

Phytotoxic property of seed methanolic extracts from *Albizia* (Fabaceae) endemic species of Madagascar

Hanitra Ranjana Randrianarivo¹, Holy Christiane Ratsimanohatra²,
Anjarasoa Ravo Razafindrakoto², Clara Fredeline Rajemiarimoelisoa³,
Lovarintsoa Judicael Randriamampianina², Lolona Ramamonjisoa⁴,
Danielle Aurore Doll Rakoto¹, Victor Louis Jeannoda¹

¹Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Department, Faculty of Sciences, University of Antananarivo, PO Box. 906, Antananarivo 101, Madagascar

²Graduate School of Life Sciences and Environment of the University of Antananarivo, PO Box. 906, Antananarivo 101, Madagascar

³Department of Pharmacy, Faculty of Medicine, PO Box 375, Antananarivo 101, Madagascar

⁴National Tree Seed Centre (SNGF), PO Box. 5091, Antananarivo 101, Madagascar

Email address:

ranjanamaso@yahoo.fr (H. R. Randrianarivo), ratsimanohatraholly@yahoo.fr (H. C. Ratsimanohatra),
anjravo@yahoo.fr (A. R. Razafindrakoto), boubalova@yahoo.fr (L. J. Randriamampianina), lolona.sngf@moov.mg (L. Ramamonjisoa),
fredeline_rajemi@yahoo.fr (C. F. Rajemiarimoelisoa), dad.rakoto@yahoo.fr (D. A. D. Rakoto), victor_jeannoda@yahoo.fr (V. L. Jeannoda)

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Abstract: Investigations on the phytotoxicity of *Albizia* species were conducted under laboratory conditions in order to assess their possible use in the control of weeds and invasive plants. The effects of seed methanolic extracts obtained from *A. androyensis*, *A. bernieri*, *A. divaricata*, *A. greveana*, *A. masikororum* and *A. viridis*, all endemic of Madagascar were evaluated against seed germination and early seedling development of vegetables (*Phaseolus vulgaris*, *Pisum sativum*, *Petroselinum crispum*, *Brassica sp.*, *Cucumis sp.*, *Allium cepa*, *Zea mays* and *Oryza sativa*). The effects of these extracts on seed germination of weeds (*Eragrostis pilosa*, and *Panicum subalbidum*) and invasive plants (*Acacia dealbata*, *Cassia rotundifolia* and *Pinus kesiya*) were also studied. Globally, all the extracts (1 mg/mL) inhibited the seed germination of all the test plants. However, the inhibitory effect varied according to both the *Albizia* extract and the target plants. Inhibition rates could reach 100%. The extracts (0.45 to 7 mg/mL) also significantly ($p < 0.05$) reduced the length of both epicotyl and hypocotyl and the effects were generally in a dose dependent manner. At the same concentration (7.2 mg/mL) with some extracts, the inhibitory effect was as high as glyphosate, a weed-killer widely used in agriculture. At low concentrations (0.45-0.9 mg/mL) a high stimulatory effect of up to 200% was observed with some extracts. Overall, the results obtained supported the probable involvement of seed secondary metabolites in the allelopathic interactions of *Albizia* species with other plants and could be exploitable in the control of undesirable plants.

Keywords: *Albizia*, Seed Methanolic Extract, Phytotoxic, Seed Germination, Seedling Growth, Weeds, Invasive Plants, Allelopathy, Herbicide

1. Introduction

In agriculture and forestry, the need to protect crops and to restrict the damaging effects of invasive plants led for about thirty years to the development of a number of broad-spectrum synthetic herbicides such paraquat and especially glyphosate and its derivatives [1, 2].

The use of these compounds had not only released millions of farmers from the duty of manual weeding but also permitted to develop sustainable and more productive new agriculture systems [3].

Even the efficiency of these pesticides in short term has been proved, their harmful effects in long term are far from to be not significant. Indeed, it was observed that the intensive

use of herbicides resulted in flora impoverishment in regularly weeded parcels, weed resistance phenomena appeared and the persistence of these compounds make worse the risks of water pollution and soil degradation [2, 4-6].

Facing these disadvantages, there is an urgent need to explore and utilize naturally occurring products for combating harmful agricultural and public health pests [7, 8].

Many plants are known to have herbicidal properties. Extracts from the fresh leaves of Tree of Heaven (*Ailanthus altissima* L.) (Simaroubaceae) showed a strong plant germination and growth inhibitory effect in laboratory bioassays against alfalfa (*Medicago sativa*) [7]. The allelopathic potential of aqueous leaf and root extracts of *Aloe ferox* Mill. was evaluated against seed germination and seedling growth of three vegetables turnip (*Brassica rapa*), beetroot (*Beta vulgaris*) and carrot (*Daucus carota*) [9].

