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Smoke dried fish quality as affected by insect and fungal colonization within storage in urban markets in Cameroon.

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Abstract
Background: *smoked dry fish is a widely consumed foodstuff throughout the world, mainly in coastal areas. However, these foods not well managed are subject to insects and Mold attacks, with dangerous consequences on consumers health. This study aims to assess insect's attacks, sensory characteristics and fungal species associated with the contamination of smoke-dried fish.* ***Methods****: survey study lead to the collection of 1200 smoke dry fish samples of three different species in four Cameroon markets, at the rate of 10 per species per record trader. The samples divided in two groups in which the first were subject to sensory (general appearance and flavor, Color, texture, and acceptability) and weevil impact analysis and the second used for fungi determination on PDA media. All the results were subjected to statistical analysis using One-way ANOVA.*
Results: *Results obtained showed thatmore than a week of storage, percentages of attacks were 92%, 94.89%, 85.71% and 90%, respectively for the Nvog-ada, Mfoundi, Youpwe and Bonassama markets. However, the attributes individually consider were significant differences between species for no hole and hole plus powder ($p<0.05$). Fungi isolate from the three species of smoke dry fish were Aspergillus flavus, Aspergillus fumigatus, A. niger, A. tamari, A. nomius, A. oryzae, A. ocraceus, Penicillum chrysogenum, Rhizopus stolonifer, Mucor and Fusarium species. Aspergillus sphad the highest rate of occurrence among the isolated fungi follow by Penicillum, Mucor, Rhizopus and Fusarium.* ***Conclusion:*** *smoked dried fishes stored for sale in four urban markets were subject to insects and fungi attacks.*
Keywords: *Smoked dry fish, insects, Fungal, Aspergillus, market.*

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I. Introduction

Fishery products including smoke dry fish are of great contribution to food security either by providing nutrients (proteins, lipids, minerals) to human body or by providing income for fishermen, processors, and countries (FAO 2006, FAO 2016a). The importance of fish and by-products is shown by an increasing annual world-wild fish consumption from 9.9 kg per capita to 20.5 kg per capita between 1960 and 2018 (FAO 2016a, FAO 2016b). Fish contributes for 17% and 6.6% respectively to animal protein intake of world-wide population and total protein consumed (FAO 2016b). Because of its perishable nature, a wide range of techniques for preserving fish have been developed including smoke dry. smoking is one of the most used conservation methods (Alhassan, 2012; Chabi et al., 2014; Kpodekon et al., 2014; Kumolu-Johnson, 2010; Yakubu & Ngueku, 2015).Smoke-dry, apart from giving the product desirable taste and odour, preserves and prolongs the shelf-life of fish products conveniently at ambient conditions through its anti-bacterial and oxidative effects, lowering of pH, imparting desirable colouration, accelerating the drying process, and acting as antagonist to spoilage agents (Akande et al., 1993, Sengor et al., 2004, Eyo, 2012, Job, M.O., Agina et al., 2016, Sani et al., 2016). Indeed, fish consumption is always scolonized by insects and microorganisms such as bacteria, yeasts, and moulds alongside with the processing units (Sani et al., 2016). In humid tropical conditions, dry smoked fish with low moisture contents are prone to insect infestation, while others in medium with high moisture contents are predisposed to both bacterial and fungal contaminations (Banwart 2004; Ayuba et al., 2013).Generally, post processing microbial and insect's contaminants are originate from poor handling

practices, air, fish source (Ayuba et al., 2013; Banwart, 2004). Improper smoke dry fish can considerably impact consumers health with drastic consequences. Numerous pathogenic agents isolated from different types of fish were toxigenic, and their toxins retained in fish flesh even after salting and storage periods which causes serious systemic dysfunctions and public health hazards (Hungerford et al., 1998; Swaminathan and Sparling, 1998; Sani et al., 2016). Despite the numerous reports on the contaminants of smoke-dried fish and its public health implications, documented reports, and studies on the mycology of smoke-dried fish in general still needed. This study was therefore conducted to assess insect's attacks, sensory characteristics and fungal species associated with the contamination of smoke dry fish in four urban markets in Cameroon.

