Preservation of Smoked African Catfish, *Clarias gariepinus* Burchell against *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) using Neem Seed Oil-iodized Salt Mixtures

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Summary

Dermestes maculatus is a major fish pest causing serious damage when left uncontrolled. This work identifies the fatty acids of Nigeria-obtained neem seed oil (NSO) and the potential of different mixtures of the NSO and iodized salt in the control of the pest. Seven fatty acids: palmitic acid (18.09%), linoleic acid (33.73%), stearic acid (14.37%), oleic acid (13.4%), octadecanoic acid (12.93%), 9, 12– octadecadienoic acid (1.47%) and arachidic acid (3.33%) were identified in the NSO. Percentage of *D. maculatus* adu1t mortality (100%) observed in fish treated with 0.00 μl NSO + 0.50 mg salt, 100 μl NSO + 0.125 mg salt and 0.00 μl NSO + 0.25 mg salt was significantly ($p<0.05$) different from mortality observed in untreated fish (31.25%). Percentage of live larvae (0.0%) in fish treated with 0.50 mg salt + 0.00 μl NSO, 100 μl NSO + 0.125 mg salt, 0.00 μ1 NSO + 0.25 mg salt and 50 μl NSO + 0.125 mg salt was significantly lower than 63.97% observed in the control. Percentage of weight loss of untreated (75.10%) and 50 μl NSO-treated fish (69.65%) was significantly higher than values obtained from fish treated with 0.5 mg salt (26.93%), 100 μ1 NSO + 0.125 mg salt (25.73%) and 0.25 mg salt (23.63%). Application of NSO-iodized salt did not change the colour and odour of treated fish. Consumers significantly rejected fish treated with ≥ 50 μl NSO.

Key words

consumers' acceptability, *Dermestes maculatus,* iodized salt, neem seed oil, smoked fish

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Introduction

Stored product insects are known in different agricultural products including smoked fish products. A number of authors (Nowsad *et al.*, 2009; Babarinde *et al.,* 2012; Lithi *et al.,* 2012; Onyuka and Ofulla, 2013; Zakka *et al.,* 2013) had reported the menace of insect pest infestation on stored smoked fish. Awoyemi (1991) examined the storability of different fish species over a period of 60 days and observed that they were reduced to mere frass and bones by *Dermestes maculatus* (De Geer). The genus *Dermestes* is known for their infestation of dried fish causing qualitative and quantitative damage. This genus accounts for about 71.5% of dried fish infestation recorded in most of the producing areas with a substantial loss in dry weights of about 43-62.7% from both larvae and adults (Osuji, 1974; Babarinde *et al.,* 2012). The need to protect smoked fish from pests is imperative when the fact that fish plays the crucial role in ensuring food security, income generation and employment opportunities are considered. The large dependence on imported fish can adversely affect the economy (mostly foreign reserves) of some developing countries. *Clarias gariepinus* (Burchell) is highly perishable and there is the need for its preservation in order to prevent spoilage. In many developing countries, the common preservative methods are drying, smoking, chilling and brining, but the most prominent fish preservation in Nigeria is smoke-drying (Akinola *et al.,* 2006; Ikenweiwe *et al.,* 2010). This is because most of the rural fishing communities do not have access to electricity to freeze their products.

The serious limitations (such as human health hazards, high cost of purchase, development of highly resistant strains etc.) offered by the use of highly persistent chemicals, as fish preservatives, had elicited interests on seeking alternative methods of controlling post-harvest fish damage. One of such promising areas is in the use of plant-derived pest control agents. Many medicinal plants and spices have been cited as pest control agents for stored grains, legumes and smoked fish (Fasakin and Aberejo, 2002; Nowsad *et al.,* 2009; Lithi *et al.*, 2012; Babarinde *et al.*, 2011, 2014, 2015; 2016). Neem seed oil (NSO) has been reported to be pesticidal against several stored products (Lale and Mustapha, 2000; Katamssadan *et al.,* 2015) and different neem formulations have been used for preservation of dried fish against insect pests (Noswad *et al.,* 2009; Lithi *et al.*, 2012). Despite its acclaimed pesticidal potentials, its bitter taste remains a concern which discourages many farmers from incorporating it into consumables. In order to reduce the bitter taste, a common form of table salt (iodized salt) available in Nigeria was incorporated into NSO formulations for trial. Although, the use of salt for preservation of animal products like fish is a traditional method in many countries (FAO, 1981), the health concerns associated with excessive salt consumption by man is a serious concern. Therefore, this research was designed with the aims of evaluating the efficacy of different formulations of NSO-iodized salt as protectant of smoke-dried African catfish, *Clarias gariepinus* and to evaluate consumer assessment of the stored fish treated with various NSO-iodized salt mixtures.

