three leaf extracts at both concentrations recorded significantly lower germination percentages (P < 0.05; Table 3) than the control, depicting their potential allelopathic effects. The lowest germination percentage was recorded by *G.sepium* (82% reduction relative to control) extract, followed by G. robusta extract (77% reduction relative to control), both at 8% concentration. Germination percentage decreased with increasing concentration across all leaf extracts.

Germination index

The germination index ranged from 0.4 in the 8% extract of *G. sepium* to 1.7 in the control (Table 3). All extracts at both concentrations showed significantly lower values for the germination rate index than the control, except for the 6% extract of *G. robusta.*

Hypocotyl and radicle growth

The hypocotyl and radicle growth were significantly ($P < 0.05$) inhibited by all three extracts at both concentrations compared to the control, and the degree of inhibition increased with increasing concentration (Table 3). All three leaf extracts at both concentrations showed more than 70% inhibition in hypocotyl elongation and more than 40% inhibition in radicle elongation. *G. sepium* at 8% concentration recorded the highest inhibitory effect on hypocotyl and radicle growth, as evidenced by the lowest hypocotyl and radicle lengths and highest percentage inhibition of hypocotyl (89%) and radicle (83.3%) elongation.

Seedling vigor index

All three leaf extracts at both concentrations recorded significantly lower seedling vigor indexes compared to the control. G. sepium recorded the lowest seedling vigor index, followed by G. robusta and E. lithosperma, each at an 8% concentration.

Pot experiment

Weed growth suppression

The highest-order interaction effect of shade tree species × concentration × weed species on the percentage reduction in plant height and dry weight of weed species relative to control was insignificant ($P > 0.05$). The effect of leaf extract on weed height and dry weight reduction was depended on its concentration ($P < 0.05$; Table 4). The highest reduction in plant height (83.8%) was recorded by *G. sepium*, followed by G. robusta (37.3%), both at 8% concentration. Similarly, *G. sepium* at 8% concentration recorded the highest reduction in weed dry weight (35.8%), which was statistically similar to its 6% concentration (32.4%) and *G. robusta* at 8% concentration (31.6%).

Table 4. *Percentage reduction in plant height and dry weight of three weed species (Ageratum conyzoides, Bidens pilosa, and Cleoma aspera) relative to the control (distilled water) at three weeks after application of different leaf extracts of three shade tree species (Erythrina lithosperma, Gliricidia sepium, and Grevillea robusta) at two concentration levels (8 and 6%; w/v).*

Leaf extract of shade tree species	Leaf Extract Concentration (%) (w/v)	Height reduction (%)*	Dry weight reduction (%)*
E. lithosperma	8	10.2^{bc}	10.6^{cd}
	6	6.3^{c}	17.3^{c}
G. sepium	8	83.8^{a}	35.8^{a}
	6	30.2^{bc}	32.4^{ab}
G. robusta	8	37.3^{b}	31.6^{ab}
	6	27.2^{bc}	26.9^{b}

Means in each column followed by the same letters are not significantly different at $P = 0.05$. * Please refer the "Eqn. 5".

Dead weed percentage

G. sepium at 8% concentration was more effective at controlling all three weed species tested, as evidenced by the highest dead weed percentages (Fig. 1). For example, there was complete weed mortality (100%) in *C. aspera* and 66.6% weed mortality in *B. pilosa* and *A. conzoydes* at three weeks after application of *G. sepium* at 8% concentration. *G. sepium* at 6% and *G. robusta* at 8% also showed good performance, resulting in 66.6% weed mortality in *C. aspera* and 33.3% weed mortality in *B. pilosa*. However, *E. lithosperma* at both concentrations did not cause weed mortality in any species tested.

