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**AMELIORATIVE POTENTIALS OF VITAMIN E ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS AND GROWTH RESPONSES OF *HETEROCLARIAS* EXPOSED TO CHLORPYRIFOS**

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**ABSTRACT**

The use of modern chemicals to control pesticides could have potential adverse effect on aquatic organisms and water quality. The effects of chlorpyrifos were investigated on biochemical, haematological and growth responses of *Heteroclaris*. Juveniles of *Heteroclaris* were assessed in aerated static renewal bioassays of chlorpyrifos using different concentrations (1, 2, 3, 4, 5 ppm) for 96 h and 0.24 ppm for 28 days which were fed on varying concentrations (50, 250, 500, 1000 mg) of vitamin E. The 96 hours LC<sub>50</sub> value was 2.4 ppm. After the exposure periods, the fish were sacrificed to collect blood, gills and liver for haematological and biochemical analyses. The levels of RBC, Hb, PCV decreased ( $P < 0.05$ ) compared to control, but increased ( $P < 0.05$ ) in vitamin E-fed groups, while, WBC level increased ( $P < 0.05$ ) in chlorpyrifos-exposed groups and decreased in vitamin E-fed groups ( $P < 0.05$ ). Activities of enzymes ALT, AST, LDH, SOD and glucose level increased ( $P < 0.05$ ) in the tissues of chlorpyrifos-exposed groups, but reduced in vitamin E- fed groups. However, the levels of AChE, MDA and total protein decreased in chlorpyrifos-exposed groups, but increased ( $P < 0.05$ ) in vitamin E-fed groups. The specific growth rate of *Heteroclaris* increased in vitamin E-fed groups. This study indicates that vitamin E is capable of minimizing stress induced by chlorpyrifos in *Heteroclaris*.

**KEY WORDS:** Biochemical, Chlorpyrifos, Haematology, *Heteroclaris*, Vitamin E

**INTRODUCTION**

Chlorpyrifos is one of the most widely used organophosphates insecticide which is

widely used in agriculture for control of insect pest on crops such as citrus, pineapple, tomato, maize, tobacco. It has

also been used as mosquito larvicide in water bodies and termiticidal barrier in around or under building (WHO, 2004). Ability of Chlorpyrifos to persisting environment could make it to have potential adverse effect on aquatic organisms and are mostly detected in streams, rivers, ponds and reservoirs (Ensminger *et al.*, 2011 and WHO, 2012). Continuous application of this pesticide in the control of insects makes large quantities of the insecticide to find their ways through surface runoff into the water bodies that later gets accumulated in aquatic organisms, particularly fish. This may have adverse effect on their metabolic activities and physiology (Varo *et al.*, 2002). Induction of biochemical and physiological changes could lead to oxidative stress that cause abnormal behaviour; alteration of erythrocytes; enzymes and growth retardation in fish. The most important tissues of fish that are exposed to contaminants are the blood, gills and liver (Sayeed *et al.*, 2003). Investigation of the effects of toxicants on these organs is therefore necessary. Gills have been the first target of contaminant due to their direct contact with the external environment (Perry and Laurent, 1993). They help to transport substances from the outside to other parts of the body via the blood. Liver is another vital organ that carries out the work of excretion, osmoregulation and detoxification. Presence of pesticides in fish body could cause liver damage that influences the release of some enzymes in to the blood stream, thereby causing disturbance of the fish metabolic activities. Fish serves as a tool for the assessment of the toxic effects of contaminants in an aquatic ecosystem as a result of their position in the food chain. *Heteroclaris* is one of the economically important fish with fast growth rate and high economic value, which can grow to a very large size as a result of their air breathing characteristics, high resistance to adverse

condition and diseases (Anoop *et al.*, 2009). To preserve these qualities and conserve this economically important fish, there is a need to develop possible ways to neutralize the toxic effects of this pesticide. Several abnormalities such as loss of equilibrium, uncoordinated movement, erratic swimming, and vertical hanging, sinking to the bottom, abnormal opercula movement and caudal bending were reported by Levin *et al.* (2004) in zebra fish, when exposed to chlorpyrifos. Physiological changes in fish could also result to alterations in both the erythrocytes and the leucocytes which in turn induced the generation of reactive oxygen species (ROS) that could lead to oxidative stress that trigger the production of both biochemical and antioxidant enzymes (Ahmad, 2012). Investigations on haematological effects of chlorpyrifos were carried out by Ishtar *et al.* (2015) on *Labeobata* and other pesticide such as deltamethrin on common carp by David *et al.* (2015). Effect of chlorpyrifos on biochemical parameters were also investigated in various fish species by different researchers. Amongst these are effect of chlorpyrifos on *Catlacatla*, *Labeorohita*, and *Cirrhinus mrigala* (Tilak *et al.*, 2005); *Oreochromis mossambicus* (Aniladevi, 2008) and *Clarias gariepinus* (Nwani *et al.*, 2013); Other pesticides such as monocrotophos on *Heteroclaris* by Abdulkareem and Owolabi, (2014); glyphosate on *Clarias gariepinus* by Abdulkareem *et al.* (2014); Dichlorvos on *Clarias gariepinus* by Abdulkareem and Uteodor, (2016) were also investigated. Out of the several investigations recorded on the effects of chlorpyrifos and other pesticides, none of these have reported on the use of vitamin E as possible preventive measures that could be used when fish are in chemical polluted environment. Vitamin E is a strong phenolic antioxidant that helps to prevent lipid peroxidation by slowing down free radicals 'formation and hyper-peroxides

(Mukai *et al.*, 2007; Traber and Atkinson, 2007). This work therefore investigated the biochemical, hematological and growth responses of *Heteroclaris* exposed to chlorpyrifos and ameliorated with vitamin E.

