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The effect of different concentrations of water extract of Sodom apple leaves (*Calotropis procera*) in the control of *Dermestes maculatus* on smoked fish (*Oreochromis niloticus* Linnaeus, 1758)

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Abstract
Dermestes maculatus is a common pest of smoked *Oreochromis niloticus.* The efficacy of aqueous leaves extracts of Sodom Apple (*Calotropis procera*) against *Dermestes maculatus* larva was evaluated in this study. Water extract of ground Sodom Apple leaves at different concentrations of 0g(control), 20g, 40g, 60g and 80g were soaked in 200ml of water. Fish samples were immersed in the aqueous extracts for one hour thirty minutes before smoking with Sodom Apple stem. Highest larva mortality was recorded in fish treated with 80g and the lowest mortality in the 0g after 24 days. The highest crude protein was recorded in fish treated with 60g and the lowest in the 0g. Weight losses were recorded in all the treatments but without significant differences. Therefore, effective concentrations to control *Dermestes maculatus* and improve crude protein content were 80 and 60g. The use of *C. procera* should be incorporated into post-harvest management strategies.

Keywords: *Oreochromis niloticus*, *Dermestes maculatus*, Sodom apple, and crude protein

1. Introduction
Fish serve as a principal source of dietary protein, which is inexpensive compared to other animal proteins (Fawole *et al*., 2007) [14]. However, fish is subject to post-harvest losses ranging from bacterial and autolytic spoilage and spoilage due to insect infestation. Fish smoking is one of the traditional methods of preserving fish in Africa. As applied to fish, Smoke curing is a method of preservation affected by a combination of drying and the deposition of naturally produced chemicals resulting from the thermal breakdown of wood (Ahmed *et al*., 2010) [6]. In Nigeria, as in several other countries, qualitative and quantitative losses of dried fish during storage, transportation, and marketing are due mainly to infestation by *Dermestes maculatus* (Coleoptera; Dermestidae, the fish beetle) (Philip-Attah, 2019; Osuji, 1974) [28, 26]. The losses due to insect infestations have been attributed to a net reduction in the number of nutrients available to the consumer (nutritive quality) resulting in declining consumer acceptability and market prices losses (Odeyemi *et al*., 2000) [22].
The losses due to this beetle account for about 71.5% in both quantity and quality of smoked fish (Johnson & Esser, 2000) [18]. Efficient control of insect pests has long been the aim of stored product entomologists worldwide due to mental torture and economic loss procured by fishmongers (Ileke *et al*., 2013) [16]. Due to a lack of reliable alternatives, synthetic chemicals have been effectively used against insect infestation, but it is only justified under approved and controlled conditions.
The chemicals are highly effective in the control of blowfly attacks during fish drying. For example, various synthetic chemicals such as carbamates, organophosphates (Ops), organochlorines (OCs), atrazine (S-triazines), polychlorinated biphenyls (PCBs), and pentachlorophenol (PCPs) are reported to be effective against the treated smoked fish insect pests (Philip-Attah, 2019) [28]. However, the general use of such chemicals to protect smoked fish has been hampered by the reports of health hazards, high cost of purchase, insecticide resistance, and lack of technical know-how (Khan and khan 2002; Philip-Attah, 2019) [19, 28]. For example, Odeyemi *et al*. (2000) [22] reported that fish treated with chemical substances adversely affect consumers, causing blurred vision and vomiting.

