EVALUATION OF THE EFFICACY OF NEEM SEED OIL (NSO) EXTRACT FOR THE CONTROL OF *DERMESTES MACULATUS* DEGEER, 1774 (COLEOPTERA: DERMESTIDAE) IN *CLARIAS GARIEPINUS* (BURCHELL, 1822)(PISCES: CLARIDAE)

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[**Mufutau, A. A.** 2012. Evaluation of the efficacy of Neem Seed Oil (NSO) extract for the control of *Dermestes maculatus* Degeer, 1774 (Coleoptera: Dermestidae) in *Clarias gariepinus* (Burchell, 1822) (Pisces: Claridae). Munis Entomology & Zoology, 7 (2): 1188-1194]

ABSTRACT: The efficacy of Neem Seed Oil (NSO) in controlling dried fish pest against *Dermestes maculatus* was investigated in the laboratory. Four concentrations of ethanolic extracts of NSO: 0.125, 0.146, 0.180 mls were rubbed on the body surface of dried *Clarias gariepinus*. The results showed that the fish were protected for 6 months against *D. maculatus*. The efficacy of treatments was however, dosage dependent. The LD_{50} was 0.125 while 0.275ml significantly halted hatchability and development. A 100% adult mortality rate was recorded at high doses within the first 10 days. NSO was therefore recommended at appropriate dosage for the prevention of insect infestation of dried fish.

KEY WORDS: Smoked fish, Neemseed oil, control, *Dermestes maculatus*.

Fish is one of the cheap and fairly accessible animal protein source. Consumption of fish provides readily available dietary nutrients to a large number of the people worldwide and makes a very significant contribution to nutrition.

Fish is a rich source of lysine, sulphur, amino acid and is therefore suitable for complementing high carbohydrate diet (Azam et al., 2004; Fasakin & Aberejo, 2002; Kent, 1994; Akinwumi, 2011).

Fish is a good source of thiamine, riboflavin, vitamins A and D, phosphorus, calcium and iron. It is high in polysaturated fatty acids which are important factor for lowering blood cholesterol level (NIFOMR, 1985; FAO, 2010).

Smoked dry fish has been confirmed as highly favoured item of many traditional dishes in Nigeria (Kumolu-Johnson et al., 2010; Adewolu & Adeoti, 2010). As a condiment it greatly enriches the flavour of various dishes, it is often a good alternative to fresh fish which in many places is scarcely available. Smoked fish are particularly popular in African countries and provided that care is taken in its processing, it is a good quality product which can be produced fairly and inexpensively (Osuji, 1974; Aderolu & Akpabio, 2009). It has been found that the total nutritional value of dried fish judged from the availability of limiting amino acids is not significantly different from that of the fresh fish. The effect of fish curing process by itself especially smoking has no known deleterious effects on the nutritional quality of fish. The liberation of certain oils add aromas and flavour, the smoking process increase the general acceptability of fish to many consumers besides its keeping quality. The loss of water which occurs during smoking and drying process naturally improves nutritionally important constituents of dried fish and thus represents an increase in the concentration of the nutritional value for the weight purchased (Akinwumi, 2011).

The African mud cat fish *Clarias gariepinus* (Buchell 1822) is the most popular, widely cultivated and mostly smoked fish in Nigeria (Kumolu-Johnson & Ndimele, 2011).

However, large-scale deterioration in quality and quantity of processed fish results from insect infestation as well as from other physical and biological agencies. Such losses in edible material may be considered very significant nutritionally.

In Nigeria *Dermestes maculatus* and *Necrobia rufipes* were generally associated with dried fish deterioration especially during its storage, transportation and marketing stages.

Significant losses in quality and quantity of smoked *Clarias gariepinus* during storage have been attributed to *Dermestes maculatus* and other dried fish pests (Aderolu & Akpabo, 2009).

Presently in Nigerian dried fish markets, fish pests had been controlled using various methods which include: salting, sun drying, smoking and chemical treatment.

An indiscriminate and uncontrolled use of insecticides may have serious health risks. While the problem of insecticide mainly affects fish for local consumption, it also affects trade between developing countries.

The choice of less toxic and environmentally friendly insecticide of plant origin as found in Azadiractin from the Neem tree *Azadrachta indica* A. Juss which is reputed for its medicinal and insecticidal properties would be more preferable to the hazardous chemicals currently in use. The NSO contained three feeding inhibitory triterprenoids: meliantriol, salamin and azadirachtin. In the ethanol extract of neem seed azadirachtin is the most effective and versatile insecticidal ingredient. In addition to being a feeding deterrent, it has profound effect on mortality and reproduction (Ivbijaro, 1983).

This work evaluates the efficacy of Neem seed oil (NSO) in the control of *Dermertes maculatus* in the common smoked *Clarias gariepinus* and proposes its adoption as an alternative to chemical treatment of smoke fish in the Nigerian markets.

