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Research Article

In Vitro Evaluation of a Commercialized Fungicide on Radial Mycelial Growth of Aspergillus Species Associated with Groundnut Seeds Collected in Niger Republic

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Abstract

Groundnut (*Arachis hypogaea L.*) is one of the most important seed in Niger republic. It plays an essential role as a food and as a cash crop. Groundnut crops face the threat of numerous toxigenic fungi and this constitute a threat too in most least-developped sahelian countries. The present study is aimed to identify the mycoflora associated with groundnut seeds collected in Niger republic and to evaluate the efficacy of a fungicide with active substance mancozeb commercialized as Manga Plus by measuring the growth rate of the isolated mycoflora at different product concentrations. Seeds associated mycoflora i.e. *Aspergillus niger* and *Aspergillus flavus* were detected and isolated from seed samples. The *in vitro* experiment revealed a noticeable reduction in the growth of both *Aspergillus niger* and *Aspergillus flavus* as the concentration of the fungicide increased. Highest levels of radial growth inhibition in all the isolates were observed at a concentration of 10g/L (stock solution diluted ten (10) times) when compared to the control, maintaining a constant inhibition diameter of 2 mm after six days of inoculation. Overall, The Manga Plus fungus treatment was found to effectively reduce the development of radial mycelial growth of *Aspergillus species*. Further complementary studies should be conducted in order to evaluate the effectiveness of the tested fungicide on sporulation and germination of *Aspergillus* species associated with groundnut seeds.

Keywords

Fungi, Groundnut, Manga Plus, Mycotoxins, Niger Republic

1. Introduction

Groundnut is an essential food and cash crop cultivated in Niger republic. Groundnut based-products might account for 25% of the total value of Niger exports, and their production or processing employed 30% of the population [1]. However, its economic importance has declined due to several numbers of challenges which include pest attacks. Field pests are one of the major challenges affecting groundnut production. There are several species of insect pests of groundnuts in the field, which are responsible for substantial yield losses. In addition to pre-harvest constraints, there are several challenges associated with post-harvest groundnut management practices. These include poor drying and storage, which lead to micro-

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bial contamination and pest attacks [2]. For African producers, the use of high-quality groundnut seed is one of the most effective ways of improving crop productivity [3]. Poor post-harvest management, i.e. the conditions under which food products are stored, processed and/or transported, could dramatically increase mycotoxin levels in foodstuffs [4]. Living organisms such as fungi, bacteria, viruses, insects, nematodes and parasitic weeds once in contact with groundnut could influence its production. The fungi responsible for contamination during food storage are generally saprophytic organisms whose growth can be prevented by influencing their water environment: numerous drying and storage techniques are known and practised to limit the development of fungi in crops [5]. Fungi like *Aspergillus niger, Aspergillus flavus, Alternari adianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillum sp.* cause several damages to groundnut seeds and or its byproducts (i.e. reduction in the germination potential of seeds; deterioration in the nutritional value of seeds due to weight loss; and the accumulation of toxic metabolites induced by infestation) [6]. Most members of the Aspergilus species could produce highly toxic and carcinogenic mycotoxins known as aflatoxines with large spectral action [7]. *Aspergillus* spp. fungal spores can grow on dried foods and feeds, such as cereals and seeds and further could result in significant health and economic problems in the affected zones/regions/nations [8].

To face these challenges and among other strategies, there is need to develop healthy and disease free seeds throughout the crop value chains. A number of chemical and biological agents are commercialized and used across the continent to control these pathogens. The "Common Regulation for the Registration of Pesticides in CILSS (Permanent Interstate Committee for Drought Control in the Sahel) Member States" is always present to ensure that pesticides used in the different countries in the Sahel are effective, of suitable quality and of low hazard to man and the environment [9]. The Sahelian Pesticides Committee (CSP) keeps a register of registrations and authorizations of pesticides authorized for sale across the countries who are members of the comity. From the latest published global list of pesticides authorized by the Sahelian Pesticides Committee, Coga 80 WP / Manga Plus with mancozeb as active substance appeared registered as fongicide of contact with preventive action against alternaria in tomato crops [10]. In Niger republic, only Coga 80 WP as brand name is registered as fungicide for fruits, food crops and flowers by the Regional Chambers of Agriculture (CRA) and the National Network of Chambers of Agriculture (RECA). However, Manga Plus with the same active substance as Coga 80 WP is also commercialized in the country as fungicide. According to the manufacturer, Manga plus is an 18g/l Mancozeb-based fungicide recommended for use against phytopathogenic crop fungi [11].

