



Antifungal Activity of Fruit Extracts of *Azadirachta indica* on Germination, Infection, Rotten Seeds and Abnormal Rice Seedlings from Chad Republic

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Authors' contributions

This work was carried out in collaboration among all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors GRTN and KJR managed the analyses of the study. Author SS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Test the effect of *Azadirachta indica* fruit extract on seed-borne fungi and seed performance of different rice varieties.

Study Design: Neem fruits harvested in the locality of Pala in Chad were used to assess the antifungal effect on rice seeds. Samples of eight rice varieties (TOX, CH3, CH8, WITA9 D6, D4, D3 and D1) were collected in Bongor, Chad to assess their performance against the aqueous extract of neem fruits.

Place and Duration of Study: This study was carried out at the Research Unit of Phytopathology and Agricultural Zoology of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang/Cameroon from June to October 2018.

Methodology: Rice seeds were dipped in *Azadirachta indica* extract at concentrations of T25 (25 mg/ml), T50 (50 mg/ml) and T75 (75 mg/ml) for 1 hour before being incubated in petri dishes containing blotting paper. Distilled water (T0) and mancozeb 80 WP (TMn1 mg/ml) were used as negative and positive controls respectively.

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Results: The results showed that the germination rate from rice seeds treated with *Azadirachta indica* extract was better (97.14%) than the rate from the negative controls (82.53%). The infection rate of rice seedlings, the number of rotten seeds and the number of abnormal seedlings were significantly ($P = .05$) lower for *Azadirachta indica* extract treatments than for the negative control (T0).

Conclusions: Neem (*Azadirachta indica*) fruit extract has a bio-fungicidal potential and a bio-stimulator of rice seed germination. This extract of *Azadirachta indica* fruit can be used to improve rice cultivation.

Keywords: Seed treatment; *Azadirachta indica*; seed germination; rice; chad.

1. INTRODUCTION

Rice (*Oriza sativa* L) is an important source of energy in human nutrition [1]. It is a staple food for more than half of the world's population, particularly in Sub-Saharan Africa. It is consumed after cooking with water, steam or in fat. It is also used to prepare porridges, pasta, biscuits and alcohol. In Chad, agricultural production contributes about 25% to the Gross Domestic Product (GDP) and employs 77.2% of the working population [2]. Rice is the 4th cereal produced after sorghum, millet and maize with a production of 257,701 tons [3]. Over time, rice cultivation has become a food empowerment strategy to reduce malnutrition and food insufficiency. Despite this, local production does not cover food needs, as 5,073 tons of rice was imported for a value of 845,000 Dollar/year to meet food needs [2]. The low rice production in Chad could be due to poor seed quality. Rice seeds are vectors of fungal diseases such as seedling meltdown and seed rot [4] which affect crop density and lead to low production. To optimize rice production, Chadian farmers use insecticide-fungicides to coat seeds before sowing. However, improper handling of these chemicals causes user poisoning and water pollution around rice growing areas. It is therefore necessary to explore control methods that are less harmful to humans and animals and to the environment. Some plants are used as chemotherapeutic means to reduce pest attacks on crops [5]. These higher plants contain biologically active secondary metabolites such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [6, 7]. These secondary metabolites have antimicrobial effects and serve as plant defense mechanisms against pathogenic microorganisms. Thus, the different organs of Neem (*Azadirachta indica*) have been identified as an effective bio-fungicide against more than 100 different species of microorganisms [8]. This study was conducted to determine the bio-

fungicidal potential of neem fruit extract on fungal growth on rice seeds. The results of this study would be used by rice farmers in pre-treatment of rice seeds before planting.

2. MATERIALS AND METHODS

2.1 Seed source and Plant Material

The rice seeds were obtained from the National Rural Development Agency (ANADER) in Bongor, Mayo - Kebbi East province, Chad. Eight (08) rice varieties were collected (TOX, CH3, CH8, WITA9 D6, D4, D3 and D1).

