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Phytotoxicity evaluation of six fast-growing tree species in South Africa

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ABSTRACT

Vachellia sieberiana, *Albizia adianthifolia*, *Buddleja saligna*, *Combretum kraussii*, *Halleria lucida* and *Rapanea melanophloeos* are fast-growing, indigenous tree species in South Africa. They are usually found growing alongside other plants in agricultural systems. In this study, the comparative phytotoxic activity of aqueous leaf extracts of these tree species at different concentrations was investigated using lettuce seeds (*Lactuca sativa* L.) in a laboratory bioassay. To simulate natural situations, seeds were germinated under 16 h light/8 h darkness in a growth chamber using distilled water as control. The results showed that germination, chlorophyll accumulation and growth indices (plumule and radicle lengths) were significantly inhibited with increasing concentration of plant extracts. The treated lettuce seedlings experienced lipid peroxidation at high extract concentrations (1.0% and 2.0%) as evidenced by increased concentration of malondialdehyde (MDA). In response to this, the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) increased at low extract concentration but significantly dropped as concentration increased. These results suggest that aqueous extracts of the studied tree species may produce growth inhibitory substances. Thus, our study revealed that these trees possess phytotoxic activity which could be exploited in the management of weeds in agroforestry systems.

1. Introduction

Tree species represent an important component of agroforestry systems in South Africa as a result of the diverse range of climatic and phytogeographic conditions the country enjoys. Virtually all the provinces are blessed with fast-growing, indigenous, pioneer tree species which produce large volumes of non-timber products with high quantities of bioactive substances including allelochemicals. These chemicals can be released into soil by exudation from roots or leaching of the aerial parts. For this reason, agroforestry systems provide an excellent opportunity to explore the properties of these species in the control of weeds, insects and nematodes (Manimegalai and Manikandan, 2010). The concept of allelopathy refers to a phenomenon involving either direct or indirect, and either beneficial or adverse effects of a plant (including microorganisms) on another plant through the release of chemicals into the environment (Rice, 1984). Interest in allelopathic studies is growing because knowledge of these interactions could provide powerful tools for a better exploitation of natural resources in the management of weeds without using herbicides.

Toxic allelochemicals may inhibit shoot/root growth, nutrient uptake or may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of nutrients. The readily visible effects include inhibited or retarded germination rates, darkened and swollen seeds, reduced radicle and coleoptile extension, swelling or necrosis of root tips, curling of the root axis, discoloration and lack of root hairs (Niakan and Saberi, 2009). Enzyme activities in receiver plants can also be affected by allelopathic compounds through increased production of reactive oxygen species (ROS) leading to oxidative stress (Gechev and Hille, 2005; Lee et al., 2007). Under this condition, the affected plants respond by increasing antioxidant defenses, notably enzymes such as superoxide dismutase (SOD) and peroxidase (POD). However, excessive ROS may cause a decrease in the activity of these enzymes (Mishra et al., 1993). Numerous studies have supported the signaling role of ROS during different environmental responses and developmental processes including biotic and abiotic stress responses as well as allelopathic plant–plant interactions (Bais et al., 2003; Apel and Hirt, 2004). In the present study, six indigenous and fast-growing tree species namely *Vachellia sieberiana*, *Albizia adianthifolia*, *Buddleja saligna*, *Combretum kraussii*, *Halleria lucida* and *Rapanea melanophloeos* were tested for their phytotoxic properties.

Although the medicinal properties of these trees have been well studied and documented; to the best of our knowledge as at the time of carrying out this research, no comparative work has been done on their phytotoxic potential. We report here the antioxidant and growth responses in *Lactuca sativa* seedlings exposed to aqueous leaf extracts of the selected fast-growing indigenous tree species. Results of this study could provide valuable suggestions for natural, effective and less polluting means of controlling weeds and pathogens in agricultural systems.

Abbreviations: CAT, catalase; MDA, malondialdehyde; NADH, Nicotinamide–adenine dinucleotide; NBT, nitroblue tetrazolium; PMS, phenazine methosulfate; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

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2. Materials and methods

2.1. Plant materials and authentication

In February 2013, fresh matured leaves of *V. sieberiana*, *A. adianthifolia*, *B. saligna*, *C. kraussii*, *H. lucida* and *R. melanophloeos* were collected from the University of KwaZulu-Natal Botanical Garden. Voucher specimens (T. Sunmonu 01 to 06 respectively) were prepared and deposited in the University Herbarium after identification by A. Young, the Chief Horticulturist.