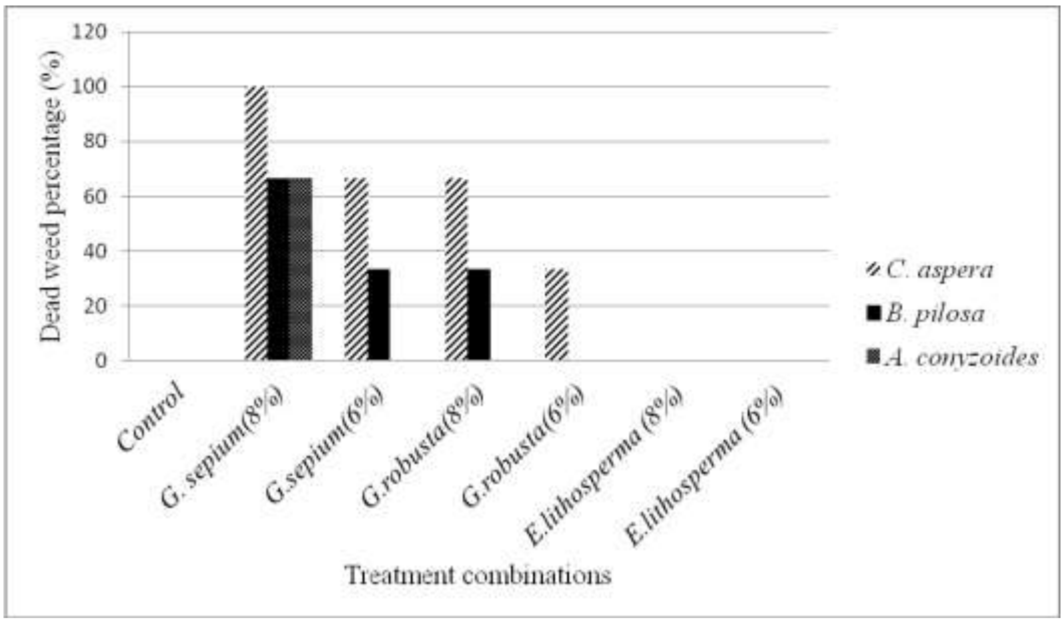

Fig. 1. *Dead weed percentage (%) of three weed species at three weeks after application of different leaf extracts of three shade tree species at two concentration levels (8 and 6%; w/v) compared to control (distilled water)*

Visual injury symptoms of weed species (wilting and chlorosis)

As depicted in Fig. 2 and 3, severe wilting (characterized by a pronounced change in leaf angle or protrusion of veins on the leaf surface with the beginning of necrosis) and chlorosis (manifested by yellow-white interveinal areas, pale green veins) of leaves were observed in *C. aspera* three weeks after the application of *G. sepium* and *G. robusta* leaf extracts at both concentration levels. A similar degree of wilting and chlorosis were observed in *Ageratum conyzoides* and *Bidens pilosa* plants applied with 8% *G. sepium* leaf extract. However, wilting or chlorosis was not observed in *Ageratum conyzoides* plants except for those applied with 8% *G. sepium* leaf extract.

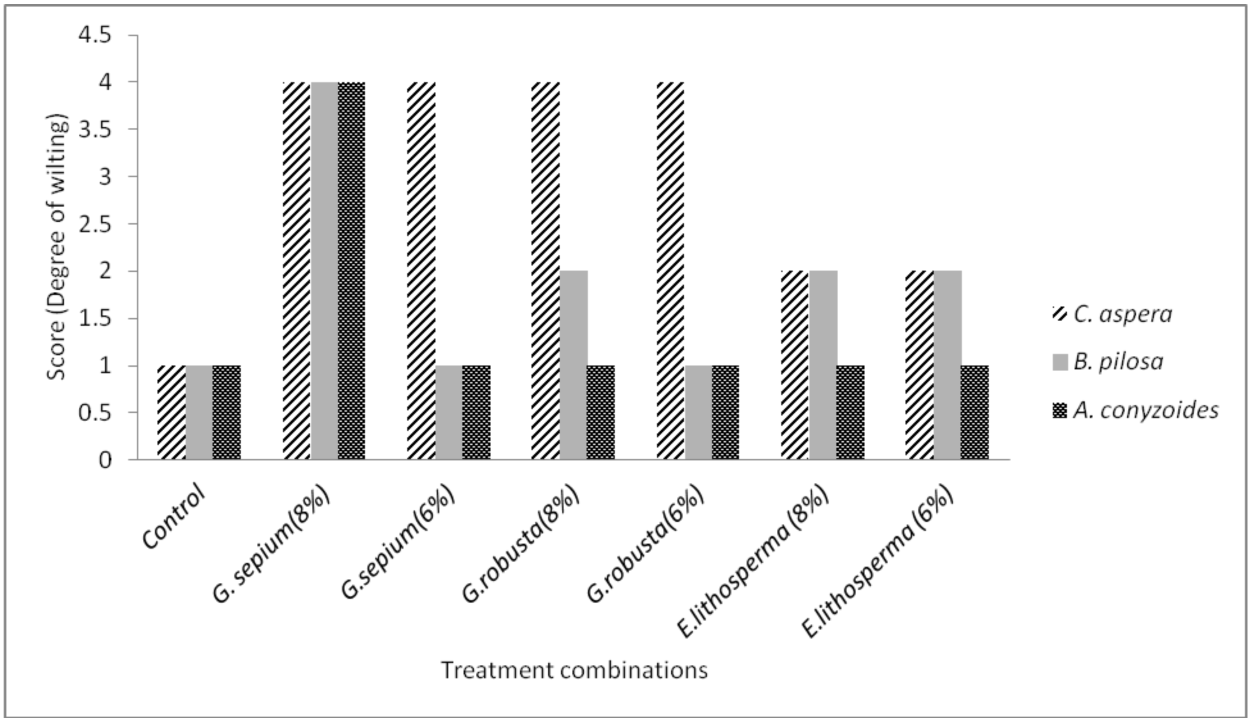

Fig. 2. *Degree of leaf wilting in three weed species at three weeks after application of different leaf extracts of three shade tree species at two concentration levels (8 and 6%; w/v) compared to control (distilled water). The higher the score, the higher the degree of wilting (see Table 1).*

