

## SHORT COMMUNICATION

### Allelopathic effects of cassava (*Manihot esculenta* crantz) on radish (*Raphanus sativus* L.) and ryegrass (*Lolium perenne* L.)

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#### ABSTRACT

We studied the mechanisms of cassava (*Manihot esculenta* crantz) allelopathy on radish (*Raphanus sativus* L.) and ryegrass (*Lolium perenne* L.). The water leachates from cassava leaves inhibited the seed germination and seedling growth of both receptor plants decreased the root vigour and chlorophyll content. However, the permeability of cell membrane and MDA content were significantly increased. The leachates spray at  $>0.025 \text{ g}\cdot\text{ml}^{-1}$  concentration decreased the soluble protein in seedlings of both receptor plants, but increase the activity of POD. Contrarily, the leachates application  $<0.0125 \text{ g}\cdot\text{ml}^{-1}$  concentration increased the content of soluble protein and reduced the activity of POD. While between  $0.05 \text{ g}\cdot\text{ml}^{-1}$  and  $0.0125 \text{ g}\cdot\text{ml}^{-1}$  concentrations, the leachates enhanced the PAL activity of both receptor plants. Spraying of leachates increased the activities of SOD and PPO in the seedlings of radish and ryegrass but decrease the PPO.

**Key Words:** Allelopathy, cassava, enzymes, *Manihot esculenta*, mechanism of action, radish, *Raphanus sativus*, ryegrass, *Lolium perenne*, seed germination, seedling growth, vigour

#### INTRODUCTION

Allelopathy became an active research field since 1974 (24) with the release of Prof. E.L. Rice *Allelopathy* book. Allelopathic potentials of many plants [wild plants (4), weeds (3), rice (8,9,24), wheat (11,27,28), maize (16) and few trees, etc] and some microorganisms (6,32) had been studied. It is very important to understand allelopathy of crops in agroecosystem (2,12 23).

Cassava (*Manihot esculenta* Crantz) potato and sweet potato are 3-major tuber crops in world. A large human population of tropical regions use cassava as their main food. Its tuber is used to make starch, ethyl alcohol, sorbic alcohol, soluble film, glucose and fructose, etc. The area under cassava is increasing in Southern China (Guangdong, Guangxi, Hainan and Yunnan) and Guangxi produces  $> 60\%$  of cassava in China (30). Currently, the cassava researches are focussing on increasing its yield (20), breeding high yielding and high starch varieties (5,21), use of growth regulators to enhance the yield and starch content (19,35), intercropping with other crops (10) studying the eco-physiology, and responses to

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environmental stresses in the tropics (13). However, allelopathy of cassava is less studied. In this study the allelopathic effects of cassava were determined on radish and rye grass.

## MATERIALS AND METHODS

Fresh leaves of donor plant Cassava (*Manihot esculenta* crantz) cv. Nanzhi 119, were collected from our campus in July, 2008. Seeds of two receptor plants radish (*Raphanus sativus* L) and ryegrass (*Lolium perenne* L.) were obtained from Guangxi Academy of Agricultural Sciences, Nanning, China. Ten fresh leaves of cassava were soaked in 100 mL sterilized water for 24 h at room temperature ( $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). The leachates were filtered through filter paper. The final leachates were preserved at  $4^{\circ}\text{C}$ .

### Receptor Plant culture

Receptor plants were cultured in clean sand in pots (10 cm dia 5 cm height). The seeds were surface disinfected with 5%  $\text{H}_2\text{O}_2$  for 10 min and rinsed many times to remove the traces of  $\text{H}_2\text{O}_2$ . Then 12 seeds were sown per pot and 25 pots each of radish and ryegrass were prepared. The plants were incubated at  $25 \pm 1^{\circ}\text{C}$ , 12 h day length and were supplied with the Hoagland nutrition solution at 3, 9, 13 and 16 d after germination. The sand was kept moist during the experimental period.

The original leachates of cassava fresh leaves were diluted with sterile water into 4-concentrations: A:  $0.1 \text{ g}\cdot\text{ml}^{-1}$ ; B:  $0.05 \text{ g}\cdot\text{ml}^{-1}$ ; C:  $0.025 \text{ g}\cdot\text{ml}^{-1}$ ; D:  $0.0125 \text{ g}\cdot\text{ml}^{-1}$ . The sterilized water was used as control. The radish and ryegrass seedling were sprayed with different leachates concentrations at 6, 11, 15 and 17 d after sowing. Radish plants received 30 ml leachates per pot and ryegrass plants received 25 ml per pot.

### Physiological indices

When receptor plants were 21 d old their root length, shoot height, fresh weight of seedling and physiological indices were measured. Membrane permeability of cell was determined as per Li (18). Root vigour was determined as per Zhang and Qu (34), chlorophyll content was determined according to Yang (31) and protein content in leaves was determined as per Zhang and Qu (34).

