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FOOD PLANT STERILIZATION AND ITS IMPACTS ON HEMOCYTE CELLS AND ACTIVITIES OF CARBOHYDRASES IN THE MIDGUT OF *ZONOCERUS VARIEGATUS* (L.)

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Abstract – Through feeding, phytophagous insects acquire their gut microbiota which assists in enzymes secretion and production of secondary metabolites. This study seeks to validate the role of microbes found on food plants by sterilizing cassava leaves eaten by *Zonocerus variegatus* and its impacts on hemocyte cells and the midgut carbohydrases (amylase, cellulase and α-glucosidase) activities. The insects were divided into two groups: insects fed with sterilized cassava leaves and others fed with unsterilized cassava leaves. T-test was used to analyze the results. Five (5) hemocytes cells (prohemocyte, plasmatocytes, granulocytes, sperulocyte and adipohemocyte) were detected in the insects' haemolymph with the granulocytes recording the highest frequencies. *Zonocerus variegatus* fed with unsterilized cassava leaves had significantly higher hemocyte cells than those that consumed sterilized leaves. Similarly, *Z. variegatus* that consumed unsterilized leaves had significantly higher carbohydrases activities than those whose feeds were sterilized. Activities of α-glucosidase were higher than other enzymes in the midgut of the experimental *Z. variegatus*. It can be concluded that removal of microorganisms on the food plants eaten by *Zonocerus* through sterilization affected its immune response and digestive physiology.

Key words: Cassava leaves, sterilization, hemocytes, midgut, carbohydrases, *Zonocerus*

Izvleček – STERILIZACIJA HRANILNIH RASTLIN IN NJEN VPLIV NA CELICE HEMOCITE IN AKTIVNOST KARBOHIDRAZ V SREDNJEM ČREVESU KOBILICE *ZONOCERUS VARIEGATUS* (L.)

S hranjenjem rastlinojede žuželke pridobijo svoje črevesne mikrobe, ki pomagajo pri izločanju encimov in produkciji sekundarnih presnovkov. V študiji smo skušali potrditi vlogo mikrobov, ki jih najdemo na hranilnih rastlinah, tako da smo sterilizirali liste kasave, ki jo jedo kobilice vrste *Zonocerus variegatus* in raziskati njihov vpliv na celice hemocite in aktivnost karbohidraz srednjega črevesa (amilaza, celulaza in α-glukozidaza). Žuželke smo razdelili v dve skupini: v eni so jedle sterilizirane liste kasave, v drugi nesterilizirane. Za analizo rezultatov smo uporabili t-test. 5 vrst hemocitnih celic (prohemociti, plazmatociti, granulociti, speruolociti in adipohemociti) smo našli v žuželčji hemolimfi in granulociti so dosegli največje gostote. Kobilice *Zonocerus variegatus,* hranjene z nesteriliziranimi listi kasave, so imele občutno več hemocitnih celic kot tiste, ki so zaužile sterilizirane liste. Prav tako so imele tiste, ki so zaužile nesterilizirane liste, občutno višjo aktivnost karbohidraz od onih, katerih hrana je bila sterilizirana. Aktivnost α-glukozidaze je bila višja od drugih encimov srednjega črevesa poskusnih živali. Lahko zaključimo, da je odstranitev mikroorganizmov s hranilnih rastlin, ki jih jedo kobilice rodu *Zonocerus* s sterilizacijo vplivalo na njihov imunski odziv in prebavno fiziologijo.

KEY WORDS: listi kasave, sterilizacija, hemociti, srednje črevo, karbohidraze, *Zonocerus*

Introduction

The variegated grasshopper, *Zonocerus variegatus* (L.) was accorded pest status in Nigeria in 1974 by Centre for Overseas Pest Regulation, due to its polyphagus nature, consuming both food and cash crops (Toye, 1982; COPR, 1974). Chiffaud and Mestre (1970) reported that *Z. variegatus* consumed more than 250 plants among which are: citrus, cocoa, banana, vegetables and coffee. Also in the neighboring country, Cameroon, *Z. variegatus* was ranked 3rd most economically detrimental insect pest of agriculture (Kekeunou, 2006).

Methods adopted for its control include – physical, cultural, chemical and integrated pest management (Toye, 1982). However, due to its numerous side effects chemical method has been discouraged and other methods are now combined for effective control. One method yet to be fully harnessed is the biological control which involves the use of natural enemies as pathogens to control pest (Toye, 1982).

The roles played by hemocytes in the insects include phagocytosis of microbes, encapsulation of foreign materials, coagulation of haemolymph and distribution of nutritive materials (Gupta, 1985). Idowu and Sonde (2004) observed that food types consumed by insects influenced the types and number of hemocyte cells.

The gut of polyphagus insects like *Zonocerus* harbors numerous microorganisms. Namely: bacteria and fungi (Chapman, 1990; Campbell, 1990). The colony forming unit (cfu) of these microbes increased as the size and age of the insect increased

(Idowu and Edema, 2004). Among other things, the microbes synthesize digestive enzymes especially cellulase which cannot be synthesized by animals (Idowu *et al*, 2009). Alcohols, vitamins and minerals are other by-products of fermentation process anchored by these microbes (Chapman, 1990). These microorganisms are not vertically transmitted from mothers to their offspring in *Zonocerus* but through the food plant consumed (Ademolu and Idowu, 2011).

