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Growth response of microorganism to powdered neem leaves (*Azadirachta indica*) and vegetable oil on smoked dried fillets of African Catfish (Chrysichthys nigrodigitatus)

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Abstract
Fillets of 50 g ± 5 treated with 30% brine and hot smoked to constant weight in an enclosed coal oven were divided into three parts; X was dusted with air dried powdered neem leaves, Y with freshly extracted vegetable oil, and Z was untreated. Products were packaged in airtight polythene containers, stored and monitored for microbial development for 12 weeks. Nutrient quality was determined.
Microbial organisms were detected from the second week of storage. Mean fungal growth on X, Y and Z were 1.5 x 10^3cfu/g ± 0.2x10^3, 5.3x10^2 cfu/g ± 0.08x10^3 and 0.9 x 10^3 cfu/g ± 0.091 x10^3 at moisture contents 22.36±0.16, 23.61±0.67 and 23.13±0.52 respectively. Population growth pattern varies with the different treatments; it doubles every fourth week in X, it continues geometrically to stabilise between the eight and tenth week in Y and less gradual in Z. Microbial development was significantly different among various treatments ($p< 0.05$). Fungi associated with powdered neem leaves on fillets were *Aspergillus flavus, Penicillium citrium, A. parasiticus* and *A. Niger* in order of importance. Though neem was found to inhibit fungal growth on mustard seeds, growth seems not to have been inhibited on *C. nigrodigitatus*, however mycotoxin production ability could have been reduced. There is need to determine the mycotoxin production level of fungi associated with dried fish preserved with powdered neem leaves.
Fillets of 50g ± 5 treated with 30% brine and hot smoked to constant weight in an enclosed coal oven were divided into three parts; X was dusted with air dried powdered neem leaves, Y with freshly extracted vegetable oil, and Z was untreated. Products were packaged in airtight polythene containers, stored and monitored for microbial development for 12 weeks. Nutrient quality was determined.
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Keywords: *Chrysichthys* sp., powdered Neem leaves, microbial growth, dried fish,

1. Introduction
Fish and fish products are important in the development strategies of many developing countries, especially the Least Developing Countries (LDCs), Small and Vulnerable Economies (SVEs) and Small Islands Developing States (SIDS). The fisheries sector is a large source of employment, a key dietary input and an important element of local livelihood [1]. One of the major courses of excessive pressure on fisheries resources is the need to make profit or at least to break even. Fishermen's response to low revenue has always been increased pressure, which has contributed to the problem of over exploitation of many stocks in the world. Losses in fish production have been estimated at between 20 and50% due to poor handling and processing and preservation especially in the humid tropics [2]. Fish deteriorate

fast after death as a result of intrinsic and extrinsic factors which cause changes of various kinds that immediately follow [3]. Many of these changes can sometimes occur simultaneously but at different rates. Certain kinds of deterioration therefore become important at different times along the processing and preservation line. Spoilage in fish is caused by living organisms, enzymes and chemical oxidation. Living organisms causing spoilage at different points from capture to marketing include bacteria, fungi and insects. Solving the problem of fish production requires a composite approach which should include better post-harvest handling and preservation strategies.

Artisanal fishermen have various means by which they try to increase the shelf life of fish, these include smoke drying, sundrying, salted sundrying and salted smoke drying. Salt performs dual roles in fish the osmotic pressure set by the salt is higher than the body fluid leading to loss of moisture and reduction in water activity of the fish tissue. In addition, salt also retards the activities of enzymes, chemicals and bacteria in the tissue of fish. It plasmolyses all bacteria cells except those that are halophilic and haloduric as it withdraws water from them. Salt has to be present in large quantity to exclude most bacteria from fish at water activity (A_w) level of less than 0.80^2.Studies have shown that these preservation methods have not been effective in extending the shelf life of fish and its products especially with respect to fungal invasion [4, 5]. Bacteria were identified as major spoilage organisms for fresh fish while dried fish are attacked by fungi [6]. Some authors however identified fragmentation, insect infestation and microbial spoilage as major contributors to losses of stored dried fish [2]. Losses caused by insects could cause as high as 50% loss in weight of dried fish. *Dermestes maculates* and *Necrobia rufipes* feed on fish flesh reducing both quantity and quality. Fragmentation occurred during transportation and is associated with bad packaging. Microbial attack in storage results from high moisture content in stored dried fish, making it putrid and dangerous for consumption. Fungi (moulds) are especially dangerous in food because they produce toxins which are detrimental to human health. Some of the fungi found to be associated with fish in the tropics include those of the *Aspergillus* sp., *Penicillium* sp., *Candida* sp. *Fusarium*.

Extracts of plants such as neem [7] garlic [8], betel leaf [9], clove, *Syzygium aromaticum* [10] have been found to effectively inhibit the growth of fungi and or the production of toxins on food crops at various concentrations. The inhibitive properties of powdered leaves of *Occimum gratissimum* [11], *Cymbopogon citratus, S. aromaticum* [12] on fungi growth and toxin production were tested by other scholars respectively. The powders were found to be effective. Neem products have been successfully used as antifungal agents in some plant food items [10].

