Environment Conservation Journal 10(3) 87-92, 2009
(ISSN 0972-3099)

Toxic effect of *Euphorbia tirucalli* (Pencil tree) sap on *Tilapia zillii* fingerlings

L.E. Akpa[1], M.N.O. Ajima[1], C.D. Nwani[2] and C.A. Onu[1]

Received: 13-04-2009 Revised: 28-06-2009 Accepted: 22-07-2009

Abstract

Fingerlings of *Tilapia zillii* (5.20 gm ±0.03) were exposed to sap extract of *Euphorbia tirucalli* at concentrations of 6.00, 3.00, 1.50, 0.75 and 0.38 mg-1 with control as 0.00 mg-1 for 96 hours. The static bioassay showed that the 96-hour LC_{50} was 1.20 mg-1 with lower and upper confidence limits of 0.78 and 1.85 mg-1 respectively. Erratic swimming, loss of balance, respiratory distress, air gulping was observed before eventually death of the fish. Opercular ventilation and tail fin counts increased with increasing concentrations of the sap extract. Histopathological examination of the gills and liver revealed damages to these organs which were directly proportional to the concentration of the sap extract while those in the control tanks remained unchanged. Phytochemical analysis of the sap extract showed the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, phenols, volatile oil, balsam and steroids. Water quality parameters monitored showed no significant difference (P>0.05) in temperature and pH while there was significant difference (P>0.05) in values obtained in dissolved oxygen, free carbon oxide and alkalinity. The implications of the findings as they affect the exposed fish and the aquatic ecosystem are discussed.

Keywords:- *Euphorbia tirucalli, Toxicity, Fingerlings, Tilapia zillii*

Introduction

In developing countries, crude extracts from plants are applied indiscriminately to water bodies with little or no regard to their detrimental effects caused to the aquatic organisms, livestock and humans. However, Kroupova *et al.* (2005) documented that the deliberate introduction of toxicants into aquatic ecosystem could eventually lead to disruption of multiple physiological disorders such as ion regulatory, respiratory, cardiovascular, endocrine and excretory processes and these could ultimately reduce their productivity capabilities. Respiratory distress as a result of fish in polluted water bodies have been reported by several authors; however, Banerjee (2007) observed that congestion of blood capillaries, periodic lifting and sloughing of respiratory epithelia of the secondary lamellae causing haemorrhage, extensive fusion of secondary lamellae and hyperplasia of the respiratory epithelia due to uncontrolled regeneration are the major causes leading to asphyxiation and eventually death of the fish if exposure is prolonged excessively.

The plant *Euphorbia tirucalli* which belongs to the family euphorbiaceae is commonly known as Barki-thohar. This plant is a native of America but has become acclimatized and grow freely in all parts of the world. It is a common medicinal plant of India (Satyarvati and Gupta, 1987). Pisicidaln activities of aqueous extracts of *Euphorbia tirucalli* were very well established, but their ultimate mode of action on fish metabolism was not yet known. Tiwari and Singh (2006) reported that exposure of fishes over 24 hr or 96 hr to sub-lethal doses (40% and 80% of LC_{50}) of aqueous extract of *E. tirucalli* stem-bark and latex, significantly (P<0.05) altered the level of total protein, total amino acids, nucleic acids, glycogen, pyruvate, lactate and activity of protease, alanine aminotransferase, aspartate aminotransferase, acetylcholineasterase and cytochrone oxidase enzyme in liver and muscle tissues of freshwater fish *Channa punctatus*. The alterations in all these biochemical

Author's Address
[1]Department of Zoology, University of Jos, Jos, Nigeria
[2]National Bureau of Fish Genetic Resources Canal Ring Road, P.O Dilkusha, Lucknow ✉

parameters were significantly ($P<0.05$) time and dose dependent. The authors documented that aqueous extracts of *E. tirucalli* adversely affect respiratory pathways of fish and cause energy crisis during stress by suppressing ATP production and that the reversibility of the action of the aqueous extracts would be additional advantage in their use. In this research the effect of *Euphorbia tirucalli* on *Tilapia zillii* was investigated under laboratory condition.

