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Study on herbicide (thiobencarb) in *Clarias gariepinus* in Kafr El-Sheikh governorate, Egypt

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Abstract

This work was conducted in order to investigate the impact of herbicide thiobencarb on ***Clarias gariepinus (C. gariepinus)*** immunity. Lethal concentration 50 (LC_{50}) was detected, it was 160 µg.l^{-1}. Four groups were distributed in 12 glass aquaria 1st water had no addition 0% control, 2nd 5%, 3rd 10% and 4th 15% of thiobencarb LC_{50}. LC_{50} 96 hours was 160 µg.l^{-1}. the highest thiobencarb residue was detected in gills followed by liver while muscles had the lowest residues. Thiobencarb chronic toxicity had an impact on SR% and it was decreased in linear manner 86.7, 73.3, 63.3 and 46.67 respectively. Haemogram of ***C. gariepinus*** RBCs WBCs HB and PCV MCV was decreased and MCHC had the same trend of HB and PCV while thiobencarb had no effect on MCH. Liver enzyme AST and ALT showed significant increase parallel to increased thiobencarb concentration and TP had decreased while Cholesterol and creatinine had a significant increase. Antioxidant activities GPx and CAT of ***C. gariepinus*** showed a significant increase along with increased thiobencarb concentration. Immune of ***C. gariepinus*** was drastically impacted as showed in glass adhesion test and serum bactericidal activities test. It was concluded that thiobencarb had an adverse effect on ***C. gariepinus*** health and anti oxidant (GPx and CAT) could be used as bioindicator for toxicity.

Keywords: ***Clarias gariepinus***, thiobencarb, immunity, GPx, and CAT.

Introduction

Fish is considered a good bioindicators for ecosystem health **(Favaru *et al.*, 2001)** where it accumulates pesticides in the tissues causing different toxic responses. Herbicides have contributed by dramatic increase in crop yields and in the quantity and variety of the diet. Also in recent years, they help to limit the large quantities of pesticides have been produced and discharged into the environment. Herbicides, a distinctive group of pesticides, are considered as selective chemical weed killer; hence they have been intensively used to destroy the unwanted plants, especially in agricultural settings **(Dutta and Meijer, 2003)**. Herbicides have harmful effects since they can cause injury to human health as well as to the environment. The range of these adverse health effects includes acute and persistent injury to the nervous system, lung

damage and injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects, and cancer **(Mansour, 2004)**.

So this study was performed to impacts of thiobencarb of ***O.niloticus*** fish.

Materials and methods

1-Investigated herbicide: Thiobencarb a thiocarbamate herbicide purchased from local market commercial product 50%. Detection of herbicide residues was performed according to **AOAC (1990)**.

2-Tested fish: A total of 210 apparently healthy ***C. gariepinus*** fish were collected from private fish farms Kafr El-Sheikh Governorate in May 2015 and previously acclimated in indoor tanks in full glass aquaria measuring (80 X 40 X 40 cm) and maintained in aerated de-chlorinated fresh water at 25 ± 2 ^{o}C for 14 days. They seemed healthy and had a uniform size and weight with average body weight 90±3.4 gram.

3-Experiment design: firstly after acclimatization of ***C. gariepinus*** in laboratory condition two experiments were conducted.

a- Determined of LC50 96 hours 90 *C. gariepinus.* At the first, 90 ***O. niloticus*** were stocked in nine glass aquaria to estimate the LC_{50}. Thiobencarb was added to aquarium water in different concentrations (0, 20, 60, 80, 120, 140, 160, 180, 200 µg.l^{-1}) and mortalities were recorded in one week. Thiobencarb solutions were prepared by diluting of a stock solution with distal water. Each concentration contained ten fish with one replicate each. The concentration of each thiobencarb caused 50% mortality in fish for 96 h was taken as the LC_{50} value. During the toxicity test, the fish were not fed. The numbers of dead fish were counted daily and removed immediately from the aquaria.

