

Original Research Article

Antifungal activity of *Securidaca longepedunculata* and *Acacia gourmaensis* hydro-ethanolic extracts against three rice seed-borne fungi

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 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 % extract after five days of culture. However, mycelial growth of *F. solani*, *F. moniliforme* and *C. lunata* was 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 fungi. In contrast, the opposite was observed for *Acacia gourmaensis* extract. Likewise, seeds infection decreased with the highest concentration (1%) of *Securidaca longepedunculata* for all fungi, 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* hydro-ethanolic 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 associated in rice all over the world are *Fusarium* spp., *Curvularia lunata*, *Bipolaris oryzae*, *Pyricularia oryzae*, *Sarocladium oryzae*, *Rhizoctonia solani*, *Microdochium oryzae*, *Sclerotium rolfsii*, *Nigrospora oryzae*, *Phoma glumarum*, and *Cladosporium* sp. [3]. They 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 [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] [9][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 the member of the Fabaceae family known in Burkina Faso. The aqueous extract of this plant is used for antifungal activity [14]. 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. MATERIAL 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 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 were 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 freeze-drying system to give the hydro-ethanolic extract.

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.

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.* [15] and Švecová *et al.* 2017 [16]. 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*. 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:

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

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 [17]. Seeds were inoculated by spraying 10^5 spores/ml of *Fusarium moniliforme*, *Fusarium solani*, and *Curvularia lunata* strains respectively for 24 hours [18]. 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 [19]. 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* hydro-ethanolic extract on the linear mycelial growth of *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata* in vitro tests is showed in Table 1. 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 ($P < 0.001$) than 0.25% and 0.5% concentrations. The same train was observed at the 10th and 15th 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 [20]. Inhibition of the growth of these fungal pathogens may be due to phenolic compounds present in *Securidaca longepedunculata*. Therefore, previous phytochemical investigation on bark of *Securidaca longepedunculata* hydro-ethanolic extract showed the presence of Flavoloids, tannins, saponins, glycosides, alkaloids, Balsams steroids and Saponin Glycosides [21]. Numerous valuable antimicrobial compounds, including xanthenes, some benzyl benzoates and triterpene saponins, amongst others, were found from various parts of *S. longipedunculata* [11].

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		
		<i>F. solani</i>	<i>F. moniliforme</i>	<i>C. lunata</i>
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	0	0a	T0a
	T1	100a	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%.

3.1.2 *In vitro* antifungal activity of *Acacia gourmaensis*

The effect of *Acacia gourmaensis* hydro-ethanolic extract on the linear mycelial growth of *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata* in vitro tests is showed 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%, 4% 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% ($P < 0.001$). This increase is more important for *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 extract against fungi is done in none concentration dependent manner. High concentrations of *Acacia gourmaensis* extract stimulated mycelium 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 [22] observed that lentil, onion, radish and garden cress extracts stimulated mycelial growth of *Phytophthora capsici*, which used these extracts as a source of energy.

Table 2. In vitro 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		
		<i>F. solani</i>	<i>F. moniliforme</i>	<i>C. lunata</i>
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%.

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 1. Three concentrations (0.25, 0.5 and 1%) of the hydro-ethanolic 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 high 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.* [23]. 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 low concentration of *S. longepedunculata* hydro-ethanolic extract.

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

Treatment	<i>Fusarium solani</i>		<i>Fusarium moniliforme</i>		<i>Curvularia lunata</i>	
	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%.

3.2.2 In vivo antifungal activity of *Acacia gourmaensis*

The effect of different concentrations of *Acacia gourmaensis* extract on rice seeds germination and infection was studied and the results are presented in Table 1. *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. moniliforme* 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. Similar increase in fresh seedling weight after treatment of rice seeds with fungal and bacterial bio-control agents have been reported [24].

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 [22].

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

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

Treatments	<i>Fusarium solani</i>		<i>Fusarium moniliforme</i>		<i>Curvularia lunata</i>	
	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%.

4. CONCLUSION

This 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* hydro-ethanolic extract phytochemical composition should be explored to determine the active fungicidal compounds.

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