

Effects Of Barley Grass Powder (*Horidium Vulgare*) On Some Biochemical Parameters In *Tilapia nilotica* Exposed To Pestban Insecticide

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ABSTRACT

Pestban (chloropyrifos) is an organophosphate insecticide widely applied in agriculture and aquaculture, induces oxidative stress due to generation of reactive oxygen intermediate and changes in the antioxidant defense system. The present study was aimed to determinate 96hrs median lethal concentration (LC₅₀) of pestban in *Tilapia* fish (*Oreochromis niloticus*) and study effect of barley grass powder on some biochemical parameters in *Tilapia nilotica* exposed to pestban. The 96hrs LC₅₀ was equal to 0.36 mg/l. The fish exposed to 1/10 LC₅₀ (0.036mg/l) and 1/20LC₅₀ (0.018mg/l) of pestban for either one, four and eight weeks showed inhibition of brain cholinesterase (ChE) and significantly increased superoxide dismutase (SOD), malondialdehyde (MDA), glutathione reductase (GR) in gills, liver and muscle comparing with control. Addition of barley grass powder 10% or 20% improve the level of antioxidant enzymes SOD, GR and MDA in gills, liver and muscle than that in exposed to pestban alone while no change in brain cholinesterase.

These findings suggest that barley grass powder is positively effective in pestban toxicity in fish.

INTRODUCTION

Health conscious consumers today are looking for more than wholesome, nutritious food to maintain good health. They also want to avoid the ever-increasing levels of pesticides and other chemicals in our environment. Periodic use of internal cleaning programs may help to eliminate pesticide residues (1).

Pestban is trade name for agriculture-use product of chloropyrifos (CPF) (48%EC) (2). CPF is a broad-spectrum organophosphate insecticide (OP) and commercially used for more than a decade to control foliar insects that affect agricultural crops (3). CPF passes via air-drift or surface runoff into natural waters, where it is accumulated in different organisms living in water, especially fish, thus making it vulnerable to several discernible effects (4).

Organophosphates are, environmental toxicants, known to inhibit acetylcholinesterase (AChE) (5). They are equally toxic to vertebrate and invertebrate aquatic animals even at low concentration (ng to µg/l levels); fish are particularly sensitive to organophosphate toxicants (6).

Generation of reactive oxygen species (ROS), which may lead to oxidative

stress, indicating the role of ROS in pesticide toxicity (7). Antioxidant defenses such as catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR) are involved to counteract the toxicity of ROS (8). So the antioxidants in fish could be used as biomarkers of exposure to aquatic pollutants (9).

Barley Grass provides balanced nutrition including an abundance of minerals (potassium, calcium, magnesium, iron, copper, phosphorus, manganese and zinc), 18 amino acids, antioxidant enzymes, beta-carotene and vitamins B1, B2, B6 and C, It is also an excellent source of Chlorophyll which helps to gently detox form the environmental pollutants (10).

MATERIAL AND METHODS

Fish

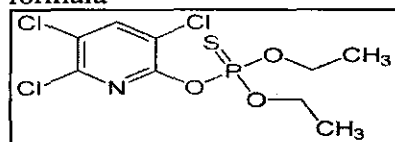
Study was conducted on *Oreochromis niloticus* freshwater fish, (40±5g, 12.5±0.5cm cm). Fish were transferred to Central Laboratory for Aquaculture Research (CLAR) Abbassa, Sharkia Governorate with in plastic bags. The bags were cut open and the fish were disinfected with 0.1% KMnO₄ solution for few seconds and acclimitized under laboratory conditions for two weeks in 1000 liter water tanks, filled with well aerated tap water and fed on basal fish diet under

laboratory condition PH 7.1-7.5 ,temperature at $26 \pm 5^\circ\text{C}$ before transferring them to the test glass aquaria .

Insecticide

Pestban 48%EC

Structural formula



Registered trade name(s): Pestban ,Dursban®, Lorsban®,Pyrinx ,DMS-0971.

Chemical name:O,O-diethyl-O-3,5 6-trichloro-2-pyridylphosphorothioate Molecular formula:C₉H₁₁Cl₃ NO₃ PS. M.W.: 350.57. Organic solvent: 79% w/w isooctane,43%w/w in methanol, readily soluble in other organic solvents (11).

Barley Grass

Barley grass green plant with 30cm length were collected from different areas of Sharkia Governorate.

