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ALLELOPATHIC EFFECTS OF *Grevillea banksii* R. BR. LEAF EXTRACTS AND ITS RHIZOSPHERIC SOIL ON GERMINATION AND INITIAL GROWTH OF THREE AGRICULTURAL CROPS IN MADAGASCAR

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Abstract

G. banksii R. Br. is a widespread species forming dense populations in the Eastern part of Madagascar. The aim of this study was to investigate the allelopathic effects of G. banksii on seeds germination and initial growth of three agricultural crops: rice (Oriza sativa L.), maize (Zea mays L.) and bean (Phaseolus vulgaris L.). The effects of leaf (leaf powder and aqueous leaf extracts obtained after shaking during 24 and 48 h) and rhizospheric soil (soil powder and aqueous soil extract shaken during 72 h) of G. banksii on seeds germination, seedling length of each tested plant were described in vitro. Results showed that the rhizospheric soil of G. banksii activates the germination of bean and maize seeds as well as the seedling length development of all crop species but inhibit rice seeds germination. However, all aqueous leaf extract inhibit seeds germination and the shoot and root length development of the three crops species tested, especially for rice and maize roots length development. This investigation therefore comparatively reveals that the leaves extract of G. banksii has more allelopathy than rhizospheric soil in seed germination and initial growth of agricultural crops tested. The results suggest some cautions when using the shoots of G. banksii as plant cover in farming systems.

***Key words**: allelopathic, crop plants, invasive plant, seed germination, root and shoot elongation.*

INTRODUCTION

G. banksii R. Br. (Proteaceae) is a plant species originated in Australia and introduced in the Eastern part of Madagascar in the 1950s in order to restore forest cover and to limit land degradation/erosion in this region (Tassin, 1995). After a few years of establishment, this exotic species was identified as an invasive species in Eastern part of Madagascar (Binggeli, 2003).This species is known for its Many research results have suggested that exotic invasive plants release some allelopathic substances into the environment (Osvald, 1948; Fletcher and Renney, 1963; Abdul Wahab and Rice, 1967; Kanchan and Jayachandra, 1979; capacity to spread rapidly especially on disturbed areas previously occupied by forest ecosystems. Moreover, *G. banksii* may affect the regeneration of native plant species by inhibiting soil biota which may have significant roles in plant development (Andrianandrasana et al., 2014). In spite of this, the farmers in the region use its biomass in farming through slash and burn farming practices. But, the interaction involved between crop plant species and this invasive plant is not well understood yet (El Ghareeb, 1991; Vaughn and Berhow, 1999; Ridenour and Callaway, 2001; Stan et al., 2018) and exploit this phenomenon to attribute their dominance success and their competitiveness (Ridenour and Callaway, 2001; Stinson et al., 2006; Jarchow and Cook, 2009). Thus, allelopathy is defined as any direct or indirect, positive or negative effect of a plant, including microorganisms, on neighbouring species through the release of biochemical compounds (Rice, 1984) that could be present in whole plant or only in some organs, like roots, rhizomes, stems, leaves, fruits and/or seeds (Zeng et al., 2008). Root exudates represent one of the largest direct inputs of plant chemical elements into the rhizosphere (Bertin et al., 2003).

Many allelopathic compounds affect crops development. Most of them are described as the main actors in the inhibition of seeds germination, overall growth and plant nutrients' uptake (Rizvi et al., 1999; Marwatand Khan, 2006). For example, Ejaz et al. (2004) found that allelopathic compounds produced by *Eucalyptus* decrease the cotton germination rate. Balicevic et al. (2015) have reported that the invasive plant, *Solidago gigantea*, decreased the germination of seeds and development of seedlings of carrot, coriander and barley.

The aim of this study was to assess allelopathic effects of leaf and rhizospheric soil of *G. banksii* on seeds germination rate and initial growth of three crops species *in vitro*.

