ISSN: 2347-5129
IJFAS 2015; 2(5): 80-83

www.fisheriesjournal.com
Received: 31-03-2015
Accepted: 18-04-2015

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Histopathological Alteration induced in gills of juvenile Nile Tilapia *Oreochromis niloticus* upon exposure to two Bio-pesticides

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Abstract
The effects of Neem and Argel leaf aqueous extracts on the histopathology of gills of the fresh water *Oreochromis niloticus* were investigated. The LC_{50} after 96 h of exposure to the aqueous extracts of Argel, *Solenostemma argel*, and Neem, *Azadirachta indica*, were 0.33 g/L and 0.15 g/L, respectively. Histopathological changes of gills were recorded when juveniles of *O. niloticus* were exposed to 20 % of 96 hrs LC50 of each aqueous extract for six weeks. Fish gills exposed to a sublethal dose of 0.07g/l. Argel showed bending of secondary lamellae, telangectiasis, cellular hyperplasia of primary filament, shortening of secondary lamellae, pyknosis, and necrosis of secondary lamellae. Gills in fish exposed to 0.03g/l Neem aqueous extract, recorded epithelial lifting of secondary lamellae, cellular hyperplasia of primary filament, necrosis of secondary lamellae, globular haematomas and shortening of secondary lamellae. The level of severity of lesions increased with incease in exposure time. Therefore the present study concluded that the aqueous extracts of, *Solenostemma argel* and *Azadirachta indica* should be used with precaution in the agricultural fields. Nevertheless these two plants can be recommended as an environmentally sound alternative for synthetic piscicides

Keywords: gills, histopathology, Neem, Argel, *Oreochromis niloticus*.

1. Introduction
Phytochemicals are botanical substances which are naturally occurring pesticides obtained from floral resources. They are sometimes used intentionally in water bodies for fishing [1] and as molluscicides [2] in the aquatic environment where non-target fish species may suffer alteration in metabolic reactions resulting in deleterious effects that harm them.

Neem, *Azadirachta indica*, has wide spectrum of biological activity. It is well known for its insecticidal properties and it is commercially exploitable [3]. Likewise Argel, (*Solenostemma argel)* as well is known for its anti-microbial activity [4]. The aqueous extracts of Argel have antifungal and antibacterial properties [5]. Both aqueous extracts of Neem and Argel used in this work have gained importance as effective natural insecticides. The use of Neem and Argel based bio-pesticides near water resources such as streams, river, and lakes may affect the non-target organisms and fish being susceptible to such water contaminants.

Histology is a mechanism which can provide an indication of fish health by determining early injury to cells. Therefore, it is an important tool to determine the effect of pollutants on fish tissues. The gills of fish are the main target organs for toxic action of chemical pollutants, as well as detoxification process. It has frequently been used in the assessment of aquatic pollutants in fresh water habitats [6].

This present study aimed at examining the acute and chronic toxicity of aqueous extracts of these two plants on the juveniles of the Nile tilapia *Oreochromis niloticus* to throw some light on the sensitivity of these extracts when used as pesticides.

2. Materials and Methods
2.1. Fish collection
Tilapia fish of the species *O. niloticus* were collected from the Shagara fisheries research station, 10 km south of Khartoum State. Fish were caught from the ponds and transported in plastic barrels half filled with pond water early morning. The size of fingerlings ranged from 4-7cm and 5-6gms.

In the laboratory the fish were left to acclimate in indoor glass aquaria measuring 80 x 60 x 60 cm with continuous aeration for two weeks. Fish were left to acclimate to de-chlorinated tap water, through mixing tap water and pond water Regular checks were made to remove the dead fish.

2.2. Collection of Neem and Argel

Azadirachta indica (Neem) leaves were obtained from the faculty of agriculture (University of Khartoum) fields at Shambat and El shagara fisheries research station. Eaves were left to dry for one week at the laboratory of the Department of Studies and Research of Natural Resources. The dried leaves were ground by an electric blender. The obtained powder was kept in plastic jars and left at room temperature. Leaves of Argel (*Solenostemma argel)* were obtained from a local market at Khartoum and ground using an electric blender. The obtained powder was stored in plastic jars and left at room condition.

