HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN *CLARIAS LAZERA* AS A RESULT OF LONG TERM EXPOSURE TO A TRIAD COMBINATION OF LEAD, MERCURY AND ARSENIC

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Abstract

Clarias lazera fish were used to study the toxicity of the triad combination of lead, mercury and arsenic at 1/100 of their LC_{50}/72 h. for 5 wekks.

All fish exposed showed decreased and unbalanced movements, appearance of red patches on the skin accompanied by inflammation laceration. Some fish showed presence of thick mucus film over the and gills. Darkening of the skin colour was also observed.

There were significant decrease in red blood cell counts from the second week of the experiment till the end, accompanied by decreased haemoglobin concentration. These resulted in macrocytic hypochromic anaemia. These results could be explained as a suppressive effect of haevy metals on both haem and globin formation.

The white blood cell counts showed significant increase in their values from the second week of exposure till the end of the experiment.

Significant decreased values of serum total protein and total cholesterol were observed from the second week of exposure. The observed results could be explained according to the toxic role of the haevy metals on the hepatic parenchyma and cholesterol synthesis, estrification and excretion from the liver.

There were significant increased values of serum glucose in the first three weeks, followed by significant decrease in the last two weeks. The hyperglycaemia observed may be due to impairment of carbohydrate metabolism, and the latter hypoglycaemia could be explained as a result of hepatocellular destruction due to exposure to heavy metals.

Activities of serum transaminases showed significant increased values during the experiment.

Liver showed the highest significant residual contents for the three heavy metals, followed by the kidney. The muscle tissue showed the lowest significant values.

INTRODUCTION

Natural water has widely physical and chemical properties. The suitability of any water as a medium for fish depends on its temperature, concentration of dissolved atmospheric gasses, salts and other minerals. However, its suitability depends on the state of adaptation of the fish to the environmental conditions. The contamination of water with various industrial and domestic wastes has rendered this water unfit for susceptible fish species (Doudoroff 1966). The dilution of water by metals and their salts (natural or experimental) can affect fish adversely. The damage to the fish is obviously influenced by the metal and the conetraton to which the fish is exposed. The main cause of death is usually suffocation due to the obstruction of the gills by mucus and injury to the gill epithelium. The presence of metallic salts in water can also be indirectly detrimental to the fish by affecting the balance between phytoplankton, zooplankton and invertebrates upon which the fish feed (Ericksen 1969).

Lead has adverse effects on nearly every tissue and organs in the body, thus, giving rise to clinical signs, biochemical and haematological changes and lesions. The clinical signs of lead poisoning are mainly those of nervous system stimulation, with gastrointestinal involvement (Hatch 1977). Pounds (1985) concluded the mechanisms by which metals exert their toxidc effects. Leàd inhibits the structural function of some enzymes such as sodium and potassium adenosine triphosphatase. Also, lead intoxication alters tissue levels of many essential elements including iron, zinc, copper and calcium .

Mercury ions produce their toxic effects by protein precipitation, enzyme inhibition and generalized corrosive action, as well as to phosphoryl, carboxyl, amide and amine functional groups. Mercury also, reacts with several enzymes and other proteins which contain these functional groups (Gossel and Bricker 1989). Gwozdzinski (1992) recorded structural changes and alterations in the internal viscosity of fish erythrocytes exposed to high concentration of mercury. He also suggested that the cause of damage of cells may be metal-protein interactions in the cells.

Arsenicals are toxic to freshwater teleosts. Thick mucus coat is secreted over the entire body surface, but especially over the gill surface, and causes suffocation due to inadequate oxygen uptake (Sorensen 1974). Arsenicals can induce iron stroage diseases in fish characterized by lysosomal accumulation of soluble and

insoluble deposits of iron (Crichton 1971), erythrocyte lysis and decreased circulating erythrocytes life span (Klaassen 1985).

The aim of the present investigation was to study the toxicity of a combination of some heavy metals together trying to simulate what happens industrially. We chose lead, mercury and arsenic as they are the major ingredients of the common used textile dyes.

MATERIALS AND METHODS

One hundred Clarias lazera fish apparently healthy and of uniform length (20-30 cm, with a mean value of 25 cm) of both sexes were collected from El-Wafa Fish farm at Giza. They were transported to the laboratory in fiberglass tanks filled with water from the same source. In the laboratory, they were finally maintained in aerated and filtered glass aquarium of 100 x 30 x 50 cm with a capacity of 200 liters of tap water. Fish were left for adaptation for two weeks and fed once daily (3% biomass) with commercial dry pellets (20 % protein) composed of fish meal (18%), cotton seed meal (30%), starch (25%), corn oil (9%), vitamin mix. (1%), mineral mix. (2 %), carboxy-methyl cellulose (1%) and cellulose (14%). It was found to be chemically free from the three studied toxic elements.

Fish were divided into 10 groups (10 fish each). Five groups for toxicity studies and the other five groups served as control. The first five groups were exposed for 5 weeks to 1/100 of the LC_{50}/72 h. of the triad combination of lead acetate (1.52 mg/L.), mercuric chloride (0.042 mg/L.) and arsenic trichloride (0.176 mg/L.) (Mary 1994). Semi-dynamic method for removal of excreta was used at regular intervals (4 days) by siphoning of a portion of water from the aquarium and replacing it by an equal volume of water containing the same conentration of the toxicants (Brown 1980). Clinical signs and mortality percentage were recorded for all experimental fish. Blood samples were collected weekly from the caudal vein of one treated group and its control group. Two blood samples were collected in 2 separate vials, the first one contained anticoagulant (dipotassium EDTA). This blood was used for erythrocyte and leucocyte count (Kanaev 1985), haemoglobin estimation (Crosby *et al.* 1954) and packed cell volume determination (Coles 1974). The second vial was for sera preparation to be used for estimation of glucose (Trinder 1969), total cholesterol (Watson 1960), total protein (King and Wooton 1959), aspartate aminotransferase "AST" and alanine aminotransferase

"ALT" activites (Reitman and Frankel 1957). Muscles, livers and kidneys were taken for residual estimation of lead, mercury and arsenic (Middleton and Stuckey 1954) using PYE-UNICAM Atomic Absorption Spectrophotometer (single beam spectrophotometer, Sp. 192).

Mean corpuscular volume (M.C.V.), mean corpuscular haemoglobin (M.C.H.) and mean corpuscular haemoglobin concentration (M.C.H.C.) were determined according to Sonnenwirth and Jarett (1980).

The obtained data were statistically analysed according to Snedecor (1971).

RESULTS

Clinical signs

The clinical signs observed were mainly in the form of dull movements of fish, red patches on the skin in the first weeks, followed by severe inflammaton and laceration at the last weeks. Darkening of the skin colour and presence of a thickened mucus film over the gills were also observed. The mortality percentage is represented in Fig.1. The results revealed that the mortality percentage increased with the increase of exposure time .