The plant organs (*Azadirachta indica* fruits) were harvested in Pala, Chad.

2.2 Plant Extracts for Seed Treatment

These harvested plant organs (fruits) were dried in an oven at a temperature of 45°C for 7 days and ground to powder using a Germany brand grinder. One hundred grams of powder plant was macerated in 500 ml of distilled water for 48 hours. The mixture was filtered using No. 1 Wattman paper and the resulting filtrate was dried in an oven at a temperature of 45°C until the water completely evaporated. After this drying time, specific weights of the paste were diluted in 100 ml of distilled water to give the following concentrations: T25 (25 mg/ml); T50 (50 mg/ml) and T75 (75 mg/ml). Distilled water (T0) and mancozeb 80 WP (TMn 1 mg/ml) were used as negative and positive controls respectively. Eight hundred seeds of rice were first washed in distilled water, the surface sterilized with 2% sodium hypochlorite for ten minutes and thoroughly rinsed with distilled water. The seeds were stored on blotting paper to remove excess moisture from the surface of the seeds. The seeds were treated by soaking separately in different concentrations of extract for one hour [9]. These seeds were then placed between two layers of blotting paper to remove

the excess moisture from the extract solution. These seeds were placed equidistant from each other in Petri dishes (9 cm in diameter) (10 seeds per dish) covered with three layers of blotting paper soaked in distilled water (6 ml) beforehand. These boxes were classified in an incubator at a temperature varying between 22-30°C with a photoperiod of 12 hours per day. The light source was from fluorescent tubes placed 40 cm above the Petri dishes. After seven days, data on sprouted seeds, the presence of rotten seeds, abnormal seedlings and mycelium-bearing seeds of fungi transmitted by the seeds were recorded. All these data were expressed as a percentage of the total number of seeds incubated. The experiment was repeated four times.

Collected data were subjected to analysis of variance (ANOVA) in a Completely Randomized Design (CRD) using SPSS Software version 17. The means for all treatments were separated using Duncan's Multiple Range Test (DMRT) at ($P = .05$)

3. RESULTS AND DISCUSSION

Table 1 shows the variation in the percentage of germination, infection, rotten seeds and abnormal seedlings of different rice varieties according to the *Azadirachta indica* extract treatments. Treating the seeds of the different rice varieties with *Azadirachta indica* fruit extract at different concentrations (T25, T50 and T75) induced an increase in the germination rate compared to negative controls (T0). Seeds of the TOX varieties treated with different concentrations of *Azadirachta indica* extract at T25, T50 and T75, induced the germination percentage of 94%; 96% and 97% respectively higher than those of positive controls (85%) and negative controls (79%). The germination percentage was better with the T75 concentration in all rice varieties (TOX, CH3, CH8, WITA9 D6, D4, D3 and D1) tested compared to the negative controls. However, no difference was observed between the germination percentage of positive and negative controls in variety D4.

The analysis showed that compared to the negative control (T0), the different extract concentrations and the positive control (TMn 1mg/ml) reduced the percentage of infection of the different rice varieties. The percentages of seed infection in WITA9 whose seeds were treated with *Azadirachta indica* extract at 75 mg/ml and positive control (TMm at 1mg/ml)

were low (3.01% and 2.59%) respectively compared to those of negative controls at 12.25%. The similar effect of reduced infection percentage was observed between positive controls and the three concentrations of *Azadirachta indica* extract.

Percentage of rotten seeds was higher for negative controls (T0) and lower for seeds from treatments with *Azadirachta indica* extract (T25, T50 and T75) and positive control (TMn at 1gm/ml). Variety CH8 showed very few rotten seeds of 1%, 0.25%, 0% and 2.75% for T25, T50, T75 and TMn 1mg/ml respectively compared to those of the negative controls (4%). However, no difference was observed between the percentage of rotten seeds at T25, TMn 1 mg/ml and T0 in the D1 varieties. Similar observation was made in CH8 when treated with TMn 1 mg/ml and T0.