2.2. Preparation of aqueous extract

The leaves of each plant were thoroughly rinsed under running tap and distilled water afterwards to remove dust and soil particles before oven drying at 50 °C for 72 h. The dried materials were ground to powders using a Retsch ZM 200 Ultra Centrifugal Mill (Germany) and stored separately in airtight containers at 4 °C until required for use. The powdery materials (2 g each) were extracted separately in 100 ml distilled water (2% w/v) for 24 h at room temperature with intermittent shaking. The mixture was passed through Whatman No.1 filter paper and the resulting filtrate was further diluted to obtain other concentrations of 1.0%, 0.5% and 0.25% for each plant. The pH value of each extract concentration was determined using a pH meter (Orion Research Digital Ionalizer 501, USA).

2.3. Seed germination experiment

The lettuce seeds employed in the study were first surface-sterilized with 0.1% mercuric chloride solution for 20 min and washed properly with distilled water before germination to ensure viability. Twenty five seeds were evenly placed in 9 cm Petri dishes lined with a double layer of Whatman No. 1 filter paper moistened with 5 ml of the respective extract concentrations or distilled water as control. The Petri dishes were firmly sealed with parafilm and incubated for 7 days at 27 ± 2 °C in a growth chamber (16 h light and 8 h dark). Initial seed germination count was done after 48 h of incubation to determine percentage germination and/or inhibition. At the end of the 7-day trial, growth was assessed based on the lengths of plumule and radicle relative to the control. Each treatment had four replicates that were laid out as a four factor experiment in completely randomized design.

2.4. Determination of response index (RI)

Response index (RI) of allelopathy was calculated using the formula described by Williamson and Richardson (1988) as follows:

$$RI = 1 - C/T \ (T \geq C); \text{ and } RI = T/C - 1 \ (T < C).$$

In the model, C represents the control response and T stands for the treatment response. The range of RI is from −1 to +1. The positive value indicates stimulation by treatment; negative value indicates inhibition by treatment whereas zero is an indication of same observation, comparing with the control.

2.5. Determination of chlorophyll content

The chlorophyll content in the treated lettuce seedlings was determined according to the method of Comb et al. (1985). Fresh leaf samples (0.1 g) was homogenized and extracted with 10 ml acetone (80%) for 24 h until the leaves turned white. The concentrations of chlorophyll a, chlorophyll b and total chlorophyll in the extracting solution were determined using a spectrophotometer (Cary 50 Conc, Australia) as follows:

$$\text{Chlorophyll a} = 13.19A_{664} - 2.57A_{647} \ \mu g/g \text{ dry weight}$$
$$\text{Chlorophyll b} = 22.10A_{647} - 5.26A_{664} \ \mu g/g \text{ dry weight}$$
$$\text{Total chlorophyll} = 7.93A_{664} + 19.53A_{647} \ \mu g/g \text{ dry weight}$$

Where A_{664} = absorbance at wavelength 664 nm;
A_{647} = absorbance at wavelength 647 nm.

2.6. Enzyme extraction

Fresh lettuce seedlings (1 g) obtained following treatment with different extract concentrations were homogenized with 4 ml of 0.1 M Tris–HCl buffer (pH 7.8) under chilled conditions using a pestle and mortar. The homogenate was filtered and the resulting filtrate was taken as enzyme extract which was used to test enzyme activity.

2.7. Membrane lipid peroxidation

Lipid peroxidation was determined by adapting the method described by Heath and Packer (1968). Enzyme extract (0.5 ml) was treated with 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA). The mixture was incubated in a water bath for 30 min, cooled immediately in ice chips and the absorbance was read at 532 nm. Lipid peroxidation was expressed in terms of malondialdehyde (MDA) content.

2.8. Assay of SOD activity

SOD (EC 1.15.1.1) activity was assayed according to the method of Kakkar et al. (1984). The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of 186 μM phenazine methosulfate (PMS), 0.3 ml of 300 μM nitroblue tetrazolium (NBT), 0.2 ml of the enzyme extract and water in a total volume of 2.8 ml. The reaction was initiated by the addition of 0.2 ml of NADH (780 μM). The mixture was left to stand for 90 s and arrested by the addition of 1.0 ml glacial acetic acid. The reaction mixture was then shaken with 4.0 ml of n-butanol and allowed to stand for 10 min. The intensity of the chromogen in the butanol layer was measured at 560 nm in a spectrophotometer (Cary 50 Conc, Australia). One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in 1 min.

