ISSN No:-2456-2165

Dichlorvos Insecticide Modulates the Antioxidant Parameters in Juveniles of African Catfish, *Clarias gariepinus*

*Anukwu, J.U. Nwamba, H.O., Nwani, C.D., Uzor, E.J. and Ogamba, E.E.
Department of Applied Biology and Biotechnology, Faculty of Applied Natural Sciences,
Enugu State University of Science and Technology.
University of Nigeria Nsukka.
PMB 1660,Enugu, Nigeria.

Abstract:- Dichlorvos is an organophosphate used commercially for the treatment of pests such as bedbug. The present study was designed to investigate the effects of dichlorvos on *Clarias gariepinus* juveniles using oxidative stress and antioxidant parameters. Fishes were exposed to 2.14 and 4.28mg/l corresponding to 1/10 and 1/5th of 96h LC_{50} of the pesticide. Each of the concentration was replicated thrice for the purpose of accuracy. The muscle tissues were sampled on day 1, 5, 10 and 15. Results showed concentration and time dependent values across the concentration. The value of catalase (CAT) declined while that of lipid peroxidation (LPO) increased significantly($p<0.05$) when compared with the control. Hepatosomatic index (HSI) and condition factor (CF) showed significant differences when compared with the control. The present study revealed that dichlorvos elicits toxic stress even at sublethal concentrations resulting in alteration in the studied parameters which is very evident in the blood, serum and muscle samples. There should be urgent education of farmers and the general public on the dangers of dichlorvos to non-target organisms and the environment.

Keywords:- *Toxicity, Dichlorvos, Fish, Antioxidant Parameters.*

I. INTRODUCTION

The United Nations Environment Program has defined pesticides as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating pest. Most developing nations of the world have found the use of pesticides as a necessary evil for agricultural purposes. This is predominant in underdeveloped or third world countries like Nigeria. Organophosphate pesticides are the most commonly used pesticides in the world due to its quick degradation. Unfortunately, organophosphates lack target specificity and can cause severe long lasting effects on the population. Irrespective of reports of health implications of these pesticides, most countries like ours still make use of it for their agricultural purposes. Dichlorvos (2, 2 dichlorovinyl dimethyl phosphate) was registered for use in 1948 but was introduced into the market in the year 1961. It has a molecular weight of 220.98g and the molecular formular is $C_4H_7Cl_2O_4P$. The trade name for dichlorvos include: DDVP, Dedevap, Sniper, Nuvan, Vapona,etc. Dichlorvos is one of the few organophosphates still registered for use. The World Health Organization has named dichlorvos as a highly hazardous chemical. Dichlorvos kills many organisms spiders, mites, caterpillars and trips (Lotti, 2001). It becomes poisonous when it gets to the body through any route. It serves as a contact and stomach poison for food and non-food crop pests (Suntio,1988 and Naqvi,1993). It is extremely toxic to aquatic organisms and hampers fish health through impairment of metabolism sometimes leading to death. As one of the few organophosphates still registered for use, dichlorvos has elicited worldwide concern for many reasons. It is specially used in the treatment of sea lice (*Lepophtheinus salmonis* and *Caligus elogatus*) on commercial salmon farms. But this pesticide usually ends up producing both lethal and sub lethal effects on the fish and even zooplanktons (Gupta,2008). At only 1mg/l or 1ppm,dichlorvos showed both acute and chronic toxicity in fish. Some other adverse effects of dichlorvos on fish have been noted by other researchers.

- Although it has been established that dichlorvos serves as a contact and stomach poison for food and non food crop pests, its toxicity to fish and other aquatic organisms need to be determined on some fish species.

Acetycholinerase in the nervous system of insects is the main target of dichlorvos. It also badly affects the genetic material of insects thereby altering the gene. Evidence for other modes of action applicable to higher animals have been presented. The safety data of dichlorvos has been severally reviewed by United States Environmental Protection Agency (USEPA, 1995). Since dichlorvos is an acetylcholinerase inhibitor, symptoms include weakness, headache, vomiting, hypotension, diarrhea, abdominal cramps, eye and skin irritation, atazia, convulsions, etc. Several researchers have been carried out to determine the effects of dichlorvos on fish. For this study, *Clarias gariepinus* was used. Fish as vertebrates contain a low-fat high quality protein. Several vitamins and elements have been identified in fish. This include riboflavin, phosphorus, omega-3-fatty acids, potassium, calcium,etc. Fish protein can be used to complement amino acid pattern and improve the overall protein quality of a mixed diet (FAO, 2005).