## MATERIALS AND METHODS

### Experimental Set-Up

Juveniles of *Heteroclaris* (average weight  $21.4 \pm 1.3$ g and average length  $11 \pm 0.8$ cm) were obtained from T.J fish farm in Ilorin, Kwara State. The fish were transported in plastic containers to the Department of Zoology laboratory, University of Ilorin, Ilorin, Nigeria in a portable well aerated black tank containing water from the farm. The fish were not fed throughout the day to avoid mortality as a result of stress. The water from the hatchery was replaced by dechlorinated borehole water and the fishes were held in a large tank of 800L capacity at a temperature of 24°C where they were acclimatized for 14 days (USEPA, 1985). Fishes were fed twice daily at 9:00pm and 5:00pm with commercial feeds (Durante) at 4 % body weight (Meyers *et al.*, 1993). The water was changed every two days to remove unconsumed feed and faecal waste to prevent pollution and to ensure good water quality (USEPA, 1996). Fishes were checked for any disease infection by monitoring their activities and observations for any physical change in behavior, feeding and swimming activities (USEPA, 1996). Feeding was stopped 24 hours before the commencement of the experiment (USEPA, 1996). During the experimental period, water was continuously monitored for temperature, pH, Biological Oxygen Demand (BOD), dissolved oxygen and conductivity following the procedure of APHA (1995). Ethical approval (UERC/ASN/2016/648) on the use of fish for the research work was given by the University Ethical Review committee of University of Ilorin, Ilorin, Nigeria.

### Acute Toxicity

Ten fish were randomly picked and introduced into 6 different aquaria containing 10L of dechlorinated water each. One aquarium was kept as control, while the remaining five contained five different concentrations (1, 2, 3, 4, 5ppm) of chlorpyrifos. The varied concentrations and the control were triplicate (Pathirantne and Athauda, 1999). The experiment lasted for 96 h, during which the behavioral change and fish mortality were observed every 24 h. The  $LC_{50}$  value was estimated using Kaper method adapted by Dede and Igbigbi (1997).

### Chronic Toxicity (Amelioration Test)

Based on the acute toxicity results, chronic experiment was set up to carry out the ameliorative effect of vitamin E in *Heteroclaris* exposed to chlorpyrifos for 28 days. Ten fish were randomly distributed into six plastic aquaria (A, B, C, D, E & F) containing 10 litres of chlorine free borehole water. The experiment was carried out in triplicate and the first aquarium A was fed with basal diet without vitamin E which served as the control group, while aquarium B was exposed to 0.24 ppm chlorpyrifos and fed on basal diet. The third aquarium C was also exposed to 0.24 ppm chlorpyrifos but fed on 50 mg vitamin E/kg diet. The fourth aquarium D was exposed to 0.24 ppm chlorpyrifos and fed on 250 mg vitamin E/kg diet, while the fifth aquarium E was exposed to 0.24 ppm chlorpyrifos and fed on 500 mg vitamin E/kg diet and the sixth aquarium F exposed to 0.24 ppm chlorpyrifos and fed on 1000 mg vitamin E/kg diet at 4% of their body weight (Pathirantne and Athauda, 1999). Each aquarium containing the fishes was cleaned and the toxicant renewed every two days, to keep the concentration of the toxicant constant.

### Biochemical Assay

Fish were randomly picked and sacrificed for analyses of Alanine Aminotransferases (ALT) and Aspartate Aminotransferase (AST) enzymes in the blood and tissue using Reitman and Frankel method (Wright *et al.*, 1970). Deutsche Gesellschaft für klinische method (DGKC, 1970) was adopted for the biochemical analysis of Lactate Dehydrogenase (LDH). Tissues used for SOD analysis were homogenized using the method by (Woolliams, *et al.*, 1983). Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS), using malondialdehyde (MDA) as standard using the method by Buege and Aust (1978). Glucose in serum was determined using Trinder (1969) method (colorimetric blood). Protein level in serum was estimated using “biuret method” (Lowry, *et al.*, 1951).

### Haematological Assay

At the end of the experiment, insulin syringe was used to collect the blood samples into an anti-coagulate (EDTA) tube for hematological evaluation after exposure and ameliorative phase. Red blood cell (RBC) and White blood cells (WBC) was estimated using Improved Neubauer counting chamber as described by Blaxhall and Daisley, (1973). The RBC and WBC were then counted under light microscope with an improved Neubauer haematocytometer. The test for haemoglobin level was carried out using the cyanometh haemoglobin method (Drabkin, 1946). The packed cell volume (PCV) was determined microhaematocrit centrifuge by Wintrobe and Westergreen method as described by (Blaxhall and Daisley, 1973). The values of RBC, HB and PCV, obtained were used to calculate the MCV, MCH and MCHC using the formulae given by Dacie and Lewis (2001).

### Growth

The specific growth rate was estimated using

$$r^3 = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1} \times 100 \quad (\text{Pack, 1991}).$$

Where  $r^3$  = the specific growth rate

$\log_e W_1$  = logarithm of average weight of fish in the aquarium at the start of the study

$\log_e W_2$  = logarithm of average weight of fish in the aquarium at the end of the study  
 $t_1, t_2$  = time in days at the start and end of the study

### Data Analysis

Analysis of variance (ANOVA) and Duncan multiple range tests were used to test for differences between different levels of treatments and to separate means respectively. Test for significant difference were at 95% ( $p < 0.05$ ) probability (Duncan, 1955)

## RESULTS

*Heteroclinarias* in the groups exposed to varying concentrations of chlorpyrifos exhibited distress behavioral responses such as erratic swimming, color change, gasping for breath and frequent surfacing, loss of equilibrium, motionlessness, erratic swimming, caudal bending and the fish stood motionless in the aquarium bottom, hanging vertically and little response to gentle probing compared to control. The qualities of water parameters during the experiments were within the standard recommended by (USEPA, 1996).

### Haematological Responses

The levels of erythrocytes (RBC, HB, and PCV) decreased ( $P < 0.05$ ) as the concentration of chlorpyrifos increased, while the levels of the leucocytes (WBC,

Table 1: Haematological parameters of *Heteroclinas* exposed to different concentrations of Chlorpyrifos for 96 hours and ameliorated with vitamin E for 28 days

