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# COMPARATIVE STUDIES ON KINETIC PARAMETERS OF DETOXIFICATION ENZYMES IN THE HIND GUT OF *Zonocerus variegatus* Linnaeus, 1758

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**Abstract:** AIMS: The grasshopper (*Zonocerus variegatus*) is a polyphagous insect that feeds on wild, cultivated and uncultivated crops, causing extensive damage during their feeding process. Understanding the presence and expression of various detoxification enzymes in the gut of *Z. variegatus* (*Z.v*) may help to understand how they cope with allelochemicals encountered in their feeding process. METHODOLOGY: The grasshoppers were collected and dissected to obtain the hindgut. The hindgut was homogenized in Tris-HCl buffer (pH 9.6) and centrifuged at 10,000 g for 1 h. The supernatant was collected and stored as crude enzyme. Assay for the presence and quantification of the following enzymes: glutathione-S-transferase (GST), peroxidase, lysozyme, catalase, and superoxide dismutase (SOD) were carried out. Specific activities and kinetic parameters of the enzymes were also determined.

RESULTS: GST expression was the lowest amongst the antioxidant enzymes assayed, with a specific activity of 2.32 ± 0.6 Units/mg protein, whereas catalase was most expressed, with a specific activity of 3827.37 ± 201.11 Units/mg protein. The order of expression from largest to lowest was catalase>SOD>lysozyme>peroxidise>GST. CONCLUSION: This study concludes that *Zonocerus variegates* contains varying amounts of detoxification enzymes, which helps the insect to eliminate both exogenous and endogenous toxins.

**Key words:** Grasshopper, Catalase, Glutathione-S-transferase, Lysozyme, Polyphagous, Peroxidase

## 1.0 INTRODUCTION

Enzyme detoxification is a biotransformation mechanism involving a functionalization process that utilizes oxygen ($O_2$) and/or other molecules, such as hydrogen peroxide ($H_2O_2$), to form reactive sites and conjugation, which causes the reactive site to gain a water-soluble group. As a result, a lipophilic compound that cannot be eliminated undergoes biotransformation to a substance that is soluble in water and readily eliminated (Prapanthadara, *et al*., 2000). Through biotransformation reactions, organisms can protect themselves from harmful toxicants (Epel, 1998; Kennedy and Tierney, 2012). Prey chemical defences and predator detoxification systems may have coevolved to produce a large portion of the multiplicity of enzymes involved in biotransformation found in insects (Gonzalaz and Nebert, 1990; Li*et al*., 2003; Saha, 2016). As a result, enzyme detoxification involves several reactions rather than a single reaction (Wang *et al*., 2020). In general, only a handful of enzymes possess broad substrate specificity for the biotransformation or detoxification of xenobiotics (Kennedy and Tierney, 2012). The process of xenobiotic biotransformation is divided into three distinct phases that work together as a cohesive unit. (Guengerich, 1990). The transformation of a non-polar, lipophilic foreign substance into a more water-soluble, less hazardous intermediate that can be removed from the cell occurs more readily during phases I and II. Phase III entails the energy-dependent translocation of foreign substances that are dissimilar in both structure and function through a range of cellular transporters through the membrane (Litman *et al*., 2001). Transport proteins prevent the retention of toxicants in cells and tissues, and they work in tandem with phase I and phase II reactions to reduce xenobiotic toxicity. Phase III metabolism of pharmaceuticals and other xenobiotics depends critically on antiporter activity. Toxic substances are pumped out of cells by an energy-dependent efflux pump known as the antiporter, which also lowers their intracellular concentrations. (Francis *et al*., 2001; Ughachukwu and Unekwe , 2012).

Living things are continuously in contact with foreign chemical species that are not nutritional. These xenobiotics may have harmful interactions with biomolecules in the organism that may result in toxic and potentially cancer-causing consequences (Ames *et al*., 1990), also they come in contact and with reactive oxygen species (ROS)like the hydrogen peroxide ($H_2O_2$) and superoxide radicals as well as plant and fungal toxins like aflatoxins and plant phenols (naturally occurring toxic compounds). Adapting biologically to withstand the dangers presented by xenobiotic and endogenously produced substances is essential for survival. (Hayes and Pulford, 1995; Hayes and Mclellan, 1999; Sheehan *et al*., 2001; Kennedy and Tierney, 2012, Piwowarska and Kiedrzyńska, 2022).