Haematological findings :

The toxic effects of long term exposure to a triad combination of the three heavy metals at 1/100 of their LC_{50}/72 h. on the haematological parameters in Clarias were illustrated in Figs. 2,3 and 4. The erythrocytic count showed decreased values than control. This decrease started to be significant from the second week (1.56 ± 0.09 million/cm3) with $P<0.05$ till the end of the experiment (1.08 ± 0.02 million/cm3) with $P<0.0005$. The haemoglobin concentration values showed gradual significant decrease from the first week (4.50 ± 0.34 g/100 ml, $P<0.005$) till the end of experiment (3.45 ± 0.31 g/100 ml, $P<0.0005$) as compared with control. The haematocrit values showed slight non-significant decrease in exposed Clarias than control during the whole period of the experiment.

Results in Fig. 3 showed higher values of MCV from the first week till the end of the experiment. These values of MCV referred to the decrease in erythrocyte volume which is known as macrocytic anaemia. Lower values of MCH and MCHC

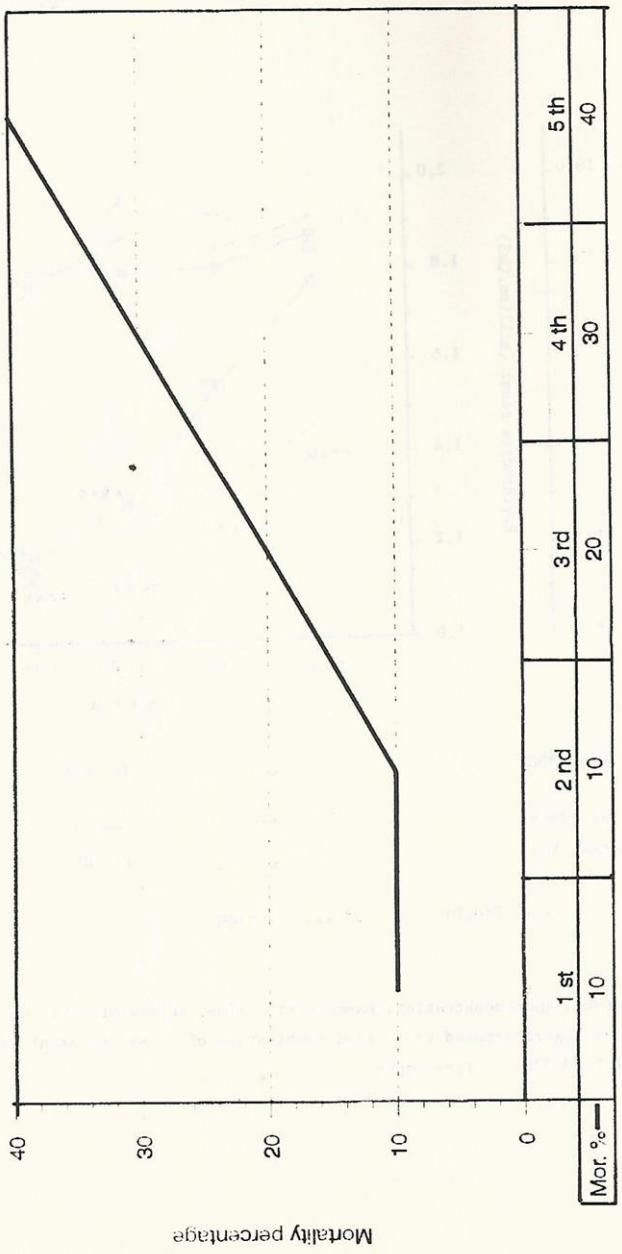


Fig. 1. Motrality percentage in *Clarias lazera* fish exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50/72 h. for five weeks.

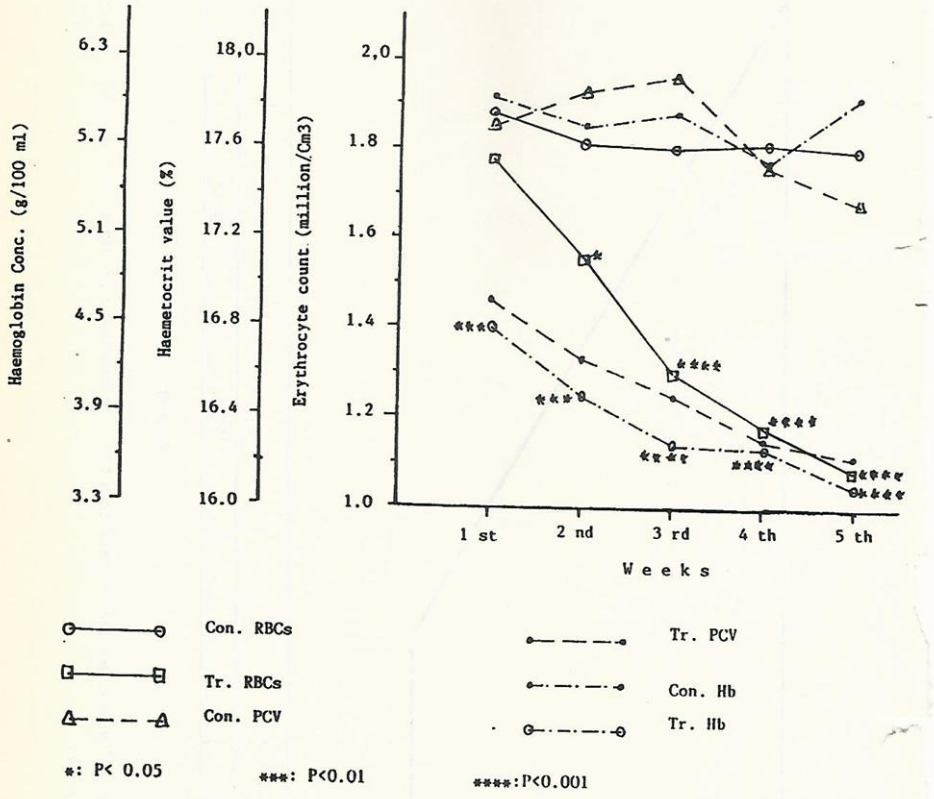
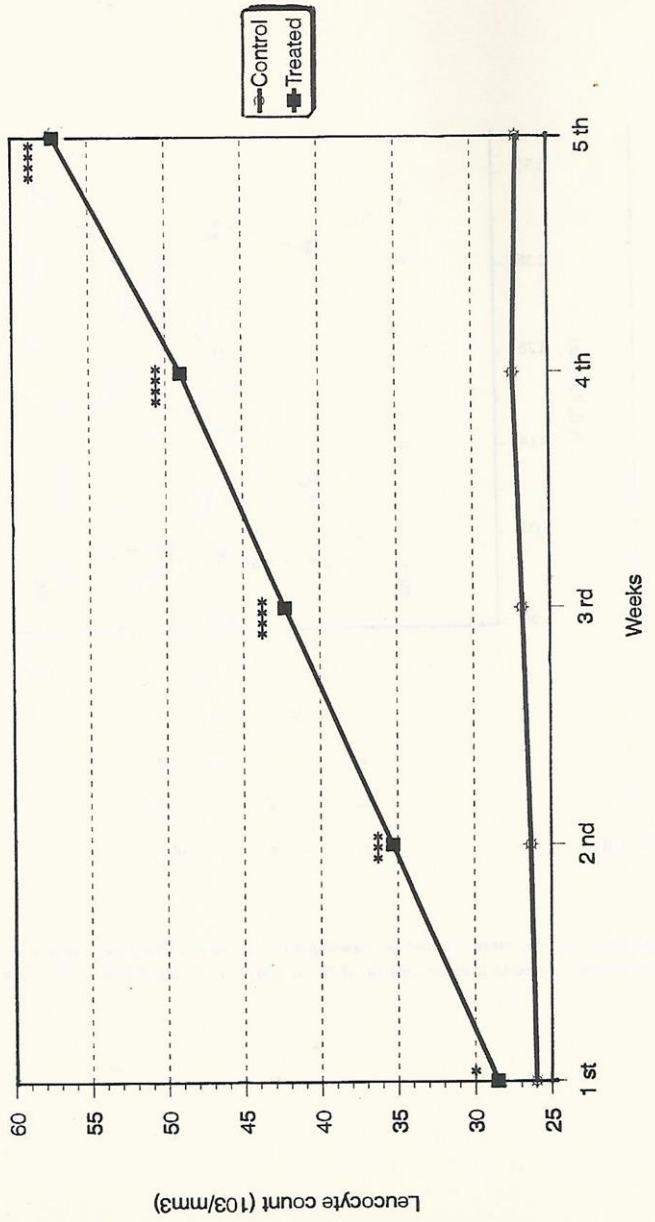