Materials and methods

Procurement of experimental materials

Un-infested *C. gariepinus* was purchased at Araada Market, Ora Gada, Ogbomoso, Nigeria. *D. maculatus* was obtained from a heavily infested *C. gariepinus* obtained from smoked fish markets in Ogbomoso. The species was carefully hand-picked into plastic jars containing uninfested smoked catfish, covered with plastic net to allow aeration and prevent insects' escape. The culture was maintained at tropical ambient storage conditions. NSO was obtained from National Research Institute for Chemical Technology, Samaru-Zaria, Nigeria.

Effect of NSO-iodized salt on biological parameters of *Dermestes maculatus* and weight parameters of fish

Ten grams *C. gariepinus* was weighed into 150 ml plastic jars. Nine treatments were administered in this experiment with four replicates for each treatment. The treatments used are presented in the Table 1. After application of each treatment into separate 150 ml plastic jars, the jar was covered with net and manually shaken together for uniform coverage of treated fish samples by the NSO-iodised salt mixtures. Four 1-2 day old *D. maculatus* were introduced into each jar. Forty seven (47) days after treatment, data were collected on adult mortality, live larvae and weight of fish, and the following were estimated.

$$Percentage\ of\ adult\ mortality = \frac{Number\ of\ dead\ adult}{Total\ number\ of\ Insects} \times 100$$

$$Percentage\ of\ weight\ loss = \frac{Initial\ weight\ -\ Final\ weight}{Initial\ weight} \times 100$$

Palatability test

Ten grams of un-infested fish obtained from the same source and treated with the same NSO- iodized salt mixtures as those used for the entomological bioassay (but no insects were added) were stored for 2 months in three replicates each. Twenty trained panellists were served with the fish samples and were asked to rank the samples in order of preference: 5 = highly preferred, 1 = least preferred and 0 = not preferred, for the following parameters: colour, odour, taste and general acceptability.

Table 1. Neem seed oil-iodized salt mixtures used for preservation of stored smoke-dried *Clarias gariepinus*

S/N	Applied Treatment	Corresponding rate[1]
1	50 µl NSO + 0.00 mg of salt	0.5% + 0.0%
2	100 µl NSO + 0.00 mg of salt	1.0% + 0.0%
3	0.00 µl NSO + 0.25 mg of salt	0.0% + 2.5%
4	0.00 µl NSO + 0.50 mg of salt	0.0% + 5.0%
5	100 µl NSO + 0.125 mg of salt	1.0% + 1.25%
6	100 µl NSO + 0.25 mg of salt	1.0% + 2.5%
7	50 µl NSO + 0.125 mg of salt	0.5% + 1.25%
8	50 µl NSO + 0.25 mg of salt	0.5% + 2.5%
9	Control without NSO and salt (untreated)	0.0% + 0.0%

[1]Volume of NSO + weight of salt per weight of fish

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Methiolation of Neem Seed Oil

The bench scale trans-esterification reaction was carried out following the modified method of Ugheoke *et al.* (2007). In this method, 450 ml of the oil was poured into the reactor and heated to 45°C to improve the NSO's mixability with alcohol. This catalyst concentration level was achieved by dissolving 4.1 g of Potassium hydroxide (KOH) in 100 ml of the alcohol and the mixture was stirred for twenty minutes to form potassium alkoxide. The resulting solution was added to the oil in the reactor and the entire content was brought to a temperature of 55°C and then held at this temperature for an hour. The reactions product mixtures were allowed to separate into phases by standing for eight hours in a separating funnel so as to separate glycerol from the NSO. Five (5) ml of acetic acid was added to the biodiesel followed by washing with water and was allowed to stand for eight hours in a separating funnel. The denser soapy mixture was carefully drained from the bottom of the separating funnel leaving behind the oil. The methylated oil obtained was dried in an oven at 100°C for 1 hr and analysed by chromatographic method.

Gas Chromatography/Mass Spectrometry

An AGILENT (19091S – 433HP – 5MS) GC interfaced with a VG Analytical 70 – 250 S, a double focusing mass spectrometer was used. Helium was the carrier gas at 1.5 ml/min. The MS operating conditions were: ionization voltage 70 ev, ion source temperature 230°C. The GC was fitted with a 25 m × 0.25 mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15 μm. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage compositions of the oil were computed in each case from GC peak areas. The identification of the components was based on the retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from Literature (Adams, 2001; NIST, 2010).

Statistical analysis

The experiment was laid out in completely randomized design. Percentage data were subjected to angular transformation prior to analysis. All entomological and consumer acceptability data were subjected to analysis of variance and where there was significant treatment effect, significant means were separated using Tukey's HSD test at 5% probability.

