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Research Paper

Non-target effects of aqueous leaf extracts of mugwort (*Artemisia vulgaris*) and herbicides: Impact on nursery tea plants (*Camellia sinensis*)

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Abstract: The weed control potential of *Artemisia vulgaris* in tea lands has been reported. This study evaluated the non-target effects of the aqueous leaf extracts of *A. vulgaris* at 25, 50, 75 and 100 g/L (soil-applied, 40 ml/pot) on the growth of 7-months-old nursery tea plants (TRI 4006 and 4046) grown in black polythene bags with 1 kg topsoil. For comparison, commercial herbicides with different modes of action, *i.e.* Glyphosate, Diuron, MCPA, Pelargonic acid, Pine oil and Glufosinate ammonium, were soil-applied at recommended dosages, with distilled water as the control. Experiments were done in CRD with five replicates, with one plant/replicate. Leaf greenness (SPAD meter) at 20 days after treatment (DAT), visual observations of root growth, and shoot and root dry weights/plant were measured with destructive sampling at 30 DAT. *Artemisia* leaf extracts did not show significant negative impacts on the aboveground parameters of the tea plant compared to the control. The highest phytotoxicity on nursery tea plants was observed with MCPA (root cell damage; 100% plant mortality). The SPAD readings showed significant leaf chlorosis in TRI 4006 and TRI 4046 with Diuron and Glufosinate ammonium, compared to the control, *Artemisia* extracts, Pelargonic acid and Pine oil. Poor root growth with lesions was observed with 100 g/L *Artemisia* leaf extract but at a comparatively low degree to that of herbicides. As the non-target effects of *A. vulgaris* on tea plants were relatively lower, further studies are warranted to determine an effective dosage for weed control with minimum impact on tea plants and the environment.

Keywords: Allelopathic effects, Eco-friendly herbicides, Nursery tea plants, Non-target impact

Introduction

Weeds, insect pests, pathogens, nematodes, and mites are the main biological constraints that affect tea crop production (Saikia *et al*., 2014). Tea productivity could reduce by 10-50% due to direct competition of weeds for water, nutrients, space, and light (Prematilake *et al*., 2010). Under favourable microclimatic conditions, insect and mite pests can cause 11-55% losses in tea plantations (Hazarika *et al*., 2009; Nadda *et al*., 2013). About 4-5 % of the total cost of production of tea is spent on weed management, which includes chemical, mechanical, manual, cultural and biological control methods practiced throughout the year as several rotations (Prematilake *et al*., 2005).

Chemical control methods are popular due to labour scarcity, as manual weed control demands more man-days for work (Premathilake, 2003). A wide range of herbicides is available to control several categories of weeds in tea land (Mohotti, 2014). Misuse of herbicides, however, has resulted in phytotoxic effects on tea plants, development of resistance weeds in the field (Marambe *et al*., 2002; 2003), presence of residuals in the end product, and contamination of chemicals in water bodies (Ekanayake, 1994).

The Office of the Registrar of Pesticides of the Government of Sri Lanka has developed policies to regulate the use of herbicides. The country banned the importation and use of total killer herbicides such as paraquat in 2014 (Note: the gazette was issued in 2014; Government Gazette No.1854/47) and glyphosate since 2015 (Government Gazette No. 1918/22 and No. 1937/35). The import ban on glyphosate for tea and rubber was temporarily lifted in 2018 for 36 months to encourage researchers and the industry to identify alternative weed control technologies. The growing concerns to prevent or minimize the misuse of agrochemicals have directed scientists to screen for effective alternate weed control techniques for tea crop that are economically viable and environmentally friendly, without leaving chemical residues in the final product.

Some weeds produce and secrete chemical substances in the environment that inhibit the germination and growth of nearby plants through a phenomenon called allelopathy (Gnanapragasam and Sivapalan, 2004). *Artemisia vulgaris* L. is one of the hedgerow shrubs grown in some upcountry (elevation above 900 m from the mean sea level) and mid-country (elevation between 300-900 m from sea level) tea estates in Sri Lanka for soil conservation and as a biological barrier in tea estates (Weerasekara, 2016).