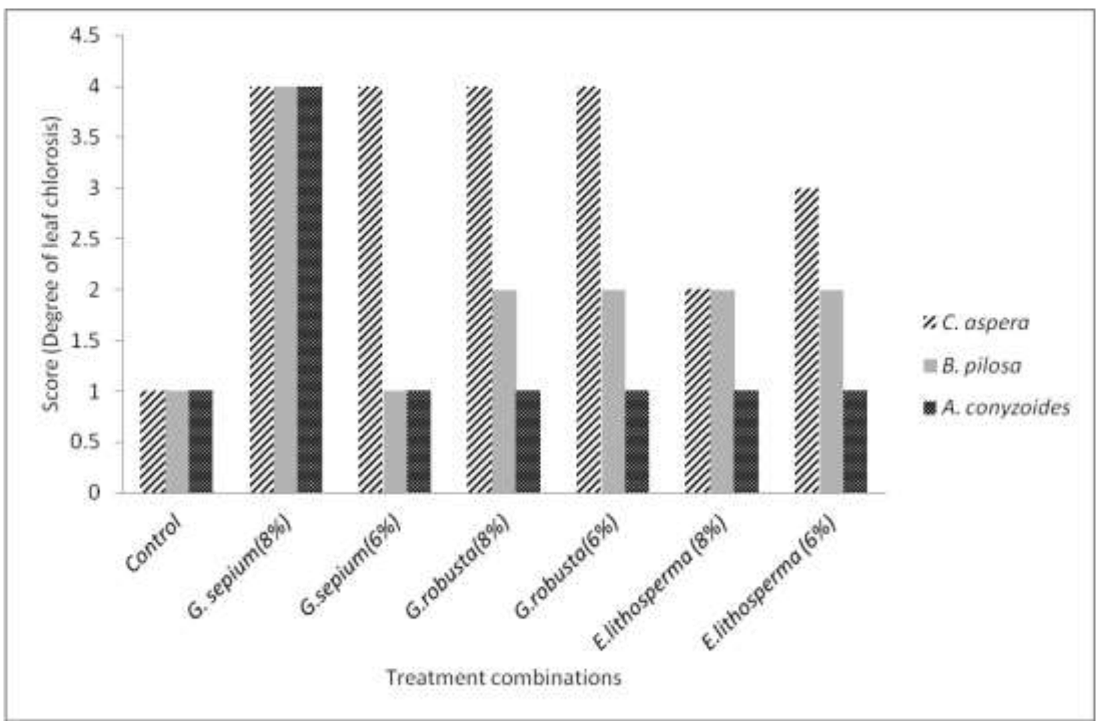

Fig. 3 *Degree of leaf chlorosis in three weed species three weeks after application of different leaf extracts of three shade tree species at two concentration levels (8 and 6%; w/v) compared to control (distilled water). [The higher the score, the higher the degree of chlorosis (see Table 1).].*

DISCUSSION

In the current study, we investigated the allelopathic potential of four shade tree species commonly planted in tea fields in Sri Lanka, namely *Calliandra calothrysus, Erythrina lithosperma, Gliricidia sepium,* and *Grevillea robusta.*

The bioassay results revealed a high allelopathic potential in leaf extracts of *G. sepium* followed by *G. robusta*, as evidenced by significant inhibition in lettuce seed germination, hypocotyl and radical elongation and lower seedling vigor index. These findings consistent with earlier studies by Oyun [40], who found an inhibitory effect of *G. sepium* leaf leachates on maize seeds. Several groups of allelochemicals have been identified in *G. sepium* such as gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, *B*-resorcyclic acid, vanillic acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, ferulic acid, sinapinic acid (trans and cis forms), coumarin, and myricetin [30] and some of these chemicals might have contributed for the inhibitory effect on seed germination and seedling growth. *G. sepium* is widely used for providing shade in tea, coffee, and other shade loving crops, and it serves as an important source of green manure, fodder, and fuelwood. It is a quick growing, nitrogen fixing tree that grows best in tropical, seasonally dry climates. *G. sepium* withstands lopping well and has good coppicing ability, allowing for multiple loppings per year depending on the growth rate [51]. These characteristics make *G. sepium* a potential candidate for suppressing weeds through its allelopathic effect. The concentration of leaf extracts significantly affected germination parameters, with 8% (w/v) concentration showing the highest inhibitory effect. These results are in accordance with the previous studies reporting that the phytotoxicity of extracts increases as their concentration increases [8, 52-55]. Interestingly, there was no significant difference between cold and hot distilled water extraction methods, indicating that either method can be used to prepare extracts.

