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Sublethal Effects of Tobacco (*Nicotiana Tobaccum*) Leaf Dust on Enzymatic Activities of *Heteroclarias* (a Hybrid of *Heterobranchus Bidorsalis* and *Clarias Gariepinus*)

Kabir Mohammed Adamu

Department of Animal and Environmental Biology, P.M.B. 001, Delta State University, Abraka, Nigeria.

Abstract

Tobacco (*Nicotiana* tobaccum) leaf dust has both piscicidal and fertilizer properties. Thus there is a need to study its effects at sub-lethal concentrations (375.60, 187.80, 93.90 0.0mg/L) on some enzymatic activities of *Heteroclarias* in a static semi-renewable bioassay system with the aim to ascertain its effect on the test fish after the 14 days exposure period. Water quality parameters and physiological parameters were monitored/determined according to standard procedures. Water quality parameters were monitored after 48hours with the exception of temperature which was monitored daily. The monitored water quality parameters such as temperature, free carbon (iv) oxide, pH and dissolved oxygen were significantly decreased while total alkalinity and conductivity increased significantly in the exposed media, compared to the control test. The effects of the plant dust on the test fish was dose-dependent, revealing insignificant difference in alkaline and acid phosphatases, aspartate and alanine aminotransferases and gamma glutamyltransferase while significant difference was observed in lactate dehydrogenase in serum, liver and kidney respectively of the fish exposed to the plant dust, compared to the control after the 14 days exposure period. From the determined enzymatic activities, the effect of the plant dust was most pronounced in the kidney, but less in the liver and in the serum. However, the monitored water quality parameters revealed that the plant dust has effects on primary productivity, and consequently the biodiversity of organisms.

Keywords: Tobacco Leaf Dust, Enzymatic Activities, *Heteroclarias*, Serum, Liver and Kidney.

1. Introduction

Aquaculture is increasingly becoming one of the fastest growing aspect of the agricultural industry worldwide (FAO, 2004). In semi-intensive system of farming, the management of water pond weeds is one of the most important aspects of a successful production system (Noga, 1996). However, the presence of predatory fishes; 'weed' fishes, such as *Chaoborus* larva, tadpoles, frogs and leeches in fish culture ponds is a serious problem in aquaculture, which is due to their faster growth rate, as they share and better utilize cultured habitats and their food (Jhingran 1983). Therefore, removal of predators/weed fishes from pond is necessary before the seed of cultured fish is added. For controlling these predators, fish farmers often use tobacco leaf in controlling these unwanted organisms/pests (Konar, 1970, Tobor, 1990). According to Aleem (1987), the use of tobacco leaf dust is due to its inexpensiveness, local availability and easier degradability. Despite, the effective use of this plant material, eco-toxicologists are interested in the ecotoxic properties of plant origin pesticides/piscicides, such that plant origin pesticides / piscicides cannot be used directly in freshwater bodies unless their toxicity and sublethal long term effect have been studied on non-target animals, sharing the habitat with the target animals.

The most valuable part / active ingredient of the plant used, is the nicotine (Hassal, 1982). Nicotine (C_5H_4N)-CH-$(CH_2)_3$-N-(CH_3) is made up of pyridine and pyrroliding ring. Nearly all the nicotine is produced in the root and transported to the leaves for storage. It is soluble in water, alcohol, chloroform, ether, kerosene and some fixed oils (Vogue, 1984). Tobacco leaf dust has been used in Nigeria as an effective insecticides and treatment of predators/pest in water (pond) since it is completely biodegradable (Aleem, 1987; Tobor, 1990).

Heteroclarias is a hybrid of the African catfish *Clarias gariepinus* and *Heterobranchus bidorsalis.* They are omnivores which are desirable as food valuable species world-wide. They are one of the commercially important species of fish for rapid aquaculture expansion in Nigeria and elsewhere in the developing world.