| RBC ( $\mu$ T)               | Hb (g/dl)                     | PCV (%)                       | WBC ( $\mu$ T)                | MCV (fl)                      | MCH (Pg)                      | MCHC (g/l)                      | PLT ( $\times 10^9$ /l)       | LYM ( $\mu$ T)                 |
|------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|--------------------------------|
| After 96 h                   |                               |                               |                               |                               |                               |                                 |                               |                                |
| 2.94 $\pm$ 0.16 <sup>d</sup> | 14.1 $\pm$ 0.46 <sup>e</sup>  | 35.40 $\pm$ 0.87 <sup>e</sup> | 2.23 $\pm$ 0.46 <sup>a</sup>  | 1.22 $\pm$ 1.96 <sup>a</sup>  | 33.7 $\pm$ 2.12 <sup>a</sup>  | 31.83 $\pm$ 3.32 <sup>ab</sup>  | 1.04 $\pm$ 1.15 <sup>a</sup>  | 97.90 $\pm$ 0.40 <sup>a</sup>  |
| 2.79 $\pm$ 0.16 <sup>d</sup> | 12.5 $\pm$ 0.35 <sup>d</sup>  | 32.47 $\pm$ 4.21 <sup>d</sup> | 2.35 $\pm$ 1.21 <sup>b</sup>  | 1.27 $\pm$ 1.10 <sup>b</sup>  | 42.5 $\pm$ 0.46 <sup>b</sup>  | 29.77 $\pm$ 4.10 <sup>a</sup>   | 1.14 $\pm$ 3.06 <sup>d</sup>  | 98.67 $\pm$ 0.37 <sup>ab</sup> |
| 2.35 $\pm$ 0.14 <sup>c</sup> | 11.3 $\pm$ 0.87 <sup>c</sup>  | 29.80 $\pm$ 1.21 <sup>a</sup> | 2.43 $\pm$ 1.27 <sup>a</sup>  | 1.31 $\pm$ 1.10 <sup>a</sup>  | 48.1 $\pm$ 1.10 <sup>c</sup>  | 36.90 $\pm$ 1.31 <sup>abc</sup> | 3.60 $\pm$ 0.58 <sup>a</sup>  | 99.00 $\pm$ 0.40 <sup>ab</sup> |
| 0.83 $\pm$ 0.07 <sup>b</sup> | 2.80 $\pm$ 0.12 <sup>b</sup>  | 10.90 $\pm$ 0.17 <sup>b</sup> | 2.49 $\pm$ 0.98 <sup>d</sup>  | 1.27 $\pm$ 1.33 <sup>b</sup>  | 50.5 $\pm$ 2.37 <sup>c</sup>  | 39.8 $\pm$ 1.10 <sup>bc</sup>   | 4.04 $\pm$ 1.15 <sup>c</sup>  | 99.20 $\pm$ 0.23 <sup>b</sup>  |
| 0.26 $\pm$ 0.04 <sup>a</sup> | 0.90 $\pm$ 0.23 <sup>a</sup>  | 3.80 $\pm$ 0.46 <sup>a</sup>  | 2.53 $\pm$ 0.98 <sup>e</sup>  | 1.26 $\pm$ 1.58 <sup>ab</sup> | 46.7 $\pm$ 1.27 <sup>ab</sup> | 41.4 $\pm$ 0.17 <sup>c</sup>    | 4.88 $\pm$ 2.81 <sup>b</sup>  | 99.50 $\pm$ 0.23 <sup>b</sup>  |
| After 28 days                |                               |                               |                               |                               |                               |                                 |                               |                                |
| 2.91 $\pm$ 0.18 <sup>c</sup> | 11.40 $\pm$ 0.92 <sup>d</sup> | 36.00 $\pm$ 0.5 <sup>c</sup>  | 10.24 $\pm$ 0.09 <sup>a</sup> | 1.12 $\pm$ 0.58 <sup>a</sup>  | 39.00 $\pm$ 0.58 <sup>c</sup> | 32.00 $\pm$ 0.5 <sup>b</sup>    | 21.00 $\pm$ 1.13 <sup>a</sup> | 55.00 $\pm$ 2.08 <sup>a</sup>  |
| 2.25 $\pm$ 0.07 <sup>a</sup> | 6.50 $\pm$ 0.23 <sup>a</sup>  | 29.00 $\pm$ 0.5 <sup>a</sup>  | 22.05 $\pm$ 0.12 <sup>c</sup> | 1.11 $\pm$ 0.58 <sup>a</sup>  | 33.00 $\pm$ 0.58 <sup>a</sup> | 30.00 $\pm$ 0.58 <sup>a</sup>   | 82.00 $\pm$ 1.13 <sup>c</sup> | 74.00 $\pm$ 0.58 <sup>c</sup>  |
| 2.60 $\pm$ 0.09 <sup>c</sup> | 6.70 $\pm$ 0.46 <sup>a</sup>  | 30.00 $\pm$ 0.5 <sup>ab</sup> | 19.30 $\pm$ 0.0 <sup>d</sup>  | 1.13 $\pm$ 1.13 <sup>a</sup>  | 36.00 $\pm$ 0.58 <sup>b</sup> | 32.00 $\pm$ 0.58 <sup>b</sup>   | 49.00 $\pm$ 1.13 <sup>c</sup> | 70.00 $\pm$ 1.13 <sup>c</sup>  |
| 2.62 $\pm$ 0.09 <sup>c</sup> | 10.00 $\pm$ 0.75 <sup>b</sup> | 33.00 $\pm$ 0.5 <sup>cd</sup> | 13.00 $\pm$ 0.0 <sup>b</sup>  | 1.18 $\pm$ 0.58 <sup>b</sup>  | 36.00 $\pm$ 1.15 <sup>b</sup> | 32.03 $\pm$ 1.15 <sup>b</sup>   | 35.00 $\pm$ 1.13 <sup>c</sup> | 69.00 $\pm$ 0.58 <sup>d</sup>  |
| 2.90 $\pm$ 0.07 <sup>d</sup> | 10.50 $\pm$ 0.46 <sup>b</sup> | 36.00 $\pm$ 1.15 <sup>c</sup> | 11.44 $\pm$ 0.8 <sup>b</sup>  | 1.23 $\pm$ 0.58 <sup>c</sup>  | 37.00 $\pm$ 1.15 <sup>c</sup> | 32.00 $\pm$ 0.58 <sup>b</sup>   | 32.00 $\pm$ 0.58 <sup>b</sup> | 58.00 $\pm$ 1.13 <sup>b</sup>  |
| 2.45 $\pm$ 0.06 <sup>b</sup> | 9.10 $\pm$ 0.64 <sup>c</sup>  | 32.00 $\pm$ 0.5 <sup>c</sup>  | 12.60 $\pm$ 0.0 <sup>b</sup>  | 1.18 $\pm$ 1.15 <sup>b</sup>  | 38.00 $\pm$ 0.58 <sup>d</sup> | 33.00 $\pm$ 0.58 <sup>bc</sup>  | 33.00 $\pm$ 1.13 <sup>c</sup> | 64.00 $\pm$ 0.58 <sup>c</sup>  |

Mean ( $\pm$  S.E, n=3) with the same superscript in the same column are not significantly different (P > 0.05)

N.B: A-Basal feed only; B-Basal feed + 0.24 mg/l Chlorpyrifos; C-50 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; D-250 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos and F-1000 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos.