These effects of using synthetic insecticides have necessitated the need to seek alternative techniques of controlling insect attacks on smoked fish (Ivbijaro, 1990) [17]. One promising alternative to synthetic insecticides is the use of plant-based materials considered safe for consumption, eco-friendly, and have insecticidal properties (Philip-Attah, 2019) [28]. Many studies (e.g., Anyaele & Amusan, 2003 [4]; Scott *et al.*, 2004; Echezona, 2006 [12]; Hassan *et al*., 2006 [15]; Sowumi, 2007 [31]; Owoade, 2008 [27]; Akinwumi, 2011 [3]; Echo *et al.*, 2012 [13]; Olayinka-Olagunju, 2014 [24]; Akinbuluma *et al.*, 2015 [2]; Philip-Attah, 2019) [28] have conducted various research to support the use of plant-based insecticides as reliable and safe alternatives to control pest threat on stored products such as smoked fish. For example, *Pipers guineense* have been found to contain chemical compounds such as *neurotoxic*, *piperamides and lignans*, which are responsible for their insecticidal properties (Scott *et al.*, 2004). Hassan *et al.* (2006) [15] reported very high larvicidal and strong repellency effects of *Detarium microcarpum* seed oil on *Dermestes lardarius*. Mossa *et al*. (1991) [21] found the phytochemical constituent of *C. procera* to have insecticide properties. Similarly, *C. procera* was effective in both inhibition of feeding and causing mortality of larvae of Egyptian alfalfa weevil and *Hyper brunneipennis* (Doghain, 2003) [11]. Therefore, the aim of this study which was to evaluate the efficacy of aqueous leave extracts of Sodom Apple (*Calotropis Procera)* as a possible protectant of smoked fish (*Oreochromis niloticus*) against *Dermestes maculatus* larval infestation, was achieved through the following objectives:
Determine the level of mortality caused by each extract on the treated smoked *Oreochromis niloticus* at different concentrations. Examine the crude protein levels and weight loss of the treated smoked *O. niloticus*.

2. Materials and Methods

2.1 Study Area

The study was conducted at the Fish Processing Unit, Department of the Fisheries University of Maiduguri, Maiduguri, which lies in the semi-arid zone of Nigeria within latitude 11 o151 north and longitude 30o051 east. It is characterized by a short rainy season (3 to 4 months) with a long dry season period. Annual rainfall ranges from 500mm to 600mm while the ambient temperature reaches 40oC and above by April and May.

2.2 Collection of Leaf Samples and Extract Preparation

Fresh leaves of *Calotropis Procera* (Apple of Sodom) were obtained within the University of Maiduguri premises. The fresh leaves were appropriately washed in clean water to remove dust and dirt, air-dried in the laboratory not to lose the active ingredients, which may be volatile or thermolabile, then ground into a fine powder using an electric grinder described by Philip-Attah (2019) [28]. The ground leaves were weighed at the varying weight of 20g, 40g, 60g and 80g using a sensitive weighing balance. They were then mixed into separate containers of 200ml of water for 24hours. The pulps obtained were left in sterile and clean glass containers with constant shaking at intervals to allow for extraction. The filtration was performed using a sterile muslin cloth to obtain the aqueous extract at different concentrations (Dienye *et al.*, 2016) [9].

2.3 Fish preparation for smoking

Fresh *O. niloticus* weighing 3500g (3.5kg) was obtained from the Custom Market of Maiduguri metropolis. The fresh fish was transported using a clean container of ice block (to retain its freshness) to the Fish Processing and Preservation Unit of the Department of Fisheries, University of Maiduguri. The procured fish was then appropriately washed in clean water, especially around the gill region of the fish, mostly loaded with microorganisms. The fish was then degutted, descaled, and then adequately washed.
The prepared fish sample was then immersed in the aqueous leaf extract of the Sodom Apple at their various concentration levels (Dienye *et al.*, 2016) [9] for one hour and 30 minutes, and the control was not treated with the aqueous solution. The soaked fish were kept under a shaded area for effective draining to ease smoking. Next, the fish samples were smoked using a modified drum smoking kiln. The wood fuel used for smoking was the stem of matured Sodom Apple. During the smoking process, the temperature of the fuelwood was controlled to prevent the burning of the fish. The smoking took three days at an interval of four hours per day. After smoking, the fish were carefully packed from the smoking kiln then allowed to cool before they were packed inside the different perforated plastic containers at room temperature. After 24 hours, the larva of *Dermestes maculatus* was then introduced.