Fig. 2. Weekly Haemoglobin concentration; haematocrit value an erythrocytic count in Clarias lazera exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50/72h for five weeks



*: P<0.05; ***: P<0.01 & ****: P<0.001

Fig. 3. Weekly leucocytic count in *Clarias lazera* exposed to a triad combination of Pb,Hg and As at 1/100 of their Lc50 /72 h. for five weeks .

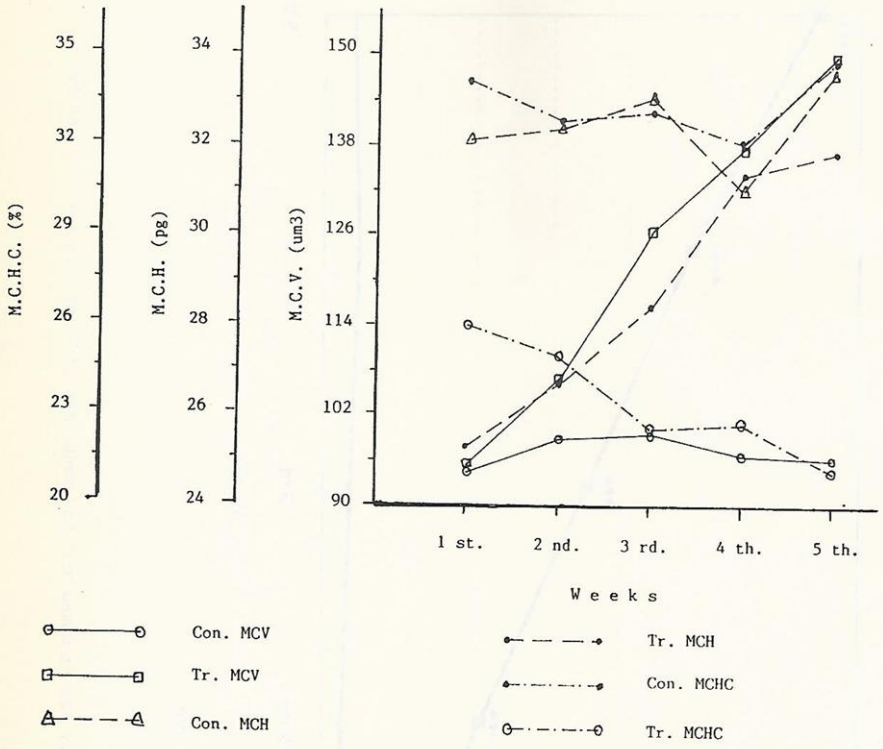
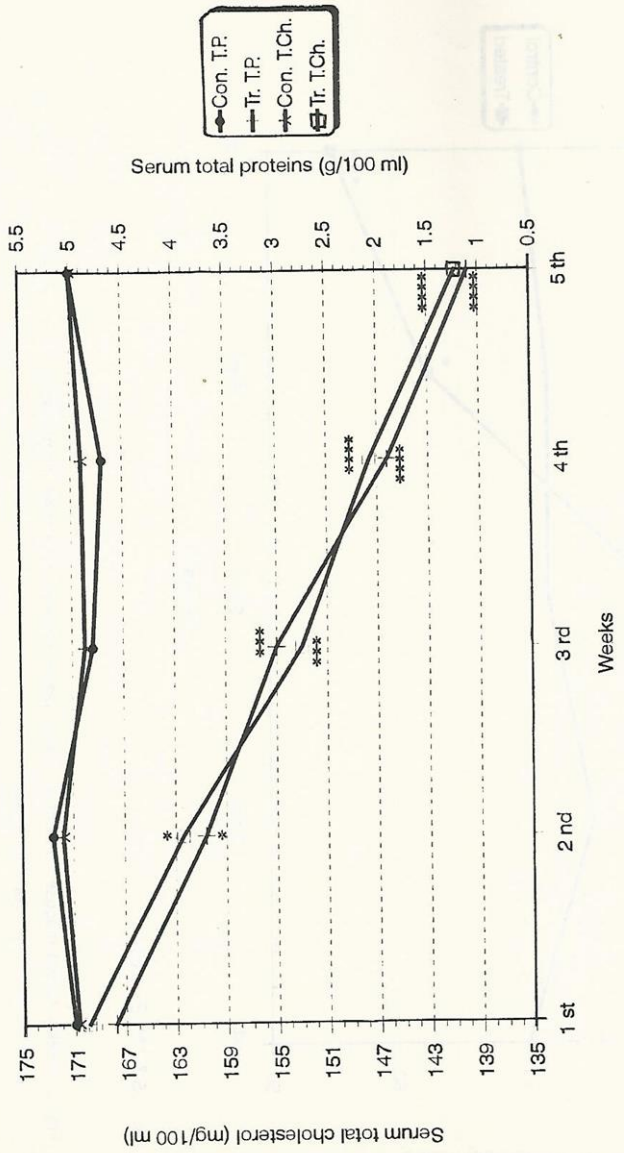
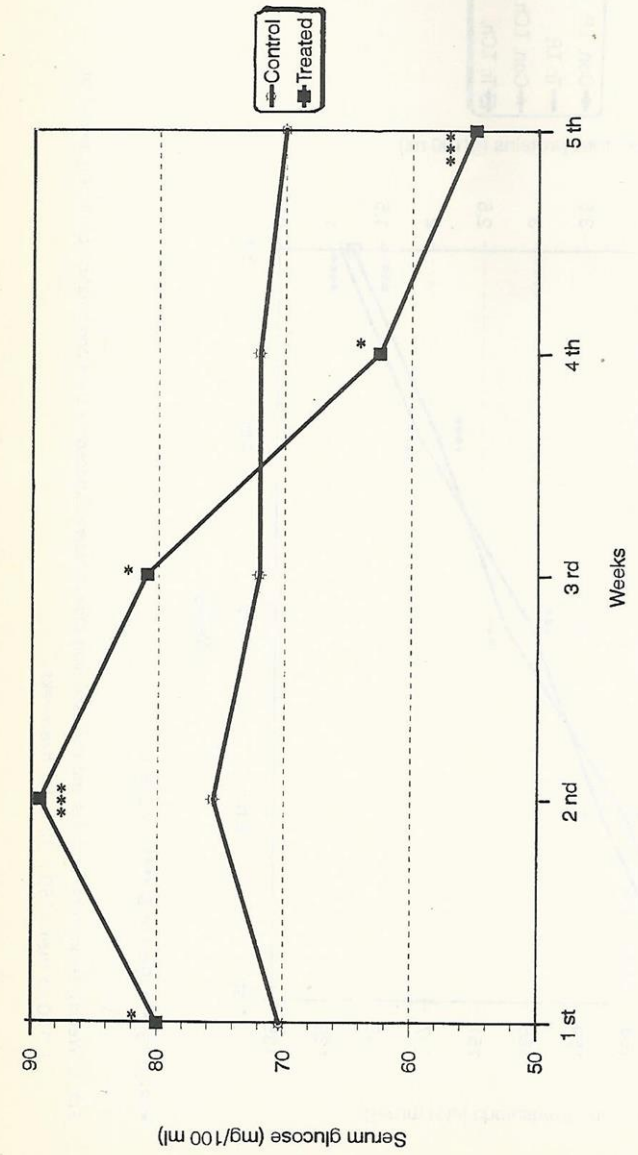