The current study is a part of a wide program on toxic plants aiming to find compounds of interest such as pesticides molecules [10-13]. It is a continuation of the previous works on the toxic activity of the endemic species of *Albizia* of Madagascar on various organisms [13-17]. It was conducted in order to determine the effects of the seed methanolic extracts from six other species (*A. bernieri*, *A. androyensis*, *A. divaricata*, *A. greveana*, *A. masikororum* and *A. viridis*) on the germination of seeds and the early growth of seedlings. Our objectives were to verify if these *Albizia* seed extracts toxic on animals were also phytotoxic and to investigate how they might be used in the control of undesirable plants.

2. Experimental

2.1. Plant Material

Albizia species are trees or treelets or shrubs. About 145 species of these plants are widely distributed in tropical regions. In Madagascar, 30 *Albizia* species are present, 24 of which are endemic [18].

2.1.1. Albizia Seeds

Origin and collection sites of *Albizia* seeds and herbarium references of the corresponding plants were already reported in a previous paper [17].

Voucher specimen of each plant was deposited in the herbarium of SNGF and in that of Plant Biology and Ecology Department of the Faculty of Sciences of the University of Antananarivo.

2.1.2. Vegetable Seeds

Seeds of bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), maize (*Zea mays*), rice (*Oryza sativa*), parsley (*Petroselinum crispum*), white tissam (*Brassica sp.*), onion (*Allium cepa*) and cucumber (*Cucumis sp.*) were provided by the Ministry of Agriculture.

2.1.3. Weed and Invasive Plant Seeds

The seeds of 5 plants considered as weeds (*Eragrostis pilosa*, *Panicum subalbidum* and *Cassia rotundifolia*) and invasive plants (*Acacia dealbata* and *Pinus kesiya*) in

Madagascar and/or elsewhere [19-21] came from SNGF. The herbarium reference numbers of these plants are presented in table 1.

Table 1. List and herbarium reference numbers of the studied weeds and invasive plants.

Plant	Herbarium reference number
<i>Eragrostis pilosa</i>	MSB 3139
<i>Panicum subalbidum</i>	MSB 3008
<i>Cassia rotundifolia</i>	13080
<i>Acacia dealbata</i>	12189
<i>Pinus kesiya</i>	13099

2.2. Seed Methanolic Extracts (SME) Preparation

Seed methanolic extracts (SME) of each *Albizia* species used for tests were prepared as previously described [17].

2.3. Assays on Seed Germination

2.3.1. Assays on Vegetable Seed Germination

The seed surface was sterilized by soaking in sodium hypochlorite solution (5%) for 1 min. Treated seeds were washed immediately and thoroughly with distilled water.

For each plant test, one lot of 10 seeds was separately soaked in the different extracts during 48 h in the dark and another lot soaked in distilled water served as control. Seeds were then transferred on to Petri dish lined with cotton or blotting paper soaked with extracts (tests) or with distilled water (control). Each treatment had four replicates. Over a period of 15 days, substrate was moistened every two days with extract (test) or water (control) and germinated seeds were counted. Results were expressed as germination percentage.

2.3.2. Assays on Weed and Invasive Plant Seed Germination

Cassia rotundifolia and *Acacia dealbata* seeds needed pretreatment consisting in soaking in boiling water and the whole was allowed to cool down during 24 h. That treatment was carried out because of the presence of inhibitors in the seed coats and the mechanical strength of seed coats against the embryo growth. The seeds of *Eragrostis pilosa*, *Panicum subalbidum* and *Pinus kesiya* were treated as the vegetable seeds.

Cassia rotundifolia, *Acacia dealbata*, *Pinus kesiya* seeds were allowed to germinate on sand substrate. Otherwise other experiment conditions were the same than described above for vegetable seed germination.

SME concentration used in all seed germination assays was 1 mg/mL. According to preliminary results, this concentration was the lowest one which was efficient on most of seeds.

2.4. Assays on Vegetable Seedling Development

Bean, pea, maize and rice were chosen as test plants because their seeds are comparatively large, germinate quickly enough and above all their hypocotyls and epicotyls were easy enough to measure.

Because the available seed amount was not sufficient *A. masikororum* was not studied and *A. greveana* was examined only on two test plants.

Seeds were soaked in water during 48 h at 30°C in the dark. Germinated seeds were transferred into Petri dishes lined with cotton or blotting paper soaked with water (control) or with extracts (tests) at increasing concentrations. Five concentrations (0.45, 0.9, 1.8, 3.6 and 7.2 mg/mL) were used except for *A. divaricata* SME on pea and maize where another range of weaker concentrations (0.06, 1 mg/mL) was used since at concentrations higher than 1 mg/mL seedling growth was totally inhibited.

Glyphosate, a specific inhibitor of the shikimate pathway which is widely utilized in agriculture as a non-selective weed killer, was used as a negative control at the concentration of 7.2 mg/mL recommended by the producer.

Substrate was moistened with extract (test) or water (control) and length measurement of epicotyls and hypocotyls were carried out every 2 days during 13 days [15]. The inhibitory effects of extracts were expressed in terms of hypocotyl and epicotyl length reduction. The relative germination ratio (RGR) was calculated as in [22] (1) and the inhibition rate was obtained from it (2):

$$\text{RGR} = \frac{\text{Germination ratio of test plant}}{\text{Germination ratio of control}} \times 100 \quad (1)$$

$$\text{Inhibition rate (\%)} = 100\% - \text{RGR} \quad (2)$$

3. Statistical Analysis

Table 2. Effects of *Albizia* SME (1 mg/mL) on vegetable seed germination.