2.4 Insect Larva Procurement and Identification

The larva of *Demestes maculatus* was obtained from many infested dried fish at the Baga fish market in Maiduguri. The insect larva of *Demestes maculatus* was identified with the following features: Thoracic segments with three pairs of jointed legs; body densely covered with hairs of varying lengths, usually the underside of the body yellowish-brown, but the upper surface of body mainly dark brown. This species was carefu1ly hand-picked into plastic jars containing the prepared smoked *O. niloticus*, covered with a plastic net to prevent the insects from escape and allow aeration. This cu1ture was maintained at tropical ambient storage conditions as done by Babarinde *et al.* (2016) [7].

2.5 Effects of Aqueous Leaf Extracts of Sodom Apple on Insect Larva

Thirty-five (35) insect larva were introduced into the perforated plastic jars containing both the extract-treated fish and the control. Each treatment was replicated thrice. The number of dead larvae was recorded daily. After 24 days of the treatment, data were collected on smoked fish weight loss using the following formula:

Weight loss = Initial weight – Final weight.

2.6 Proximate Analysis of Smoked and Fresh Fish

The proximate composition of both the fresh, extract-treated and untreated smoked fish was analysed according to the Association of Official Analytical chemist procedure (AOAC, 2002) [5].

2.6.1 Protein Content

The Protein content was obtained through the determination of total nitrogen by micro Kjeldahl's method. The value of nitrogen obtained was then multiplied by 6.25g to get the crude protein value as follows:

$$\%N = \left(\frac{\text{mLHCL x NHCLx14008}}{Mg\ of\ sample}\right) \times 100$$

% protein = % N x 6.25

Where
NHCL: Normality of standard, HCl (0,02N)
mLHCL: Standard volume HCl for titration
14,008: Weight from a nitrogen atom
6.25: Amount of nitrogen base in fish protein

2.6.2 Moisture content
The moisture content of the samples was determined by the Clucas (1981) method, in which 2g of the samples (fish muscles) were oven-dried at 110°C for 24 hours to a constant weight. Thus, loss in weight was equal to the moisture content of the original sample.

2.6.3 Ash content
The ash content was determined by heating the samples to a temperature of 550°C, and the residue was equivalent to the ash content.

2.6.4 Fat (Lipid) content
The Soxhlet method determined the fat content used, where the principal lipid separation from the material is conducted using organic solvent chloroform. Lipid was extracted in a flask flowing N_2 for the purpose to evaporate the organic solvent in the flask (AOAC, 2002) [5].

2.6.5 Dry matter
According to the AOAC method (AOAC, 2002) [5]. The dry matter was obtained as follows:

$$\text{Dry matter (\%)} = \left(\frac{\text{Weight of dried sample}}{\textit{Original wet weight}}\right) \times 100$$

3. Statistical Analysis
All data collected were subjected to analysis using one-way analysis of variance (ANOVA) at a 5% significance level using statistix 9.0 Statistical package. Where significant differences occurred, means were separated using the Least Significance Difference (LSD).

4. Results and Discussions
Results of mortality of insect larva on both the extract-treated and control *O. niloticus* at intervals of four days for 24 days are presented in Table 1.

The results revealed that at day 4, the mortality values of the insect larva were 2.00± 0.00, 2.33 ± 0.33, 2.67 ± 0.33, 2.33 ± 0.33, 3.0 ± 0.00 for the control, 20, 40, 60, and 80g of Sodom Apple/200ml of water treated fish, respectively. The treated fish at a concentration of 80g of Sodom Apple/200 ml of water had the highest mortality of the insect larva. The control had the lowest mortality value.

