

# Efficacy of *Balanites aegyptiaca* (L.) Del. Hydro-Ethanollic Extract against Three Rice Seed-Borne Fungi

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**Abstract:** Seed-borne fungi are an important source of rice (*Oryza sativa* L.) diseases and causing crop yield reduction worldwide. This study was undertaken to investigate the antifungal activities of *Balanites aegyptiaca* bark hydro-ethanollic extract against *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata* and to evaluate the percentages of germination and infection of rice seeds. 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. The extract exhibited antifungal activity in terms of inhibition of mycelial growth of all fungi in a concentration-dependent manner. The extract concentration inducing 100% inhibition of mycelial growth was 0.5% for *Curvularia lunata* and 1% for *Fusarium solani* and *Fusarium moniliforme*. It has been observed that when *Balanites aegyptiaca* hydro-ethanollic extract concentration increased up to 1%, seeds germination percentage decreased for all fungi. However, seeds infection decreased with the highest concentration (1%) for all fungi. In this study, the results show that *Balanites aegyptiaca* hydro-ethanollic bark extract can be used as a potential antifungal agent in the management of rice fungal diseases caused by pathogenic *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata*. The hydro-ethanollic extract of *Balanites aegyptiaca*, at low concentration, could be used as an alternative biopesticide to control rice seed-borne fungi.

**Keywords:** Antifungal, *Balanites aegyptiaca*, Rice seed-borne fungi, Germination.

## 1. Introduction

Rice (*Oryza sativa* L.) is an important food crop and a source of income for farm households in Africa. The demand for rice in sub-Saharan Africa is grown faster than production [1]. In Burkina Faso, rice needs are increasing rapidly with population growth, especially in urban areas [2]. One of the main issues and challenges is to increase rice productivity. Unfortunately, many factors affect rice yield in Burkina Faso. These include low soil fertility, pests and plant diseases [3], [4], [5]. Among these diseases, seed-borne fungal diseases have a wide geographical distribution. Fungi are the main pathogens responsible for the majority of diseases observed on rice crops [3]. In addition, the privileged mode of transmission of fungal diseases is most often by seeds [6]. Seeds, apparently unharmed, often include fungi on the surface, in the coat or even in the embryo and attacking the seedling upon germination [7]. The most destructive seed-borne fungal diseases of rice are Brown spot (*Bipolaris oryzae*), Blast (*Pyricularia oryzae*), Sheath rot (*Sarocladium oryzae*), Sheath blight (*Rhizoctonia solani*), Leaf scald (*Microdochium oryzae*), Seed rot and Seedling blight (*Bipolaris oryzae*, *Sclerotium rolfsii* and *Fusarium* spp.), Grain spot (*Curvularia lunata*, *Nigrospora oryzae*, *Phoma glumarum*, and *Cladosporium* sp.) [8]. These pathologies have led to poor germination, stunted plants, seedling blight, inflorescences and leaves diseases [9], [10]. On the other hand, seed-borne fungi produce mycotoxins that pose risks to humans and animals health [11]. The use of healthy seeds provides better protection of crops against fungal diseases and preserves humans and animals health [12].

A particular interest is then focused on local medicinal plants with the aim to use them as seeds treatment products to control fungal diseases. Being natural products, the use of the medicinal species is safe for producers, the environment and the cost is low compared to synthetic pesticides [13]. Plant extracts, used as natural pesticides, could reduce the incidence of seed-borne fungi and increase the percentage of germination and seedling emergence [8], [14], [15]. Plant extracts like aqueous extract of *Balanites aegyptiaca* leaves, rich in phenolic compounds is known to reduce the mycelial growth of seed-borne fungal diseases [16], [17].

*Balanites aegyptiaca* (L.) Delile, known as desert dates belonging to the Zygophyllaceae family, is one of the most common wild plant species to drylands of Africa and South Asia [18]. The plant (leaves, roots and bark, fruit) is used in phytotherapy for its potential antimicrobial effect [19], [20].

Plant extract preparation using hydro-alcoholic solvents allows the extraction of more biologically active compounds [21]. In this study, hydro-ethanollic extract of *Balanites aegyptiaca* was tested for its efficacy against tree seed-borne fungi (*Fusarium moniliforme*, *Fusarium solani* and *Curvularia lunata*) of rice seeds and the percentages of seeds germination and infection were evaluated.