Determination of the 96-hrs median lethal concentration (96-hrs LC₅₀) of Pestban insecticide

Fish were exposed to varying levels of the toxicant for 96hrs.First trail (0.1, 0.2 ,0.3, 0.6), second trail (0.06, 0.07, 0.08, 0.09) and Third trail (0.0, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg/l) of Pestban were freshly prepared and distributed in test glass aquaria; each one contains 10 fish (*O.niloticus*).Control fish were maintained simultaneously in separate aquaria without Pestban. All aquaria were kept for 96 hrs under the same conditions of aeration, temperature, PH and photoperiod as during acclimatization period .Mortality was assessed along the experimental period (17).

Barley grass powder

Barley grass was cut with 30cm length then slowly dried at Muffle Furnace without delay (within 60 minutes of being cut) at temperature 38°C to preserve all nutrients and to maintain the maximum potency of its vitamins, amino acids, enzymes, chlorophyll and other

ingredients contents. Then grind, using electric blender, resulting in a new generation micro-fine powder(13). Chemical analysis of barley grass powder,was estimated (14) (Table 1)

Table 1. Nutritional analysis of barley grass powder

Protien	Fat	Fiber	Ash
30%	7%	15%	16%

The basal control diet was formulated (15) from practical ingredients to satisfy all known nutrient requirements of Nile tilapia . Barley grass diet 10% or 20% barley grass powder experimental diets were formulated to meet the requirement of experimental fish (16).Both types of the experimental diets (control diet and barley grass diet) were prepared at the Fish Nutrition Department ,Central laboratory for Aquaculture Research.

Experimental design

Four hundred and fifty of *O. niloticus* fish were divided into four groups (Table 2).

At the end of expermental peiods (Table 2) tissue samples (brain ,gills,liver and muscle) for biochemical studies were taken at the end of first, fourth and eighth weeks and then were preserved at -20°C till analysis.

Laboratory analysis

Biochemical tests

Tissue samples (Brain, gills ,liver and muscle) were homogenized for 1.5 min in cold 0.25M pH 7.4 sucrose buffer(1:5, w/v) using a glass-teflon homogenizer (Heidolph S01 10R2RO) and then centrifuged at 9500 \times g for 30 min at Sorvall RC2B centrifuge. All processes were carried out at 4°C . Supernatants were used to determine brain cholineesterase (ChE) activity and the superoxide dismutase (SOD), malondialdehyde (MDA), glutathione reductase (GR) of gills , liver and muscle by using a spectrophotometer (Shimadzu UV-mini 1240).

Brain ChE : ChE kit was purchased from SPINREACT Kit . (AChE) activity was estimated in brain (17) and measured at 405

nm. SOD in gills, liver, muscle: SOD kit was purchased from Bio diagnostic Kit . SOD was estimated (18) and measured at 560 nm.MAD in gills, liver and muscle: MDA kit was purchased from Bio diagnostic Kit.The tissues

MDA concentration was determined (19,20) and measured at 534 nm.GR in Gills, liver, muscle : GR kit was purchased from Bio diagnostic Kit GR the enzyme was assayed and measured at 340 nm (21).

Table 2. Experimental Design

Groups	Sub groups	No. of fish	Treatment	Duration
I		90	Control (-ve) basal diet	1 , 4 and 8 weeks
II		90	Control (+ve) barley grass powder diet (10%, 20%)	1 , 4 and 8 weeks
III	a	30	1/10 96-hrs LC ₅₀ of pestban	1 week
	b	60	1/20 96-hrs LC ₅₀ of pestban	4 and 8 weeks
IV	a 1	30	1/10 96-hrs LC ₅₀ of pestban +10% barley grass powder diet	1 week
	a 2	30	1/10 of 96-hrs LC ₅₀ of pestban +20% barley grass powder diet	
	b1	60	1/20 96-hrs LC ₅₀ of pestban +10% barley grass powder diet	4 and 8 weeks
	b2	60	1/20 96-hrs LC ₅₀ of pestban +20% barley grass powder diet	

Statistical analysis

Statistical analysis was performed using the one way analysis of variance (ANOVA) of SPSS (22).

RESULTS AND DISCUSSION

The application of various pollutants like pesticides in the aquatic environment is known to cause several structural and functional changes in the biota. A major part of the world’s food is being supplied from fish source, so it is essential to secure the health of fishes (23). Recent years marked a major shift in human understanding of the role f food products in mantaining their proper health status (24).