It is within this framework that the present study intervenes, with a view to contribute to the identification of mycoflora associated with groundnut seeds and to evaluate the efficacy of MANGA PLUS in Niger republic.

2. Material and Methods

2.1. Study Area

The study was carried out at the Plant Protection Laboratory of the Regional Center for Agronomic Research (CERRA) of Niamey in Niger republic.

2.2. Collection of Materials

Groundnut seeds: Raw groundnut seeds were purchased from local farmers of the states of Dosso, Maradi and Zinder; conditioned in sterile gunny bag and sent to the Regional Center for Agronomic Research and stored at room temperature of the Plant Protection Laboratory till experimentation.

Manga Plus: The fungicide Manga Plus (Mancozeb 800g/Kg) was purchased from the Savana Niger Company a branch of the main company Savana (http://www.savana-france.com) located in France.

2.3. Fungal Isolation, Species Identification

Seed samples of groundnut were assayed for the detection of seed-borne fungi by using Standard Agar plate method, in which disinfected seeds with 1% bleach (1 min soaking followed by rinsing with distilled water) were plated on Potato Dextrose Agar (PDA) media, five (5) per Petri dish. Plated seeds were then incubated at 28 $\pm 2^{o}$C for three (3) to five (5) days in order to permit the fungi to develop and emit characteristic structures (sporulation) that enable them to be identified under a microscope, using the key of Barnet and Hunter [12].

2.4. Preparation of Poisoned Culture Media and Assessment of Radial Growth

Fungicide was tested against target pathogens at various concentrations (100g/L; 10g/L; 1g/L and 0,1g/L) following a poisoned food technique [13] on PDA medium. Desired concentration of the fungicide was prepared by dissolving one gram (1g) in 10 ml distilled water. The well-mixed and homogenized solution thus obtained is called the stock solution (Sm). From the stock solution, a series of dilutions, to the tenth (Sm1), to the hundredth (Sm2) and to the thousandth (Sm3) were produced. PDA medium is poured into Petri dishes, each containing 1 ml of the product at the desired concentration and allowed to solidify. After that, five mm diameter agar disc of pure fungus culture were removed by using sterile cork-borer and placed in the center of the Petri dishes containing different concentrations of the fungicide. A total of 10 Petri dishes including the control (PDA without

addition of Manga Plus), *i.e*. two Petri dishes for each strain of fungus. The plates were then incubated at 28 ℃ for 8 days. The radial growth of the fungus on the treated medium was measured on the 2nd, 4th and 6th Day after seedling. The percentage inhibition of mycelia growth over the control was calculated using the formula [14]:

$$\text{Inhibition percentage} = \frac{C-T}{C} \times 100$$

Where,

C = Colony diameter in mm in control Petri dishes.

T = Colony diameter in mm in treated Petri dishes.

3. Results

3.1. Identification of Mycoflora Associated with Groundnut Seeds

The seed borne mycoflora was identified on the basis of cultural and morphological characteristics observed by visual inspection and under optic microscope. On the basis of microscopic images and using the identification key of Barnett and Hunter [12], two fungi of the same genus associated with groundnut seeds were identified as: *Aspergillus niger* and *Aspergillus flavus*.

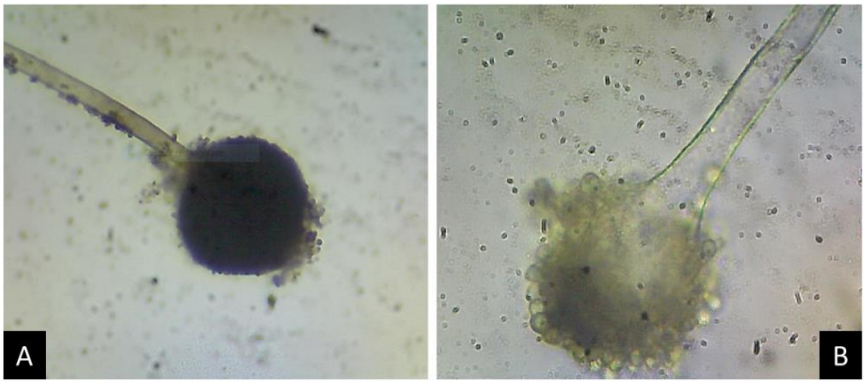
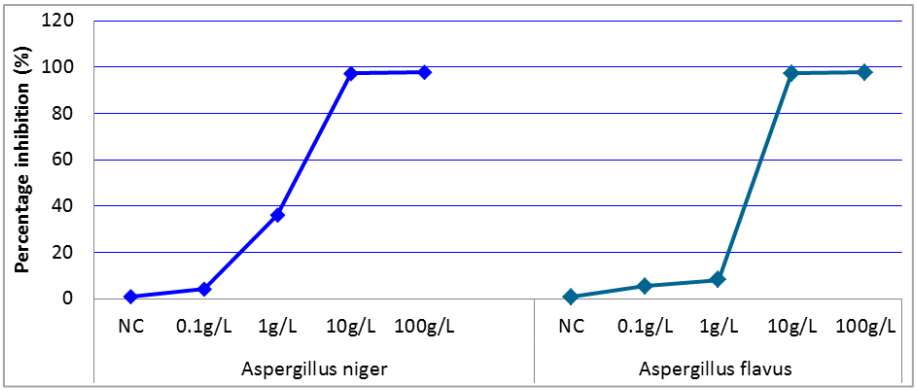