Fig. 4. Weekly mean corpuscular volume; mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in *Clarias lazera* axposed to triad combination of Pb, Hg and As at 1/100 of their LC50 / 72 h. for five weeks .



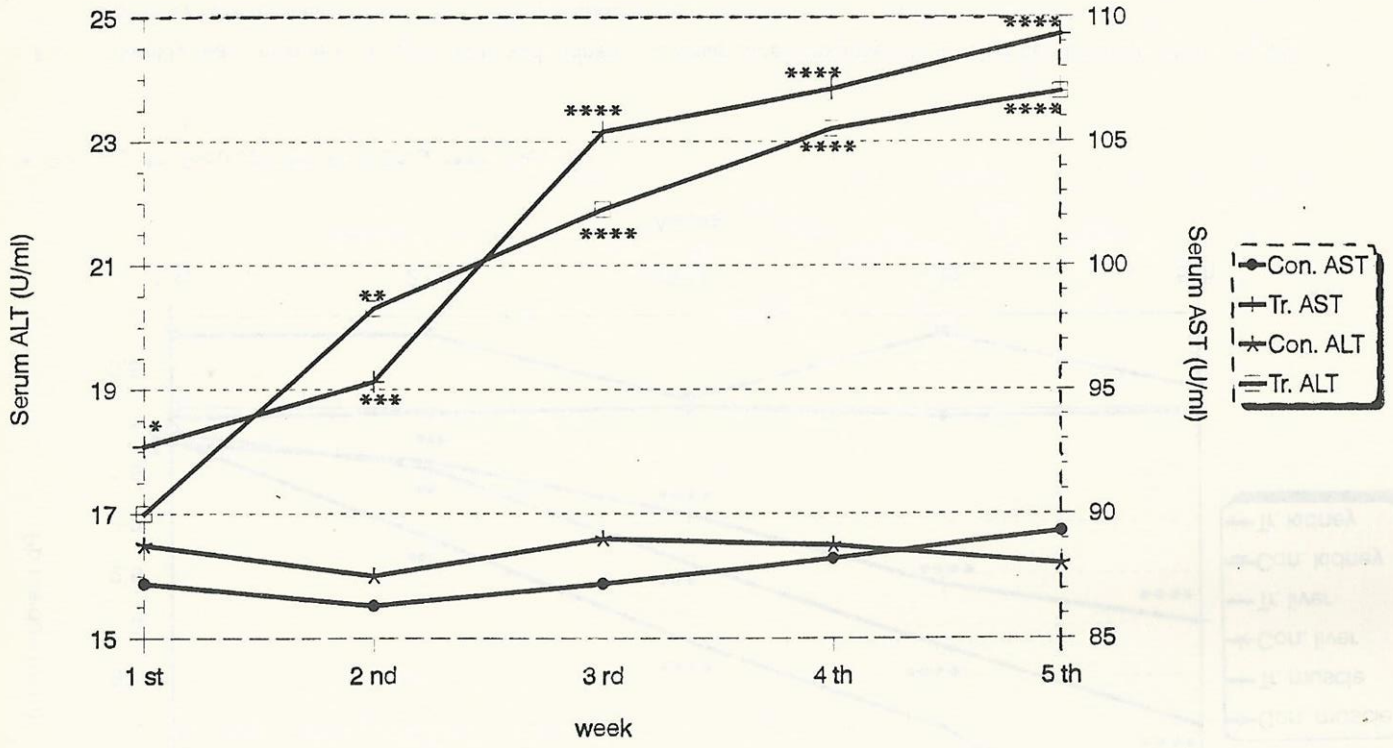
*: P<0.05; ***: P<0.01 & ****: P<0.001

Fig. 5. Weekly serum total proteins and cholesterol in *Clarias lazera* exposed to triad combination of Pb, Hg and As at 1/100 of their LC50 / 72 h. for five weeks .



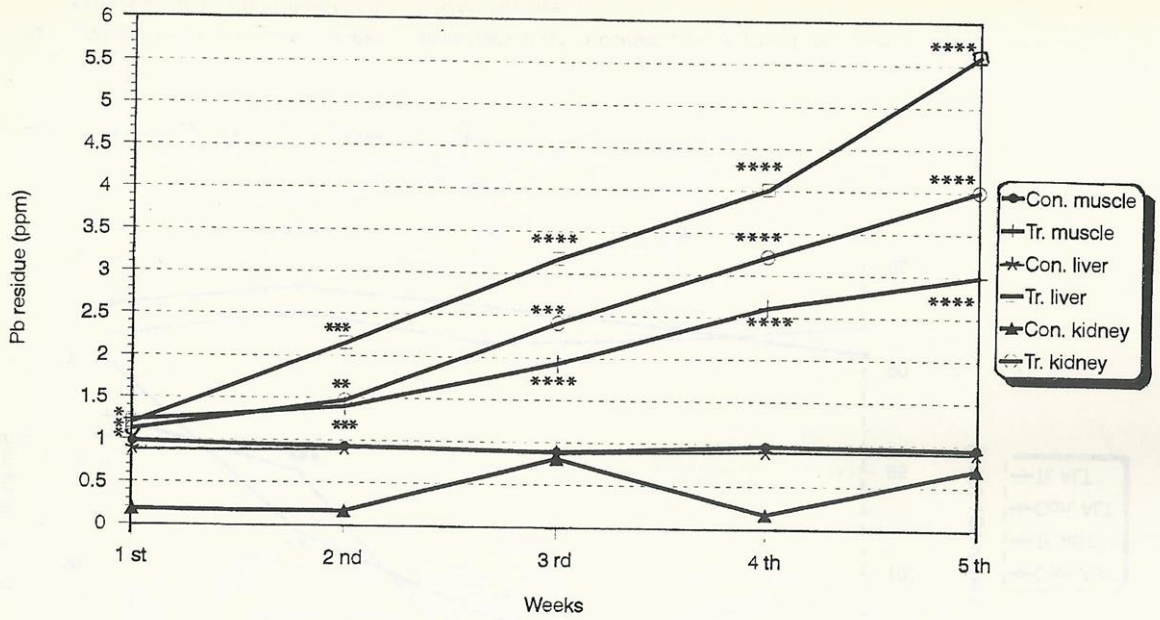
*: P<0.05 & ***: P<0.01

Fig. 6. Weekly serum glucose content in *Clarias lazera* exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50 / 72 h. for five weeks .



