International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 44 No. 3 Jan. 2025, pp. 629-642

http://www.ijias.issr-journals.org/

Activité antifongique de la poudre de feuille de Neem (Azadirachta indica) sur les contaminants fongiques isolés des graines de Niebe (Vigna unguiculata) pendant le stockage après la récolte

[Antifungal activity of Neem (*Azadirachta indica*) leaf powder on fungal contaminants isolated from Cowpea (*Vigna unguiculata*) seeds during postharvest storage]

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ABSTRACT: In sub-Saharan Africa in general and in Côte d'Ivoire in particular, post-harvest losses of agricultural products are still a major problem. It is with this in mind that this study was conducted to identify the fungal flora of cowpea seeds intended for human consumption and to evaluate the activity of neem leaf powder on this flora. Thus, four batches of cowpea were purchased in the three main markets of Korhogo. At T = 0 week, nine Petri dishes with Dichloran Rose Bengal Chloramphenicol were inoculated with the batch of untreated cowpea seeds. Each Petri dish was then incubated at 30 ° C for five days. We also proceeded in the same way, with the seeds treated at a rate of one culture per week for 21 days. At T = 5 days, three fungi were isolated and identified at varying percentages of occurrence: *Aspergillus flavus* (34.79%), *Aspergillus niger* (32.95%) and *Rhizopus solinifer* (6.79%). The evaluation of neem leaf powder showed its ability to inhibit the fungal growth of pathogens isolated from cowpea seeds. The leaf powder therefore has properties that can be used for the conservation of cowpea seeds.

KEYWORDS: Cowpea, fungal, neem, biopesticides, post-harvest losses.

RESUME:: En Afrique subsaharienne en général et en Côte d'Ivoire en particulier, les pertes post-récolte des produits agricoles constituent toujours un problème majeur. C'est dans cette optique que cette étude a été menée pour identifier la flore fongique des graines de niébé destinées à la consommation humaine et évaluer l'activité de la poudre de feuilles de neem sur cette flore. Ainsi, quatre lots de niébé ont été achetés dans les trois principaux marchés de Korhogo. A T=0 semaine, neuf boîtes de Petri au Dichloran Rose Bengal Chloramphénicol ont été inoculées avec le lot de graines de niébé non traitées. Chaque boîte de Petri a ensuite été incubée à 30°C pendant cinq jours. Nous avons également procédé de la même manière, avec les graines traitées à raison d'une culture par semaine pendant 21 jours. A T=5 jours, trois champignons ont été isolés et identifiés à des pourcentages d'occurrence variables: Aspergillus flavus (34,79%), Aspergillus niger (32,95%) et Rhizopus solinifer (6,79%). L'évaluation de la poudre de feuilles de neem a montré sa capacité à inhiber la croissance fongique des agents pathogènes

isolés des graines de niébé. La poudre de feuilles possède donc des propriétés qui peuvent être utilisées pour la conservation des graines de niébé.

MOTS-CLEFS: Niébé, champignon, neem, biopesticides, pertes après récolte.

1 INTRODUCTION

Diet helps to preserve health and fight against nutritional diseases. Although abundant food is produced for all humans, the coexistence of extreme and opposite forms of malnutrition still globally exists. If in one part of the world there is a high incidence of malnutrition due to excess, obesity, and non-communicable diseases, in the other there is an increasing number of people afflicted by chronic food deprivation [1]. Many people suffer from what is called “hidden hunger”; malnutrition due to a lack of micronutrients which prevents them from leading a healthy life [2]. In this context, the availability of a food supply rich in nutrients and stable at low cost and offering a level of safety and quality acceptable to all is necessary. In this regard, pulses represent a cheaper source of nutrients for low-income countries and a healthier and more sustainable option than animal-based proteins in developing countries [3]. Thus, the global area and total legume production increased steadily during the 2000s, especially during the 2001s [4]. Nowadays, legumes share an area of around 81 million ha with production of more than 92 million tons globally [5]. Among these legumes, cowpea is a legume with nutritional and soil fertilizing properties. It is one of the most important grain legumes, with an annual global production of about 5.59 million tonnes from a cultivated area of over 12.61 million hectares Africa produces 64% of this production. It is a plant with very high economic value for developing countries. Its protein content is 2 to 3 times higher than that of cereals [6]. This high protein content has earned it the nickname “poor meat” [7]. Cowpea is important for food security and the livelihoods of millions of smallholder farmers who rely on it for economic and nutritional well-being [8].

