Chronic Effect Of Fenitrothion On Health Of *Oreochromis niloticus* and Oxidative Stress Biomarkers

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ABSTRACT

Serious problems of pollution and health hazards accompanied the wide range of production and application of pesticides during last few years have occurred. Ninety *Oreochromis niloticus* were exposed to $1/_{10}$ and $1/_{20}$ 96 hrs LC_{50} to assess its chronic injurious effect on growth performance, biochemical analysis and histopathological alteration.The results revealed that a significant decrease in the final body weight, weight gain and body gain percentage, condition factor was decreased. The usual inhibition of Acetylcholine esterase (AChE) was detected that result in the detected behavioral changes. Significant reduction in serum glutathione (GSH) content and significant increase in superoxide dismutase enzyme activity (SOD), malondialdehyde (MDA), alanine aminotransferase (ALT), creatinine and urea levels. In the other a significant decrease in total serum Immunoglobulon M (IgM). Histopathological alterations in liver, kidney and gills related to concentration and duration of exposure.

Keywords: Fenitrothion insecticide, *Oreochromis niloticus*, health and growth, oxidative stress, prooxidant activity

INTRODUCTION

For centuries, pesticides have been used in agriculture for enhancement of food production by eradication of unwanted insecticides and controlling disease vector especially organophosphorous (OP) compounds such as fenitrothion (FNT) ***(1)***. In chronic (low) dose tests, unexpectedly only the lowest concentration (0.011 microgram/liter) of Fenitrothion depressed the growth of an algae, though all of the chronic dose levels used were toxic in other ways to the algae ***(2)***. Just half of FNT's minimally effective dose altered the thyroid structure of a freshwater murrel (the snakehead fish) ***(3)***. In an unusual demonstration of resistance to pesticides, 8% of insects in farm fields were found to carry a symbiotic gut microbe that can metabolize and detoxify FNT; after in-vitro tests showed that the microbe significantly increased the survival of FNT-treated insects ***(4)***. Due to the probability of their being discharged into the aquatic system, great attention has to be paid to their degradation, to diminish their harmful effect (***5***). It considered a common river pollutant and its residues in natural water undergo photo degradation, resulting in the release of many toxic metabolites, some being more toxic than the parent compound to aquatic organisms (***1,6,7***) in addition fishes serve as a biomarkers of this environmental pollution (***8***). Primary effect of OPC on both in vertebrate and vertebrate organisms, including humans, was the inhibition of the acetyl cholinesterase (AChE). However, the toxic effects of OPC are not restricted to the Ach inhibition only but may be directed toward the induction of oxidative stress and reactive oxygen species ***(9)***.So, this study was done to

illustrate the effect of chronic exposure of FNT for Nile tilapia through determining the growth performance, behavioral alterations, pro-oxidant activity, alterations in serum biochemical parameters and histological examination of liver, kidneys and gills.

MATERIAL AND METHODS

Fish: A total number of 90 *Oreochromis niloticus* fingerlings were obtained from the Abbassa Fish Hatchery, Sharkia province with an average body weight 9±0.5 g. Fish were randomly divided into glass aquaria (96 L for each 15fish) and allowed to be acclimated for laboratory conditions for 10 days before the beginning of the experiment. During the experiment, fish were maintained under constant and continuous aereation, dechlorinated tap water and temperature (25°C). Fish were fed daily with commercial pellets.

Chemicals: FNT was obtained from analytical standard grade (CAS number :122-14-5) and purchased from Sigma–Aldrich Chemical Corporation(Egypt).

Experimental design: The 96 hrs LC_{50} (4.7 mg/L) of FNT for *O .niloticus* fingerlings previously determined ***(10)*** was used. The experiment was applied to determine the sub lethal effects of FNT chronic exposure, so two sub lethal concentrations of 0.47 mg/L and 0.235 mg/L which corresponding to $1/_{10}$ and $1/_{20}$ 96 hrs LC_{50} respectively were used. After acclimatization fish were randomly divided into three groups each with 30 individuals. Each group with two replicates containing 15 fish per replicate. Group 1 was reared in pesticide free tap water and treated as control. Groups 2 and 3 were exposed to the mentioned sub lethal concentrations (0.47 mg/l and 0.235 mg/l) of FNT respectively for eight weeks. Throughout the experimental period fish were fed 4 times daily with commercial food at a rate of 4% of their body weights. Food was not given for 24 h prior to experiment and dissection. Aquaria water was completely changed every 48 h to maintain water quality with the appropriate pesticide amount.