Table 1: Mortality of Insect Larva at Four Days Interval for 24 days

Concentration of Sodom apple in g/200 ml of water					
Days	Control	20	40	60	80
4	2.0±0.00^{b}	2.33±0.33^{ab}	2.67±0.33^{ab}	2.33±0.33^{ab}	3.00±0.00^{a}
8	7.0±0.58^{c}	8.33±0.89^{bc}	10.33±0.31^{a}	8.67±0.30^{abc}	9.67±0.06^{ab}
12	17.0±0.55^{b}	17.33±0.67^{b}	18.33±0.88^{ab}	18.33±1.2^{ab}	20.33±0.33^{a}
16	23.7±0.67^{bc}	23.3±0.88^{c}	25.67±0.33^{ab}	24.67±0.67^{abc}	26.00±0.57^{a}
20	26.67±0.67^{bc}	26.0±0.33^{c}	27.70±0.33^{ab}	27.00±0.57^{abc}	28.33±0.33^{a}
24	26.79±0.88^{bc}	26.0±0.58^{c}	28.0±0.57^{ab}	27.33±0.57^{abc}	28.67±0.33^{a}

The values with different superscript letters are significant different (P<0.05).

At day 8, the mortality values of the insect larva were 7.0 ± 0.58, 8.33 ± 0.89, 10.33 ± 0.33, 8.67± 0.30, 9.67 ± 0.66 for the control, 20, 40, 60, and 80g of Sodom apple/200ml of water treated fish, respectively. The treated fish at a concentration of 40g of Sodom apple/ 200ml of water had the highest mortality of the insect Larva.

On day 12, the mortality values of the insect larva were 17.0±0.58, 17.33±0.67, 18.33±0.88, 18.33±1.20, 20.3±0.33, for the control and the treated fish at a concentration of 20, 40, 60, and 80g of Sodom apple/200ml of water, respectively. There was no significant difference between the control and 20g of Sodom apple/200ml of water treated fish.

On day 16, the mortality values of the insect larva were 23.7±0.67, 23.30±0.88, 25.67±0.33, 24.67±0.67, 26.0±0.57, for the control and the treated fish at 20, 40, 60, and 80g of Sodom apple/200ml of water, respectively. There were significant differences in all the treatments. 80g of Sodom apple/200ml of water treated fish had the highest mortality of the insect larva. 20g/200ml of water treated fish had the lowest mortality of the insect larva.

On day 20, the mortality values of the insect larva were 26.67±0.67, 26.0±0.33, 27.70±0.33, 27.33±0.57, 28.67±0.33, for the control and treated fish at 20, 40, 60, 80g of Sodom apple /200ml of water, respectively. There were significant differences in all the treatments. Fish treated with 80g/200ml of water had the highest mortality of insect larva.

On day 24, the highest mortality of the insect larva was recorded with fish treaded with 80g/200ml of water. There was no significant difference in the mortality of the insect larva between day 20 and day 24 in all the treatments.

As expected, the experiments demonstrated that the mortality of the insect larva was because of the presence of high alkaloid concentration and critical enzymes in the Sodom Apple plant. The results of this study are consistent with the previous result of Romas *et al*. (2007) [29]. Romas *et al*. (2007) [29] reported that the latex of Sodom Apple posses a high concentration of chitinase and proteases that acted as defensive molecules and are responsible for insecticidal activities. Also, the Sodom apple stem used in the smoking processes contributed to the mortality of the insect larva both in control and in the treated fish. Smoke from the fuelwood (Sodom apple) deposited some chemical particles like phenols and carbonyl. These chemical particles are deposited on the fish muscle and acted as repellants and antifeedants of insect larva. These findings are in complete agreement with Odour-Odole (2010) [23], who reported that Neem wood and Acacia wood smoke delayed and deterred insect infestation on smoked catfish for 56 days and 48 days, respectively. Therefore, it can be postulated that the Neem wood smoke contains bioactive ingredients which when deposited on the fish muscle during smoking prevent infestation (Battachaya *et al*., 2007). In this study, the mortality of the insect larva correlates favourably with Vikash (2003) [34]. Vikash (2003) [34] reported that latex of Sodom apple at the rate of 1.5ml and 2.0ml evoked 100% mortality of adult *Callosobruchus maculatus* after four days of application. The present result is also consistent with that of Begum *et al.* (2010) [8], who reported that at 200ppm of ethanol leaf extract of Sodom apple, 20% mortality of *Musca domestica* when exposed for 48 hours was recorded, and 100% mortality was recorded when 500ppm leaf extract of *Calotropis procera* (Sodom apple) was used. Similarly, Romas *et al*. (2007) [29] reported that the number of *Callosobruchus maculatus* was reduced when the seed are precoated with Sodom apple latex.