In the present study calculated 96-hrs LC₅₀ of pestban to adults *O. niloticus* and it was 0.36 mg/l (Table 2) by the following equation:

$$96\text{-hrs LC}_{50} \text{ (mg/l)} = \frac{\text{the highest concentration} \sum (A \times B)}{N}$$

Where:

A=The difference between two successive doses.

B= The mean of dead fish in two successive groups.

N = Number of fishes in each aquarium.

Concerning the result of 96h/LC₅₀of pestban tabulated in Table 3 is not on the harmony with (25, 26, 27) *O. niloticus* sub adults (0.26mg/l) for lorsban,(1.57 µg/L) for CPF-methyl in larvae , (10µg/l) for CPF in fingerlings.This may be belonged to difference in fish life stagesin addittion to chemical formula of CPF respectively.

Table 3. Determination of the 96-hrs median lethal concentration (96-hrs LC₅₀) of Pestban insecticide.

Pestban conc.(mg/l)	No. of fish/group	No. of dead fish	A	B	A×B
0.00	10	0	0	0	0
0.07	10	0	0.07	0	0
0.08	10	0	0.01	0	0
0.09	10	1	0.01	0.5	0.005
0.1	10	3	0.01	2	0.02
0.2	10	4	0.1	3.5	0.35
0.3	10	5	0.1	4.5	0.45
0.4	10	6	0.1	5.5	0.55
0.5	10	6	0.1	6	0.6
0.6	10	10	0.1	8	0.8

Our result showed that brain cholinesterase of *O.niloticus* recorded significant decrease in both short-term exposure 1/10 LC₅₀ (0.034 mg/L) and long-term exposure 1/20 LC₅₀ (0.018 mg/L) of

pestban treated groups during four and eight weeks treatment, while there was no changed after addition of barely grass powder (10%,20%) treatment in both exposure time comparing with control groups (Figs1,2).

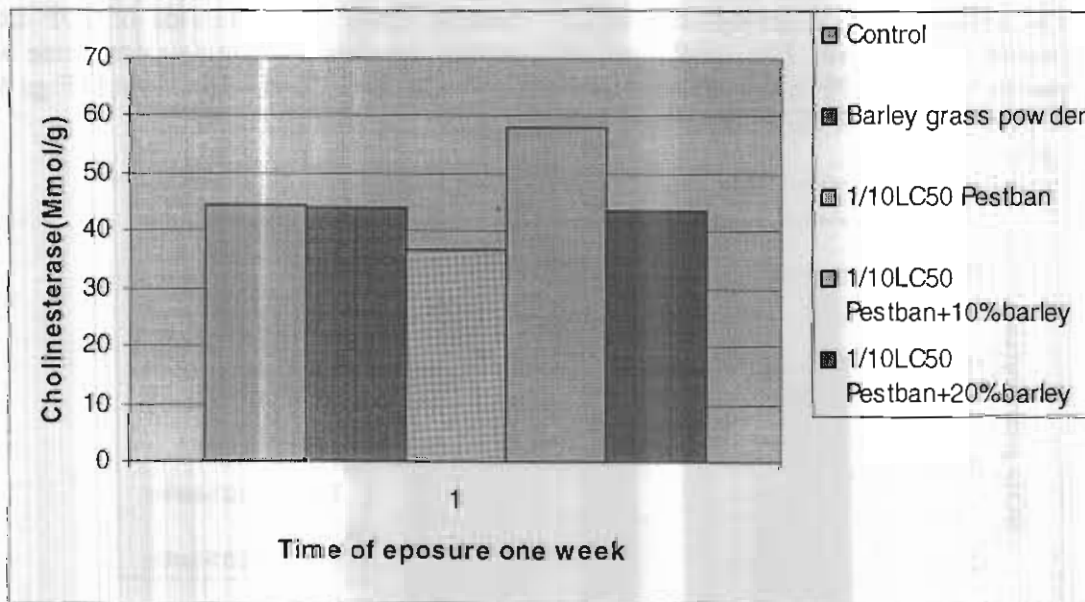


Fig. 1. Effects of short-term exposure 1/10 LC₅₀ of pestban on brain choline esterase (Mmol/g) of *Tilapia nilotica* for 1 week.