II. Materials and methods

2.1. Study Area and sample collections

The study was conducted in two main cities in Cameroon including Yaoundé (3° 52′ N, 11° 31′ E) and Douala (4° 3′ 0″ N, 9° 42′ 0″ E). Those cities have environmental characteristics close to the humid tropical rain zone, favorable to the growth of microorganisms. Four targeted markets including 'Nvog-ada' and 'Mfoundi' at Yaoundé, 'Bonassama' and 'Youpwe' at Douala, were involved in this study. Short survey initially conducted credited those markets with their regular supplies of smoked dry fish of various species. They did not only provide this food to local populations but also to the entire sub region of central Africa. Ten traders from each market were selected according to the capacity of their store, who was supposed to have permanently three packs of 1000 individual smoke dry fish. Samples were randomly selected from the stock of each trader, purchased, and keep aseptically in well-labeled sterile polyethene bags before transported to the Laboratory for processing, sensory and weevil impact analysis, exploration of fungi. A total of 1200 smoke dry fish samples at the rate of 10 per species per trader in each market were collected. Fish species included "Catfish" (*Clarias gariepinus*), "Stock fish" (*Gadus morhua*) and "Bonga fish" (*Ethalmosa fimbriota).* Finally, samples were divided in two groups in which one were used for sensory and weevil impact analysis and the other for fungi determination.

2.2 Sensory evaluation

Sensory evaluation of the smoke dry fish samples after one week of storage was carried out at the Laboratory of Valorization and Quality Control of Aquatic Products, Institute of Fisheries and Aquatic Products, University of Douala, by an untrained panel of 30 members that included both men and women with age groups ranging from 25 to 40 years. Sensory attribute such as flavor, color, texture (friability), general appearance and overall acceptability of the smoked dry fish were used. Prior to the test, brief explanation of each parameter was addressed to the panel. Products were scored on a 5 point hedonic scale range as follow: 4 - Excellent, 3 - Good, 2 - Fair, 1 - Poor, and 0 - Bad (Eyo, 2001). Impact of damage cause by insect attacks was evaluated as follow: 0 - No hole in the fish skin, 1 - Presence of holes in the fish skin and 2 - Holes and powder in the fish skin.

2.3Exploration of smoke dry fish fungal flora.

The fish samples were aseptically processed for fungal isolation following the procedure described by Oyebamiji and Oyebimpe (2013). Smoke dry fish samples was blended, and 5 g suspended in 10 ml of sterile distilled water. One milliliter of the suspension of blended smoke dry fish samples was inoculated on Sabouraud Dextrose Agar plates. The plates were sealed with masking tape to prevent contamination by crawling insects and were then incubated at room temperature (25℃±3) by placing the inoculated plates on a clean bench in the Laboratory. The plates were then examined daily for fungal growth for at least 1 weeks. Isolates were identified using macroscopic (cultural characteristics), microscopic morphologies and comparison with already identified fungal species (Hungerford *et al.*, 1998; Madigan *et al.*, 2009).

2.4 Data analysis

The data collected was subjected to statistical analysis using one way Analysis of variance (ANOVA) and Duncan Multiple Range Test was used to appreciate the difference between means. The statistical analysis was conducted by using SPSS version 18 software.

III. Results

3.1 Insects attack of smoke dry fish per markets

The distribution of insect impacts per market are shown in Table 1. It appears that all the smoke dry fish collected in the involved urban markets in Cameroon are covered by insects' impacts. These impacts appear with a variable frequency depending on the storage time and the different markets. About one week of storage, a growing attack by insects of smoke dry fish was recorded in regard of the markets of origin. More three weeks of storage, the rate of insect attacks were considerably high with percentages of attacks ranging as follow: 92%, 94.89%, 85.71% and 90%, respectively for the Nvog-ada, Mfoundi, Youpwe and Bonassama markets.

Table 1: impact of insect attack on smoke dry fish species after storage in four local market in Cameroon.

Markets	Insect attack	
	One Week later	**Three weeks later**
Nvog-ada	8%	92%
Mfoundi	5.11%	94.89%
Youpwe	14.28%	85.71%
Bonassama	10%	90%

3.2 Insects attack of smoke dry fish per species

The proportion of insect attacks of three species of smoke dry fish are shown in Fig 1. It appears that all the smoked-dried fishes sold are subject to insect's attacks at variable levels. The species *Clarias gariepinus* and *Ethmalosa fimbriata*are the most susceptible as they record the entire attributes involved for the evaluation in this study. The species *Gadus morhua* is less susceptible to insect attacks as he did not show all the attributes at the same time on individual sample. Gradually, the proportion of individual fish damage is highest on *Clarias gariepinus*, follow by *Ethmalosa fimbriata* and *Gadus morhua*. While consider attributes individually, they were significant differences between species for no hole and hole plus powder($p<0.05$).