Omoniyi *et al.,* (2002) and Agbon *et al.,* (2002) had reported the effect of tobacco leaf dust on *Clarias gariepinus*. Control of mollusk in fish pond can be accomplished by using tobacco waste (FAO, 1970). In Taiwan, tobacco waste dust is applied at 1 ton acre as a pesticides and fertilizer in fish ponds (Jhingran, 1975).

* Corresponding author. kabrmoh@yahoo.com.

The choice of the test fish *Heteroclarias* is attributed to the report of Rand *et al.,* (1995) that in order to extrapolate meaningful, relevant and ecological significant results from aquatic toxicity tests, not only appropriate test but also appropriate organism should be used, whenever possible, species should be studied if indigenous to, or representative of the ecosystem that may be impacted; thus the choice of the *Heteroclarias* which constitutes one of the main fish species is of economic importance in the Niger Delta as an abundant cultural fish species in Nigeria and is very popular with fish farmers and consumers.

The knowledge of sublethal effects of xenobiotic compounds on enzymatic activities is very important to delineate the health of fish status and to provide a future understanding of ecological impacts (Radhaiah *et al.,* 1987). The activities of enzymatic activities are useful 'markers' physiological of damage to the organs such as the liver and hepatocytes (Chapatwala *et al.,* 1982; Ngaha, 1982; and Akanji *et al.,* 1993), thus are needed to be assayed in the test fish.

The aim of this research is to ascertain the assumption whether tobacco leaf dust (*Nicotina tobaccum*) in a sublethal concentration and in a medium exposure time can influence changes in the values of enzymatic activities (alkaline and acid phosphatases, aspartate and alanine aminotransferases, lactate dehydrogenase and gamma glutamyltransferase) in serum, liver and kidney of the test fish – *Heteroclarias* and which of the examined tissue/organs (serum, liver and kidney) determined enzymatic activities after the sublethal exposure is most affected by the pesticide/piscicides – tobacco leaf dust after the 14-day exposure period.

2. Materials And Methods

2.1. Experimental Fish

Juvenile of the fish *Heteroclarias* of mixed sex and the same brood stock: mean weight (29.07±0.34g) and length (17.35±0.23cm) were obtained from Asaba fish farm in Emede, Isoko Local Government Area of Delta State, Nigeria. They were transported to the Department of Animal and Environmental Biology Research Laboratory, Delta State University at the early hours of the morning (6.00 – 8.00 hour) in a large plastic container. The fishes were acclimatized for 14 days during which they were fed to satiation with commercial fish feed pellets (copens 2.0mm) twice daily. Left-over feed and faeces were siphoned off promptly and dead fish were promptly removed to avoid contamination. The percentage of death recorded during acclimatization was less than 2% as such the fishes were accepted as being adapted to the laboratory conditions. They were then transferred to the experimental plastic aquaria (ten (10) fish/40L aquaria).

2.2. Tobacco Leaf Dust

The leaves of tobacco (*Nicotiana tobaccum*) were obtained from Apinko garden, Lokoja, Kogi State, Nigeria, which was identified by Dr. S. M. Ayodele of the Department of Botany, Delta State University, Abraka, Nigeria. The collected sample leaves where sun dried for 14 days (as has been practiced by the fish farmers) and grounded into powder with the use of laboratory mortar, and pestle, and sieved before been stored in a sealed plastic container until required. The concentrations of tobacco used for the experimentation were calculated as 50% 96 h LC_{50}, 25% 96 h LC_{50} and 12.5% 96 h LC_{50} (96 h LC_{50} of tobacco leaf dust on the *Heteroclarias* obtained from preliminary investigation was 751.20mg/L). Thus 375.60, 187.80 and 93.90mg of tobacco leaf dust were measured and homogenously mixed in 1 liter of water to give 375.60, 187.80, 93.90 0.0mg/L concentration of the tobacco leaf dust. These concentrations were introduced into four (4) sets of aquaria with one replication.

