

Full Length Research Paper

Allelopathic potential of green manure, cover crops, mulching plants, and weeds found in tea plantations

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The allelopathic activity of cover crops, green manure, mulching plants, and weeds commonly found in tea plantations was evaluated employing laboratory bioassays, greenhouse experiments and field trials. The results of laboratory and greenhouse experiments concluded that the green manure plants, *Gliricidia sepium*, *Erythrina lithosperma*, *Eupatorium inulifolium*, *Tithonia diversifolia*, *Artemisia vulgaris* and mulching plants, *Chrysopogon zizanioides*, *Tripsacum luxum* and *Pennisetum purpureum* × *Pennisetum americanum* exhibited phytotoxicity against *Lactuca sativa*. According to the findings of the field tests, these plants significantly exhibited phytotoxicity toward weeds present at the experimental sites. In combination with integrated weed management, these plants could be utilized to manage weeds in sustainable agriculture.

Key words: Allelopathy, field experiments, greenhouse experiments, laboratory bioassay, phytotoxicity.

INTRODUCTION

The management of weeds has been a major problem from the beginning of tea plantations. Weed infestation in tea plantations results in a reduction in the quality and quantity of the crop productivity. In general, expenditures related to weed control in tea represent approximately 10-14% of the cost of all field operations, including chemical and labour costs (Prematilake, 2003). At present, integrated weed management is used on tea fields. Integrated weed management involves utilizing a range of weed control methods, including weeding, herbicides, mulching materials, and etc. However, excessive use of synthetic herbicides has had a negative impact on the environment and has increased the presence of herbicide-tolerant weeds (Vyvyan, 2002; Hong et al., 2004). Consequently, efforts are being made to use naturally occurring plant materials, biologically

active secondary metabolites isolated from plants as well as ecofriendly chemicals to overcome these problems to the best extent possible (Dayan et al., 2009). In this regard, allelopathy has been accepted as another possible way to control weeds in sustainable agriculture (Dayan et al., 2009).

Allelopathy is a natural approach and this concept has been presented as a viable option for alternative weed management in sustainable farming (Weiner, 2001). Use of allelopathy to control weeds includes the use of live cover crops, crop rotation, intercropping and residues of cover crops, or the use of natural herbicides (Chung et al., 2001).

However, there are many limitations on the use of plant residues as mulch or soil incorporation because of heavy fieldwork involving massive amounts of plant residues

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(Bhowmik, 2003). There are natural herbicides that are being marketed right now. Most bioactive secondary metabolites are water soluble with a shorter half-life in the environment (Duke et al., 2002; Duke et al., 2000).

Due to geographic and climate conditions, Sri Lanka has a diverse flora (Ashton et al., 1997). The introduction of allelopathic active plants from the Sri Lankan flora may lead to the production of more environmentally friendly and effective natural herbicides. The objective of this study was the evaluation of the allelopathic activity of 75 plants including cover crops, green manure, mulching plants, and commonly found weeds in tea plantations using lettuce seed germination bioassay, greenhouse, and field experiments with the hope of identification of phytotoxic plants as a viable option for alternative weed management in tea plantations.

MATERIALS AND METHODS

Preparation of plants extracts

The total number of 75 plant species including problematic weeds, cover crops, green manure, and mulching plants was collected from low (0-600 m), mid (600-1,200 m), and up (>1200 m) elevations in Sri Lanka. The fresh plant material was cleaned and washed thoroughly with water and the plant parts were separated, air dried and pulverized. Aqueous extract (2 %w/v) of the relevant plant material was prepared using distilled water followed by 24 hours of soaking of plant material (Singh et al., 1989).

Lettuce (*Lactuca sativa*) seeds germination bioassay

The lettuce seeds were treated with sodium hypochlorite (NaOCl) solution (5%) for 10 min, thoroughly rinsed with distilled water and the immature seeds were removed afterwards. Lettuce seeds were put onto the filter paper (Whatman No. 4) and aligned in a petri dish that contained plant extract in distilled water (5 mL). Four replicates were used and incubated for 5 days. Distilled water and pine extract (*Pinus* sp.) were used for both negative and positive controls. Germinated lettuce seeds were counted and the lengths of the root and hypocotyl were measured (Piyasena and Dharmaratne, 2013).