ISSN No:-2456-2165

Ayanda and Egbamuno (2005) reported that most fish contain significant amount of all amino acids. The African Catfish is generally accepted by many people as a source of meat (Prusynski, 2003) and it is mostly smoked and used in soups throughout Africa and beyond. Streams, ponds, swamps, rivers and lakes are aquatic habits of African catfish (Ayanda and Egbamuno, 2012). According to Nwamba (2008), catfish is recognized by its long dorsal and anal fins, has a slender body, a flat bony head, and a broad terminal mouth.

Lakra and Nagpure (2009) reported that catfish is at the risk of being contaminated in the environment through the use of pesticides in agricultural areas and linear food relationship among organisms.

II. MATERIALS AND METHOD

❖ *Materials:*

The materials used for this analytical work include the test samples, equipment and various chemicals.

A. Collection and Transport of Experimental Fish and Chemicals:

The juveniles of African catfish, *Clarias gariepinus* were collected from Rogeny Game Village Idemiri LGA of Anambra State. The fishes were transported in a 500 litres capacity of aquaria tank to the experimental site-Heldin Fishery Unit,Old airport road,thinkers corner. A total 300 juveniles of *Clarias gariepinus* of average weight 200±0.7g and length of 27±1.2cm were used for the experiment. The experimental chemical-dichlorvos (1000EC) was purchased from Ogbetemarket, Enugu.

B. Acclimitation of Experimental Fish

The acclimatization of the fish was done for a period of 14 days under laboratory conditions. The water was changed after few They were placed in a fifteen acclimatization tank of 25litres and filled with 10 litres of tap water. The fish were fed daily at 2% body weight during the period of acclimatization. The tanks were checked daily for fish mortality at time intervals as recommended by Sprague (1973).

Range finding test was carried out to determine the concentration of the test solution for definitive test.

C. Acute Toxicity Bioassay

Acute 96h bioassay was conducted in the laboratory as described by Sprague (1973) to determine the toxicity of dichlorvos to *Clarias gariepinus*. The fish were divided into experimental and control groups. The control group had no treatment while the experimental group had six different concentrations. The experiment was carried out according to the guideline of U.S EPA (1994) and replicated thrice for the purpose of accuracy. The six different concentrations were 18mg/l, 20mg/l,22mg/l, 24mg/l, 26mg/l, 28mg/l and the control. A total of 10 fish were used per replicate. The acute toxicity test lasted for four days (96h) in which the mortality rate as well as survival of the fresh water fish under each concentration of dichlorvos was recorded. The readings were taken after 24h, 48h,72h and 96h exposure time. The water was not changed (static system) until after 96h but dead fish were removed to avoid the pollution of the water. Death rate was not recorded in the control.

D. Sublethal Concentrations

Two sublethal concentrations were determined from 96h LC_{50} following the result obtained from probit analysis method as described by Finney(1971). The 96h LC_{50} value from probit is 21.4mg/l. The first sublethal concentration I(SL-I) was divided by 5 ($1/5^{th}$ of 96h LC_{50}=4.278) while the second sublethal concentration II(SL-II) was divided by 10($1/10^{th}$ of 96h LC_{50} =2.139). Fish are then exposed to these two sublethal concentrations and a control. Each treatment group was further randomized into three replicates (ten fish per replicate) in 10 litres of water. The exposure period was 15 days during which the fish was fed with small quantity of food approximately 1% of total body weight about an hour before the test solution was recommended to avoid catabolism and subsequent mortality. One fish from each replicate was sacrificed to get blood,liver and muscles. To avoid stress, the fish were anaesthetized using tricainemethana sulfonate. Other morphological indices such as weight of fish, fork length, total length, weight of liver were determined before the carcass was finally disposed.

E. Antioxidant Parameter:

➢ *Determination of Catalase Activity (CAT):*

CAT was determined according to the method as described by (Aebi, 1983).10% homogenate was prepared in 0.9% NaCl and centrifuged at 1500rpm at 15 minutes. The supernatant was used for the analysis. 25ul of sample and a typical reaction mixture containing 1ml 50Nm Potassium phosphate buffer (7.4) was added to the mixture. The reaction was then initiated with the addition of dichromate acetic acid and 1ml of H_2O.