2.3. Experimental Procedure

Forty (40) liters capacity aquaria were maintained throughout the exposure period. Ten (10) juveniles each were placed in the 40L plastic aquarium. Bore-hole water was used during the acclimatization and exposure period. Feeding regime (0.800 and 1800 hours) during exposure period was the same as that of acclimatization period. In order to monitor the toxicant strength, level of dissolved oxygen, the effects of evaporation; ammonia concentration and reduce stress during experimentation, the test media were replaced by 50% prepared – concentrations of the same quantity after removing its equivalent along with undigested food and defaecation every 48 hours to maintain the requisite level and potency of the concentration. The exposure period lasted for fourteen (14) days during which some water quality parameters were monitored after 48 hours with the exception of temperature which was monitored daily by using the method described by APHA (1998). At the start (0 hour) of the experiment, the sum total of ten (10) fishes was sacrificed and analyzed for the enzymatic activities.

2.4. Sampling Techniques

Blood was obtained from randomly selected fifteen (15) fish from the control and the exposed test after the 14 days exposure period, using 2.0ml plastic syringe. This was done as described in Kori-Siakpere, (1998). The blood was transferred into a lithium heparin anticoagulant tube and allowed to clot at room temperature for 30 – 40 minutes (Mahoba, 1987). Serum was thereof obtained by centrifugation, using Hawkley centrifuge for 10 minutes at 3,000rpm (Ogbu and Okechukwu, 2001). The serum was transferred into anticoagulant free test-tube and stored at refrigerator until analyses.

After blood collection, the fishes were sacrificed. The desired organs (liver and kidney) were removed from the fish and pulverized in a laboratory mortar and pestle while extractions were prepared by adding 2ml of 10% sucrose solution before been centrifuged (Mahoba, 1987) and stored in another test-tube in the refrigerator until analyses.

2.5. Data Analysis

2.6. All data were presented as means ± standard error, the data from the 14-day tobacco leaf dust exposure was first analyzed using a one-way (concentration) analysis of variance, after which individual means were compared, using Bonferoni multi-sample correction/test. Control values obtained at the beginning and the end of the 14-day exposure period were not significantly different and were therefore combined as one control. In all cases, differences were considered statistically significant at either $p<0.01$ or $p<0.05$. All statistical analyses were performed, using the software (GraphPad Prism® Software version 5.0, San Diego, CA – Trial version).

3. Results

3.1. Water Quality Parameters

The mean values of the water quality parameters of the different sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust and control media to which the test fish *Heteroclarias* were exposed over the 14 days exposure period is as presented in Table 3.1. The value of temperature and free carbon (iv) oxide, pH and dissolved oxygen were found to significantly (p<0.05) and (p<0.01) decreased as the concentrations of tobacco leaf dust increased. However, the values of total alkalinity and conductivity in the exposed media were found to be significantly (p<0.01) increased as the concentrations of tobacco (*Nicotiana tobaccum*) leaf dust increased, compared to the control test.

3.2. Enzymatic Activities

The changes in enzymatic activities (gamma, lactate dehydrogenase, acid phosphatase, alkaline phosphatase, aspartate aminotransferase glutamyltransferase and alanine aminotransferase) recorded in the test fish *Heteroclarias* following exposure to the various sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust over a 14 days exposure period are as presented herein.

The activities of acid phosphatase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period are as presented in

Table 3.1. Mean values[#] (SE) of Water Quality Parameters of the different sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust and control to the test fish *Heteroclarias* during the 14 days exposure period.

CTLD (mg/L)	Water Quality Parameters					
	Temp (^{O}C)	FCO (mg/L)	TAL (mg/L)	pH	DO (mg/L)	Cond (µs/cm)
0.00	30.63 (0.34)	1.85 (0.12)	0.25 (0.02)	7.15 (0.06)	6.62 (0.00)	146.47 (0.37)
93.90	29.60 (0.60)	1.50 (0.13)	0.35 (0.03)	6.60 (0.02)	5.57 (0.08)	150.75 (0.38)*
187.80	28.77 (0.34)*	1.25 (0.17)*	0.47 (0.06)*	6.27 (0.02)**	4.20 (0.06)**	154.80 (0.90)**
375.60	28.77 (0.34)*	0.87 (0.05)*	0.57 0.09)**	6.07 (0.02)**	3.21 (0.08)**	167.42 (1.23)**

- mean value obtained from 14 sampling with replicate; SE- standard error; CTLD- Concentration of Tobacco (*Nicotiana tobaccum*) leaf dust; Temp- Temperature; FCO- Free Carbon IV Oxide; TAL- Total Alkalinity; DO- Dissolved Oxygen; Cond- Conductivity; * P < 0.05 and ** P < 0.01.