Samples to determine the MDA content, activities of SOD and POD were prepared by freezing 0.5 g leaves in liquid nitrogen (to prevent proteolytic activity), followed by grinding with 5 mL extraction buffer (0.1 mol/L phosphate buffer, pH 7.5, containing 0.5 mmol/L EDTA, 10mg/mL PVP and 1 mmol/L ascorbic acid) in a chilled pestle and mortar, the homogenate was centrifuged at  $13\,000 \times g$ ,  $4^{\circ}\text{C}$  for 20 min and the supernatant was used for analysis.

MDA content was determined as per Zhang and Qu (34). 1 mL supernatant was added to 3 mL of 5 mg/mL thiobarbituric acid (TBA) in 0.2 g/mL trichloroacetic acid (TCA). The mixture was heated at  $100^{\circ}\text{C}$  for 20 min in a sealed tube and then cooled in ice bath. After centrifugation at  $5000 \times g$  for 10 min, the absorbance of supernatant was recorded at 450, 532 and 600 nm with a spectrometer. The MDA content was calculated as under:

$$C (\text{mmol} \cdot \text{g}^{-1} \text{FW}) = [6.452 \times (OD_{532} - OD_{600}) - 0.559 \times OD_{450}] \times \frac{V \times V_t}{W \times V_s}$$

Where,  $V$ : Total volume mL;  $V_l$ : Volume of leachates mL;  $V_s$ : Volume of leachates determined mL;  $W$ : sample fresh weight (g).

SOD activity was determined as per Giannopotitis and Ries (15); one unit of SOD activity was defined as the amount of enzyme inhibiting photochemical reduction of NBT by 50% per min. POD activity was determined by monitoring the increase in absorbance at 470 nm as guaiacol was oxidised, as per method of Zhang and Qu (34).

Activity of phenylalanine ammonia lyase was determined as per Xue (29). 0.5 g fresh leaves were grinded with 5 mL boric acid buffer (containing 5 mmol/L mercaptoethanol) and 0.5 g vinyl pyrrolidone, a few quartz sand, at 4°C. The homogenate was centrifuged at 11100 ×g, 4°C for 15 min. The supernatant was used for analysis. Two mL supernatant was added to 2 mL of 0.02 mmol/L phenylalanine ammonia acid and 2 mL sterilized water, kept at 30°C for 30 min in water bath, the reaction was stopped by 0.2 mL 6mol/L HCL, then activity was recorded at 290 nm with spectrometer.

Activity of Polyphenol Oxidase (PPO) was determined as per Zhang and Qu (34). Fresh leaves (1.0 g) were grinded with 5 mL of 0.05 mol/L pH 8.0 phosphate buffer and some quartz sand in ice-bath, the homogenate was centrifuged at 999 ×g, 4°C for 15 min, the supernatant was used to analyze. 1 mL supernatant was added to 3 mL 0.02mol/L catechol and 1.5 mL 0.05 mol/L phosphate buffer (pH 8.0), 30°C for 30 min in water bath. The liquid was recorded at 398 nm with spectrometer.

**Statistical analysis:** SPSS 15.0 for Windows XP was used for statistical analysis. The data were analyzed with one-way analysis of variance with the significant differences among means identified by Duncan's multiple range test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

Cassava fresh leaf leachates inhibited the seedlings growth of both radish and ryegrass (Figs. 1,2), the higher the of leachates concentration, the more was the inhibition i.e. the inhibition was concentration dependent. The 0.05 g·ml<sup>-1</sup> leachate significantly inhibited the root and shoot growth of ryegrass and radish. The inhibition was not significant below 0.025 g·ml<sup>-1</sup> concentration.

**Membrane permeability and root vigour:** The leachates application promoted the root vigour at low concentration, but inhibited at high concentration in both radish and ryegrass (Fig. 4). The root vigour of radish and ryegrass increased significantly at 0.025 g·ml<sup>-1</sup> leachate i.e. at lower concentration. However, the vigour in rye grass and radish decreased at 0.05 and 0.1 0.05 g·ml<sup>-1</sup> leachate, respectively. The leachates < 0.025 g·ml<sup>-1</sup> reduced the vigour, but it was increased > 0.05 g·ml<sup>-1</sup> concentration. Higher concentrations of leachate increased the cell permeability (Fig. 3), thereby causing exosmosis; consequently inhibiting the growth of receptor plants.