Vegetable	Inhibition rate (%)						
	DW	<i>A. androyensis</i>	<i>A. bernieri</i>	<i>A. viridis</i>	<i>A. divaricata</i>	<i>A. greveana</i>	<i>A. masikororum</i>
Bean	0	50	40	55	40	100	42.1
Pea	0	0	7.5	25	90	85	43.8
Parsley	0	70	100	100	90	60	90.9
White tissam sp.	0	10	2.5	20	25	30	0
Cucumber	0	20	52.5	100	100	30	58.3
Onion	0	100	80	100	90	90	80
Maize	0	30	20	5	10	50	47.4
Rice	0	30	50	10	50	40	21.4

DW: distilled water

4.2. Effects of *Albizia* SME on Weed and Invasive Plant Seed Germination

Inhibition rates of the invasive plant seed germination by the *Albizia* SME were shown in table 3.

All weed and invasive plant seeds were sensitive to all *Albizia* SME. Among the tested plants, *Eragrostis pilosa* seemed to be the most sensitive with inhibition rate ranged between 96.67% (with *A. bernieri* SME) and 100% (with *A. greveana* SME), and *Pinus kesiya* the least sensitive with inhibition rate ranged between 12.50% (with *A. viridis* SME) and 37.50% (with *A. masikororum* SME). Effect of the same

One-way analysis of variance (ANOVA) which was followed by Newman Keuls comparison test with Statistix® software was used for statistical analysis. Statistical estimates were made at confidence interval of 95%.

4. Results

4.1. Effects of *Albizia* SME on Vegetable Seed Germination

When compared with a water control, most of *Albizia* SME exerted an inhibitory effect on the germination of vegetable seeds (table 2). Except for pea and white tissam seeds which could normally germinate in the presence of respectively *A. androyensis* and *A. masikororum* SME at 1 mg/mL, all the other treated vegetable seeds were sensitive to the *Albizia* SME but with variable responses. In most cases, the extent of the inhibitory effect could get as high as 100%. The lowest and the highest susceptibility were respectively observed on seeds of white tissam and onion where inhibition rates ranged between 0 and 30% for the former and 80 and 100% for the latter. Besides, the response of the test plants to the same extract treatment was different. As an example, *A. viridis* SME inhibited the germination of parsley, cucumber and onion by 100% but that of maize and rice only by 5 and 10% respectively.

It should be noted that some of non-germinating seeds decayed.

Albizia SME varied according to the test plants. As an example *A. viridis* SME inhibited the germination of *Cassia rotundifolia* by 100% and *Acacia dealbata* and *Pinus kesiya* by 40 and 12.50% respectively.

4.3. Effects of *Albizia* SME on Vegetable Seedling Growth

The effects of *Albizia* SME on the test plants growth monitored at the 13th day of experiment are presented in tables 4-9. Examples showing the evolution of hypocotyl and epicotyl elongation of the test plants every two days throughout the experiment are presented in fig. 1-6.

Table 3. Effects of *Albizia* SME (1 mg/mL) on weed and invasive plant seed germination.

Plant species	Inhibition rate (%)				
	Distilled water	<i>A. bernieri</i>	<i>A. greveana</i>	<i>A. masikororum</i>	<i>A. viridis</i>
<i>Eragrostis pilosa</i>	0	96.67	100.00	99.71	98.57
<i>Panicum subalbidum</i>	0	73.33	86.79	81.47	66.79
<i>Cassia rotundifolia</i>	0	70.00	90.00	67.50	100.00
<i>Acacia dealbata</i>	0	35.00	30.00	15.00	40.00
<i>Pinus kesyia</i>	0	20.00	22.50	37.50	12.50

Table 4. Inhibition rates (%) of bean hypocotyl (H) and epicotyl (E) elongation at the 13th day of treatment with the *Albizia* SME.

<i>Albizia</i> SME (mg/mL)		<i>A. androyensis</i>	<i>A. bernieri</i>	<i>A. viridis</i>	<i>A. divaricata</i>
0	H	0	0	0	0
	E	0	0	0	0
0.45	H	41.8	11.4	32.3	-3.5*
	E	43.9	18.2	-7.7*	-173.5*
0.9	H	52.6	19.8	13.7	68.5
	E	51.0	33.5	-14.1*	18.0
1.8	H	58.5	11.9	24.9	80.1
	E	59.4	14.5	-9.0*	79.1
3.6	H	70.5	52.2	30.4	95.7
	E	90.3	69.9	18.8	89.8
7.2	H	83.6	56.5	33.2	100.0
	E	94.1	62.4	44.4	100.0

* : a negative sign before a number indicates a stimulatory effect.

Table 5. Inhibition rates (%) of maize hypocotyl (H) and epicotyl (E) elongation at the 13th day of treatment with the *Albizia* SME.

