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Comparative toxicity of thiobencarb and penoxulam in Clarias gariepinus with referring to residues and public health hazards

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Abstract

The present experiment was conducted to study the impact of herbicides thiobencarb and penoxsulam on catfish ***(clarias gariepinus c. gariepinus)***. Four groups were distributed in 12 glass aquaria, G1 water had no herbicide (control), G2 exposed to thiobencarb by dose 1mg/L for 12 days, G3 exposed to penoxsulam by dose 10mg/L for 12 days and G4 exposed to half dose of both herbicides (5mg/L penoxsulam+ 0,5mg/L thiobencarb) for 12 days. Hematological and biochemical parameters were significantly changed. Glutathione peroxidase enzyme (GSH.Px) was significantly increased in all groups compared with group1 (control negative) on 5 and 10 days after exposure to pesticides and on 5 days after stop the exposure to pesticides and there was non significant increase on 10 and 20 days after stop the exposure to pesticides. All hematological parameters: hemoglobin (Hb), Red blood cells (RBCs), Packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and white blood cell (WBCs) showed significant decrease; as well as total protein compared with group1 (control negative) on 5 and 10 days after exposure to pesticides and on 5 days after Stop the exposure to pesticides. Biochemical parameters: ALT,AST, uric acid and creatinine showed significant increase in all groups compared with group1 (control negative) on 5, 10 days after exposure to pesticides and on 5 days after Stop the exposure to pesticides and there was non significant increase on 10 and 20 days after Stop the exposure to pesticides. It was concluded that thiobencarb and penoxsulam had an adverse effect on ***C. gariepinus*** health and antioxidant (GPx) could be used as bioindicator for toxicity. There were increases in residues of both thiobencarb and penoxulam in fish muscles during exposure period as thiobencarb has the greatest cumulative character than penoxulam in examined muscle samples following exposure for 10 days. After 10 days of stopping exposure penoxulam residues became lower than MRL (Maximam residual level) while thiobencarb residues still higher than MRL. Penoxulam residues after heat treatment by boiling for 30 min. (cooking) could not be detected in all examined samples while in case of thiobencarb residues, it markedly decreased by reduction percent of 88%.

Keywords: *Thiobencarb, penoxulam, clarias gariepinus, residues, heat treatment.*

Introduction

Enviromental pollution caused by pesticides, especially in aquatic ecosystems, has become a serious problem. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish **(Velisek *et al.,* 2011)**. Aquatic toxicity is an important parameter in evaluating the potential adverse environmental impact of synthetic chemicals in

water discharges. The presence of toxic substances in receiving waters and water treatment plants is a major environmental concern. Traditional acute toxicity measurements are time consuming, requiring lengthy exposure times as well as requiring an abundance of equipment for exposing the organisms to several concentrations of the tested toxicant **(Indorato *et al.*, 1984)**. 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 contributed by dramatic increase in crop yields and in the quantity and variety of the diet. Also, they have helped to limit the spread of certain diseases but they 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 nervous system, lung damage, injury to the reproductive organs, dysfunction of the immune and endocrine systems, birth defects and cancer **(Mansour, 2004).**

Thiobencarb is being widely used to control argulus disease and to eradicate the larvae of mosquitoes also, extensively used in rice fields to control weeds. Contamination of water bodies adjacent to rice fields by thiobencarb, mainly through run off, is quite possible. Thus, it can be toxic to aquatic organisms. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fish management and disease **(Adhikari *et al,* 2004)**. The suspected accumulation of herbicides residues in the fish flesh has a public health hazards for the human consumers **(Aly *et al.*, 2008).**