Fig 3.2.1. There was an insignificant ($p>0.05$) decrease and increase in serum and (liver and kidney) acid phosphatase as the concentration of tobacco leaf dust increased. Statistically, the activity of acid phosphatase was most pronounced in the liver, less in the serum and least in the kidney of the exposed fish after 14 day exposure period.

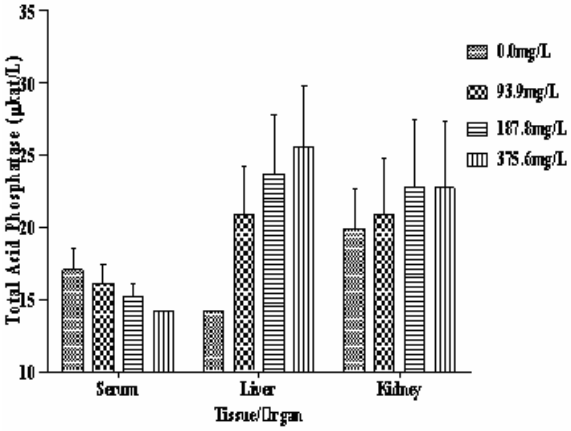

Fig 3.2.1. Acid Phosphatase activities of test fish *Heteroclarias* following 14-days exposure to various sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust. Each column represents mean value while the bar represents standard error

The activities of alkaline phosphatase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period are as presented in Fig 3.2.2. There were insignificant ($p>0.05$) decrease and increase in (serum and liver) and kidney alkaline phosphatase as the concentration of tobacco leaf dust increased. Statistically, the activity of alkaline phosphatase was most pronounced in the liver, less in the kidney and least in the serum of the exposed fish after 14day exposure period.

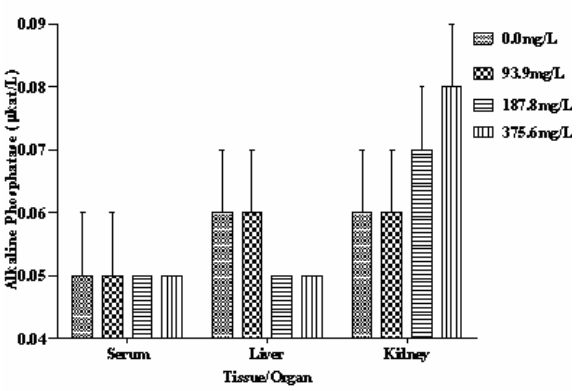

Fig 3.2.2. Alkaline phosphatase activities of test fish *Heteroclarias* following 14-days exposure to various sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust. Symbols as in Fig. 3.2.1

The activities of alanine aminotransferase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period are as presented in Fig 3.2.3. There were insignificant ($p>0.05$) decrease and increase in (serum and liver) and kidney alanine aminotransferase as the concentration of tobacco leaf dust increased. Statistically, the activity of alanine aminotransferase was most pronounced in the kidney, less in the liver and least in the serum of the exposed fish after 14-day exposure period.

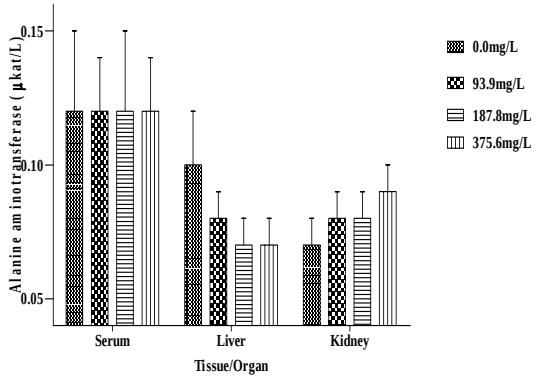

Fig 3.2.3.Alanine aminotransferase activities of test fish *Heteroclarias* following 14-days exposure to various sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust. Symbols as in Fig. 3.2.1

