



Incidence and Types of Fungal Contaminations of Some Locally Produced Cigarette Tobacco Sold in Niger State

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ABSTRACT

Cigarette smoking is a social behavior still practiced in Niger State. Reports have shown that some toxic fungi have been isolated from some cigarette tobacco. This research was therefore aimed at investigating the incidence and type of fungal contamination of locally produced cigarette tobacco sold and consumed in Niger State. Twenty-one different brands of cigarettes produced in Nigeria were obtained from supermarkets in Lapai and Minna in Niger State, Nigeria. The cigarette tobacco was cultured on potato dextrose agar and observed for fungal growth for 72 hours. Isolates of the fungi were sub-cultured on fresh media until pure cultures were obtained. The pure isolates were identified morphologically. The most prevalent genera isolated from the cigarette samples was the *Aspergillus*. Five different fungi; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans* and *Chrysonilia sitophila* were isolated and identified from the cigarette tobacco, with *A. fumigatus* being the dominant fungal contaminants of the cigarette tobacco. The presence of these fungi in the locally produced cigarette tobacco suggests another risk factor associated with cigarette smoking, as some of these fungi are known to produce very toxic secondary metabolites, which are not combusted by smoking and are harmful to human health.

Keywords: Cigarette, Tobacco, Fungi, *Aspergillus*, Smoking, Niger State

INTRODUCTION

Nigeria has one of the leading tobacco markets in Africa, with over 18 billion cigarettes sold annually (Adeloye *et al.*, 2019). In spite of the age restriction and regulations imposed by government that forbids smoking in public places, cigarette manufacturers have continued to churn out new cigarette variants to further appeal to the emotions of their expanding consumers leading to an increase in demand by Nigerian consumers (Adewunmi, 2018). In Nigeria, about 80 percent of consumed tobacco products are produced by three registered tobacco companies (Centre for the Study of the Economies of Africa [CSEA], 2019).

Smoking is the inhalation and exhalation of the fumes of burning tobacco, and cigarette is the most popular method of smoking (Musa and Gbodi, 2020). Smoking, a health risk pattern of behaviour usually acquired during adolescence (Oyewole *et al.*, 2018) is a growing public health problem in the developing world including Nigeria (Salawu *et al.*, 2009).

The health risks associated with cigarette smoking could be accounted for by the composition of the cigarette smoke, as well as some microorganisms that contaminate during its manufacture, processing and storage (Gomina *et al.*, 2019). Some researcher such as Forgacs and Carll (1996); Pauly and Paszkiewicz (2011) and Verweij *et al.* (2000) have reported the identification of toxic microbes in tobacco products such as bacteria and fungal spores. Fungi are involved in the curing process of tobacco, a critical step in the manufacture of cigarettes, and the final product could be contaminated by fungi. (Musa and Gbodi, 2020).

Fungi are ubiquitous and are primarily saprophytic pathogen that causes a number of

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human, animal and plant diseases. Over 100 species of fungi have been shown to produce poisons called mycotoxins during their growth (Christensen and Tuthill, 1985). conjugated

Although a group of Dutch researchers also reported no trace of fungi in the fume of cigarette, containing fungal contaminants, smoked down by a smoking machine (New Scientist, 2002). The real danger and threat to the health of smokers is posed by those fungi spores that are able to elaborate mycotoxins in the cigarette as the mycotoxins are not heat labile (Musa and Gbodi, 2020).

This research was therefore aimed at determining if there are fungal contamination of cigarette tobacco manufactured in Nigeria and sold in Niger state and to identify the fungal contaminants.

METHODOLOGY

Sample Collection

Two sealed packs each of twenty-one different commercial brands of cigarettes, locally produced in Nigeria, were acquired from various supermarkets in Lapai and Minna, Niger State of Nigeria. They were labelled as Samples A1 to A21.

Preparation of Potatoes Dextrose Agar

The tobacco from the cigarette samples were cultured on Potato Dextrose Agar (PDA). The PDA was prepared by heating 500 g of thinly sliced peeled white potatoes with 1000ml distilled water at about 1 hour and then filtered using muslin cloth. Exactly 20 g of glucose, 15g of agar agar and 0.5 g of chloramphenicol was added to the potato broth while stirring the mixture, which was then made up to 1000 ml with distilled water. The mixture was shaken to homogeneity and then autoclaved. Cooled PDA broth (20 ml) was pipetted into sterile Petri dishes using sterile pipettes in a previously fumigated inoculating hood. It was then allowed to cool and gel.