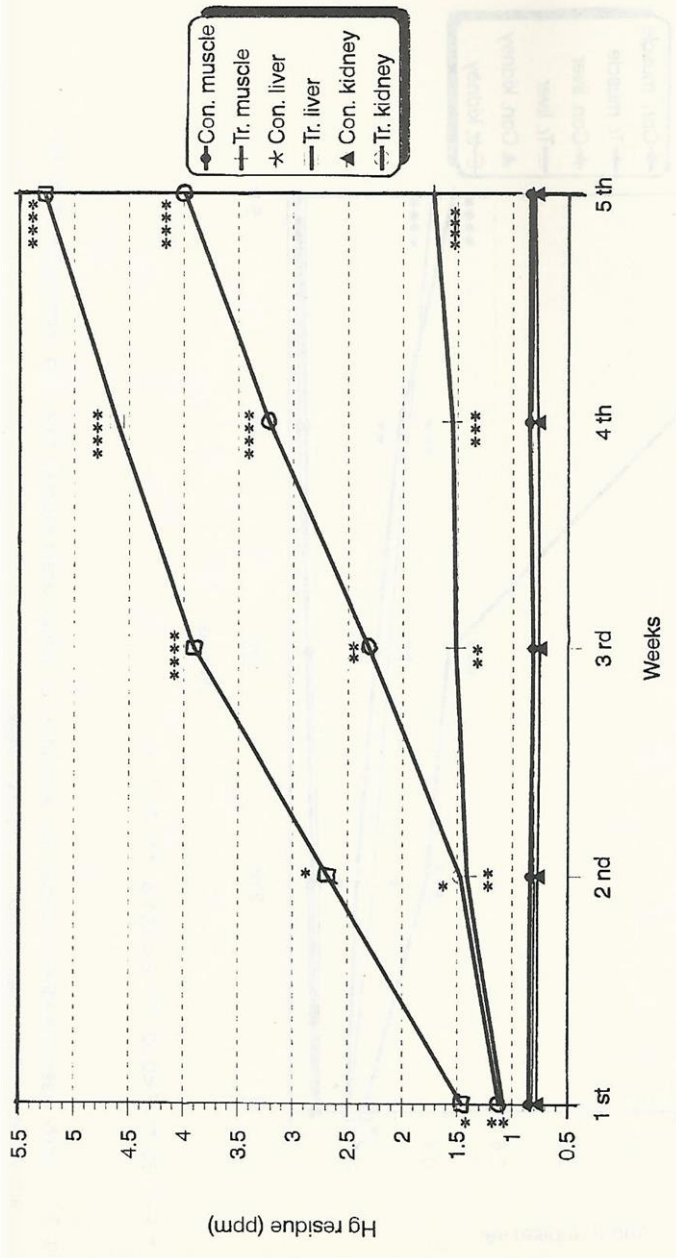
*: P<0.05; **: P<0.02; ***: P<0.01 & ****: P<0.001

Fig. 7. Weekly serum aspartate and alanine aminotransferase in *Clarias lazera* exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50 / 72 h. for five weeks .



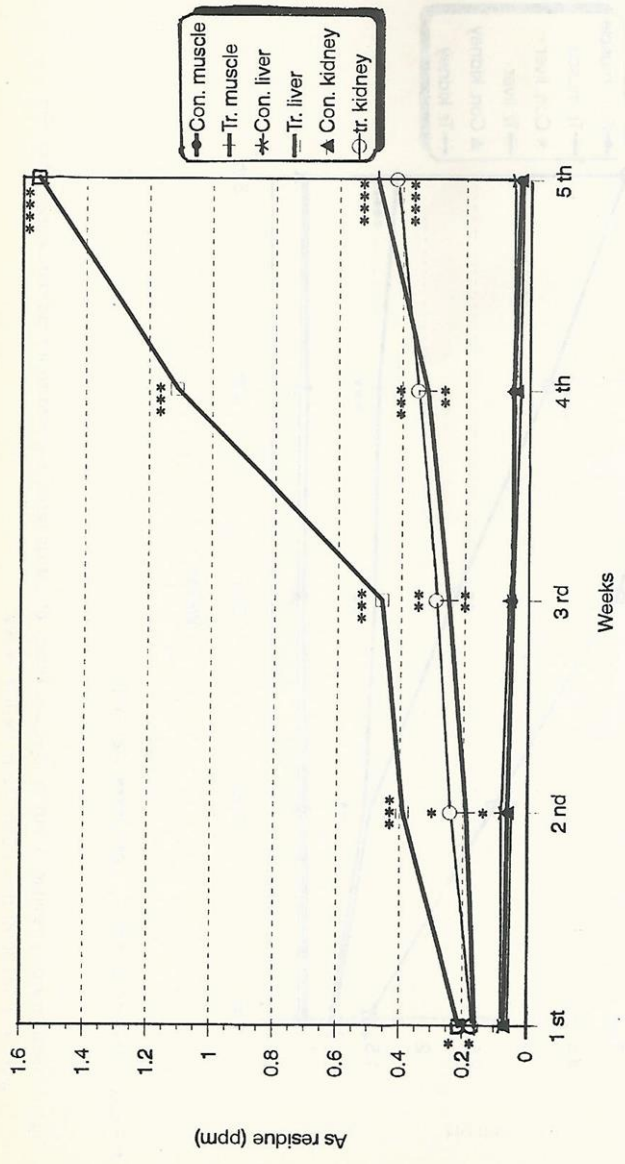
*: P<0.05; **: P<0.02; ***: P<0.01 & ****: P<0.001

Fig. 8. Weekly lead residue in muscle, liver and kidney of *Clarias lazera* exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50 / 72 h. for five weeks .



*: P<0.05; **: P<0.02; ***: P<0.01 & ****: P<0.001

Fig. 9. Weekly mercury residue in muscle, liver and kidney of *Clarias lazera* exposed to a triad combination of pb, Hg and As at 1/100 of their LC50 /72 h. for five weeks .



*: P<0.05; **: P<0.02; ***: P<0.01 & ****: P<0.001

Fig. 10. Weekly arsenic residue in muscle, liver and kidney of *Clarias lazera* exposed to a triad combination of Pb, Hg and As at 1/100 of their LC50/72 h. for five weeks .

were also shown in *Clarias* exposed to the triad combination of Pb, Hg and As than control.

The leucocyte count in Fig. 4 showed gradual significant increase from the first week (28.5 ± 1.2 thousand/mm3, $P<0.05$) till reaching the double (57.3 ± 1.0 thousand/mm3 $P<0.0005$) at the end of the experiment.