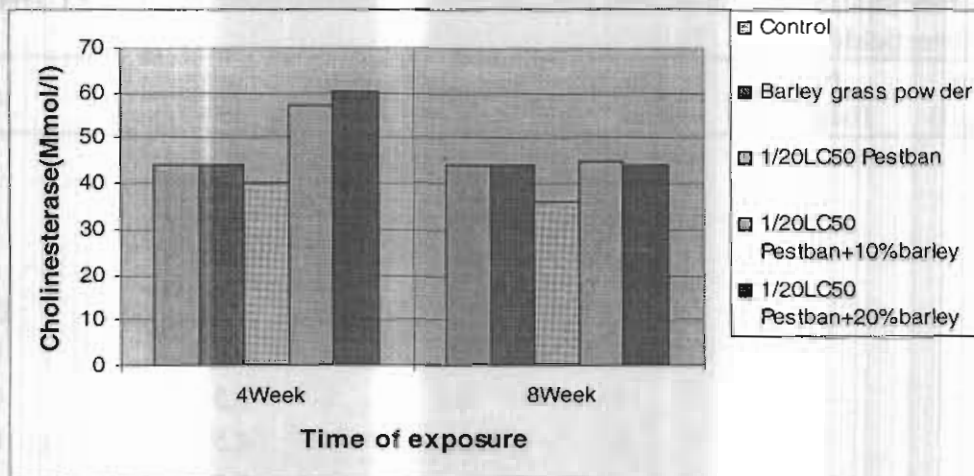


Fig. 2. Effects of long-term exposure 1/20 LC₅₀ of pestban on brain cholinesterase of *Tilapia nilotica* for 4 and 8 weeks.

The inhibition of brain AChE activity was similarly recorded (4, 27,28) during exposure to CPF in different fish species. Moreover it has been found that the inhibited brain AChE is greater than that in gill tissue euryhaline fish, (*O. mossambicus*) after exposure to CPF (29). Similarly in brain and muscle, AChE inhibited in common carp (*Cyprinus carpio L.*) after exposure to CPF(30). The inhibition of ChE is mainly due to CPF oxon which is the metabolic desulfuration of the compound in fish liver (31-33).

The present study, showed significant increased of SOD activity in gills, liver and muscle of *O. niloticus* treated with pestban during exposure for one week to (1/10 LC₅₀) and for four and eight weeks to (1/20 LC₅₀). Addition of barley grass powder had slight affect on SOD activity in gills, liver and muscle tissue at all periods of CPF exposure, while no changes in gills during one week of exposure comparing with control (Figs 3-5).

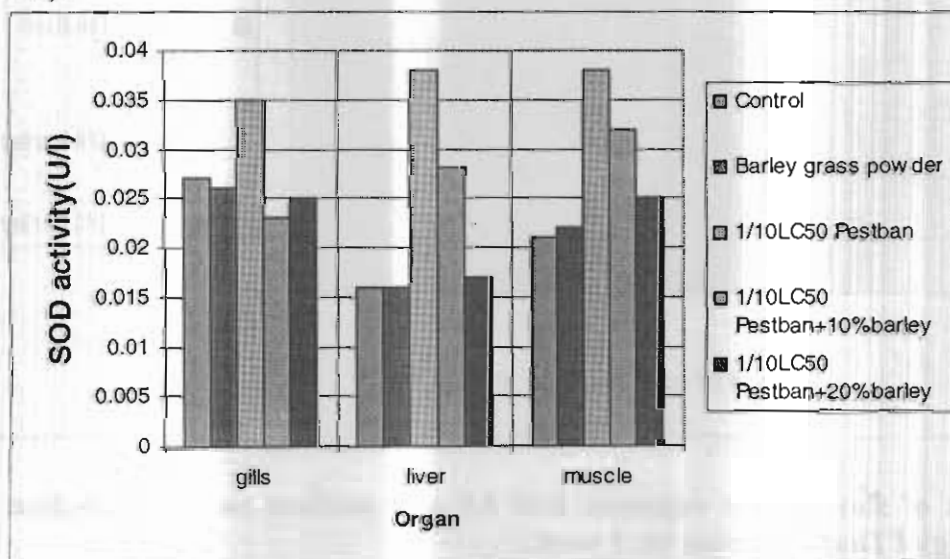


Fig. 3. Effects of exposure to 1/10 LC₅₀(0.036 mg/L) of pestban for one week on SOD activity in gills, liver and muscle of *Tilapia nilotica*.