b- Long term exposure last for 2 month (60 days) 120 of ***C. gariepinus*** fish were randomly distributed equally 30 per group in four groups, the 1st was the control no addition 0%, 2nd 5%, 3rd 10% and 4th 15% of LC_{50}

4-Survival rate (SR %):- SR%= (No. of fish at end / No. of fish at the start) ×100**.**

5-Blood sample collection and Heamatogram:- Red blood cell (**RBCs**) and White blood cell (**WBCs**) counts were counted by haemocytometer according to **Stoskopf (1993).** Blood film was prepared according to the method described by **Lucky (1977)**. Differential leukocytic count was calculated according to **Schalm (1986)**. Blood hemoglobin (**Hb**) was assessed by cyanometahemoglobin method (**Drubkin, 1964)**. In addition, **M.C.V.** Mean Corpuscular Volume, **M.C.H.** Mean Corpuscular hemoglobin and **M.C.H.C.** Mean Corpuscular hemoglobin concentration were calculated according to the formula mentioned by **Dacie and lewis (1975)**.

M.C.V. = (PCV / RBCs) x 10 as m/mm^3.

M.C.H. =(HB content gm/100ml/ RBCs) x 10 as m/mm^3.

M.C.H.C. =(HB content gm/100ml / PCV) x100 as %.

6-Liver enzymes, Total protein, Cholesterol and creatinine: Other blood samples for serum separation were collected without the addition of anticoagulants and then centrifuged at 3500 rpm for 10 min. The activity of liver enzyme Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) were determined colorimetrically according to **Reitman and Frankel (1957)**. Serum total protein was determined according to **Weich-sellbaum (1946)**. Serum total cholesterol was determined according to **Watson (1960)**. The activity of the serum creatinine was determined according to **Henry (1974).**

7- Neutrophils glass- adhesion assay and serum bactericidal activity:

Neutrophils glass- adherent, using nitroblue tetrazolium NBT assay, was determined according to **Anderson *et al.* (1992)**. Briefly, one drop of heparinized blood sample was placed onto cover slip. The cover slips were incubated for 30 minutes at room temperature (25 ^{o}C) in humid chambers to allow the neutrophils to stick to the glass. Cover slips were gently washed with phosphate buffer saline PBS (pH 7.4) and the cells were transferred to a microscope slide containing a 50 µl drop of 0.2% filtrated NBT solution (Fluka Buchs, Co. Switzerland). The positive, dark-blue stained cells were counted under the microscope.

Serum bactericidal activity according to **Kajita *et al.* (1990)**, Equal volumes (100µl) of serum and bacterial suspension 2x10^{8} (CFU) of ***Aeromonas hydrophila*** kindly obtained from fish diseases department animal Health Reaserch institute according to the Mc Farland scale were mixed and incubated for 1 h at 25 ^{o}C. Blank control was also prepared by replacing serum with sterile PBS. The mixture was then diluted with sterile PBS at a ratio 1:10. The serum-bacterial mixture (100µl) was plated in blood agar and plates were incubated for 24 h at 37 ^{o}C. The number of viable bacteria was determined by counting the colonies grown in nutrient agar plates.

8-Glutathione peroxidase GPx and catalaze CAT activities: GPx activity was assayed by the method **Pagila *et al.* (1967).** Catalase activity was determined according to **Xu *et al.* (1997).**

9-Statistical analysis: Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range **Duncan (1955)** was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SPSS program (**SPSS, 2004**).

Results and Discussion

This study was conducted to evaluate the impacts of thiobencarb on ***C. gariepinus*** health status. LC_{50} 96 hours was detected by exposure of ***C. gariepinus*** to serial thiobencarb concentration and it was 160 µg.l^{-1} these Data concerning the LC_{50} of thiobencarb agreed with those obtained by **Abdel-Halim and Massoud (2014)**

mentioned that LC_{50} of thiobencarb 96hrs was 185µg.l^{-1} on mosquito fish ***Gambusia affinis***. While our results were differed with those obtained by **Abbas *et al.*** (**2007)** as they recorded that the LC_{50} of thiobencarb for 96hrs was 720µg/l these differences maybe attributed by differences in fish species which was ***Oreochromis niloticus,*** body weight and experimental condition.

