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# Antifungal Activity of Securidaca longepedunculata and Acacia gourmaensis Hydro-ethanolic Extracts against Three Rice Seed-borne Fungi

Léon W. Nitiema<sup>1\*</sup>, Fabrice W. Nikiema<sup>1</sup>, Drissa Sérémé<sup>1</sup> and Pierre A. E. D. Sombié<sup>1</sup>

<sup>1</sup>Centre National de la Recherche Scientifique et Technologique (CNRST), Institut de l'Environnement et de Recherches Agricoles (INERA), 01 BP 476 Ouagadougou 01, Burkina Faso.

#### Authors' contributions

This work was carried out in collaboration with all authors. Author LWN designed the study, managed the literature searches. Authors LWN and PAEDS conducted sample collections. Authors LWN, FWN and PAEDS conducted the bench work. Author LWN performed the statistical analysis and wrote the first draft of the manuscript. Authors LWN, FWN, PAEDS and DS corrected the manuscript. All authors read and approved the final manuscript.

#### Article Information

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#### **ABSTRACT**

**Aims:** This study was undertaken to investigate the antifungal activities of *Securidaca longepedunculata* and *Acacia gourmaensis* bark hydro-ethanolic extract against *Fusarium solani, Fusarium moniliforme* and *Curvularia lunata* and to evaluate the percentages of germination and infection of infected rice seeds.

**Methods:** Different extract concentrations ranging from 0.25, 0.5 and 1% were tested during 15 days using poisoned food technique method for *in vitro* antifungal activity against above three fungal strains. The same concentrations of extract were used to evaluate *in vivo* antifungal activity on rice seeds infected by these three fungal strains.

**Results:** The extract of Securidaca longepedunculata had antifungal effect on Fusarium solani and Fusarium moniliforme and completely inhibited its mycelial growth at all tested concentrations (0.25, 0.5 and 1%). Curvularia lunata mycelial growth was inhibited of 84.7% by 1% Securidaca

longepedunculata extract after five days of culture. However, mycelial growth of *F. solani*, *F. moniliforme* and *C. lunata* were increased with increasing concentration of *Acacia gourmaensis* extract. It has been observed that when *Securidaca longepedunculata* hydro-ethanolic extract concentration increased up to 1%, seeds germination percentage decreased for all infection. In contrast, the opposite was observed for *Acacia gourmaensis* extract. Likewise, seeds infection decreased with the highest concentration (1%) of *Securidaca longepedunculata* for all infection, whereas the opposite was observed with *Acacia gourmaensis* extract.

**Conclusion:** This study showed that *S. longepedunculata* hydro-ethanolic extract has more antifungal activity against seed-borne fungi (*F. solani, F. moniliforme* and *C. lunata*) than *A. gourmaensis in vitro* and *in vivo*. Results show that *Securidaca longepedunculata* hydroethanolic bark extract can be used as a potential antifungal agent in the management of some rice fungal diseases. *Acacia gourmaensis* extract, at low concentration, could be used in rice seed treatment to increase seed germination.

Keywords: Antifungal; Securidaca longepedunculata; Acacia gourmaensis; rice seed-borne fungi; germination.

#### 1. INTRODUCTION

Rice is an important staple food crop worldwide. In Burkina Faso, losses due to fungal infections range from 20 to 80% [1]. During seedling establishment, rice may be infected by fungal pathogens [2]. The most common fungal species associates in rice all over the world are Fusarium spp., Curvularia lunata, Bipolaris oryzae, Pvricularia orvzae. Sarocladium orvzae. Rhizoctonia solani. Microdochium oryzae, Sclerotium rolfsii, Nigrospora oryzae, Phoma glumarum, and Cladosporium sp. [3]. There are causing rice pre- and post-infections and considerable quality losses, seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value reduction [4,2].