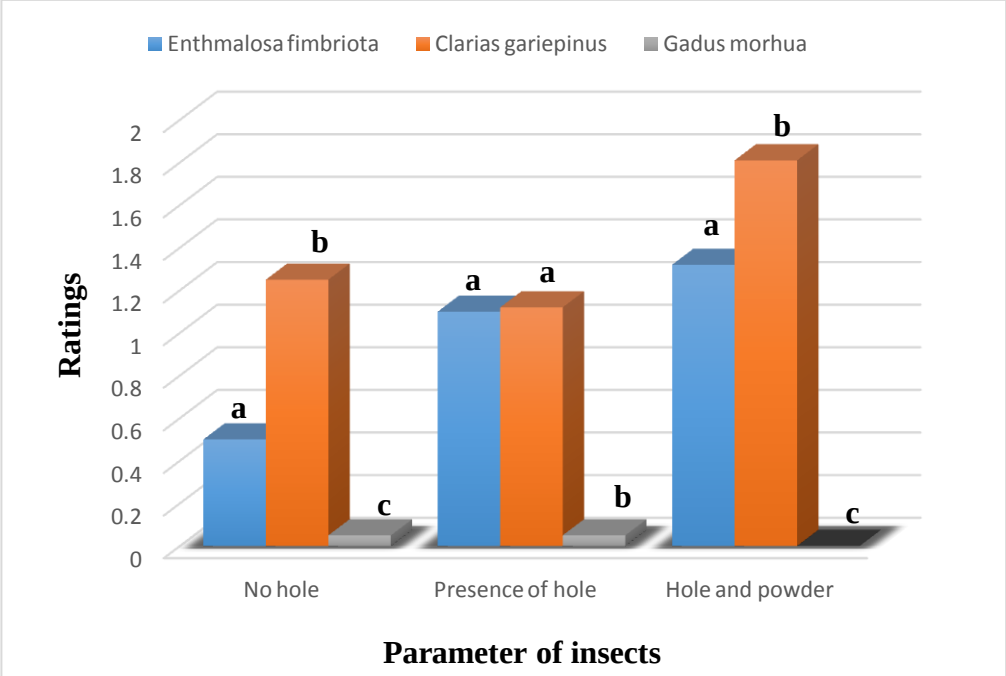

Fig 1: Level and frequencies of insect's attacks of three smoke dried fish species in four urban markets in Cameroon.

The histogram bars bearing the same letters and reflecting the same attributes are not significantly different ($p<0.05$).

3.3 Sensory analysis

Sensory attributes of three species of smoke dry fish variation are record in figure 2. We notice that the scores resulting from the appreciation of the different sensory attributes of the three species of smoke dry fish are highly variable. For the color attribute, no significant variation was record in all three species of smoke dry fish studied ($p<0.05$). For texture attribute, significant difference was record between species involved with *Clarias gariepinus* having the best score follow by *Ethmalosa fimbriata* and finally *Gadus morhua.* For the general appearance attributes, flavor and overall acceptability, the scores obtained from the appreciation of the three species of smoke dry fish show the lowest score for the species *Clarias gariepinus.* However, this observation is mitigated for the two other smoke dry fish species as it shows the best score to the species *Gadus morhua* in regard of the attribute general appearance. The best score shift to the species *Ethmalosa fimbriata* in regard of the attribute flavor and overall acceptability. All the differences record within species in regard of attributes were significant ($p<0.05$).