Figure 1. *Microscopic appearance of mycoflora associated with groundnut seeds. Aspergillus niger (A): It appears as large conidiophores, brownish, measuring 1.5 to 3 mm in height and widening to form the vesicle; Apergillus flavus (B): It appears as long conidiophores, greenish-yellow to light yellow in color, widening upwards to form vesicles.*

3.2. Effect of Fungicide Against Isolated Fungi

By using Manga Plus (mancozeb) at different concentration, maximum mycelia growth inhibition after 6 days incubation was observed in Sm1 (10g/L) in case of *Aspergillus niger* (97.26%) and *Aspergillus flavus* (97.30%). In terms of concentration scales, inhibition (%) was increased with the increase of concentrations of applying fungicide (Figure 2). The increase in average colony diameter during the day two (2), four (4) and six (6) of cultivation of *Aspergillus niger* (A), and of *Aspergilus flavus* (B) under treatments with the fungicide is presented in Figure 3 below. Growth diameters were found to be constant throughout the first six days with nearly zero increase in average colony diameter at concentration Sm (100g/L) and Sm1 (10g/L) for *Aspergilus sp*.



NC: Negative Control

Figure 2. *Radial mycelia growth inhibition percentage of the tested isolates of mycoflora associated with groundnut seeds after six days of incubation.*

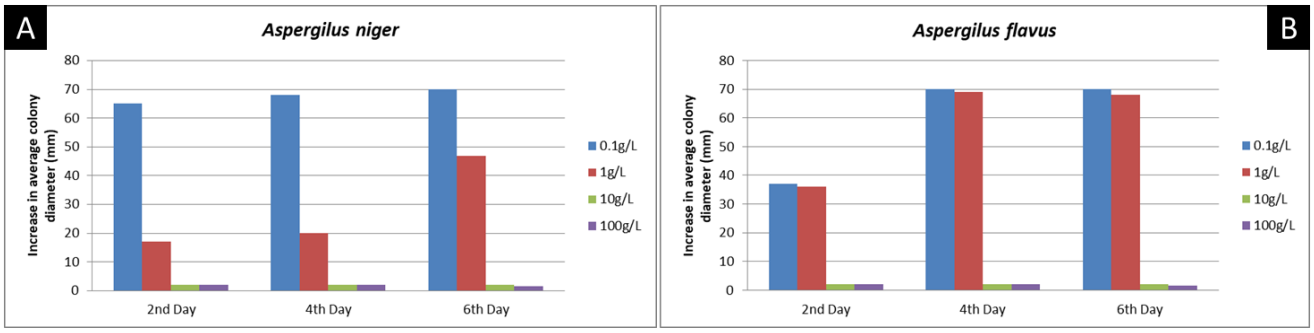


Figure 3. *Increase in average colony diameter during the first six days of cultivation of Aspergilus niger (A) and of Aspergilus flavus (B).*