<i>Albizia</i> SME(mg/mL)		<i>A. androyensis</i>	<i>A. bernieri</i>	<i>A. viridis</i>	<i>A. divaricata</i>	<i>A. greveana</i>
0	H	0	0	0	0	0
	E	0	0	0	0	0
0.45	H	71.0	-12.1*	-206.9*	-204.3*	68,4
	E	91.5	40.7	52.4	40.4	95,2
0.9	H	80.3	-37.9*	-145.7*	-183.6*	89,9
	E	97.3	25.1	68.0	29.4	92,2
1.8	H	86.4	-37.1*	-69.8*	11.2	82,8
	E	99.1	55.6	57.1	88.1	91,1
3.6	H	94.5	58.6	-9.5*	56.0	91,4
	E	99.1	89.2	86.0	92.6	97,8
7.2	H	98.6	72.4	42.2	100.0	91,4
	E	99.3	87.7	92.2	100.0	97,8

* : a negative sign before a number indicates a stimulatory effect

Table 6. Inhibition rates (%) of pea hypocotyl (H) and epicotyl (E) elongation at the 13th day of treatment with the *Albizia* SME.

<i>Albizia</i> SME(mg/mL)		<i>A. androyensis</i>	<i>A. bernieri</i>	<i>A. viridis</i>	<i>A. greveana</i>
0	H	0	0	0	0
	E	0	0	0	0
0.45	H	91.8	72.2	55.1	85,4
	E	74.6	-16.4*	-24.6*	76,7
0.9	H	95.4	75.4	61.3	83,7
	E	90.3	-16.8*	18.5	86,2
1.8	H	96.6	79.5	59.0	84,6
	E	81.7	53.3	4.9	84,9
3.6	H	96.9	85.4	66.4	87,1
	E	87.9	81.9	13.2	91,5
7.2	H	97.3	87.7	68.7	89,0
	E	93.7	83.5	6.5	95,7

* : a negative sign before a number indicates a stimulatory effect

Table 7. Inhibition rates (%) of rice hypocotyl (H) and epicotyl (E) elongation at the 13th day of treatment with the *Albizia* SME.

<i>Albizia</i> SME(mg/mL)		<i>A. androyensis</i>	<i>A. bernieri</i>	<i>A. viridis</i>
0	H	0	0	0
	E	0	0	0
0.45	H	37.3	88.5	55.1
	E	29.3	-16.4*	87.0
0.9	H	46.3	90.2	58.2
	E	57.1	-16.8*	85.7
1.8	H	51.0	91.3	77.4
	E	63.4	53.3	79.7
3.6	H	58.0	88.0	68.3
	E	75.7	81.9	88.4
7.2	H	82.7	91.5	76.7
	E	95.0	83.5	91.6

As compared to control, all the *Albizia* SME had an effect on all the test plants. However, the sensitive test plant(s) and the nature (stimulation and/or inhibition) and the extent of the effect were variable.

Table 8. Inhibition rates (%) of rice and pea hypocotyl (H) and epicotyl (E) elongation at the 13th day of treatment with the *A. divaricata* SME.

<i>A. divaricata</i> SME (mg/mL)		Pea	Rice
0	H	0	0
	E	0	0
0.06	H	38.3	12.5
	E	3.7	58.6
0.125	H	52.3	23.0
	E	24.7	64.3
0.5	H	60.4	69.3

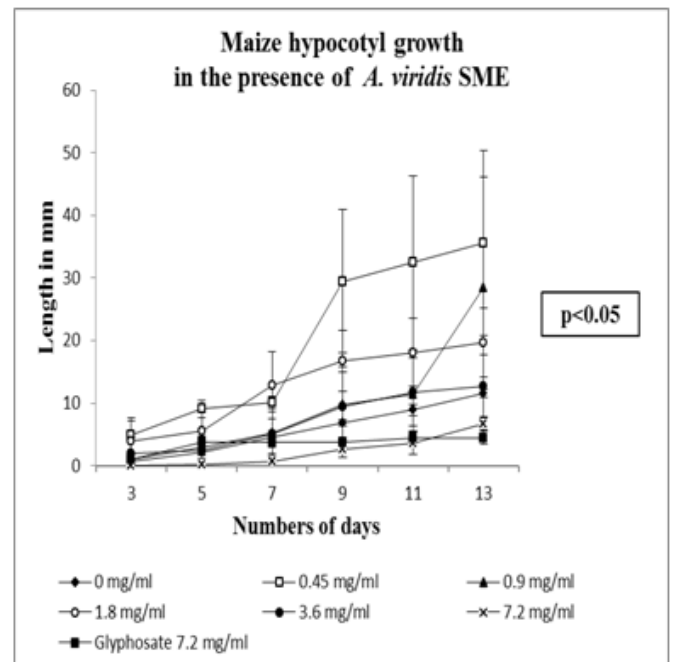
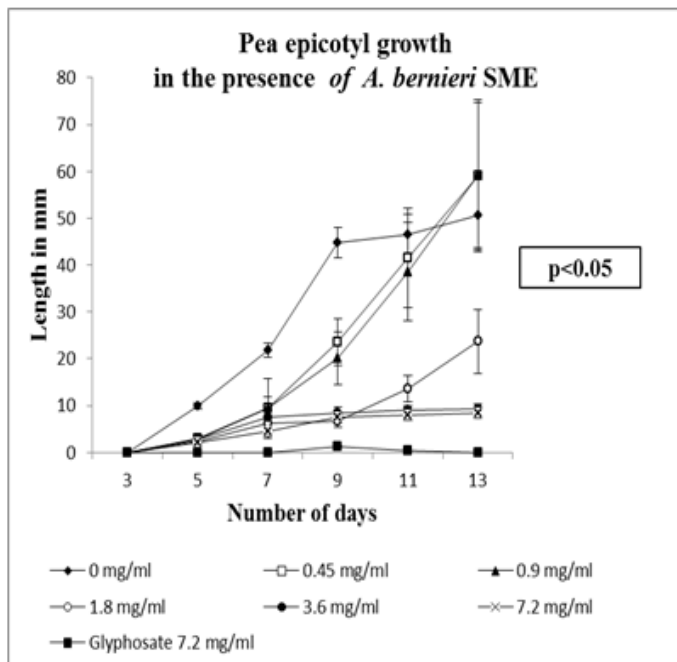
<i>A. divaricata</i> SME (mg/mL)		Pea	Rice
1.25	E	58.6	60.0
	H	76.1	93.0
	E	74.8	74.3
1	H	99.7	100.0
	E	74.8	81.6