The allelopathic effects are closely and best evaluated during early plant development stages under controlled conditions, as external factors such as water, nutrients, light, competition, and other field interferences can confound allelopathic responses [56]. Consequently, the efficacy of the six most promising leaf extracts, selected based on the bioassay results, was tested on three weed species in a pot study under controlled conditions. Pot study further supports the findings of the bioassay. As evident from the pot study *G. sepium* at 8% (w/v) emerged as the most effective leaf extract in suppressing the growth of the tested weed species, especially *C. aspera. G. robusta* at 8% concentration also exhibited significant performance, causing 66.6% mortality in *C. aspera* and 33.3% mortality in *B. pilosa.* Among the 15 allelochemicals identified in *G. sepium* [30], coumarins were considered the main allelopathic compound [57]. Ashraf et al. [58] also found 15 phytochemicals in *G. robusta* extract with a high percentage of coumarin (30.64%). Coumarin is well-known for its phytotoxic potential [59] and has been reported to cause growth inhibition and cell death by inducing overproduction of reactive oxygen species [60]. Therefore, we can hypothesize that the observed weed mortality caused by *G. sepium* and *G. robusta* extracts may be attributed to the activity of coumarins. Previous literature also supports the notion that coumarin possesses potential as a natural herbicide for eco-friendly weed control [61]. Moreover, our study noted significant growth retardation in weeds treated with different plant extracts, particularly *G. sepium and G. robusta* compared to the control. Allelochemicals present in these extracts may have disrupted various physiological processes in weeds, including cell division, hormone biosynthesis, and mineral uptake/transport [62], membrane permeability [63], stomatal oscillations, photosynthesis [64], respiration, protein metabolism [65] and plant water relations [8]. These disruptions likely contributed to the significant suppression of weed growth observed in our study.

We observed severe chlorosis characterized by yellow-white interveinal areas and pale green veins in all three tested weed species (Cleoma aspera, Ageratum conyzoides, and Bidens pilosa) three weeks after application of *G. sepium* extract at 8% concentration. Inostrosa and Fournier [66] also found an inhibitory effect on *Bidens pilosa* by *G. sepium* as evident in our study, and suggested that the inhibition may be due to a phenolic substance known as protocatechuic acid. Similarly, *G. robusta* leaf extracts at both 6 and 8% concentration caused chlorosis in *Cleoma aspera* (Severe chlorosis) and *Bidens Pilosa* (slightly chlorosis). This could be attributed to the inhibitory effect of phenolic allelochemicals present in both species [30] on Mg-chelatase activity and the accumulation of chlorophyll and porphyrin contents in the leaves [67]. *A. conyzoides* appeared to be less sensitive to leaf extracts and was only suppressed by *G. sepium* extract at 8% concentration. These findings highlight the potential of using these types of shade tree species in developing selective bio-herbicides by adjusting the type of extract and concentration.

Based on the above findings, we emphasized the potential of employing allelopathy for sustainable weed management in tea plantations. Plant materials from these allelopathic shade tree species could serve various purposes, including surface mulching [10], soil incorporation [68], preparation of aqueous extracts [13, 69], or combined application of aqueous extracts with lower synthetic herbicide doses [70, 71] to effectively control weeds. However, further investigation is warranted to identify the best application methods for effective weed control in field conditions. In some cases, mixtures of different allelochemicals have exhibited synergistic effects, resulting in a greater allelopathic impact than individual compounds alone [72]. Therefore, combined application of allelopathic extracts may prove more effective in weed control than individual applications [73-75]. Similarly, we anticipate that the combined application of *G. sepium* and *G. robusta* extracts could lead to more effective weed suppression than their individual applications. Nevertheless, additional studies are necessary to validate this hypothesis.

Although our study yielded positive results, it is crucial to recognize its limitations. For instance, the allelopathic effects observed in controlled laboratory settings may not fully represent the complexities of interactions within natural ecosystems. It is important to note that biodegradation, type, and concentration of allelochemicals released into the environment are influenced by a combination of factors, including plant characteristics and environmental conditions [38]. Controlling these environmental factors can present challenges, potentially affecting the study's outcomes. Although numerous plant species exhibit significant allelopathic potential, not all of these species or their byproducts may be appropriate for practical use. Issues such as cost, limited environmental stability, availability, and low herbicidal activity of numerous natural compounds have served as the primary constraints in allelopathy research [35]. Despite numerous challenges associated with implementing the allelopathy concept for weed management, there exists significant potential for exploring allelopathy as a novel tool in this regard. Future investigations should prioritize understanding the mechanisms underlying allelochemical selectivity, their modes of action, their interactions with different species, and strategies for their effective implementation [35].