**Chlorophyll:** The chlorophyll a, chlorophyll b and total chlorophyll contents in leaves of receptor plants increased significantly at lower concentration (0.0125 g·ml<sup>-1</sup>) of leachates but decreased at > 0.025 g·ml<sup>-1</sup> concentration (Table 1). However, there were differences in

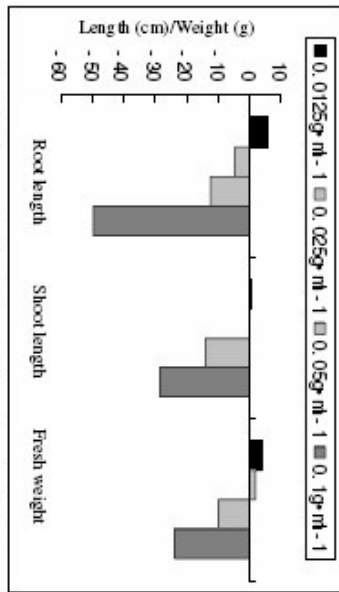


Figure 1. Inhibitory and stimulatory effects of water leachates of cassava leaves on seedling of *Raphanus sativus*

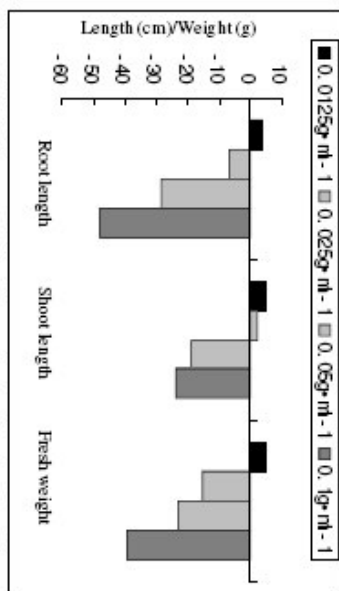


Figure 2. Inhibitory and stimulatory effects of water leachates of cassava leaves on seedling of *Lottium perenne*

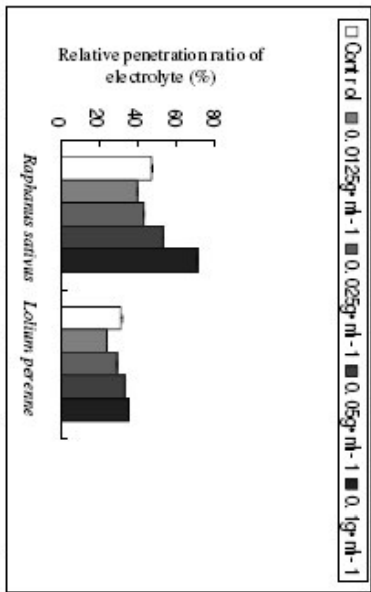


Figure 3. Effects of water leachates of cassava leaves on membrane permeability in seedlings of *Raphanus sativus* and *Lottium perenne*

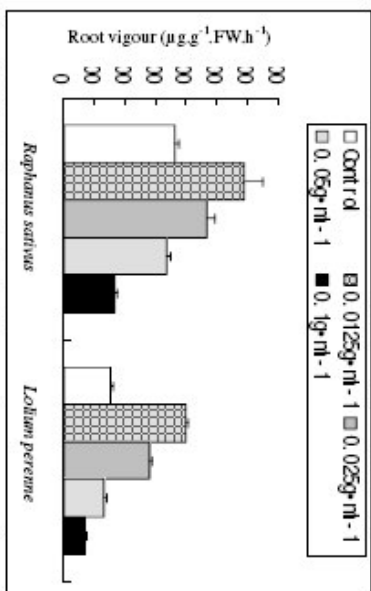


Figure 4. Effects of water leachates of cassava leaves on root vigour in seedlings of *Raphanus sativus* and *Lottium perenne*

Table 1. Effects of the water leachates from cassava leaves on the concentration of chlorophyll in seedlings of radish and ryegrass

		Chlorophyll (mg·g <sup>-1</sup> FW)			
		a	b	Chl a / Chl b	a + b
Radish	Control	0.83±0.12b	0.40±0.02b	2.08	1.23±0.13b
	0.0125	0.86±0.15a	0.42±0.09a	2.05	1.28±0.25a
	0.025	0.81±0.19c	0.40±0.16b	2.03	1.21±0.06c
	0.05	0.81±0.08c	0.39±0.09c	2.03	1.20±0.18d
	0.1	0.52±0.07d	0.27±0.07d	1.79	0.790.11e
Ryegrass	Control	1.17±0.01b	0.47±0.01b	2.46	1.63±0.02b
	0.0125	1.36±0.01a	0.54±0.01a	2.50	1.90±0.02a
	0.025	1.16±0.01b	0.47±0.01b	2.48	1.62±0.01bc
	0.05	1.15±0.01bc	0.46±0.01bc	2.48	1.60±0.02bc
	0.1	1.13±0.02c	0.45±0.02c	2.53	1.59±0.01c

the changes in chlorophyll a/b contents between the radish and ryegrass. With increase in leachate concentration, the radish seedlings chlorophyll a/b ratio was decreased, however at higher concentration, there were slight changes in ryegrass seedlings i.e. the mechanism of allelopathy varies in different receptor plants.