The activities of aspartate aminotransferase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period are as presented in Fig 3.2.4. There were insignificant ($p>0.05$) increase and decrease in (serum and kidney) and liver aspartate aminotransferase as the concentration of tobacco leaf dust increased. Statistically, the activity of aspartate aminotransferase was most pronounced in the kidney, less in the serum and least in the liver of the exposed fish after 14-day exposure period.

The activities of lactate dehydrogenase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust

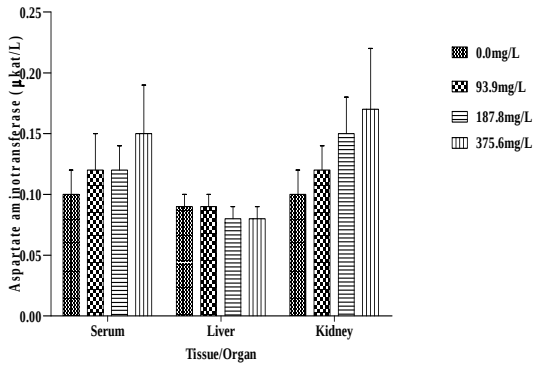

Fig 3.2.4. Aspartate aminotransferase activities of test fish Heteroclarias following 14-days exposure to various sublethal concentrations of Tobacco (Nicotiana tobaccum) leaf dust. Symbols as in Fig. 3.2.1

after the 14-days exposure period are as presented in Fig 3.2.5. The activity of serum lactate dehydrogenase was significantly ($p<0.01$) decreased, while liver and kidney lactate dehydrogenase were noticed to decrease insignificantly ($p>0.05$) when compared to the control. Statistically, the activity of lactate dehydrogenase was most pronounced in the serum, less in the liver and least in the kidney of the exposed fish after 14-day exposure period.

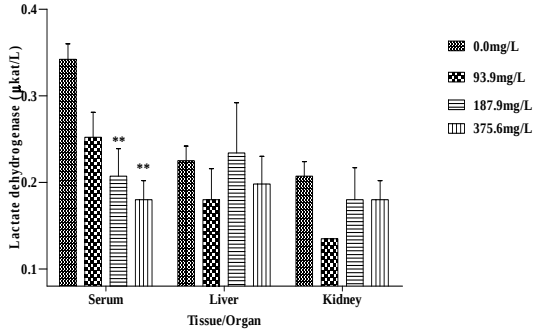

Fig 3.2.5: Lactate dehydrogenase activities of test fish *Heteroclarias* following 14-days exposure to various sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust. Symbols as in Fig. 3.2.1, ** - $p<0.01$

The activities of gamma glutamyltransferase in the sampled tissue and organs of the *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period are as presented in Fig 3.2.6. There was generally insignificant ($p>0.05$) decrease in the activity of gamma glutamyltransferase in serum, liver and kidney of *Heteroclarias* exposed to sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust after the 14-days exposure period. Statistically, the activity of gamma glutamyltransferase was most pronounced in the kidney, less in the serum and least in the liver of the exposed fish after 14-day exposure period.

4. Discussion

Water quality parameters such as temperature, dissolved oxygen, free carbon (iv) oxide, pH, alkalinity and conductivity are parameters that are paramount to the