CONCLUSION

The current study highlights that the aqueous leaf extracts of *Gliricidia sepium* and *Grevillea robusta*, at 8% concentration, exhibit a significant allelopathic effect capable of suppressing the growth of succulent broad-leaf weeds in tea plantations. Notably, this inhibitory effect is concentration-dependent, with the 8% (w/v) concentration demonstrating the highest efficacy. Moreover, our findings indicate that there is no significant difference in allelopathic effect between hot and cold-water extraction methods. This suggests that either extraction method can be utilized without compromising the allelopathic potential of the extracts.

Acknowledgments. This study was funded by the National Research Council of Sri Lanka (Grant No: NRC EWC18-05)

REFERENCES

[1] Prematilake, K.G. (2003): Weed management in tea–Recent development. Tropical Agricultural Research and Extension 6: 98–107

[2] Nelum, K.G., Piyasena, P., Liyanage, M.G.S., Weerakoon, N.C., De Silva, M.S.D.L. (2023): Allelopathic potential of green manure, cover crops, mulching plants, and weeds found in tea plantations. African Journal of Agricultural Research 19(10): 962-968

[3] Buhler, D.D., Liebman, M., Obrycki, J.J. (2002): Review: theoretical and practical challenges to an IPM approach to weed management. Weed Science 48:274–280

[4] Chauhan, B.S., Johnson, D.E. (2010): The role of seed ecology in improving weed management strategies in the tropics. Advances in Agronomy 105:221–262

[5] Jabran, K., Farooq, M. (2013): Implications of potential allelopathic crops in agricultural systems. In: Cheema, Z., Farooq, M., Wahid, A. (eds.) Allelopathy, Springer, Berlin, Heidelberg, pp 349-385. https://doi.org/10.1007/978-3-642-30595-5_15.

[6] Zeng, R.S. (2014): Allelopathy-the solution is indirect. Journal of Chemical Ecology 40(6): 515–516

[7] Kostina-Bednarz, M., Płonka, J., Barchanska, H. (2023): Allelopathy as a source of bioherbicides: Challenges and prospects for sustainable agriculture. Reviews in Environmental Science and Bio/Technology 22(2): 471-504

[8] Rice, E.L. (1984): Allelopathy. Academic Press, New York

[9] Cheema, Z.A., Sadiq, H.M.I., Khaliq, A. (2000b): Efficacy of Sorgaab (sorghum water extract) as a natural weed inhibitor in wheat. International Journal of Agriculture and Biology 2: 144–146

[10] Cheema, Z.A., Asim, M., Khaliq, A. (2000a): Sorghum allelopathy for weed control in cotton (*Gossypium arboretum* L.). International Journal of Agriculture and Biology 2: 37–40

[11] Irshad, A., Cheema, Z.A. (2004): Effect of sorghum extract on management of barnyard grass in rice crop. Allelopathy Journal 14: 205–212

[12] Cheema, Z., Khaliq, A., Saeed, S. (2004): Weed control in maize (*Zea mays* L.) through sorghum allelopathy. Journal of Sustainable Agriculture 23(4): 73–86.

[13] Jabran, K., Cheema, Z.A., Farooq, M., Basra, S.M.A., Hussain, M., Rehman, H. (2008): Tank mixing of allelopathic crop water extracts with pendimethalin helps in the management of weeds in canola (*Brassica napus*) field. International Journal of Agriculture and Biology 10:293–296

[14] Cheema, Z.A., Khaliq, A., Akhtar, S. (2001): Use of sorghum water extract (sorghum water extract) as a natural weed inhibitor in spring mungbean. International Journal of Agriculture and Biology 3: 515–518

[15] Asad, S., Bajwa, R. (2005): Allelopathic effects of *Senna occidentalis* L. on Parthenium weed. Proceedings of the 6th National Weed Science Conference March 28-30, 2005. NWFP Agricultural University, Peshawar.

[16] Tehmina, A., Bajwa R. (2005): Allelopathic potential of sunflower (*Helianthus annus* L.) as natural herbicide. Proceedings of the 6th National Weed Science Conference March 28-30, 2005, NWFP Agricultural University, Peshawar.

[17] Ashrafi, Z.Y., Sadeghi, S., Mashhadi, H.R., Hassan, M.A. (2008): Allelopathic effects of sunflower (*Helianthus annuus*) on germination and growth of wild barley (*Hordeum spontaneum*). Journal of Agricultural Technology 4(1): 219–229

[18] Alsaadawi, I.S., Sarbout, A.K., Al-Shamma, L.M. (2012): Differential allelopathic potential of sunflower (*Helianthus annuus* L.) genotypes on weeds and wheat (*Triticum aestivum* L.) crop. Archives of Agronomy and Soil Science 58: 1139–1148.