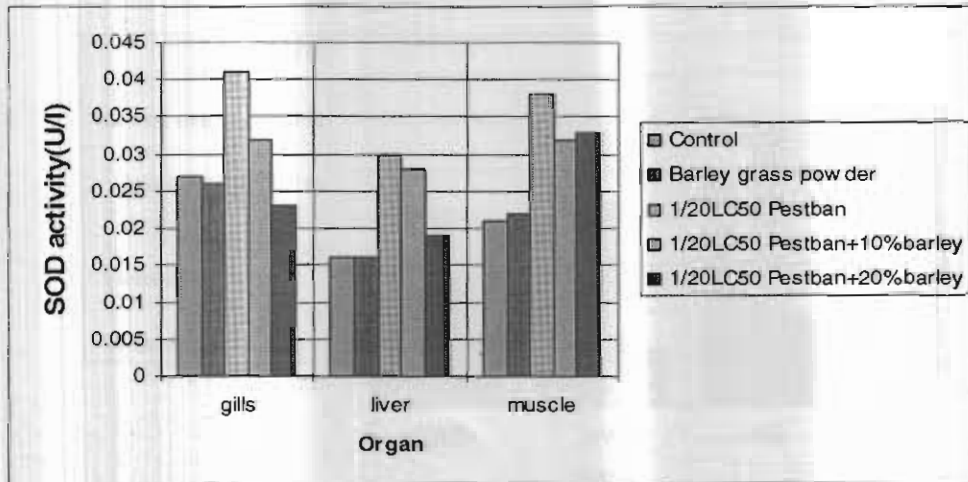


Fig. 4. Effects of exposure to 1/20 LC₅₀(0.018 mg/L) of pestban for 4 weeks on SOD activity in gills, liver and muscle of *Tilapia nilotica*.

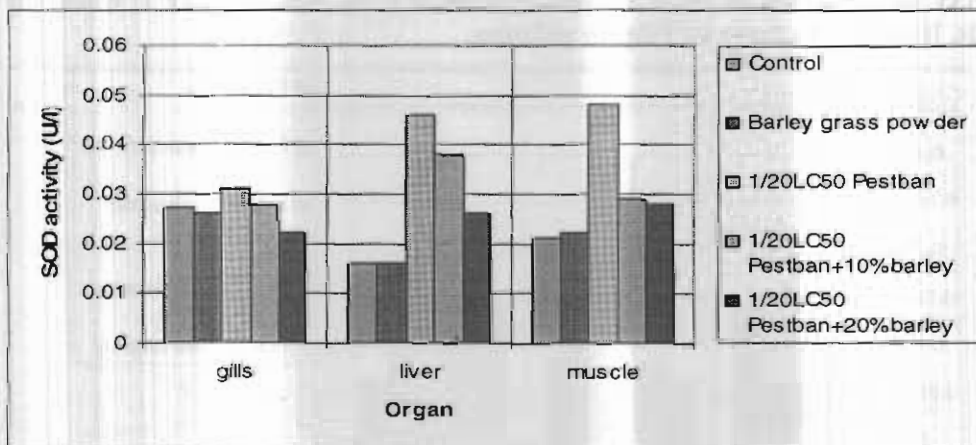


Fig. 5 . Effects of exposure to 1/20 LC₅₀(0.018 mg/L) of pestban for 8 weeks on SOD activity in gills, liver and muscle of *Tilapia nilotica*.

It has been reported that when the euryhaline fish, *Oreochromis mossambicus* was exposed to sub-lethal concentration (30 µg/L) of profenofos for 28 days GST and SOD activities showed transient increases in gill, viscera and muscle, but decreased in brain (34). An increased of SOD is attributed to increased ROS generation and alterations in antioxidants or free oxygen radicals scavenging enzyme systems in aquatic organisms due to pesticide toxicity (35). Also this increase indicates an increase in O₂⁻ production (36). The elevated levels of antioxidant enzymes demonstrate a pollutant-induced adaptive response in fish, and is an attempt to protect the body against the generated ROS (37,38). The apparent increase in SOD

activity in the fish may be due to the production of superoxide anions which led to the induction of SOD, to convert the superoxide radical to H₂O₂ (39). An increase in the activity of CAT and SOD is usually observed in the case of exposure to environmental pollutants since SOD-CAT system represents the first line of defense against oxidative stress(40).

Our result revealed that gills,liver,muscle MDA of *O. niloticus* increased significantly during both exposure to 1/10 or 1/20LC₅₀with pestban while slight improvement in barley grass powder treated groups was observed specially in muscle varied between increases, decreases and no changed comparing with control groups (Figs 6-8).