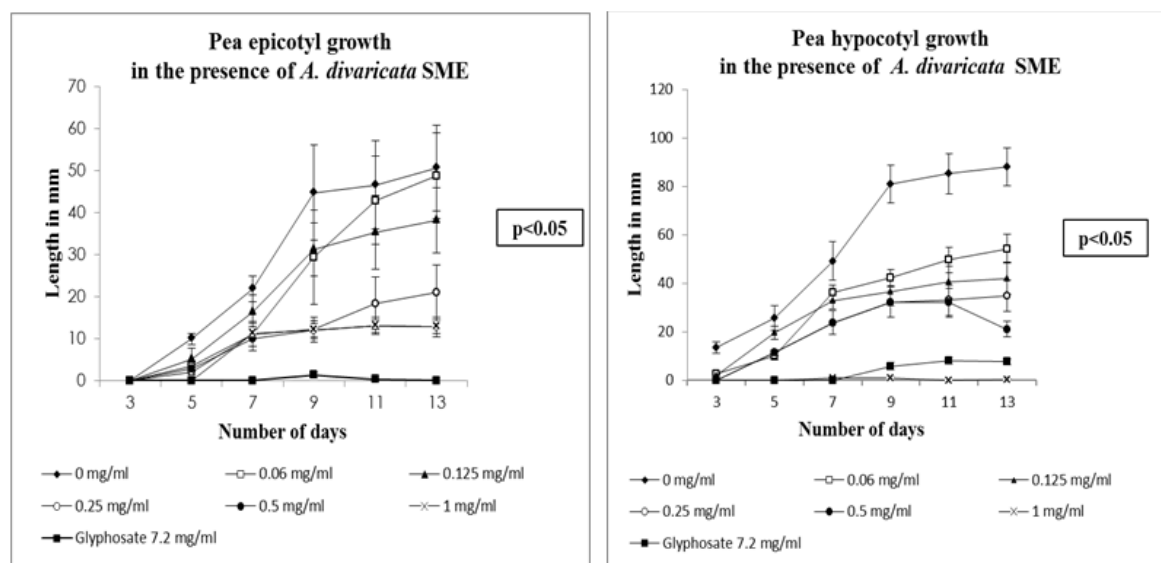
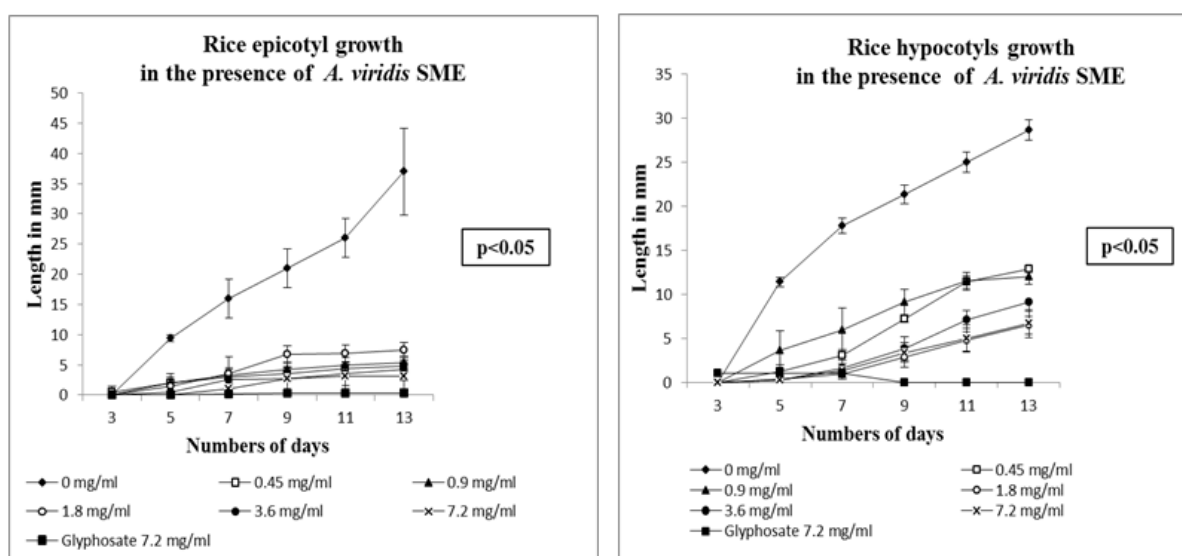
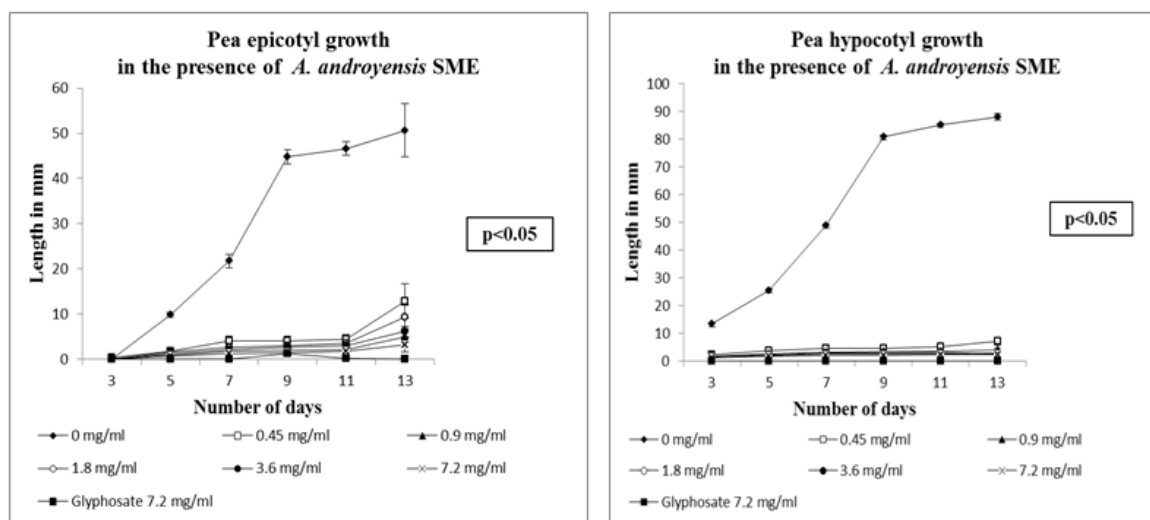
4.3.1. Stimulatory Effects *Albizia* SME