Artemisia vulgaris, commonly known as mugwort, belongs to the family Asteraceae. It is a perennial plant that has spread around the globe and is well known to tolerate harsh environmental conditions and for the secretion of its secondary metabolites (Barney *et al*., 2005). The plant is also known as a medicinal herb used in traditional medicine for several ailments. It is also grown as a hedgerow plant in many agricultural landscapes. The species is also used as an insect-repellant hedgerow plant in tea estates in Sri Lanka and is accepted by the Rain Forest Alliance for its certification. Researchers have found several allelopathic chemicals using the leaf extract of mugwort, analyzed *via* gas chromatography coupled with mass spectrometry (Barney *et al*., 2005). These compounds include volatile fatty acids such as terpenes, camphor, eucalyptol, ά-pinene, β –and pinene (Judžentien and Buzelyte, 2006). Those volatile fatty acids are phytotoxic for other plants and affect seed germination (Onen *et al*., 2002). They could be used effectively as alternatives to synthetic commercial herbicides (Soltys *et al*., 2016).

Extracts of *A. vulgaris* have shown insect-repellent properties (Hwang *et* al., 1985) and reduced red mites in tea, showing promise for their use in integrated pest management. The leaf extracts of *A. vulgaris* were more effective than root and stem in pest management in tea cultivation in Sri Lanka (Weerasekara, 2016). However, the impact of such extracts, despite having herbicidal and insecticidal properties, has yet to be scientifically evaluated on the growth and yield of the tea plant.

This research focused on assessing the non-target effects of aqueous leaf extracts of *A. vulgaris* on 7-month-old nursery tea plants despite their proven potential for pest control, including weeds.

Materials and Methods

Leaves of *A. vulgaris* plants were collected from the hedgerows planted by the roadside of upcountry tea estates. The moisture content of the plant leaves was measured after oven drying at 65 °C until constant weight. Three soil samples were collected from the *Artemisia*-growing sites, each at 5 cm depth from the soil surface, made into a composite soil sample, divided into three equal parts, and oven-dried at 100 °C until constant weight to measure the soil moisture content. The different concentrations of Artemisia leaf extracts were then prepared, assuming that the total leaf biomass of the plants falling into the soil would dissolve in available soil water.

For the experimental purposes, the plant density of *A. vulgaris* was considered as one plant per 900 cm^2. The test concentration of the plant leaf extracts was prepared based on the amount of fresh matter of leaves mixed with the total moisture content in the top 5 cm soil layer covering an area of 30 cm × 30 cm and considered as the stock solution. The required fresh leaves were blended with 100 ml of distilled water using a mechanical blender. The blended mixture of leaves was filtered using Whatman® filter paper (190 mm). The natural concentration for an extract of leaves prepared on a dry weight basis was approximately 18 g of leaves in 1330 mL of distilled water. Hence, for experimental purposes, the above concentration was considered as 100%. Accordingly, the leaf extracts of *A. vulgaris* were prepared on a fresh weight basis to have a series of concentration gradients, including 25 g/L, 50 g/L (100% concentration), 75 g/L and 100 g/L). These rates of the aqueous extract of *A. vulgaris* controlled different weeds in our preliminary experiments carried out at the Tea Research Institute (TRI) of Sri Lanka in tea plantations in Talawakelle (data not shown) and, thus, were selected for this study.

The experiment was conducted at the net house of the Entomology and Nematology Experimental Area of the TRI, Talawakelle, Sri Lanka. Similar-sized, seven-month-old nursery tea plants of the cultivars TRI 4006 and TRI 4046 were grown using single nodal cuttings in standard black polythene bags (23 cm height × 10 cm diameter, 150 gauge) filled with 1 kg of treated and untreated soil (control). The bottom end of the bag was kept open with a few holes punched on the sides to facilitate drainage.