MATERIALS AND METHODS

Plants materials and soil collection

Leaves and rhizospheric soil were collected from mature individuals of *G. banksii* growing in the Eastern part of Madagascar (18°57'48.0''S, 048°45'51.3''E). The collected samples were brought into the Laboratory of Environmental Microbiology of National Center of Environment Research (CNRE) Madagascar.

Seeds of rice (*Oriza sativa* L.), maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) were used as test plants in all experiments to assess the allelopathic effects of *G. banksii*. Seeds of *O. sativa* (variety Botrafotsy) and *Z. mays* were taken from farmers' stock in the eastern part of Madagascar.

Seeds of *P. vulgaris*, variety Ranjonomby, were provided by the FOFIFA (National Center for Applied Research on Rural Development in Madagascar).

Aqueous extract preparation

Leaf of *G. banksii* was dried at room temperature and cut into 1-2 cm pieces. Leaf was pounded using electrical stainless material. Aqueous leaf extracts were prepared by soaking 10 g leaf powder of *G. banksii* with 200 ml sterile distilled water. Each container was shaken separately for 24 and 48 hours at room temperature. The resulting aqueous extracts were filtered with Whatman No.1 filter paper. Rhizospheric soil extract was prepared by soaking 10 g of soil in 200 ml sterile distilled water at room temperature for 72 h and filtered.

Seed germination and seedling growth

Effect of aqueous extracts. Five millilitre (5 ml) of each aqueous extracts types (two leaf extracts and one soil extract) were tested on the three test plant seeds (rice, maize and bean) which were respectively deposited on filter paper contained insterilized Petri dishes. Sterile distilled water was used as control. For each treatment, four replicates, each with 10 seeds, were made. The Petri dishes were incubated at 25°C. After 5 to 10 days, seeds germination and initial growth (shoot and root length) of each plant were noted.

Effect of leaf and soil powder. Five grams of leaf powder and 2 g of rhizosphere soil powder were placed separately in Petri dish and topped with a single sheet of filter paper. Ten seeds of crop species were placed on it. The dishes were moistened with 10 ml sterile distilled water. For the control, fine pieces of filter paper were used. The bioassay was run as mentioned above.

Data analyses

The results were quantified as germination capacity, root and shoot length development. Germination percentage G (%) was calculated using the following formula:

$$\mathbf{G\ (\%) = \frac{N}{Nt}\ X\ 100}$$

Where: **N** is germinated seeds in each treatment and **Nt** - number of seeds used in bioassay.

The relative inhibition (**I**) or stimulation (**S**) of seed germination and shoot and root length development affected by the allelopathic substance was calculated according to Chung et al. (2001) and Ladhari et al. (2013) as following:

$$I (\%) \text{ or } S (\%) = \frac{E - C}{C} X\ 100$$

Where: **E** is extract (growth parameter measured in presence of *G. banksii* leaf or soil extract and powder) and **C** - control (growth parameter measured in presence of sterile distilled water).

All statistical analyses were done using XLSTAT 2008 software. Differences among the treatment means were assessed according to ANOVA test.

Significant differences among the means were found through Fisher (LSD) significant difference test at $P < 0.05$.

RESULTS AND DISCUSSIONS

1. Allelopathic effects of leaf of *G. banksii* on crop plants species

1.1. Germination percentage

The effects of *G. banksii* leaf extracts on the germination capacity of rice, maize and bean are shown in Table 1. The results showed that leaf affected seed germination of rice, maize and bean except for the 24 h leaf extract on rice and leaf powder on maize. Notable inhibition was found on rice germination with 48 h leaf extract (-24.88%) and leaf powder (-36.15%). However, rice seed germination stimulation was found with 24 h leaf extract (+23.94%) and a little stimulation on maize germination by both 48 h leaf extract and leaf powder was recorded (Figure 1).