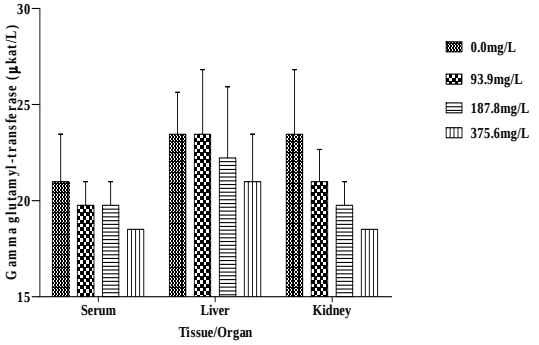

Fig 3.2.6. Gamma glutamyltransferase activities of test fish *Heteroclarias* following 14-days exposure to various sublethal concentrations of Tobacco (*Nicotiana tobaccum*) leaf dust. Symbols as in Fig. 3.2.1

many factors which affect fish health, growth and reproduction (Camus *et al.*, 1998 and Hill, 1955). However, Richards (1977) reported that the main cause of mortality in aquarium fish is the adequate maintenance of the water environment. In this study, the monitored parameters were noted to be significantly different from the control test after the 14-days exposure period which invariably means that tobacco leaf dust has an effect on the water chemistry. The study of Omoniyi *et al.,* (2002) on the sublethal effects of tobacco leaf dust on the haematological parameters of the *Clarias gariepinus* revealed insignificant increase and decrease in the monitored water quality parameters. The variation in the reported result of monitored parameters may be associated to the exposure period and the concentration of tobacco leaf dust used. Noga, (1996) and Richards (1977) have recommended the pH for fresh water fish to be 6.5 to 8.5, the value of pH in the highest concentration of tobacco leaf dust was found to be lower than the recommended value. Thus the significance decrease in pH value as the concentrations of tobacco leaf dust increased revealed that the toxicant resulted in acidic condition. This was supported by the findings of Omoniyi *et al.,* (2002) who reported acidic condition in water of *Clarias gariepinus* exposed to tobacco leaf dust. The decline in pH with time may be due to the production of acidic metabolites (Delyan *et al.,* 1990). Odokuma and Okara (2004) attributed the pH of various treatments to be a function of the chemical composition of the treatment, which was related to the nature of the carbon source and other nutrients present, and thus signifying that tobacco leaf dust as the potential of fertilizing the pond as reported by Aleem (1987). The acidic condition of the water had resulted in the decrease in the level of dissolved oxygen, free carbon (iv) oxide and temperature with a corresponding increase in the values of total alkalinity and conductivity. Omoniyi *et al.,* (2002) had also reported decrease in temperature, dissolved oxygen with increase in conductivity values respectively. The decrease in the available free carbon (iv) oxide may affect the survival of plants, and thus reduction of dissolved oxygen results in hypoxia in animals which is as a result of degradation of the tobacco leaf dust. According to Almeida-Val *et al.*, (1993) low dissolved oxygen environments are found in many tropical plain lakes, ponds, swamps and other eutrophic water xenobiotes when nutrient potential is introduced.

Enzymes catalyze physiological reactions by lowering the activation energy level that the reactant (substrate) must reach for the reaction to occur. The effect of toxicant on enzymatic activity is one of the most important biochemical parameters, which is affected under stress. When an organ is diseased due to the effect of a toxicant, enzymatic activity appears to be increased or inhibited due to the active site being either denatured or distorted. Since some enzymes catalyze some steps in the metabolism of carbohydrate and protein, they are present in most tissues. The increase or decrease in their level may be sufficient to provide information of diagnostic values.