Aqueous leaf extracts of *A. vulgaris* prepared as described previously were applied to healthy nursery plants at a rate of 20 mL per pot once a week for two consecutive weeks. Distilled water was used as the control treatment. Irrigation was done at 30 mL per plant on alternate days. Commercially available synthetic herbicides, namely, Glyphosate (36% SL, two formulations were used), Diuron (80% WP), MCPA (50% SL) and Glufosinate ammonium (28% SL) and two natural herbicides, namely, Pelargonic acid (72% EC) and pine oil were also used at dosages recommended by the TRI of Sri Lanka, to the nursery soil to compare the non-target effects of the *Artemisia* leaf extracts. Pots using distilled water to replace the treatments served as the control.

All treatments were applied to soil, assuming that weed control is done only between rows by applying herbicides in an actual situation. Every effort was taken so that the leaves or stems of the nursery tea plants would not come in direct contact with the treatments. Apart from these treatments, nursery management practices recommended by the TRI were adopted. The experiment was conducted in CRD with five replicates (1 plant per replicate) of two cultivars of nursery tea plants.

The initial changes in plants among treatments were observed once a week during the experimental period (calculated after the second application of treatments). The chlorophyll content of tea leaves as a physiological parameter was measured by using a chlorophyll meter (SPAD-502Plus) in three locations of the two uppermost mature leaves to determine chlorosis as a non-target effect. The average SPAD value was taken as an indicator of the chlorophyll content of the leaf in 20 days after the second application of soil treatments. At the end of the first month from treatment, the tea nursery plants were removed through destructive sampling to measure the fresh and dry weight of shoots and roots after oven drying at 105 °C until constant weight. Visual observations of the root systems were recorded after destructive sampling.

The Analysis of Variance was conducted to test the statistical significance of the treatment impacts ($P=0.05$) using the SAS software (university version). The treatment means were compared using Duncan's Multiple Range Test at $P=0.05$.

Results and Discussion

The soil-applied MCPA killed the nursery tea plants due to phytotoxicity of the herbicide. Similarly, Glufosinate ammonium showed a significant negative effect ($P<0.05$) on the nursery plants of both tea cultivars at seven months of age (Table 1). The results revealed the significant soil residual activity of MCPA (half-life 1-14 weeks; Helweg, 1987) on young tea plants. Though Glufosinate ammonium is known for its rapid microbial degradation in soil (Takano and Dayan, 2020), changes in the environmental conditions would determine the soil residual activity of the herbicide in the field conditions. The aqueous leaf extracts of *A. vulgaris* did not significantly impact (P>0.05) the shoot dry weight at all concentrations when applied to the soil in pots with 7-month-old tea seedlings. The root dry weight of nursery tea plants was also affected (P<0.05) by Glufosinate ammonium one month after treatment. However, other herbicide treatments (except MCPA), including the four concentrations of aqueous extracts of *Artemisia*, did not significantly impact the root growth of nursery tea plants (Table 1).

Table 2 presents the chlorophyll contents (SPAD meter reading) of the uppermost matured leaves of two tea cultivars measured 20 days after the second application of soil treatments. As explained previously, plant mortality was reported in all replicates in both cultivars in plots where soil was treated with MCPA. Diuron and glufosinate ammonium showed a significant negative impact in terms of the chlorophyll content in the nursery tea plants of both cultivars. Diuron is absorbed quickly through plant roots, translocates through the xylem and phloem to leaves, and leads to lipid peroxidation, resulting in loss of chlorophyll (Moncada, 1998). Symptoms of yellowing and cupping of tomato leaves have occurred within five days after treatment of Glufosinate ammonium into soils (You and Barker, 2004), indicating the significant adverse impacts of the soil application of the herbicide on plant chlorophyll content.

Table 1. Impact of aqueous leaf extracts of *Artemisia* and other herbicides on young tea plants.