[19] Ejaz, AK., Khan, M.A., Ahmad, H.K., Khan, F.U. (2004): Allelopathic effects of Eucalyptus leaf extract on germination and growth of cotton (*Gossypium hirsutum* L.). Pakistan Journal of Weed Science Research 10: 145–150.

[20] Alireza, D., Asaadi, M.A. (2010): Allelopathic effects of *Eucalyptus camaldulensis* on seed germination and seedling growth of *Acroptilon rapens, Plantago laceolata* and *Portulaca oleracea*. Research Journal of Biological Sciences 5(6): 430–434.

[21] Rezaeinodehi, A., Khangholi, S., Amindehaghi, M., Kazemi, H. (2006): Allelopathic potential of tea (*Cammellia sinensis* (L.) kuntze) on germination and growth of *Ameranthus retroflexus* L. and *Setaria gluca* (L.) P.Beauv. Journal of Plant Diseases and Protection 20: 447–454.

[22] Waris, A., Waris, L., Khan, M.A., Shad, A.A. (2016): Allelopathic effect of methanol and water extracts of *Cammellia sinensis* L. on seed germination and growth of *Triticum aestivum* L. and *Zea mays* L. Journal of Bioresource Management 3(1). https://doi.org/10.35691/JBM.6102.0043

[23] Kong, C.H., Wang, P., Zhang, C.X., Zhang, M.X., Hu, F. (2006): Herbicidal potential of allelochemicals from *Lantana camara* against *Eichhornia crassipes* and the alga *Microcystis aeruginosa*. Weed Research 46(4): 290–295.

[24] El-Rokiek, K.G., El-Masry, R.R., Messiha, N.K., Ahmed, S.A. (2010): The allelopathic effect of mango leaves on the growth and propagative capacity of purple nutsedge (*Cyperus rotundus* L.). Journal of American Science 6(9): 151–159.

[25] Prematilake, K.G., Liyanage, M.G.S. (2011): The potential of exploitation of Ginisapu (*Mechelia champaca* L.) seeds as a natural herbicide, Proceedings of the 6th World Congress on Allelopathy December 15-19, 2011, Guangzhou, China

[26] Ghimire, B.K., Ghimire, B., Yu, C.Y., Chung, I.M. (2019): Allelopathic and autotoxic effects of Medicago sativa—Derived allelochemicals. Plants 8(7): 233. doi:10.3390/plants8070233

[27] Khanam, H., Rahman, M.S., Islam, M.J., Fancy, R., Hosain, M.B., Rima, R.S.N. (2020): Allelopathic Effect of neem (*Azadirachta indica*) leaf extracts on germination and seedling growth of some vegetable crops. Asian Journal of Research in Botany 4(4): 113–121

[28] Weston, L.A., Alsaadawi, I.S., Baerson, S.R. (2013): Sorghum allelopathy from ecosystem to molecule. Journal of Chemical Ecology 39: 142–153.

[29] Kremer, R.J., Reinbott, T.M. (2021): Allelopathy of sorghum residues on weed establishment is affected by hybrid, phenolic acid contents and environment. Journal of Plant Sciences 9(1): 25–31.

[30] Ramamoorthy, M., Paliwal, K. (1993): Allelopathic compounds in leaves of *Gliricidia sepium* (Jacq.) Kunth ex walp. and its effects on *Sorghum vulgare* L. Journal of Chemical Ecology 19(8): 1691–1701.

[31] Macias, F.A., Torres, A., Galindo, J.L.G., Varela, R.M., Alvarez, J.A., Molinillo, J.M.G. (2002): Bioactive terpenoids from sunflower leaves cv. *Peredovick.* Phytochemistry 61: 687–692

[32] Anjum T, Bajwa R (2005): A bioactive Annuionone from sunflower leaves. Photochemistry. 66: 1919–1921

[33] Duke, S.O., Romagni, J.G., Dayan, F.E. (2000): Natural products as sources for new mechanisms of herbicidal action. Crop Protection 19: 583–589.

[34] Haig, T., Pratley, J., An, M., Haig, T., Hildebrand, S. (2005): Using allelopathy to search for new natural herbicides from plants. In Proceedings of the 4th World Congress on Allelopathy. Harper, J., An, M., Wu, H., Kent, J.H. (eds.). Charles Sturt University, Wagga, Australia. pp. 565–568.

[35] Khamare, Y., Chen, J., Marble, S.C. (2022): Allelopathy and its application as a weed management tool: A review. Frontiers in Plant Science 13:1034649. https://doi.org/10.3389/fpls.2022.1034649

[36] Kaliyadasa, P.E., Jayasinghe, S.L. (2018): Screening of allelopathic activity of common weed species occurring in agricultural fields. African Journal of Agricultural Research 13 (47): 2708-2715.