Materials and Method

Experimental fish

Fingerlings (mixed sex) of *Tilapia zillii* mean weight (5.20 gm ±0.03) were collected from Renaji Integrated Fish Farm in Rayfield Jos, Plateau state. They were transported to University of Jos Fisheries Research Laboratory with the aid of well aerated oxygen bag. The fish were held in plastic tanks and acclimated to laboratory condition for a period of two weeks.

Experimental design

Euphorbia tirucalli (pencil tree) was obtained from the rocks along Bauchi Ring Road Angwarukuba area Jos plateau state. The plant was identified by the Botany Department, University of Jos, Nigeria. The plant sap was collected by means of an incision into the stem and the sap was collected into small clean transparent plastic bottles. Based on the preparation and preliminary tests, serial dilutions were made and the concentrations of the extracts used were 6.00, 3.00, 1.50, 0.75 and 0.38 mg^{-1} while dechlorinated tap water without sap extract (0.00 ml^{-1}) served as the control. For the set up of the experiment, eighteen circular plastic tanks (40cm x 5cm x 24cm) were used as each concentration was in triplicate in order to minimize experimental errors.

The fish were not fed for 48 hours prior to and during the exposure period. Dechlorinated well aerated municipal tap was used. Each tank was stocked with ten fish. The tanks were examined on a daily base and dead fish were removed and recorded immediately from test solutions to avoid polluting the test media.

Phytochemical analysis to determine the active ingredients present in the extract was performed using the procedure described by Sofowora (1982). The physico-chemical analysis of the test water; temperature, dissolved oxygen, alkalinity, free carbon dioxide, pH were determineded using analytical method described in APHA (1995) and Khanna and Bhutiani (2004). The 96-hour LC_{50}, lower and upper confidence limits were estimated using the method for acute toxicity tests as recommended by UNEP (1989). Histopathological examinations of the gills, liver and kidney after exposure period were done using method described by Buck and Wallington (1972). The results obtained from this investigation were subjected to statistical analysis using two-way analysis of variance (ANOVA) to test for level of significant between the various levels of the sap extract of *E. tirucalli* concentrations.

Results and Discussion

The result of the physico-chemical parameters of the experimental media (Table-1) indicated a significant difference ($P<0.05$) in the values obtained for dissolved oxygen content, free carbon dioxide and alkalinity with the control while there were no significant different ($P>0.05$) between the values of temperature and pH with the control. The result indicated a reduction in level of dissolved oxygen content and an increase in alkalinity of the test media. The abnormal behavior observed in fish exposed to the extract before death included respiratory distress, loss of balance, gulping of air, settling at the bottom motionless, sluggish movement and erratic swimming. The abnormal behaviors displayed by the exposed fish increased with increasing concentrations of the sap extract.

The mortality rate of *Tilapia zillii* exposed to *E. tirucalli* sap extract showed l00% mortality at 6.00 mg^{-1} concentration, 90% at 3.00mg^{-1}, 70% at 1.50 mg^{-1}, 40% mortality at 0.75 mg^{-1} concentration. No mortality was observed in the group of fish in the control group experiment and 0.38 mg^{-1} concentration during the exposure period. Observed mortality increased with increasing concentration of the sap extract, showing both time as well as dose dependent relationship. The first 24-hr of the exposure was critical for the survival of the fish as most of mortalities were recorded during the period. No fish survived

Table-1: Mean values of Physico-chemical parameters measured during 96 hour exposure of various concentrations of sap extract of *E. tirucalli* to *Tilapia zillii*

Parameters	Concentrations (mg/l)					
	6.00	3.00	1.50	0.75	0.38	0.00
Temperature (^{o}C)	23.28±0.12	23A5±0.05	23.55±0.15	23.56±0.25	23.40±0.15	23.83±0.36
DO (mg/l)*	2.32±0.22	2.87±0.33	4.29±0.28	4.69±0.25	5.00±0.16	6.20±0.16
pH	6A6±1.02	6.55±1.32	6.60±1.22	6.89±1.l0	6.63±1.20	7.50±.1.04
FCD (mg/l)**	5.35±0.26	4.27±0.30	3.75±0.13	3.60±0.32	3.39±0.38	3.30±0.24
Alkalinity (mg/l)	13.93±0.12	l2.58±0.3l	10.68±0.63	7.73±0.71	l2.35±0.85	4.78±0.35