In general, phytopathogenic fungi are controlled by synthetic fungicides. However, their use is increasingly restricted due to the harmful effects of pesticides on human health and the environment [5]. The regulations on the use of chemicals and the emergence of pathogens resistant to the products justify the search for novel active molecules and new control strategies.

Currently, a particular interest is then focused on local biologically based compounds of plant extracts with the aim to use them as seeds treatment products to control fungal diseases. They constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinones, tannins, alkaloids, saponins, and sterols [6]. Being natural products, the use of plant extract is safe for producers, the environment and the cost is low compared to

synthetic pesticides [7]. Plant extracts, used as natural pesticides, could reduce the incidence of seed-borne fungi and increase the percentage of germination and seedling emergence [8-10].

Securidaca longepedunculata Fres., commonly known as violet tree, is a savanna grown medicinal plant belonging to the Polygalaceae family. It is commonly used as a medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache and various other inflammatory conditions [11]. Dried roots powder is also used as a pest control agent in storage, and methanol extracts of the roots have the potential to protect against insect pests and microbial agents [12,13].

Acacia gourmaensis A. Chev. is a member of the Fabaceae family present in Burkina Faso, Côte d'Ivoire, Niger and Nigeria [14]. The aqueous extract of this plant is used for antifungal activity [15]. Particularly, Acacia gourmaensis antimicrobial activity study is limited.

Plant extract preparation using hydro-alcoholic solvents allows the extraction of more biologically active compounds [6]. In this study, the aim was to test hydro-ethanolic extract of *Securidaca longepedunculata and Acacia gourmaensis* for its efficacy against tree seed-borne fungi (*Fusarium moniliforme*, *Fusarium solani* and *Curvularia lunata*) of rice seeds and evaluated the percentages of seeds germination and infection.

# 2. MATERIALS AND METHODS

## 2.1 Plant Material

Fresh stem bark of Securidaca longepedunculata and Acacia gourmaensis was harvested from

different trees during May 2018 in Mogtedo localized in the Plateau-Central region of Burkina Faso. The plant material was washed with tap water to remove debris and dust particles and then rinsed with sterile distilled water. It was dried under shade at 25°C, pulverized with a pestle and mortar, finally kept in a sterile transparent polyethylene bag and stored at 4°C until used.

Samples of healthy rice (*Oryza sativa* L.) seeds of the popular variety FKR19 were kindly provided by the Rice Program of the Institute of Environment and Agricultural Research (INERA) in 2018.

#### 2.2 Preparation of Extract

Fifty grams (50 g) of the plant material powder was extracted by using 500 mL of ethanol (70%) under mechanical agitation at room temperature during 24 hours. The mixture was filtered, concentrated and lyophilized by using a freezedrying system to give the hydro-ethanolic extract [16].

## 2.3 Fungal Pathogens

The isolates of *Fusarium moniliforme*, *Fusarium solani* and *Curvularia lunata* used in this study are from the fungal collections of INERA. The pathogens were isolated from infected rice seeds collected in Burkina Faso, stored in the laboratory and activated on potato dextrose agar (PDA) before use. *In vitro* and *in vivo* experiments were performed using 7-day-old cultures of three pathogens [17].

## 2.4 In vitro Antifungal Assays

Food poison technique was used to determine the antifungal effects of different concentrations of the extract, according to the procedure described by Abou-Jawdah et al. [18] and Švecová et al. [19]. The plant extract, sterilized by filtration through 0.2 µL Millipore filter, was added to the culture medium (potato dextrose agar) after autoclaving when the temperature of the medium reached 50°C and mixed thoroughly. The final volume of the extract in 20 ml of the culture medium per each Petri dish was adjusted to three different final concentrations (0.25, 0.5 and 1%). The culture medium plates unamended with plant extract were used as controls. Mycelial growth inhibition tests were performed by placing in the center of each plate one piece of 5 mm mycelial agar disc cut from the margin of seven days old cultures of Fusarium solani, Fusarium moniliforme and Curvularia lunata [20]. The

diameter of colonies was measured after incubation for 5, 10 and 15 days in the dark, at 22°C. All treatments were replicated three times. The percentage of inhibition was calculated by comparing the treated plates with the control, whose inhibition was established as 0%. Percentage inhibition of mycelia growth was calculated by using the formula [21]:

$$% Inhibition = \frac{dc - dt}{dc} X 100$$

where:

dc = Average increase in mycelium growth in control:

dt = Average increase in mycelium growth in treatment.