Health hazards from exposure to toxic chemicals and economic considerations make natural plant extracts ideal alternatives to protect food and feed from fungal contamination [10]. In search of alternatives to chemical antimicrobial agents as a response to the growing health concerns and economic reasons, this study investigates the efficacy of powdered neem as a growth inhibitor to fungi and bacteria on the fillets of *Chrysichthys nigrodigitatus*.

2. Materials and Methods

Neem leaves were collected fresh from matured fruiting trees, air dried and then made into powder while freshly prepared groundnut oil was purchased from groundnut cake producers for the study. *Chrysicthys nigrodigitatus* weighing between 250 and 500g were purchased from fishermen combing lower section of the Ogun River in South western Nigeria. The fish species were stunned, killed and made into fillets weighing 50g ± 5. The fillets were treated with 30% brine, allowed to drain and then hot smoked to constant weight in an enclosed coal oven. Smoke was supplied from hard wood. Smoked samples were divided into three parts; X, Y and Z. Sample X was dusted with air dried powdered neem leaves, Y with freshly extracted vegetable oil, and Z was untreated. Products were packaged in airtight polythene containers for storage. Each pack was monitored fortnightly for microbial development for a period of 12 weeks. Dried samples were analysed for nutrient quality at biweekly intervals [13]. Statistical data were analysed using SPSS 15 for windows. Prevailing environmental conditions at the time were observed from the university weather station.

3. Results

The mean values of prevailing environmental conditions monitored during the period were temperature 28^0C, relative humidity 60%, rainfall 58mm and wind speed 3.1Km/hr.

The mean values of the nutritional components of the products are presented on Table 1. Protein level was found to have increased between the first and the fourth week, there after which the level started reducing. Lipid and fibre contents decreased with increasing length of storage while the moisture content increases. At the end of the twelve week, the level of protein, fibre and lipid were highest in Z which was not treated, X (which was dusted with leave powder) and lowest in Y sample sprayed with vegetable oil. Moisture content followed a reversed order (Table 1).

Table 1: mean values of nutrient composition of the different products (%)

Nutrient	X	Y	Z
Protein	55.47 ±1.7	54.75±1.04	55.15±1.73
Lipid	5.87±0.21	5.81 ±0.21	5.77±0.21
Fibre	1.64±0.19	1.60±0.19	1.59±0.19
Ash	14.47±0.60	14.24±0.60	14.26±0.60
Moisture	22.46±2.57	23.62±1.97	23.13±2.56

3.1 *Microbial population*

Weekly mean population for the microbes and the moisture conditions are presented on Tables 2. In treatment X, Fungi population almost doubled between the second and the fourth week and between the eight and tenth week after which there was a reduction in the rate of multiplication. In Y growth doubled between second and fourth week as well as between the fourth and sixth week. Fungal growth was more gradual in treatment Z. Mean fungal population was observed to be highest in treatment X ($1.5x10^3$± $0.2x10^3$) and lowest in Y ($5.30x10^2$± $0.08x10^2$) Mean populations of fungi were significantly different from one another.

The multiplication of bacterial population was gradual in the early weeks in all the treatments. Geometric growth occurred between sixth ($2.92x10^3$ ± $0.66x10^2$, $8.60 x 10^3$ ± $0.22x10^2$) and eighth ($3.90x10^4$ ± $1.12x10^2$, $1.09x10^4$ ± $0.14x10^3$) week in treatment X and Z respectively, while growth was sporadic between eighth ($6.10x10^3$ ± $1.10x10^2$) and tenth ($4.70x10^4$ ± $1.20x10^3$) week in Y. Gradual growth resumed in all the treatment afterwards. The least mean bacterial population was observed in Z and the highest was in X. Mean bacteria populations differ significantly from one another (Table 2).

Table 2: Bi-weekly mean population of microbes (cfu/g)

