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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