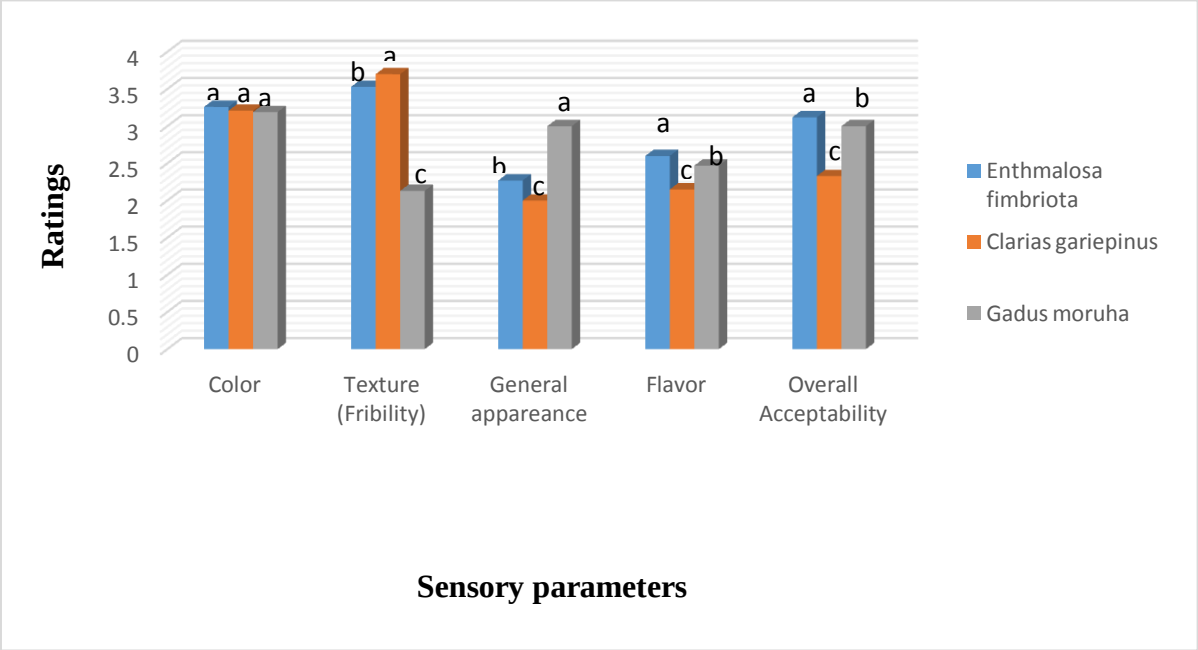

Fig 2.: Frequencies of quality attributes of three smoke dried fish species from four urban markets in Cameroon**.** *The histogram bars bearing the same letters and reflecting the same attributes are not significantly different (p<0.05).*

3.4Proportion of fungal in four local markets

Samples from 85 of the 200 smoke-dried fish examined, showed fungal contamination (Table 2). The rates of fungal contamination of smoke-dried fish sold in Nvog-ada, Mfoundi, Bonassama and Youpwe markets in Cameroon were ranging from 38% to 46%. This finding reveals that smoke-dried fish sold in the study markets in Cameroon are contaminated by fungi. The data record show significant variation of the proportion of fungal contamination in regard of markets.

Table 2. Distribution of fungal contaminants of smoke-dried fish in four markets in Cameroon

Markets	**N^0 Samples**	**No. (%)* positive**
Mvog-ada	50	23^a (46)
Mfoundi	50	22^a (44)
Bonassama	50	21^b (42)
Youpwe	50	19^c (38)
Total	**200**	**85 (85)**

* = % of total number sampled for each row.

The macroscopic and microscopic features of the fungi isolated are presented on Table 3. The difference between *A. flavus, A. fumigatus, A. ochraceus, A. oryzae, A. tamari, A. nomius* and *A. niger* is in the form of colony. While A. flavus has yellowish green colonial color with scattered spores, *A. niger* is dark in color, *A. fumigatus* has green in color, *A. ochraceus* as chamois color, *A. oryzae* has olive green color, *A. tamari* has dark bronze green colonial color, *A. nomius* has clear colonies with ball head. *Penicillium chrysogenum*, has dark-green colonial color, *R. stolonifer* has a brownish wooly colony, Mucor spp has a white colonial color and the Fusarium spp as brown colonial color.

Table 3. Macro and Micro features of the fungi isolated from smoke dry fish samples from four urban markets in Cameroon.

Close to	Colonial macroscopic characteristics	Microscopic characteristics
Aspergillus niger	Black colony with hallow region. Powdery Surface and regular colonies.	-Septate hyphae. Conidiophores terminate in a swollen vesicle
Aspergillus flavus	Yellow-green colonies with whitish powdery edge, reserves of colonies are colorless	Uniserial heads, globular vesicles, swollen Conidiophores, phialides carried by vesicle
Aspergillus fumigatus	Dark green to grayish-black colonies.	compact column conidian heads, blue-green colony surrounded with withe mycelia
Aspergillus ochraceus	Powdery colonies, Buff colored colonies, reserves of colonies are colorless.	biseriate conidial heads, rough conidiophores, globular vesicles, phialides carried by mutules.
Aspergillus oryzae	fluffy colonies, Powdery and olive-green Colonies, reserves of colonies are colorless.	Bruns uniserial heads, hyaline conidiophores, phialides inserted directly in the vesicle.
Aspergillus nomius	clear and very flaky Colonies, low growth rate colonies,	Colourless conidiophores, globose vesicles. echinulate conidia, uniserial phialides
Aspergillus tamarii	dark bronze to green Colonies, reverse is orange-yellow	heads bear divergent spore chains, broad and rough conidiophores, spherical vesicle, large and round spores
Penicillium chrysogenum	green diamond to dark green colonies.	- Globe-like close to glass beads conidia, branching hyphae, erect septate conidiophores
Fusarium species	Snow white colony. Aerial mycelium growth. Colonies turns pink as it ages, reverse is purple	Septate hyphae. Spindle form of conidia, branch conidiophores, Rod like and slightly bent sporangia, scattered macro-phialides
Rhisopusstolonifer	White to gray-brown colonies, white on the reverse. Fast growth rate.	Dark pigment sporangium, branched hyphae, Large saclike containing sporangiophores inter-connected with septate hyphae.
Mucor species	White colony surrounded with irregular hallo zone, fast hyphal growth.	Thick non septate hyphae. laterally departing sporangiospores, sporangia filled with spores