Greenhouse experiment

Pot experiments were conducted at the Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka in 2019. In this experiment, 27 plants including seven green manure plants, five mulching plants, four cover crops, and 12 weeds were tested. The pots contained loamy soil (6.5 kg). A residue experiment was conducted in a greenhouse at a mean temperature of $26 \pm 2^\circ\text{C}$. In this greenhouse experiment, a 420 g (35 t ha^{-1}) mulch was integrated into the soil (6.5 kg) in the trays. Each pot was prepared in randomized complete block designs (RCBD) and three replicates were used (Rueda-Ayala et al., 2015). Pots containing only loamy soil were used for the negative control and *Artemisia vulgaris* incorporated soil was used for the positive control. Lettuce seeds (100) were planted with a depth of 12.7 mm. The number of emergences of lettuce seedlings per week was recorded for four consecutive weeks.

Field experiment

The field experiments were conducted in St. Coombs tea estate, Field No. 8, Talawakelle, Sri Lanka, and performed during the wet season (from April to July) in 2019. Under this experiment, eight plants including five green manure plants and three mulching plants were tested as mulch. All weed seedlings inside a quadrat placed randomly in the centre part of each plot, were identified and recorded. The tea field was divided into plots with an area of 4 x 4 m and the plots received no previous herbicide treatment or any special treatment for the soil. Treatments have been imposed on weeds growing between rows of tea in a mature tea field in its tender phase. The weed screening assessment was completed before mulching using one quadrat (0.3 x 0.3 m) on each plot. Following the imposition of treatments, samples of weeds were randomly collected two, four, six, and eight weeks after application. A quadrat sample (0.3 x 0.3 m) was taken from each plot and the weedy species available were identified. Fresh weed weights were taken and the dry weight was recorded at 85°C for 16 h. The experimental design for this trial was a fully randomized block design with three replicates.

Statistical analysis

A lettuce seed germination percentage, dry weight assessment, shoots, and root lengths of lettuce seedlings were analyzed using the one-way analysis of variance by using SAS software (9.0 version) followed by Dunnett's comparisons. The means were separated based on the least significant difference at a 0.05 probability level.

RESULTS AND DISCUSSION

Lettuce seeds germination bioassay

Laboratory bioassays have been performed to evaluate the phytotoxicity of 75 plant aqueous extracts (Annexure 1). The plants were selected in this study mainly using field observations, including those that are used for green manure, cover crops, mulch, and weed or pest management, plants that do not easily decompose, plants that are free of pest attacks, invasive plants, and plants that have a lesser natural weed density when compared with the other plants in their ecosystem.

According to the lettuce seed germination assay, 27 plant extracts showed the highest phytotoxicity that is, completely inhibiting the germination of lettuce seeds compared to the control.

These plants included seven green manure plants (*Gliricidia sepium*, *Cassia spectabilis*, *Erythrina lithosperma*, *Eupatorium inulifolium*, *Tithonia diversifolia*, *Sesbania sesban* and *Artemisia vulgaris*), three cover crops (*Oxalis barrelieri*, *Hydrocotyle javanica* and *Drymaria cordata*), five mulching plants (*Vetiveria zizanioides*, *Cymbopogon flexuosus*, *Tripsacum laxum*, *Cymbopogon confertiflorus* and *Pennisetum purpureum* x *Pennisetum americanum*), and 12 problematic weeds (*Commellina diffusa*, *Ipomoea indica*, *Crassocephalum crepidioides*, *Anredera cordifolia*, *Persicaria capitata*, *Ageratum Conyzoides*, *Amaranthus viridis*, *Clidemia*

Table 1. Percentage of sprouting of lettuce after treatment with different types of plant materials (weeds) in the greenhouse.