2.9. Assay of CAT activity

The activity of CAT (EC 1.11.1.6) was determined following the method of Luck (1974). Hydrogen peroxide–phosphate buffer (3 ml, 0.067 M, pH 7.0) was taken followed by the addition of an aliquot of 40 μl of enzyme extract and mixed thoroughly. The time required for a decrease in absorbance by 0.05 units was recorded at 240 nm using a spectrophotometer (Cary 50 Conc, Australia). The enzyme solution containing hydrogen peroxide–free phosphate buffer served as control. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240 nm by 0.05 units.

2.10. Assay of POD activity

POD (EC 1.11.1.7) was assayed following the method described by Koroi (1989). The enzyme extract (0.1 ml) was added to the assay mixture containing 2 ml acetate buffer (0.2 M, pH 5.0), 0.4 ml of 3% H_2O_2 and 0.2 ml of 0.01 M bezidin solution in 50% alcohol. Absorbance was read at 530 nm using a spectrophotometer (Cary 50 Conc, Australia) and the experiment was performed in chilled condition to preserve the activity of the enzyme.

2.11. Statistical analysis

The experiment was carried out in completely randomized design with four replicates. One-way analysis of variance (ANOVA) was

Table 1
pH values of different concentrations of aqueous leaf extract of selected tree species.

Tree species	Extract concentrations			
	0.25%	0.5%	1.0%	2.0%
Vachellia sieberiana	5.62	5.60	5.59	5.58
Albizia adianthifolia	5.88	5.86	5.85	5.85
Buddleja saligna	6.06	6.03	6.03	6.01
Combretum kraussii	4.91	4.89	4.87	4.85
Haleria lucida	6.13	6.13	6.10	6.08
Rapanea melanophloeos	6.03	6.01	6.00	5.98

employed to assess the significance of treatment means. In all cases, the confidence coefficient was set at 0.05.

3. Results

3.1. pH values of aqueous extracts

The pH values of the aqueous leaf extracts of the studied tree species ranged between 4.80 and 6.13 for *C. kraussii* at 2.0% and *H. lucida* at 0.25% respectively (Table 1). This clearly indicates that all the extracts were acidic at all tested concentrations, with no significant alteration in pH values as concentration increased.

3.2. Effect of extracts on germination of lettuce seeds

All the seeds germinated after 48 h although at different magnitudes dependent on extract concentration (Table 2). *V. sieberiana* at 1.0% and 2.0% as well as *C. kraussii* at 0.5%, 1.0% and 2.0% concentrations exhibited significant inhibition on germination with the least germination of 7% recorded for *C. kraussii* at 2.0% concentration. However, there was no significant effect on the germination of lettuce seeds exposed to aqueous leaf extracts of the remaining tree species at all the concentrations tested. The control seedlings experienced 100% germination which clearly indicated that the lettuce seeds used in this study were viable and of high quality.

3.3. Effect of extracts on growth indices of lettuce seedlings

Compared to the control, aqueous extracts of all the tested species significantly reduced ($p < 0.05$) the plumule length of lettuce seedlings in a concentration dependent manner (Table 3). Evidently, plumule length was significantly reduced in lettuce seedlings exposed to aqueous extracts of *C. kraussii*, *H. lucida* and *R. melanophloeos* at all concentrations tested, whereas *V. sieberiana*, *A. adianthifolia* and *B. saligna* did not show any effect on plumule length except at the highest tested concentration of 2.0%. *C. kraussii* at 2.0% produced the most pronounced effect on plumule length with a reduction of about 4-fold compared to the control. A similar reduction trend was observed in the radicle length of lettuce seedlings exposed to different concentrations of aqueous extract of the tested species (Table 3). The only exception was *H. lucida* which significantly promoted radicle length as concentration increased. However, the elongation (3.83 cm) at 2.0% concentration did not match up with the radicle length recorded for the control (5.50 cm) at the end of the 7-day trial. Again, lettuce seedlings were most sensitive to *C. kraussii* at 2.0% concentration, producing about 11-fold reduction in radicle length compared to the control.