Results

Fatty acid composition of methyl esters of NSO used for the study

From the result of chromatographic analysis of the methyolated NSO, seven fatty acids were identified with linoleic acid (33.73%) being the predominant fatty acid. Others were palmitic acid (18.09%), stearic acid (14.37%), oleic acid (13.4%), and arachidic acid (3.33%), octadecanoic acid (12.93%), and 9, 12–octadecadienoic acid (1.47%) (Table 2).

Table 2. Fatty acid composition of the methyl esters of NSO evaluated for protectant ability of smoke-dried fish against *Dermestes maculatus*

S/N	Fatty acid	% Composition[1]
1	Palmitic	18.09
2	Linoleic	33.73
3	Stearic	14.37
4	Oleic	13.4
5	Octadecanoic	12.93
6	9, 12 – Octadecadienoic	1.47
7	Arachidic	3.33
	Total	97.32

[1] % composition peak area relative to total peak area obtained from TIC peak report

Influence of neem oil + iodized salt on percentage of adult mortality and live larvae of *Dermestes maculatus* and percentage of weight loss of *Clarias gariepinus*

Percentage of adult mortality of *D. maculatus* was significantly affected by treatment. Mortality (100%) observed in fish treated with 0.50 mg salt, 100μl NSO + 0.125 mg salt and 0.25 mg salt was significantly (df = 8, 27; F value = 3.00; p = 0.015) different from mortality observed in untreated fish (control) which was 31.25% (Table 3). Percentage of live larvae of D. *maculatus* was significantly affected (df= 8, 27 F= 6.05 p= 0.0002) by the treatments. Values (0.00%) observed in fish treated with 0.50 mg salt + 0.00 μl NSO, 100 μl NSO + 0.125 mg salt, 0.00 μl NSO + 0.25 mg salt and 50 μl NSO + 0.125 mg salt was significantly lower than 33.75% observed in fish treated with 50μl NSO + 0.125 mg salt and 63.97% observed in the untreated fish sample (Table 3). The effect of NSO + iodized salt on percentage of weight loss of treated fish followed the same pattern as observed in the percentage of live larvae. Percentage of weight loss of stored fish was not significantly different in control (untreated) which had 75.10% and 50 μl NSO (69.65%) but was significantly (Df = 8, 27; F value = 4.54; p = 0.0014) different from values obtained from fish treated with 0.5 mg salt (26.93%), 100 μl NSO + 0.125 mg salt (25.73%) and 0.25 mg salt (23.63%) (Table 3).

The influence of NSO-iodised salt on taste, colour, odour and consumer acceptability of *Clarias gariepinus*

Taste was not significantly different in untreated fish, 0.25 mg salt, 0.50 mg salt and 50 μl NSO + 0.25 mg salt but was significantly affected by 100 μl NSO, 50 μl NSO + 0.25 mg salt, 100 μl NSO + 0.125 mg salt and 100 μl NSO + 0.25 mg salt. The influence of the treatment was not significantly (p>0.05) different from each other. Application of NSO - iodized salt did not change the colour and odour on treated smoked fish. Consumers accepted untreated fish, and fish treated with 0.25 mg salt, 0.50 mg salt and 50 μl NSO + 0.125 mg salt but significantly rejected fish treated with 100 μl NSO, 50μl NSO + 0.25 mg salt, 100 μl NSO + 0.125 mg salt and 100μl NSO + 0.25 mg salt (Table 4).

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Table 3. The influence of Neem seed oil + iodized salt on percentage of adult mortality, percentage of live larvae of *Dermestes maculatus* and percentage of weight loss of *Clarias gariepinus*

Treatment	% adult mortality	% live larvae	% weight loss of fish
0.00 µl NSO + 0.50 mg salt	100 (90.00)a	0.0 (0.00)b	26.93 (30.56)b
100 µl NSO + 0.125 mg salt	100 (90.00)a	0.0 (0.00)b	25.73 (29.40)b
0.00 µl NSO + 0.25 mg salt	100 (90.00)a	0.0 (0.00)b	23.63 (28.47) b
50 µl NSO + 0.125 mg	100 (90.00)a	0.0 (0.00)b	23.63 (28.47) b
100 µl NSO + 0.25 g salt	93.75 (82.5)ab	41.98 (10.62)a	41.95 (40.61)ab
100 µl NSO + 0.00 mg salt	75 (63.75) ab	26.32 (23.46.) ab	56.10 (48.54) ab
50 µl NSO + 0.00 mg salt	68.75 (63.75)ab	17.85 (23.31.) ab	54.13 (47.77) ab
50 µl NSO + 0.125 mg salt	62.5 (56.25) ab	33.75 (45.75) a	69.65 (58.61)a
Control	31.25 (30) b	63.97 (55.50) a	75.10 (61.07) a
ANOVA result	Df = 8, 27; F value = 3.00; p = 0.015	Df = 8, 27; F value = 6.05 p = 0.0002	Df = 8, 27; F value = 4.54 p = 0.0014