## 2. Material and methods

### Plant material

Fresh stem bark of *Balanites aegyptiaca* 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. They were dried under shade at 25°C, pulverized with a pestle and mortar, then 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.

#### 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.

#### 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.

#### *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. [22] and Švecová et al. [23]. 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.

#### *In vivo* antifungal assays

Rice seeds were disinfected with sodium hypochlorite 15% for 10 minutes [24]. Seeds were inoculated by spraying  $10^5$  spores/ml of *Fusarium moniliforme*, *Fusarium solani*, and *Curvularia lunata* strains respectively for 24 hours [25]. 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 *B. aegyptiaca* extract for 24 hours [26]. 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.

#### 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

#### *In vitro* antifungal assays

The mycelial growth inhibition effect of *Balanites aegyptiaca* hydro-ethanolic extract was assessed for *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata*. Five days after incubation, at concentrations of 0.25%, 0.5% and 1% of extract the percentages of mycelial growth inhibition were respectively 60%, 77.49% and 100% for *Fusarium solani*; 23.98%, 47.94% and 100% for *Fusarium moniliforme* and 75.5%, 100% and 100% for *Curvularia lunata*. Results indicate that all concentrations had a positive effect on reducing the linear mycelial growth of the pathogens compared to the control ( $P < 0.05$ ). The percentage values of mycelial growth inhibition increase when the concentration of the extract increases from 0.25 to 1%. The high inhibition of mycelial growth of *Fusarium solani* and *Fusarium moniliforme* was obtained with 1% of extract, while *Curvularia lunata* mycelial growth high inhibition was obtained with 0.5% of extract (Fig. 1, 2, 3). However, a significant difference was observed between mycelial growth inhibitions of three extract concentrations for *F. moniliforme* only (Fig. 2).

It is known that plant extracts inhibited the *in vitro* growth of several strains of *Aspergillus niger*, *Aspergillus flavus*, *Colletotrichum graminicola*, *Phoma sorghina* [27], [28]. Recently, antifungal activity of *Balanites aegyptiaca* was reported [17], [29]. Indeed, phytochemical investigation of *Balanites aegyptiaca* stem bark hydro-ethanolic extract revealed the presence of some major bioactive compounds, namely alkaloids, tannins, triterpenoids, saponins, amino acids and carbohydrates, flavonoids, glycosides and sterols [20], [30]. These phytochemicals are known to be biologically active. Tannins, flavonoids, alkaloids, saponin play a role in antifungal, antibacterial, astringent and antibiotic activities [29], [31], [32].

The effect of the concentration of the extract on mycelial growth inhibition in our study showed a dose-dependent inhibition of mycelial growth of *F. solani*, *F. moniliforme* and *C. lunata* caused by *Balanites aegyptiaca* hydro-ethanolic extract. A quite similar result was observed by Kabbashi [33]. He reported that the effect of *B. aegyptiaca* ethanolic extract against *Aspergillus niger* increased with concentration.

The effect of extract on incubation time indicates that the suppression of mycelia growth was the same. The hydro-ethanolic extract of *B. aegyptiaca* was active up to 10 and 15 days against *F. solani*, *F. moniliforme* and *C. lunata*. There is an increase in the percentage of inhibition with the concentrations 0.25 and 0.5% at 10 and 15 days for the different strains (Fig. 1, 2, 3). It is particularly important for *F. moniliforme* at the concentrations of 0.25 and 0.5% at 10 and 15 days (Fig. 2). However, Hussain et al. [34] reported that *B. aegyptiaca* aqueous and organic extracts were effective against fungi (*Microsporum gypseum* and *Trichophyton rubrum*) after 21 days of incubation.