3.4. Effect of extracts on response index of allelopathy in lettuce seedlings

The response of lettuce seedlings to allelochemicals present in the aqueous leaf extracts of the studied tree species is presented in Table 4. The data clearly revealed that the extracts at all tested concentrations inhibited plumule and radicle growth when compared with the control except for *A. adianthifolia* at 0.25% and 0.5% concentrations. *C. kraussii* at the highest concentration of 2.0% manifested the most devastating effect on both the plumule and radicle with values of −0.75 and −0.91 respectively.

3.5. Effect of extracts on chlorophyll content of lettuce seedlings

With the exception of *B. saligna*, aqueous extracts of all tested plants significantly reduced ($p < 0.05$) the concentrations of chlorophyll a, chlorophyll b and total chlorophyll in the lettuce seedlings at the end of the 7-day trial (Table 5). The reduction in the levels of these parameters followed a concentration dependent pattern with the 2.0% extract concentration producing the most pronounced reduction compared to the control.

3.6. Effect of extracts on MDA content and antioxidant enzyme activity in lettuce seedlings

Lipid peroxidation was observed in lettuce seedlings treated with higher concentrations (1.0% and 2.0%) of all the extracts. This was evident from the accumulation of MDA which was significantly higher when compared with the control and lower concentrations (Table 6). Considering that antioxidant enzymes are stress indicators in plants, we assayed for the activities of these enzymes in lettuce seedlings (Table 7). In response, the activities of SOD, CAT and POD in the germinated lettuce seedlings were significantly affected by the aqueous leaf extracts of the tested plants particularly at higher concentrations (1.0% and 2.0%). The activities of these enzymes in lettuce seedlings watered with aqueous extracts of *V. sieberiana*, *A. adianthifolia* and *R. melanophloeos* showed upward and later downward trend as concentration increased. However, for *B. saligna*, *C. kraussii* and *H. lucida*, there was continuous reduction in the activities of the enzymes with increase in concentration.

4. Discussion

Tree species are an important component of agroforestry systems with far reaching economic values. This is attributable to their medicinal uses that should be balanced with their possible phytotoxic properties. Results from our study clearly revealed that aqueous extracts of the tested tree species namely *V. sieberiana*, *A. adianthifolia*, *B. saligna*, *C. Kraussii*, *H. lucida* and *R. melanophloeos* exhibited various degrees of phytotoxicity including inhibition of seed germination, reduction of radicle and plumule lengths as well as alterations in chlorophyll content and enzyme activities.

Table 2
Percentage germination of lettuce seeds exposed to different concentrations of aqueous leaf extract of selected tree species.

VS	AA	BS	CK	HL	RM	
Control	100 ± 0.00^{a}					
0.25%	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	97 ± 2.03^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
0.5%	96 ± 2.80^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	89 ± 1.38^{b}	100 ± 0.00^{a}	100 ± 0.00^{a}
1.0%	88 ± 3.53^{c}	100 ± 0.00^{a}	100 ± 0.00^{a}	26 ± 3.16^{c}	100 ± 0.00^{a}	95 ± 2.83^{a}
2.0%	11 ± 1.36^{e}	100 ± 0.00^{a}	100 ± 0.00^{a}	7 ± 1.83^{d}	100 ± 0.00^{a}	97 ± 2.30^{a}

Means ± SE followed by different superscripts along the same column indicate that values are significantly different at $p < 0.05$.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

Table 3
Effect of different concentrations of aqueous leaf extract of selected tree species on growth indices (cm) of lettuce seedlings.