**The concentration of soluble protein and Malondialdehyde (MDA):** At the low leachate concentration (0.0125 g·ml<sup>-1</sup>), the soluble protein content in radish seedlings increased significantly but did not change in ryegrass seedlings (Table 2). At 0.05 g·ml<sup>-1</sup> concentration the soluble protein content decreased in both receptor plants, further increase in leachate concentration caused reduction. The MDA content in radish seedling leaves increased significantly, when sprayed with leachate upto 0.1 g·ml<sup>-1</sup> concentration. However, the ryegrass seedlings were more sensitive to leachates and the MDA content increased significantly at 0.05 g·ml<sup>-1</sup> leachate concentration.

Table 2. Effects of water leachates of cassava leaves on the concentration of soluble protein and MDA in seedlings of radish and ryegrass

	Radish		Ryegrass	
	Soluble protein (mg·g <sup>-1</sup> FW)	MDA (mmol·g <sup>-1</sup> FW)	Soluble protein (mg·g <sup>-1</sup> FW)	MDA (mmol·g <sup>-1</sup> FW)
Control	10.38±0.06b	4.47±0.46bc	13.36±0.15ab	8.82±0.36d
0.0125	11.04±0.11a	4.24±0.04c	13.59±0.10a	8.82±0.03d
0.025	10.25±0.06b	4.68±0.06bc	12.95±0.07b	9.09±0.06c
0.05	9.57±0.11c	5.07±0.07b	12.11±0.20c	9.53±0.06b
0.1	8.76±0.06d	5.47±0.14a	10.66±0.64d	9.97±0.13a

**Phenylalanine ammonialyase (PAL):** The activity of phenylalanine ammonialyase increased significantly in seedlings of both radish and ryegrass sprayed with leachates < 0.05 g·ml<sup>-1</sup> concentration over the control (Table 3). Nevertheless, the leachates at higher concentration (0.1 g·ml<sup>-1</sup>) did not affect its activity.

Table 3. Effects of water leachates of cassava leaves on the activity of PAL in seedlings of radish and ryegrass

	Activity of PAL (U·ml <sup>-1</sup> min <sup>-1</sup> )	
	Radish	Ryegrass
Control	1.43±0.02c	2.02±0.11b
0.0125	1.66±0.03a	2.35±0.11a
0.025	1.61±0.03b	2.28±0.12a
0.05	1.59±0.01b	2.27±0.09a
0.1	1.39±0.01c	2.09±0.01b

**Protective enzymes:** The activity of peroxidase (POD) and polyphenol oxidase (PPO) increased synchronously with increase in leachates concentration. The superoxide dismutase (SOD) activity was increased more in treated seedlings than control, however, the magnitude of increase in activity became lower with enhanced leachate concentration (Table 4,5).

Table 4. Effects of water leachates of cassava leaves on the activity of protective enzymes in radish seedlings

Leachate conc. (g·ml <sup>-1</sup> )	SOD (U·g <sup>-1</sup> FW)	POD (U·g <sup>-1</sup> FW·min <sup>-1</sup> )	PPO (U·g <sup>-1</sup> FW·min <sup>-1</sup> )
Control	34.21±0.24d	6.38±0.51c	0.32±0.02c
0.0125	50.42±0.55a	11.55±1.67b	0.33±0.02bc
0.025	38.46±0.45b	11.82±1.10b	0.35±0.09ab
0.05	36.73±0.39c	16.72±0.81a	0.37±0.01a
0.1	34.28±0.05d	17.45±1.05a	0.38±0.01a

Table 5. Effects of water leachates of cassava leaves on the activity of protective enzymes in ryegrass seedlings

Leachate conc. (g·ml <sup>-1</sup> )	SOD (U·g <sup>-1</sup> FW)	POD (U·g <sup>-1</sup> FW·min <sup>-1</sup> )	PPO (U·g <sup>-1</sup> FW·min <sup>-1</sup> )
Control	95.53±0.80e	123.95±3.87d	0.51±0.13c
0.0125	144.81±1.43a	115.46±0.47e	0.54±0.01b
0.025	120.54±0.76b	133.66±1.28c	0.56±0.01ab
0.05	112.34±5.48c	143.60±1.21b	0.57±0.02ab
0.1	106.63±0.66d	160.14±2.12a	0.59±0.01a

Allelochemicals inhibit the plant growth mostly through multiple mechanisms: damaging cell membrane (1), reducing photosynthesis, disturbing protein synthesis, changing enzyme system and the gene expression (17,22,26,33). This study showed that cassava exerts allelopathic effects by disturbing many physiological processes of receiver plants.

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