Plant material	Germination percentage of lettuce			
	1st week	2nd week	3rd week	4th week
<i>Commellina diffusa</i>	44.33 ^b	44.67 ^c	44.67 ^c	44.67 ^c
<i>Ipomoea indica</i>	8.33 ^e	10 ^{i,h}	9.67 ^h	9.67 ^h
<i>Crassocephalum crepidioides</i>	26.67 ^c	29.33 ^e	29.33 ^e	29.33 ^e
<i>Anredera cordifolia</i>	9.67 ^d	11 ^h	10.67 ^h	10.67 ^h
<i>Persicaria capitata</i>	10.33 ^d	12 ^{g,h}	13.33 ^{g,h}	13.33 ^{g,h}
<i>Ageratum conyzoides</i>	NG	7 ⁱ	16.33 ^{f,g}	16.33 ^{f,g}
<i>Amaranthus viridis</i>	NG	11.67 ^h	17.67 ^f	17.67 ^f
<i>Clidemia hirta</i>	NG	NG	11 ^h	11 ^h
<i>Achyranthes aspera</i>	NG	16.67 ^f	33 ^{c,d}	33 ^{c,d}
<i>Borreria latifolia</i>	NG	49.67 ^b	49.67 ^b	49.67 ^b
<i>Galinsoga parviflora</i>	NG	31.67 ^b	31.67 ^{d,e}	31.67 ^{d,e}
<i>Axonopus compressus</i>	NG	15.33 ^{f,g}	15.33 ^{f,g}	15.33 ^{f,g}
Negative control	93.33 ^a	93.33 ^a	93.33 ^a	93.33 ^a

Means with the same letter within a column are not significantly different at 0.05 probability level.

NG-Not germinated.

Table 2. Percentage of lettuce germinated after green manure treatment in greenhouse.

Plant material	Germination percentage of lettuce			
	1st week	2nd week	3rd week	4th week
<i>Gliricidia sepium</i>	58.67 ^b	67 ^{b,c}	59.33 ^b	59.33 ^b
<i>Tithonia diversifolia</i>	27.67 ^c	30 ^d	29.33 ^c	29.33 ^c
<i>Erythrina lithosperma</i>	72 ^b	80 ^b	80 ^b	80 ^b
<i>Euphorbia inulifolium</i>	33.67 ^c	37.67 ^{c, d}	36.67 ^c	36.67 ^c
<i>Sesbania sesban</i>	56.67 ^b	62.67 ^b	62.67 ^b	62.67 ^b
<i>Cassia spectabilis</i>	NG	45.67 ^d	45.67 ^c	45.67 ^c
<i>Artemisia vulgaris</i>	NG	NG	32 ^c	32 ^c
Negative control	93.33 ^a	93.33 ^a	93.33 ^a	93.33 ^a

Means with the same letter within a column are not significantly different at 0.05 probability level.

NG-Not germinated.

hirta, *Achyranthes aspera*, *Borreria latifolia*, *Galinsoga parviflora* and *Axonopus compressus*).

Greenhouse experiment

The greenhouse experiment was initiated for plants that showed the highest phytotoxicity in the lettuce seed germination bioassay. In this experiment, 27 of the aforementioned plants were employed. All the plant materials were air-dried and incorporated into the loamy soil, followed by watering for one week prior to planting lettuce seeds. The germinated lettuce seeds were counted once a week for four consecutive weeks and the germinated lettuce seeds in each treatment were compared to those in plain loamy soil, which was used as

a negative control (Rueda-Ayala et al., 2015). A one-way analysis of variance (ANOVA) and Dunnett's comparisons were performed (Table 1). The results showed that all plant materials tested in this study exhibited significant phytotoxicity to the germination of lettuce. *A. conyzoides*, *A. viridis*, *C. hirta*, *A. aspera*, *B. latifolia* and *G. parviflora* strongly inhibited the germination of lettuce seeds during the first week, and lettuce seeds germinated to some extent during the second week. The largest decline in the germination of lettuce seeds was observed in weeds *I. indica*, *A. cordifolia*, and *C. hirta*. For green manure plants, all plant materials tested in this study showed significant phytotoxicity (Table 2 and Figure 1). The greatest decrease in germination of lettuce seeds was observed in *Artemisia vulgaris* during the first two weeks.