Mortality rate and behavioral responses of tested *O.niloticus*: The mortality rate and behavioral responses of tested fish were investigated ***(11,12)***.

Growth performance: Fish of all replicate were counted and weighted individually after 2, 4, and 6 and 8 weeks of the experiment and body gain ***(13)***, body gain % ***(14)*** and condition factor ***(15)*** were calculated.

Sampling: Blood samples from all fish were taken from caudal vein and processed immediately ***(16)***. Sera were separated and frozen at -80 till investigation of biochemical parameters. At the end of the experimental period fish were killed immediately through neck incision and the tissues (liver, kidneys and gills) were collected for histopathological examination ***(17)***.

Biochemical investigation: Serum samples were used for detection of Superoxide dismutase (SOD) activity ***(18)***, Malondialdehyde (MDA) concentration as a marker of lipid peroxidation ***(19)***, reduced glutathione (GSH) content ***(20)***. Serum AChE ***(21)***, serum alanine aminotranseferase (ALT) was determined colorimetrically ***(22)***, serum urea level ***(23)***, serum creatinine ***(24)***. Finally, IgM levels were determined according to ***(25)***. Protein levels estimation were determined ***(26)*** using bovine serum albumin as standard.

Statistical analysis: The statistical significance of the data has been determined using one way analysis of variance (ANOVA-LSD) using SPSS statistical software package version 18. The level of significance was taken as $p < 0.05$.

RESULTS AND DISCUSSION

The chronic toxic effects of FNT on the health of *O. niloticus* still unclear, therefore, the present study aimed to assess the chronic harmful effect of FNT insecticide on *O. niloticus* fingerlings.

Effect of chronic FNT intoxication on growth performance of O. niloticus fingerlings

The results demonstrated in (table 1) showed a significant decrease in final body weight, weight gain, condition factor and body gain percentage in groups 2 and 3 compared with group 1. These results are nearly agreed with those previously recorded in *Macropondus Cupanus(27)*.

Table 1. Effect of FNT chronic exposure concentrations of ($1/_{10}$ and $1/_{20}$ 96 hrs LC_{50}) on growth performance on *Oreochromis niloticus* fingerlings

	Control	Chronic FNT exposure ($1/_{10}$ 96 hrs LC_{50})	Chronic FNT exposure ($1/_{20}$ 96 hrs LC_{50})
Initial body weight (g)	10.14±0.49 [a]	10.2 ± 0.24 [a]	10.33± 0.23 [a]
Initial total body length (cm)	8.5 ± 0.7 [a]	8.5 ± 0.41 [a]	8.5 ± 0.2 [a]
*Final body weight (g)	32.41±1.4 [a]	24.5 ± 0.35[b]	26.4± 0.45[b]
**Final total body length (cm)	12.3± 0.4[a]	12± 0.5[a]	12.1± 0.42[a]
Weight gain (gm)	22.58± 0.31[a]	14.45± 0.25[b]	16.05± 0.22[b]
body gain %	217	140	156
Condition factor			
a. at start	1.65	1.66	1.68
b. at end	1.72	1.42	1.49
c. percent of change condition factor (% of initial value)	+7	-24	-19

Means within the same row bearing different subscripts are significant at $p \leq 0.05$.

*Final body weight after 8 week.**Final total body length after 8 week.

Mortality rate and Behavioral changes

The results showed in (table 2) revealed that, the mortality rate of groups 2 and 3 were 36.6% and 26.6 % respectively. The exposed fish appeared sluggish and not respond to tested reflexes. Dark coloration, presence of thick mucus and severe congestion in internal organs were observed.

Behavior represents the animal's response to physiological and environmental factors and specific to one organism from another *(28)*; in addition, behavioral alteration may be one mechanism at which fish adapt to environmental changes, including contaminants *(29)*. Thus it can be considered a useful biomarker to evaluate chronic chemical exposure *(30)*.

