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SOME STUDIES ON THE DEFENSE MECHANISM OF OREOCHROMIS NILOTICUS FISH EXPOSED TO ACROLEIN POLLUTION AND EXPERIMENTALLY INFECTED BY AEROMONAS HYDROPHILA

(With 2 Tables & 3 Figs.)

By

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بعض الدراسات علي الامكانيات الدفاعيـــة
في سمكة البلطي النيلية المعرضة للتلوث بمبيد الحشائش المائيـــة
الأكرولين عند تعرضها للاصابة بميكروب الايروموناس
هيدروفيـــلا

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صممت الدراسة الراهنة بهدف التعرف علي تأثير التلوث بمبيد الحشائش المائيـــة الاكرولين بالجرعات تحت المميتة (٣ر ، ٦ر جزء في المليون) لمدة ٣ أسابيع في أسماك البلطي النيلي علي العد الكلي والعد التصنيفي لكرات الدم البيضاء . وقد أظهرت النتائـــج فروقا معنوية في زيادة عدد كرات الدم غير المحببة ونقص معنوي في العد الكلي لكريات الدم البيضاء وعدد كريات الدم المحببة . كما اتضح من النتائج استجابة أسماك البلطي المعروضة للتركيزيين السابقيين للحقن البر يتوني بميكروب الايروموناس هيدروفيلا بالصورة الاكلينكية والصفات التشريحية وزيادة نسبتي العدوي والنفوق في هذه الأسماك غير المعرضة للمبيـــد. وقد يعزي هذا نتيجة لتأثير التلوث ـ كعامل ضاغط ـ بمبيد الحشائش المائية (الاكروليـــن) علي الأجهزة المناعية والمكونة لخلايا الدم وخاصة نقص كريات الدم البيضاء المحببة والتـــي تكون خطا دفاعيا هاما في زيادة المقاومة ضد الأمراض البكتيرية للأسماك .

SUMMARY

The present study revealed that the chronic exposure (3 weeks) to sublethal concentrations (0.3 and 0.6 ppm) of the aquatic herbicide Acrolein which is considered as a drastic stress factor on Boltifish Oreochromis niloticus when subjected to the experimental infection with Aeromonas hydrophila intraperitoneally leading to high morbidity (with typical septicemic lesions) and mortality rates in comparison to the un-exposed fish to pollution.

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The total leucocyte count, granulocyte and agranulocyte counts were determined in fishes given Acrolein as an index for alteration in defense mechanism of fishes given two concentrations of Acrolein. In both concentrations, there was significant increase in agranulocyte counts while significant decrease was found in both total leucocytes and granulocyte counts.

The present work proved that the long term toxicity may affect the defense mechanism of O.niloticus fish and subject them to the infection by bacterial diseases.

INTRODUCTION

The drastic and quick spread of aquatic weeds and plants in vast areas in the Nile River leads to major problems such as consuming large amounts of freshwater and nutrients with consequent hindering of navigation; restriction the movements of fishes and limiting space and prevnting light penetration into water with consequent reduction in photosynthesis leading to oxygen depletion (JOHNSON, 1965 and KAMEL, 1974).

Aquatic herbicides are widely used for controlling of undesirable weeds. Magnacides (Acrolein) is considered the main aquatic herbicide used - nowadys - by Ministry of Irrigation in Egypt. ALBIN (1963) reported that Acrolein kills the aquatic plants through its sulfhydryl reactivity of enzyme systems in the plants. However,the NATIONAL RESEARCH COUNCIL (1981) considered Acrolein is quite embryotoxic substance. Moreover, SCHMID, et al. (1981) claimed that Acrolein have teratogenic effect in rats. BROWN (1978) and SANTHANAM, et al. (1987) recorded that Acrolein is very toxic for fishes.

From the pollution picture of view, fishes are damaged in different ways where they are killed or stressed and weakened with suppression of immune response (SNIESZKO, 1974). Consequently they easily become victims to infectious and noninfectious diseases (POST, 1983; KABATA, 1985 and SHEPHERD and BROMAGE, 1988).