[37] Motmainna, M., Juraimi, A.S.B., Uddin, Md.K., Asib, N.B., Islam, A.K.M.M., Hasan, M. (2021): Assessment of allelopathic compounds to develop new natural herbicides: A review. Allelopathy Journal 52: 21–40. https://doi.org/10.26651/allelo.j/2021-52-1-1305

[38] Scavo, A., Mauromicale, G. (2021): Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. Agronomy 11: 2104. https://doi.org/10.3390/agronomy11112104

[39] Tea Research Institute, Advisory Circular (2003): Integrated Weed Management in Tea. Serial No:9/03. The Tea Research Institute of Sri Lanka.

[40] Oyun, M.B. (2006): Allelopathic potentialities of *Gliricidia sepium* and *Acacia auriculiformis* on the germination and seedling vigour of maize (*Zea mays* L.). American Journal of Agricultural and Biological Science 1(3): 44–47.

[41] Kaboneka, S., Nsavyimana, G., Nkurunziza, M., Ntezukwigira, G. (2020): Allelopathic effects of *Calliandra calothyrsus* Meisn, *Senna siamea* L. and *Gliricidia sepium* (Jacq.) Walp Leaves on maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) root and shoot growth. International Journal of Advances in Scientific Research and Engineering 6(2): 47–59.

[42] Subtain, M.U., Hussain, M., Tabassam, M.A.R., Ali, M.A., Ali, M., Mohsin, M., Mubushar, M. (2014): Role of allelopathy in the growth promotion of plants. Scientia Agriculturae 6 (3): 141–145.

[43] Dahiya, S., Kumar, S., Khedwal, R.S., Jakhar, S.R. (2017): Allelopathy for sustainable weed management. Journal of Pharmacognosy and Phytochemistry 6: 832–837.

[44] Al-Samarai, G.F., Mahdi, W.M., Al-Hilali, B.M. (2018): Reducing environmental pollution by chemical herbicides using natural plant derivatives–allelopathy effect. Annals of Agricultural and Environmental Medicine 25(3): 449–452.

[45] Hong, N.H., Xuan, T.D., Eiji, T., Hiroyuki, T., Mitsuhiro, M., Khanh, T.D. (2003): Screening for allelopathic potential of higher plants from Southeast Asia. Crop Protection 22(6): 829–836.

[46] Carpýcý, E.B., Celýk, N., Bayram, G. (2009): Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. African Journal of Biotechnology 8(19): 4918–4922

[47] Hou, Q.Q., Chen, B.M., Peng, S.L., Chen, L.Y. (2014): Effects of extreme temperature on seedling establishment of nonnative invasive plants. Biological Invasions 16:2049–2061

[48] Abdul-Baki, A.A., Anderson, J.D. (1973): Vigor determination in soybean seed by multiple criteria 1. Crop Science 13(6): 630–633.

[49] Engelbrecht, B.M., Tyree, M.T., Kursar, T.A. (2007): Visual assessment of wilting as a measure of leaf water potential and seedling drought survival. Journal of Tropical Ecology 23(4): 497–500.

[50] Sudahono, D.H.B., Rouse, R.E. (1994): Greenhouse screening of citrus rootstocks for tolerance to bicarbonate-induced iron chlorosis. HortScience 29(2): 113–116

[51] Tea Research Institute, Advisory Circular (2003): Shade in Tea. Serial No:3/03. The Tea Research Institute of Sri Lanka.

[52] Putnam, A.R. (1994): Phytotoxicity of plant residues. In: Unger, P.W. (ed.) Managing agricultural residues, Lewis Publishers, Boca Raton, pp 285–314

[53] Bora, I.P., Singh, J., Borthakur, R., Bora, E. (1999): Allelopathic effects of leaf extracts of *Acacia auriculiformis* on seed germination of some agricultural crops. Annals of Forestry 7: 143–146.

[54] Sinkkonen, A. (2001): Density-dependent chemical interference, an extension of the biological response model. Journal of Chemical Ecology 27: 1513–1523

[55] Peng, SL., Wen, J., Guo, Q.F. (2004): Mechanism and active variety of allelochemicals. Acta Botanica Sinica 46: 757–766

[56] Kaboneka, S., Kwizera, C., Nijimbere, S., Irakoze, W., Nsengiyumva, P., Ndihokubwayo, S., Habonimana, B. (2021): Yield responses of maize (*Zea mays* L.) and successive potato (*Solanum tuberosum* L.) crops to maize stover co-composted with *Calliandra calothyrsus* Meisn green manure. International Journal of Advances in Scientific Research and Engineering 7(4): 1–15.

[57] Takemura, T., Kamo, T., Sakuno, E., Hiradate, S., Fujii, Y. (2013): Discovery of coumarin as the predominant allelochemical in *Gliricidia sepium*. Journal of Tropical Forest Science 25(2): 268–272.