Increasing agricultural production is urgent. Therefore, reducing post-harvest losses and deteriorating quality are the most crucial objectives for boosting food availability from established production, which might also improve food security and spur local economic growth. In the case of sub-Saharan African countries, there is more than 30% loss of production between harves t and storage for consumption. This is believed to be due to a high infestation of stored food, which is caused by insufficient storage techniques for agricultural products over a long period of time [9]. These high rates of post-harvest losses contribute to insufficient food supply for the population and reduce agricultural incomes [10]. Fungi acidify, discolor, ferment and make food products unpleasant or even dangerous [11]. In fact, their development in seeds can generate low-dose toxic compounds such as mycotoxins, responsible of poisoning (chronic or acute) in vertebrates (humans and animals). Several fungi genera including Aspergillus, Penicillium and Fusarium are known to be contaminants of agricultural products and/or for their ability to produce secondary toxic metabolites [12]. Thus, Storage of harvest products according to techniques respecting the environment and protecting the user remains one of the key factors, given that synthetic plant protection products expose manipulators more to multiple intoxications and also to environmental pollution. The use of these chemicals for post-harvest control of food increases the risk of toxic residues in food products [13]. The increasing sensitivity of consumers to environmental pollution and the toxic effects of synthetic fungicides leads to the use of natural alternatives [14]. For this purpose, the use of biopesticide plants such as *Azadirachta indica* for food storage will be a practice that could protect both users and consumers in order to reduce pressure on human health and the environment. The neem, also called neem or «lilac of Persia» [15], is a tree measuring about 15 m in height of the family of the meliacees. Extracts from its various parts have high antimycotic activity [16].

Although studies have been conducted on the evaluation of post-harvest practices of this legume to reduce losses [7], little scientific data exists on the use of *Azadirachta indica* as a source of biocontrol. It is in this context that this study was initiated to determine the antifungal activity of neem leaves during cowpea seed conservation. The overall objective of this study is therefore to contribute to the reduction of post-harvest losses of cowpea seeds in order to ensure consumer food security. Specifically, this will include: identifying invasive mycofungi in legume seeds and assessing the antifungal effect of neem leaf powder on the *in vitro* growth of these mycofungi.

2 MATERIALS AND METHODS

2.1 BIOLOGICAL MATERIAL

The material used in this study consists of cowpea seeds (Figure 1) purchased from three markets in Korhogo, namely the Korhogo Grand Market (GMK), the Haoussabougou Market (MHB), and the Sinistré Market (MSI). The neem leaves (Figure 2) were collected from the Peleforo Gon Coulibaly University in Korhogo

Fig. 1. A sample of cowpea seeds obtained at Korhogo Grand Market

Fig. 2. Neem (Azadirachta indica) leaves

2.2 COWPEA SEED SAMPLING

The city of Korhogo includes six markets, including the Grand Maket of Korhogo (GMK), the market of Sinistré (MSI), the market of Belle Ville (MBV), the market of Cocody (MCC), the market of Petit Paris (MPP), and the market of Haoussabougou (MHB). The choice of collection of cowpea seeds was based on geographical location and the fact that the selected markets are the points of unloading and supply of food from the peripheral villages of Korhogo. Precisely, the seeds were collected from the market of Hausabougou, the market of Sinistré, and the Grand Market of Korhogo (Figure 3). Cowpea seeds were collected randomly from the shops of three traders in the main markets of the study. A sample of 500 g of seeds was taken from each trader, with three samples per shop. Total of nine samples were collected in all three markets. Once collected, the samples were individually packaged in stomacher plastic bags, labelled, sealed, and then kept in a bag. Subsequently, the samples were transported to the laboratory for analysis.