Percentages of abnormal seedlings of the different rice varieties decrease with treatments with *Azadirachta indica* extract (T25, T50 and T75) and positive control (TMn 1mg/ml) compared to the negative control (T0). Indeed, the treatments (T25, T50 and T75) recorded percentages of abnormal seedlings in the TOX variety as 2.04%, 1.36% and 0.73% respectively compared to 5.68% for negative controls. The positive control treatment effect (TMn 1mg/ml) was similar to that of *Azadirachta indica* extract treatments.

Fig. 1 shows the variation in the average percentage of germination and infection with *Azadirachta indica* extract treatment. It can be seen from this figure that treatments at different concentrations have induced an increase in the average percentage of germination. These percentages were higher for the different extract concentrations compared to the negative controls. Similarly, for the average percentage of infection, the lowest values come from different extract concentrations and the positive control compared to the negative control. The average percentage of infection gradually decreases with extract concentration. These values were 6.50% for the T25 treatment, 3.36% for T50 and 1.90% for T75.

The mean percentage variation in abnormal seedlings and rotten seeds is presented in Fig. 2, as the mean percentages of abnormal seedlings from *Azadirachta indica* extract treatments and positive control were low compared to those from negative controls. The same effect was observed for the average percentage of rotten seeds.

Treating rice seeds with *Azadirachta indica* fruit extract significantly improved germination and seedlings in the different rice varieties.

Table 1. Variation in the percentage of germination, infection, rotten seeds and abnormal seedlings of different rice varieties *Azadirachta indica* extract treatments

Treatments	TOX	WITA9	D6	D1	CH8	CH3	D4	D3
Percentage seed germination								
T25	94±3a*	91±0,9b	91±1ab	91±1b	88±1c	87±1c	93±0,01b	94±23b
T50	96±3a	92±2b	92±1,9ab	94±0,9ab	91±1b	93±1b	100±0a	97±3ab
T75	97±3a	94±1a	94±0,6a	96±1a	98±1a	96±1a	100±0a	99±0,02a
TMn(1mg/ml)	85±1b	85±0,4c	89±1,7b	86,5±1c	85±1d	85±1d	90±0,001c	86±3,41c
T0	79±1c	81±0,9d	83±3,8c	83,3±3d	81±1e	80±0,05e	90±0,05c	81±1d
Percentage seed infection								
T25	12,04±2,49b	5,58±0,9b	5,37±1,7b	8,25±1,25b	10,03±2,9b	4,12±0,85b	4,5±1,9b	2,12±0,75b
T50	1,5±0,5c	3,08±0,8bc	1,68±0,85c	5,75±0,95c	10,11±2,6b	1,69±0,87c	2,5±1,29c	0,52±0,05c
T75	1,08±0,57c	3,01±1,47bc	0,65±0,5c	4±1de	3,7±2,06c	0,01±0,004c	2,33±1,15c	0,36±0,35c
TMn(1mg/ml)	0,75±0,73c	2,59±0,6c	4,11±1,3b	2,37±1,27e	6,13±1,2c	1,62±1,2c	0,75±0,95c	1,56±0,9bc
T0	20,75±0,05a	12,25±2,87a	17,36±1,85a	14±2,34a	28,5±1,29a	10,25±2,75a	10±0a	12±1a
Percentage of rotten seeds								
T25	0,75±0,95b*	1,75±0,5c	6,37±1,7b	1,75±0,95a	1±0,8b	1,5±1ab	3,25±0,5c	1,50,4c
T50	0,5±0,57b	1,25±0,5c	5,43±1,2b	0,25±0,5b	0,25±0,5b	1±0b	0±0d	0,63±0,48c
T75	0,6±0,57b	1±0c	4,7±0,9b	0,33±0,57b	0±0b	0,33±0,5b	0±0d	0,36±0,05c
TMn(1mg/ml)	1,57±0,3b	4,9±1,4b	7,71±1,64ab	2±0a	2,75±0,95a	1,5±0,57ab	4,75±0,75b	3,34±1,4b
T0	3,4±1,12a	7,5±1,12a	11,17±5,2a	1,75±0,04a	4±1,5a	3±1,6a	8,5±1,3a	6±1,96a
Percentage of abnormal seedlings								
T25	2,04±1,39b*	6,5±1,29b	2,12±0,85bc	8,25±1,25b	10,03±2,9b	8,7±1,79b	2,25±0,5c	4,12±1,1c
T50	1,36±0,53b	4±1,1c	2,06±0,96bc	5,75±0,95c	10,11±2,6b	8,13±2,6b	0±0d	1,75±1,32d
T75	0,73±0,8b	3,260,45c	0,83±0,28c	4±1cd	3,7±2,06c	1,33±0,57c	0±0d	1±0d
TMn(1mg/ml)	1,18±0,5b	6,69±0,47b	3,04±0,39b	2,37±1,27d	6,13±1,23c	9,49±1,4b	4±0,81b	9,21±3,8b
T0	5,68±0a	10,65±0,92a	5,33±1,73a	14±2,34a	28,5±1,29a	17,5±3,18a	6,25±1,5a	16,5±5,41a