However, when Sodom apple is serially diluted in water, its deterrent activity in *Callosobruchus maculatus* oviposition diminishes (Romas *et al*., 2007) [29]. Furthermore, the result concurs well with *Salunke et al.* (2005) [30], *who* reported the control of *Callosobruchus chinnensis* reared in unguiculate seeds by Sodom apple. The proximate compositions of both the smoked dried and fresh fish samples were presented in Tables 2 and 3, respectively. The crude protein in table 2 was highest in fish treated with 60g of Sodom apple/200ml of water with a Crude Protein value of 80.2±0.15 as compared to the fresh fish sample in table 3, which had the lowest crude protein value of 42.24±0.24. In table 2, treated fish with 80g of Sodom apple/200ml of water had the highest fat content value of 16.00±0.04. The fresh sample had the lowest ash content value of 1.0±0.20. There was no significant difference in the ash content of the smoked fish treated at concentrations of 20, 40, and 80g of Sodom apple/200ml of water. Smoked fish treated at a concentration of 80g of Sodom apple had the lowest moisture content value of 3.8±0.16, while the fresh fish sample in table 3 had the highest moisture content value of 73.34±0.10. Treated fish at the concentration of 40g of Sodom apple/ 200ml of water had the highest Dry matter value of 96.5±0.16 but had no significant difference with treated fish at the concentration of 80g of Sodom apple/ 200ml of water with Dry matter value of 96.2±1.47. The fresh sample fish had the lowest dry matter value of 26.67±0.12.

The effect of Sodom apple extract on the proximate composition of the smoked *O. niloticus* was presented in table 2. The data in the table indicates that the fresh fish sample has the lowest protein content. This finding was strong evidence of high moisture content. The protein contents of both the treated and the untreated smoked fish were higher than that of the fresh sample. The sharp increase in protein content in the two samples increased dry matter per unit of weight. The finding in the present study is consistent with the finding of Doe and Olley (1983) [10] that smoking results in an increased concentration of nutrients due to low residual moisture content. The high ash content obtained was due to the mineral content of the Sodom Apple, which is in complete agreement with Turan and Sonmez (2002) [33] finding. The highest crude protein was obtained at 60g of apple Sodom/200ml of water. It may be the case that Sodom apple (*Calotropis procera)* has a total crude protein of 27-36% (Khonzada *et al*., 2008) [20].

Table 2: Proximate Composition of Fish Smoked with Sodom Apple

Concentration of Sodom apple in g/200 ml of water					
Proximate composition	**Control**	**20**	**40**	**60**	**80**
Protein	69.15±0.12^{d}	64.25±0.04^{e}	72.13±0.10^{c}	80.2±0.15^{a}	76.16±0.13^{b}
Fat	7.0±0.004^{b}	6.00±0.12^{c}	6.06±0.06^{c}	5.0±0.08^{d}	16.00±0.04^{a}
Ash	5.0±0.12^{b}	6.05±0.04^{a}	6.08±0.05^{a}	5.0±0.20^{a}	6.05±0.04^{a}
Moisture	7.7±0.98^{b}	4.6±0.92^{d}	3.5±0.24^{e}	5.0±0.08^{c}	3.8±0.16^{e}
Dry matter	92.3±0.16^{c}	95.4±1.16^{b}	96.5±0.16^{a}	95.0±0.04^{b}	96.2±1.47^{a}
Fiber	9.00±0.06^{e}	34.0±0.04^{a}	8.0±0.08^{f}	11.00±0.12^{c}	28.00±0.09^{e}