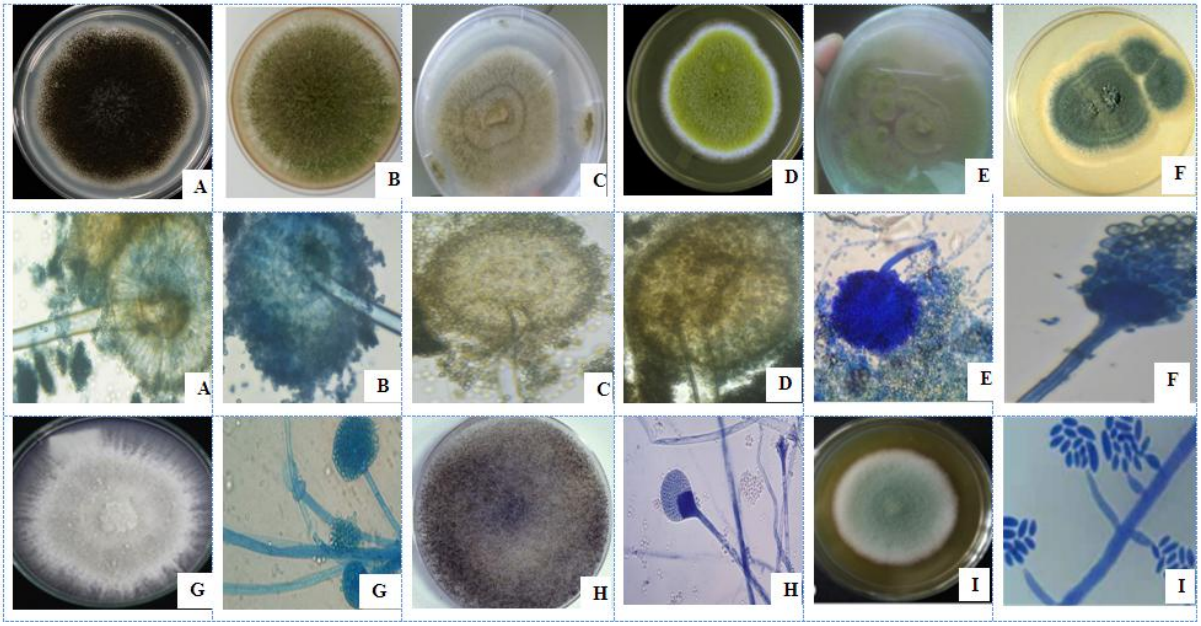

Fig 3: Macro and micro structures of fungi isolated from *Ethmalosa fimbriata* species from 4 urban market in Cameroon. A, B, C, D, E, F: Aspergillus sp; G: Mucor sp; H: Rhizopus sp, I: Fusarium sp