Data presented in table (1) and demonstrated in fig (1) concerning residues of thiobencarb in ***C. gariepinus*** tissues showed the highest residue was detected in gills followed by liver while muscles had the lowest residues. Also, from data presented it was noticed that residues level was parallel to thiobencarb concentration in water. The highest residues were in 4th group 17, 18 and 10 µg.g^{-1} in liver, gills and muscles respectively. Our results agreed with those obtained by **Stoliar and Lushchak (2012)** noticed that the residues of thiobencarb in ***C. gariepinus*** increased along with thiobencarb level in water. Increased influents of agricultural and industrial wastes in aquatic environment induce accumulation in tissues. the bioaccumulation of herbicides concentrations in fish was higher than other species **(Suter, 2007)**. Also, in agreement with our results **Abdel-Halim and Massoud (2014)** examined the impact of thiobencarb on mosquito fish ***Gambusia affinis*** under sublethal concentrations and the compounds revealed an accumulation reached 67-folds more than those of the surrounding environment.

From data presented in table (2) demonstrated in fig (2)it was obvious that exposure of 120 ***C. gariepinus*** to different thiobencarb concentration (1st water had no addition control 0%, 2nd 5%, 3rd 10% and 4th 15% of LC_{50}) had an impact on SR% and it was decreased in linear manner 86.7, 73.3, 63.3 and 46.67 respectively. Also, **Finlayson and Faggella (1986)** mentioned that acute toxicity tests with the rice herbicides molinate (Ordram (R)) and thiobencarb (Bolero (R)) and with thiobencarb-molinate mixtures on juvenile steelhead ***Salmo gairdneri***, chinook salmon ***Oncorhynchus tshawytscha***, channel catfish ***Ictalurus punctatus***, and striped bass ***Morone saxatilis*** produced median lethal concentrations (96-h LC_{50} values) indicating that thiobencarb (0.76-1.8 mg.l^{-1}) was 11 to 19 times more toxic than molinate (8.1-34 mg/L); thiobencarb-molinate mixtures at 1:1 LC_{50}-value ratios had additive toxic effects on steelhead, chinook salmon, and channel catfish.

In table (3) haemogram analysis of ***C. gariepinus*** exposed to different thiobencarb concentration showed that RBCs was decreased in 3rd and 4th groups 2.96 and 2.88 x10^{6} respectively while WBCs only the 4th group was effected 11.88x10^{3}. HB and PCV all groups were affected and decreased significantly as compared with control group. Also, MCV and MCHC had the same trend of HB and PCV while thiobencarb had no effect on MCH. Our findings agreed with **Oluah and Nwosu (2003)** stated that several studies

involving exposure of fish species to herbicides/pesticides indicated that exposed fish species showed poor health status demonstrated by adverse effects on measured haematological variables. Also, inagreement, **Gabriel and Sunday (2010)** mentioned that exposure to chronic of ***C. gaiepinus*** levels of Roundup (herbicide) caused a reduction in the values of PCV, HB, ESR, thrombocytes, WBC, reticulocytes, RBC, MCV and MCH relative to the control. haematological and biochemical changes may be attributed to the chemical structure of the tested herbicide thiobencarb that may its accumulation in the tissue because of its lipophilic nature **(El-Said and Radwan, 2004).** Also, **El-Deen and Rogers (1992)** demonstrated that, white catfish, ***Ictalurus catus*** and common carp sampled from field basins receiving molinate and thiobencarb had normal range of blood values probably due to the low levels of the herbicides compared to the level in their laboratory study. In agreement, **Mgbenka *et al.* (2003)** stated that ***C. albopunctatus*** exposed to gammalin 20 a herbicide fish had suffered from anaemic as the herbicide has great implications for the fish with respect to oxygen exchange and transport which may be greatly impaired.