VS	AA	BS	CK	HL	RM	
Plumule length						
Control	3.28 ± 0.11[a]					
0.25%	3.05 ± 0.12[a]	3.28 ± 0.12[a]	3.14 ± 0.08[a]	1.09 ± 0.08[b]	0.94 ± 0.09[b]	2.14 ± 0.04[b]
0.5%	3.05 ± 0.11[a]	3.28 ± 0.10[a]	3.11 ± 0.05[a]	1.05 ± 0.05[b]	0.95 ± 0.07[b]	2.02 ± 0.05[bc]
1.0%	3.04 ± 0.09[a]	2.97 ± 0.13[a]	3.06 ± 0.07[a]	0.97 ± 0.05[b]	0.99 ± 0.05[b]	1.99 ± 0.02[c]
2.0%	2.06 ± 0.18[b]	1.88 ± 0.12[b]	2.96 ± 0.10[a]	0.81 ± 0.02[c]	1.03 ± 0.09[b]	1.94 ± 0.03[c]
Radicle length						
Control	5.50 ± 0.28[a]					
0.25%	2.54 ± 0.30[b]	3.32 ± 0.15[b]	5.48 ± 0.11[a]	3.03 ± 0.11[b]	2.61 ± 0.14[b]	4.48 ± 0.09[b]
0.5%	2.15 ± 0.06[b]	2.53 ± 0.11[c]	4.76 ± 0.04[b]	2.19 ± 0.06[c]	2.70 ± 0.14[b]	2.22 ± 0.12[c]
1.0%	1.51 ± 0.18[c]	1.92 ± 0.23[d]	4.08 ± 0.02[c]	1.87 ± 0.03[d]	2.85 ± 0.08[b]	1.85 ± 0.15[cd]
2.0%	1.16 ± 0.09[c]	1.35 ± 0.32[d]	2.57 ± 0.09[d]	0.48 ± 0.05[e]	3.83 ± 0.08[c]	1.57 ± 0.08[d]

Data are Mean ± SE. Values with different superscripts along the same column for each parameter indicate significant difference at $p < 0.05$.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

The pH values of the different extracts fell within the acidic range and remained fairly constant irrespective of concentration. Previous studies have reported that changes in extract concentrations are not likely to cause significant changes in pH values but the responses of test targets could be dramatic (Al-saadawi et al., 1986; Hartung et al., 1990). Thus, our findings in this study with regards to pH values correspond with earlier submissions. However, it is possible that the acidic pH of the extracts probably contributed to their phytotoxic properties.

Seed germination is a valuable index in allelopathic studies (An et al., 1979). Exposure of seeds to plant extracts or essential oils often results in physiological effects on germination and seedling growth (Mungole et al., 2010). Generally in studies with aqueous extracts, the observed inhibitory effects are attributed to change in pH raising concerns about allelopathy and its ecological existence and relevance (Conway et al., 2002; Sisodia and Siddiqui, 2009). Studies have also implicated phenolic content of plant extracts in allelopathic activity (Weston, 1996). Consequently, the significant reduction in percentage seed germination observed in this study with respect to *C. kraussii* (0.5% to 2.0%) and *V. sieberiana* (1.0% and 2.0%) may be attributed to the very acidic nature of their aqueous extracts and presence of allelochemicals. During germination, the action of gibberellic acid which induces the production of α-amylase (an enzyme responsible for degradation of reserved carbohydrate to soluble sugars) is disrupted by phytotoxic chemicals. Alterations in the enzymatic activity of seeds have also been reported to affect the mobility of stored compounds thus leading to reduced germination (Einhellig, 1995). Therefore, the observed reduction in percentage seed germination in *C. kraussii* and *V. sieberiana* treated groups relative to the control might be attributed to these conditions.

Aqueous extracts of all tested tree species inhibited plumule and radicle growth in lettuce seedlings particularly at higher concentrations except for *H. lucida* which enhanced radicle elongation. This observation is in agreement with earlier studies which reported that responses of receiver plants to allelochemicals occur in a concentration dependent manner (An et al., 2005; Batlang and Shushu, 2007). The lettuce seeds employed in this experiment were viable and adequately spaced in the Petri dishes; thus eliminating competition for resources as young seedlings withdraw nutrients from seeds during developmental stage (Ashrafi et al., 2008). Therefore, the inhibition of growth indices of lettuce seedlings is likely due to the presence of allelochemicals in the aqueous extracts of the tree species. The extracts also exhibited greater growth inhibition in radicle than plumule of the lettuce seedlings. This may be attributed to the permeability of allelopathic substances to root tissues arising from direct contact with the phytotoxic compounds present in the extracts (Turk and Tawaha, 2002). This might inhibit cell division which is highly active at meristematic regions of the growing root tip (Javaid and Anjum, 2006; Nishida et al., 2005; Salam and Noguchi, 2010). The elongation of radicle recorded with respect to *H. lucida* aqueous extract is not surprising as it may be an indication of a stimulatory effect of allelopathy (Ferguson and Rathinasabapathi, 2009). Also the response index (RI) of allelopathy in the plumule and radicle of lettuce seedlings clearly revealed the fact that all the studied trees possess growth inhibitory substances as indicated by the negative values.