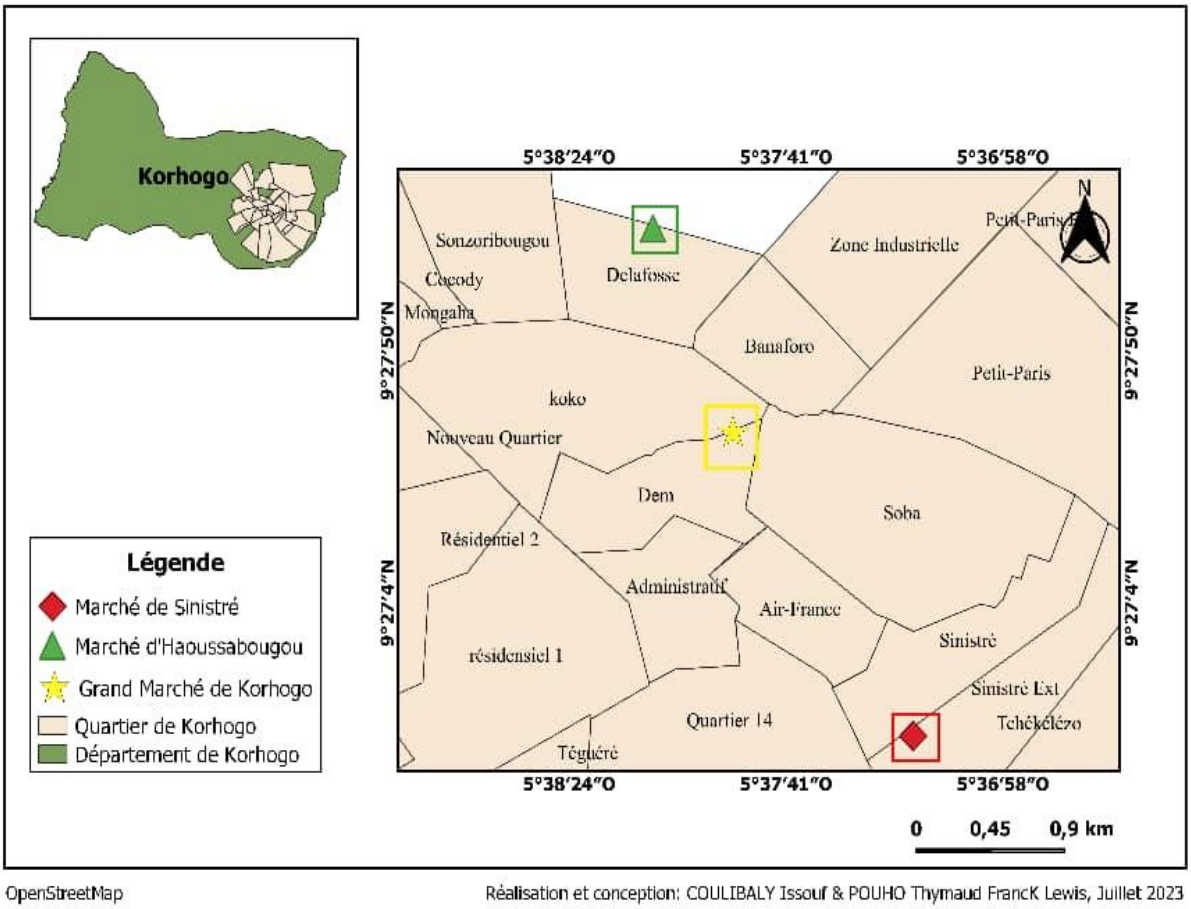


Fig. 3. Map of the city of Korhogo with the different study markets

2.3 PREPARATION OF COWPEA SEED ALIQUOTS FOR TESTING

A total of 36 cowpea seed aliquots was used for testing. In each market of Korhogo (Grand market, Market of Sinister, Market of Haoussabougou), three sellers were randomly chosen. From each seller, four cowpea seed aliquots were taken. One lot is composed of nine aliquots. We obtained 36 aliquots, which labelled as follows (Table 1). Once in the laboratory, 20 seeds from each sample were put into five closed jars at the rate of five seeds per jar

Tableau 1. Cowpea seed aliquots for testing

Lots	Grand market of Korhogo			Market of Sinistré			Market of Haoussabougou			Total
	Seller 1	Seller 2	Seller 3	Seller 1	Seller 2	Seller 3	Seller 1	Seller 2	Seller 3	
1	GMK C1-1	GMK C2-1	GMK C3-1	MSI C1-1	MSI C2-1	MSI C3-1	MHB C1-1	MHB C2-1	MHB C3-1	9
2	GMK C1-2	GMK C2-2	GMK C3-2	MSI C1-2	MSI C2-2	MSI C3-2	MHB C1-2	MHB C2-2	MHB C3-2	9
3	GMK C1-3	GMK C2-3	GMK C3-3	MSI C1-3	MSI C2-3	MSI C3-3	MHB C1-3	MHB C2-3	MHB C3-3	9
4	GMK C1-4	GMK C2-4	GMK C3-4	MSI C1-4	MSI C2-4	MSI C3-4	MHB C1-4	MHB C2-4	MHB C3-4	9
Total	4	4	4	4	4	4	4	4	4	36

GMK: Grand Market of Korhogo, MHB: Haoussabougou Market, MSI: Sinistré Market

2.4 MICROBIOLOGICAL ANALYSES OF COWPEA SEEDS

2.4.1 PREPARATION OF CULTURE MEDIUM

Dichloran Rose Bengal Chloramphenicol (DRBC) agar was prepared according to the manufacturer's instructions and poured into Petri dishes.

2.4.2 SEEDS TREATMENTS

The methods used to treat seeds [17]. The aliquots 2, 3, and 4 of the lots of all the trials of each of the cowpea traders contained in the jars, respectively, received a quantity of neem powder according to the following ratio: 0.5 mg of powder per gram of seeds, while aliquots from lot 1 have not received a biopesticide.

2.4.3 ISOLATION OF FUNGAL STRAINS BY DIRECT METHOD

At T = 0 weeks, each aliquot from each trader's lot was placed in one Petri dish containing DRBC agar. The Petri dishes were then incubated at 30°C for five days. To obtain a pure strain, each colony obtained was transplanted onto fresh DRBC agar and then identified on the basis of macroscopic and microscopic characteristics using identification keys [18].

2.4.4 TEST WITH NEEM LEAF POWDER

At T = 1 week, each aliquot of lot 2 of each trader was placed in one Petri dish containing DRBC agar. The Petri dishes were then incubated at 30°C for five days. To obtain a pure strain, each colony obtained was transplanted on DRBC agar and then identified on the basis of macroscopic and microscopic characteristics from identification keys [18]. The tests at T = 2 weeks and T = 3 weeks were done using the same process

2.4.5 MACROSCOPIC REVIEW

The macroscopic examination took into account the dorsal and ventral sides of the Petri dishes

The macroscopic criteria were:

- On the surface (dorsal side), the characteristics observed include the appearance of the colonies (powdery, cottony, downy, etc.), the shape (domed, starry), the color (white, green, black), and the size (small, extensive, invasive).
- On the reverse side (ventral side), the ability of the mycelium to penetrate the agar, the colour of the reverse, the presence or absence of pigment was observed.