Treatment	Mean Shoot Dry Weight (g per plant)		Mean Root Dry Weight (g per plant)	
	TRI 4046	**TRI 4006**	**TRI 4046**	**TRI 4006**
Untreated control	2.5[a]	2.4[a]	1.4[a]	1.3[a]
Artemisia 25 g/L	3.5[a]	2.7[a]	1.2[a]	1.3[a]
Artemisia 50 g/L	2.6[a]	4.2[a]	1.5[a]	1.2[a]
Artemisia 75 g/L	3.2[a]	3.3[a]	1.5[a]	1.1[a]
Artemisia 100 g/L	3.6[a]	2.8[a]	1.5[a]	1.3[a]
Glyphosate*	3.3[a]	3.3[a]	1.4[a]	1.3[a]
Diuron	2.9[a]	2.8[a]	1.4[a]	1.3[a]
Glufosinate Ammonium	1.9[b]	0.8[b]	0.5[b]	0.4[b]
2-methyl-4-chlorophenoxyacetic acid (MCPA)	0.0	0.0	0.0	0.0
Pelargonic acid	3.3[a]	3.2[a]	1.8[a]	1.9[a]
Pine oil	3.3[a]	3.7[a]	0.9[b]	1.2[a]

* Average of two formulations. Within a column, means followed by the same letter are not significantly different by DMRT at P=0.05. Except for *Artemisia*, all herbicides were applied at recommended dosages, but as a soil application

Table 2. Chlorophyll (SPAD meter reading) content of nursery plants of TRI 4046 and TRI 4006

Treatment	TRI 4046	TRI 4006
Untreated control	36.77[bc]	39.91[ab]
Artemisia 25 g/L	33.75[d]	39.41[ab]
Artemisia 50 g/L	38.13[ab]	41.18[a]
Artemisia 75 g/L	42.29[ab]	43.91[a]
Artemisia 100 g/L	39.52[ab]	41.97[a]
Glyphosate*	41.35[ab]	42.38[a]
Diuron	28.17[d]	28.72[bc]
Glufosinate Ammonium	4.59[e]	19.95[d]
2-methyl-4-chlorophenoxyacetic acid (MCPA)	0.0	0.00
Pelargonic acid	44.83[a]	47.20[a]
Pine oil	44.37[a]	44.93[a]

* Average of two formulations. Within a column, means followed by the same letter are not significantly different by DMRT at P=0.05.

Interestingly, the two naturally-occurring chemicals used in the study recorded the highest chlorophyll content in nursery tea plants of both cultivars. All concentrations of aqueous leaf extracts of *Artemisia* leaf extracts tested did not show a significant impact (P>0.05) on the chlorophyll content of mature leaves of the nursery tea plants of both cultivars.

The visual observations recorded on the root growth of TRI 4046 and TRI 4006 after soil application of treatments are illustrated in Figures 1 and 2, respectively.