Note:- *Dissolved oxygen, ** Free Carbon dioxide

longer than 48 hr during exposure to 3.00 mg^{-1} and above. The mean value of 96 hr LC_{50} of the sap extract to the test fish was calculated to be 0.87 mg^{-1} with lower and upper confidence limits of 0.60 and 2.40 mg^{-1} respectively (Fig. l). The dead fish in all the sap extract concentration showed grey red coloration of the skin with brownish gill. The exposed fish exhibited higher opercular ventilation rate and tail frequency compared to the values obtained for the control group (Table-2). The result obtained from this research from water quality (dissolved oxygen) of the test media is not sufficient for the survival of living organism. At the concentrations used in this investigation, the extract led to the significant reduction in the dissolved oxygen and an increase in alkalinity of the test media. The air gulping observed in the exposed fish during exposure period was an indication of insufficient amount of dissolved oxygen in the experimental media. This can be attributed to the adverse effects of concentration of sap extract in the media. Stickney (1979) had reported that insufficient amount of dissolved oxygen is one of the contributing factors to mortality and poor growth of freshwater fish species. The general oxygen starvation can lead to the gasping behavior as observed in this investigation as a result of the introduction of the sap extract of *E. tirucalli* in the experimental media. Aggergaard and Jensen (2001) reported similar observation n when they exposed nitrite to difference fish species. The behavioral changes which were characterized -respiratory distress, loss of balance, settling at the bottom motionless and erratic swimming as reported in this investigation compared favorably with the observation of Pascual *et al.* (1994), Kroupova *et al.* (2005), Svecevicius (2006), Jain *et al.*(2007) when they exposed some species of fish to different toxicants.

Respiratory distress noticed in exposed fish could be caused by mucous precipitation and neurological

Table-2: Mean values of opercular ventilation rate per minute of *Tilapia zillii* exposed to varying concentrations of sap extract of *E. tirucalli* for 96 hours

Concentration (mg/l)	Exposure period (h)				
	Start (0)	24	48	72	96
6.00	l40.00±0.20	l40.00±1.05	-	-	-
3.00	138.00±0.06	136.00±1.l0	128.00±0.28	l24.00±0.10	l22.00±0.01
1.50	132.00±0.02	l26.00±1.20	l18.00±0.40	115.00±0.14	107.00±0.23
0.75	l28.00±1.21	l22.00±0.06	110.00±0.33	102.00±0.22	100.00±0.12
0.38	112.00±0.58	110.00±0.08	109.00±0.02	105.00±0.13	92.00±0.02
0.00	86.00±0.02	85.00±0.01	87.00±0.07	88.00±0.04	89.00±0.08

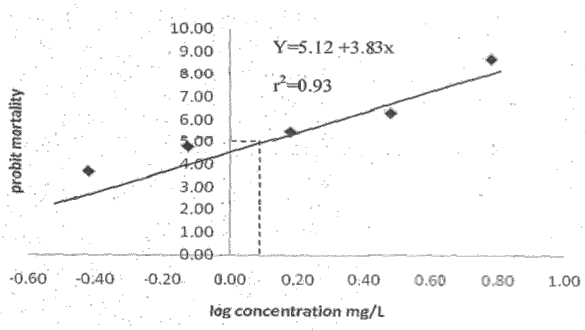
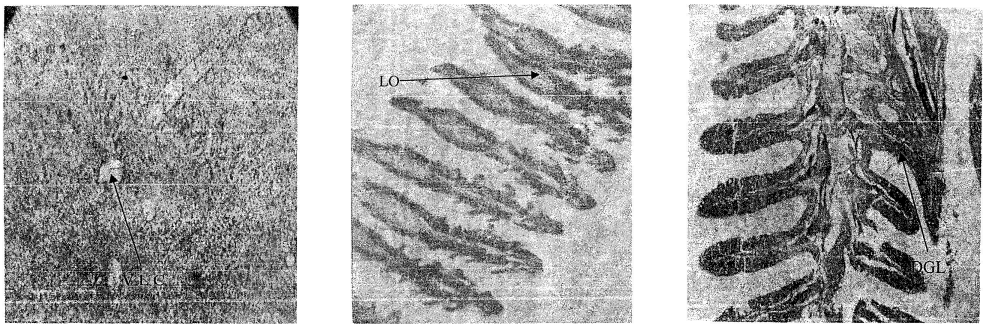