Treatment	Week	Fungi	Bacteria	Moisture %
X	2	$5.12x10^2 \pm 0.22x10^2$	$1.86x10^3 \pm 0.17x10^2$	21.83±0.1
	4	$9.30x10^2 \pm 2.20x10^2$	$2.46x10^3 \pm 0.66x10^2$	21.69±0.6
	6	$1.14x10^3 \pm 1.10x10^2$	$2.92x10^3 \pm 0.66x10^2$	22.01±0.05
	8	$1.64x10^3 \pm 5.90x10^2$	$3.90x10^4 \pm 1.12x10^2$	22.23±0.07
	10	$2.32x10^3 \pm 1.50x10^2$	$4.84x10^4 \pm 0.66x10^2$	23.44±0.06
	12	$2.44x10^3 \pm 0.70x10^2$	$5.06x10^4 \pm 0.41x10^2$	23.55±0.02
	Mean total	$1.5x10^3 \pm 0.2x10^{3a}$	$3.50x10^4 \pm 1.34 \times 10^{3a}$	22.36±0.16
Y	2	$1.30x10^2 \pm 0.22x10^2$	$1.50x10^3 \pm 0.22x10^2$	21.48±0.05
	4	$3.20x10^2 \pm 0.50 \times 10^2$	$2.52x10^3 \pm 0.50 \times 10^3$	21.43±0.07
	6	$6.20x10^2 \pm 0.13x10^2$	$4.20x10^3 \pm 0.13x10^2$	21.40±0.06
	8	$7.10x10^2 \pm 1.10x10^2$	$6.10x10^3 \pm 1.10x10^2$	22.06±0.65
	10	$8.35x10^2 \pm 1.30x10^2$	$2.10x10^4 \pm 1.30x10^3$	23.23±1.33
	12	$9.70x10^3 \pm 1.20x10^2$	$4.70x10^4 \pm 1.20x10^3$	23.53±2.04
	Mean total	$5.30x10^2 \pm 0.08x10^b$	$1.73 \times 10^4 \pm 0.87x10^{3b}$	23.61±0.67
Z	2	$4.10x10^2 \pm 0.22x10^2$	$4.10x10^3 \pm 0.22x10^2$	21.41±0.06
	4	$5.90x10^2 \pm 0.90x10^2$	$5.90x10^3 \pm 0.90x10^2$	21.41±0.09
	6	$8.60x10^2 \pm 0.22x10^2$	$8.60 \times 10^3 \pm 0.22x10^2$	21.81±0.62
	8	$1.09x10^3 \pm 0.14x10^2$	$1.09x10^4 \pm 0.14x10^3$	22.73±1.33
	10	$1.14x10^3 \pm 0.40x10^2$	$3.14 \times 10^4 \pm 0.40x10^3$	23.35±1.45
	12	$1.34x10^3 \pm 0.80x10^2$	$4.34x10^4 \pm 0.80x10^3$	23.55±0.05
	Mean total	$0.9x10^3 \pm 0.09x10^{3c}$	$2.27x 10^4 \pm 0.81 \times 10^{3c}$	23.13±0.52

** Mean total on the same column with different superscripts are significantly different from each other*

4. Discussion

The data on environmental factors collected indicated high relative humidity and warm temperature, which is typical of tropical environment. These conditions very much favour the growth and development of spoilage organisms. Organisms that have been identified as responsible for spoilage thrive best under warm environmental conditions. The initial values of each of the nutrients estimated in the three products (Table 1) were higher than those estimated for smoked *C. nigrodigitatus* in previous studies [4]. The nutrient values of the fish samples were similarly higher than those of other animals such as beef and pork [14] and were within the ranges recommended by US/RDA [15]. This observation confirms the assertion that fish has nutrients that are comparable to other animals and infact may be a richer source of nutrients of animal origin.

Fungi found to be associated with smoked *C nigrodigitatus* in all the treatments were *Penicillium citrium, A. parasiticus, Aspergillus flavus,* and *A. niger*, similar fungi were identified in various smoked fish species displayed for sale in some West African markets [4, 5]. Those associated with fillets treated with powdered neem leaves were *Aspergillus flavus, Penicillium citrium, A. parasiticus* and *A. niger* in the order of importance. Fungi population growth pattern varies with the different treatments, the higher the moisture content the higher the mean population; it doubles every fourth week in X, it continues geometrically to stabilise between the eight and tenth week in Y and was gradual in Z (Table 3). Bacterial population was more rapid in X and Z than in Y (Table 3), microbial population was significantly different among various treatments ($p< 0.05$).

Table 3: Mean population of fungi

Fungi			Bacteria	
Treatment	Population	Moisture	Population	Moisture
X	$1.5x 10^3 \pm 0.2 \times 10^{3a}$	22.36±0.16,	$3.50x10^4 \pm 1.34 \times 10^{3a}$	22.36±0.16
Y	$5.3x10^2 \pm 0.08x10^{3b}$	23.61±0.67	$1.73 \times 10^4 \pm 0.87x10^{3b}$	23.61±0.67
Z	$0.9x 10^3 \pm 0.09x10^{3c}$	23.13±0.52	$2.27x 10^4 \pm 0.81 \times 10^{3c}$	23.13±0.52

** Values with different superscripts are significantly different*

The mean population of bacteria was higher in X than Y and Z (Tables 3). Oil film possibly blocks the air passages thus impairing the respiratory activities that slow down the growth of microbes in Y. while microbial growth in Z seem to have been better slowed down than those of X by the brine. Treatment X was found to have encouraged the growth of microbes better than the other treatments because the powdered leaves possibly provided additional nutrient for the growth and survival of the microbes.

5. Conclusion

The effectiveness of neem in preventing fungi growth on smoked *C. nigrodigitatus* was not clearly detected from this study. However neem has been found to effectively slow down fungal growth in some plants products. It was also proven to have effectively hinder the production of aflatoxins associated with *Aspergillus spp*. Though neem was found to inhibit fungal growth and toxins production on mustard seeds and when inoculated into their culture media [7, 15], growth seems not to have been inhibited on *C. nigrodigitatus*, however mycotoxin production ability could have been reduced [16]. There is need for further studies to determine the mycotoxin production level of fungi associated with dried fish preserved with powdered neem leaves.

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