The herbicide penoxsulam belongs to the group of triazolopyrimidine sulphonamides, acting by the inhibition of acetolactate synthetase, an enzyme that is responsible for the formation of branch- chained aminoacids (valine, leucine, isoleucine). These aminoacids are necessary for the synthesis of new plant tissues **(Koschnick *et al.*, 2007)**. This product has a broad spectrum of weed control and a low octanol/water partition coefficient, indicating that it is not likely to bioaccumulate in organs or tissues. According to residual soil activity, it has become one of the most widely used herbicides in rice crops around the world **(Jabusch and Tjeerdema 2005)**. Due to the widespread use of penoxsulam, it is important to evaluate its possible adverse effects on aquatic life. Environmental pollutants can cause oxidative damage in fish and other aquatic organisms by leading to the increased production of reactive oxygen species (ROS), increasing lipid peroxidation and/or protein oxidation **(Van der Oost *et al.* 2003)**. It is known that some herbicides, including penoxsulam, inhibit AChE activity in fish **(Cattaneo *et al.* 2011)**, even though the inhibition of this enzyme is a primary toxic response for carbamate and organohosphate insecticides. In fish species, the hydrolysis of acetylcholine in the synaptic cleft is very important to the neuromuscular junction and general neurotransmission. Altered AChE activity causes disturbances of functions such as feeding, equilibrium, movement and reproductive behavior **(Saglio and Trijasse, 1998)**.

Fish and fish products are considered as the cheapest important feedstuffs of high protein quality, minerals and vitamins contents. The method of culturing fish in rice fields provides an additional income as well as food for farmers and reduces the risk of rice crop failure **(Huat and Tan, 1980).**

Fish consumption could be considered as one of the major sources of human toxicity because fish exposed to all environmental contaminants **(Storelli, 2008).**

In this respect, the present study aimed to study the impacts of thiobencarb and penoxsulam on *clarias gariepinus (c. gariepinus)* fish.

Materials and Methods

1- Investigated herbicide: Thiobencarb (Saturn) herbicide purchased from local market

commercial product 50% and used in a dose of 1mg/liter for 12 days .Detection of herbicide residues was performed according to **AOAC (1990)**. The herbicide penoxsulam belongs to the group of triazolopyrimidine sulphonamides purchased from local market and used in a dose of 10mg/liter for 12 days.

2- Tested fish: A total of 200 apparently healthy ***C. gariepinus*** fish were collected from private fish farms in El-Sharkia Governorate 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°C for 14 days. They seemed healthy and had a uniform size and weight with average body weight 200-225 gram.

3- Experiment design: Two hundred (200) apparently healthy ***C. gariepinus*** fish were divided into four equal groups after 2 weeks.

- Group 1(G1): provided with basal diet without treatment (control).
- Group 2(G2): provided with basal diet and exposed to thiobencarb by dose (1/10 of LC_{50}) 1mg/L for 12 days. **(EPA, 2008)**
- Group 3(G3): provided with basal diet and exposed to penoxsulam by dose (1/10 of LC_{50}) 10mg/L for 12 days **(EPA, 2006)**
- Group 4(G4): fed the basal diet and exposed to half dose of both herbicides (5mg/ L penoxsulam+ 0,5mg/L thiobencarb) for 12 days **(Joshua et al, 2011).**

4- 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)**. 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/mm3.

M.C.H. = (HB content gm/100ml/ RBCs) x 10 as m/mm3.

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

5- Liver enzymes, Total protein 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)**. The activity of the serum creatinine was determined according to **Henry (1974).**

6- Glutathione peroxidase GSH.Px: GPx activity was assayed by the method **Miller** and **Slebodzinska (1993).**

7-Sample collection for residues content:

After 5, 10 days of exposure and 5, 10, 20 days post stop exposure following clearance test 5 fish from each herbicide groups were collected to determine the residues in muscle.

5 muscle samples of each group were subjected to heat treatment (boiling for 30 min.) to determine the effect of high temperature on the herbicide stability.

8- HPLC:

The extraction procedure was performed according to **AOAC (1990)**, dealt with pesticide extracts that were evaporated at 30°C to dryness. After clean up and dissolution in 1 ml methanol they were directed to HPLC analysis. These extracts were chromatographed with UV spectrophotometer detector and C18 stem less

column 25 mm.