2.3. The setup of Experiment

The experiment setup consisted of glass aquaria with vigorously aerated water kept at room temperature and diffused day- light. The test media were changed every twenty four hours to avoid chemical degradation, volatilization, adsorption to the container or reaction with fish excreta. Total length of the fish to the nearest 0.1 cm were measured, the weights of individual fish were taken using an electronic balance sensitive to the nearest 0.1 gm. Measured and weighed fish were then introduced to the experimental system 30 minutes after introduction of the prepared aqueous extracts. Experiments were carried out in triplicates with regular feeding for both experimental and control fish. Fish were fed with wheat bran and groundnut cake in the ratio 3:1 (35% crude protein) Fingerlings were fed once daily at ratio of 3% of their body weight.

2.4. Acute toxicity tests (Determination of LC_{50})

Prior to the acute toxicity experiments, preliminary tests were carried out to determine a convenient and logarithmically spaced range of concentration to be used. These were conducted over a wide range of concentrations using only five fish at each concentration in small plastic containers. Screened Neem concentrations were (2.5, 2.0, 1.0, 0.5, 0.25, 0.125 g/L) while Argel concentrations were (2.0, 1.0, 0.5, 0.25, 0.125, 0.062 g/l).

Acute toxicity tests were conducted within 96 hours. Within this period observations on fish mortality were recorded every 3, 6, 12, 24, 48, 72 and 96 hours. From these mortality observations LC_{50} (the concentration of insecticide that is lethal to 50% of the tested population) was then calculated using the equations as described by [7]

2.5. Histopathological Tests

Acclimated fish fingerlings were exposed to a sub-lethal dose of 20% LC50 of Neem (group1) and 20 % LC50 Argel (group 2) aqueous extracts for a period of 45 days, under natural light conditions and at room temperature. Controls for the tested fish were set parallel to the experiment (group 3). At time intervals of 2 weeks, 4 weeks and 6 weeks of exposure, 5 fish from each group were sacrificed. From each the gills were removed, fixed in formalin and then processed routinely for histological studies according to [8].

3. Results

3.1. Acute Toxicity Tests

Results showed that no mortalities occurred in all controls throughout the 96 hours of the experiment. There was irregular but progressive rise in the rate of mortality of fingerlings with increase in concentration of Neem and Argel.

Table 1: Variation 0f LC50 (g/l) of Neem and Argel aqueous extract within each time interval for *Oreochromis niloticus* fingerlings.

Time in hours	Half lethal dose (LC50) of Neem	Half lethal dose (LC50) of Argel
24	0.48	0.88
48	0.30	0.52
72	0.18	0.47
96	0.15	0.33

3.2. Histopathological tests

Histopathological results revealed that chronic exposure (six weeks) of juvenile *Oreochromis niloticus* to both aqueous extracts of Neem and Argel caused observable effects on gills.

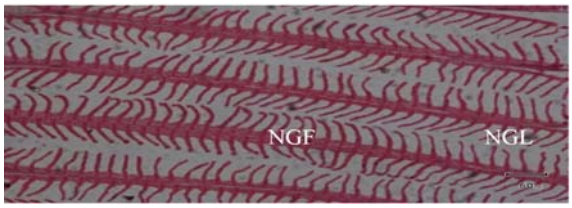


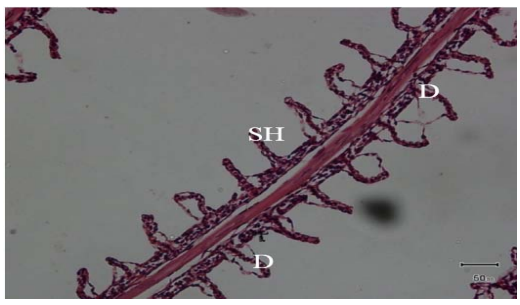
Plate 1: Gill of *Oreochromis niloticus* under control conditions. Note: normal gill filaments (NGF), normal gill lamellae (NGL) H&EX10,