2.5 MICROSCOPIC REVIEW

For microscopic analysis, a portion of the colony to be identified was removed with forceps and placed in a drop of methylene blue on a slide. The needles were sterilized with a Bunsen beak, and used to pick small portions of fungal cultures (with bits of media gelled with the fungus) from the edges of colonies. The fungal portions were then spread on a blade with the aid of a second needle that was used to disentangle fungal structures [19]. The resulting preparation was covered with a lamella and then observed under an optical microscope with the lens at x 40 magnification [20]. The characteristics observed were the appearance of the mycelium (cloisonne or not), the shape of conidia or spores, conidian heads and conidiophores, the presence of metules, and the arrangement and shape of phialides.

2.6 PURIFICATION OF FUNGAL ISOLATES

The different fungal isolates obtained were transplanted by successive cultures on PDA medium until obtention of a pure culture. These cultures were incubated for 7 days at 30°C and then stored at 4°C on sloping agar for further study. The isolates obtained were described macroscopically and then observed under an optical microscope with the lens at x 40 magnification [19] (Zeiss, Germany) to identify microscopic aspects. Subsequently, they were identified by macroscopic and microscopic characters using [20] identification keys.

2.6.1 FUNGAL DNA EXTRACTION

The extraction of DNA was performed using the method described by Aamir et al [21]. Genomic DNA was extracted from five to seven days old fungal cultures grown either in PDA culture plates. The fungal mass from the culture plate was scraped out with the help of a fine spatula and placed in a 2 ml tube. one volume of 0.2 ml of extraction buffer consisting of 3% SDS (w/v), 50 mM EDTA, 1.0 M NaCl, 100 mM hydroxymethyl hydrochloride (Tris-HCl) at pH 8.0 was placed in a 2 ml microtube, then 300 mg of microbeads were added and the mixture was shaken vigorously. for 5 minutes. Then, the suspension was centrifuged at 13000 for 10 minutes and the supernatant was transferred in a new microtube. An equal volume of phenol/chloroform (25: 25, v/v) mixture was slowly added and centrifugated at for 10 min (the aqueous phase was recovered in a new microtube and this step was repeated again). Afterwards, the aqueous phase was transferred to a new microtube, and an equal volume of 100% isopropanol was added. The contents were carefully mixed by turning and stored at -20°C for 30 minutes to precipitate the total DNA. The mixture was then centrifuged at 12000 rpm for 10 minutes. The pellet was washed twice 13 000 rpm at 4°C with 70% ethanol and centrifuged at 12000 rpm at 4°C for 5 minutes. The supernatant was discarded, and the pellet was dried and dissolved in 75 µL of TAE 1X buffer.

2.6.2 DNA AMPLIFICATION BY PCR

Amplification of the ITS1-rDNA-ITS2 gene 5.8 S-ITS region was performed using the method described by White et al. [22], using primers ITS1 (forward) (5' TCCGTAGGTGAACCTGCGG 3') and ITS2 (reverse) (5' TCCTCCGCTTATTGATATGC 3'). PCR amplification was performed using a thermocycler (Sordalab, France). A 50 µL reaction volume contained 2 µL of DNA extract, 25 µL PCR Master Mix 2X (Promega, USA); 0.2 µM of each primer (Sigma, Belgium), and the volume was adjusted to 50 µL with Nuclease-Free Water. PCR amplification was performed according to the program below:

- initial denaturation at 95 ºC for 5 min;
- 35 cycles of: denaturation at 94 ºC for 1 min, primer priming at 55.5 ºC for 1 min and 72 ºC for 2 min for DNA chain elongation;
- final extension at 72 ºC for 10 min.

2.6.3 AGAROSE GEL ELECTROPHORESIS

The PCR amplification products were analyzed by 1% (w/v) agarose gel electrophoresis. Electrophoretic migration is performed in a 0.5 X TAE buffer (consisting of 0.25% (w/v) Tris-base, 0.057% (v/v) acetic acid, and 0.1% (w/v) 0.5 M EDTA pH 8) at 80 V for 1 h. A 100 pb DNA size marker was used. DNA amplification fragments were fixed with GelRed (Biotium, Germany) solution, and visualized by trans-UV illumination.

2.7 STATISTICAL ANALYSIS

For the analysis, a test of the homogeneity of the variances was carried out using the tests of Hartley Cochran and Bartlett. The degree of homogeneity of the fungi studied were assessed with the Statistica 7.5 software through an analysis of variance and mean values compared according to the Newman-Keuls test at the 5% threshold.

3 RESULTS

3.1 PREVALENCE OF FUNGAL CONTAMINANTS OF COWPEA SEEDS

Key fungal contaminants were identified by the market during storage (Figure 4). Three species were isolated in the Korhogo Grand Market, Haoussabougou Market and the Sinistré Market. The three species were *Aspergillus flavus, Aspergillus niger* and *Rhizopus solonifer*.