Data followed by the same alphabet along the column are not significantly different (p<0.05) using Tukey's HSD test. Data in parenthesis are transformed values using angular transformation

Table 4. The influence of neem seed oil on taste, colour, odour and consumer acceptability of *Clarias gariepinus*

Treatment	Taste	Colour	Odour	General acceptability
Control	5.00 a	5.00a	5.00a	5.00a
50 µl NSO + 0.00 mg salt	3.33 c	5.00a	5.00a	3.67b
100 µl NSO + 0.00 mg salt	4. 00 b	5.00a	5.00a	4.00b
0.00 µl NSO + 0.25 mg salt	5. 00 a	5.00a	5.00a	5.00a
0.00 µl NSO + 0.50 mg salt	5.00 a	5.00a	5.00a	5.00a
50 µl NSO + 0.125 mg salt	5.00a	5.00a	5.00a	5.00a
50 µl NSO + 0.25 mg salt	4.00 b	5.00a	5.00a	5.00b
100 µl NSO + 0.125 mg salt	4 .00b	5.00a	5.00a	4.00b
100 µl NSO + 0.25 mg salt	4.00 b	5.00a	5.00a	4.00b
ANOVA result	Df = 8, 18; F value = 32.50; p=<0.001	Df = 8, 18; NS	Df = 8, 18; NS	Df = 8, 18; F value = 26.50; p=<0.001

Data followed by the same alphabet along the column are not significantly different (p<0.05) using Tukey's HSD test.

Discussion

The use of botanicals to control *Dermestes* species is not new especially in developing countries where these botanicals are cheaply available. Several scientists (Fasakin and Aberejo, 2002; Akinwumi *et al*., 2006, Nowsad *et al*., 2009; Akinwumi and Fesobi, 2010; Babarinde *et al*., 2016) have reported the bio-efficacy of different plant formulations against *D. merculatus*. The selection of NSO in this study was based on the fact of the availability of neem plant and its various medicinal properties wherein its safety in oral administration by human has been postulated. For instance, neem leaf is a popular herbal medicine for treatment of malaria in Nigeria.

The result of GC-MS analysis of NSO used for the study has some similarities with literature data. Uko *et al.* (2008) had earlier reported high percentage of oleic acid (48.08%) from Nigeria-obtained neem seed oil with stearic, palmitic, linoleic, arachidic and acids being other dominant identified fatty acids, in order of abundance. Oleic, stearic, linoleic and palmitic acids were the principal fatty acid in Senegalese NSO, with oleic acid (44.61- 43.61%) being the most abundant one (Faye *et al*., 2010). The result of Katamssadan *et al.* (2015) who studied NSO obtained from Cameroonian neem seed followed similar pattern, with oleic acid being 50.02-51.83%. Similarly, the result of NSO

obtained from The Republic of Benin had the four principal fatty acids in abundance with oleic (43.5%) being the most abundant fatty acid (Djenontin *et al*., 2012). In the present study, linoleic acid (33.73%) was the most predominant acid. Others were palmitic acid (18.09%), stearic acid (14.37%), oleic acid (13.4 %), and octadecanoic acid (12.93%). Some of the factors responsible for the differences in the chemical composition of NSO were variations in geographical location of neem seed collection, method of extraction of NSO, and age/storage condition of the NSO. Incidentally, linoleic, palmitic, stearic and oleic acids have been identified in various botanical oils reported to possess insecticidal properties against stored product insects (Abd El-Salam, 2010; Katamssadan *et al.,* 2015).

From the results of this study, incorporation of iodized salt into botanical treatment shows better protectant ability than single application of botanical. Salt had traditionally been used for preservation of animal products, especially fish (FAO, 1981; Tuara, 1997; Onyuka and Ofulla, 2013). Salt preserves by extracting water, this happens because water from inside the fish is drawn out into the strong salt solution outside the fish. As the water moves out, the salt moves in, penetrating deep into the flesh of the fish (Tuara, 1997). The extremely low moisture

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content of salted stored fish reduces the suitability of such fish as breeding substrate for *D. maculatus*. Secondly, the principle of insect control cou1d be desiccation which killed both the immature and adu1ts that had contact with the salt.

In conclusion, irrespective of the treatment used, the result of the palatability test shows that there was no change in colour and odour. General acceptability was also improved with addition of iodized salt to 50 µl NSO (0.5% v/w). Selection of NSO was based on its availability and many professed insecticidal activity of the plant species. Its insecticidal potentials against *D. maculatus* have been reported by Mufutau (2012). The result of the palatability test suggests that consumers may be sceptical to accept the most effective level of NSO reported in this study (100 µl, corresponding to 1.0% v/w) due to bitter taste of treated smoke-dried fish. It is certain that the treated smoke-dried fish with bitter taste wou1d lose its bitterness after an extended storage period.

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