3.2.1 Histopathological lesions in gills under exposure to Neem leaf aqueous extract

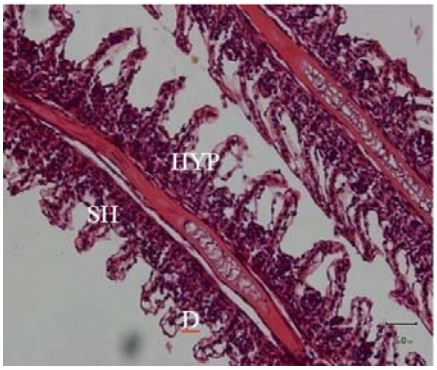
After two weeks of exposure to (20 % of LC50) Neem aqueous extract the gills showed moderate detachment of epithelial layer form pillar cells and shortening of the secondary lamellae as shown in Plate (4). In four weeks of exposure these lesions became more severe. Also gill filament showed blood congestion and mild hyperplasia. In six weeks of exposure shortening of secondary lamellae were severe and globular haematomas were noted (Plate 2).

3.2.2 Histopathological lesions in gills under exposure to Argel leaf aqueous extract

After two weeks of exposure to Argel aqueous extract (20 % of LC50), the gills showed bending of the distal extremities of secondary lamellae, telangectiasis, and shortening of secondary lamellae. In four weeks of exposure mild cellular hyperplasia of chloride cells in gill filaments, mild detachment of epithelial layer form pillar cells were observed. In six weeks shortening of secondary lamellae became more obvious. (Plate 3).



(A)



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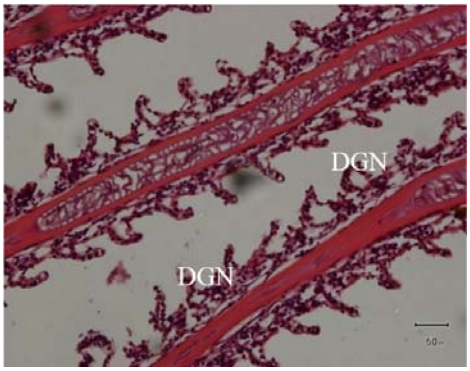
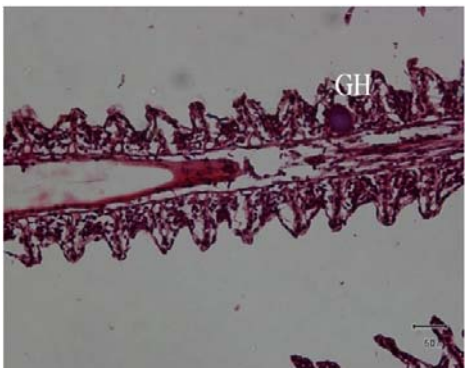
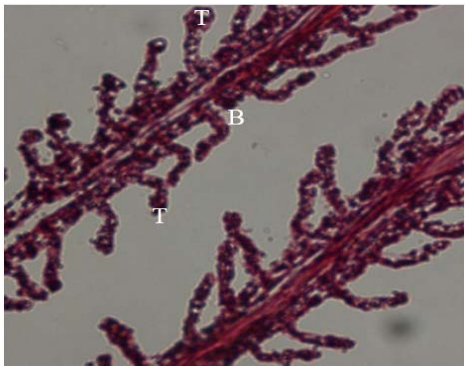


(C)

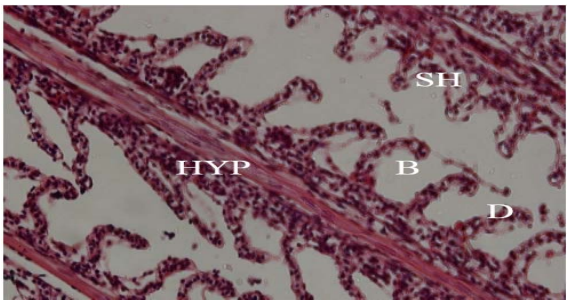


(D)