*Means in a column for each variety followed by the same letters are not significantly different according to Duncan New Multiple Range Test (P = .05). T0 = untreated control; T25 = 25 mg/ml; T50 = 50 mg/ml; T75 = 75 mg/ml; TMn (1mg/ml) = Mancozeb (1mg/ml). Data given are means of four replicates

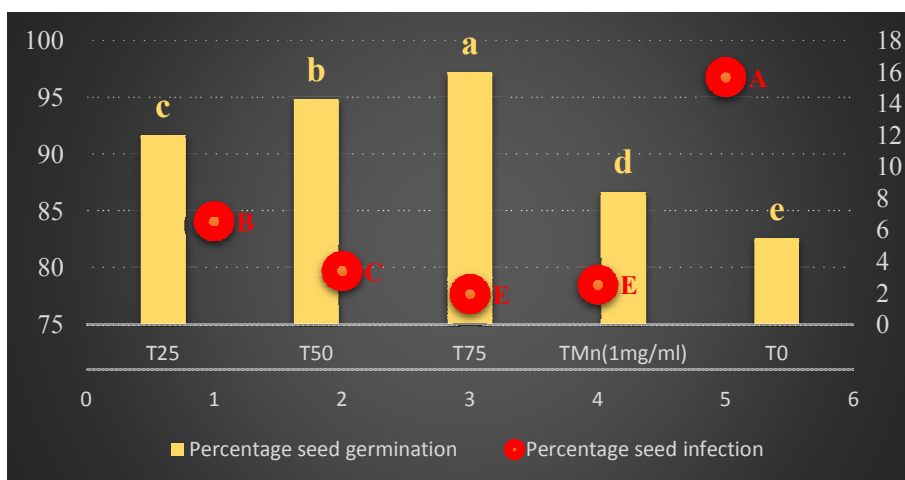


Fig. 1. Variation in the mean percentage of germination and infection with *Azadirachta indica*
 *Means by the same letters are not significantly different according to Duncan New Multiple Range Test (P = .05). T0 = untreated control; T25 = 25 mg/ml; T50 = 50 mg/ml; T75 = 75 mg/ml; TMn (1mg/ml) = Mancozeb (1 mg/ml). Data given are means of four replicates