Chlorophyll molecules are the core component of pigment–protein complexes embedded in the photosynthetic membranes and play a major role in photosynthesis (Siddiqui and Zaman, 2005). In this study, the reduction in chlorophyll a, chlorophyll b and total chlorophyll content in lettuce seedlings treated with aqueous extracts of the test plants agrees with earlier reports. Ilori et al. (2007) reported a reduction in these photosynthetic pigments in *Amaranthus cruentus* L. and *Oryza sativa* L. seedlings treated with aqueous extract of *Tithonia diversifolia*. Similarly, Ahmed et al. (2004) reported that the root and shoot extracts of *Chenopodium murale* reduced chlorophyll and protein contents of *Melilotus indicus*, *Trifolium alexandrium*, *Triticum pyramidal*, *Lycopersicon esculentus* and *Cucumus sativa*. The authors further confirmed that inhibition was a function of extract concentration and plant tissue type. These reports have clearly shown that allelochemicals in plant extracts are capable of impairing chlorophyll synthesis thereby reducing chlorophyll accumulation. According to Yang et al. (2002) as well as Morgan and Overholt (2005), allelochemicals in plant extracts may reduce chlorophyll accumulation in three ways viz inhibition of chlorophyll biosynthesis, stimulation of chlorophyll degradation or both. Therefore, the allelochemicals present in the aqueous extracts of the tested tree species must have exerted growth inhibitory effects in the plumule and radicle of lettuce seedlings through reduction in chlorophyll synthesis

Table 4
Response index (RI) of allelopathy on the growth indices of lettuce seedlings exposed to different concentrations of aqueous leaf extract of selected tree species.

	VS	AA	BS	CK	HL	RM
Plumule length						
0.25%	−0.07	0.00	−0.04	−0.67	−0.71	−0.35
0.5%	−0.07	0.00	−0.05	−0.68	−0.71	−0.38
1.0%	−0.07	−0.10	−0.07	−0.70	−0.70	−0.39
2.0%	−0.37	−0.43	−0.10	−0.75	−0.69	−0.41
Radicle length						
0.25%	−0.54	−0.40	−0.01	−0.45	−0.53	−0.19
0.5%	−0.61	−0.54	−0.14	−0.60	−0.51	−0.60
1.0%	−0.73	−0.65	−0.26	−0.66	−0.48	−0.66
2.0%	−0.79	−0.76	−0.53	−0.91	−0.30	−0.72

Negative values indicate inhibition while zero is an indication of same observation when compared with the control.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

Table 5
Effect of different concentrations of aqueous leaf extract of selected tree species on chlorophyll content (μg/g) of lettuce seedlings.

VS	AA	BS	CK	HL	RM	
Chlorophyll a						
Control	47.12 ± 1.55[a]					
0.25%	46.78 ± 1.85[a]	46.87 ± 1.65[a]	46.81 ± 2.03[a]	24.43 ± 1.58[b]	23.72 ± 2.09[b]	31.65 ± 1.88[b]
0.5%	46.72 ± 2.02[a]	46.84 ± 1.96[a]	46.81 ± 1.33[a]	24.42 ± 1.18[b]	23.70 ± 1.99[b]	31.06 ± 1.62[b]
1.0%	46.35 ± 1.95[a]	46.09 ± 1.77[a]	46.72 ± 1.86[a]	23.71 ± 1.65[b]	23.78 ± 1.96[b]	31.00 ± 1.82[b]
2.0%	31.43 ± 2.18[b]	28.46 ± 2.01[b]	46.09 ± 1.98[a]	22.97 ± 2.03[b]	23.81 ± 1.93[b]	28.33 ± 2.02[b]
Chlorophyll b						
Control	15.87 ± 1.02[a]					
0.25%	15.10 ± 1.20[a]	15.13 ± 1.49[a]	15.04 ± 1.11[a]	7.87 ± 0.91[b]	7.94 ± 0.88[b]	10.20 ± 0.82[b]
0.5%	14.88 ± 1.29[a]	15.09 ± 1.29[a]	15.00 ± 1.09[a]	7.91 ± 0.89[b]	7.97 ± 0.92[b]	9.92 ± 0.90[b]
1.0%	14.47 ± 1.08[a]	14.17 ± 1.99[a]	14.85 ± 1.88[a]	7.97 ± 0.91[b]	7.99 ± 0.80[b]	9.88 ± 0.98[b]
2.0%	9.83 ± 0.89[b]	8.89 ± 1.08[b]	14.20 ± 1.80[a]	7.99 ± 0.96[b]	8.00 ± 0.97[b]	8.80 ± 1.09[b]
Total chlorophyll						
Control	63.02 ± 2.53[a]					
0.25%	61.88 ± 2.88[a]	61.94 ± 1.90[a]	61.85 ± 2.58[a]	32.30 ± 1.20[b]	31.65 ± 1.78[b]	41.85 ± 0.78[b]
0.5%	61.62 ± 2.99[a]	61.99 ± 1.99[a]	61.81 ± 2.42[a]	33.33 ± 1.31[b]	31.69 ± 1.48[b]	41.02 ± 0.72[b]
1.0%	60.82 ± 2.96[a]	60.26 ± 2.06[a]	61.56 ± 2.65[a]	31.68 ± 1.68[b]	31.77 ± 1.64[b]	40.95 ± 0.53[b]
2.0%	41.26 ± 2.39[b]	37.39 ± 2.93[b]	60.30 ± 2.03[b]	30.98 ± 1.89[b]	32.01 ± 1.46[b]	37.18 ± 0.98[c]