LYM & PLT) increased ( $P < 0.05$ ) as the contraction of chlorpyrifos increased during the acute toxicity experiment. At the end of 28 days of exposure, the values of RBC, HB, and PCV in group B exposed to chlorpyrifos and fed on basal diet only without amelioration are significantly lower ( $P < 0.05$ ) compared to control, while those in the groups (C, D & E) exposed to chlorpyrifos and fed on diet formulated with varying concentrations of vitamin E are significantly higher ( $P < 0.05$ ) compared to those in group B. However, the values of RBC, HB and PCV increased with increase in the concentration of vitamin E except in the highest concentration (1000mg/kg) diet where a reduction occurred.

### Biochemical Responses

At the end of 96 h of exposure to lethal concentrations (1.00, 2.00, 3.00 and 4.00 ppm) of chlorpyrifos, activities of enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in the blood, gill and liver of the fish significantly increased ( $P < 0.05$ ) in the groups exposed to CPF compared to control. While the activities of acetylcholinesterase (AChE) significantly decreased ( $P < 0.05$ ) in the blood, gills and liver of the groups exposed to CPF compared to control. The levels of activities of the antioxidant enzymes superoxide dismutase (SOD) in the groups exposed to CPF were significantly higher ( $P < 0.05$ ) than the control group. The levels of Malondialdehyde (MDA) in the blood, gills and liver were significantly higher ( $P < 0.05$ ) compared to control, the level increased as the concentration of the toxicant increased. The level of Total protein significantly decreased ( $P < 0.05$ ) in the liver and blood, but decreased in the gill. While the glucose level in the blood decreased significantly ( $P < 0.05$ ) as the concentration of chlorpyrifos increased (Table 1). After 28 days of exposure to

chlorpyrifos, activities of enzymes ALT and AST and LDH and AChE in the blood, gills and liver of *Heteroclinus* group B exposed to Chlorpyrifos only without vitamin E supplementation in their diet increased significantly ( $P < 0.05$ ) compared to control group A, that was fed on basal diet only without exposure to chlorpyrifos. However, the level of the enzymes decreased significantly ( $P < 0.05$ ) as the concentration of vitamin E increased from 50, 250 & 500 mg/kg diet in groups C, D & E respectively. While a significant ( $P < 0.05$ ) increase occurred in group F fed on diet incorporated with highest concentration of vitamin E (1000 mg/kg diet). The level of antioxidant enzyme SOD in the blood, gills and liver of fish in group B was significantly ( $P < 0.05$ ) higher than that of group A, while Chlorpyrifos-exposed groups that were fed on diet with varying concentrations of Vitamin E recorded significant decrease ( $P < 0.05$ ) as the concentration of vitamin E inclusion in the diet increased. A great decrease was recorded in the level of SOD activity in group E that was fed on 500 mg vitamin E/kg diet with a significant increase ( $P < 0.05$ ) in group F. The level of total protein in the blood, gills and liver of Chlorpyrifos-exposed group B increased compared to the control group A, but decreased with increase in the concentration of Vitamin E. While the glucose level in the blood of Chlorpyrifos-exposed group B was lower ( $P < 0.05$ ) compared to the control group. However, the level of glucose increased as the concentration of vitamin E inclusion increased with decrease ( $P < 0.05$ ) in group F. There was a significant increase ( $P < 0.05$ ) in the level of MDA in the blood, gills and liver of fish in group B compared to those fish in group A, while decrease ( $P < 0.05$ ) in the level of MDA was recorded in the ameliorated groups with a great reduction ( $P < 0.05$ ) in group E, except in group F with a significant increase

**Table 2: Enzyme activities in the blood of *Heteroclinus* exposed to varying concentrations of Chlorpyrifos for 96 h and ameliorated with vitamin E for 28 days**