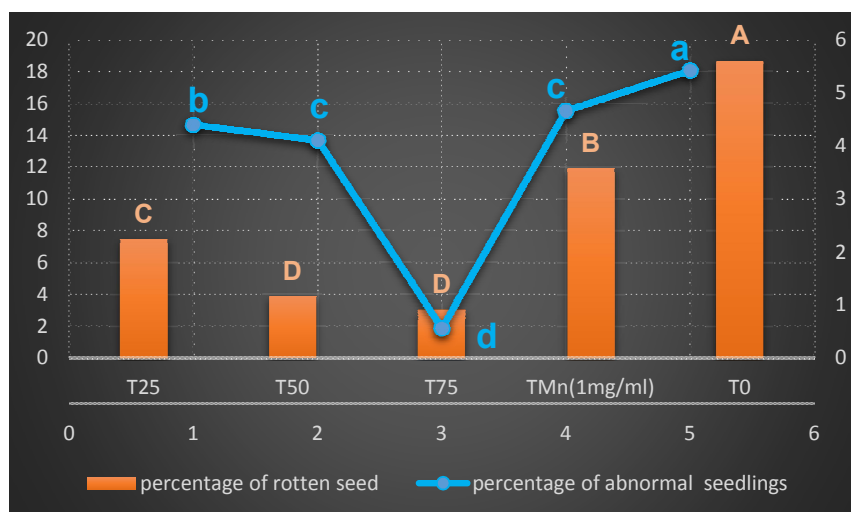


Fig. 2. Variation in the mean percentage of rotten seed and abnormal seedlings with *Azadirachta indica*

*Means by the same letters are not significantly different according to Duncan New Multiple Range Test ($P = .05$). T0 = untreated control; T25 = 25 mg/ml; T50 = 50 mg/ml; T75 = 75 mg/ml; TMn (1mg/ml) = Mancozeb (1mg/ml). Data given are means of four replicates

Treating rice seeds with neem extract at different concentrations (T25, T50 and T75) resulted in a reduction and inhibition of fungi on rice seeds compared to negative control (T0). Also, the percentage of infection of seedlings of the different rice varieties from mancozeb-treated seeds is identical to that from *Azadirachta indica* fruit extract treatments. *Azadirachta indica* extract may contain molecules capable of preventing the development of fungi on seeds and seedlings. These results are consistent with those of [10] who noted a decrease in the percentage of infection of *Dioscorea sp* seedlings when the seeds were treated with *Azadirachta indica* leaf extracts. Similar results have been demonstrated by [11] because *Azadirachta indica* leaf extract inhibited the development of fungi associated with *Sorghum bicolor* and *Arachis hypogaea* seeds. According to [12], *Azadirachta indica* extract has antifungal properties on the development of *Alternaria alternata*. Previous work by [13] has shown that *Azadirachta indica* seed extract contains antifungal substances such as nimbidine, gedunin and cyclic trisulfide, which would act as fungicides.

The results indicated that the best germination rates were obtained with the different extract concentrations compared to the negative control. This stimulation of the germination of different varieties of rice comes from the bioactive substances contained in the extract of Neem

fruits. Indeed, these substances would be at the origin of the activation of seed germination and therefore healthy quality. The stimulating effect of *Azadirachta indica* extract on seed germination and seedling vigour has been reported by [14] on cotton seeds. For these authors, the healthy quality of cotton seedlings is attributed to the antifungal and stimulant substances contained in the neem extract. According to [15], extracts of certain plants contain nutrients such as sodium, potassium, calcium, magnesium, iron, zinc and manganese. These substances would act on the metabolism of the seedling by stimulating cellular elongation at the beginning of germination and therefore make the seedlings more vigorous.

The results of the seed treatments of the different rice varieties showed that abnormal seedlings and rotten seeds were lower for seeds treated with *Azadirachta indica* extract. The positive effect of this extract on seedling performance resulted from metabolites such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [6]. In fact, these substances act as the origin of the stimulation of seed germination, therefore the more vigorous and healthier the seedlings.

4. CONCLUSION

Evaluation of the effect of *Azadirachta indica* extract on fungal infections transmitted by rice seed showed that this extract improved the

germination rate of seeds and reduced fungal infection. The use of neem fruit extract in the control of fungal pathogens in rice is proving to be an opportunity to reduce the use of environmentally toxic chemicals and improve rice cultivation.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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