Liver enzyme AST and ALT presented in table (4) demonstrated in fig (3) showed significant increase parallel to increased thiobencarb concentration and the highest values were in 4^{th} group 84.7 and 64 $u.l^{-1}$ respectively. TP had decreased in the 3^{rd} and 4^{th} groups 4.8 and 4.37 $g.dl^{-1}$ respectively. Cholesterol and creatinine had a significant increase in the 3^{rd} and 4^{th} groups 155, 196.7 and 1.2, 1.34 respectively. In agreement, **Ibrahim (2006)** mentioned that the effects of catfish ***Clarias lazera*** exposure to the herbicide thiobencarb in concentrations of (1.0 & 2.0 pp m) on liver and kidney functions Liver function tests:The exposure of ***C. lazera*** to both concentrations of thiobencarb resulted in a significant increase in serum levels of AST, ALT and ALP along the course of the experiment (14 days). Whereas the activities of these enzymes were decreased during the recovery period (7 days).2- Serum total proteins, albumin & globulins:Serum total proteins, albumin and globulins showed a marked increase during the entire period of exposure. Determination of enzyme activity in plasma or serum and tissues has proven to have diagnostic application in fish health studies **(Bouk *et aL,* 1978).** AST and ALT are normally found in low concentrations in blood; so if liver cells are damaged, they may leak them into the plasma causing an increase in catalytic activity **(Heath, 1987). Shalaby *et al.* (2005)** mentionrd that the increase of plasma creatinine and uric acid may be attributed to the action of thiobencarb herbicide on the glomerular tissues as well as deficiency of oxygen on the glomerular filtration rate which cause pathological changes in kidneys, due to the accumulation of herbicide in kidneys. Increasing levels of creatinine and uric acid above normal values indicate several disturbances in the kidney **(Maxine and Benjamin, 1985).**

In table (4) and fig (4) antioxidant activities of ***C. gariepinus*** showed a significant increase along with increased thiobencarb concentration. GPx 3rd and 4th groups were significantly increased 4.7 and 5.2 while catalase activities were significantly increased in all groups and the lowest was the 4th group 4.8comparing with control 1.02. Also, **Dickinson and Forman** (**2002**) mentioned that fish antioxidants enzymes and oxidative stress could be used as biomarkers for aquatic pollution, thus helping in the diagnosis of pollution as the role of thiobencarb as a stressor on some antioxidant enzymes such as CAT which have a minor role in H2O2 metabolism, where detoxifies it in biological systems and the activity was found to be lower than those of untreated fish. Also they added, GPx and GST activities play a central role in maintaining cellular redox status and protecting cells from oxidative injury. **Abdel-Halim and Massoud (2014)** GPx activity in the exposed animals to thiobencarb increased compared with control group. increased lipid peroxidation and significant changes in stress-responsive antioxidant enzymes (GST, CAT and GPx) were detected chronic poisoning of herbicide **(Nunes *et al.*, 2015).**

Immune of ***C. gariepinus*** was evaluated by conducting two tests namely glass adhesion test and serum bactericidal activities in table (5) and fig (5). Results in table (5) emphasis the impact of thiobencarb on ***C. gariepinus*** as glass adhesion test showed significantly linear decrease with increased thiobencarb concentrations and the lowest was the 4th 4.3 compared with control 8.33. Also, serum bactericidal activity had significantly decreased and the lowest was the 4th 32.3 compared with control 55. It was noticed that also there was no difference in WBCs between different groups except for the 4th there was significant immune suppression.

These results could be explained as changes in circulating WBC in fish are characteristic of exposure to culture stress and xenobiotics **(Ellsaesser Lohner *et al.* 2001).** Low activities of immune cells could be due to the failure of the bone marrow to produce the normal number of red cells, white cells and platelets **(Seiverd, 1983).**

From our results concerning haemogram, liver enzymes and antioxidant of ***C. gariepinus*** it was obvious the drastic impact of thiobencarb on health status and subsequently immune status (glass adhesion test and serum bactericidal activities). Also, there was a possibility of using GPx and CAT as biomarkers for thiobencarb toxicity. The fish should be under hygienic protocol to insure free from intoxication comply with Egyptian hygienic standard.