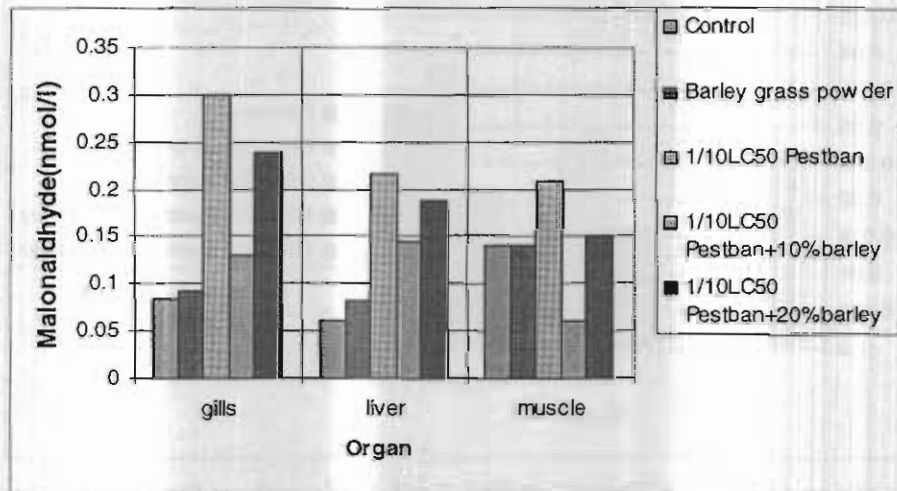


Fig. 6. Effects of exposure to 1/10 LC₅₀(0.036 mg/L) of pestban for 1 week on MDA in gills, liver and muscle of *Tilapia nilotica*.

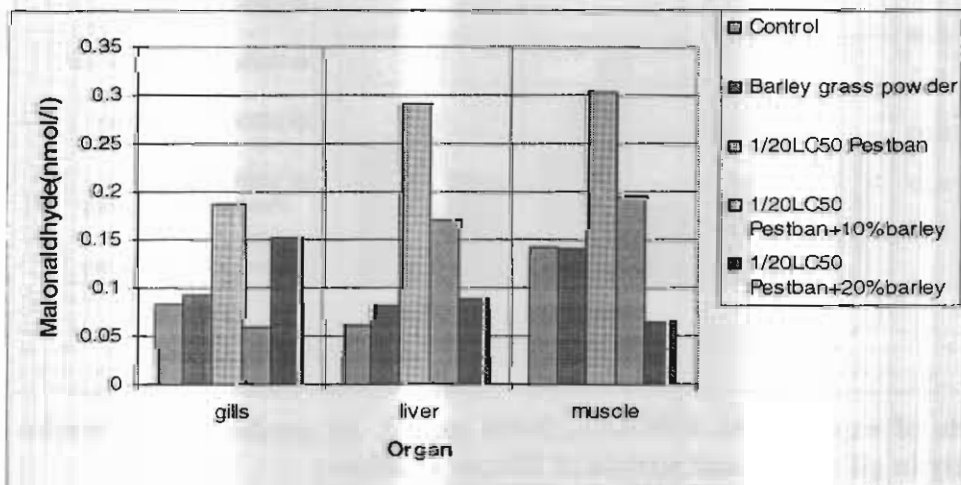


Fig 7. Effects of exposure to 1/20 LC₅₀ (0.018mg/L) of pestban for 4weeks on MDA in gills, liver and muscle of *Tilapia nilotica*.

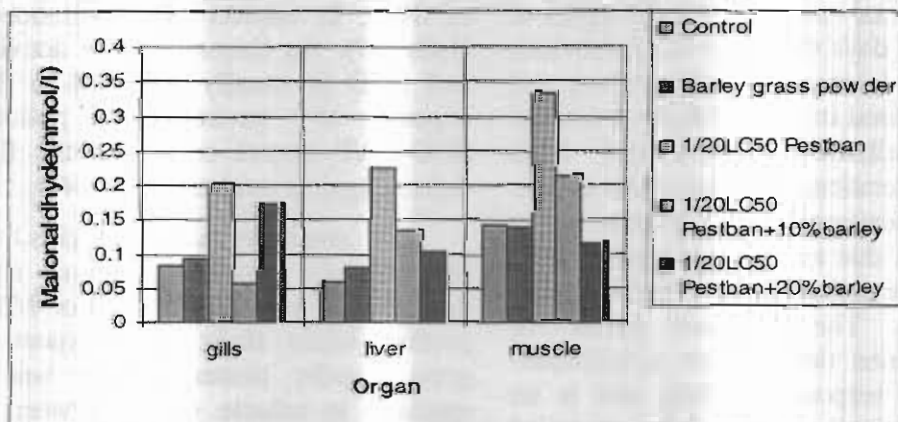


Fig. 8. Effects of exposure to 1/20 LC₅₀(0.018mg/L) of pestban for 8weeks on MDA in gills, liver and muscle of *Tilapia nilotica*.