Table 1. Seeds germination capacity (%) of rice, maize and bean in presence of leaf of *G. banksii*

Treatments	Seeds germination capacity (%)		
	Rice	Maize	Bean
24 h leaf extract	82.50 ± 17.50 (a)	72.50 ± 17.50 (b)	85.00 ± 7.50 (b)
48 h leaf extract	50.00 ± 5.00 (a)	95.00 ± 5.00 (a)	90.00 ± 5.00 (ab)
Leaf powder	42.50 ± 4.20 (a)	97.50 ± 3.75 (a)	95.00 ± 5.00 (ab)
Control	66.56 ± 5.31(a)	93.75 ± 9.39 (a)	98.00 ± 2.00 (a)

Means with the same letter in a column are not significantly different at $p < 0.05$ according to Fisher's LSD test.

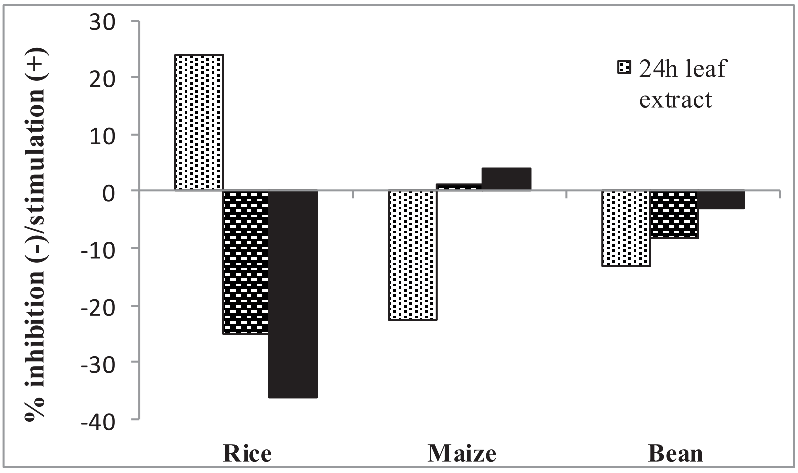


Figure 1. Inhibition (-)/stimulation (+) of germination seeds of rice, maize and bean in presence of leaf of *G. banksii*

1.2. Root and shoot length development

Effects of leaf of *G. banksii* on root and shoot length development of rice, bean and maize are shown in Table 2. Root and shoot length development of rice, maize and bean were reduced by leaf of *G. banksii* except for the 24 h leaf extract on rice root length development. The highest inhibition was found on maize root length development with 24 h leaf extract (-81.44%) and on maize shoot length development with 24 h and 48 h leaf extracts (-100%) (Figure 2). Generally, the leaf of *G. banksii* proved to be toxic to roots and shoots length development of the three tested plant species.

Table 2. Root and shoot length development of rice, maize and bean in presence of leaf of *G. banksii*

	Treatment			
	24 h leaf extract	48 h leaf extract	Leaf powder	Control
Root length (cm)				
Rice	2.21 ± 1.46 (a)	1.66 ± 1.66 (a)	0.93 ± 0.90 (a)	1.78 ± 1.65 (a)
Maize	1.06 ± 0.46 (c)	4.79 ± 0.48 (ab)	3.68 ± 0.56 (b)	5.73 ± 1.65 (a)
Bean	3.48 ± 1.47 (b)	3.83 ± 0.97 (b)	4.55 ± 1.35 (ab)	6.55 ± 1.24 (a)
Shoot length (cm)				
Rice	0.78 ± 0.50 (a)	0.63 ± 0.62 (ab)	0.01 ± 0.02 (b)	0.78 ± 0.12 (a)
Maize	0.00 ± 0.00 (b)	0.00 ± 0.00 (b)	0.13 ± 0.02 (b)	1.14 ± 0.72 (a)
Bean	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)

Means with the same letter in a line are not significantly different at $p < 0.05$ according to Fisher's LSD test.