Calculation: Δ OD of test x total volume x 1000/43.6 x 0.05ml sample x mg protein.

➢ *Determination of Oxidative Stress*

- *Determination of Lipid Peroxidation*

Lipid peroxidation (LPO) was determined by estimation of the thiobarbituric acid reactive substance as described by (Wallin *et al*., 1993). 0.5ml of sample was added to test tubes immediately followed by 0.5ml of 10% TCA (Trichloroacetic acid). Add 0.5ml of 10% TBA in the 10% TCA then boil for 30 minutes and cool.

The blank was prepared as follows:

1ml of distilled water and 0.5ml of TCA were added with 0.5 ml of TBA,
This was boiled for 30 minutes and allowed to cool.
1ml of distilled water was added and allowed to centrifuge for 30 minutes
The absorbance was read at 532nm and 600nm against the blank.
Calculation: LPO =(532-600 *2* 10/0.066)mg/100g .

ISSN No:-2456-2165

In concentrations. No mortality was observed in the control group after 96h of the test. In 18mg/l concentration, 3 fishes died out of the exposed 30 after 96h showing that the concentration was mild. The case was different when there was an increase in the concentrations. At 20mg/l, 9 fish died out of the exposed 30. There was a double increase in the death of fish from 20mg/l to 22mg/l. 24, 27 and 30 fish died at 24mg/l, 26mg/l and 28 mg/l respectively. This showed that these concentration became very toxic to the fish. The highest level of death was recorded at 28mg/l where all the fish died.

III. RESULT

A. Effect of Dichlorvos on Condition Factor (CF) and Hepatosomatic Index (HSI)

Table 1 and 2 showed the hepatosomatic index and condition factor of *Clarias gariepinus* exposed to dichlorvos. On day 1, the lowest value of HSI was observed at 4.28(a)mg/l while the highest value was observed at 2.14(a)mg/l. The lowest value of HSI in day 5 was observed at 2.14(b)mg/l while the highest value was observed at 4.14(a)mg/l. On day 10, the highest value of HSI was obtained at 2.14(a)mg/l while the lowest value was observed at 4.28(a)mg/l. When compared with the control, 4.28(a)mg/l recorded the lowest value of HSI while the highest value was observed at 4.28(b)mg/l.

Parameter	Conc.	Day 1	Day 5	Day 10	Day 15
Hapatosomatic index	0.00 2 .14 4.28	1.23 ± 0.29^{b_1} 0.68 ± 0.41^{a_1} 0.61 ± 0.08^{a_1}	0.97 ± 0.25^{b_1} 0.55 ± 0.24^{b_2} 0.47 ± 0.19^{b_2}	0.92 ± 1.04^{b_1} $0.41\pm.0.08^{b_2}$ 0.38 ± 0.46^{b_2}	0.87 ± 0.07^{b_1} 0.32 ± 0.07^{b_2} 0.28 ± 0.28^{b_2}

Table 1:- Record of Hepatosomatic Index at Different Concentrations.

Means with different alphabetical superscripts differ significantly across the duration while means with different numerical figures differ significantly across the concentration ($p< 0.05$).

	Conc.	Day 1	Day 5	Day 10	Day 15
Condition factor	0.00 2 .14 4.28	1.87 ± 0.40^{b_1} 1.47 ± 0.60^{b_2} 1.31 ± 0.33^{b_2}	1.83 ± 0.00^{b_1} 1.26 ± 0.02^{b_2} 1.14 ± 0.41^{b_2}	1.77 ± 0.03^{b_1} 1.07 ± 0.65^{b_2} 0.99 ± 0.22^{b_2}	1.72 ± 0.31^{b_1} 0.73 ± 0.00^{b2} 0.66 ± 0.12^{b_2}

Table 2:- Record of Condition Factor at Different Concentrations.

Means with different alphabetical superscripts differ significantly across the duration while means with different numerical figures differ significantly across the concentration ($p< 0.05$).

B. Record of Mortality After Exposure to Dichlorvos

Table 3 clearly showed that there was increase in mortality with a corresponding increase in concentrations. No mortality was observed in the control group after 96h of the test. In 18mg/l concentration, 3 fishes died out of the exposed 30 after 96h showing that the concentration was mild. The case was different when there was an increase in the concentrations. At 20mg/l, 9 fish died out of the exposed 30. There was a double increase in the death of fish from 20mg/l to 22mg/l. 24, 27 and 30 fish died at 24mg/l, 26mg/l and 28 mg/l respectively. This showed that these concentration became very toxic to the fish. The highest level of death was recorded at 28mg/l where all the fish died.