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

The inappropriate handling of pesticides may lead to contamination of aquatic systems, causing adverse effects to various organisms that inhabit these resources, including populations of fish **(Jiraungkoorskul *et al.*, 2002)**. The results of hematological parameters presented in tables (1, 2, 3) showed that ***C. gariepinus*** exposed to thiobencarb and penoxsulam showed significant decrease in RBCs, HB, WBCs, PCV, MCV, MCH and MCHC. Our findings agreed with **Oluah and Nwosu (2003)** who stated that several studies involving exposure of fish species to herbicides indicated that exposed fish species showed poor health status demonstrated by adverse effects on measured. Haematological variables. haematological changes may be attributed to the chemical structure of the tested herbicide thiobencarb and its accumulation in the tissue due to its lipophilic nature **(El-Said and Radwan, 2004).** Also **Sherif *et al.* (2015)** mentioned that ***C. gariepinus*** exposed to thiobencarb showed decrease in RBCs, HB, WBCs, PCV, MCV and MCHC. The present findings also agreed with **EPA (2004)** who stated that rats exposed to penoxsulam showed decrease in erythrocyte parameters (erythrocyte count, hemoglobin and hematocrit).

Determination of enzyme activity in plasma or serum and tissues has proven to have diagnostic application in fish health studies **(Bouk *et aL*, 1978)**. Many pollutants have been shown to act specifically by inhibiting certain enzymes, thus interfering with metabolic processes in development **(Weis *et al.*, 1981).**

Marie (1994) stated that transamination represents one of the principal pathways for the synthesis and deamination of amino acids. Thereby, allowing an interplay between carbohydrate, fat and protein metabolism during fluctuating energy demands of the organism in various adaptive relations. Therefore, attention has been focused on the changes in the amino transferees, (AST) and (ALT) which promotes gluconeogensis from amino acids and relates changes in their activities to the liver condition **(Marie, 1994)**. AST and ALT are normally found in low concentrations in blood; so if liver cells are damaged, they may leak into the plasma causing an increase in catalytic activity **(Heath, 1987)**. In the present experiment fish that exposed to thiobencarb and penoxsulam showed a significant elevation in AST and ALT activity (Tables 6,7,8) .An increase of AST and ALT activities in serum is a sensitive indicator of even minor cellular damage **(Palanivelu *et al.*, 2005)** and indicates stress-based tissue impairment. Our findings in agreement with **Sherif *et al.* (2015)** they mentioned that exposure of ***C. lazera*** to thiobencarb resulted in a significant increase in serum levels of AST and ALT whereas the activities of these enzymes were decreased during the recovery period. **Ibrahim (2006)** observed significant increase AST and ALT activities in fish exposed to thiobencarb.

This fluctuation in AST and ALT activities could be attributed to a number of factors such as leakage from liver and muscle into the blood, liver enzyme inhibition by the effect of pollutant, and/or disturbances in kreb's cycle **(Salah El-Deen, 1991).** The present result was in agreement with the results of **Jee *et al.* (2005)** in fish *Sebastes schlegeli*, and **Borges *et al.* (2007)** in fish Rhamdia quelen who observed increased activity of serum ALT, AST and LDH in fish, exposed to pesticides.

In the present study Serum total proteins showed a marked decrease during the entire

period of exposure. Our result is in agreement with results observed by **(Sherif *et al.*, 2015)** Creatinine is a waste product largely from the muscle breakdown. Increasing levels of creatinine and uric acid above normal values are considered as kidney dysfunction indicators **(Khaled *et al.*, 2015).** Plasma creatinine and uric acid can also be used as a rough index of the glomerular filtration rate (GFR) and kidney dysfunction **(Hernandez and Coulson, 1967)**. Increasing levels of creatinine and uric acid above normal values indicate several disturbances in the kidney **(Maxine & Benjamin, 1985).**