## 2.5 In vivo Antifungal Assays

Rice seeds were disinfected with sodium hypochlorite 15% for 10 minutes [22]. Seeds were inoculated by spraying 10<sup>5</sup> spores/ml of Fusarium moniliforme, Fusarium solani and Curvularia lunata strains respectively for 24 hours [23]. The control is treated in the same way with each fungus. Four hundred rice seeds pre-inoculated with each fungus were soaked in 20 ml suspensions of different concentrations (0.25, 0.5 and 1%) of S. longepedunculata and A. gourmaensis extracts for 24 hours [24]. Seeds were dried in the laminar flow chamber on sterile blotter papers for 2 hours. Treated seeds were then spread on blotter paper in Petri dishes (25 seeds per Petri dish) and incubated at 25°C for seven days under alternating cycles of light and darkness of 12 hours each and examined for percentages of seeds germination and infection.

## 2.6 Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 20.0 software. Means for all treatments were separated using Tukey's multiple analysis test (P < 0.05) to determine the significant differences between treatments.

#### 3. RESULTS AND DISCUSSION

## 3.1 In vitro Antifungal Assays

# 3.1.1 *In vitro* antifungal activity of *Securidaca longepedunculata*

Effect of Securidaca longepedunculata hydroethanolic extract on the linear mycelial growth of

Fusarium solani, Fusarium moniliforme and Curvularia lunata in vitro tests is showed in Table The inhibitory effect of Securidaca longepedunculata on fungi was assessed at 0.25, 0.5 and 1% concentrations during 5, 10 and 15 days incubation. Results indicated that all treatments were positively effective in reducing the linear mycelial growth of the pathogens comparing with the control. The minimum inhibitory concentration of Securidaca longepedunculata extract was 0.25% for Fusarium solani and Fusarium moniliforme after five days of incubation. At this concentration, 100% of these fungi were inhibited. Similar observations were performed at the 10th and 15th day of fungal culture incubation. However, Curvularia lunata was inhibited by 84.7% at the concentration of 1% after five days of incubation. The inhibitory effect on C. lunata at 1% was significantly higher (P < 0.05) than 0.25% and 0.5% concentrations. The same train was observed at the 10<sup>th</sup> and 15<sup>th</sup> day of *C. lunata* culture incubation. Curvularia lunata required extract concentration higher than 1% to reach the minimum inhibitory concentration. It is evident that the inhibitory effect of the Securidaca longepedunculata hydro-ethanolic extract on mycelial growth of fungi varied among the fungal species [25]. Inhibition of the growth of these fungal pathogens may be due to phenolic compounds present Securidaca in longepedunculata. Therefore. previous phytochemical investigation on the bark of Securidaca longepedunculata hydro-ethanolic extract showed the presence of flavonoids, tannins, saponins, glycosides, alkaloids, balsams, steroids and saponin glycosides [26]. Numerous valuable antimicrobial compounds, including xanthones, some benzyl benzoates and triterpene saponins, amongst others, were found from various parts of S. longepedunculata [11].

# 3.1.2 In vitro antifungal activity of Acacia gourmaensis

The effect of *Acacia gourmaensis* hydroethanolic extract on the linear mycelial growth of *Fusarium solani, Fusarium moniliforme and Curvularia lunata in vitro* tests is shown in Table 2. Five days after incubation, at concentrations of 0.25%, 0.5% and 1% of extract the percentages of mycelial growth inhibition were respectively 34.2%, -6.9% and -41.7% for *Fusarium solani*; 100%, 40% and 37.5% for *Fusarium moniliforme* and 88.9%, 74.1% and 72.2% for *Curvularia lunata*.