Fig. 1: Linear relationship between probit mortality and log concentration of sap extract of *E. tirucalli* exposed to *T. zillii* for 96



A **B** **C**

Fig. 2: Histopathological section of liver and gill (x 100) of *Tilapia zilli* exposed to different lethal concentrations of *T. tirucalli* for 96 hours. (A) and (B) exposure to 6.00mg/L (C) exposure to 3.00mg/L (VLC = Vacuolation ofliver cell, LO = Lamellar oedema, DOL = Degeneration of gill lamellar)

dysfunction of gill epithelia in response to the toxicant which resulted in high respiratory rate as reported by Lin and Lin (1990) and Banerjee (2007). The effectiveness of the gill epithelium not only as an organ of respiration but as the mediator of osmoregulation and associated processes may be severely impaired by the sub-lethal quantities of toxic substances David *et al.* (1976). Generally, fish and crustaceans in response to either environmental hypoxia or impaired dissolved oxygen diffusion at the gills are to increase ventilation rates (Morris *et al.*, 2005). A hyperventilation in response to sap extract of *E. tirucalli* exposure to *T. zillii* may be a survival mechanism to endure severe degradation of water quality and consequent impairment of dissolved oxygen uptake. Increased ventilation rate could be as the result of the toxicant in the test media as it reduced the amount of oxygen present in the media. The fish could have increased ventilation rates in an attempt to make up for the loss in oxygen content in the gill. High opercular ventilation has been reported by Sprague (1993) as an index of stress when fish come in contact with an unfavorable environmental condition. Therefore, sap extract of *E. tirucalli* induces significant hyperventilation in *Tilapia zillii*. The 96-hour LC_{50} which was estimated as 1.20 ml^{-1} with upper and lower confident limits of 1.85 and 0.78 mg^{-1} means that the concentration of the extract

which is close or in excess of 1.20 mg^{-1}, can cause mortality to *T. zillii*.

Histopathological examination of the test fish showed some pathological disruptions (Fig. 2). The liver cells revealed necrosis and vacuolation of the liver cells while gills showed oedema of the gill lamellar. The histopathological disruption done to the Tsed fish as observed in this investigation are harm caused by the *E. tirucalli.* Cardoso *et al.* (1996)) and Cengiz *et al.* (2001) reported that histopathological studies of fish exposed to pollutants revealed that organs were efficient indicators of water quality. This is because gills are important organs in fish for respiration, osmotic regulation, acid base balance and nitrogenous waste excretion. The damage done to these organs as the result of the toxicant correlates with the concentrations of the toxicant in each experimental tank. The phytochemical analysis of the leaf extract revealed the presence of alkaloid, tannin, saponin, cardiac glycoside, rotenone, steroids, balsam, phenol and volatile oil. Francis (2001) reported that these chemical substances affect the productivity and health of fish. Rotenone in aquatic environment can reduce amount of dissolved oxygen in water. Robertson and Smith-vaniz (2008) observed that when fish are poisoned with rotenone, they swim erratically and move to shallower water or come to the surface gasping for air. After that, their ventilation rate slows and they sink to the bottom where they remain until death. From the data obtained from this investigation, it is evident that *E. tirucalli* is toxic to *Tilapia zillii* and could possibly affect other aquatic animals. However fishermen should be discouraged and also be enlightened on the effect of using this plant on fish. Relevant authorities should be adequately informed to set quality criteria on the wise use of plants especially *E. tirucalli* in the aquatic ecosystem.

Acknowledgement

The authors acknowledge the assistance rendered by the Department of Zoology, University of Jos, Jos Nigeria for the provision of equipment and reagents used for the investigation. The data reported herein forms part of the Degree research project undertaken by Mr. C.D. Onu.