In the present study, ***C. gariepinus*** exposed to thiobencarb and penoxsulam showed significant increase in uric acid and creatinine on 5, 10 days after exposure to herbicides and on 5 days after Stop the exposure to pesticides .Our results agree with that reported by **Sherif *et al.* (2015)** mentioned that fish exposed to thiobencarb showed significant increase in plasma creatinine level., also **Shalaby *et al.* (2005)** stated that the increase of plasma creatinine and uric acid may be attributed to the action of thiobencarb 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. Increasing levels of creatinine and uric acid above normal values indicate several disturbances in the kidney **(Jacobs *et al.*, 2002).** The significant rise in the serum creatinine level might be due to the impairment of glomerular function, tubular damage in the kidney **(Mansour and Mossa, 2010)**. Our results agree with that reported by **EPA (2004)** stated that penoxsulam causes Renal tubular degeneration in male rats and this manifested by increase blood urea, reduced urine osmolality and increased urine volume were noted as secondary effects, these findings may be attributed to limited solubility of penoxsulam and its metabolites in urine which lead to formation of crystals in the kidney and lumen of urinary bladder.

In tables (6, 7, 8) antioxidant activities (GSH-Px) of ***C. gariepinus*** showed a significant increase due to exposure to thiobencarb and penoxsulam. Our results in agreement with **Dickinson and Forman (2002)** reported that fish antioxidants enzymes and oxidative stress could be used as biomarkers for aquatic pollution, thus helping in the diagnosis of pollution. Also they added that GSH.PX activities play a central role in maintaining cellular redox status and protecting cells from oxidative injury. Furthermore, **Monteiro *et al.* (2006); Üner *et al.* (2006)** stated that many pesticides are known to cause oxidative stress in aquatic organisms since these contaminants may induce the formation of reactive oxygen species (ROS) and alterations in the antioxidant system. ROS are produced during normal cellular functioning. Fish, as all aerobic organisms, are susceptible to the attack of ROS, and their cells have an efficient antioxidant defense system **(Trenzado *et al.*, 2006)**. In normal conditions, a balance exists between the formation and elimination of ROS. However, if there is an imbalance in the formation of ROS or a deficiency in the antioxidant defense, cell oxidative stress occurs.

Our results in agreement with that of **Abdel-Halim and Massoud (2014)** stated that the activity of GSH.PX in the exposed fish to thiobencarb increased compared with control group. **Nunes *et al.*, (2015)** stated that changes in antioxidant enzyme (GSH.PX) were detected in chronic poisoning of herbicide. Also, **Sherif *et al.*, (2015)** reported that ***C. gariepinus*** showed a significant increase in GSH.PX activity along the experimental period with increased thiobencarb concentration. Moreover, **Moraes *et al.* (2009)** stated that fish exposed to penoxsulam showed changes in the activity of antioxidant enzymes and this may be attributed to damage of antioxidant and detoxificant systems in liver.

The obtained data of residual analysis in table (11) showed that thiobencarb has the greatest cumulative character than penoxulam in exam-

ined muscle samples following exposure for 10 days. The level of residues depending on the concentration of herbicide in water, the fat content of the tissues, dose, period of exposure and the degree of damage in tissues caused by the direct toxic effect **(Aly *et al.*, 2008)**
This accumulation of herbicides in fish may be 10 to 10,000 times greater than their original concentrations in water **(Kannan *et al.* 1997)**.

The mean values of penoxulam residues increased gradually from 0.6588 to 0.8356 ppm for 5 and 10 days exposure, respectively; while, during clearance period the residues were decreased to reach below the MRL 0.01 ppm **EPA(2007)** after 10 days and could not be detected in the examined samples after 20 days. These results are in agreement with those recorded by **EPA (2004)** and **(Sondiha, 2014).**

The prolonged exposure to sub lethal concentrations of herbicides led to increase in the accumulation of residues. The residues are accumulated in different tissues, causing toxicity to the fishes which ultimately resulted in biomagnifications through the food chain **(Tilak, 2007)**.

There is marked increase in mean values of thiobencarb residues in fish muscles from 0.753ppm in the day 5 of application to 1.826 ppm in the day 10.These results are similar to those by **(Abd El-Azzim, 2001** and **Marzouk *et al.*, 2006**) who stated that the tissue structural damages caused by long period of exposure lead to accumulation of Saturn (thiobencarb) in the gills, brain, skin, muscles and ovaries. These results 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 & Radwan, 2004)**.