Data are Mean ± SE. Values with different superscripts along the same column for each parameter indicate significant difference at $p < 0.05$.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

Table 6
Effect of different concentrations of aqueous leaf extract of selected tree species on MDA content (mmol/g fresh weight) of lettuce seedlings.

VS	AA	BS	CK	HL	RM	
Control	4.05 ± 0.09[a]					
0.25%	4.06 ± 0.07[a]	4.08 ± 0.03[a]	4.07 ± 0.01[a]	3.98 ± 0.05[a]	4.01 ± 0.01[a]	4.07 ± 0.02[a]
0.5%	4.10 ± 0.06[a]	4.12 ± 0.04[a]	4.08 ± 0.02[a]	4.00 ± 0.01[a]	4.03 ± 0.02[a]	4.08 ± 0.03[a]
1.0%	4.96 ± 0.06[b]	4.98 ± 0.03[b]	5.08 ± 0.04[b]	5.56 ± 0.08[b]	5.98 ± 0.04[b]	5.01 ± 0.01[b]
2.0%	5.33 ± 0.04[c]	6.37 ± 0.06[c]	5.09 ± 0.03[b]	5.61 ± 0.09[b]	6.85 ± 0.05[c]	5.98 ± 0.02[c]

Data are Mean ± SE. Values with different superscripts along the same column for each parameter indicate significant difference at $p < 0.05$.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

and/or stimulation of chlorophyll degradation and consequently reduction in photosynthesis.

In general, various types of environmental stresses mediate their impact through oxidative stress caused by generation of ROS (Blokhina et al., 2003). Studies of modern plant physiology further indicated that the amount of these species such as singlet oxygen, hydroxyl radical and hydrogen peroxide will increase in plants under adverse conditions (Li et al., 2013). ROS are highly reactive and toxic molecules that can cause oxidative damage to membranes, DNA, proteins, photosynthetic pigments and lipids. To avoid cellular damage due to ROS generation, plants produce a number of antioxidant enzymes that are induced and provide secondary protection against oxidative stress (Apel and Hirt,

Table 7
Effect of different concentrations of aqueous leaf extract of selected tree species on antioxidant enzyme activities (U/g fresh weight) of lettuce seedlings.