MATERIAL AND METHODS

Preparation of plants extract

Neem plant kernels were collected around Ilorin, Kwara state Nigeria. The seeds were grounded into powder and transferred into soxhlet extractor by first placing glass wool beneath and above. Absolute ethanol was carefully poured and the set up is heated using a steam bath. The vaporization process was carried out severally and the solvent finally distilled. The greenish yellow oil with neem characteristic odour is the extracted Neem Seed Oil (NSO).

Fish collection and insect culture

Fresh samples of smoked African mud cat fish *C. gariepinus* were obtained from a reputable dried fish market in Dugbe dried fish market Ibadan, weighed and oven dried at 60^{o}c for 1hr and allowed to cool to room temperature.

The initial insect pest culture was obtained from infested smoked *C. gariepinus* collected from stalls of a dried fish market at Ilorin, Nigeria. The cultures were maintained separately in jars covered with muslin cloth under laboratory condition and kept at temperature 30 + 2^{o}c and relative humidity of 75 + 5%. All jars were first disinfected using standard procedure by heat treatment at 70^{o}c and then cooled at room temperature. New generation of pests were prepared by removing adult insects and placing them on fresh uninfected fish, then removing the parent adults after 2-3 weeks oviposition period, water was supplied with pieces of soaked cotton wool in the jars. This forms the stock for the study.

Bioassay of Neem Seed Oil (NSO) on oviposition of *D. maculatus*

Uninfested 25g dried Clarias fish was intensely rubbed with 0.125ml of Neem seed oil (NSO) in a specimen jar and covered with muslin material for aeration but prevention of escape of the experimental animal. A pair of newly emerged male and female *Dermertes maculatus* was placed in the jar. A piece of wet cotton wool was placed at sides in the jars to induce oviposition (Okorie et al., 1990). The set up was kept on the laboratory table at temperature of 30°-3°c and 78-98% relative humidity.

The experiment was repeated using in turns NSO concentrations of 0.150ml, 0.180ml, and 0.225ml per 25g dried *C. gariepinus*. Each treatment was replicated thrice. Untreated fish were used as control. Eggs laid in dried *C. gariepinus* were counted every 24hours for 14 days. The number of dead adults was noted in each case.

Effect of Neem Seed Oil (NSO) on the hatchability of different age group of eggs of *D. maculatus*

The eggs laid were grouped according to time period within which they were laid 0-6hr, 19-24hr and 37-42hr. /25g. Dried *C. gariepinus* were treated separately and was replicated three times. Twelve eggs from each group were placed on the treated fish. The incubation period and percentage hatchability of the eggs were noted. Untreated fish were used as control.

Effect of Neem Seed Oil (NSO) on the larvae and adult of *D. maculatus*

25g each of dried *C. gariepinus* were treated with 0.125ml and 2.25ml concentrations of NSO. Thirty newly hatched first in-star larvae of *D. maculatus* were placed on each of the treated fish in specimen jars and covered with muslin mesh of 1mm to prevent the escape of the larvae. This same process was repeated using 20 adults in specimen jar. Untreated fish was used as control in both cases.

Both larvae and adult were allowed to feed on the treated fish for 35 days. The number of the dead and living larvae and adults as well as their growth and development were monitored.

Effects of NSO treatment of the fish kept with fish mongers against *D. maculatus* under natural market condition

125g of fish was purchased from five fish mongers and weighed into 25grammes each. Each portion was treated with NSO 0.175ml, 0.200ml, 0.275ml and 0.325ml on dried *C. gariepinus*. The last 25 grammes were untreated and served as control experiment. *D. maculatus* was introduced is each of the treated fish and fish mongers were instructed to help keep separately. After 6 months (Oct-April 2010) the fish were collected and inspected against *D. maculatus* infestation.

The number of *D. maculatus* living, dead, and concentration of the NSO were calculated and recorded.

RESULTS AND DISCUSSIONS

The result of the toxicity bioassay of ethanol extract of NSO against smoked fish pest *D. maculatus* revealed significant reduction in the number of live larvae and adults of the pests when compared with untreated fish (control experiment).

The result of this study revealed that the crude extract of Neemseed oil (NSO) was found to be highly toxic to *Dermestes maculatus* at all concentrations (Table 1).

LD_{50} was 0.125ml, LD_{70} 0.175ml LD_{99} 0.250ml while LD_{100} was 0.275ml in both laboratory and natural market conditions. The result obtained for adult is similar to those got for larvae, 35% mortality was recorded under 0.125ml in the first 24hr while 60% was recorded after 8-14 days. Under 0.224ml concentration 50% was recorded for the first 24hr but 30% for 8-14 days. These findings are in line with those of Okoye (2000), Fasakin & Aberejo (2002), Ofuya (2003) and Akinwumi (2011) who similarly reported the efficacy of various plant-derived pest control agents for grains, smoked fish, hides and skin.