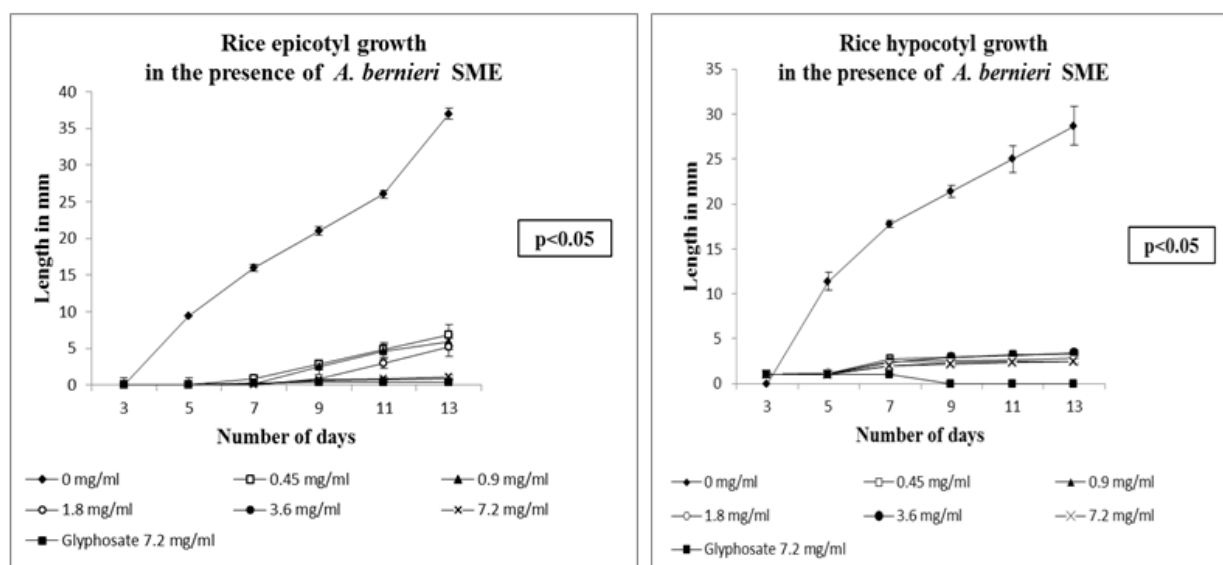
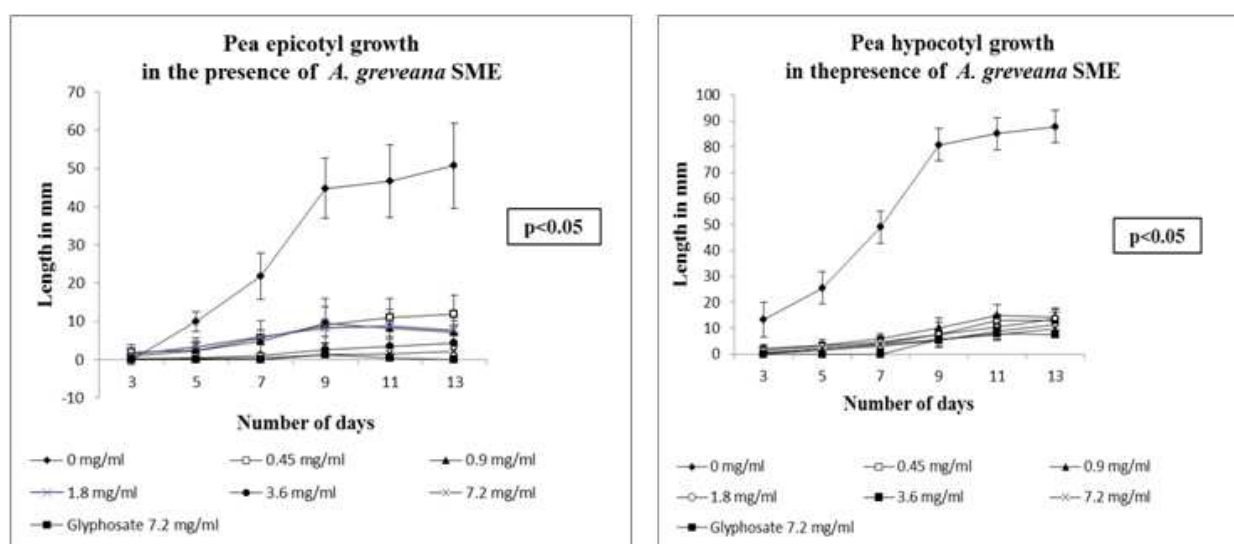
Except for *A. androyensis* and *A. greveana* SME the three other *Albizia* SME exhibited a stimulatory effect on some test plants. Stimulation was generally observed at low concentrations (0.45 and 0.9 mg/mL) but in maize treated with *A. viridis* (table 6) it still demonstrated at 3.6 mg/mL. In a same test plant stimulation concerned the elongation of either only hypocotyl (e.g. Maize treated with *A. bernieri*, *A. divaricata* and *A. viridis*) (table 6 and fig. 1) or only epicotyl (e.g. pea and rice treated with *A. bernieri*) (table 8 and fig. 1).