Results showed that the mycelial growth increase with increasing concentration of the extracts from 0.25%, 0.5% and 1% (P < 0.05). increase is more important This Fusarium solani with negatives inhibitions than other fungi. Inhibition rates of all the tested fungi decreased through the end of incubation. These results suggest that the optimal concentration of Acacia gourmaensis extracts was 0.25%. Antifungal activity of the extract against fungi is done in a none concentration dependent manner. High concentrations of Acacia extract stimulated mycelium gourmaensis growth.

The mechanisms of action of Acacia gourmaensis against Fusarium solani, Fusarium moniliforme and Curvularia lunata are unknown. There is no data available in the literature on phytochemical aspects of A. gourmaensis. Nevertheless, some authors have observed the negative impact of plant extracts on fungal growth. Demirci and Dolar [27] observed that lentil, onion, radish and garden cress extracts stimulated mycelial growth of Phytophthora capsici, which used these extracts as a source of energy.

#### 3.2 In vivo Antifungal Assays

# 3.2.1 *In vivo* antifungal activity of *Securidaca longepedunculata*

The effect of different concentrations of Securidaca longepedunculata extract on rice seeds germination and infection was studied and the results are presented in Table 3. Three concentrations (0.25, 0.5 and 1%) of the hydroethanolic extract were tested on rice seeds germination and infection. In general, seeds germination decreased with increasing extract concentration. This reduction in germination is more important with high concentration (1%) for three fungi (P < 0.05). On the other hand, seeds infection slightly decreased with concentrations compared to low concentrations of extract (P < 0.05 for Fusarium solani). In vivo assay confirmed the results of in vitro tests since the efficacy of Securidaca longepedunculata extract at the highest concentration (1%) also inhibited mycelial growth.

The inhibitory effect of Securidaca longepedunculata on seeds germination was confirmed by Ogukwe et al. [28]. Indeed, they found that the high concentration of *S. longepedunculata* roots ethanol extract inhibited

Sorghum bicolor seeds germination comparing to control ones. Likewise, Mongalo et al. [11] in toxicity studies revealed that *S. longepedunculata* extracts are only toxic at relatively high concentrations both *in vivo* and *in vitro*.

Seed-borne fungi (*F. solani*, *F. moniliforme*, *C. lunata*) can be controlled by seeds treatment with a low concentration of *S. longepedunculata* hydro-ethanolic extract.

# 3.2.2 In vivo antifungal activity of Acacia gourmaensis

The effect of different concentrations of *Acacia* gourmaensis extract on rice seeds germination an infection was studied and the results are presented in Table 4. *Acacia gourmaensis* hydro-

ethanolic extract used in the experiment increased rice seeds germination slightly with increasing concentration compared to untreated control (P < 0.05 for Fusarium moniliforme).

However, seeds infection increased slightly with increasing concentration (P < 0.05 for F. solani and C. lunata).

The stimulatory effect of *Acacia gourmaensis* extract in our study was confirmed by Zida, et al. [14]. They found that *A. gourmaensis* aqueous extract used in Sorghum and Pearl Millet seeds treatment enhanced seedling emergence and promoted plant growth. A similar increase in fresh seedling weight after treatment of rice seeds with fungal and bacterial bio-control agents have been reported [29].