VS	AA	BS	CK	HL	RM	
SOD						
Control	135.02 ± 4.48[a]					
0.25%	130.13 ± 3.01[a]	137.09 ± 2.98[a]	136.08 ± 3.10[a]	112.09 ± 3.37[b]	110.94 ± 1.36[b]	148.14 ± 1.30[b]
0.5%	150.05 ± 4.28[b]	145.21 ± 1.09[b]	138.05 ± 3.09[a]	110.10 ± 3.45[b]	109.95 ± 1.83[b]	128.02 ± 1.05[c]
1.0%	152.12 ± 4.12[b]	125.30 ± 2.69[c]	128.11 ± 1.69[b]	91.79 ± 1.12[c]	96.85 ± 1.28[c]	112.09 ± 1.25[d]
2.0%	118.11 ± 3.69[c]	110.24 ± 2.89[d]	125.14 ± 1.98[b]	90.81 ± 1.18[c]	89.11 ± 1.02[d]	96.13 ± 1.18[e]
CAT						
Control	73.32 ± 1.01[a]					
0.25%	75.25 ± 2.54[a]	86.33 ± 1.03[b]	78.54 ± 1.04[b]	88.30 ± 1.23[b]	80.26 ± 1.26[b]	85.44 ± 1.84[b]
0.5%	84.21 ± 2.15[b]	92.25 ± 1.01[c]	78.47 ± 1.10[b]	87.21 ± 1.19[b]	79.27 ± 1.07[b]	79.22 ± 1.22[c]
1.0%	64.15 ± 2.10[c]	66.19 ± 2.53[d]	70.08 ± 0.96[c]	67.18 ± 0.98[c]	64.25 ± 2.01[c]	68.18 ± 1.02[d]
2.0%	49.11 ± 2.16[d]	40.13 ± 2.35[e]	65.07 ± 0.81[d]	54.08 ± 0.86[d]	50.38 ± 2.83[d]	60.15 ± 1.01[e]
POD						
Control	45.17 ± 2.52[a]					
0.25%	42.11 ± 2.12[b]	47.15 ± 1.53[a]	46.11 ± 2.03[a]	38.22 ± 1.36[b]	39.18 ± 1.03[b]	52.31 ± 2.01[b]
0.5%	57.16 ± 1.64[b]	54.16 ± 2.36[b]	47.14 ± 1.05[a]	37.23 ± 1.83[b]	38.24 ± 1.01[b]	39.43 ± 2.11[c]
1.0%	60.22 ± 2.53[b]	41.21 ± 2.12[c]	38.20 ± 1.01[b]	29.30 ± 2.12[c]	30.37 ± 1.25[c]	32.40 ± 1.03[d]
2.0%	38.35 ± 2.46[c]	33.22 ± 1.21[d]	37.21 ± 1.06[b]	28.29 ± 1.20[c]	29.30 ± 1.13[c]	24.25 ± 1.00[e]

Data are Mean ± SE. Values with different superscripts along the same column for each parameter indicate significant difference at $p < 0.05$.
VS = *Vachellia sieberiana*; AA = *Albizia adianthifolia*; BS = *Buddleja saligna*; CK = *Combretum kraussii*; HL = *Haleria lucida*; RM = *Rapanea melanophloeos*.

2004). Recently, allelochemicals have been proposed to cause oxidative stress in target tissues and induce antioxidant mechanisms (Li and Hu, 2005; Singh et al., 2006).

Malondialdehyde (MDA) is a membranous peroxidation end product which can serve as an important index to determine the degree of membrane lipid peroxidation and plant response to adverse conditions (Zhang et al., 2006). In the present study, elevation of MDA concentration in lettuce seedlings treated with aqueous extracts of test plants is an indication of a stress signal which can stimulate the expression of stress-tolerance genes and gradually establish defense system (Bayr, 2005). The response of plants to damaging adverse circumstances is closely related to their SOD activity (Liang et al., 2003) while POD could restrain the peroxidation of cell membrane under adverse condition and reduce the injury that cell membranes suffered (Zhi-hui et al., 2011). CAT is another important antioxidant enzyme in plants which functions as an oxidoreductase. Our findings showed an initial increase in the activities of SOD, POD and CAT in response to stress at low extract concentrations. This may be an indication of enzyme induction as a secondary defense mechanism in response to allelopathic compounds present in the plant extracts. This was possible because the stress of allelopathy was not strong enough and the amount of ROS generated was minimal at lower extract concentrations. As a result, the scavenging effects of SOD, POD and CAT could still protect the cell membrane. This also accounted for the non-significant difference in MDA content in the extracts at lower concentrations when compared with the control. At higher concentration, the trend was reversed when MDA concentration increased dramatically indicating high concentration of ROS which was beyond the threshold of scavenging by the antioxidant enzymes. These findings are consistent with previous studies which reported up/down regulation in antioxidant enzyme activities under allelopathic stress of aqueous extracts from *Eremochloa ophiuroides* (Li et al., 2013).

The present study clearly showed that aqueous leaf extracts of the tested tree species possess phytotoxic properties ranging from growth inhibition to alterations in antioxidant enzyme activities. In addition, the results indicated that the phytotoxic activity is concentration dependent in all studied plants. We therefore conclude that these trees may be explored in the development of bio-herbicides for environmentally friendly and sustainable agricultural systems.

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