Elevated levels of lipid peroxidation observed in the present study, was also recorded in mosquitofish, (*Gambusia affinis*) exposed to CPF (4) and on carp (*Cyprinus carpio*) and catfish (*Ictalurus nebulosus*) exposed to dichlorovos (6). An increased MDA is liberated as the end product of lipid peroxidation. Lipid peroxidation is a complex process resulting from free radical reactions in biological membranes, which are rich in polyunsaturated fatty acids. It forms lipid hydroperoxides which decompose double bonds of unsaturated fatty acids and destructs membrane lipids (41,42). It is known that OH⁻ can initiate lipid peroxidation in tissues (43).

Improvement MDA in fish treated with barley grass powder treated groups may be

due to 2''-O-GIV glycosylisovitexin Flavonoids which present only in young barley grass, which is an extremely effective as antioxidant and preventing free-radical oxidation of lipids found in the skin and blood, enhances the antioxidant actions of vitamin C and also helps in preventing the formation of the toxic compounds as malonaldehyde and acetaldehyde, which are known to denaturate proteins and DNA (44).

Our result revealed that gills, liver GR of *O. niloticus* during exposure to 1/10 or 1/20 LC₅₀ to pestban increased significantly while decrease in muscle specially at eight weeks and also in barley grass powder treated groups with little significant increase comparing with control (Figs 9-11).

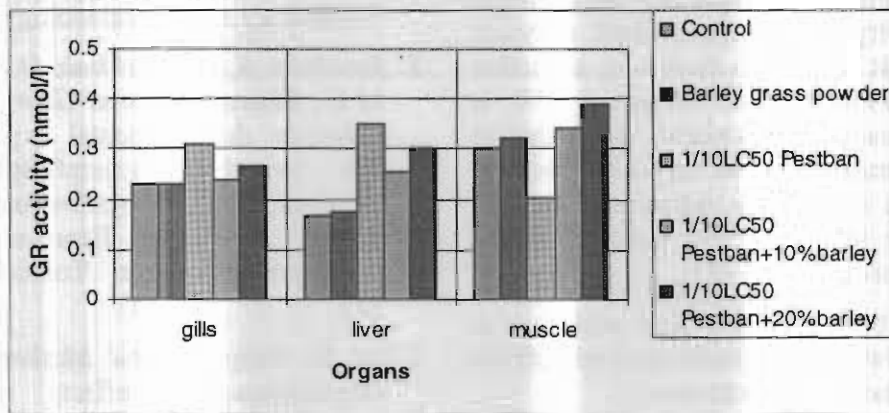


Fig. 9. Effects of exposure to 1/10 LC₅₀(0.036mg/L) of pestban for 1 week on GR activity in gills, liver and muscle of *Tilapia nilotica*.

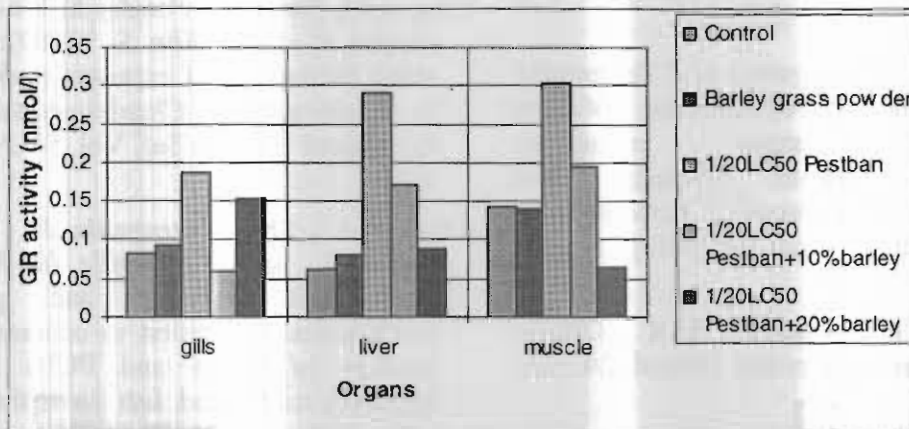


Fig. 10. Effects of exposure to 1/20 LC₅₀(0.018mg/L) of pestban for 4 weeks on GR activity in gills, liver and muscle of *Tilapia nilotica*.