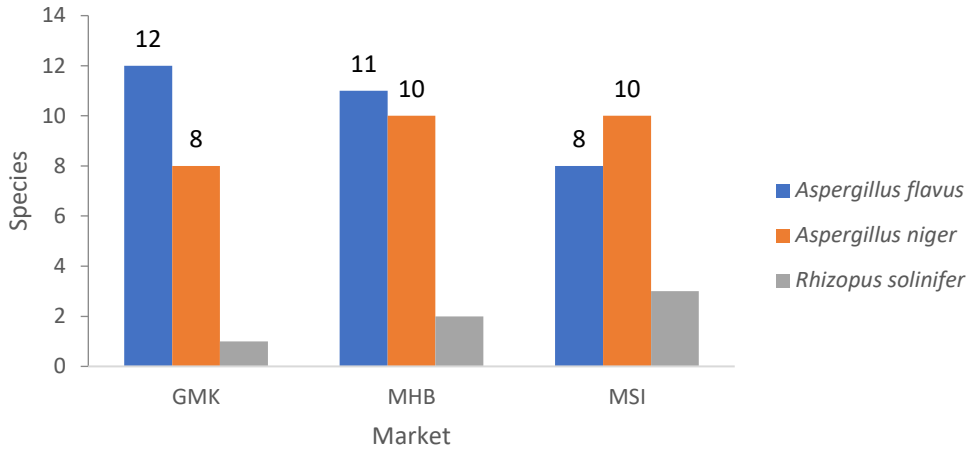


Fig. 4. Key fungal contaminants identified by market during storage. GMK: Grand Market of Korhogo, MHB: Haoussabougou Market, MSI: Sinistré Market

3.2 Frequency Of Fungal Species Isolated From Cowpea Seeds

The isolation frequencies of fungal species from cowpea seeds sold in the three markets are presented in Table1. The genus *Aspergillus* recorded the highest frequency with 77.27% followed by the genus *Rhizopus* which recorded a frequency of 22.72%. The species *Aspergillus flavus* recorded the frequency of 50% in the Korhogo Grand Market, 37.5% in the Haoussabougou Market and 37.5% in the Sinistré Market. *Aspergillus niger* recorded 33.33% in the grand Market of Korhogo, 37.5% in the Haoussabougou Market and 37.5% in the Sinistré Market. *Rhizopus solinifer* was identified at 16.66% at the Korhogo Grand Market, at a frequency of 25% in the Haussabougou and Sinistré Markets

Tableau 2. Isolation frequency of the main contaminating genera and fungal species of untreated cowpea seeds sold in the different markets

Markets	Isolated germs	Number of isolates per species	Gender frequency of isolation (%)	Species	Number of isolates per species	Frequency of isolation (%)
GMK	*Aspergillus*	17	77.3	*Aspergillus flavus*	3	50.0
MHB				*Aspergillus flavus*	3	37.5
MSI				*Aspergillus flavus*	3	37.5
GMK				*Aspergillus niger*	2	33.3
MHB				*Aspergillus niger*	3	37.5
MSI				*Aspergillus niger*	3	37.5
GMK	*Rhizopus*	5	22.7	*Rhizopus solinifer*	1	16.2
MHB				*Rhizopus solinifer*	2	25.0
MSI				*Rhizopus solinifer*	2	25.0

GMK: Grand Market of Korhogo, MHB: Haoussabougou Market, MSI: Sinistré Market

3.3 Macroscopic And Microscopic Characteristics Of Fungal Genera Isolated From Cowpea Seeds Sold In The Three Study Markets

Figure 5 presents the macroscopic and microscopic characteristics of fungal contaminants isolated from untreated cowpea seeds during storage. Based on the identification keys, taking into account macroscopic and microscopic characters, three fungal species were isolated.

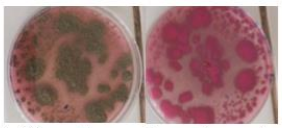
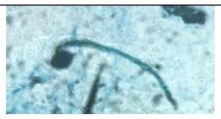
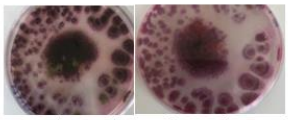
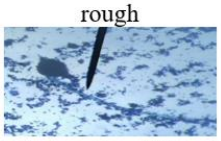
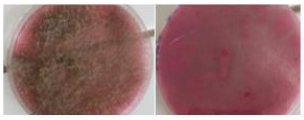
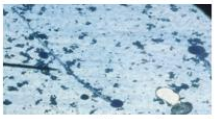
Markets	Fungal species	Macroscopic aspect Surface Reverse	microscopic appearance
GMK MHB MSI	*Aspergillus flavus*	 Fluffy cotton colony, invasive, green, diffuse green reverse in agar	 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore, long and rough
GMK MHB MSI	*Aspergillus niger*	 Powder colony, invasive, of color becoming black, black reverse diffuses in the agar	 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore long and smooth
GMK MHB MSI	*Rhizopus solinifer*	 Colony filamentous, invasive, gray color, fast growing, gray lapels	 Hyphae not partitioned. Columella rounded

Fig. 5. Macroscopic and microscopic characteristics of fungal contaminants isolated from untreated cowpea seeds sold during storage

3.4 GROWTH OF FUNGAL CONTAMINANTS IN COWPEA SEEDS STORED THREE WEEKS AFTER TREATMENT

During storage, the main fungal contaminants were identified by market as a function of time (Figure 6). Two weeks after treatment, *Rhizopus solinifer* disappeared; however, *Aspergillus flavus* species and *Aspergillus niger,* decreased slightly. The microbial population decreases by half from the first (T0) to the third week (T3).