Inoculation of Sample

Two cigarette sticks from each of the two packs of the same cigarette brands, making a total of four sticks from each sample were emptied into a sterile porcelain dish in a previously fumigated inoculation hood. A forcep was used to mix the tobacco leaves together and then inoculated to an appropriately labeled PDA plates. Triplicates were inoculated for each sample and later transferred to another previously fumigated

inoculation hood and left at room temperature. Three plates of uninoculated PDA plates were kept as control. The plates were then observed after 72 hours for fungal growth.

Isolation of Pure Cultures

Plates that had growth with multiple fungal colonies were further separated into different plates to obtain pure cultures of the fungi. A sterile inoculating loop was used to touch the PDA close to the colony to be separated to make it sticky. The sticky end was used to touch the colony to be separated such that some spores or mycelia stick on to the loop. This was then carefully transferred to an appropriately labeled freshly prepared PDA plate. This was repeated until all the different colonies have been transferred into separate plates. The newly inoculated plates were then transferred to another previously fumigated inoculation hood together with three uninoculated plates which served as control. The plates were then observed for fungal growth after 24 hours. The plates that still had mixed colonies growing on it were further separated until pure culture were obtained.

Identification of Pure Isolate

The pure cultures obtained were identified using morphological identification. This was done by examining each isolate under the microscope for Macroscopic and macroscopic characteristics to identify the fungi with references to Kenaga *et al.* (1971); Lucas *et al.* (1992) and Barnett and Hunter (1998).

RESULTS

The inoculation was carried out in triplicates. There was no growth on the control plates, but the plates inoculated samples produced fungal growths except for eight plates. Twenty-one plates had multiple fungal colonies growing on them while 34 plates had single colonies of fungal growth, as seen in Table 1.

Table 2 Shows that most of the samples were contaminated with different types of fungal isolates except for Samples A1, A6, A12 and A21. The incidence of the contaminating fungi is as shown in Table 3. *A. fumigatus* occurred a total of 38 times, *A. niger*, *A. flavus*, *C. sitophila* and *A. nidulans* occurred 30, 11, 4 and 2 times respectively.

Table 1: Occurrence and type of fungi isolates from cigarette tobacco culture

S/N	Sample	Plate I			Plate II			Plate III		
		Isolate I	Isolate II	Isolate III	Isolate I	Isolate II	Isolate III	Isolate I	Isolate II	Isolate III
1	A1	-	-	-	<i>A. niger</i>	-	-	<i>A. niger</i>	-	-
2	A2	-	-	-	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. niger</i>	-	-
3	A3	<i>A. niger</i>	-	-	<i>A. fumigatus</i>	-	-	<i>A. niger</i>	-	-
4	A4	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. niger</i>	-
5	A5	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	-	-
6	A6	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	-	-
7	3	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	-	-	<i>A. niger</i>	-	-
8	A8	<i>A. fumigatus</i>	<i>A. flavus</i>	-	<i>A. niger</i>	-	-	<i>A. fumigatus</i>	<i>A. flavus</i>	-
9	A9	<i>A. fumigatus</i>	-	-	<i>A. niger</i>	-	-	<i>A. fumigatus</i>	-	-
10	A10	<i>A. flavus</i>	<i>A. niger</i>	<i>A. nidulans</i>	<i>C. sitophila</i>	-	-	<i>A. fumigatus</i>	<i>A. niger</i>	-
11	A11	<i>A. fumigatus</i>	<i>A. nidulans</i>	-	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	-	-
12	A12	-	-	-	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	-	-
13	A13	<i>A. fumigatus</i>	<i>A. niger</i>	-	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	<i>A. niger</i>	-
14	A14	<i>A. niger</i>	-	-	<i>A. fumigatus</i>	-	-	-	-	-
15	A15	-	-	-	<i>A. fumigatus</i>	<i>A. niger</i>	-	<i>C. sitophila</i>	-	-
16	A16	<i>A. fumigatus</i>	<i>A. niger</i>	-	<i>C. sitophila</i>	-	-	-	-	-
17	A17	<i>A. fumigatus</i>	-	-	<i>A. fumigatus</i>	-	-	<i>C. sitophila</i>	-	-
18	A18	<i>A. fumigatus</i>	-	-	<i>A. niger</i>	-	-	<i>A. fumigatus</i>	<i>A. niger</i>	-
19	A19	<i>A. fumigatus</i>	<i>A. niger</i>	-	<i>A. fumigatus</i>	<i>A. niger</i>	-	<i>A. niger</i>	-	-
20	A20	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. fumigatus</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. niger</i>	-	-
21	A21	-	-	-	<i>A. fumigatus</i>	-	-	-	-	-