FNT as any organophosphate insecticide has neurotoxic effects by inhibition of AChE activity (a standard biomarker of organophosphate poisoning), and this confirmed by our results which clears the inhibitory effect of FNT on AChE **(table 3)** and this suggest that, cholinesterase inhibition can induce sub lethal effects on a variety of parameters with implications for organism's fitness *(31)*, thus this may be a cause of behavioral alterations observed in *O. Niloticus* fingerlings after chronic exposure of FNT. More specifically escape reflex can be influenced by AChE impairment *(32)*. Regarding to the mortality rate of the fish exposed chronic ($1/_{10}$ and $1/_{20}$ 96 hrs LC_{50}) of FNT, that may be due to the accumulative toxic effect of FNT in long exposure*(33)*.

Table 2. Effect of chronic FNT exposure (1/10 and 1/20 96 hrs LC_{50}) on behavioral changes and mortality rate of Nile tilapia fingerlings.

Group	Concentration of FNT (mg/l)	Observation (week)																Mortality rate at the end	
		1st		2nd		3rd		4th		5th		6th		7th		8th		No	%
		ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR	ER	MR		
1	Control	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	+++	0	0	0
2	Chronic FNT exposure ($1/_{10}$ 96 hrs LC_{50})	+++	0	+++	1	++	1	++	2	++	1	+	3	+	2	+	1	11	36.6
3	Chronic FNT exposure ($1/_{20}$ 96 hrs LC_{50})	+++	0	+++	0	+++	1	++	1	++	2	++	2	+	1	+	1	8	26.6

ER= escape reflex MR= mortality rate

+++ = fish respond well to escape reflex and showing normal activity and movement.

++ = fish moderately respond to escape reflex and showing sluggish activity and movement.

Serum biochemical parameters

The results in (table 3) presents that, the chronic exposure ($1/_{10}$ and $1/_{20}$ 96 hrs LC_{50}) FNT of *O. niloticus* fingerlings results in inhibition of A.Ch.E activity that significantly decreased in the treated group when compared to control one. Relating to the effect of chronic ($1/_{10}$ and $1/_{20}$ 96 hrs LC_{50}) sub lethal intoxication of *O. niloticus* fingerlings to FNT insecticide on oxidative stress parameters, the data demonstrated in (Table 3) declared a significant decrease of serum reduced glutathione content (GSH), significant increase in the serum SOD activity and in lipid peroxidation biomarker (MDA) after FNT insecticide exposure in compare to control groups ($p<0.05$). This refers to the disturbance in oxidant and antioxidant status in the treated groups. Also the recorded results revealed the damage state of liver that was indicated by a significant increase in the serum levels of ALT after treatment with FNT in different groups than the control ones. Furthermore, there was significant increase in serum levels of cortisol, creatinine, urea which indicate renal damage with a significant decrease in IgM of fish groups after chronic exposure to FNT insecticide compared with the control ones at $p < 0.05$.

Beside the classical inhibitory effect of FNT insecticide to AChE that detected in this work and results in behavioral changes, FNT can induce its toxic effects through induction of oxidative stress that detected by evaluation of thiobarbituric acid reactive substances (TBARS) MDA level a marker for lipid peroxidation (LPO) that significantly increased by chronic exposure to FNT insecticide. With a significant reduction in GSH contents (table 3). The observed depletion in the glutathione GSH in this study is considered as an early consequence of FNT induced oxidative stress as GSH molecules scavenges free radicals resulted from oxidative metabolism *(34)*. Consequently depletion in GSH content in this study is due to oxidation of GSH to

glutathione disulfide GSSG by free radicals produced by FNT insecticide*(35)*.

The increase in MDA levels reflects one mechanism of cell damage manifested by increase in lipid peroxidation (LPO). OP pesticides can lead LPO either by direct interaction with cellular plasma membrane *(36)* or by reactive oxygen accumulation *(37)*.Increase of MDA in the serum of *O. Niloticus* fingerlings reflects the increase in the reactive species produced due to chronic FNT exposure that not eliminated effectively due to suppression of antioxidant enzymes activities and reduction in GSH levels leading to insufficient neutralization of reactive species.