Grasshopper (*Zonocerus variegatus*) is among the most widespread and damaging pests. Out of about 150 grasshopper species, 90% damage crops, trees, and shrubs, and they became very destructive during outbreaks. Grasshoppers and locusts are members of the Locustidae family, and they cause extensive damage in Sub-Saharan Africa (van Huis, 2022). A wide range of detoxification enzymes exists in *Z. variegatus*, among which are Glutathione-S-transferases, Catalase, Peroxidase, and Lysozyme (Bessie and Agboola, 2013; Adeyi*et al*., 2015; Adeleke *et al*., 2021). A good understanding of the roles played by these enzyme systems that function adequately to minimize the potential changes in *Zonocerus variegatus* that feed on these plants will involve determining their relative abundance in the insect, studying the resulting isoforms, their catalytic and kinetic efficiencies, as well as possible strategies to control the damage effect of the insect. This study was therefore designed to determine the relative abundance and investigate the kinetic parameters of some of this enzyme

(Glutathione-S-transferases, Catalase, Peroxidase, Lysozyme, and Superoxide dismutase (SOD)) in the gut of *Zonocerus variegates* to further understand their role.

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

1-chloro-2, 4-dinitobenzene (CDNB), Glutathione (GSH), Ethylenediaminetetraacetic acid (EDTA), glycerol, hydrogen peroxide *O*-dianisidine, *and Micrococcus lysodeikticus* were purchased from Sigma Chemicals, St. Louis, USA. Glycerol, 2-mercaptoetanol, sodium hydrogen phosphate, and sodium dihydrogen phosphatewere obtained from Merck Chemical Ltd New Jersey, USA. Protein assay reagents were purchased from Bio-Rad Ltd. (Hercules, CA, USA.

### 2.2 Animal source

Grasshoppers (*Zonocerus variegatus* Linnaeus, 1758) were harvested from a farmland within Obafemi Awolowo University, Ile-Ife, Nigeria and were identified in the Zoology Department, Obafemi Awolowo University, Ile-Ife.

### 2.3 Crude enzymes preparation

Thirty percent (30%) homogenate was prepared from a weighed mass of the gut of adult grasshoppers in a buffer: 50 mM Tris-HCl (pH 9.6) containing EDTA (1 mM), Mercaptoethanol (2 mM), and glycerol (10 %) for stability of the enzymes. The homogenate was subjected to centrifugation (Centurion Scientific LTD. 8880, R-Series) at 10,000 g for 1hr to eliminate cellular debris. The collected supernatants were stored as crude at 4 ºC.

### 2.4 Detoxification enzyme activity

The experiments to determine the expression and catalytic parameters of the enzymes were carried out at room temperature. The absorbance was also measured using a standard spectrophotometer (Biobase UV-visible spectrophotometer BK-D5 series).

#### 2.4.1 GST Activity Assay

The method of Habig *et al*., (1974) was used to determine GST activity in the homogenate. Kinetic-based activity was carried out by monitoring the absorbance at 340nm with a Biobase UV-visible spectrophotometer BK-D5 series following the thioether formation between reduced glutathione and CDNB. Briefly, the reaction mixture a total of 1 ml contains 0.7 ml of the 0.1 M phosphate buffer (pH 7.0), 0.1 ml of 10 mM GSH, 0.1 ml of 30 mM CDNB, 0.095 ml of distilled water and 5µl of the enzyme that would initiate the change in absorbance of between 0.02 and 0.05 nm upon reaction. A blank containing the entire reagent except the enzyme was also carried out. A unit of enzyme activity was defined as the amount of enzyme catalysing the formation of 1 µmol of product per minute under the conditions of the specific assay, with an extinction coefficient as 9.6 $mM^{-1}cm^{-1}$. Specific activity was defined as the units of enzyme activity per mg of protein.

#### 2.4.2 Catalase Activity Assay

Hydrogen peroxide degradation at 240 nm was observed spectrophotometrically to assess catalase activity. following the assay protocol of Chance and Maehly (1955). The assay mixture was 3 ml containing 1.4 ml of 50 mM potassium phosphate buffer (pH 7.0 (assay buffer) at 25 °C, 1.5 ml of 26 mM hydrogen peroxide and 100µl of the crude catalase enzyme. One unit of catalase is defined as the amount of enzyme that decomposes 1.0 micromoles of hydrogen peroxide to oxygen and water per minute at pH 6.5 - 7.0 at 25 ºC at a substrate concentration of 10 mM hydrogen peroxide.