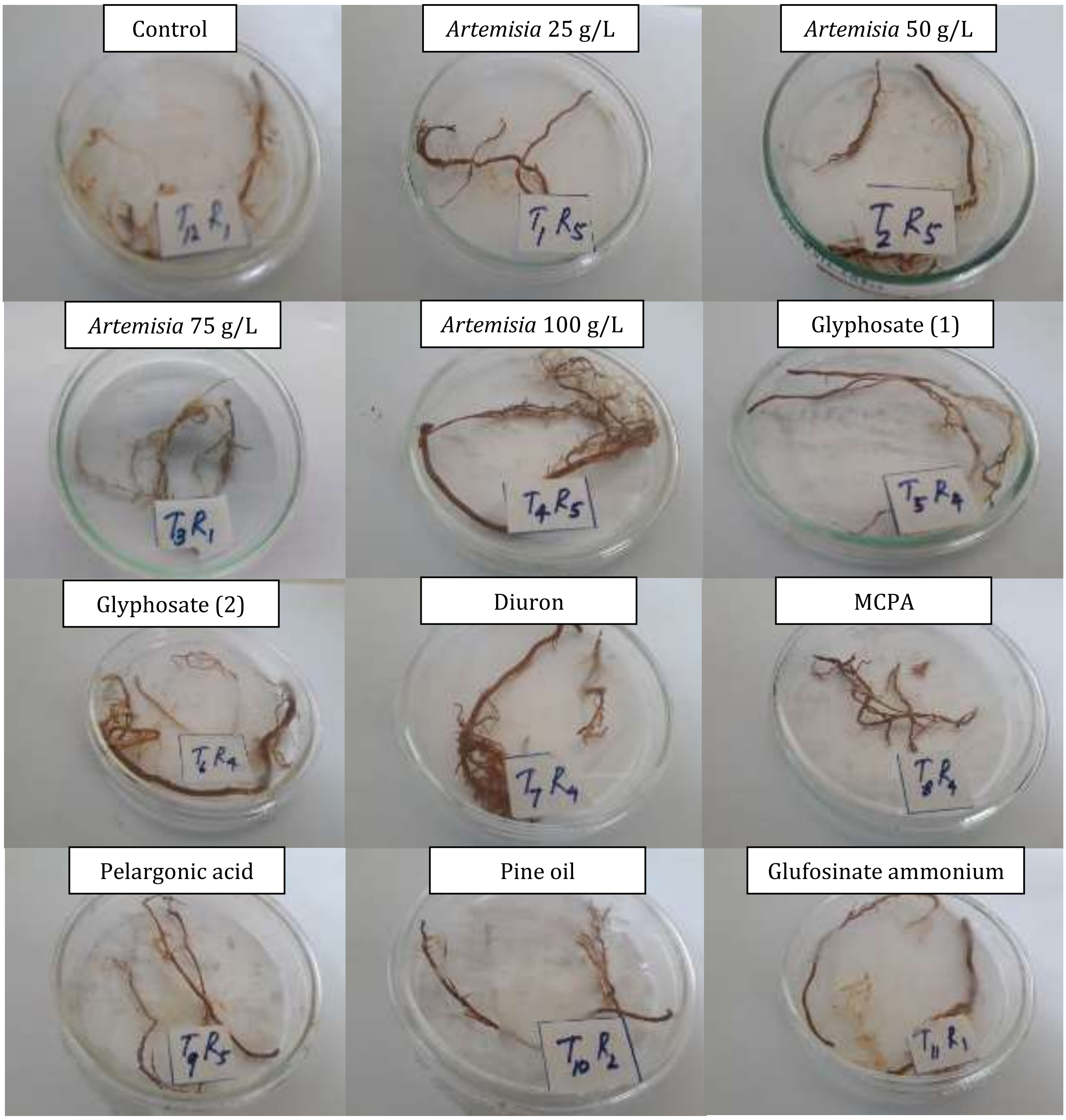

Figure 1. Observations of root growth of cultivar TRI 4046

The root growth of nursery plants of tea cultivar TRI 4046 was visually the best visual in the untreated control. All other treatments resulted in brown lesions in the root systems. Soil application of aqueous leaf extracts of *Artemisia* at 100 g/L showed the highest number of thin new roots (T4; Figure 1). No signs of new root growth was observed MCPA-treated pots after one month (T8; Figure 1), as the plants died due to the residual phytotoxicity of the herbicide. All other treatments showed signs of new root growth at various degree. Similar observations were reported in the case of the tea cultivar TRI 4006, too (Figure 2).

Figure 2. Observations of root growth of cultivar TRI 4006

Conclusion and Recommendations

The results revealed that soil application of aqueous leaf extracts of *A. vulgaris*, even at 100 g/L, has no significant negative non-target effects on the young nursery tea plants of TRI 4006 and TRI 4046. The herbicides MCPA and Glufosinate ammonium, despite been well-known for their high weed control efficacy, had negative impacts on 7-months old nursery tea plants. Mortality of nursery tea plants was observed in the former when applied at recommended dosages to the soil in pot experiments. As the non-target effects of aqueous leaf extracts of *A. vulgaris* on tea plants, even at the highest concentration used, were relatively lower compared to the other treatments used, further studies are recommended to determine an effective dosage for weed control with minimum impact on tea plants and the environment.

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