The present work was planned to study the blood leucocyte alterations of the cichlid tilapia Oreochromis niloticus fish associated with the stress state induced by the sublethal concentrations of the aquatic weed killer Acrolein. Experimental infection with Aeromonas hydrophila was conducted on both fish who were exposed to chronic toxicity with Acrolein and those which were away from Acrolein in order to discover the role played by water pollutents on fishes.

MATERIAL and METHODS

Handling of fish:

The experimental fish were Bolti-fish "Oreochromis niloticus", they were procured from Fish Hatcharies at Abbasa, Sharkia Governorate. 120 live and clinicaly healthy fish with average body weight 30±5g, were kept in prepared glass aquaria with areated chlorine free tap water. Water temperature was thermostatically adjusted at 25±1C. A sponge filter was fixed to clarify water from fish waste and food residues. All fish were left in aquaria for seven days for acclimatization.

Acrolein (Magnicide):

Magnicide is commercial form supplied by Aqualin Magna Corporation, California, USA. The used Acrolein was of 97% minimum purity as 2-propenal acrylaldhyde.

Estimation of LC_{50}:

50 fish were divided equally into five aquaria used for the estimation of LC_{50} of Acrolein which is calculated according to SPERMAN (1954) and BARTLY (1976). Mortality rate was recorded, during the experimental period (48 hrs).

Clinico-pathological examinations:

30 fish were divided into 3 equal groups. The first and second groups were treated with 0.3 and 0.6 ppm of Acrolein respectively. The drug was injected in the aquaria by means of a glass pippet immersed under water surface. The third group was left as a control and all groups of fish were put under observation for one week, then caught for haematological examinatios. Fresh blood samples from each group were collected through heart puncture method according to LIED, et al. (1975). EDTA was used as an anticoagulant (1.5 mg/ml) according to MCKNIGHT (1966). Total leucocytic counts, granulocytes and agranulocytes were determined according to the methods adopted by LUCKY (1977).

Experimental infection:

40 fish were divided into 4 equal groups. The first and second groups were treated with the same concentrations (0.3 and 0.6 ppm) of Acrolein. The third and fourth groups were untreated (served as control). After 3 weeks the first three groups were experimentally infected intraperitonealy (I.P) with a well identified pure culture of Aeromonas hydrophila according to LUCKY (1977) at a dose of 0.5 ml from 24 hrs. broth culture (total bacterial count, $5x10^6$/ml). The fourth group was kept as a control. The identification of the bacteria was carried out using the morphological features, colonial and growth appearance as well as biochemical reactions according to LENNETTE, et al. (1980) and KRIEG and HALT (1984). Reisolation of the same bacteria was attempted. Besides, recording of the clinical signs, the P.M. lesions and morbidity and mortality rates.

Statistical analysis:

The obtained results of all data of blood leuocytes examinations were statistically carried out according to SNEDECOR and COCHRAN (1969).

RESULTS

The detection of LC_{50} of Acrolein in O.niloticus fish after 48 hours was 0.80 ppm.

The total leucocyte counts, granulocytes and agranulocytes counts of healthy and treated O.niloticus fish with both sublethal concentrations (0.3 and 0.6 ppm) of Acrolein are showed in Table 1, and Fig. 3.

Table 2 revealed the morbidity and mortality rates in treated and untreated O. niloticus fish with the same concentrations of Acrolein and experimentally infected with Aeromonas hydrophila intraperitoneally. Reisolation of A.hydrophila was obtained from all the dead and sacrificed experimentally infected O.niloticus fish.

The clinical signs of toxicity 3 weeks post experimental infection revealed that affected fish manifested the following clinical signs: dark skin, bilateral exophthalmia, gasping, rolling in circular manner, erythema in mouth, opercula, base of the fins and tail rot (Fig. 1 and 2). The post mortem examination showed severe congested internal organs, friable muscles, distension of gall bladder, the gills are hyperemic, enlarged liver, petecheal haemorrhage in the kidneys. While, the unexposed fish showed low morbidity rate and mild form of septicaemia (Table 2).