SOD is one of the most important defense mechanisms against toxic effects of oxygen metabolism. SOD catalyzes the conversion of superoxide radicals to hydrogen peroxide therefore; maintain low steady-state concentrations of the ROS and alleviate their toxic effects *(35)*. Oxygen radical production was increased parallel to increase in MDA and this appeared by an increase in SOD activity observed in this experiment (table 3) as an adaptive response to get rid of oxygen species free radicals*(38)*. On the other hand, the activity of the antioxidant enzymes could be increased or inhibited by xenobiotic exposure depending on the intensity and the duration of the stress applied, as well as the susceptibility of the exposed species *(39)*.

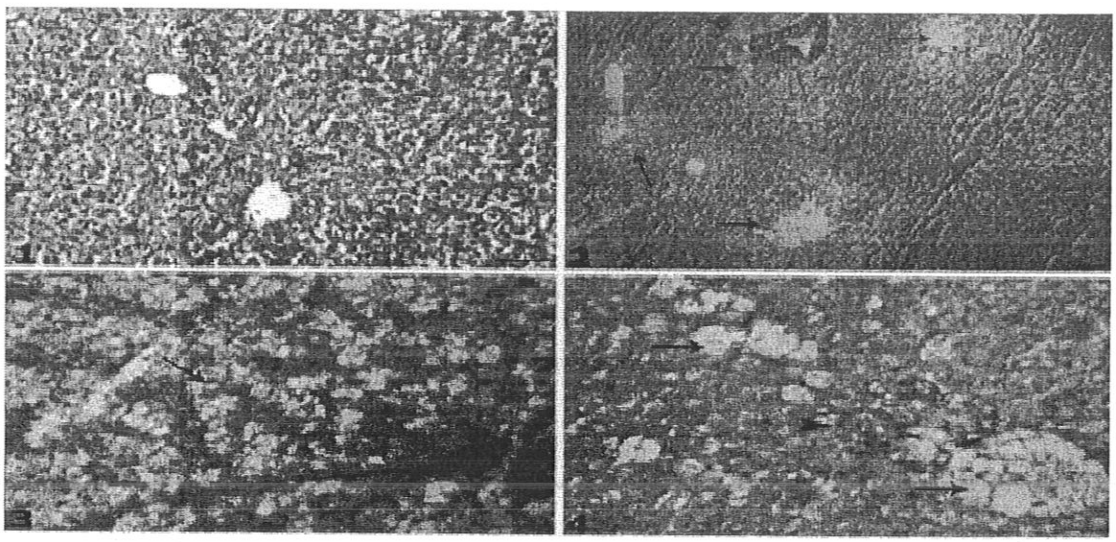
Concerning to the toxic effect of FNT insecticide on liver and kidneys, FNT chronic exposure showed that, there were a significant increase in the level of ALT, serum urea and serum creatinine which reflects the deleterious effect against liver and kidneys. This increase in liver enzymes may be due to liver cell damagewhich confirmed by our histologic examination of liver presented in **figure** 1 or due to alteration in the permeability of cell membrane due to increase in free radicals production.With regards to the increase in serum urea and creatinine, this may be due the decrease in glomerular filtration of kidney or tubular dysfunction *(40)*; this also is confirmed by the obtained histopathological changes in the kidneys of O. Niloticuse. In respects to IgM, our search demonstrated that there is a significant decrease in total circulating IgM.

Table 3. Biochemical changes after chronic exposure of (1/10 and 1/20 96 hrs LC50) concentrations of FNT insecticides on *O.niloticus fingerlings*

	Control	**Chronic FNT exposure ($1/_{10}$ 96 hrs LC_{50})**	**Chronic FNT exposure ($1/_{20}$ 96 hrs LC_{50})**
A.Ch.E (U/ml)	415.3± 40.3 ^{a}	115.4±39.6^{c}	271.6±37.8^{b}
GSH (ng/ml)	9.91±0.3 ^{a}	5.59±0.26^{c}	7.56±0.51^{b}
SOD (unit/l)	48.31±1.35 ^{e}	74.68±1.91^{c}	61.9±1.4 ^{d}
MDA (nmol/l)	33.21±0.44 ^{d}	44.4±1.7^{b}	36.4±0.6 ^{c}
ALT (IU/dl)	17.67±0.33^{a}	28.67±0.67^{a}	25.00±0.57^{b}
Creatinine (mg/dl)	0.15±0.005^{c}	0.87±0.03^{a}	0.74±0.02^{b}
Urea (IU/dl)	7.67±0.33^{c}	17.00±0.57^{a}	15.33±0.33^{b}
IgM value(µg /ml)	23.38±0.33^{a}	10.33±0.08^{b}	8.38±0.11^{c}