*Balanites aegyptiaca* hydro-ethanolic extract against *F. solani*, *F. moniliforme* and *C. lunata* had a positive impact on inhibition of mycelia growth. *Curvularia lunata* was 100% inhibited at a concentration of 0.5%, while *F. solani* and *F. moniliforme* were 100% inhibited at a concentration of 1% (Fig. 1, 2, 3). This is in agreement with the findings of Bhuiyan et al. [35] who found that plant extracts (Garlic, allamanda, neem, marigold and bishkatali) reduced *Bipolaris oryzae*, *Alternaria padwickii*, *Sarocladium oryzae*, *Curvularia lunata*, *Aspergillus niger* and *Fusarium* spp. seed-borne infections of rice.

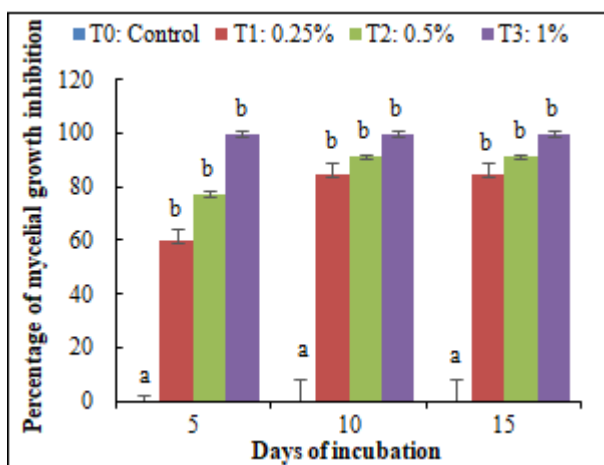


Figure 1: In vitro inhibition of *Fusarium solani* mycelial growth by *B. aegyptiaca* hydro-ethanolic extract

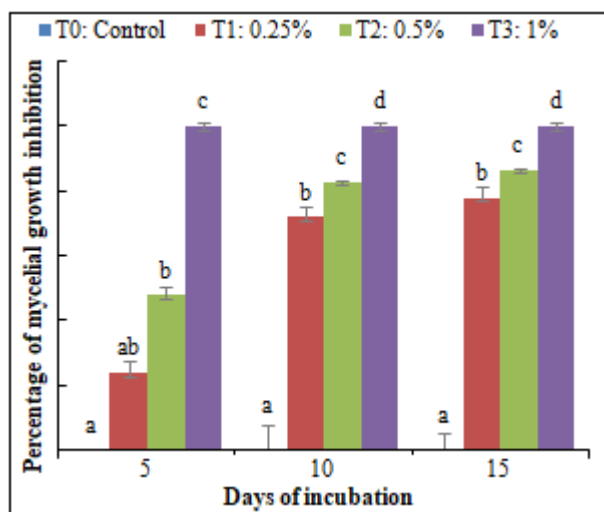


Figure 2: In vitro inhibition of *Fusarium moniliforme* mycelial growth by *B. aegyptiaca* hydro-ethanolic extract

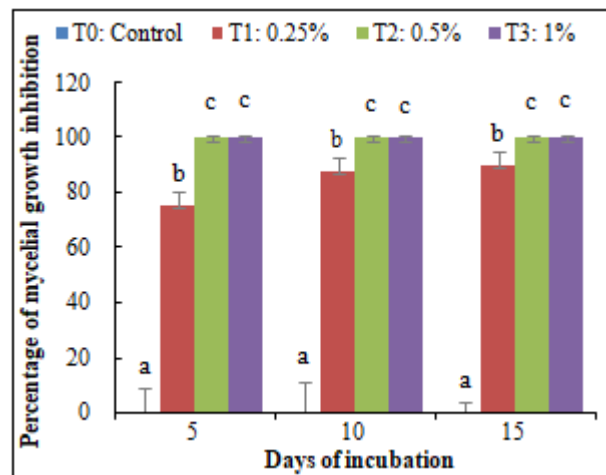


Figure 3: In vitro inhibition of *Curvularia lunata* mycelial growth by *B. aegyptiaca* hydro-ethanolic extract

### In vivo antifungal assays

The effect of different concentrations of *B. aegyptiaca* extract on rice seeds germination 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. In general, seeds germination decreased with increasing of extract concentration. This reduction in germination is significant with seeds infected by *F. solani* and *C. lunata* and non-significant with *F. moniliforme*. The application of lower concentration (0.25%) of *B. aegyptiaca* extract induced an increase in germination of rice seeds infected with *F. solani* compared to control. However, non-significant increase of germination was observed in rice seeds infected with *F. moniliforme* and *C. lunata* at a lower concentration of extract (0.25%) compared to control. High concentrations of the hydro-ethanolic extract of *B. aegyptiaca* (0.5 and 1%) had a negative effect on the germination of rice seeds.