Thiobencarb residues started to decrease with the herbicide withdrawal but it was still higher than MRL in the 10 th day and declined to below the MRL0.02ppm **(EPA, 2001)** after 20 days of using free herbicide water. These results are in agreement with that reported by **Aly *et al.* (2008)** and may be attributed to the ability of fish to eliminate thiobencarb residues from their tissues by an enzyme related detoxification in liver.

The small amounts of herbicides that remain in the food supply will cause no immediate reaction but could cause health problems if routinely consumed over a long period. Residues of pesticides and herbicides affect the central nervous system, respiratory and gastro intestinal system of human beings. **(Kumari *et al.*, 2014)**

The results tabulated in table (12) revealed that penoxulam residues after heat treatment by boiling for 30 min.(cooking) could not be detected in all examined samples while in case of thiobencarb residues, it markedly decreased by reduction percent of 88%. These results are in correspondence to that mentioned by **Bajwa and Sandhu (2014)** who stated that processing such as washing , heating and boiling caused marked reduction in herbicides, resulting in complete removal of residues in some cases. Some residual herbicides passed into cooking water from the food materials according to their water solubility.

Foods are invariably subjected to heat treatment during preparation and preservation. The heat treatment is given in many ways including pasteurization, boiling, cooking etc. The loss of herbicides residues during heat processing may be due to evaporation, co-distillation and thermal degradation which vary with the chemical nature of the individual herbicides **(Sharma *et al.* 2005)**.

Conclusion

It could be concluded that thiobencarb and pe-

noxulam herbicides have harmful effects on the physiology and biochemistry structure of fish which in turn leads to deterioration of meat quality of the exposed fish to the point that it can be hazardous to humans at certain levels in water.

Herbicides residues in fish muscle after recommended use for control of weeds are relatively high initially, and residues are often reduced and become not detectable after 21 days of maintaining in free herbicides water.

The small amounts of herbicides that remain in the food supply will cause no immediate reaction but could cause health problems if routinely consumed over a long period.

It is clear that herbicides residues remain in fish muscle, as a result of herbicides application are affected by washing, preparatory steps, heating or cooking, processing during product manufacturing .The extent of reduction varies with nature of pesticide molecule

Recommendations

It can be recommended that fresh water should be maintained, after application of the selected herbicides, for at least 21 days for the two herbicides. This time, is very important to release the herbicide residues in fish before marketing to avoid the susp-ected hazards on public health.

There is urgent need to monitor the herbicides residues to standardize the application doses. Equally important is to develop or find new pesticide molecules with high effectiveness and fast degrading capabilities. All concerted efforts should be made to ensure food safety as it has a direct bearing on human health.

Table (1). Effects of thiobencarb and penoxsulam on some heamatological parameters of fish 5 days post exposure to herbicides. (n= 5)

Group	HB (g/dl)	RBCs×10^6mm^3	PCV%	MCH (pg)	MCHC%	MCV (fl)	WBCs× 10^3mm^3
G1	9.2±0.29 a	2.54±0.27 a	29.52±0.20 a	36.2±0.86a	34.4±0.66a	117.16±2.04a	12.45±0.10a
G2	7.6±0.40 ab	2.19±0.11 b	23.6±0.48 b	33.5±0.83b	31.5±0.77b	106.8±2.5b	10.94±0.33b
G3	7.2±0.43 ab	2.12±0.08 bc	22.6±0.84 bc	33.1±0.75bc	30.7±1.5b	105.7±1.9b	10.85±0.10bc
G4	6.8±0.59 b	2.10±0.12 c	21.7±0.40 c	32.1±0.93c	30.4±0.38b	104.2±2.8b	10.27±0.05c

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (2). Effects of thiobencarb and penoxsulam on some heamatological parameters of fish 10 days post exposure to herbicides. (n= 5).