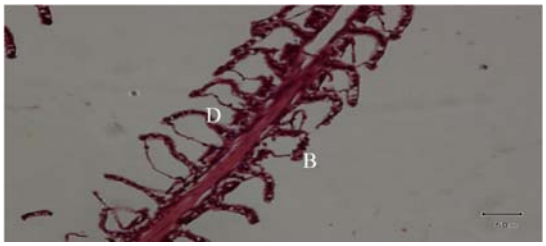
Plate 2: Gill filament from *Oreochromis niloticus* kept under a sublethal dose of Neem aqueous extract for 2 weeks (A) 4 weeks (B) and 6 weeks (C and D). D= detachment of epithelium.SH= Shortening of secondary lamellae, HYP=hyperplasia of gill filaments, DGN= degenerated and necrotic lamellae, GH= globular haematomas.



(E)



(F)



(G)

Plate 3: Gills from *Oreochromis niloticus* kept under a sublethal dose of Argel aqueous extract for 2weeks (E), 4 weeks (F) and 6 weeks (G). Showing B=bending of lamellae, D = detachment of epithelium, SH= shortening of secondary lamellae, T= telangectiasis, HYP= hyperplasia of gill filament.

The severity of lesions was time dependant as shown in tables (1 -2) below.

Table 1: Prevalence intensity of histopathological lesions in the gills of *Oreochromis niloticus* exposed to Neem for different time intervals.

Histological lesion	Exposure time (in weeks)			
	control	Week 2	Week 4	Week 6
Detachment of secondary lamellae.	–	+ +	+ +	++
Cellular hyperplasia of primary filament.	–	–	+	–
Necrosis of secondary lamellae	–	–	–	++
Globular haematomas	–	–	–	+
Shortening of secondary lamellae.	–	+	+ +	+ + +

No lesion (-) mild lesion (+/ 25% of the field) moderate lesion (++/ 75% of field) Severe lesion (+++/ all field)

Table 2: Prevalence intensity of histological lesions in the gills of juvenile Oreochromis *niloticus* exposed to Argel aqueous extract for different time interval.

Histological lesion	Exposure time (in weeks)			
	Control	Week 2	Week 4	Week 6
Bending of secondary lamellae	–	+	++	++
Telangectiasis	–	+	–	–
Cellular hyperplasia of primary filament.	–	+	+	+
Shortening of secondary lamellae.	–	-	+	+
Necrosis of secondary lamellae	–	–	–	+

No lesion (-), mild lesion (+/ 25% of the field), moderate lesion (++/ 75% of field), Severe lesion (+++/ all field)

4. Discussion

Many investigators stated that gills, which participate in many important functions, remain in close contact with the external environment. They become particularly sensitive to changes in water and are considered to be the first organs to be affected by the contaminants [9]. Findings of this work showed early lesions in gills of juvenile tilapia after sublethal exposure to both Neem and Argel aqueous extracts. In two weeks of exposure gills showed moderate bending, mild hyperplasia, moderate lifting of epithelium and shortening of secondary lamellae. These lesions are explained as a defense mechanism where gills try to increase the distance between the external environment and the blood, thus serving as a barrier to the entrance of contaminants [9]. Similar results were shown [10] working with Catfish *Heteropneustes fossilis* exposed to Purified Neem and [11] working with carps (*Cyprinus Carpio*) and tilapias (*Oreochromis mossambicus)* exposed to the effluents of a waste water. [12] Observed epithelial lifting in rainbow trouts (*Oncorhynchus mykiss*) exposed to petroleum residues. Some investigators working with heavy metals [13, 14]. [15] Working with organic pesticides. [16] Working with Phenol and after exposure to radiation [17].

Severity of lesions in gills was found to be time dependant. Therefore, with time stress increase causing more damage in secondary lamellae resulting in necrosis of gill lamellae. These findings agreed with above mentioned investigations. The histopathological changes observed in *O. niloticus* were of significant diagnostic value to both aqueous extracts of, *Solenostemma argel* and *Azadirachta indica*. Therefore they should be used with precaution in the agricultural fields. They can also be recommended as an environmentally sound alternative for synthetic piscicides.

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