| Conc (ppm)           | ALT (IU/L)                | AST (IU/L)                | LDH (IU/L)                 | SDS (units/g)              | ACHE (units/g)            | NDA (units/g)            | TPST (µM/g)               | GLU (gms/dl)             |
|----------------------|---------------------------|---------------------------|----------------------------|----------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
| <i>After 96 h</i>    |                           |                           |                            |                            |                           |                          |                           |                          |
| 0.00                 | 6.06 ± 1.12 <sup>a</sup>  | 21.60 ± 0.38 <sup>a</sup> | 81.08 ± 1.12 <sup>a</sup>  | 40.08 ± 1.12 <sup>a</sup>  | 26.86 ± 0.67 <sup>a</sup> | 1.21 ± 0.06 <sup>a</sup> | 13.33 ± 1.12 <sup>a</sup> | 1.38 ± 0.06 <sup>a</sup> |
| 1.00                 | 7.88 ± 0.58 <sup>a</sup>  | 23.61 ± 1.12 <sup>a</sup> | 82.5 ± 1.12 <sup>a</sup>   | 75.08 ± 1.12 <sup>a</sup>  | 30.86 ± 1.72 <sup>a</sup> | 1.90 ± 1.72 <sup>a</sup> | 19.60 ± 1.85 <sup>a</sup> | 2.75 ± 0.09 <sup>a</sup> |
| 2.00                 | 14.17 ± 0.58 <sup>a</sup> | 23.82 ± 1.72 <sup>a</sup> | 86.08 ± 1.12 <sup>a</sup>  | 80.08 ± 0.58 <sup>a</sup>  | 12.21 ± 1.12 <sup>a</sup> | 2.42 ± 0.06 <sup>a</sup> | 24.67 ± 0.38 <sup>a</sup> | 4.13 ± 0.08 <sup>a</sup> |
| 3.00                 | 21.25 ± 1.12 <sup>a</sup> | 28.73 ± 0.38 <sup>a</sup> | 123.75 ± 0.38 <sup>a</sup> | 126.08 ± 0.89 <sup>a</sup> | 10.86 ± 1.12 <sup>a</sup> | 3.63 ± 0.05 <sup>a</sup> | 48.60 ± 0.38 <sup>a</sup> | 4.49 ± 0.14 <sup>a</sup> |
| 4.00                 | 28.19 ± 1.12 <sup>a</sup> | 31.37 ± 1.12 <sup>a</sup> | 165.86 ± 0.04 <sup>a</sup> | 168.08 ± 1.12 <sup>a</sup> | 6.67 ± 0.89 <sup>a</sup>  | 4.83 ± 1.12 <sup>a</sup> | 33.33 ± 1.72 <sup>a</sup> | 5.58 ± 0.07 <sup>a</sup> |
| <i>After 28 days</i> |                           |                           |                            |                            |                           |                          |                           |                          |
| A                    | 6.06 ± 1.12 <sup>a</sup>  | 21.60 ± 0.38 <sup>a</sup> | 86.08 ± 1.12 <sup>a</sup>  | 75.08 ± 1.12 <sup>a</sup>  | 10.86 ± 1.12 <sup>a</sup> | 1.90 ± 0.06 <sup>a</sup> | 19.60 ± 1.85 <sup>a</sup> | 4.4 ± 0.07 <sup>a</sup>  |
| B                    | 17.08 ± 0.58 <sup>a</sup> | 21.60 ± 1.12 <sup>a</sup> | 89.08 ± 0.58 <sup>a</sup>  | 96.08 ± 0.58 <sup>a</sup>  | 16.86 ± 0.58 <sup>a</sup> | 2.90 ± 0.02 <sup>a</sup> | 32.60 ± 0.38 <sup>a</sup> | 2.3 ± 0.08 <sup>a</sup>  |
| C                    | 14.88 ± 0.58 <sup>a</sup> | 29.80 ± 0.58 <sup>a</sup> | 96.08 ± 1.12 <sup>a</sup>  | 94.08 ± 1.12 <sup>a</sup>  | 14.86 ± 0.58 <sup>a</sup> | 2.70 ± 0.08 <sup>a</sup> | 28.60 ± 1.12 <sup>a</sup> | 3.6 ± 0.09 <sup>a</sup>  |
| D                    | 12.88 ± 1.12 <sup>a</sup> | 27.60 ± 1.12 <sup>a</sup> | 94.08 ± 1.12 <sup>a</sup>  | 96.08 ± 1.72 <sup>a</sup>  | 12.86 ± 1.12 <sup>a</sup> | 2.20 ± 0.05 <sup>a</sup> | 27.60 ± 1.12 <sup>a</sup> | 3.7 ± 0.08 <sup>a</sup>  |
| E                    | 8.06 ± 0.58 <sup>a</sup>  | 23.60 ± 1.12 <sup>a</sup> | 89.08 ± 0.58 <sup>a</sup>  | 89.08 ± 0.58 <sup>a</sup>  | 10.86 ± 0.58 <sup>a</sup> | 2.00 ± 0.05 <sup>a</sup> | 24.60 ± 1.72 <sup>a</sup> | 4.2 ± 0.09 <sup>a</sup>  |
| F                    | 10.88 ± 1.12 <sup>a</sup> | 26.86 ± 0.38 <sup>a</sup> | 90.88 ± 1.72 <sup>a</sup>  | 96.08 ± 1.12 <sup>a</sup>  | 14.86 ± 1.12 <sup>a</sup> | 2.10 ± 0.02 <sup>a</sup> | 26.60 ± 1.12 <sup>a</sup> | 3.9 ± 0.07 <sup>a</sup>  |

Mean (S.E., ppm) with the same superscript in the same column are not significantly different ( $P > 0.05$ )

N.B: A-Basal feed only; B-Basal feed + 0.24 mg/l Chlorpyrifos; C-60 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos; D-250 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos and F-1000 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos.

( $P < 0.05$ ) (Table 2). The erythrocytes such as RBC, HB, and PCV of *Heteroclinus* exposed to sub-lethal concentration (0.24 ppm) of chlorpyrifos showed significant reduction ( $P < 0.05$ ) compared to control, while the groups (C, D & E) ameliorated with varying concentrations of vitamin E recorded significant increase. The level of erythrocytes increased as the concentrations of the vitamin E increased except in group F

that was fed on the highest concentrations of vitamin E compared to control (group A). The leucocytes such as WBC, LYM and NEUT in group B exposed to 0.24 ppm of chlorpyrifos only increased significantly ( $P < 0.05$ ), while that of ameliorated group C, D and E significantly decreased compared to control (Table 4).

**Table 3: Enzymes activities in the gills of *Heterochelone* exposed to varying concentrations of Chlorpyrifos for 96 h and a molluscated with vitamin E for 28 days**