Table 2: Number and Type of Isolates isolated from Cigarette Tobacco samples

S/N	Sample	Number of Isolates	Type of Isolates
1	A1	2	1
2	A2	4	3
3	A3	3	2
4	A4	8	3
5	A5	7	3
6	A6	3	1
7	A7	3	2
8	A8	5	3
9	A9	3	2
10	A10	6	5
11	A11	6	4
12	A12	2	1
13	A13	5	2
14	A14	2	2
15	A15	3	3
16	A16	3	3
17	A17	3	2
18	A18	4	2
19	A19	5	2
20	A20	7	3
21	A21	1	1

Table 3: Incidences of Fungi Isolated From 21 Samples of Cigarette Tobacco

Fungi	Incidence
<i>Aspergillus fumigatus</i>	38
<i>Aspergillus niger</i>	30
<i>Aspergillus flavus</i>	11
<i>Chrysonilia sitophila</i>	4
<i>Aspergillus nidulans</i>	2

DISCUSSION

Result shows that cigarette produced locally in Nigeria are capable of being contaminated by fungi. This finding agrees with the findings of Rubenstein (1992), Forgacs and Carll (1996) and Warke *et al.* (1999) who reported the presence of fungal spores in tobacco products. It also agrees with the findings of Okechi *et al.* (2014), Onuorah and Orji (2016) and Ogidi *et al.* (2018), who observed and reported the presence of fungal contamination of tobacco products sold in Nigeria.

The genus *Aspergillus* was the most preponderant of the fungal contaminant, accounting for 81 of the 85 total fungal isolates that contaminated the cigarette samples. This

agrees with the finding of some other researchers who reported that the prominent fungi that have been isolated from tobacco products are of the Genus *Aspergillus* (Papavassiliou *et al.*, 1971; Welty and Nelson, 1971; Varma, 1991). This could probably be a result of *Aspergillus* being ubiquitous in nature.

The highest occurring species was *Aspergillus fumigatus*. Its presence in cigarette tobacco could be another associated risk for cigarette smoking as it is capable of eliciting several secondary metabolites such as fumagillin, fumitremorgin, gliotoxin, restrictocin and verruculogen, which could cause deleterious health effects. Gliotoxin particularly is ciliotoxic and can cause damage to the respiratory epithelium of humans (Hassan, 2019).

Aflatoxin, the most potent carcinogen yet discovered (Christensen and Tuthill, 1985) is produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Musa and Gbodi, 2020). Thus, the presence of *A. flavus* as a contaminant of locally produced cigarette tobacco could be a worrying sign.

Chrysonilia sitophila was the only non *Apergillus* contaminant found in the cigarette samples. *C. sitophila* has a cosmopolitan, saprobiological spread, contaminating the bread, some pastries and various vegetables, and is considered a contaminant without pathogenicity, but may have implications in triggering asthmatic disease (Rapuntean *et al.*, (2018). The presence in cigarette sample may not pose any harm as the spores are likely to be destroyed during the smoking process.

Sterigmatocystin is the major metabolite of *Aspergillus versicolor* and *Aspergillus nidulans*, and is similar to aflatoxins, and it is also of hepatotoxic and hepatocarcinogenic potentials (Hassan, 2019). Two of the cigarette samples were contaminated with *A. nidulans* and this raises the issue of the potentials for mycotoxin associated toxicity with cigarette smoking in Nigeria.

Conclusion

All the cigarette samples investigated were contaminated with one or more fungal isolates. *Aspergillus* species was the most predominant fungal contaminant of the cigarette tobacco. Some of the contaminants isolated are known toxicogenic fungi, and it raises the concern for

mycotoxin associated risks for smoking of locally produced cigarettes sold in Niger State.

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