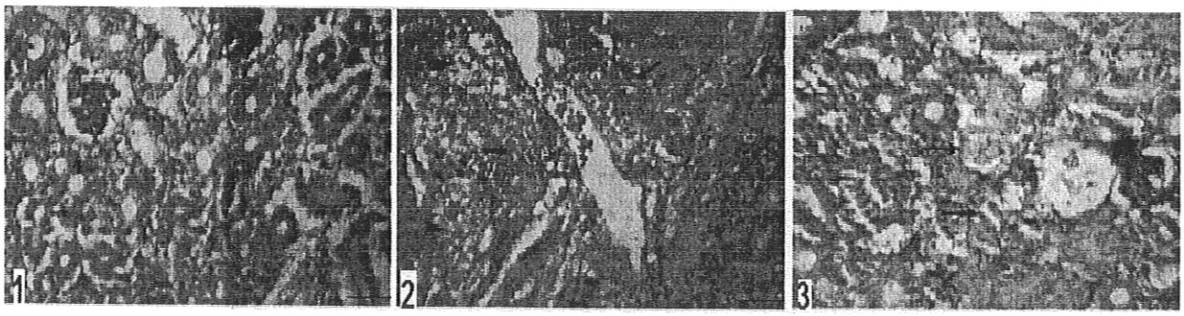
Means in the same row carrying different superscript were significantly different (P< 0.05).

Histopathological findings



Figs 1. Light micrograph of liver section of Nile tilapia showing:1.1) Normal typical hepatocytes and sinusoidal architectures. 1.2) Periportal vacuolations of hepatocyte 1.3) Diffuse vacuolations with pyknotic or disappeared nuclei. 1. 4) Focal fatty change of large clear vacuoles. **HE (Bar = 100 µm).**

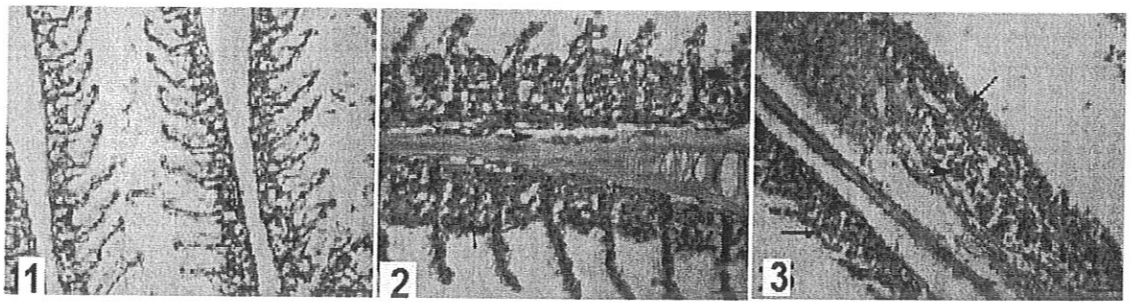
Liver section of control group showed normal liver architecture with the central vein and radiating cords of normal hepatocytes with central rounded nuclei. Normal blood sinusoids appeared between the liver cords(**Fig 1.1**). Meanwhile, the liver of chronic FNT exposure showed peri-portal vacuolations of hepatocytes (**Fig 1.2**). These vacuolations became diffuse with pyknotic or disappeared nuclei (**Fig 1.3**). Focal fatty changes of large clear vacuoles were noticed, particularly with high FNT level (**Fig 1.4**). Dark brown pigments of bile were seen in the cytoplasm of hepatocytes. Moderate congestion and hemorrhage were detected.



Figs 2. Light micrograph of kidney of Nile tilapia showing: 2.1) Kidney of control with normal glomerular and tubular structures. 2.2) Moderate vacuolation of the renal tubular epithelium and few interstitial lymphocytic infiltrations. 2.3) Coagulative necrosis in the renal epithelium. **HE (Bar = 100 µm).**

Kidney section of control group revealed normal cortex showing normal renal corpuscles with Bowman's capsules and renal glomeruli are made of tuft of blood capillaries. Sections of the proximal and distal convoluted tubules showed normal cuboidal epithelial lining (**Fig. 2.1**).On the other hand, the kidney of chronic exposure to FNT revealed moderate vacuolation of the renal tubular epithelium and few interstitial lymphocytic infiltrations (**Fig 2.2**). Focal interstitial hemorrhage was noticed among the

degenerated renal tubules. Coagulative necrosis was seen and represented by coagulated eosinophilic cytoplasm and absent nuclei (**Fig 2.3**). Eosinophilic hyaline droplets were focally accumulated in the tubular epithelial cells.