Biochemical changes :

The toxic effects on the biocemical parameters were demonstrated in Figs. 5,6 and 7. Fig.5 showed gradual significant decreased values of serum total protein and total cholesterol from the second week of the experiment (3.7 ± 0.2 g /100 ml and 162.4 ± 3.5 mg/100 ml, respectively) with $P < 0.05$ till it reached 1.1 ± 0.04 g/100 ml with $P<0.005$ for total proteins and 140.8 ± 1.8 mg/100 ml with $P< 0.0005$ for total cholesterol at the last week of the experiment .

Serum glucose values showed significant increased values in the first three weeks of the experiment with the maximum value at the second week (89.3 ± 2.5 mg/100 ml). This was followed by significant decreased values in the last two weeks.

Significant higher values in AST (92.7 ± 2.5 U/ml) and ALT (20.3 ± 0.8 U/ml) activities were observed from the first and second week, respectively. They reached their highest values at the last week (109.3 ± 1.5 and 23.8 ± 0.5 U/ml for AST and ALT, respectively) with $P<0.0005$.

Bioaccumulation residues :

The results graphed in Figs, 8,9 and 10 showed that the residual contents of Pb, Hg, and As in muscle, liver and kidneys of the experimental group were significantly higher than those of the control group from the first week and up to the end of the experiment .

The residual amounts of the three heavy metals were mostly concentrated in liver. Kidneys were considered the second organ, and finally, muscle tissues contain the least amount.

DISCUSSION

Signs and symptoms observed on *Clarias lazera* as a result of the long term exposure to lead, mercury and arsenic were in the form of dull movements of fish,

red patches, followed by inflammation and laceration of the skin. Dark colouring of the body and thickened mucus film over the gills were also recorded. This observed pattern of clinical signs and symptoms were similar to those previously mentioned by Metelev *et al.* (1971), Helmy *et al.* (1978), Hodson *et al.* (1982) and Elsa (1991) for fish exposed to lead. They noted that the fish were suffering from restlessness, increased rhythm and depth of respiration, sluggish movement, loss of equilibrium, black tails pigmentation, darkening of the body colour, coagulated mucus film covering gills and skin, symptoms of neurotoxicity, followed by reduction in frequency of respiration, and death finally occurred. For fish exposed to mercury, Matida *et al.* (1972), Helmy *et al.* (1978) and Kaviraj and Konar (1982) described haemorrhage of gills and buccal epithelium, rolling swim with loss of equilibrium, precipitation of mucus over gills, darkening of the body colour with formation of vertical bars, respiratory distress, visual disturbances and opalescence of eyes. Gossel and Bricker (1989) described dark brown pigmentation and a thickening of the kertin layer of skin of fish exposed to arsenic toxicity.

The increased mortality percentage with the prologation of time of exposure to the triad combination are in agreement with Daoust (1981) who mentioned that higher dose and long period of exposure will increase the degree of toxicity which led to increased mortality percentage.

Our results referred to the decrease in both Hb concentration and RBCs count, which is responsible to the case of hypochromic anaemia induced. So, we obtained a case of macrocytic hypochromic anaemia (Pernicious anaemia).

The available literature concerning the fish haematology in cases of heavy metals poisoning described the changes occurred as a result of exposure to single heavy metal, and few of them described the effect of exposure to more than one metal. Our results are in agreement with those obtained by Metelev *et al.* (1971), Hard *et al.* (1973) and Hodson *et al.* (1978) that the chronic poisoning with lead in fish had caused sever anaemia due to haemolysis and destruction of the erythrocytes. Helmy *et al.* (1978), Salmeron *et al.* (1990) and Rao *et al.* (1990) described significant lower values of RBCs count, PCV% and Hb concentration induced by lead poisoning. On the other hand, Santos and Hall (1990) mentioned that eels exposed to 300 ug organic lead/L for 30 days showed no differences in Hb% and RBCs count when compared with control values.

Regarding mercuric toxicity in fish, our haematological results are in

agreement with the findings of Helmy *et al.* (1978), Panigrahi and Misra (1978, 1980) and Hilmy *et al.* (1980b).They described lower values of Hb %, RBCs and count and PCV %, while, the leucocytes were more than control values.

Klaasen (1985) and Gossel and Bricker (1989) agreed with our results and attributed the decrease in RBCs count in cases of arsenic poisoning to the lysis of the cirulating RBCs which decreased their life span. Also, Cockell *et al.* (1991, 1992) described mild to moderate anaemia in the rainbow trout exposed to 33-60 mg disodium arsenate/g diet for 8-24 weeks.

The aforementioned results could be explained through the toxic role of lead, mercury of arsenic either separately or in their triad combination on the haematological parameters. Lead had a suppressive effect on both haem and globin formation. It inhibits amino levulinic acid-synthetase (ALA-S), amino levulinic acid-dehydratase (ALA-D) and ferrocheltase enzymes resulting in reduction of haem formation. Lead also prevents the incorporation of glycine into globin (Gossel and Bricker 1989). The same author explained the mode of action of mercury on the living tissues. Mercury precipitates proteins and inhibits enzymes. It binds to sulfhydryl groups, phosphoryl, carboxyl, amide and amine functional groups. Mercury also reacts with several enzymes and other proteins which contain these functional groups. Thus, mercury interferes with cellular enzymes reactions. Gwozdzinski (1992) also mentioned that, when fish RBCs exposed to different concentrations of mercury ranging from 0.05-0.3 mmol/L, they were damaged due to the metal protein interactions inside the cells. Gossel and Bricker (1989) mentioned that arsenic is a general protoplasmic poison. Toxicity results when it combines with sulfhydryl (-SH) groups, particularly those contained with enzymes (specifically the pyruvate dehydrogenase complex).

Also, significant higher values of WBCs count were described by Helmy *et al.* (1978) and Rao *et al.* (1990).

The mean values of serum total proteins in control groups ranged from 4.7 ± 0.2 to 5.2 ± 0.6 g % (Fig. 5). Bentick-Smith *et al.* (1987) mentioned that the serum total protein level in the different species of Catfish ranged from 3.27 to 4.50 g %. Significant hypoproteinaemia was observed in the treated groups from the 2nd week of the experiment and onward. Our results are in agreement with the earlier results reported by Panigrahi and Misra (1978, 1980) in their studies on the experimental mercuric nitrate intoxication in fish with different doses and times. Hilmy *et al.*

(1980 c) described different patterns of serum total protein in the form of an initial hyperproteinaemia after exposure to mercury for 96h. This was followed by transient hypoproteinaemia for few days ending with another hyperproteinaemia in chronic exposure to mercury. On the other hand, Santos and Hall (1990) and Salmeron *et al.* (1990) described non-significant changes in serum total protein of lead poisoned fish. The observed hypoproteinaemia could be explained by the the demonstrated toxic effects of the heavy metals on the hepatic parenchyma (Olson *et al.* 1973, Holcombe *et al.* 1976 and Sorensen *et al.* 1979).

The mean values of serum total cholesterol in control groups ranged from 170.0 ± 3.6 to 171.8 ± 1.4 mg % (Fig. 5). Bentick-Smith *et al.* (1987) mentioned that the serum total cholesterol level in the different species of Catfish ranged from 152.0 to 212.0 mg %. Gradual significant hypocholesterolaemia were observed in the treated groups from the 2nd week till the end of the experiment. Our results are in agreement with those reported by Kirubagaran and Joy (1992) who described a significant hypocholesterolaemia (the esterified fraction) in Catfish exposed to 0.04 mg methyl mercuric chloride/L for 90 days. On the other hand, Hilmy *et al.* (1980c) described an increased level of serum total cholesterol in fish exposed to mercury. Santos and Hall (1990) didn't find any changes in the total cholesterol level in lead exposed fish as compared with the controls. Holcombe *et al.* (1976). and Kendall (1977) explained the toxic role of the heavy metals on liver concerning the cholesterol synthesis, estrification and excretion.