Table 1. In vitro inhibition of Fusarium solani, Fusarium moniliforme and Curvularia lunata mycelial growth by Securidaca longepedunculata hydro-ethanolic extract

Time (Days)	Treatment	Percentage of mycelium growth inhibition				
		F. solani	F. moniliforme	C. lunata		
5	T0	0a	0a	0a		
	T1	100b	100b	77bc		
	T2	100b	100b	69.4b		
	T3	100b	100b	84.7c		
10	T0	0a	0a	0a		
	T1	100b	100b	78.9bc		
	T2	100b	100b	76.3b		
	T3	100b	100b	87.1c		
15	T0	0a	0a	0a		
	T1	100b	100b	78.9b		
	T2	100b	100b	76.3b		
	T3	100b	100b	87.8c		

T0: Control; T1: S. longepedunculata extract 0.25%; T2: S. longepedunculata extract 0.5%; T3: S. longepedunculata extract 1%.

Table 2. *In vitr*o inhibition of *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata* mycelial growth by *Acacia gourmaensis* hydro-ethanolic extract

Time (Days)	Treatment	Percentage of mycelium growth inhibition				
		F. solani	F. moniliforme	C. lunata		
5	T0	0c	0a	0a		
	T1	34.2d	100c	88.9c		
	T2	-6.9b	40b	74.1b		
	T3	-41.7a	37.5b	72.2b		
10	T0	0c	0a	0a		
	T1	-11.8b	100b	65.8c		
	T2	-11.8b	33.7a	65.8c		
	T3	-76.5a	33.1a	63.2b		
15	T0	0b	0a	0a		
	T1	-20b	100c	64c		
	T2	-18.3b	61b	57.9b		
	T3	-66.7a	12a	57.9b		

T0: Control; T1: A. gourmaensis extract 0.25%; T2: A. gourmaensis extract 0.5%; T3: A. gourmaensis 1%.

Table 3. Effect of Securidaca longepedunculata hydro-ethanolic extract treatment on rice seeds germination and infection

Treatment	Fusarium solani		Fusarium moniliforme		Curvularia lunata	
	Germination	Infection	Germination	Infection	Germination	Infection
	(%)	(%)	(%)	(%)	(%)	(%)
T0	76b	16b	60ab	10a	88b	36a
T1	76b	6a	80c	6a	80b	18a
T2	64ab	2a	66b	0a	60a	6a
T3	50a	0a	54a	0a	72ab	8a

T0: Control; T1: S. longepedunculata extract 0.25%; T2: S. longepedunculata extract 0.5%; T3: S. longepedunculata extract 1%.

Table 4. Effect of *Acacia gourmaensis* hydro-ethanolic extract treatment on rice seeds germination and infection

Treatment	Fusarium solani		Fusarium moniliforme		Curvularia lunata	
	Germination	Infection	Germination	Infection	Germination	Infection
	(%)	(%)	(%)	(%)	(%)	(%)
T0	76a	16a	60a	10a	88a	36a
T1	76a	20a	80b	6a	86a	20a
T2	80a	36b	88b	6a	96a	40b
T3	80a	38b	82b	8a	92a	98b

T0: Control; T1: A. gourmaensis extract 0.25%; T2: A. gourmaensis extract 0.5%; T3: A. gourmaensis 1%.

The effect of *A. gourmaensis* extracts on seeds germination could be favored by their antifungal effect and mostly by extract phytochemical compounds used as an energetic source [27].

In the present study, *A. gourmaensis* is involved in the seeds germination process and not efficient in controlling seed pathogens *in vitro*.

#### 4. CONCLUSION

study showed that Securidaca longepedunculata hydro-ethanolic extract has more antifungal activity against seed-borne fungi (Fusarium solani, Fusarium moniliforme and Curvularia lunata) than Acacia gourmaensis in vitro and in vivo. High concentrations of S. longepedunculata extract decrease rice seeds germination and decrease fungal infection while the opposite was observed with A. gourmaensis. Therefore, S. longepedunculata plant extract can be considered as an alternative to control rice fungal diseases while A. gourmaensis can be used for seeds treatment. However, our S. longepedunculata and A. gourmaensis hydroethanolic extract phytochemical composition should be explored to determine the active fungicidal compounds.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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