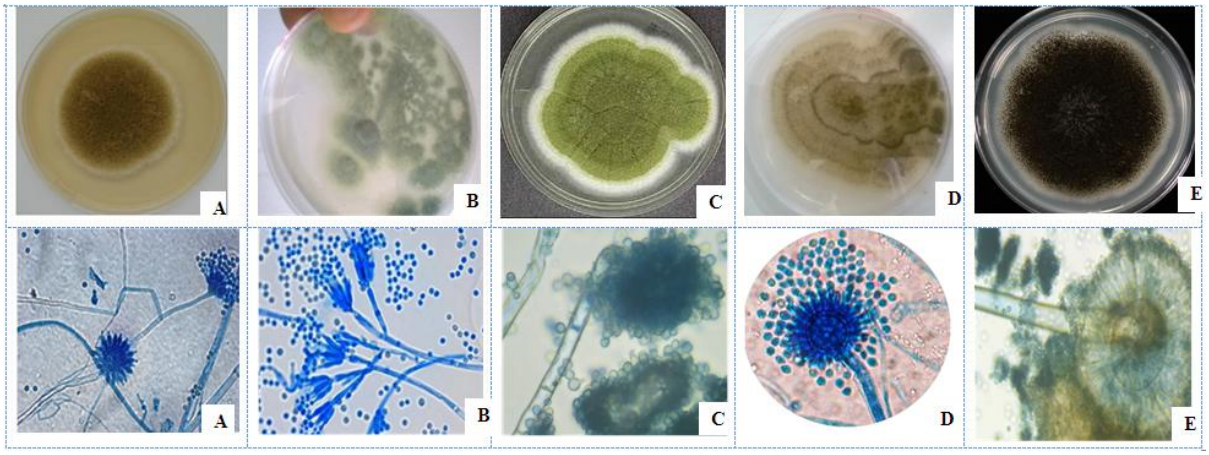

Fig 4: Macro and micro structures of fungi isolated from *Gadus morhua* species from 4 urban market in Cameroon; A: Rhizopus sp;B: Penicillium sp;C, D et E: Aspergillus sp.

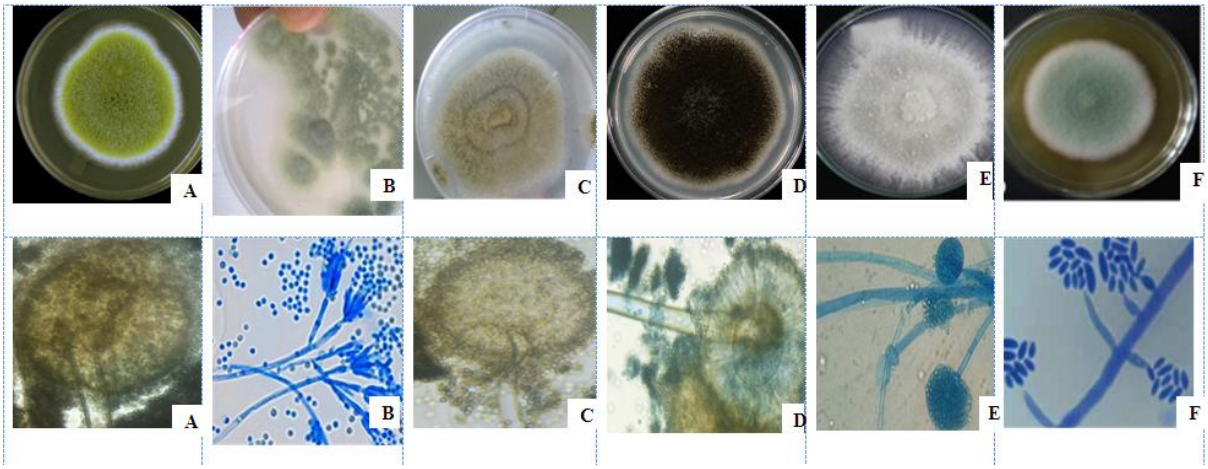

Fig 5: Macro and micro structures of fungi isolated from *Clarias gariepinus* species from 4 urban market in Cameroon; A, C, D: Aspergillus sp. B: Penicillium sp;E: Rhizopus sp and F: Fusarium sp

A total of 91 fungal isolates were recorded from the 85 positive smoke-dried fish samples examined (Table2; Table 4). Youpwe market show the less fungal contamination while Bonassama market show the highest fungal contamination of the three smoke dry fish species involved in this study. The other two markets (”Mvog-ada”, “Mfoundi”) show a fungal contamination of the three species of smoke dry fish but with numerical values ranging between those record from the “Bonassama” and “Youpwe” markets (Table 4). another outcome from this table is that all the fungi isolate are found on the smoke dry fish of "Mvog-ada" market while the three other markets have an absent species of which *Rhisopus stolonifer* for the "Mfoundi and" Youpwe "market and *Fusarium* sp for the "Bonassama" market.

Table 4. Frequency of occurrence of fungi isolate on smoke dry fish samples from four urban markets in Cameroon.