No of deaths

Conc.	**No exposed**	**24**	**48**	**72**	**96**	**% Mortality**	**% Survival**
Control	30	0	0	0	0	0	100
18	30	0	0	1	3	10	90
20	30	0	3	6	9	30	70
22	30	2	6	10	18	60	40
24	30	4	10	18	24	80	20
26	30	5	10	17	27	90	10
28	30	7	15	27	30	100	0

Table 3:- Cumulative Mortality of *Clariasgariepinus* Exposed to Different Concentrations of Dichlorvos

C. Effects of Different Concentrations of Dichlorvos on Oxidative Stress and Antioxidant Parameters in Clariasgariepinusat Different Exposure Periods.

Table 4 shows the effects of different sublethal concentrations of dichlorvos on lipid peroxidation and catalase (CAT). The result obtained in day 1, 5 and 10 showed that the lowest value (34.55±1.90) of CAT was obtained at 4.28mg/l while the highest value (41.25 ± 0.63) was obtained at 2.14mg/l. The case was different for day 15 where equal value (35.00 ± 0.14) was obtained in the two concentrations. The result obtained in LPO showed that the lowest value (268.95 ± 2.19) was obtained on day 1 at 4.28mg/l while the highest value (297.65 ± 3.60) was obtained on day 15 at 2.14mg/l.

Parameter Conc. (mg/l)	Concentration	Day 1	Day 5	Day 10	Day 15
Catalase	0.00	42.95 ± 0.77^{b_1}	41.15 ± 1.48^{b_1}	39.55 ± 0.49^{b_1}	37.95 ± 1.20^{b_1}
	2.14	41.25 ± 0.63^{b_2}	39.30 ± 0.70^{a_1}	36.80 ± 0.98^{a}	33.95 ± 0.35^{a1}
	4.28	39.95 ± 0.21^{a_1}	39.00 ± 0.98^{a_1}	34.55 ± 1.90^{a_1}	32.00 ± 0.14^{a_1}
LPO	0.00	290.65 ± 3.60^{b_1}	304.10 ± 3.65^{b_1}	330.65 ± 2.05^{b_1}	351.70 ± 1.41^{b_1}
	2 .14	294.65 ± 2.19^{a_1}	313.85 ± 0.63^{a_1}	345.85 ± 0.63^{a_1}	362.25 ± 4.17^{a_1}
	4.28	298.95 ± 0.35^{a_1}	325.55 ± 0.77^{a_1}	348.55 ± 0.77^{a_1}	370.70 ± 3.22^{a_1}

Table 4:- Effects of Different Concentrations of Dichlorvos on Oxidative Stress and Antioxidant Parameters in *Clariasgariepinus* at Different Exposure Periods.

Means with different alphabetical superscripts differ significantly (p<0,05) across the duration while means with different numerical figures differ significantly (p<0.05) across the concentration

IV. DISCUSSION

This study investigated the toxic effects of different concentrations of dichlorvos on the juveniles of African catfish, *Clarias gariepinus*. Toxicity of various toxicants are known to be dependent on various factors such as developmental stages,concentration, sex and duration of exposure (Pandey *et al*., 2011). There were behavioural changes in *Clarias gariepinus* treated with different concentrations of dichlorvos compared to the control. Among the behavioural changes were hyperactivity, equilibrium status, increased erratic swimming and jerky movements. This type of activities were reported by Matsumura (1975) to be the primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities in non target organisms.

Pal and Koner (1987) opined that disruption of the functioning of the nervous system of fish might be the cause of slow and agitated swimming, erratic movement and loss of equilibrium. Increase in the production of mucus over the body as a result of toxicant may interfere with the gaseous exchange, secretion and waste products and osmoregulation (Hossain, 1987). Similar observations were made by Shafieland Costa (1990), Balogun and Auta (2001), who studied the effects of pesticides on different species of fish.The tested concentrations of 2.14 and 4.28mg/l could be environmentally relevant since dichlorvos is commercially used in the control of pest. In view of the repeated application and sources from other anthropogenic agents, dichlorvos concentration maybe present in the environment beyond the safe level of 1ppm. This could pose potential health hazards to both vertebrate and invertebrate populations in the ecosystem (Suzawa and Igraham, 2008; Bye *et al*., 2011).