4. Discussion

Proper agricultural production in any farming system is intimately related with the use of good seeds with high germination potential and free to seed-borne pathogens such as fungi. In the present study, groundnut seed samples were investigated in order to isolate and identify the seed mycoflora associated with seeds and to test the effect of a commercialized fungicide (Manga Plus) against two isolates of fungi of the same strain recorded as *Aspergillus flavus* and *Aspergillus niger*. According to the findings of many authors, *Aspergilus* species are frequently encountered in consumed foods or crops. Dedi and Diomande [15] reported the presence of *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp.* and even other fungi such as *Fusarium spp* in maize kernels intended for the preparation of compound poultry feed. Asma et al. [16] in there study aimed to assess the risks of mycotoxins on human health, reported the presence of several fungi in wheat, with a predominance of the genus *Aspergillus*. Ephrem et al. [17] reported a proportion of seed contamination by *Aspergillus* species varying from 30% in seed collected from vendors to 85% in seed samples from farmers in there survey which was aimed to determine the levels of infection of groundnut seed by *Aspergillus* species along the groundnut value chain in different agro-ecological zones of eastern Ethiopia. Of the several *Aspergillus* species isolated from the groundnut seed samples, *Apergilus flavus* and *Aspergilus niger* were the most prevalent mycotoxigenic fungi across the farmers' fields, farmers' stores, market retailers and vendors. Bonzi [18] identified 40 species of pathogenic and saprophytic fungi in 19 genera after assessing the mycoflora associated with sorghum seeds, with *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium spp*., *Penicillium spp*., and *Rhizopus spp*. as the most predominant ones. Savary [19] conducted a survey of fungal diseases of groundnut (*Arachis hypogaea L.*) in Ivory Coast. A list of 16 fungal pathogens has been published and included *Aspergillus flavus*, *Aspergillus niger*. Mohamed EL-Maraghy [20] conducted an investigation which was focussed on the thermophilic fungi associated with peanut seeds and their ability for production of mycotoxins. They found that *Aspergillus* was the most common genus and was represented in 92.5% of the samples comprising 72.9% of total fungi. In May 2021, a group of researchers in Niger republic published a report titled: '*Alert: Aflatoxine, a silent killer in Niger*' through a national network of the Agricultural Chambers of Niger (RECA). From there findings, of the 418 samples collected from maize, sorghum and groundnuts, aflatoxins were detected in all these crops and 100% of them were contaminated, with *Aspergillus flavus* [21]. To reduce aflatoxin contamination, it is important to control the spread of aflatoxin-producing fungi at vulnerable stages of crop development. A preventive contact fungicide that inhibits spore germination by acting on the fungi's respiratory and food chains could be a perfect response to combat to slow or stop the burden. In this study, a commercialized fungicide (Manga Plus) in Niger republic was tested against *Aspergilus* species that were isolated from the groundnut seeds. The growth of *Aspergillus flavus* and *Aspergillus niger* as a function of the concentration of Manga Plus fungicide shows that the recommended commercial dose provides good protection against fungal attack. In fact, none of these fungi were able to grow on the stock solution, i.e. when Manga Plus was applied at 10g/L to 100g/L. Similar results were reported by Abalo et al. [22] in a more recent study which was aimed to evaluate the efficiency of three contact fungicides on the developmental stages of isolated pathogen in vitro. A very good efficiency of Mancozeb (PI=100%) commercialized as Manga Plus in Burkina Faso on mycelial growth, sporulation and germination of conidia of *Curvularia lunata*. In the absence of the fungicide, the growth rate six days after inoculation was 7.4 cm in diameter for *Aspergillus spp*. Asma et al. [16] and Dongmo et al. [23] have reported the same growth rates when these fungi are grown on a culture medium without fungicide. In contrast, Lina et al. [24] have reported respective growth rates of 4.5 cm and 4.3 cm/week for *Aspergillus spp.* on PDA. Adamou et al. [25] on the other hand have reported growth rates on PDA of 6.8 cm for *Aspergillus flavus* and 2.9 cm for *Aspergillus niger*.

5. Conclusion

Seed-borne fungi constitute a serious problem which adversely affects the health of seeds. Various alternatives have been used to control infectious diseases affecting seeds across the world and Niger in particular. Contamination by

fungi is unfortunately correlated with the development of toxins, making it dangerous to consumers of contaminated foodstuffs or crops such as that of groundnut. However, it is possible to reduce contamination by protecting seedlings from fungal attack during and after germination, by treating seeds with products such as Manga plus, which was found effective to reduce radial mycelial growth when used at the dose recommended by the manufacturer. However, complementary studies on the efficacy of the tested fungicide on sporulation and germination of *Aspergillus* species associated with groundnut seeds should be conducted in near future.

Abbreviations

PDA	Potato Dextrose Agar
Sm	Stock Solution
CILSS	Permanent Interstate Committee for Drought Control in the Sahel
CSP	Sahelian Pesticides Committee
CRA	Regional Chambers of Agriculture
RECA	National Network of Chambers of Agriculture
CERRA	Regional Center for Agronomic Research

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Author Contributions

Boubacar Abdou Hassane: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – review & editing

Maman Manzo Lawaly: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing

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Basso Adamou: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing

Conflicts of Interest

The authors declare no conflicts of interest.

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