The values with different letters superscript are significant different ($P<0.05$)

Table 3: Proximate Composition of Fresh *Oreochromis niloticus*

Parameters	**% Composition**
Protein	42.24±0.24^{f}
Fat	5.0±0.04^{d}
Ash	1.00±0.20^{c}
Moisture	73.34±0.10^{a}
Dry matter	26.67±0.12^{d}
Fiber	10.00±0.09^{d}

The weight losses of treated *O. niloticus* following infestation with *D. maculatus* are presented in table 4. Fish treated with 20g of Sodom apple/200ml of water had the highest weight loss value of 8.50±0.40, while the fish treated with 80g of Sodom apple/200ml of water had the lowest weight loss value of 8.00±0.40.

There was no significant difference in the weight loss of smoked fish in all the treatments and control. The highest value of weight loss recorded in 20g of Sodom Apple per 200ml of water treated fish may be the case that there is low mortality of the insect larva. Similarly, the lowest value of weight loss recorded in 80g of Sodom Apple per 200ml of water treated fish may be due to the high mortality of the insect larva, which is expected to reduce the insect infestation. The findings were not unexpected because insect infestation can drastically reduce the quantitative value (weight) of dried fish (Osuji, 1974) [26].

Therefore, the degradation of fish tissue by *D. maculatus* has led to the loss in weight of treated smoke-dried *O. niloticus*. However, a more apparent weight loss was also observed in the control sample. The findings in the present study are consistent with the previous findings of Ajayi *et al.* (2019) [1] and Philip-Attah (2019) [28] that *D. maculatus* plays a critical role in weight loss of smoked dried *C. gariepinus* and its tissue degradation.

Table 4: Weight Loss of Treated *O. niloticus* following Infestation with *D. maculatus*

Concentration of Sodom apple in g/200 ml of water					
	Control	**20**	**40**	**60**	**80**
Initial W.(g)	50.5±0.40^{a}	50.33±0.47^{a}	50.16±0.62^{a}	51.0±0.81^{a}	49.83±0.63^{a}
Final W.(g)	42.06±0.44^{a}	41.8±0.62^{a}	42.07±0.82^{a}	42.83±0.84^{a}	41.83±0.81^{a}
Weight loss (g)	8.43±0.26^{a}	8.50±0.40^{a}	8.10±0.29^{a}	8.16±0.23^{a}	8.00±0.46^{a}

The values with the same superscript letters are not significantly different ($P<0.05$)

5. Conclusion

This study shows that the aqueous leaf extracts of the Sodom Apple plant (*Calotopis Procera*) were effective against *D. maculatus* infestation on *O. niloticus*. Hence, the variation in the level of extract concentrations can be a fundamental clue in designing appropriate effective control strategies for *D. maculatus* on *O. niloticus*, thus enhancing its shelf-life and crude protein and reducing its weight loss and food poisoning among consumers.

Furthermore, the highest potency of the extracts to do these was recorded at both 80 and 60g concentrations. Therefore, the effectiveness of *C. Procera* in controlling *D. maculatus* infestation and reducing the damage done on smoked fish samples was excellent and could serve as a possible means of ensuring a steady supply of good quality smoked fish. *C. Procera* will also serve as an affordable substitute for synthetic insecticides, which proved to be detrimental to the health of stored fish consumers. Besides, the use of Sodom apple extracts should be incorporated into post-harvest management strategies. There is however, the need for more research to evaluate the efficacy of the flowers and root extracts of Sodom Apple in controlling the larva of *D. maculates* infestation on smoked fish.

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