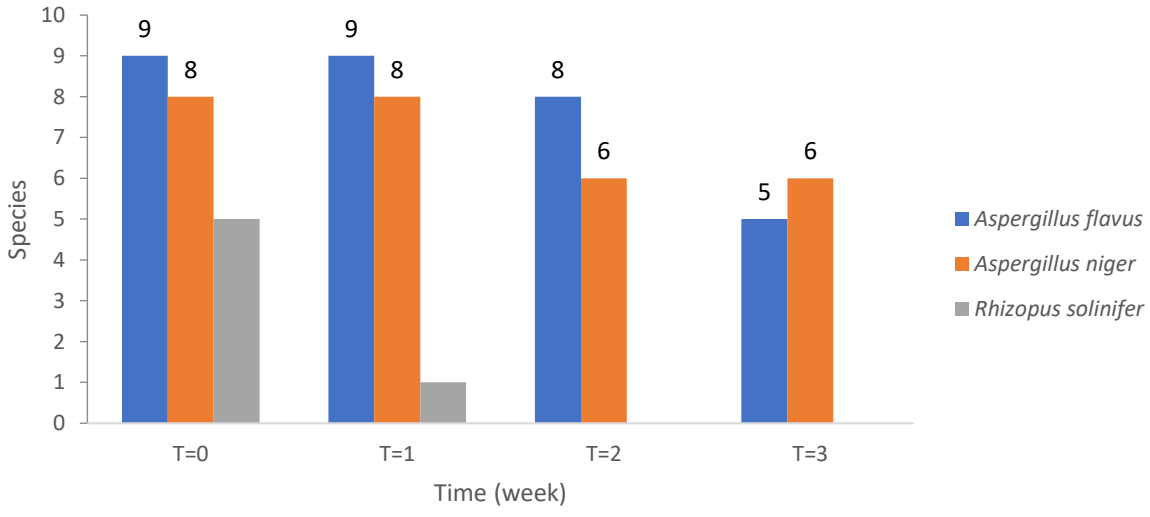


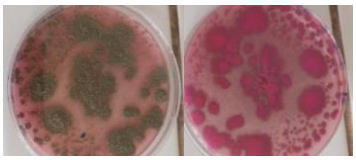
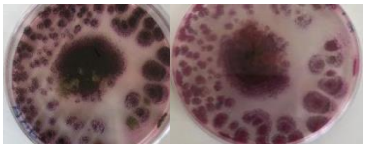
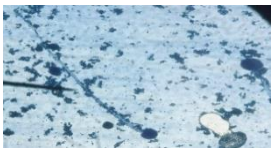
Fig. 6. Key fungal contaminants identified after treatment

3.5 GROWTH OF FUNGAL CONTAMINANTS IN COWPEA SEEDS STORED THREE WEEKS AFTER TREATMENT

The isolation frequencies of the main genera and fungal species contaminating cowpea seeds from the three markets treated after three weeks are presented in Table 3. Only the genus Aspergillus was observed, representing 100% of the isolated

fungal germs. The species *Aspergillus flavus* were recorded with a frequency of 75% in the grand market of Korhogo, 50% in the market of Haoussabougou, and 0% in the market of Sinistré. *Aspergillus niger* recorded 25% in the large market of Korhogo, 50% in the market of Haoussabougou, and 100% in the market of Sinistré**.**

Tableau 3. ***Isolation frequency of major genera and fungal species contaminating cowpea seeds from the three markets treated for three weeks after treatment***

Markets	Fungal species	Macroscopic aspect		microscopic appearance
		Surface	Reverse	
GMK MHB MSI	*Aspergillus flavus*	 Fluffy cotton colony, invasive, green, diffuse green reverse in agar		 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore, long and rough
GMK MHB MSI	*Aspergillus niger*	 Powder colony, invasive, of color becoming black, black reverse diffuses in the agar		 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore long and smooth
GMK MHB MSI	*Rhizopus solinifer*	 Colony filamentous, invasive, gray color, fast growing, gray lapels		 Hyphae not partitioned. Columella rounded

GMK: Grand Market of Korhogo, MHB: Haoussabougou Market, MSI: Sinistré Market

3.6 Percentage Of Occurrence Of Key Fungal Contaminants Isolated During Treatment

For the majority of fungi, the rate of occurrence was lower when leaf powder was in contact with fungal contaminants for three weeks (21 days). These rates are between 40.09 % and 0 % (Table 4).