References

Aggergaard, S. and Jensen, F.B., 2001. Cardiovascular changes and physiological response during nitrite exposure in rainbow trout. ***J. of Fish Biol.***, 59: 13-27.

APHA, 1995. *Standard methods for the treatment of water waste APHA/* AWW*A/WPCE/* American Public Health Association Washington DC.

Banerjee, T.K., 2007. Histopathology of respiratory organs of certain air-breathing fishes of India. ***J.Fish Physiol. and Biochem.***, 33: 441-454.

Buck, D. and Wallington, R.A., 1972. *Some histological techniques applicable to fish tissues*. In. Maudeshy Thomas B.E.(eds), Disease of fish proceeding of symposium No 30 Zoology Society of London academic Press and Zoological Society of London.

Cardoso, E.I., Chiarini-Garcia, H., Ferreira, R.M.A. and Poli, C.A., 1996. Morphological changes in the gills of *Lophiosilurus alexandri* exposed to unionized ammonia. ***J. of Fish Bioi.***, 49: 778-787.

Cengiz, E.I.,Unlu, E. and Balci, K., 2001. The histopathological effects of Thiodan on the liver and gut of mosquito fish, *Gambusia affinis*. ***J. of Environ. Sci. and Hlth.***, 36:75-85.

David, L.B., Robert, W.B. and Moti, L.S., 1976. Respiratory stress in yellow perch induced by subtoxic concentrations of Diquat. ***Ohio J. of Science***, 2: 76-87.

Francis, G., 2001. Effect of important nutrients in some commonly used alternative fish feed ingredients. ***Aquaculture***, 199: 197-227.

Jain, K.L., Gupta, R.K., Mitta1, Vinta and Kumar, Dushant., 2007. Chronic toxicity of arsenic in combination with cobalt and nickel in *Cirrihinus mrigal*. ***J. Ecophysiol. Occup. Hlth.***, 7: 1-4.

Khanna, D.R. and Bhutiani, R., 2004. *Water analysis at a glance*. Pub. ASEA. pp: 1-115.

Kroupova, H., Machova, J. and Svobodova, Z., 2005. Nitrite influence on fish: a review. ***Vet. Med.-Czech***, 50: 461-471.

Lin, C.C. and Lin, C.I., 1990. *Test for ammonia toxicity of cultured hybrid Tilapia*. Asian Fisheries Society Manila, Philippines. pp: 457-459.

Morris, S., van Aardt, W.J and Ahem, M.D., 2005. The effect of lead on the metabolic and energetic status of the Yabby, *Cherax destructor,* during environmental hypoxia. ***Aquatic Toxicology***, 75: 16-31.

Pascual, F.C., Tayo, G.T. and Cruiz-lacierda, E.R., 1994. Acute toxicity of formalin to sea Bass *(Lates calcarifler)* fry. *Asian fisheries Society* 346-348.

Robertson, D.R. and Smith-vaniz, W.F., 2008. Rotenone: An essential but demonized tool for assessing marine fish diversity. *Bioscience,* 58,165-170.

Satyarvati, G.V and Gupta, D.K., 1987. Medicinal plants of India. I.C.M.R. New Delhi.

Sofowora, E.A.,1982. Medicinal plants and traditional medicine in Africa. John Wiley and Sons New York 405pp.

Sprague, J.B. 1993. Measurement of pollutant toxicity to fish. (111). Sub-lethal effects and safe concentration. ***Water Research,*** 5: 245-266.

Stickney, R.R., 1979. Principle of warm water Agriculture New York. John Wiley and Sons Incorporate U.K. pp: 350.

Svecevicius, G., 2006. The use of fish avoidance response in identifying sublethal toxicity of heavy metals and their mixtures. International conference on ecotoxicology trends and perspectives 17-20 September 2006 Wilsa Poland.

Tiwari, S. and Singh, A., 2006. Biochemical stress response in freshwater fish *Channa punctatus* induced by aqueous extracts of *Euphorbia tirucalli* plant. ***Chemosphere,*** 64: 36-42

UNEP., 1989. Estimation of pollutants to marine fish and invertebrates. United Nations Environmental Program, Reference methods for marine pollution studies. No 43.