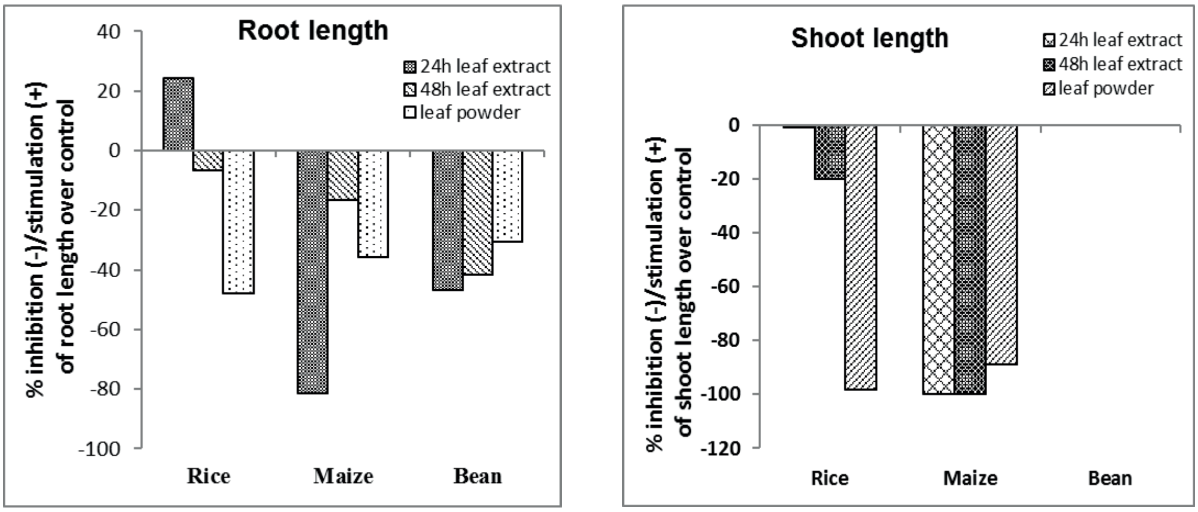


Figure 2. Inhibition (-)/stimulation (+) of root and shoot length development of rice, maize and bean in presence of leaf of *G. banksii*

2. Allelopathic effect of the rhizospheric soil of *G. banksii* on crop plants species

2.1. Germination percentage

Compared to control treatment, soil powder and soil extract did not significantly ($p < 0.05$) reduced seeds germination percentage of rice, maize and bean (Table 3).

Besides, inhibition/stimulation analyses revealed that maize seeds germination was stimulated by rhizospheric soil powder and rhizospheric soil extract respectively +6.66% and + 4%.

But, rice seed germination was inhibited by rhizospheric soil powder and rhizospheric soil extract respectively -17.37% and -2.34%. However, bean seeds germination was stimulated with rhizospheric soil powder (+2.04%) but inhibited by rhizospheric soil extract (-5.80%) (Figure 3).

Table 3. Germination capacity (%) of seeds of rice, maize and bean in presence of rhizospheric soil of *G. banksii*

Treatment	Seeds germination capacity (%)		
	Rice	Maize	Bean
Soil powder	55.00 ± 4.50 (a)	100.00 ± 0.00 (a)	100.00 ± 0.00 (a)
Soil extract	65.00 ± 10.00 (a)	97.50 ± 3.70 (a)	92.31 ± 7.70 (a)
Control	66.56 ± 5.30 (a)	93.75 ± 9.30 (a)	98.00 ± 2.00 (a)

Means with the same letter in a column are not significantly different at $p < 0.05$ according to Fisher's LSD test.