Table (1): Residues of thiobencarb in *C. gariepinus* tissues exposed to different concentrations (µg.g^{-1}).

Item	**1st** Control	**2nd** 5%=8 µg.l^{-1}	**3rd** 10%=16 µg.l^{-1}	**4th** 15%=24 µg.l^{-1}
Liver	0	4.5	12	17
Gills	0	8	14	18
Muscles	0	3	9	10

Table (2): Survival rate of *C. gariepinus* exposed to different thiobencarb concentrations.

Item	**1st** Control	**2nd** 5%=8 µg.l^{-1}	**3rd** 10%=16 µg.l^{-1}	**4th** 15%=24 µg.l^{-1}
NO	30	30	30	30
SR	8.67^{a}	7.33^{b}	6.33^{b}	4.67^{c}
SR%	86.7	73.3	63.3	46.67
MR	1.33	2.67	3.67	5.33
MR%	13.3	26.7	36.7	53.33

NO= number of fish and SR= Survival Rate. Each reading represents mean±SD of and different letter indicate significant change.

Table (3): Heamogram of *C. gariepinus* exposed to different thiobencarb concentrations.

Item	**1st** Control	**2nd** 5%=8 µg.l^{-1}	**3rd** 10%=16 µg.l^{-1}	**4th** 15%=24 µg.l^{-1}
RBCs x10^{6}	3.43^{a} ±0.12	3.23^{a} ±	2.96^{b} ±0.03	2.88^{b} ±0.05
WBCs x10^{3}	14.7^{a} ±0.3	14.3^{a} ±0.3	13.7^{a} ±0.15	11.88^{b} ±0.4
HB g.dl^{-1}	10.6^{a} ±0.4	9.7^{b} ±0.2	8.87^{c} ±0.09	8.3^{c} ±0.15
PCV%	36^{a} ±1.2	31.57^{b} ±0.6	27.9^{c} ±0.5	25.2^{d} ±0.5
MCV fl	104.9^{a} ±0.2	97.6^{b} ±0.08	94.35^{c} ±0.99	87.6^{d} ±0.03
MCH pg	31^{a} ±0.001	31^{a} ±0.001	30^{a} ±0.001	29^{a} ±0.01
MCHC g.dl^{-1}	29.5^{d} ±0.06	30.7^{c} ±0.03	31.8^{b} ±0.34	33.1^{a} ±0.01

Each reading represents mean±SD of and different letter indicate significant change.

Table (4): liver enzyme, cholesterol, creatinine and antioxidant activities of *C. gariepinus* exposed to different thiobencarb concentrations.

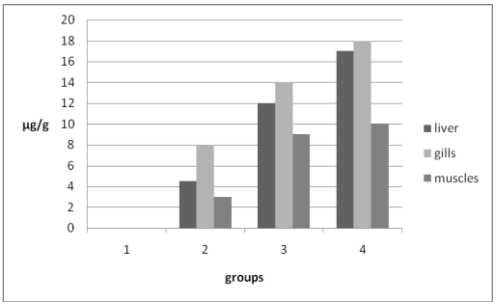
Item	**1st** Control	**2nd** 5%=8 µg.l^{-1}	**3rd** 10%=16 µg.l^{-1}	**4th** 15%=24 µg.l^{-1}
AST u.l^{-1}	27.67c ±0.88	33.7b ±1.9	52.3a ±1.4	84.7a ±2.9
ALT u.l^{-1}	16d ±0.6	21.7c ±1.2	42.3b ±1.45	64a ±2.3
TP g.dl^{-1}	5.23a ±0.03	5.1a ±0.06	4.8b ±0.03	4.37c ±0.09
Cholestrol mg.dl^{-1}	132.3c ±1.45	138.3c ±1.2	155b ±2.9	196.7a ±8.8
Creatinine mg.dl^{-1}	0.92c ±0.01	1.1bc ±0.06	1.2b ±0.06	1.34a ±0.03
GPx µmol	2.7c ±0.2	3.7b ±0.1	4.7a ±0.2	5.2a ±0.09
Catalase k/gHb	1.02d ±0.14	2.27c ±0.07	3.6b ±0.26	4.8a ±0.09

Each reading represents mean±SD and different letter indicate significant change.