Group	HB (g/dl)	RBCs×10^6m m_3	PCV%	MCH (pg)	MCHC%	MCV (fl)	WBCs× 10^3mm^3
G1	9.13±0.34a	2.45±0.12a	26.8±0.76a	38.4±0.73 a	35.1±0.84 a	112.7±0.92 a	12.68±0.15 a
G2	7.3±0.63b	2.12±0.08b	21.9±0.49b	33.8±1.5 b	31.6±1.3 b	103.4±3.4 b	10.74±0.20 b
G3	7.2±0.46b	2.1±0.05b	22.7±0.67b	34.1±1.1b	31.1±1.8 b	107.46±2 b	10.7±0.23 b
G4	6.06±0.14b	2±0.09b	21.5±1.2b	31.4±1.1 b	28.2±0.78 b	106.5±3.1 b	10.16±0.04 b

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (3). Effects of thiobencarb and penoxsulam on some heamatological parameters of fish 5 days post stop exposure to herbicides. (n= 5)

Group	HB (g/dl)	RBCs×10^6m m_3	PCV%	MCH (pg)	MCHC%	MCV (fl)	WBCs× 10^3mm^3
G1	9±0.27 a	2.9±0.20 a	29.1±0.46 a	31.8±0.76 a	32.5±0.40 a	101.7±1.08 a	12.7±0.16 a
G2	7.5±0.25 b	2.6±0.14 b	26±0.88 ab	27.3±1.8 b	28.1±0.80 b	97.4±3.2 b	11±0.24 b
G3	7.3±0.29 b	2.5±0.14 b	23.9±1.2 ab	28.7±1.4 b	29.5±0.97 b	95.9±3.5 b	10.9±0.44 b
G4	6.7±0.29 b	2.4±0.17 b	23±1.6 b	27.2±1.6 b	28.5±0.72 b	95.6±2.6 b	10.6±0.29 b

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (4). Effects of thiobencarb and penoxsulam on some heamatological parameters of fish 10 days post stop exposure to herbicides. (n= 5).

Group	HB (g/dl)	RBCs×10^6m m_3	PCV%	MCH (pg)	MCHC%	MCV (fl)	WBCs× 10^3mm^3
G1	9±0.32	3.1±0.20	28.5±0.69	30.7±1.13	33.1±0.80	93.7±0.92	12.8±0.38
G2	8.9±0.24	2.9±0.11	27.6±0.26	30.1±0.83	32.2±0.90	93.1±1.3	12.4±0.55
G3	8.9±0.20	3±0.29	28.1±0.46	29.2±1.3	31.4±0.17	92.3±1.1	12.1±0.34
G4	8.6±0.24	2.95±0.32	27.5±0.34	29.1±0.63	31.7±0.35	93.3±1.13	12±0.36

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (5). Effects of thiobencarb and penoxsulam on some heamatological parameters of fish 20 days post stop exposure to herbicides. (n= 5).

Group	HB (g/dl)	RBCs×10^6m m_3	PCV%	MCH (pg)	MCHC%	MCV (fl)	WBCs× 10^3mm^3
G1	8.7±0.29	3.05±0.20	27.7±0.62	29.1±1.1	32.5±1.1	93.4±1.1	12.6±0.13
G2	8.1±0.40	3±0.17	27.1±0.32	28.4±0.72	30.2±0.72	91.3±1.3	12.2±0.10
G3	8.2±0.26	2.91±0.12	27±0.56	28.2±0.26	29.7±0.26	93.2±0.57	12.3±0.13
G4	8±0.32	2.85±0.25	26.9±0.76	28±0.47	29.5±0.47	92.6±0.98	12.4±0.22

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (6). Effects of thiobencarb and penoxsulam on some biochemical parameters of fish 5 days post exposure to herbicides. (n= 5).