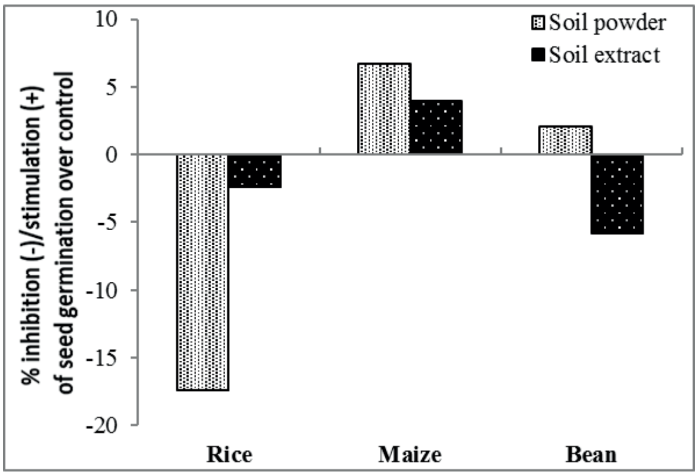


Figure 3. Inhibition (-)/stimulation (+) of germination seeds of rice, maize and bean in presence of rhizospheric soil of *G. banksii*

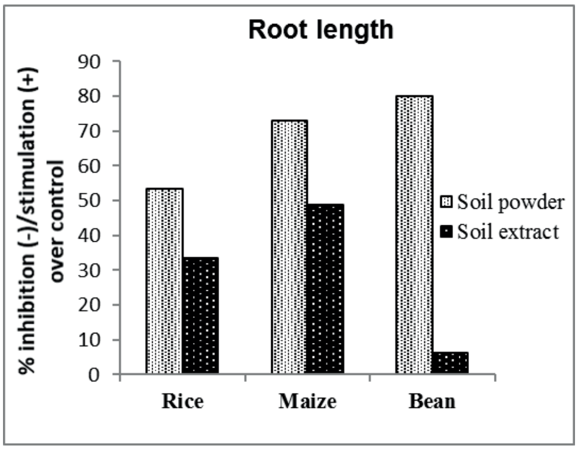
2.2. Root and shoot length development

Root length development of rice, maize and bean was significantly ($p < 0.05$) stimulated by both rhizospheric soil powder and extract. Shoot length development of maize was also stimulated by these two treatments (Table 4). The highest stimulation was found on bean root length development with rhizospheric soil powder (+80.03%) and on maize shoot length development with the same treatment (+228.94%) (Figure 4).

Table 4. Root and shoot length development of rice, maize and bean in presence of rhizospheric soil of *G. banksia*

	Treatment		
	Rhizospheric Soil extract	Rhizospheric Soil powder	Control
Root length (cm)			
Rice	2.73 ± 0.62 (a)	2.38 ± 0.70 (a)	1.78 ± 0.18 (a)
Maize	9.91 ± 0.98 (a)	8.53 ± 0.81 (a)	5.73 ± 1.65 (b)
Bean	11.79 ± 1.86 (a)	6.96 ± 1.58 (b)	6.55 ± 1.24 (b)
Shoot length (cm)			
Rice	0.59 ± 0.56 (a)	0.73 ± 0.37 (a)	0.78± 0.12 (a)
Maize	3.75 ± 0.85 (a)	1.93 ± 0.75 (b)	1.14 ± 0.72 (b)
Bean	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)	0.00 ± 0.00 (a)

Means with the same letter in a line are not significantly different at $p < 0.05$ according to Fisher's LSD test.



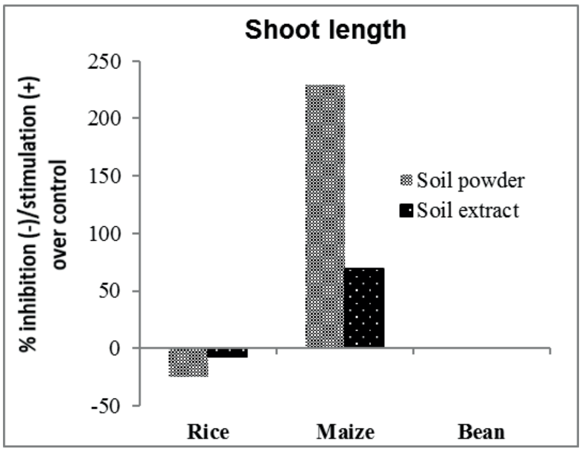


Figure 4. Inhibition (-)/stimulation (+) of root and shoot length development of rice, maize and bean in presence of rhizospheric soil of *G. banksii*

In this study, leaf of *G. banksii* and its rhizospheric soil were used to evaluate their effect on seeds germination and growth of both roots and shoots of rice, maize and bean in vitro. Our results showed that, the allelopathic effect of this exotic plant was varied according to the aqueous extract type (leaf extract and rhizospheric soil extract) and tested plant species. Thus, the response of germination, seedling growth of test plant species towards the same extract (Hisashi et al., 2009) and the toxicity of allelochemical in different part of same plant (Hussain et al., 2010) are variables.