Phosphatases are important enzymes of animal metabolism, which play important roles in the transport of metabolites across the membrane (Vorbrodt, 1959). Alkaline phosphatase diagnosis is important in the bone disease and hepatobiliary disease thus employed to assess the integrity of plasma membrane and endoplasmic reticulum (Akanji *et. al.,* 1993 and Wright and Plummer, 1974) while acid phosphatase diagnosis is intended to detect the carcinoma of the prostate. Verma *et al.,* (1984) had reported an increase in serum acid phosphatase in *Mystus vittatus* (Bloch) exposed to different pesticides. The activity of serum acid phosphatase in the test fish *Heteroclarias*, exposed to sublethal concentrations of tobacco leaf dust, revealed an insignificant decrease which differs from the report of Verma *et al.,* (1984) while the activity of liver and kidney acid phosphatase was found to insignificantly increase. The insignificant difference recorded in acid phosphatase may be an indication of no serious or mild effect on bone and hepatobiliary disease as well as the integrity of the plasma membrane. However, the activity of alkaline phosphatase was found to insignificantly decrease in the serum and liver with a corresponding insignificant increase in the activity of kidney acid phosphatase in *Heteroclarias* exposed to sublethal concentrations of tobacco leaf dust after the 14-days exposure period. The dose-dependent inhibition observed in this investigation is in agreement with the report of many authors. Ogueji and Auta (2007) reported reduced value of serum alkaline phosphatase in African catfish *Clarias gariepinus*, exposed to lambda-cyhalothrin. Sastry and Sharma, (1980) reported alkaline phosphatase inhibition after 96h exposure to diazinon. Goel, *et al.,* (1982), reported serum alkaline phosphatase inhibition by 15% in *Heteropneutes fossilis* resulting from the effect of malathion. Similarly, Das and Mukherjee (2003) reported depletion of alkaline phosphatase due to sublethal exposure of *Labeo rohita* fingerlings to cypermethrin. Rashatwar and Ilyas (1983) had reported significance decrease in alkaline phosphatase activity in fresh water fish *Nemachelius denisonii* (day) exposed to sublethal concentrations of Basalin. Due to the resulting activity values of alkaline phosphatase, it may be assumed that the liver tissue of the experimental fish was not markedly affected by tobacco leaf dust. The inhibition in protein level may also be due to the decrease in alkaline phosphatase activity as it plays an important role in protein synthesis (Pilo *et. al.,* 1972). The insignificant difference recorded in alkaline phosphatase may be an indication of no serious or mild effect on the carcinoma of the prostate.

Aminotransferases are gainfully used in the diagnosis of disease and tissue damage. It functions as a link between carbohydrate and protein metabolism by catalyzing the inter conversion of strategic compounds respectively (Martin *et. al.,* 1983). They are normally localized within the cells of the liver, heart, gill, kidney, muscles and other organs. The enzymes are important in assessing and monitoring liver cytolysis (Wells and Snell, 1962). They are intracellular enzymes which exist in only a small amount of the serum. Their presence in the serum may give information on organ dysfunction (Wells *et al*., 1986). The aminotransferases occupy a central position in the amino acid metabolism as they help in retaining amino group (to form a new amino acid) during the degradation of amino acid; and are also involved in the biochemical regulation of intracellular amino acid pool. They also help in providing necessary intermediates for gluconeogenesis. Alanine aminotransferase is remarkably specific for liver function since aspartate aminotransferase is mostly present in kidney (Witthawaskul *et al*., 2003). The activities of alanine aminotransferase in the test fish *Heteroclarias*, exposed to sublethal concentrations of tobacco leaf dust, were insignificantly decreased and increased in (serum, liver) and kidney. The liver is especially rich in alanine aminotransferase, being the enzyme measurement used primarily as a test for infectious and toxic hepatitis. The reduction of liver alanine aminotransferase may be attributed to reduced rate of synthesis of the liver enzyme. It may also be that the plant dust had caused leakage of the enzyme into the blood where alanine aminotransferase altered membrane permeability (Wroblewski and La Due, 1956). The sublethal effects of tobacco leaf dust on the activity of aspartate aminotransferase of the test fish (*Heteroclarias*) after the 14-days exposure period revealed insignificant increase and decrease in serum, kidney and liver. Jee *et al*., (2005) had reported increase in serum aspartate aminotransferase in Korean rock fish (*Sebastes schlegeli*) exposed to cypermethrin. The increase in serum aspartate aminotransferase may be attributable to the process of either deamination or transamination due to the effect of the plant dust (tobacco leaf dust). The decrease in aspartate and alanine aminotransferases in the liver of experimental fish revealed that the plant extract has an effect on the parenchymatous tissue and skeletal musculature which probably may disturb the permeability and integrity of cell organelles as supported by Adamu and Iloba, (2008). Yakubu *et al.,* (2005) reported significant increase ($p<0.05$) in serum aspartate aminotransferase and liver aspartate aminotransferase, increased significantly ($p<0.05$) in rats exposed to *Khaya senegalensis* during the 18-days exposure period. Oruc and Uner, (1999), reported inhibition in seral aspartate aminotransferase and alanine aminotransferase enzyme activity following 2 and 30 days of exposure to 2, 4-Diamin. Similarly, Sadhu, *et al.,* (1985) had reported decreased aspartate aminotransferase and alanine aminotransferase activities in the serum of *Channa striatus*, following exposure to Malathion for 10 days. The pattern of alanine aminotransferase and aspartate aminotransferase activities observed in this study are biochemical symptoms tending towards liver cytolysis, indicating disturbance in the structure and integrity of cell organelles like endoplasmic reticulum and membrane transport system (Dere and Polat, 2001). Alterations in their activities may have an adverse effect on the amino acid metabolism of the tissues and consequently the intermediates needed for gluconeogenesis. The activities of serum aspartate aminotransferase and alanine aminotransferase did not show any significance change probably due to the low damaging effect of the tobacco leaf dust used as supported by Al-Salahy and Mahmoud (2003).