### 2.4.3 Peroxidase Activity Assay

The method adopted for peroxidase quantification was the audited method of Edwin and Derek (1974).In the reaction mixture, 1.3 ml of 100 mM sodium phosphate buffer (pH 6.0), 0.1 ml of 1 mM O-dianisidine, 0.05 ml of 30 mM hydrogen peroxide ($H_2O_2$) and 50 µl of the enzyme were added together. There was a brownish color change due to the oxidation of O-dianisidine in the presence of hydrogen peroxide, and the optical density was read at 460 nm. One unit of enzyme activity is defined as the amount of enzyme that oxidizes 1 µmol o-dianisidine per min ($\varepsilon_{460}$ = 11.3 $mM^{-1}cm^{-1}$).

### 2.4.4 Lysozyme Activity Assay

The enzymatic activity of Lysozyme in the crude enzyme preparation was measured using the adjusted method of Shugar (1952). *Micrococcus lysodeikticus* (0.0015%) was used as the substrate. The reaction volume added up to 1.6 ml and contained 1.5 ml of substrate prepared in the assay buffer (0.1 M Sodium phosphate, pH 7.0) and 0.1 ml of lysozyme crude enzyme extract. The substrate was equilibrated at 25 °C followed by standardizing at 450 nm until its absorbance reading was constant. The decrease in absorbance was monitored at 15 sec intervals for 150 sec. One unit is equal to a decrease in turbidity of 0.001 per minute at 450 nm at pH 7.0 and 25°C under the specified conditions.

### 2.4.5. SOD activity Assay

SOD activity was assayed spectrophotometrically at 550 nm using a xanthine and xanthine oxidase system. Briefly, the reaction mixture (1 ml) which contain in final assay concentration, appropriate amount of homogenate, 0.5 mM hypoxantine, 0.5 mM hydroxylamine, and 0.01 units of xanthine oxidase was incubated at 37 °C for 40 min in an incubator and terminated with an appropriate volume of 16% (v/v) acetic acid solution, which had 38.6 mM naphthyl ethylenediamine and 2.6 mM sulfanilic acid. In order to calculate the SOD activity, the absorbance at 550 nm was measured. Under these conditions, one unit of SOD activity was defined as the amount of SOD required for 50% inhibition of the enzymatic reaction in 1 ml enzyme extraction of per milligram of protein.

### 2.5. Measurement of protein concentrations

The protein content of the crude homogenate was determined according to the method of Bradford (1976) with slight modification. The standard protein used was bovine serum albumin. Pipetting from 0 to 1.0 ml of BSA (10 µg/ml) into labeled test tubes, which corresponds to 0–10 µg BSA concentration. The test tube was then filled with distilled water to a volume of 1.8 ml. Each test tube was then filled with 0.2 ml of Bradford reagent, mixed, and incubated for a short time at room temperature. This allowed for the determination of the protein standard curve. Every test tube's absorbance was measured at 595 nm and compared to a blank sample that included every other ingredient in the mixture besides BSA. The standard curve was then created by plotting absorbance against the equivalent mass (in milligrams) of the standard protein. Using extrapolation from the standard protein, the amount of protein in the supernatant was calculated.

### 2.6 Determination of the kinetic parameters

The assay to determine the kinetic parameters of the various enzymes investigated was carried out by varying the concentration of the various substrates of the enzymes while monitoring their enzyme activity. The non-linear regression analysis software of GraphPad Prism 7 was used to analyze the data to determine the Kinetic parameters ($K_m$and $V_{max}$) of the enzymes.

## 3.0 RESULTS

The specific activities of the detoxification enzymes are presented in Table 1. Catalase had the highest specific activity, whereas glutathione S-transferase had the lowest. The apparent kinetic parameters (Table 2) revealed GST and peroxidase had the lowest Michealis-Menten constant ($K_m$) and catalase have the highest $K_m$. The highest maximum velocity ($V_{max}$) was obtained for peroxidase, and GST had the lowest $V_{max}$.