Isolats of fungi	**Markets**				**Total (%) ****
	Nvog-ada	**Mfoundi**	**Bonassama**	**Youpwè**	
Aspergillus niger	4	3	4	3	14 (15.4%)
Aspergillus ochraceus	2	2	3	2	9 (9.9%)
Aspergillus oryzae	1	2	2	2	7 (7.7%)
Aspergillus flavus	7	5	6	5	23 (25.3%)
Aspergillus fumigatus	2	2	4	2	10 (11%)
Aspergillus nomius	2	1	2	1	6 (6.6%)
Aspergillus Tamarii	2	3	1	1	7 (7.7%)
Penicillium chrysogenum	2	1	2	1	6 (6.6%)
Fusarium species	1	1	0	1	3 (3.3%)
Mucor species	1	1	1	1	4 (4.4%)
Rhisopusstolonifer	1	0	1	0	2 (2.2%)
Total *	**25 (27,47)**	**21 (23,07)**	**26 (28,57)**	**19 (20,87)**	**91 (100)**

* = % of total number of isolates for each market; ** = % of overall number of isolates (91).

The present study revealed that the fungal genera contaminated smoke dry fish sold in four local markets in Cameroon were *Aspergillus*, *Penicillum*, *Mucor*, *Rhizopus*, *Fusarium* species (Table 4). *Aspergillus* (83.6%) was the dominant fungi recorded colonized the smoke-dry fish contamination. *Penicillium* (6.6%), *Fusarium* (3.3%), *Mucor* (4.4%), and *Rhizopus* (2.2%) occurred less frequently. Members of Aspergillus species, taken as a group (Fig 6), recorded the highest isolation rate (83.6%). Aspergillus isolates obtained in this study were identified, as shown in Table 4, as *A. niger* 14 (15.4%), *A. ochraceus* 9 (9.9%), *A. oryzae* 7 (7.7%), *A. flavus* 23 (25.3%), *A. fumigatus* 10 (11%), *A. nomius* 6 (6.6%), *A. tamarii* 7 (7.7%). *A. flavus*, *A. nigerand A. fumigatus* were the dominant species.

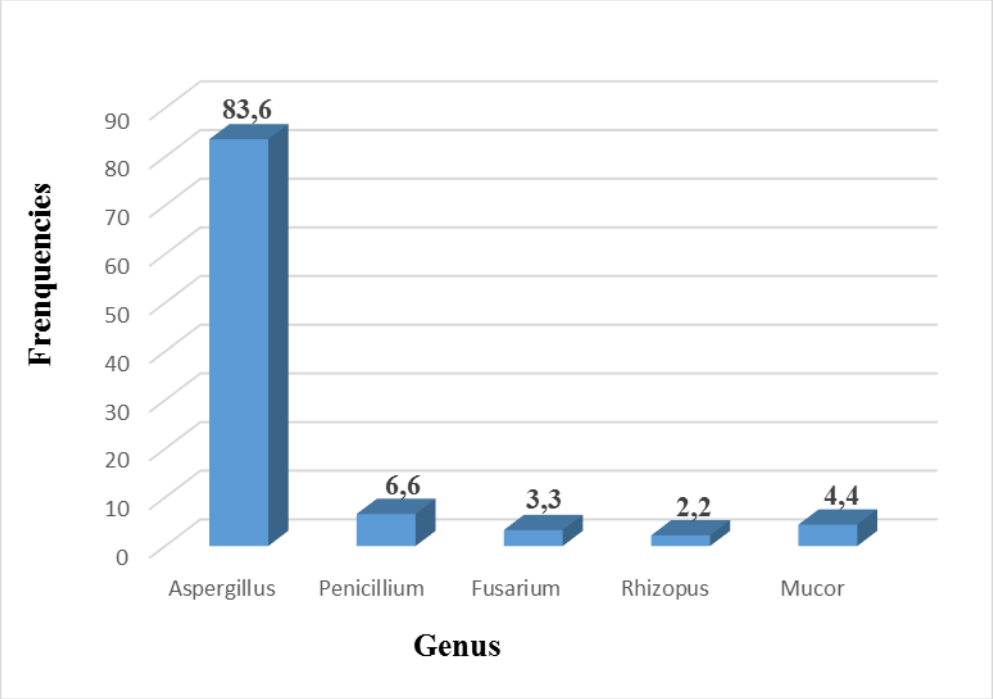

Fig 6. Percentage occurrence of fungal isolates in smoke-dry fish in four local markets in Cameroon.