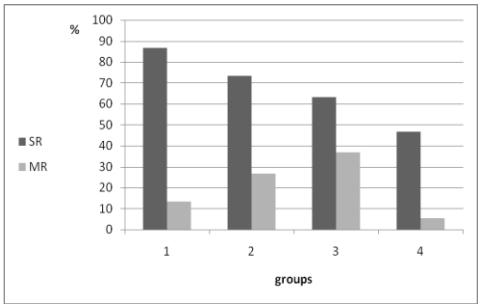
Table (5): immune items of *C. gariepinus* exposed to different thiobencarb concentrations.

Item	**1st** Control	**2nd** 5%=8 µg.l^{-1}	**3rd** 10%=16 µg.l^{-1}	**4th** 15%=24 µg.l^{-1}
Glass adhesion test	8.33^{a} ±0.3	6.7^{b} ±0.7	5.3^{bc} ±0.3	4.3^{c} ±0.3
Serum bactericidal Activity %	55^{a} ±2.9	47.7^{b} ±1.5	40^{c} ±1.2	32.3^{d} ±1.45

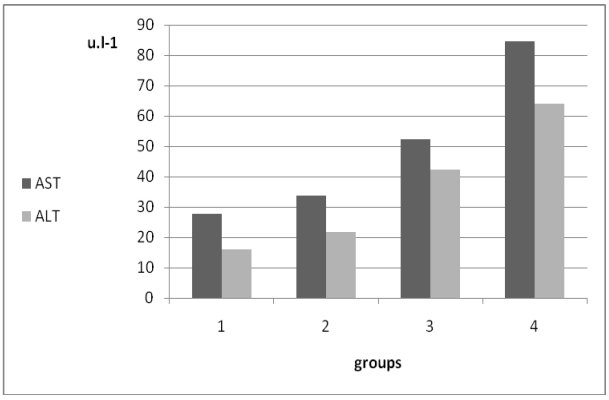
Each reading represents mean±SD of 3 fish and different letter indicate significant change



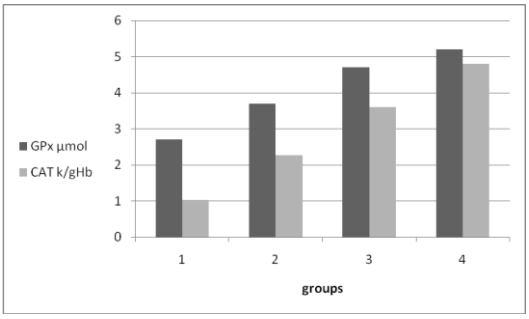
fig(1)



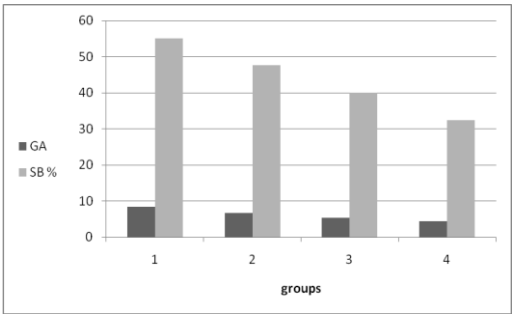
fig(2)



Fig(3)



fig(4)



fig(5)

Legend of figures:

fig(1) : Residues of thiobencarb in ***C. gariepinus*** exposed to different concentrations.

fig(2) : Survival rate of ***C. gariepinus*** exposed to different thiobencarb concentrations.

fig(3) : AsT and ALT ***C. gariepinus*** exposed to different thiobencarb concentrations.

fig(4): Antioxidant activities of ***C. gariepinus*** exposed to different thiobencarb concentrations

fig(5) : Immune items of ***C. gariepinus*** exposed to different thiobencarb concentrations

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