Figs 3. Light micrograph of gills of Nile tilapia showing 3.1) Gills of control with Normal filaments and respiratory epithelium. 3.2) Diffuse proliferation and fusion of the respiratory epithelium were hemorrhage and leukocytic infiltrations. 3.3) Focal sloughing of the secondary lamellae with aggregations of lymphocytes and congestion lamellar capillaries. **HE (Bar = 100 µm).**

As regards to gills of Nile tilapia fingerlings, the gills of control groups showed normal filaments and respiratory epithelium (**Fig 3.1**). The gills of chronic exposure to FNT revealed diffuse focal epithelial and mucous cells proliferations, with excessive hemorrhage and leukocytic infiltrations (**Fig 3.2**). Focal sloughing of the secondary lamellae with aggregations of lymphocytes and congestion lamellar capillaries (**Fig 3.3**).

All the results of the histopathological alterations were completely agree with *(41 - 43)*

CONCLUSION

The results of this work showed a significant decrease in final body weight, weight gain, weight gain percent and condition factor. Significant increase in serum SOD, MDA, ALT, creatinine and urea. Significant decrease in GSH and IgM in fish exposed to $1/_{10}$ then $1/_{20}$ 96 hrs LC_{50} of FNT compared to control group. FNT produced histopathological alteration on the fish species.

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الملخص العربي

التأثير المزمن للمبيد الحشري فينتروثيون على صحة اصبعيات البلطي النيلي والمؤشرات الحيوية للأكسدة

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ان التوسع في انتاج و استخدام المبيدات الحشرية يصحبه مشاكل عديدة من التلوث و التأثير على الصحة العامة. و لذلك ركزت الدراسة الحالية على تقييم التأثير المزمن الضار للمبيد الحشري فينتروثيون (احد مركبات الفوسفور العضوي) على اصبعيات البلطي النيلي من خلال استخدام ٩٠ سمكة من اصبعيات البلطي النيلي قسمت الي ثلاث مجموعات متساوية كل مجموعة تحتوي على ١٥ سمكات في تكرارين. المجموعة الاولى كانت ضابطة و المجموعة الثانية تعرضت الى ١٠/١ من التركيز النصف مميت (٩٦ ساعة) بينما المجموعة الثالثة تعرضت الى ٢٠/١ من التركيز النصف مميت (٩٦ ساعة). تم دراسة معدلات النمو، نسبة النفوق، التغيرات السلوكية للأسماك، نشاط الاكسدة من خلال تحديد كمية انزيم الجلوتاثيون (GSH)، نشاط انزيم السوبراوكسيد ديسميوتاز (SOD) و تركيز الميلانوالدهيد (MDA) كمؤشر على اكسدة الدهون. كذلك تم تحديد تركيز الألانين امينو ترانسفيريز (ALT) ، اليوريا، الكرياتنين و الاميونو جلوبيولين (IgM). كذلك تم تحديد التغيرات المرضية في انسجةالكبد، الكلى و الخياشيم.لوحظ انخفاض معنوي في الوزن النهائي، الوزن المكتسب، النسبة المئوية للوزن المكتسب و معامل الحيوية و تغيرات سلوكية للأسماك. انخفاض معنوي في (GSH) و زيادة معنوية في (SOD)، (MDA)، (ALT)، الكرياتنين و اليوريا في المقابل لوحظ انخفاض معنوي في (IgM) كما لوحظ تغيرات مرضية في انسجة الكبد و الكلى و الخياشيم. من خلال ما سبق يمكن استنتاج التاثير السيئ للفينتروثيون على معدلات النمو للبلطي النيلي كذلك على صحة الاسماك من خلال انتاج العوامل المؤكسدة او تثبيط العوامل المضادة للأكسدة في الاسماك