The mean values of serum glucose in the control *Clarias lazera* fish ranged from 70.0 ± 3.2 to 75.6 ± 3.0 mg % (Fig. 6). Bentick - Smith *et al.* (1987) mentioned that the normal glucose level in Catfish species ranged from 36.5 to 104.4 mg %. In treated *Clarias lazera* groups, serum glucose level showed significant increased values in the 1st three weeks reaching their peak at the 2 nd week. This hyperglycaemia was followed by significant hypoglycaemia at the last 2 weeks, reaching its bottom at the 5 th week (55.0 ± 2.0 mg %). Our results are in agreement with those of Hilmy *et al.* (1980c). They described hyperglycaemia in fish during the first 48 hours in chronic mercury exposure. This was followed by a decrease after 8 days, and then, steadily decreased during the rest of the exposure period. On the other hand, Salmeron *et al.* (1990) mentioned that lead exposure in fish caused inhibitoy effect on glucose level. From the aforementioned data, we suggest that the hyperglycaemia observed in the first 3 weeks may be due to decrease in the serum insulin required to ocntrol the glucose level either through a reduction in the rate of production or the effectiveness of the available insulin. This

could be attributed to alterations in the rate and degree of digestion, absorption and utilization of glucose due to the exposure to the heavy metals, i.e. impaired carbohydrate metabolism due to exposure with heavy metals. The latter hypoglycaemia in the last 2 weeks could be explained as a result of hepatocellular destruction. Well (1962) mentioned that the hepatic cellular damage as a results of exposure to toxic substances was accompanied by hypoglycaemia. Hillmy *et al.* (1980c) stated that, in chronic exposure to mercury, fish thyroxin level was altered and stimulated insulin production leading to depletion of blood glucose.Ramalingam (1988) and Kramer *et al.* (1992) recorded that the normal carbohydrate metabolic pathway was altered when fish was exposed to mercury. They also mentioned that fish exhibited an increase in the glycolytic activity in response to mercury treatment.

The mean values of serum AST and ALT activities in the control *Clarias lazera* varied from 86.3 ± 1.6 to 89.3 ± 1.2 and 16.0 ± 1.3 to 16.6 ± 10 units/ml, respectively (Fig. 7). Bentick-Smith *et al.* (1987) reported the normal serum AST activity (95.0 to 143 U/L) and ALT activity (17.5 U/L). The AST and ALT activities in treated *Clarias lazera* showed gradual significant higher values from the 1st week (AST) and 2nd week (ALT). Our results are in agreement with the previous results of Hilmy *et al.* (1980a) and Suresh *et al.* (1991). They described increased activities of both serum AST and ALT in fish exposed to mercury. On the other hand, Salmeron *et al.* (1990) showed that there were non-significant changes in the activities of serum AST and ALT in fish exposed to 188 mg/Pb/L. Reeves (1981) mentioned that the serum transaminases levels are normally low, and they were liberated into the serum after extensive tissue destruction.

Results of the residual contents of Pb, Hg and As in the soft tissues of the experimental *Clarias lazera* were significntly higher than those present in control ones (Figs. 8, 9 and 10). Liver tissue was found to contain the highest amounts of residual elements, followed by kindneys, while, muscles were the least tissues. Bohn and Fallis (1978) found that residues of arsenic were mainly concentrated in muscle tissue, followed by liver tissue in the *Shorthorn. sculpins.* Crespo *et al.* (1986) found that lead residues were concentrated in kidneys, followed by liver in the rainbow trout fed lead at concentration of 10 mg/kg/day for 15 days.

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Table 2 showed the effect of different rice entries at flowering stage on the biology of the rice stem borer; *Chilo agamemnon* Bles .

As the larval mortality parameter was used, the highest percentages were obtained from rearing the larvae on diets containing powder of GZ 4071-16-1 (42.6%), while the lowest larval death was obtained from rearing the larvae on diets containing GZ 4255-3-1 (2.0 %).

As for the larval duration, it ranged from 27.5 to 34.6 days. The longest mean of larval period occurred in case of GZ 4071-16-2-1 (34.6 days) and Kagahi-kari (33.2 days), while the shortest was obtained from GZ 4255-3-1 (27.5 days) and GZ 4255-6-1 (27.5 days).

Table 2. Effect of some rice entries at flowering stage on biology of *C.agamemnon* .

Entries	Larval Mortality %	Larval duration (day)	Pupal duration (day)		Pupal weight (mg)		Adult longevity (day)	
			Female	Male	Female	Male	Female	Male
Todorokiwase	35	33.2 a	7.2 b	7.5 bc	40.0 ef	26.7 bc	4.2 c	4.3 cd
Gz 4294-10-2	10	32.7 bc	8.1 a	8.9 a	41.0 d-f	22.2 d	5.6 a-c	6.2 a-c
Giza-176	13	31.9 b-d	7.0 bc	7.3 b-d	43.7 c-f	24.5 cd	7.2 a	6.1 a-c
Giza-172	42.6	34.6 a	6.0 f	6.2 cf	38.1 f	25.1 cd	5.0 c-c	6.8 ab
GZ 4122-23-4-2	12	30.0 dc	7.0 bc	7.3 ef	39.8 ef	26.0 bc	4.2 e	4.5 cd
Gz 4071-16-2-1	12.2	30.1 dc	6.9 b-d	7.3 b-d	40.0 ef	26.3 bc	5.0 c-c	5.3 b-d
Kagahikari	11.5	31.0 b-d	7.0 bc	7.4 bc	43.7 c-f	27.3 bc	5.4 b-c	5.7 a-d
Giza - 171	10.8	29.8 c	7.1 bc	7.8 b	44.6 ab	26.4 bc	4.3 e	3.8 d
Gz 4255-3-1	20.5	30.7 c-c	6.6 a	7.1 cd	47.8 a-c	28.8 ab	6.6 ab	5.6 a-d
Gz 4255-6-1	29.8	32.7 bc	6.6 c-c	7.3 bc	41.0 d-f	26.7 bc	6.1 a-d	4.8 b-d
Giza - 181	12.2	27.5 f	6.2 cf	6.0 f	41.3 c-f	25.1 cd	5.0 c-c	6.8 ab
Gz 4255-6-1	9.8	29.1 ef	6.6 c-e	6.7 de	44.6 a-e	27.3 ab	6.8 ab	6.2 ac
Gz 4256-7-4-7	15.0	31.9 b-d	6.4 d-f	6.7 dc	44.6 a-c	27.5 bc	6.7 ab	6.2 a-c
Toridel	32.0	30.2 de	6.7 b-d	7.5 bc	47.8 a-c	28.8 ab	6.1 a-d	6.2 a-c
Gz 4386-34-3	5.9	27.5 f	6.2 ef	6.7 dc	48.9 ab	31.5 a	7.2 a	6.7 ab
Tell Hamsa	2.0	27.5 f	6.0 f	6.1 ef	49.4 a	31.7 a	7.3 a	6.8 ab
Mean	17.1	30.6	6.7	7.1	43.5	27.0	5.8	5.8

Values followed by the same letter are not significantly different at the 5% level of DMRT.