| Conc.(ppm)           | ALT(IU/L)                  | AST(IU/L)                 | LDH(IU/L)                 | SOD (units/mg)            | ACHE (units/mg)           | SOD4 (units/mg)             | T-PROT (mM/mg)             |
|----------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|-----------------------------|----------------------------|
| <b>After 96 h</b>    |                            |                           |                           |                           |                           |                             |                            |
| 0                    | 44.00 ± 0.58 <sup>a</sup>  | 7.00 ± 33.41 <sup>a</sup> | 1.29 ± 0.58 <sup>a</sup>  | 67.00 ± 1.13 <sup>a</sup> | 68.00 ± 0.58 <sup>a</sup> | 11.00 ± 0.58 <sup>a</sup>   | 12.00 ± 0.58 <sup>a</sup>  |
| 1                    | 47.00 ± 0.58 <sup>ab</sup> | 7.00 ± 1.73 <sup>ab</sup> | 1.36 ± 1.13 <sup>a</sup>  | 70.33 ± 0.88 <sup>a</sup> | 67.00 ± 1.13 <sup>a</sup> | 13.00 ± 0.58 <sup>a</sup>   | 13.00 ± 0.58 <sup>a</sup>  |
| 2                    | 50.00 ± 0.58 <sup>b</sup>  | 7.02 ± 1.15 <sup>ab</sup> | 1.40 ± 1.73 <sup>a</sup>  | 85.00 ± 1.13 <sup>a</sup> | 63.00 ± 1.73 <sup>a</sup> | 17.00 ± 0.58 <sup>a</sup>   | 13.00 ± 0.58 <sup>ab</sup> |
| 3                    | 58.00 ± 0.58 <sup>c</sup>  | 7.25 ± 2.60 <sup>b</sup>  | 1.42 ± 1.73 <sup>ab</sup> | 88.00 ± 1.73 <sup>a</sup> | 54.00 ± 1.13 <sup>a</sup> | 17.00 ± 0.58 <sup>ab</sup>  | 15.00 ± 0.58 <sup>a</sup>  |
| 4                    | 44.00 ± 2.08 <sup>d</sup>  | 7.46 ± 1.73 <sup>b</sup>  | 1.43 ± 2.31 <sup>a</sup>  | 93.00 ± 0.98 <sup>a</sup> | 52.00 ± 1.13 <sup>a</sup> | 20.00 ± 0.58 <sup>b</sup>   | 16.00 ± 0.58 <sup>d</sup>  |
| <b>After 28 days</b> |                            |                           |                           |                           |                           |                             |                            |
| A                    | 49.00 ± 1.13 <sup>a</sup>  | 6.56 ± 1.73 <sup>a</sup>  | 1.26 ± 1.13 <sup>a</sup>  | 65.00 ± 1.73 <sup>a</sup> | 69.00 ± 2.58 <sup>a</sup> | 12.00 ± 119.01 <sup>a</sup> | 10.00 ± 1.13 <sup>a</sup>  |
| B                    | 56.00 ± 1.13 <sup>a</sup>  | 7.49 ± 4.91 <sup>a</sup>  | 1.48 ± 1.13 <sup>a</sup>  | 74.00 ± 0.98 <sup>a</sup> | 82.00 ± 1.13 <sup>a</sup> | 30.00 ± 0.58 <sup>a</sup>   | 17.00 ± 1.13 <sup>a</sup>  |
| C                    | 54.00 ± 1.13 <sup>a</sup>  | 7.32 ± 1.73 <sup>a</sup>  | 1.46 ± 1.73 <sup>a</sup>  | 72.00 ± 1.13 <sup>a</sup> | 78.00 ± 1.13 <sup>a</sup> | 16.00 ± 1.38 <sup>a</sup>   | 16.00 ± 1.13 <sup>a</sup>  |
| D                    | 52.00 ± 1.73 <sup>a</sup>  | 7.15 ± 1.15 <sup>a</sup>  | 1.38 ± 1.73 <sup>a</sup>  | 70.00 ± 1.13 <sup>a</sup> | 78.00 ± 1.73 <sup>a</sup> | 14.00 ± 1.13 <sup>a</sup>   | 14.00 ± 1.73 <sup>a</sup>  |
| E                    | 50.00 ± 1.73 <sup>b</sup>  | 6.65 ± 1.15 <sup>a</sup>  | 1.32 ± 0.98 <sup>a</sup>  | 65.00 ± 1.13 <sup>a</sup> | 72.00 ± 1.73 <sup>a</sup> | 13.00 ± 1.13 <sup>a</sup>   | 10.00 ± 1.13 <sup>a</sup>  |
| F                    | 53.00 ± 1.13 <sup>a</sup>  | 7.02 ± 0.58 <sup>a</sup>  | 1.42 ± 1.13 <sup>a</sup>  | 68.00 ± 1.13 <sup>a</sup> | 76.00 ± 1.73 <sup>a</sup> | 15.00 ± 0.58 <sup>a</sup>   | 12.00 ± 0.58 <sup>a</sup>  |

Mean (± S.E, n=6) with the same superscript in the same column are not significantly different ( $P > 0.05$ )

N, B: A-Rated feed only; B-Rated feed + 0.24 mg/l Chlorpyrifos; C-40mg vitamin B/Rg diet + 0.24 mg/l Chlorpyrifos; D-200 mg vitamin B/Rg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin B/Rg diet + 0.24 mg/l Chlorpyrifos and F-1000 mg vitamin B/Rg diet + 0.24 mg/l Chlorpyrifos.

**Table 4: Enzyme activities in the liver of *Heteroclitus* exposed to varying concentrations of Chlorpyrifos for 96 h and unsaturated with vitamin E for 28 days**