Reduction in Dissolved Oxygen after treatment is an dictates water pollution. There were fluctuations in the values obtained for temperature. A general decrease in pH was observed across the concentration. The reduction in pH values as observed is an indication that the water became acidic after treatment.

The mortality table shows that the survival of fish exposed to Dichlorvos is dose dependent. The highest level of mortality was recorded at the highest concentration. The increased mortality could be as a result of increased toxicity of the pesticides to the fish.

The over all health condition of the fish is ascertained by hepatosomatic index and condition factor. These two have been widely used as indicators of stress due to environmental contamination (Kleinkauf, 2004). The significant decrease in the hepatosomatic index (HIS) and condition factor (K) at the highest concentration of 4.28mg/l throughout the duration of the experiment suggests that stress has been induced due to histopathological disorders in the liver. Jordan and Ahmed (2013) have reported similar case in hepatosomatic index and condition factor.

The LPO induction was dependent on time and concentration. The increase in the level of lipid peroxidation suggests that there is increased production of ROS. Reactive Oxygen Species can react with biological biomolecules and cause increase in LPO, DNA damage and protein oxidation resulting to disturbance in the physiological processes (Tejeda, 2007). This is consistent with the report of Blahova *et al*. (2013) and Guilherme (2012). It is therefore suggested that the different responses probably are functions of species, the time of exposure, type and concentration of stressors. The observed increased

LPO as reported by (Nwani *et al*., 2010) resulting from the ROS generated by atrazine may lead to cell apoptosis (a process of programmed cell death). Reactive Oxygen Species production as a result of oxidative stress has been demonstrated to be triggers of cell apoptosis.

V. CONCLUSION

The present findings show that fishes showed a marked change in their behavior and responses when exposed to various concentrations of dichorvos. Mortality as observed is both time and concentration dependent**.** The result also supports the hypothsis that high concentration of dichlorvos beyond safe levels induces oxidative stress and alteration in antioxidant parameters. High concentrations of dichlorvos above its safe level of 1ppm is highly toxic to non target organisms. The application and use of dichlorvos for the control of pest should be highly monitored by relevant agencies.

RECOMMENDATIONS

- There should be urgent education of farmers and the general public on the dangers of dichlorvos to the environment.
- Manufacturers should be compelled to categorically state the effects of dichlorvos on non target organisms.
- Manufacturing industries should brain storm and come up with brilliant ideas on ways of reducing the potency of dichlorvos to non target organisms.
- The federal ministry of environment and state ministries should as a matter of urgency set up committees that would monitor and control the use of dichlorvos.

REFERENCES

[1]. Aebi, H.E. (1983). Catalase: Methods of Enzymatic Analysis. 3rd Edition. Weinheim Deerfield Beach. Pp. 273-285

[2]. Ayande, O. I. and Egbamuno, E. A. (2012). Histhopathological Examination of Gills of *Clariasgariepinus* treated with Glyphosate. *Environ. Res. Journal,* **6(**3**):** 228-234.

[3]. Ayande, O. I. and Egbmuno, E. A. (2005). Methods for the Examination of Amino Acids Contents in *Clariasgariepinus*. *Environ. Res. Journal, 5:* 234-240

[4]. Balogun, J.K. and Auta, J. A. (2001). Fisheries Resources and Development Potentials of Lake Kangimi, Kaduna State. *Nig. Journal of Science*, **1**: 50-56.

[5]. Blohova, J., Plhalova, L. and Hostovsky, M. (2013). Oxidative Stress Responses in Zebra Fish *Danio rerio* after Subchronic Exposure to Atrazine. *Food Chem. Toxicol*., **61**: 82 – 85.

[6]. Bye, J.D. and Struger, J. (2011). Spatial and Seasonal Variations in Atrazine and Metalochlor in Surface Water Concentration in Onatario (Canada) using ELISA. *Chemosphere,* **82:** 1155-1160.

[7]. Gupta, A. and Das, S. (2012). Effect of Malathion (EC_{50}) on Gill Morphology of Inian Flying Barb, *Esomusdanricus*. *World Journal of Fish and Marine Sciences.*, **4:** 626-628.