Cassia spectabilis showed strong suppression of the

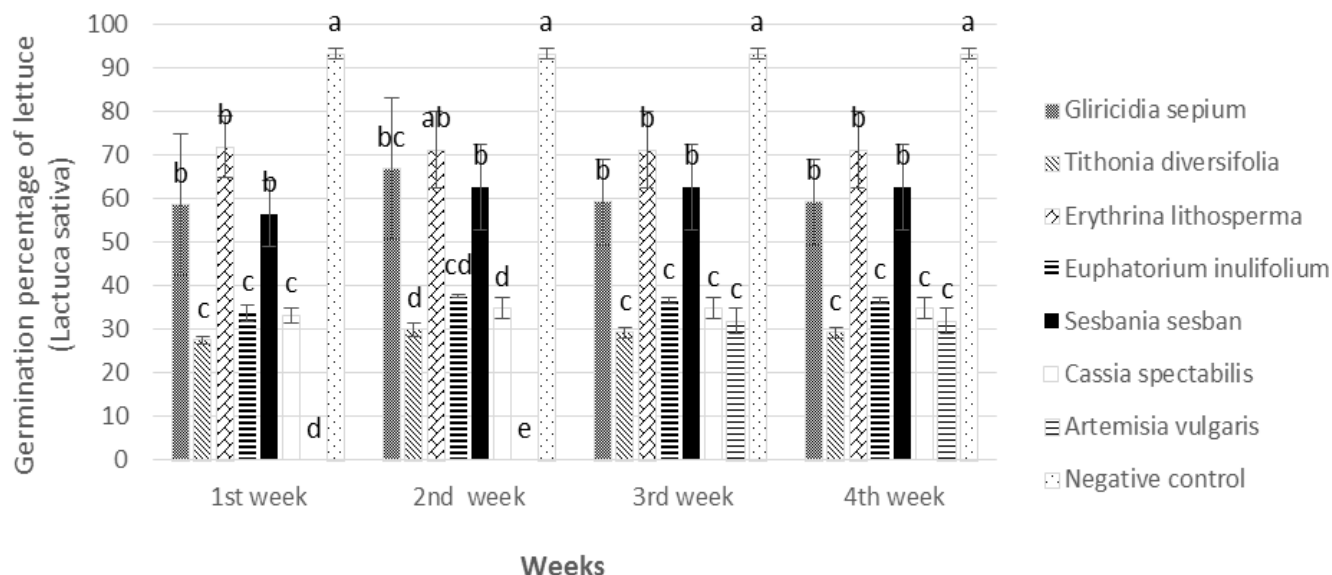


Figure 1. Germination percentage of lettuce after treating with green manure plants in greenhouse. Means with the same letter within a column are not significantly different.

Table 3. Percent germination of lettuce following mulched plant treatment.

Plant material	Germination percentage of lettuce			
	1st week	2nd week	3rd week	4th week
<i>Cymbopogon confertiflorus</i>	NG	30.67 ^b	30.67 ^b	30.67 ^b
<i>Tripsacum laxum</i>	NG	22.33 ^c	22.33 ^c	22.33 ^c
<i>Cymbopogon flexuosus</i>	NG	34.67 ^b	34.67 ^b	34.67 ^b
<i>Chrysopogon zizanioides</i>	15.67 ^a	21 ^c	21 ^c	21 ^c
<i>P. purpureum</i> x <i>P. americanum</i>	NG	8.67 ^d	8.67 ^d	8.67 ^d
Negative control	93.33 ^a	93.33 ^a	93.33 ^a	93.33 ^a

Means with the same letter within a column are not significantly different exhibited significant.