The result of the study revealed that NSO substantially reduced oviposition in *D. maculatus* at lowest concentration few eggs were laid while at highest concentration no eggs were laid. Neem seed oil inhibited the hatching of eggs. At 0.225ml an average of 2 eggs hatched in most cases. The statistical analysis showed that the incubation period of the eggs that hatched from treated fish was significantly longer than that of the control (P=0.001) (Table 2). This showed that percentage mortality was a function of time. For the first 24hr, 20% of the used larvae were found dead under 0.125ml while it increased to 53% under same concentration after 8-14 days. 23.3% mortality was recorded for larvae under 0.225ml/ 25g of fish in the first 24hr while it increased to 46.6% for the same concentration after 8-14 days (Table 3).

The result of the effectiveness of NSO concentrations under natural market condition at the dried fish market Ilorin showed that the percentage mortality of *D. maculatus* treated with different concentrations of NSO steadily increases from 68.8% recorded under 0.175ml to 82.28 for 0.200ml to 100% for 0.275ml and 0.325ml respectively; while 2.0%(mean) was recorded under control set up (Table 4).

Neem seed oil (NSO) is highly effective under natural market condition. LD_{70} was 0.175ml, LD_{99} was above 0.250ml while LD_{100} is 0.275ml. The regression analysis result showed a significant positive relationship between dosage rates and adult and larval mortalities (r=0.93099, and r=0.9605) (% Adult mortality=0.90399; % Larval mortality=0.9605, Number living=-0.1159).

Neem Seed Oil (NSO) was found to be effective on dried *C. gariepinus* under market condition. Similar result was obtained by Egwunyenga et al. (1998), Ivbijaro (1983a), Okorie et al. (1990) using neemseed powder as a protectant for dried Tilipia fish against *Dermestes maculatus*. The percentage effectiveness on adult *D. maculatus* at 0.125ml was 53% while at 0.225ml it stood at 99% in the laboratory investigation. Concentrations of 0.225ml and 0.275ml protect dried fish for up to and above six months and no single progeny emerged from the treated fish under natural market condition. Its residual effectiveness lasts for a minimum of 28 weeks. This probably suggests that neem seed oil (NSO) could afford protection for dried fish against *D. maculatus* and other pests for the upward of six months. It is simple to apply; by using a simple camel's hair brush to rub the oil intensely on the fish and no toxicity hazard in the edible gelatin produced from trimmings, crumbs etc. it readily satisfied all the conditions of an envisaged safe insecticides on edible animal materials and does not affect the quality of the treated fish.

CONCLUSIONS

In view of the fact that most pesticides and insecticides are becoming unpopular as awareness of their danger to health of man and environment unfolds and their unmonitored use on the control of edible material is still rampant, the recommendation of less hazardous and environmentally friendly alternatives such as ethanol extract of Neemseed oil is imperative. NSO with its characteristic high mortality rates on both the most notorious adult and larvae of the smoked fish pests, and their reproductive inhibitory effects, as fish protectant is promising and could play a major role in post-harvest management strategies of consumed dried animal material's pests, most especially *D. maculatus* on *C. gariepinus*.

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Table 1. Effect of varying concentration of Neem seed oil (NSO) on adult oviposition of *D. maculatus*.

Treatment	Conc. (ml) /25g fish	Total No of adults used	Eggs (mean)	No of dead adults	% Mean mortality
NSO	0.125ml	20	1	10	50
	0.146ml	20	1	12	65
	0.180ml	20	0	16	80
	0.225ml	20	0	20	100
Control	-	20	136	02	14

Table 2. Effect of NSO on hatchability of eggs of *D. maculatus*.

Age range of egg, (hrs)	**Conc/ml**	**No of eggs**	**No that hatched**	**% hatchability**
0-6hr	0.150	12	2	16.6
	0.225	12	0	0
	Control	12	10	83.33
19-24hr	0.150	12	2	16.6
	0.225	12	0	0
	Control	12	11	91.63
37-42hr	0.150	12	3	24.63
	0.225	12	2	16.6
	Control	12	11	91.63

Table 3. Effects of NSO on larvae and adults of *D. maculatus* per unit time.

Days after initial no treatment of larva used	No of larvae used	Mortality/ conc.(larvae)				Initial no of adult used	Mortality/conc. (adults)					
		0.125/25g fish		0.225/25g fish			0.125ml/25g fish		0.225ml.25g fish		Control	
		No	%	No	%		No	%	No	%	No	%
1st 24hrs	30	6	20	7	23.3	20	7	35	10	50	Nil	0
2-7days	30	13	43.3	18	60	20	15	75	17	85	Nil	0
8- 14 days	30	16	53.3	14	46.6	20	12	60	6	30	Nil	0

Table 4. Number living, percentage mortality and weight loss per concentration of NSO used on *D. maculatus* under natural market condition.

Conc. (ml)	% Mortality	No living	% Weight loss
0.175	68.28	6	12.04
0.200	82.28	4	11.23
0.275	100.0	0	3.30
0.325	100.0	0	1.32