As the pupal weight measure was used, the mean pupal weight for males was lightest in case of Todorokiwase and Giza 181 (24.5 and 22.2 mg) and for females in case of GZ 4255-8-1 and GZ 4071-16-2-1 (39.8 and 38.1 mg). The pupal weight was heaviest, in both sexes, when the larvae were reared on GZ 4255-3-1 (49.4 and 31.7 mg for females and males, respectively) .

The pupal duration was prolonged to 7.2 and 7.5 days for females and males, respectively when larvae were reared on Kagahikari, while it decreased to 6.0 and 6.1 days for females and males, respectively in case of GZ 4255-3-1 entry.

The longevity of adult emerging from larvae reared on GZ 4255-3-1 (7.3 and 6.8 days for females and males, respectively) was the longest, while it was shortest in case of Kagahikari (4.2 and 4.3 days for females and males, respectively).

These data indicated that wide differences existed among the tested entries in their ability to retard growth and development of the borer. This phenomenon could have been due to the unsuitability of the plants (toxic, deterrent and antifeedant substances) for larval survival and development, thereby resulting in reduced population on the resistant entries. These unsuitability characters seem to exist in Todorokiwase, Giza 176 and Kagahikari entries.

As for the effect of the plant growth stage on the insect biology, the response of the borer to most of the tested entries differed as the growth stage differed. Kagahikari caused lower larval mortality at the tillering stage (14%) than at flowering (35 %). While Giza 176 caused higher larval mortality at the tillering stage (55.6 %) than at flowering (20.5 %). These results were confirmed by Israel (1967) who reported that factors responsible for resistance to damage at the different plant stages may be independent. Such factors were stage specificity and the structure of the plant that differ at different stages, (Dhaliwal *et al.*, 1993). Some entries reacted in similar manner for the borer at both stages. GZ 4255-8-1 caused 12.8 and 12.0% larval mortality at the tillering and flowering stages, respectively. The life cycle and weight of pupae were almost similar at both stages, Ukwungwu (1990).

As general view, the resistant entries caused higher mortality, smaller body weight, prolonged larval and pupal periods. The slower rate of development could have two disadvantages to rice stem borer larvae; 1) the longer the period of development the greater the chance that the larvae may fall victim to environment stresses such as predators, parasites and/or adverse weather and 2) the lengthened total growth period for insects on resistant entry may result in late emerging adults

that have diffculty in finding a mate. With resistant entries, all these effects should result in cumulative reduction in rice stem borer subsequent generation, Ukwugwu (1990).

It is important to mention that varietal resistance based on field, chemical and biological evaluation will be used to introduce to the breeder rice entries based on whole plant resistance.

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المقاومة الصنفية : تقييم بيولوجى وانتخاب لبعض أصناف وسلالات الأرز ضد ثاقبة ساق الأرز

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معهد بحوث وقاية النباتات - مركز البحوث الزراعية بالجيزة - الدقى .

تم معمليا تقييم المقاومة البيولوجية فى ١٦ صنف وسلالة أرز مبشرة - فى مرحلتى نمو - لتغذية يرقات ثاقبة ساق الأرز ، وأوضحت الدراسة الآتى :-

١) فى مرحلة التفريع :

بحساب نسبة الموت اليرقى كمقياس للمقاومة، أثبتت السلالات والأصناف جيزة ١٧٦ ، جى زد (٤٢٩٤ - ٤ - ١٠ - ٢) ، تودوروكيـواز ، جى زد (٤٢٥٦ - ٧ - ٤ - ٧) ، جى زد (٤١٢٢-٢٣-٤-٢) انهـا الاكثر مقاومة ، حيث أعطت أعلى نسبة موت تراوحت بين ٢٠ - ٢٥,٦ ٪.

وعند حساب مدة الطور اليرقى، كانت الأصناف والسلالات تودوروكيواز ، جى زد (٤٢٩٤ - ١٠ - ٢)، جيزة ١٧٦ هى الاكثر مقاومة ، حيث ادت الى اطالة مدد الطور اليرقى فتراوح ما بين ٣٢,٣ : ٣٥,٢ يوما عند التربية عليها .

بتقدير فترة التعذر ، كان تودوروكيواز ، جيزة ١٧٦ هما الأعلى مقاومة مسببة أطول فترات تعذر تراوحت بين ٧ : ٧,٧ يوما حسب جنس العذراء ونوع النبات.

عند تقدير وزن العذارى ، اظهر تودوروكيواز ، جيزة ١٧٦ اعلى مقاومة كمسببة لأقل الاوزان العذرية والتى انخفضت الى ما بين ٤٠,٢ : ٢٤,٦ مجم.

بتقدير مدة الطور البالغ للحشرة ، كان تلى همسا ، توريد-١ هما الاكثر مقاومة حيث سببا طول مدة الاطوار البالغة والتى تراوحت ما بين ٩ : ٧,٦ يوما.

ب) فى مرحلة الإزهار :

عند حساب نسبة الموت اليرقى ، أوضحت السلالات جى زد (٤٠٧١ - ١٦ - ٢ - ١)، كاجاهيكارى انها الاكثر مقاومة حيث أعطت اعلى نسبة موت وصلت الى ما بين ٤٢,٦ : ٣٥٪.

وبالنسبة لمدة الطور اليرقى ، كان جى زد (٤٠٧١ - ١٦ - ٢ - ١)، كاجاهيكارى هما أيضا الاكثر مقاومة حيث ادت الى طول الاطوار اليرقية والتى وصلت الى ما بين ٣٤,٦ : ٣٣,٢ يوما.

بقياس فترة التعذر ، كان جيزة ١٨٠ ، كاهيكارى هما اكثر الاصناف مقاومة حيث ادت الى طول مدة العذراء الى ما بين ٨,٩ : ٧,٢ يوما.

عند تقدير وزن العذارى ، كان جى زد (٤٢٥٥ - ٣ - ١) ، جى زد (٤٠٧١ - ١٦ - ٢ - ١) هما اكثر مقاومة حيث نتج عنها اقل العذراى وزنا فيما بين ٢٢,٢ : ٤١ مجم.

عند حساب مدة الطور البالغ، كان جى زد (٤٢٥٥ - ٣ - ١) . جى زد (٤١٢٢ - ٢٣ - ٤ - ٢) هما اكثر الاصناف مقاومة مسببة طول اعمار الاطوار البالغة والتى وصلت الى ما بين ٧,٣ : ٦,٧ يوما.

وبصفة عامة ، فقد اختلفت درجة المقاومة البيولوجية كثيرا باختلاف الصنف ومرحلة النمو النباتية وكذلك الجنس فى الحشرة، وكان جيزة ١٧٦، تودوروكيواز ، كاجاهيكارى هى اعلى الاصناف والسلالات المختبرة مقاومة ، كما كانت النباتات فى مرحلة التفريغ اكثر مقاومة منها فى مرحلة الإزهار.