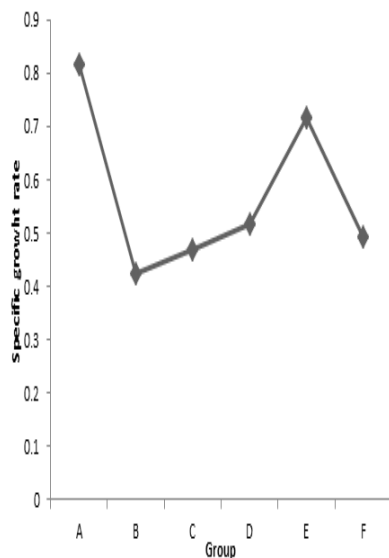
| Conc. (ppm)   | AST (U/mg)                 | AST (U/mg)               | LDH (U/mg)                | SDH (units/mg)            | ACoA (units/mg)           | SDA (units/mg)            | γ-GT (units/mg)            |
|---------------|----------------------------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| After 96 h    |                            |                          |                           |                           |                           |                           |                            |
| 0             | 68.00 ± 0.58 <sup>a</sup>  | 1.10 ± 0.37 <sup>a</sup> | 1.85 ± 0.31 <sup>a</sup>  | 0.98 ± 0.31 <sup>a</sup>  | 98.00 ± 1.15 <sup>a</sup> | 50.00 ± 0.58 <sup>a</sup> | 23.00 ± 1.15 <sup>a</sup>  |
| 1             | 76.00 ± 1.15 <sup>ab</sup> | 1.27 ± 0.38 <sup>a</sup> | 1.90 ± 0.48 <sup>a</sup>  | 1.02 ± 0.38 <sup>ab</sup> | 95.00 ± 2.31 <sup>a</sup> | 21.00 ± 1.15 <sup>a</sup> | 23.00 ± 1.15 <sup>a</sup>  |
| 2             | 84.00 ± 1.15 <sup>a</sup>  | 1.32 ± 0.64 <sup>a</sup> | 2.03 ± 0.31 <sup>ab</sup> | 1.13 ± 0.35 <sup>a</sup>  | 94.00 ± 2.31 <sup>a</sup> | 23.00 ± 0.38 <sup>a</sup> | 26.00 ± 0.38 <sup>ab</sup> |
| 3             | 93.00 ± 0.38 <sup>a</sup>  | 1.34 ± 0.38 <sup>a</sup> | 2.18 ± 0.04 <sup>a</sup>  | 1.28 ± 0.47 <sup>a</sup>  | 84.00 ± 0.38 <sup>a</sup> | 27.00 ± 0.38 <sup>a</sup> | 26.00 ± 0.38 <sup>a</sup>  |
| 4             | 96.00 ± 0.38 <sup>a</sup>  | 1.34 ± 0.31 <sup>a</sup> | 2.28 ± 0.31 <sup>a</sup>  | 1.24 ± 0.73 <sup>a</sup>  | 76.00 ± 0.38 <sup>a</sup> | 29.00 ± 0.38 <sup>a</sup> | 18.00 ± 0.38 <sup>a</sup>  |
| After 28 days |                            |                          |                           |                           |                           |                           |                            |
| A             | 68.00 ± 1.73 <sup>a</sup>  | 1.56 ± 0.37 <sup>a</sup> | 2.25 ± 1.73 <sup>a</sup>  | 1.66 ± 0.35 <sup>a</sup>  | 1.62 ± 1.15 <sup>a</sup>  | 19.00 ± 1.15 <sup>a</sup> | 15.00 ± 1.15 <sup>a</sup>  |
| B             | 76.00 ± 1.15 <sup>a</sup>  | 1.35 ± 0.35 <sup>a</sup> | 2.42 ± 1.73 <sup>a</sup>  | 2.02 ± 0.35 <sup>a</sup>  | 1.54 ± 0.38 <sup>a</sup>  | 20.00 ± 0.38 <sup>a</sup> | 23.00 ± 0.38 <sup>a</sup>  |
| C             | 76.00 ± 1.73 <sup>a</sup>  | 1.34 ± 0.73 <sup>a</sup> | 2.40 ± 1.15 <sup>a</sup>  | 1.84 ± 0.38 <sup>a</sup>  | 1.32 ± 2.38 <sup>a</sup>  | 24.00 ± 1.15 <sup>a</sup> | 18.00 ± 0.38 <sup>a</sup>  |
| D             | 74.00 ± 1.73 <sup>a</sup>  | 1.34 ± 0.73 <sup>a</sup> | 2.38 ± 0.01 <sup>a</sup>  | 1.75 ± 0.38 <sup>a</sup>  | 1.30 ± 0.38 <sup>a</sup>  | 24.00 ± 0.38 <sup>a</sup> | 18.00 ± 1.15 <sup>a</sup>  |
| E             | 76.00 ± 2.31 <sup>a</sup>  | 1.36 ± 0.35 <sup>a</sup> | 2.30 ± 1.73 <sup>a</sup>  | 1.76 ± 0.35 <sup>a</sup>  | 1.64 ± 0.38 <sup>a</sup>  | 20.00 ± 0.38 <sup>a</sup> | 16.00 ± 0.38 <sup>a</sup>  |
| F             | 72.00 ± 1.15 <sup>a</sup>  | 1.29 ± 0.38 <sup>a</sup> | 2.36 ± 1.15 <sup>a</sup>  | 1.73 ± 0.35 <sup>a</sup>  | 1.66 ± 1.73 <sup>a</sup>  | 22.00 ± 1.73 <sup>a</sup> | 18.00 ± 0.38 <sup>a</sup>  |

Mean (± S.E., n=5) with the same superscript in the same column are not significantly different ( $P > 0.05$ )

N.R. A-Rated feed only; B-Rated feed + 0.24 mg/l Chlorpyrifos; C-20 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos; D-200 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos; and F-1000 mg vitamin B/kg diet + 0.24 mg/l Chlorpyrifos.

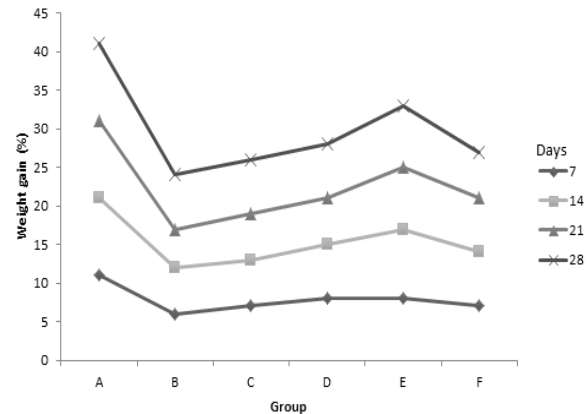
### Growth Responses

After 28 days of exposure of *Heteroclaris* to chlorpyrifos and amelioration with vitamin E, the highest percentage weight gain (41 %) and specific growth rate (0.817) was recorded in the control group (A) with no toxicant, while the lowest percentage weight gain (24 %) and specific growth rate (0.425) was revealed in group B that was exposed to chlorpyrifos only (Figures 1 & 2). However, the groups (C, D&E) fed on feed that was supplemented with varying concentrations of vitamin E recorded increase in their percentage weight gain (26, 28 & 33 % respectively). This increased as the concentration of vitamin E increased, except in group F that was ameliorated with the highest concentration of vitamin E with low weight gain (27 %) compared to control (Figure 1)



**Figure 1: Specific growth rate of *Heteroclaris* juvenile fed on varying concentrations of Vitamin E diet for 28 days.**

N.B: A-Basal feed only; B-Basal feed + 0.24 mg/l Chlorpyrifos; C-50 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; D-250 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos and F-1000 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos.



**Figure 2: Mean weight gain of *Heteroclaris* fed on varying concentrations of vitamin E diet for 28 days.**

N.B: A-Basal feed only; B-Basal feed + 0.24 mg/l Chlorpyrifos; C-50 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; D-250 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos; E-500 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos and F-1000 mg vitamin E/kg diet + 0.24 mg/l Chlorpyrifos.

### DISCUSSION

The decreased levels of the erythrocytes in the groups exposed to chlorpyrifos could be as a result of the toxic effect of chlorpyrifos that destroyed the red blood cells or inhibition of the production of more of the cells which lead to reduction in the value of PCV (Adeyemo, 2007). This result is in conformity with the reports of Abdulkareem and Owoloabi, (2014) and Ishtar *et al.* (2015). The significant increase in the erythrocytes of the groups ameliorated with vitamin E could be attributed to the ameliorative ability of vitamin E as reported by Mukai *et al.* (2007). This result conforms with the report of Abdulkareem and Tudor, (2016) who reported elevation in the erythrocytes of *Clarias gariepinus* ameliorated by *Moringa oleifera* as a result of high percentage of vitamin E in Moringa leaves. The significant increase in the level of white blood cells (WBC) and lymphocytes in the groups exposed to