[58] Ashraf, A., Qureshi, N.A., Shaheen, N., Iqbal, A., Fatima, H., Afzal, M., Alhewairini, S.S., Qureshi, M.Z. (2020): Termiticidal and protozocidal potentials of eight tropical plant extracts evaluated against *Odontotermes obesus* (Rambur) (Blattodea; Termitidae) and *Heterotermes indicola* (Wasmann) (Blattodea; Rhinotermitidae). Polish Journal of Environmental Studies 29(5): 3493–3507.

[59] Araniti, F., Mancuso, R., Lupini, A., Giofrè, S.V., Sunseri, F., Gabriele, B., Abenavoli, M.R. (2015): Phytotoxic potential and biological activity of three synthetic coumarin derivatives as new natural-like herbicides. Molecules 20(10): 17883–17902.

[60] Yan, Z., Wang, D., Cui, H., Zhang, D., Sun, Y., Jin, H., Li, X., Yang, X., Guo, H., He, X., Le Pan, Ren, X., Guo, K., Qin, B. (2016): Phytotoxicity mechanisms of two coumarin allelochemicals from *Stellera chamaejasme* in lettuce seedlings. Acta Physiologiae Plantarum 38: https://doi.org/10.1007/s11738-016-2270-z

[61] Niro, E., Marzaioli, R., De Crescenzo, S., D'Abrosca, B., Castaldi, S., Esposito, A., Fiorentino, A., Rutigliano, F.A. (2016): Effects of the allelochemical coumarin on plants and soil microbial community. Soil Biology and Biochemistry 95: 30–39.

[62] Rizvi, S.J.H., Haque, H., Singh, V.K., Rizvi, V. (1992): A discipline called allelopathy. In: Rizvi, S.J.H., Rizvi, V. (eds.) Allelopathy: Basic and Applied Aspects, Springer, Dordrecht. https://doi.org/10.1007/978-94-011-2376-1_1

[63] Harper, J.R., Balke, N.E. (1981): Characterization of the inhibition of K+ absorption in oat roots by salicylic acid. Plant Physiology 68: 1349–1353

[64] Einhellig, F.A., Rasmussen, J.A. (1979): Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. Journal of Chemical Ecology 5: 815–824

[65] Kruse, M., Strandberg, M., Strandberg, B. (2000): Ecological effects of allelopathic plants-A review, National Environmental Research Institute, Silkeborg, Denmark. 66 pp. – NERI Technical Report No. 315

[66] Inostrosa, S.I., Fournier, O.L.A. (1982): Allelopathic effect of *Gliricidia sepium* (Jacq.) Steud (Madero Negro) Steud (Madero Negro). Revista de Biologia Tropical 30 (1): 35-39

[67] Yang, C.M., Lee, C.N., Chou, C.H. (2002): Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: I. Inhibition of supply-orientation. Botanical Bulletin of Academia Sinica 43: 299–304

[68] Matloob, A., Khaliq, A., Farooq, M., Cheema, Z.A. (2010): Quantification of allelopathic potential of different crop residues for the purple nut sedge suppression. Pakistan Journal of Weed Science Research 16:1–12

[69] Iqbal, J., Cheema, Z.A. (2007): Effect of allelopathic crops water extracts on glyphosate dose for weed control in cotton (*Gossypium hirsutum* L.). Allelopathy Journal 19:403–410

[70] Iqbal, J., Cheema, Z.A. (2008): Purple nut sedge (*Cyperus rotundus* L.) management in cotton with combined application of *Sorgaab* and S-Metolachlor. Pakistan Journal of Botany 40:2383–2391

[71] Razzaq, A., Cheema, Z.A., Jabran, K., Farooq, M., Khaliq, A., Haider, G., Basra, S.M.A. (2010): Weed management in wheat through combination of allelopathic water extracts with reduced doses of herbicides. Pakistan Journal of Weed Science Research 16:247–256

[72] Chotsaeng, N., Laosinwattana, C., Charoenying, P. (2017): Herbicidal activities of some allelochemicals and their synergistic behaviors toward *Amaranthus tricolor* L. Molecules 22(11): 1841. doi:10.3390/molecules22111841

[73] Cheema, Z.A., Khaliq, A., Mubeen, M. (2003): Response of wheat and winter weeds to foliar application of different plant water extracts of sorghum (*S. bicolor*). Pakistan Journal of Weed Science Research 9:89–97

[74] Jamil, M., Cheema, Z.A., Mushtaq, M.N., Farooq, M., Cheema, M.A. (2009): Alternative control of wild oat and canary grass in wheat fields by allelopathic plant water extracts. Agronomy for Sustainable Development 29:475–482

[75] Farooq, M.K., Jabran, Z.A., Cheema, A., Wahid, Siddique, K.H.M. (2011): The role of allelopathy in agricultural pest management. Pest Management Sci 67: 493–506