ND: Not germinated.

sprouting of lettuce seeds during the first week, and they germinated during the second week. During the observations in the third and fourth weeks, seven green manure plants significantly reduced the germination of lettuce seeds. The greatest reduction in lettuce seedling growth was observed in *A. vulgaris*. This may be due to the fact that artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* L., is a patent plant growth inhibitor; also, 1, 8 coneole, a monoterpene, has been recognized as the most active phytotoxic compound released by *Artemisia* spp.; and a synthesized analog, cinmethylin, is being sold as an herbicide in Europe (Javaid and Anjum, 2006).

The benefits of mulching for the growth and yield of annual and perennial crops have long been recognized (Kasirajan and Ngouajio, 2012). Consequently, greenhouse experiments were conducted on mulching plants currently

used in plantations in order to assess their phytotoxicity (Table 3 and Figure 2).

It was observed that all plant materials tested in this study showed significant phytotoxicity against lettuce seedlings. The greatest reduction in lettuce seedling growth was observed in *C. confertiflorus*, *T. laxum*, *Cymbopogon flexuosus* and *P. purpureum* x *P. americanum* (CO-3 grass) in the first week. According to Dunnett's comparison, Figure 2 clearly shows that *P. purpureum* x *P. americanum* showed the highest percentage reduction of the germination of lettuce seedlings in four consecutive weeks, whereas the lowest reduction of lettuce seeds was shown in *Cymbopogon confertiflorus* in four consecutive weeks.

The results indicated that the cover crops tested in this study were phytotoxic to lettuce compared to the negative control (Table 4). The largest reduction in the germination

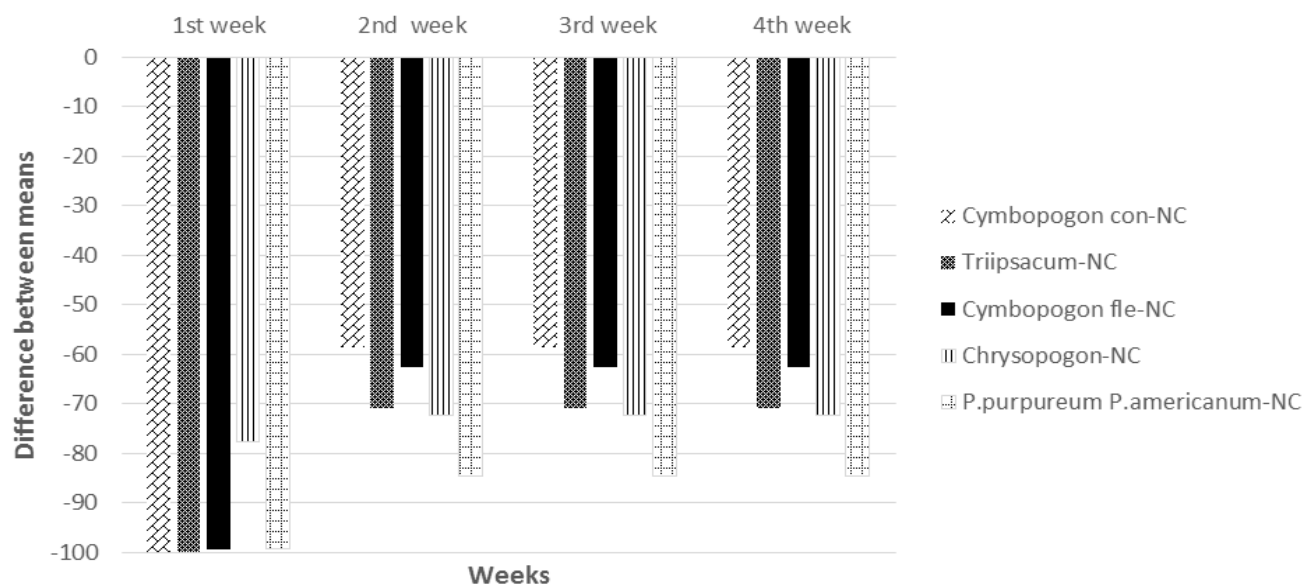


Figure 2. Dunnett's comparison of mulching plants compared to the negative control (difference between means compared to the negative control).