4.3.2. Inhibitory Effects of *Albizia* SME

Except for the stimulatory effect at low concentrations, significant inhibitory effect ($p < 0.05$) was observed at the other concentrations. Globally, inhibitory potential significantly ($p < 0.05$) increased with increasing concentrations of *Albizia* SME (fig. 2, 3, 5). However, the extent of the inhibitory effect varied according to both *Albizia* SME and test plant (tables 5-8 and fig. 2-6). For example, on pea hypocotyl growth inhibition rates varied between 55.1 and 68.7% when treated with *A. viridis* SME and between 91.8 and 97.3% when treated with *A. androyensis* SME (table 6); on hypocotyl growth treated with *A. bernieri* SME inhibition rates varied between 11.4 and 56.5% in bean (table 5) and 88.5 and 91.5% in rice (table 7).

**Fig. 1.** Stimulatory effects of *Albizia* SME on hypocotyl and epicotyl of test plants.

Fig. 2. Effects of *A. divaricata* SME on pea seedling growth.Fig. 3. Effects of *A. viridis* SME on rice seedling growth.Fig. 4. Effects of *A. androyensis* SME on pea seedling growth.

Fig. 5. Effects of *A. bernieri* SME on rice seedling growth.Fig 6. Effects of *A. greveana* SME on pea seedling.

The hypocotyl and epicotyl length was both reduced (tables 5-8 and fig. 2-6). In most cases the hypocotyl length was more affected than the epicotyl length whereas in other cases the opposite result was observed as in bean treated with *A. androyensis* SME (hypocotyl inhibited by 83.6% and epicotyl by 94.1%) and with *A. viridis* SME (hypocotyl inhibited by 33.2 % and epicotyl by 44.4% (table 4).