**Table 1: Levels of detoxification enzymes in grasshoppers**

| ENZYME | Specific Activity Units/mg protein |
|---|---|
| Glutathione-S-transferase | 2.32 ± 0.60 |
| Peroxidase | 50.05 ± 12.14 |
| Lysozyme | 293.54 ± 79.37 |
| Catalase | 3827.80 ± 201.73 |
| Superoxide Dismutase | 921.67 ± 19.22 |

**Table 2: Kinetic properties of detoxification enzymes in grasshoppers**

| ENZYMES | Apparent$K_m$ (mM) | $V_{max}$ (U/min) |
|---|---|---|
| Glutathione-S-transferase | $K_m^{GSH}$ 1.138 ± 0.092 | 0.49 ± 0.15 |
| | $K_m^{CDNB}$ 1.258 ± 0.13 | |
| Peroxidase | $K_m^{H2O2}$ 0.8682 ±0.09 | 643.25 ± 120.11 |
| | $K_m^{O\text{-}dianisidine}$ 0.6562± 0.152 | |
| Lysozyme | 74.09 ± 18.94 | 400.29 ± 108.43 |
| Catalase | 251.53 ± 48.17 | 126.55 ± 22.08 |
| Superoxide Dismutase | 0.38 ± 0.08 | 453.86 ± 109.47 |

## 4.0 DISCUSSION

In other to understanding the roles of detoxification enzymes (chemical defence) in the feeding habits of Z. *variegatus* Linnaeus, 1758, it is necessary to study the relative abundance of these enzymes which help the insect to cope with allelochemical encountered in its feeding process.

This study established that *Zonocerus variegatus* contains varying amounts of detoxification enzymes that it to eliminate both exogenous and endogenous toxins. GST was the least abundant detoxification enzyme with specific activity of 2.32 ± 0.6 Units/mg protein, a value that is lower than 3.33± 0.2 Units/mg protein for GST from grasshoppers published by Adewale and Afolayan, (2006). GST is an inductive enzyme, known to be induced by various compounds, food (Sherratt and Hayes 2001), xenobiotics (Higgins & Hayes, 2011) and even metal ions (Agunbiade *et al*., 2014). The Apparent$K_m^{GSH}$1.138 ± 0.092 mM and $K_m^{CDNB}$ 1.258 ± 0.13 mM) obtained from the gut of *Z. Variegates* for this study is also found to be much higher than those previously published by Adewale and Afolayan, (2006) and Famutimi and Adewale, (2021). This may be due to the feeding habits of the insects. It was previously found that GST expression is altered when grasshoppers were feed on grass of different types. This may explain why they obtained results that differ from our study.

Peroxidase ranks next in the rating order with a specific activity of 50 ± 12 U/mg protein, which is higher than values obtained for peroxidase isolated from fruits, as reported by Prestamo and Mangano (1993). However, when compared with the report of peroxidise obtained from the gut of grasshoppers under metal toxicity, it falls

between the range of the values obtained (Zhang *et al*., 2011). The value obtained is also within the range of peroxidase expression in other insects (Augustyniak, 2009).

Catalase, whose main function is to protect cells from the toxic effects of hydrogen peroxide, was found to be most abundant, with a specific activity of 3827 ± 201 U/mg protein, which is higher than that obtained from microbial sources as reported by Marinka *et al*., 2001. The value obtained from the catalase activity is higher than the activity of catalase found in various other insects and from grasshoppers previously published by Zhang *et al*., 2011.The reason for this is unknown and may be due to the feeding habits of the insect. The high apparent Km of $H_2O_2$ for catalase suggests that it has a high potential for biotechnological applications.

Lysozyme in the gut of *Z. Variegates* cannot be understated. Lysozymes are involved in many cellular functions. They disintegrate extraneous or worn-out cell components. They can be applied to eliminate bacterial and viral invasions. This suggests that the presence of lysozyme may deter the invasion of diseases causing pathogens to the pest. The digestive tracts of insects offer unique conditions for the colonization of microorganisms, and the bacteria found there may offer numerous advantageous functions to their hosts, and the presence of lysosomes may be due to the presence of various microbes in the gut (Engel and Moran, 2013). High levels of lysozymes in the gut of Z. *Variegates* indicate that grasshoppers have rich sources of microbial flora.

SOD expression in this study was found to be 921.67 ± 19.22 Units/mg protein with $K_m$ and $V_{max}$ of 0.38 ± 0.08 mM and 453.86 ± 109.47 Units/mg protein respectively. The value obtained for the expression of SOD in this study is significantly greater than that obtained by Zhang *et al*., 2011. The increase in the amounts of antioxidants may help coop with the burden of free radicals generated during the digestion of the various foods and allelochemical the pest is subjected to.

In conclusion, the relative abundance of these detoxification enzymes may help *Z. Variegates* eliminateallelochemical. Inhibition studies will provide insights into the stability of these enzymes, and how they can be exploitedfor pest control. Further studies on the resulting isoenzymes of each enzyme will provide a regulatory mechanism that can be used to formulate an insecticide as an effective biological control strategy against the damaging effects of insects on farm produce.

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