Table 4. Germination percentage of lettuce after treating with cover crops in greenhouse.

Plant material	Germination percentage of lettuce			
	1st week	2nd week	3rd week	4th week
<i>Oxalis barrelieri</i>	NG	30.33 ^b	30.33 ^b	30.33 ^b
<i>Drymaria cordata</i>	21 ^b	24 ^c	24 ^{c,d}	24 ^{c,d}
<i>Hydrocotyle javanica</i>	20 ^b	21.67 ^c	22 ^d	22 ^d
Negative control	93.33 ^a	93.33 ^a	93.33 ^a	93.33 ^a

Means with the same letter within a column are not significantly different at 0.05 probability level.

of lettuce seeds occurred in *O. barrelieri* during the first week.

The results revealed that the phytotoxicity of mulched cover crops was considerably higher than that of green manure in greenhouse experiments. The highest phytotoxicity was seen in the weeds used in this study. These harmful weeds may contain powerful phytotoxic compounds that could be used in the development of herbicides (Khan et al., 2019). However, allelopathic active plants, *C. spectabilis*, *S. sesban*, *D. cordata*, *H. javanica*, and *O. barrelieri* were not used for the field trials due to the unavailability of a bulk quantity of plant materials.

Field trails

For field trials, eight of the plants with the highest phytotoxicity in greenhouse experiments were utilized. Plant materials (leaves and branches), including five green manure plants (*G. sepium*, *E. lithosperma*, *E.*

inulifolium, *T. diversifolia*, and *A. vulgaris*), and mulching plants (*C. zizanioides*, *T. laxum*, and *P. purpureum* x *P. americanum*) were incorporated into the field as mulch. Plastic mulch was used as a positive control.

The following weed species were heavily infested at the trial site: *Ageratum conyzoides*, *Anredera cordifolia*, *Bidens pilosa*, *Borreria latifolia*, *Clidemia hirta*, *Crassocephalum crepidioides*, *Desmodium uncinatum*, *Digitaria sanguinalis*, *Eleusine indica*, *Erigeron* sp., *Eupatorium riparium*, *Hydrocotyle sibthorpioides*, *Oxalis* sp., *Paspalum* sp., *Phyllanthus niruri*, *Polygonum* spp., *Setaria barbata*, *Sida rhombifolia*, *Solanum nigrum*, *Vigna* sp., *Viola betonicifolia*, and *Youngia japonica*. No significant difference was observed among the dry weight of weeds collected for pre-weed assessment compared with the negative control.

Following the imposition of treatments, weed samples were randomly collected every two weeks. A significant difference was observed in the dry weights of weeds collected every two weeks compared to the negative control (Table 5). *A. vulgaris* has observed a significantly

Table 5. Dry weight of weeds after treating with different plant materials in the field.

Plant material	BA	2nd Week	4th Week	6th Week	8th Week
<i>Gliricidia sepium</i> **	19.99 ^{a,b,c}	1.88 ^{c,d}	0.93 ^b	2.90 ^{d,e}	7.42 ^{b,c,d}
<i>Erythrina lithosperma</i> **	14.77 ^c	3.77 ^{b,c,d}	4.60 ^b	7.46 ^{c,d,e}	13.22 ^{b,c}
<i>Eupatorium inulifolium</i> **	17.18 ^{a,b,c}	1.36 ^{c,d}	4.70 ^b	18.37 ^b	9.19 ^{b,c,d}
<i>Tithonia diversifolia</i> **	19.80 ^{a,b,c}	3.25 ^{b,c,d}	3.59 ^b	12.06 ^{b,c,d}	9.24 ^{b,c,d}
<i>Artemisia vulgaris</i> **	15.63 ^{b,c}	7.05 ^b	2.55 ^b	7.65 ^{c,d,e}	11.42 ^{b,c,d}
<i>Chrysopogon zizanioides</i> *	21.16 ^{a,b,c}	3.33 ^{b,c,d}	5.19 ^b	9.70 ^{b,c,d}	5.95 ^{c,d}
<i>Tripsacum luxum</i> *	18.79 ^{a,b,c}	4.70 ^{b,c,d}	4.86 ^b	14.58 ^{b,c}	6.62 ^{c,d}
<i>P. purpureum</i> × <i>P. americanum</i> *	22.09 ^{a,b}	5.96 ^{b,c}	3.52 ^b	10.57 ^{b,c,d}	14.89 ^b
Negative Control	24.35 ^a	30.71 ^a	18.07 ^a	31.85 ^a	25.93 ^a
Positive Control	21.41 ^{a,b,c}	0.00 ^d	0.00 ^b	0.00 ^e	3.817 ^d