The most strongly inhibitory effect was observed on pea and rice treated with *A. divaricata* SME at 1 mg/mL where hypocotyl length was reduced by 99.7% and 100% respectively (table 8 and fig. 2). The inhibitory potential of *A. androyensis* SME on maize (table 5) and pea (tab. 6) was also worth pointing out: the hypocotyl and epicotyl development was indeed strongly affected with inhibition rate ranging from 71.0% to 98.6% and 91.5% to 99.3% respectively at the used concentrations (0.45-7.2 mg/mL).

At the same concentration (7.2 mg/mL) the inhibitory effect of some *Albizia* SME was as high as glyphosate: for example

on maize (table 5) and bean (table 4) treated with *A. divaricata* SME the inhibition rate reached 100% for both hypocotyl and epicotyl.

5. Discussion

As far as we know, except our previous investigations [15], *Albizia samans*, seemed to be the only species in the *Albizia* genus studied on phytotoxicity point of view [22].

The results demonstrated that *Albizia* SME contain some phytochemicals capable of affecting both germination and seedling growth.

The response of various test species to the same extract treatment was different. This may be due to physiological or morphological difference in them [22].

It was reported that root is higher sensitive to phytotoxic compounds than shoot [24]. Root tissue is more permeable to allelochemicals than shoot tissue [25]. By contrast, several

Albizia SME exhibited more pronounced inhibitory effect on epicotyl than on hypocotyl.

In some cases, *Albizia* SME exhibited inhibition rates as high as pure glyphosate at the same concentration (7.2 mg/mL). Pure active principles from these extracts could be expected to be as efficient if not more so as glyphosate.

The inhibitors involved in the *Albizia* SME phytotoxicity might interact with basic physiological and biochemical processes. Hormonal balance, protein synthesis, photosynthesis, respiration, chlorophyll production, plant-water relations and permeability may be disturbed by allelochemicals [26, 27]. The allelochemicals might have also prevented water uptake [28] or caused an alteration in the synthesis or activation of gibberellic acid, a growth promoting hormone [29].

Stimulatory effect on seedling growth at low concentrations was considered as a common phenomenon in several plant extracts [30]. This was also observed in some *Albizia* SME but not in others where inhibitory effects were recorded at the same concentrations. Such result was reported about the action of a phenolic compound isolated from *Carya cathayensis* on the growth of different plant seedling [31]. A stimulatory effect up to 200%, much higher than that exerted by reglone, the principal component of walnut husk, on lettuce and radish growth (160%) [32] was observed.

At the same concentration a same *Albizia* SME had a stimulatory effect on some test plants but an inhibitory effect on others. Moreover, the level of its stimulation or inhibition activity was variable. Those results suggested that the *Albizia* SME effects were selective.

Although phenolic compounds were the allelochemicals often found to be involved in seed and seedling growth inhibition [30, 34-36], they could not be responsible of the *Albizia* SME phytotoxicity. Indeed, according to phytochemical study on *Albizia* species SME, except for *A. bernieri* SME, phenolic compounds were absent [17]. Alkaloids and saponins the most frequent secondary metabolites in most of these *Albizia* seeds and other chemical groups such as terpenoids and heterosides might be involved in their phytotoxic activity. Other potential inhibitors of germination and seedling growth such as terpenes, glucosides, alkaloids, amino acids and sugars were reported [37-42]. Total alkaloids from seeds of the medicinal plant *Peganum harmala* L. were found to possess strong growth inhibitory effect on lettuce, wheat, amaranth and ryegrass [23].

Preliminary results so far obtained on weeds and invasive plants here studied were encouraging. Thus the phytotoxic compounds of Malagasy *Albizia* seeds could be further explored as natural herbicides and plant growth regulators. Natural products would likely be very biodegradable, thus posing less risk to the environment [23]. There have been successful examples of using natural products, including allelochemicals, as sources to develop commercial herbicides [43]; for instance, mesotrione, a synthesized analogue of leptospermane that is produced by *Callistemon citrinus* [44], and cinmethylin, a derivative of 1,4-cineole that is a natural phytotoxin found in the essential oils of a number of plants

[45]. More importantly, natural phytotoxins were found to act on a large number of unexploited herbicide target sites, which can be used to deal with the rapid evolving resistance to synthetic herbicides [46].

For the *Albizia* plants, the seed secondary metabolites may represent an efficient tool for self-defense against predation and play an important role in allelopathic interactions with other plants. There is need to examine the chemical nature of inhibitors and carry out field experiments to ascertain the ecological role of allelopathic potential of the secondary metabolites in *Albizia* seeds.

6. Conclusion

All the *Albizia* SMEs were found to inhibit the seed germination of vegetables, weeds and invasive plants. They also inhibited the early seedling growth of test plants and in some cases, inhibition was as high as glyphosate a widely weed-killer used in agriculture. Their phytotoxicity was likely involved in a defense process against animal predation and in allelopathic interactions with other plants and therefore their use as herbicide might be explored. On the other hand, some of them strongly stimulated the seedling growth up to 200% at low concentrations and under this condition they might also be used to promote seedling growth.

Purification of active principles from each *Albizia* extract and determination of their chemical nature and mode of action are the priority studies required to complete those preliminary works. They are in progress in our laboratory.

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