Group	AST(U/L)	ALT(U/L)	T. Protein (g/dl)	urea (mg/dl)	Creatinine (mg/dl)	GSH-Px (M/g %)
G1	29±1.7 b	24±2.02 c	6.8±0.17 a	27±2.02b	1±0.14 b	2.07±0.19 b
G2	45±3.1 a	40±2.7b	5.2±0.39b	37±2.7a	1.5±0.22a	3.3±0.30a
G3	47 ±1.5a	42±1.7b	5.3±0.24b	38±2.08a	1.5±0.17a	3.1±0.29a
G4	56±3.1a	50±1.45a	4.6±0.29b	40±2.3a	1.7±0.13a	3.4±0.17a

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (7). Effects of thiobencarb and penoxsulam on some biochemical parameters of fish 10 days post exposure to herbicides. (n= 5)

Group	AST (U/L)	ALT (U/L)	T. Protein (g/dl)	urea (mg/dl)	Creatinine (mg/dl)	GSH-Px (M/g %)
G1	29±2.9 b	26±1.4 b	6.8±0.3 a	27±3.2 b	0.86±0.05 b	2.3±0.29 b
G2	50±3a	43±3.2 a	4.7±0.21 b	41±2.08 a	1.49±0.13a	3.2±0.17 a
G3	53±3.1a	46±4.4a	4.4±0.23 b	42±1.2 a	1.42±0.12 a	3.4±0.05 a
G4	57±2a	51±3.7a	4.8±0.20 b	44±1.7 a	1.5±0.15 a	3.5±0.14 a

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (8). Effects of thiobencarb and penoxsulam on some biochemical parameters of fish 5 days post stop exposure to herbicides. (n= 5)

Group	AST (U/L)	ALT(U/L)	T. Protein (g/dl)	urea (mg/dl)	Creatinine (mg/dl)	GSH-Px (M/g %)
G1	28±1.4 b	26±2.3 b	6.9±0.43a	28±1.8 b	1±0.11 b	2.28±0.28 b
G2	39±2 ab	39±2 a	5.3±0.12 b	36±2 a	1.3±0.13 ab	3.1±0.16 a
G3	37±2.1 ab	38±2 a	5.5±0.21 b	37±2.08 a	1.29±0.17ab	3.2±0.15 a
G4	41±1.4 a	40±1.7 a	5.2±0.12 b	39±2.3 a	1.45±0.03 a	3.3±0.23 a

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (9). Effects of thiobencarb and penoxsulam on some biochemical parameters of fish 10 days post stop exposure to herbicides. (n= 5)

Group	AST(U/L)	ALT(U/L)	T. Protein (g/dl)	urea (mg/dl)	Creatinine (mg/dl)	GSH-Px (M/g %)
G1	26±1.4	28±1.7	7.6±0.52	25±1.7	1.1±0.11	2.3±0.32
G2	30±2	31±1.4	6.6±0.31	29±2	1.23±0.03	2.6±0.12
G3	31±1.7	30±2	6.8±0.24	30±1.7	1.21±0.07	2.4±0.20
G4	30±1.3	31±1.8	6.6±0.38	31±1.1	1.26±0.03	2.6±0.11

Different letters at the same column means that there was a significant change at $p<0.05$.

Table (10). Effects of thiobencarb and penoxsulam on some biochemical parameters of fish 20 days post stop exposure to herbicides. (n= 5)

Group	AST (U/L)	ALT (U/L)	T. Protein (g/dl)	urea (mg/dl)	Creatinine (mg/dl)	GSH-Px (M/g %)
G1	28±3.3	25±1.7	7.2±0.17	24±2	1±0.49	2.4±0.17
G2	30±1.4	26±2.9	7.1±0.29	26±1.8	1.3±0.87	2.4±0.27
G3	31±1.5	25±1.4	7±0.11	25±1.7	1.21±0.95	2.3±0.23
G4	30±2.4	27±2	6.9±0.29	27±2.4	1.32±0.12	2.5±0.32

Different letters at the same column means that there was a significant change at $p<0.05$.