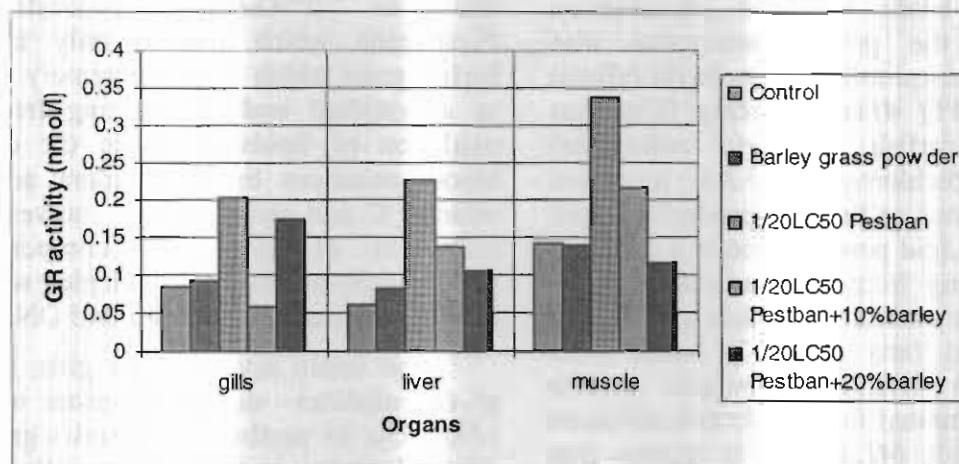


Fig. 11. Effects of exposure to 1/20 LC₅₀ (0.018mg/L) of pestban for 8 weeks on GR activity in gills, liver and muscle GR of *Tilapia nilotica*.

On the other hand mosquito fish, (*Gambusia affinis*) after exposure to 297mg/L CPF the activity of the antioxidant enzymes SOD, CAT, GR in viscera. However, induction in lipid peroxidation and increased GR is attributed to increased oxidative stress due to excessive generation of free radicals generated by CPF(4). The increase in antioxidant enzyme activities were related to the maintenance of lipoperoxide concentration (43).

It could be concluded that addition of barley grass powder can ameliorate toxic effects of pestban on the tested parameter.

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الملخص العربي

تأثير بودرة الشعير الأخضر على بعض التغيرات الكيميائية لاسماك البلطى النيلية
المعرضة لمبيد الحشرى البستبان

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البستيبان هو احدى المبيدات الفسفورية العضوية المستخدمة فى الزراعة والاستزراع السمكى ويسبب الاكسدة الناتجة عن تكوين بعض الايونات الحرة والتي تسبب فى ظهور الانزيمات المضادة للاكسدة. اوضحت الدراسة ان الجرعة النصف مميتة لمبيد البستبان خلال ٩٦ ساعة هي ٠,٣٦ ملجرام/لتر. تم دراسة دور بودرة الشعير الاخضر فى الحماية من التأثير السام لمبيد البستبان على اسماك البلطى النيلية (٥ ± ٤٥ جم و $٥,٥ \pm ١٢,٥$ سم) المعرضة ١٠/١ من الجرعة النصف مميتة لمدة اسبوع و ٢٠/١ من الجرعة النصف مميتة لمبيد البستبان لمدة ٤ و ٨ اسابيع وكذلك المضافة لها بودرة الشعير الاخضر مقارنة بالمجاميع الضابطة. تم دراسة تأثير المبيد و بودرة الشعير الاخضر على انزيم الكولين استيرز فى المخ وانزيم السوبر اوكسيد ديسميوتيز ومالونداالدهيد وانزيم الجلوتاثيون ريدكتيز فى الخياشيم والكبد والعضلات. اوضحت الدراسة ان الاسماك المعالجة ببودرة الشعير الاخضر لم يتغير مع انزيم الكولين استيرز فى المخ مع تحسن طفيف فى كلا من وانزيم السوبر اوكسيد ديسميوتيز و مالونداالدهيد وانزيم الجلوتاثيون ريدكتيز فى الخياشيم والكبد والعضلات. الاسماك المعرضة للمبيد فقط تبين نقص فى انزيم الكولين استيرز فى المخ مع زيادة ملحوظة فى انزيم السوبر اوكسيد ديسميوتيز ومالونداالدهيد وانزيم الجلوتاثيون ريدكتيز فى الخياشيم والكبد والعضلات .

لذلك نوصى باضافة بودرة الشعير الاخضر الى عليقة اسماك البلطى لما لة من دور ايجابى فى الحماية من تأثير

المبيد.