Tableau 4. ***Percentage of major fungal contaminants isolated during storage***

Markets	Isolated germs	Number of isolates per species	Gender frequency of isolation (%)	Species	Number of isolates per species	frequency of isolation (%)
GMK				*Aspergillus flavus*	3	75
MHB				*Aspergillus flavus*	2	50
GMK				*Aspergillus niger*	1	25
MHB	*Aspergillus*	11	100	*Aspergillus niger*	2	50
MSI				*Aspergillus niger*	3	100

3.7 Macroscopic And Microscopic Characteristics Of Fungal Genera Isolated From Cowpea Seeds Treated Three Weeks After Storage

The results of the macroscopic and microscopic characteristics of fungal genera isolated from cowpea seeds treated three weeks after storage are recorded in Figure 7. Based on the identification keys, taking into account the macroscopic and microscopic traits, a fungal genus has been isolated. It is called *Aspergillus.*

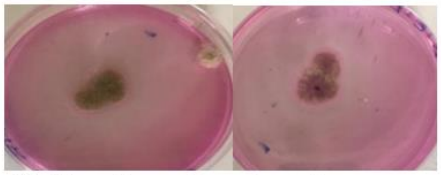
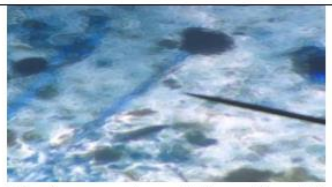
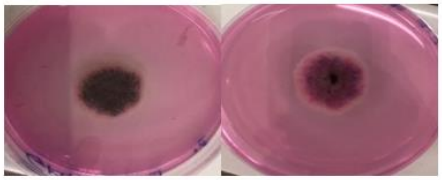
Fungal species	Macroscopic aspects Surface	Reverse	Microscopic appearance
Aspergillus flavus	 Fluffy cotton colony, invasive, green, diffuse green reverse in agar		 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore, long and rough
Aspergillus niger	 Powdery colony, invasive, of color becoming black, black reverse diffuse in agar		 Hyphae, not partitioned, with spherical conical head or rounded conical club, Conidiospore long and smooth

Fig. 7. Macroscopic and microscopic characteristics of fungal genera isolated from cowpea seeds after 3 weeks of treatment

3.8 CONFIRMATION OF FUNGAL SPECIES BY PCR

Fungal isolates were confirmed by PCR using amplification of the ITS region (Figure 8). It revealed three species namely *Aspergillus niger, Rhizopus solonifer* and *Aspergillus flavus.*

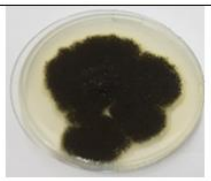
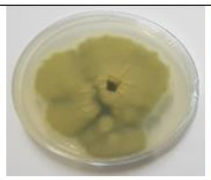
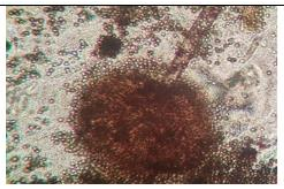
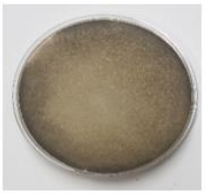
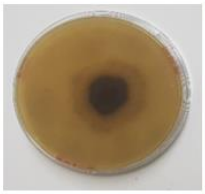
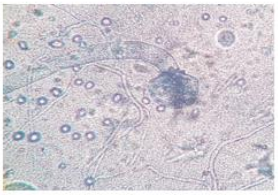
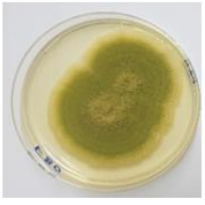
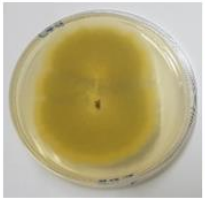
Number	Surface	Reverse	Microscopic aspect	Identification
1				*Aspergillus niger*
2				*Rhizopus solonifer*
3				*Aspergillus flavus*

Fig. 8. Macroscopic and microscopic characteristics of fungal isolate

3.9 ELECTROPHORETIC ANALYSIS OF AMPLICON ON AGAROSE GEL

Electrophoretic analysis showed that the size of 500 pb and 550 pb of fungal amplicons were present (Figure 9).

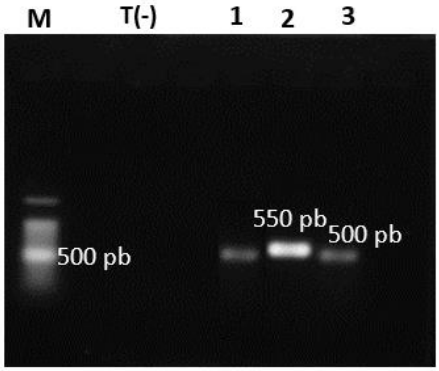


Fig. 9. Gel electrophoresis of the ITS1-rDNA-ITS2 gene of fungal amplicons. Column M: molecular weight marker; T (-) negative control; Column 1 and 3: strains of fungal; Column 2: strains of fungal