Firstly, all aqueous leaf extracts of *G. banksii* were negatively affected the percentage of seed germination and root and shoot length development of all tested agricultural species. Lara-Núñez et al. (2006; 2009; 2015) explain this phenomenon that allelochemical can alter enzymatic activities during seed germination and radicle growth. Indeed, the high inhibition of seed germination was found on rice with 48h leaf extract.

The results indicate that by increasing the shaken duration, the efficiency of extracts is also increased, especially on rice seed germination. These findings agree with those of Barkat et al. (2010), Hussain et al. (2010) and Ehsan et al. (2012). Contrary for bean, shaken leaf extract during 24 h was more inhibitory than 48 h leaf extract. This might be due to denaturation of phytotoxic substances capable to inhibit particularly bean seed germination. Samreen et al. (2009) and Batlang and Shushu, (2007) suggested that allelopathic stress depends upon concentration of allelopathic material.

Generally, plant litter is known to increase soil fertility during decay. However, our results showed that leaf of *G. banksii* reduce remarkably the seed germination of rice. Similar results were also reported in other studies. For example: *Ageratina adenphora* litter reduced growth of *Lantana camara* (Kaul and Bansal, 2002). Moreover, leaf extracts of *G. banksii* inhibit shoot elongation of maize and rice, respectively -100% and -98.3%. Many studies explained that decrease in root and shoot length development may be attributed to inhibiting or reducing rate of cell division and elongation due to the presence of allelochemicals compound which inhibit probably the hormones such as gibberellin and indoleacetic acid function in the plant species (Tomaszewski and Thimann, 1966).

Secondary, allelopathic effect of rhizospheric soil of *G. banksii* was evaluated using an aqueous extract and powder of soil on germination and shoot length development of three crops plants. Samedani et al. (2013) explain that allelochemicals activities in soil are depend on complex interactions betwen soil and plant factors. In this way, soil properties are the dominant factors determining the activity of allelochemicals in soil (Inderjit, 2002). As other species of Proteaceae family, *G. banksii* has proteoid roots (Purnell, 1960; Andrianandrasana et al., 2014). This root type is known for its ability to produce acids, such as malates and citrates (Dinkelaker et al., 1995; Shane and Lambers, 2005) and enzymes such as phosphatases (Miller et al., 2001; Gilbert et al., 2000) into the rhizosphere. This root type may affect rhizospheric soil properties of *G. banksii* and subsequently affects germination and growth of other plant species. Our results showed that rhizospheric soil of *G. banksii* inhibited rice and bean seeds germinations but stimulated maize seed germination. Besides, all rhizospheric soil generally activated shoot elongation on the three agricultural crops tested. Therefore, the stimulation growth of crop species by rhizospheric soil could probably be due to the induction of growth promoting hormones that were described by Tomaszewski and Thimann (1966).

CONCLUSIONS

In summary, our results demonstrated that allelopathic effect of *G. banksii* as inhibitory or stimulatory was depending on the extract and on the/or crop species. Indeed, the degree of seeds germination and plant initial growth inhibition were higher in aqueous leaf extract of *G. banksii* than rhizospheric soil on the three crops species tested. Also, rice seed germination was more affected than maize and bean. Remarkable stimulation was observed in maize development with rhizospheric soil. However, this study needs further evaluation at field level. Also, allelopathic potential of *G. banksii* will be tested to it herbicidal activity on weeds species associated with crop plant.

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