chlorpyrifos could be due to oxidative stress induced by the toxic effects of chlorpyrifos. Elevation of platelet in the group exposed to chlorpyrifos could probably indicate repair of cells result of damage caused by accumulation of the toxicant in the fish tissues. This is similar to the reports of Ishita *et al.* (2015). However, decrease in the levels of these leucocytes in the groups ameliorated with vitamin E indicates that vitamin E could help to prevent oxidative stress in *Heteroclinas*. Similar report was given by Abdulkareem and Utuedor (2016) on *Clarias gariepinus* ameliorated with *Moringa oleifera*. Accumulation of this toxicant causes injury to the liver, and this may lead to release of tissue specific enzymes into the blood stream. Significant increase in the transaminase (ALT and AST) activities observed in the blood could be due to possible leakage of these enzymes across damaged plasma membrane of liver. The increase in the activities of serum AST, ALT & LDH of fish exposed to the chlorpyrifos therefore indicates liver damage. Similar results were observed in *Cyprinus carpio* and *Oncorhynchus mykiss* (Banaee *et al.*, 2011) exposed to organophosphorus pesticides. The significant increase in the Transaminases (AST and ALT) activities observed in gill and liver could be due to increased utilization of amino acids for energy synthesis as a result of hyperactivity induced by chlorpyrifos. This indicates that accumulation of this pesticide induces oxidative stress which increases the metabolic activities in the tissues by raising energy resources through release of enzymes that synthesize energy. Similar studies have been reported by Yildirim *et al.* (2006) in *Oreochromis niloticus* exposed to deltamethrin and Abdulkareem and Owolabi (2014) in *Heteroclinas* exposed to Monochrotophos and Abdulkareem and Utuedor (2016) in *Clarias gariepinus* exposed to Dichlorvos. The increased activity of LDH in fish

exposed to Chlorpyrifos could be as a result of the increased rate of conversion of lactate to pyruvate and also to glucose due to oxidative stress that resulted to oxygen depletion. This could be in compensation of the incapability of oxygen consumption by the fish under stress condition. Similar findings of increased LDH have been recorded in *Oreochromis Mossambicus* (Aniladevi, 2008); *Catlacatla*, *Labeorohita* and *Cirrhinus mrigala* (Tilak *et al.*, 2005); exposed to chlorpyrifos and *Clarias gariepinus* exposed to glyphosate (Abdulkareem *et al.*, 2014). The decreased level of AchE in the blood, gills and liver of fish exposed to chlorpyrifos could be attributed to inhibitory effect of Ache which leads to reduced synthesis of the enzyme. This could be attributed to the sluggish and uncoordinated movement in the chlorpyrifos-exposed fish; inhibition in the activity of AchE in various tissues of the fish has been reported (Joseph & Raj, 2011). Malondialdehyde (MDA) is a biomarker of membrane lipid peroxidation resulting from the interaction of ROS and the cellular membrane (Asian *et al.*, 1997). The increased level of MDA is an indication that Chlorpyrifos has induced oxidation stress on the fish which trigger the production of reactive oxygen species (ROS) that cause damage to cellular membrane, hence induction of lipid peroxidation as reported by Adams, *et al.*, (1990). The toxic effect of chlorpyrifos on the fish could generate the reactive oxygen species which induced oxidative stress that trigger the production of antioxidant enzymes. The increased level of SOD could therefore be an indication of oxygen depletion which help to transform the hydroxyl radicals ( $O_2$ ) to hydrogen peroxide ( $H_2O_2$ ) that is metabolized to  $O_2$  in stressed fish (Yonar *et al.*, 2012). The result is in agreement with the reports of Aniladevi (2008) and Abdulkareem and Owolabi (2014). Reduction in the activities of both

the biochemical enzymes and antioxidant enzymes in the group of fish fed on varying concentrations of vitamin E could probably be attributed to the preventive ability of vitamin E. Vitamin E is therefore capable of ameliorating the toxic effect of chlorpyrifos and reduce the production of reactive oxygen species, hence reduction in the oxidative stress. Protein plays a crucial role in virtually all biological processes in living organisms, increased in the levels of total protein in the blood and in the gills could be as a result of production of more protein as an alternative source of energy due to hyperactivity induced by the toxic effect of chlorpyrifos (Ganeshwade, 2011). Decrease in the total protein in the liver could probably be due to inhibition of protein synthesis due to liver damage or increase in the rate of its conversion to amino acid to cope with high energy demands to meet the stress situation (Lavanya et al. 2011). The reduction in the total protein in the blood and gills of fish ameliorated with vitamin E is an indication that vitamin E is capable of neutralizing the toxic effect of the toxicant and reducing the oxidative stress in the fish. Increase in blood glucose of *Heteroclaris* exposed to chlorpyrifos indicates an increased need for energy to cope up with the stress condition. Hyperglycemia or elevated blood glucose levels indicate impaired glucose, lipid metabolism and degradation of glycogen in liver. Increase in the level of blood glucose has been reported in *Heteroclaris* (Abdulkareem and Owolabi, 2014). The glucose level in the blood was minimized in ameliorated group and this indicated that vitamin E has the ability to reduce hyperactivity and oxidative stress in fish. This is similar to the reports of Abdulkareem and Utuedor, (2016). Reduction in specific growth rate with increase in chlorpyrifos could be due to suppressive effect in the food consumption by the toxicant or increased activity in an

attempt to avoid polluted water. The increased percentage weight gains and specific growth rate in the groups ameliorated with vitamin E could be as a result of prevention in the production in the reactive oxygen species by vitamin E which eventually reduced the high metabolic activity induced by the toxic effect of chlorpyrifos. This result is in agreement with that of Abdulkareem and Utuedor, (2016).

## CONCLUSION

The results of this work revealed that 2.4 ppm of Chlorpyrifos caused 50 % mortality in juveniles of *Heteroclaris*. Both lethal and sub-lethal concentrations of Chlorpyrifos induced oxidative stress and cause considerable alterations in erythrocytes and enzymes activities of juveniles of *Heteroclaris*, however, 500 mg/kg vitamin E supplemented in diet was able to improve the erythrocytes levels and reduced the enzymes activities after 28 days. This therefore, implies that 500 mg of vitamin E per kg diet is capable of ameliorating the toxic effect of Chlorpyrifos and reduced oxidative stress in juveniles of *Heteroclaris*.

**DISCLAIMER** – None

**CONFLICT OF INTEREST** – None

**SOURCE OF FUNDING** - None

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