4 DISCUSSION

The study of the fungal microflora of cowpea seeds revealed the existence of two fungal genera, including *Aspergillus,* a potential producer of mycotoxins. The species *Aspergillus flavus, Aspergillus niger,* and *Rhizopus solinifer* were isolated and identified at different percentages in the samples from nine traders from three Korhogo markets. In addition to being present in all samples, fungi strains like A. flavus and A. niger also have very high infection rates compared to the others. Our results are similar to those of Gyasi [23], who reported infection rates of A. flavus ranging from 3.0% to 57.0% and a percentage of infected samples of 54.5% on cowpea seeds produced in Ghana. In the same line, Ouili [24] have reported 56.55% infection rates of Aspergillus strains from cowpea seed samples collected in the three agro-ecological zones of Burkina Faso. Moreover, Theses two species (*A. niger* and *A. flavus*) were found to be associated with cowpea seed infection (with rates of 23.57% and 16.42%, respectively) in India by Zanjare [25]. The presence of these fungal strains could be due to the hot and humid environmental conditions of Côte d'Ivoire. Indeed, the hot and humid environmental conditions of tropical regions of Africa are ideal for the growth of moulds contaminating foodstuffs such as cowpea and its derivatives. Contamination from field, during post-harvest operations, or storage could also explain the presence of *A. flavus* and *A. niger* strains in the tested seeds. According to Degraeve [26] and Badd [27], inappropriate harvesting, drying, and storage practices contribute to the development of fungi, mainly the genus Aspergillus. Numerous plant and agricultural product illnesses, ranging from harvest to processing transformation, are caused by Aspergillus. Ours Aspergillus strains presented similar morphological characteristic to those isolated by Abdallah [28], Okayo [29], and Ono [30] with ability to produce aflatoxins and ochratoxin A (OTA), two toxins that could be the cause of liver cancer [26]. The ability of these fungi to adapt to a wide temperature range may be the reason for their prevalence in all three agro-ecological zones. Aspergillus is widely spread geographically but is more frequently found in areas with warm temperature [31]. The majority of Aspergillus species prefer temperatures between 25˚C and 40˚C for optimum growth. For this reason, they grow very well in the so-called "dry" food products like cowpea. This point was also raised by Hocking [32], who pointed out that fungi of the genus *Aspergillus,* belonging to the sub-branch Ascomycotina and having a sexual mode of reproduction, easily colonize food products when storage conditions are not adapted. In addition to cowpea, other authors have isolated strains of *Aspergillus* from other food samples, including peanuts [33], wheat silage, sorghum, and cassava [34]. Thus, precautions must be taken during post-harvest activities and storage to avoid contamination of cowpea crops by these ubiquitous moulds.

Rhizopus was isolated at a low prevalence rate. Species of the genus Rhizopus are highly present in the soil, and contact of the pods with their spores during harvesting could explain their presence in the analyzed samples. In addition, there is a lack of good dehulling, drying, and seed storage practices by the farmers. Shahnaz [35] also isolated species of the genus Rhizopus from cowpea seeds produced in Pakistan with infection rates of 30.8%. Fungi of the genus Rhizopus are classified in the order Mucorales. They rapidly colonize decaying plants and fruits where they develop as filaments. *Rhizopus* can rapidly colonize decaying plants and fruits and cause soft rot in some high-moisture crops, such as sweet potatoes [36]. Apart from *Aspergillus flavus* and *A. niger* that were isolated from cowpea samples, *Rhizopus solonifer* was also isolated. It was reported by [37] that *Rhizopus* colonizes cereals, fruits, and legumes

The evaluation of the antifungal activity of neem leaf powder on fungal contaminants of cowpea showed that neem leaf powder has an inhibitory effect on fungal growth. The lowest rate of appearance is reached after seven days of treatment. Botanical control using neem leaf powder in vitro showed the ability of this plant to inhibit the growth of fungi such as Rhizopus and Aspergillus. The results obtained in this study are consistent with those of Fotso et al. [38] who reported that powdered plant parts could adequately protect stored grains against insects and storage pests. The presence of the active ingredient azadirachtin in the leaves may explain the reduction in fungal activity in seeds treated with neem leaf powder. Azadirachtin could be used as an insect repellent, growth retardant, and sterilant. Azadirachtin has both direct and systemic action on the mycelial filaments of fungi thereby reducing their survival. Moreover, their visual examination under a magnifying glass did not show any mycelial filaments unlike the negative control seed lots. The advantages associated with the use of these products include, among others, their availability near farmers, their preparation and simple use techniques, they are less expensive natural substances with very little or no harmful effects on the user and the environment [39].

5 CONCLUSION

Our present work is part of the enhancement of the storage of legume seeds through the use of botanical pesticides such as neem leaf powder. The identification of fungal contaminants in cowpea seeds during storage revealed the presence of several fungal genera, including *Mucor, Rhizopus,* and *Aspergillus*. Thus, in view of the altered action of cowpea seeds by the genera *Mucor* and *Rhizopus* and the ability of the genus *Aspergillus* to produce toxins such as aflatoxin, these moulds were considered to be potentially pathogenic to cowpea seeds. The evaluation of neem leaf powder has shown its ability to inhibit the growth of fungal pathogens isolated from cowpea seeds. This powder, therefore, has properties that can be used for the conservation of cowpea seeds.

ACKNOWLEDGMENTS

The authors thank the sellers of cowpea seeds in the markets of Korhogo. This study was self-funded.

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