DISCUSSION

As a fact, fish are the most important indicators for water pollution. According to the classification of HODGE and STRENER (1956) Acrolein is one of the highly toxic substances to fish. From this point, the present work delt with the alterations in the defense mechanisms of our native breed "Nile Bolti" Oreochromis niloticus fish with sublethal concentrations of the aquatic herbicide "Acrolein" in chronic toxicity. This was evaluated by investigating the changes of the total number of blood leucocytes and their differential count. The significant increase of agranulocytes and the significant decrease of both total leucocytes and granulocytes in both concentrations may indicate the toxic effects of Acrolein on the haemopoietic organs (Anterior kidney, liver, spleen and thymus). This suggestion is supported by BARNHART (1969), JACKIM, et al. (1970) and REHWOLDT (1978). In addition, WOTTON and FREEM (1982) recorded that Acolein leads to degeneration of kidneys. Moreover, DRAMINSKI and HENCHLER (1983) reported that hepatic glutathion was depleted in mice injected I/P with Acrolein. Glutathions

act as protective agent against mutagenic chemicals. They added also that the reaction of Acrolein with thiols to form thiol ether is irreversible.

As SNIESZKO (1974) reported that the disease is not a simple result of contact between host and pathogen, there was a complex interaction between host, pathogen and environment. Thus, the second experiment was designed as such base, consequently treated O.niloticus fish with both concentrations revealed high morbidity and mortality rates when experimentally infected (I.P) with A.hydrophila in comparison to unexposed fish with Acrolein. The clinical signs and the septicaemic lesions were more severe as the concentration of Acrolein increases. This may be indicative to the immune suppression in O.niloticus fish with Acrolein, in chronic toxicity and the decrease in granulocytes may give a great chance for bacterial invasion.

As A.hydrophila bacteria is normally inhabitant in fish (POST, 1983). Thus, the pollution by the aquatic herbicide played as a stress factor enhancing and favouring the infection by A.hydrophila. This was cleared in the (I.P) experimental infection where the experimental fish nearly showed similar clinical signs and P.M. lesions like observed in natural infection with redmouth disease. The pathogenicity of A.hydrophila to fish was attributed to the production of proteolytic enzymes (KOU, 1972). Moreover, WAKABAYASHI, et al. (1981) reported that A.hydrophila produce extracellular enzymes (elastase, staphylolytic and bacillolytic), cytotoxins and haemolysins.

In conclusion, the present study suggests that we must look forward to the natural and non-pollutant substances to eradicate the undesirable aquatic weeds for protecting fish and man.

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Table (1) : Blood leucocytes changes in healthy O.niloticus fish and those with chronic Acrolein toxicity (3 weeks).

Acrolein	No.	Total leucocytes (X $10^3/\mu l$)	Granulocytes %	Agranuloctes %
0.3 ppm	10	5.20±0.43*	29.70±1.36**	71.30±1.42**
0.6 ppm	10	3.45±0.63**	20.10±3.20**	79.90±3.85**
control	10	6.60±0.40	37.80±1.68	62.20±1.90

* $P < 0.05$ ** $P < 0.001$

Table (2) : Morbidity and mortality rates in healthy and experimentally treated fish (O.niloticus) with Acrolein and those infected by A.hydrophila after 3 weeks.

Group	No.	Treatment	Route & dose per each fish	Temperature	Morbidity rate	Mortality rate
1	10	0.3 ppm	I/P 0.5 ml ($5X10^6$ml)	25±1 °C	80.0 %	20.0 %
2	10	0.6 ppm	I/P 0.5 ml ($5X10^6$ml)	25±1 °C	60.0 %	40.0 %
3	10	--	I/P 0.5 ml ($5X10^6$ml)	25±1 °C	20.0 %	00.0 %
4	10	--	0.5 ml sterile broth	25±1 °C	00.0 %	00.0 %