IV. Discussion

The smoke dry fish species used in our study were *Clarias gariepinus*, *Ethmalosa fimbriata* and *Gadus morhua*, which are fish commonly encountered in the smokee dry fish chain in coastal areas. All of these fish were attacked by insects in all markets studied at varying frequencies. Insects are often found infesting smoke dry fish after processing. In this study, no identification was made on beetles and all were lumped as "insects".It seems that the perpetuation of the marketing activity of the smoke dry fish maintains the inoculum of these insects in the storage places, which in turn would lead to a rapid attack of the fish newly obtained by the traders (Banwart2004;Ayuba et al., 2013). This may justify why the fish were attacked in such large numbers about three weeks of storage ago.In this study, assessment three species of smoke dry fish from four urban markets of Cameroon revealed insect impacts(table 1). These impacts appear with variable frequency depending on the duration of storage and the markets involved. Several studies on smoke dry fish show that they are subject to attack by insects in regard of the storage periods (Oduor-Odote et al., 2010; Sani et al., 2016)

The smoke dry fish was subject to insect attacked in varying proportions in regard of the species involved. *Gadus morhua* species was the least attacked fish compared to the two others, both stocked in the same environment. It is possible that genetic factors linked to the species could justify such variation.

In the four markets involved in our study, the sensory analysis of the smoke dry fish of our three studied species presents acceptable assessment scores for the various attributes, thus reflecting their initial good quality. However, these attributes show lower and lower scores with storage time. This is noticeable with the reduction in the score of the texture attribute (figure 2).Similar finding was record in study performed on the Italian market realizing that the hygienic quality of the smoked salmon is poor at the expiry date (Vergara et al., 2001)

The appreciation of the sensory quality of the three species of fish studied on four markets clearly shows that it is less and less good as the products last in storage. The assessment of quality has been essentially macroscopic so far. it is important to explore the microscopic aspect for a better appreciation

Our results show that all collected smoke dry fish samples are contaminated with microscopic fungi. This contamination being at variable frequency according to the markets and the species of fish involved. A range of study point out contamination of smoke dry fish by fungi flora across markets precisely within storage (Junaid et al., 2010; Akande and Tobor, 2012; Eyo et al., 2012; Ayuba et al., 2012; Ayuba et al., 2013; Jimoh et al., 2014; Sani et al., 2016). We do believe that high fungal contamination of smoke dry fish in the study areas could be associated with the handling of fish which could engender microbial contamination especially in artisanal fishery due to unhygienic methods as record previous studies (Akande and Tobor, 1993; Sani et al., 2016). In addition, post-processing handling and storage, may be responsible for the presence of high numbers of fungi in the smoked fish samples at the points of sale (Okiya and Akeodi, 1998). Another possibility could also be a result of survival of the spores of these organisms during the heat treatment and their subsequent germination afterward (Edema et al., 2010). Thus, the growth of fungi in smoke dry fish can be affected by the storage method employed. It was observed, during sampling for this study that smoke dry fishes were stored either in baskets, wooden boxes or metal containers with covers. Storage containers play a vital role in the preservation and shelf-life of smoked dry fish, and recommended the use of airtight storage containers for smoke fish (Osibona et al., 2018).

The present study revealed that the fungal genera colonizing three smoke dry fish species sold in four local markets in Cameroon were Aspergillus, Penicillum, Mucor, Rhizopus, Fusarium. This finding is in accordance with that of Sani et al., 2016 who reported a number of fungi genera similar to those obtain in this study. The fungi isolated in this study are all opportunistic pathogens (Hungerford 1998) of medical importance. The presence of toxigenic fungi, for example some species of Aspergillus and Penicillium in foods as contaminants increases the risk for mycotoxins production which could induce gastrointestinal and metabolic disturbances when contaminated foods like smoke dry fish are consumed (Martin 2008; Bennett et al., 2003).
Results of this work show that the diversity of fungi recorded on the fish studied varies according to the target market. This is the case with the genus Rhizopus present on fish from two out of four markets. Similar studies on smoke dry fish have shown that the diversity of opportunistic fungal species varies depending on the sample collection environment (Akwuobu et al., 2019; Sani et al., 2016).

V. Conclusion

The present study did to assess insects' attacks, sensory characteristics and fungal population associated with the contamination of smoke dry fish in four urban markets in Cameroon showed that all the three species of fish study have insect impact on their skin. The rate of these impact increase with the storage time. The sensory characteristics of the fish were good at the beginning but decrease with storage time. At least 4 genera of fungi were isolated from fish sampling in the survey market with the predominance Aspegillus.Important measures need to be taken to train local populations in hygienic practices as well as good practices within trading activities of smoke dry fish

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