In terms of seeds infection, the highest percentage was recorded in control treatment. Seeds infection decreased with high concentration (1%) compared to low concentrations (0.25 and 0.5%) of extract. However, this decrease is significant only for seeds infected with *F. Solani* (Table 2). *In vivo* assay confirmed the results of *in vitro* tests since the efficacy of *B. aegyptiaca* extract at the highest concentration (1%) also inhibited mycelial growth of *F. solani*, *F. moniliforme* and *C. lunata*. However, higher extract concentrations had an inhibitory effect on the germination of rice seeds. *In vivo* results indicated that *B. aegyptiaca* hydro-ethanolic extract probably contains some fungicidal compounds that could inhibit the growth of pathogenic seed-borne fungi. The extract was more effective at high concentration to reduce the incidence of fungi, indicating that active plant compounds are easily extracted by the hydro-ethanol (70%) solvent. Many studies have shown the importance of plant extracts from organic solvents in controlling seed-borne fungi of rice [36], [37]. It has been shown that plant extracts from hydro-alcoholic solvents provide more consistent antimicrobial activity than those from water and pure organic solvents [21], [38].

Seeds treated with higher concentrations of the hydro-ethanolic extract had a lower germination percentage than those treated with lower concentrations. However, they



showed a lower infection percentage with higher concentration of extract. Higher concentrations of the extract could be phytotoxic to the seeds. This indicates that ethanol (70%) solvent extracts more active plant compounds that could inhibit seeds germination. This result corroborates those of Fritz et al. [39]. They found that *Lactuca sativa* germination percentage in the most concentrated ethanolic extracts of *Hypericum myrianthum* and *H. polyanthemum* was significantly reduced compared to the control. Indeed, some plant extracts have an inhibitory effect on seeds germination [40].

Seed-borne fungi (*F. solani*, *F. moniliforme*, *C. lunata*) can be controlled by seeds treatment with low concentration (0.25%) of *B. aegyptiaca* hydro-ethanolic extract.

**Table 1:** Effect of *Balanites aegyptiaca* hydro-ethanolic extract treatment on rice seeds germination

Seeds germination percentage			
Treatments	<i>Fusarium solani</i>	<i>Fusarium moniliforme</i>	<i>Curvularia lunata</i>
T0	76 ab	80 a	88 b
T1	92 b	84 a	88 b
T2	78 ab	82 a	78 ab
T3	64 a	64 a	72 a

T0: control; T1: *B. aegyptiaca* extract 0.25%; T2: *B. aegyptiaca* extract 0.5%; T3: *B. aegyptiaca* extract 1%.

**Table 2:** Effect of *Balanites aegyptiaca* hydro-ethanolic extract treatment on rice seeds infection

Seeds infection percentage			
Treatments	<i>Fusarium solani</i>	<i>Fusarium moniliforme</i>	<i>Curvularia lunata</i>
T0	8 b	16 a	36 a
T1	2 ab	10 a	12 a
T2	2 ab	8 a	10 a
T3	0 a	4 a	6 a

T0: control; T1: *B. aegyptiaca* extract 0.25%; T2: *B. aegyptiaca* extract 0.5%; T3: *B. aegyptiaca* extract 1%.

#### 4. Conclusion

This study showed the effect of *Balanites aegyptiaca* hydro-ethanolic extract as an antifungal agent against seed-borne infections caused by *Fusarium solani*, *Fusarium moniliforme* and *Curvularia lunata* in a concentration-dependent manner. In opposite, with increasing the concentration of *Balanites aegyptiaca* hydro-ethanolic extract, rice seeds germination decreased. Therefore, this plant extract can be considered as an alternative to control rice seed-borne pathogens. However, the phytochemical composition of hydro-ethanolic extract should be explored to identify the *Balanites aegyptiaca* fungicidal compounds.

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