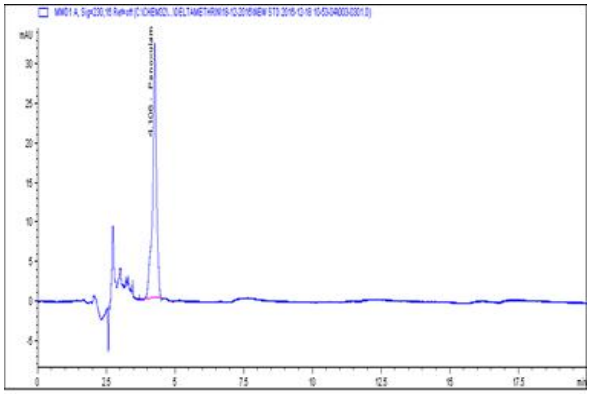
Table (11). Residual analysis of Penoxulam and Thiobencarb herbicides in muscles of catfish.

Herbicide type	Control	Residues after application (ppm)		Residues after clearance periods (ppm)		
		5 days	10 days	5 days	10 days	20 days
Penoxulam	ND	0.658±0.022 (a)	0.835±0.065 (a)	0.290±0.029 (b)	0.009±0.001 (c)	ND
Thiobencarb	ND	0.753±0.039 (b)	1.826±0.308 (a)	0.434±0.030 (c)	0.108±0.021 (c)	0.011±0.001 (a)

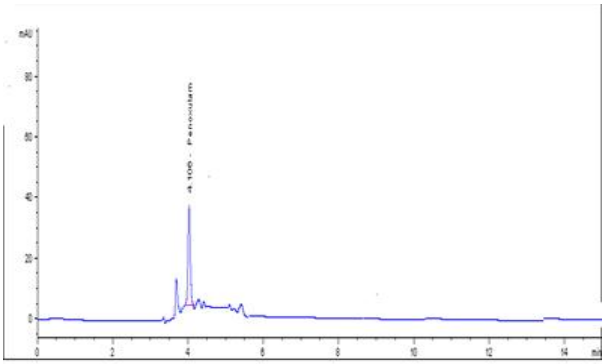
ND : not detected
Different letters in the same raw means significant differences at ($p\leq 0.5$)

Table (12). Effect of heat treatment (boiling for 30 min.) on the penoxulam and Thiobencarb herbicides residues in muscles of catfish

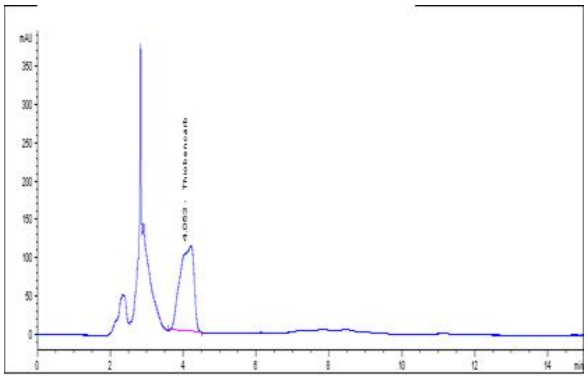
Herbicide type	Before treatment	After heat treatment	Reduction %
Penoxulam	0.8356±0.065	ND	100%
Thiobencarb	1.826±0.308*	0.219±0.006	88%



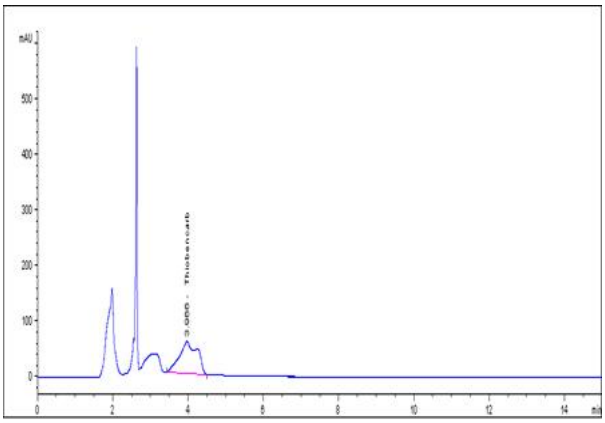
Chromatogram of (control) penoxulam



Chromatogram of penoxulam residue in fish muscle



Chromatogram of (control) Thiobencarb



Chromatogram of Thiobencarb residue in fish muscle

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