Lactate dehydrogenase catalyses the conversion of pyruvic acid to lactic acid in aerobic condition; thus acts as an indicator of hepatobiliary disease. The activity of lactate dehydrogenase in the test fish (*Heteroclarias*) exposed to sublethal concentrations of tobacco leaf dust revealed significant decrease and insignificant decrease in serum and liver and kidney lactate dehydrogenase activity. Rashatwar and Ilyas (1983) had reported significant decrease in lactate dehydrogenase activity in fresh water fish *Nemachelius denisonii* (day) exposed to sublethal concentrations of Basalin. The reduction in the activity of liver lactate dehydrogenase, cytosolic enzyme (Wroblewski, 1955) without corresponding increase in the serum enzyme may also be adduced to inhibition of the enzyme activity at the cellular level such a radiation may have consequential effect on the glycolytic pathway thus, affirmed by the low level of dissolved oxygen in the test water. The sluggish movement of the test fish can also be explained by the reduction of the lactate dehydrogenase activity. The reduced lactate dehydrogenase in liver and kidney may have occurred, due to the stress- induced increase in the rate of glycolysis.

Gamma glutamyltransferase catalyses the conversion of pyruvic acid to lactic acid in aerobic condition; thus acts as an indicator of hepatobiliary disease. The activity of gamma glutamyltransferase in serum, liver and kidney of the test fish (*Heteroclarias*) exposed to sublethal concentrations of tobacco leaf dust after the 14-days exposure period was insignificantly decreased which is an indication that affirm the activity of lactate dehydrogenase and the tendency towards hepatobiliary disease as a result of low level of dissolved oxygen in the test water.

5. Conclusion

Tobacco dust is locally available, inexpensive, easily degraded and serves as piscicides and an organic fertilizer. Tobacco leaf dust may be a useful substitute of synthetic piscicides in killing weed fish from culture pond. This is environmentally safe because their toxic effect is reversible within 3 days after application. Its moderate effect and the rapid rate of degradation make it attractive for aquaculture purposes, as a substance to control the pest and subsequently as an organic fertilizer. This study confirms that the extracts of tobacco leaf exert piscicidal and fertilizer properties. The result revealed that tobacco leaf dust at studied concentrations had slight effect on the intermediary metabolism of *Heteroclarias.* It again suggests that sublethal concentrations of tobacco leaf dust at the tested concentrations have some mild effects on some basic function of the serum, liver and kidney of *Heteroclarias.* Therefore, the determined enzymatic activities can be suitably used to determine the effect of toxicant on the physiology of fish under sublethal condition prior to sudden death of the fish. The activities

of determined enzymatic activities showed mild damage in kidney, liver, plasma membrane, endoplasmic reticulum as well as any hepatobiliary and bone disease and carcinoma of the prostate enzymes. However, it has been known to affect the chemistry of the water thus may primarily affect primary productivity and the biodiversity of organisms as a result of decrease in the level of dissolved oxygen, free carbon iv oxide, temperature and increase in total alkalinity and conductivity.

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