Means with the same letter within a column are not significantly different at 0.05 probability level.

*Mulching plants, **Green manure, BA= before application.

lower reduction in weed biomass, while *G. sepium* and *E. inulifolium* experienced the most significant biomass reductions during the second week. The significantly lowest biomass reduction of weeds was seen by *E. inulifolium*, whereas the significantly highest biomass reduction of weeds was seen by *G. sepium* in the sixth week. The lowest and most significant phytotoxicity was observed in *P. purpureum* × *P. americanum*, and *C. zizanioides* and *T. luxum*, respectively, for eight weeks. It is reported that mulching plants could be used to prevent soil evaporation, preserve moisture, control soil temperature, reduce weed growth, and improve microbial activities (Iqbal et al., 2020). The findings of this study indicated that the mulches used in this study were the most phytotoxic compared to green manure plants.

Experiments conducted in greenhouses clearly demonstrated that after weeks three and four of mulch application, the same proportion of lettuce germination was seen. And also, in the field trials, it was found that after the fourth week, weeds began to grow. This may be due to plant residues that contain or produce chemicals which responsible for the phytotoxic activity for a certain extent, and these toxic effects disappear within two to three weeks. The degree of toxicity is dependent on the type of residue, its maturity, and the extent of the alteration (Kimber, 1973). It is reported that after four weeks, the allelochemicals in the leaves of *Litchi chinensis* were degraded and the inhibitory activity of the aqueous extract was greatly reduced (Wang et al., 2013). Weeds are the major negative factor affecting yield loss in agricultural systems around the world (Kural and Özkan, 2020). It is reported that allelopathic mulches of sorghum (*Sorghum bicolor*), sunflower (*Helianthus annuus*), rice (*Oryza sativa*), and maize (*Zea mays*) may be used to control herbicide-tolerant *Phalaris minor* due to their high phytotoxic potential. In this study, *G. sepium*, *E. lithosperma*, *E. inulifolium*, *T. diversifolia*, *A. vulgaris*, *C. zizanioides*, *T. luxum* and *P. purpureum* × *P. americanum* were identified as phytotoxic plants. These

plants may contain allelopathic substances that may also be present in decomposing leaf residues and may act as natural herbicides. In addition, it is reported that the biological activity of natural plant extracts may be demonstrated through synergistic activity between several compounds (Gorlenko et al., 2020).

CONCLUSION

In this study, the allelopathic effects of green manure, cover crops, and mulched plants used in tea plantations were assessed through laboratory bioassay, greenhouse, and field trials. Five green manure plants (*Gliricidia sepium*, *Erythrina lithosperma*, *Eupatorium inulifolium*, *Tithonia diversifolia*, *Artemisia vulgaris*) and three mulching plants (*Chrysopogon zizanioides*, *Tripsacum luxum* and *Pennisetum purpureum* × *Pennisetum americanum* (CO-3 grass)) exhibited the highest phytotoxicity. These plants can be used as a viable alternative to manage weeds in the tea plantations. Further chemical investigations are necessary to identify relevant phytotoxic compounds from the allopathic active plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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