[8]. Finney, D.T. (1971). Probit Analysis. Cambridge University Press, London **1964**: 333-360

[9]. Food and Agriculture Organization (FAO, 2005). Cultured Aquatic Species Information Programme-*Clariasgariepinus.*

[10]. Gupta, R. (2008). Hepatic Toxicity Biomarkers. Biomarkers in Toxicology Academic Press: Burlington, MA, USA, pp. 103-112.

[11]. Lakra, W.S.andNagpure, N.S.(2009). Assessment of Tissue Specific Effects of Cadmium on Antioxidant Defence System and Lipid Peroxidation in Freshwater Murrel, *Channa punctatus*. *Fish Physiol. Biochem.*, **38**: 469-482

[12]. Lotti, M. (2001). Clinical Toxicology of AnticholineraseAgents in Humans . Handbook of Pesticide Toxicology. Volume 2. 2nd ed. Academic Press; Diego: Pp. 1043-1085.

[13]. Matsumura, F. (1975). Toxicology of Insecticides. Plenum Press, New York. Pp, 503-506.

[14]. Naqvi, S.M. and Vaishnavi, C. (1993). Mini Review, Bioaccumulative Potential and Toxicity of Dichlorvos insecticide to non-target organisms. *Comp. Biochem. Physiol.*,**105**: 347-361.

[15]. Nwamba, H.O and Ugwu, L.C. (2008). Oil Injection of *Heterobranchusbodorsalis* Adults and its Effects on Aspartate Transaminase Activity. *African Journal of Agriculture*, **4**: 60-69

[16]. Nwani, C.D., Lakra, W.S. and Kumar, R. (2010). Toxicity of *Atrazine*. Effects on Lipid Peroxdation and Activities of Antioxidant Enzymes in the Freshwater Fish *Channapunchants* (Bloch). *International Journal of Environmental Research Public and Health*, **7**: 3298-3312

[17]. Pal, A. K. and Konar, S.K. (1987). Long-term Effects of Organophosphate Insecticide. *Journal of Environmental Ecology*, **513**: 564-517.

[18]. Pandey, A.K., Nagpure, N.S. and Trivedi, S. (2011). Profenofos Induced DNA Damage in Fresh Water *Channa punctatus* (Bloch) using Alkaline Single Gel Electrophoresis. *Mut. Res.*,**726**: 209-214.

[19]. Pruszynski, T. (2003). Effects of Feeding on Ammonium Excretion and Growth of the African catfish (*Clariasgariepinus*) fry. *Czech Journal of Animal Sciences*,**48**(3): 106-112.

[20]. Shafar, M and Bueltner,C. (2006).Effects of Chronic Exposure to Primextra on the Oxidative Stress and Antioxidant Response in Common Carp. *Environ.Toxicol. Pharmacol.*,**34**: 334-343

[21]. Sprague, J. B. (1971). Measurement of Pollutant Toxicity to Fish. Bioassay Methods for Acute Toxicity. *Water Resources*, **3**:793-821.

[22]. Sprague, J. B. (1973). Measurement of Pollutant Toxicity to Fish Part III. Sublethal Effects and Safe Concentration. *Water Resources*, **5**:245-266.

[23]. Suntio, L. R., Shiu, W.Y. and Glotfelty, D. (1988). Critical Review of Henry's Law Constants for Pesticides. *Rev.Environ.Toxicol*. **103**: 1-59

[24]. Suzawa, M. and Ingraham, H.A. (2008). The Herbicide Atrzine Activates Endocrine Gene Network via Non-Steroidal NR5A Nuclear Receptors in Fish and Mammalian Cells. *Plos One*, **3**:1-11
[25]. Tejada, S., Sureda, A. and Roca, C. (2007). Antioxidant Response and Oxidative Damage in Brain Cortex after High Dose of Pilocarpine. *Brain Res. Bull.*, **71**: 372 – 375.
[26]. US EPA (1994). Environmental Protection Agency. Rules and Regulations. *Fed.Register.*,**56**: 35-52
[27]. Varo, I. and Amat, F. (2003). Effects of Dichlorvos on the Cholinerase Activity of the European Sea Bass (*Dicentrarchuslabrax*). *Pesticide Biochem. and Physiol.*,**75**: 61-72.
[28]. Wallin, B., Rosengren, B., Shertzer, H.G. and Camejo, G.(1993). Lipoprotein Oxidation and Measurement of TBARS formation in a Single Microlitre Plate; its use for Evaluation of antioxidants. *Analytical Biochemistry*, **208**: 10-15.