The roles played by microbes in the physiology of insects are numerous, however, their significant roles at micro level (enzymes and immune response) has not received full attention in literature. The study aims to investigate the role of microbes in digestion process and effects on immune response. The focus of this study is to evaluate the effect of food (leaves) sterilization on gut enzymes and hemocytes number in *Z. variegatus.*

Materials and methods

INSECT COLLECTION AND MAINTENANCE

Variegated grasshoppers (*Z. variegatus*) of various stages of development were collected from uncultivated farmland near the Health Centre of Federal University of Agriculture, Abeokuta, (FUNAAB) Nigeria early in the morning (6:30 am – 7:30 am). The insects were sorted by sex and later instars (5^{th} and 6^{th} instars) were maintained on fresh cassava (*Manihot esculenta*) leaves until they reached the adult stage. Male adults of 3 days old were recruited for this study.

EXPERIMENTAL PROCEDURE

Forty (40) male adults *Z. variegatus* were separated into two groups (A and B) of twenty individuals each. Group A were fed with sterilized cassava leaves while insects in group B were fed with unsterilized cassava leaves. The two groups of insect were fed *ad-libitum* for 6 weeks.

The sterilization of the cassava leaves was done following procedures described by Rutala *et al.* (2008) and Ademolu *et al.* (2015).

HEMOCYTE ESTIMATION

Hemocytes estimation was done by making a thin smear of the insect haemolymph, air dried and hemocyte cells were detected with drops of Leishman's stain (Gupta, 1979). Freshly prepared slides were used for identifying cell types under microscope (x 400). Estimates of cell counts were made by counting number of different cells in three different fields of view (Jibir,1981). Quantification was performed in triplicate for each animal.

GUT PREPARATION

Three individual insects per group were frozen in a deep freezer for 30 minutes after which their guts were dissected out and the midguts were carefully removed and homogenized in 0.05M KCl and centrifuged at 500 rpm for 30 minutes at 5 ^{0}C. The enzymes extract was decanted into bottles and kept for enzyme analysis.

GUT ENZYMES ANALYSIS

The activities of carbohydrases (α-glucosidase, amylase and cellulase) in the midgut of *Z. variegatus* were found out following methods described by Adedire and Balogun (1995). The enzymes activities were determined quantitatively by using Dinitrosalicyclic Acid Reagent (DNSA). The amount of reducing sugar produced at the end of incubation period was estimated spectrophotometrically at 550nm. The experiment was replicated three times.

STATISTICAL ANALYSIS

Data collected from the experiments were analyzed using t-test ($t = 0.05$).

Results

The number and types of hemocytes in the *Zonocerus* fed sterilized cassava leaves are presented in Table 1. Five (5) different types of hemocyte cells were detected in the insect haemolymph with Granulocytes representing the highest number of cells. Also, insects fed unsterilized cassava leaves had significantly higher number of hemocyte cells than those fed sterilized cassava leaves.

Table 1: Estimate of hemocyte cells in *Zonocerus variegatus* (L) fed cassava leaves ($x10^5$ per ml of haemolymph)

HEMOCYTE CELLS	INSECTS FED STERILIZED LEAVES	INSECTS FED UNSTERILIZED LEAVES
Prohemocyte	8	10
Plasmatocyte	7^b	12^a
Granulocyte	10^b	15^a
Oenocytoid	0	0
Sperulocyte	3^b	10^a
Adipohemocyte	7^b	11^a
Total	35	58

*Means values in the same row having different superscript are significantly different at ($p<0.05$)

The activities of carbohydrases in the midgut of *Zonocerus* fed sterilized cassava leaves are shown in Table 2. Insects fed unsterilized leaves had significantly higher cellulase and α-glucosidase activities than those fed sterilized leaves. The highest enzymatic activity in the midgut was detected for α-glucosidase, while amylase activity was the lowest.

Table 2: Carbohydrases activities (Abs/min) in the midgut of *Zonocerus variegatus* fed cassava leaves

S/N	SAMPLE	CELLULASE	AMYLASE	α - GLUCOSIDASE
1	**Grasshopper fed sterilized leaves**	0.678^b	0.215	0.861^b
2	**Grasshopper fed unsterilized leaves**	0.712^a	0.221	0.868^a

*Means values in the same column having different superscript are significantly different at ($p<0.05$).

Discussion

The number and types of hemocyte cells present in the haemolymph of *Z. variegatus* and in other insects generally varies with the physiological state of the insects (Mullin, 1985 and Idowu and Sonde, 2004). *Zonocerus* fed with unsterilized leaves had more hemocytes than their counterparts fed with sterilized leaves. Ademolu *et al* (2010) observed that the number of hemocytes increased as *Z. variegatus* increased in age (during embryonic development) and this is attributed to the increase in food consumption as the insect ages. The food plants consumed by *Zonocerus* harbor microorganisms which elicit or activate host defense system of the insect (Eslin and Provost, 1998). Hence, the lower hemocytes in the insect fed with sterilized leaves might be due to reduced/low microbial load on the leaves which elicit less of the insect defense system.

Five hemocyte types were detected in the haemolymph of the experimental insects namely prohemocyte, plasmatocyte, granulocyte, sperulocyte and adipohemocyte. Oenocytoids were not detected in the experimental insects and this runs contrary to the six (6) hemocyte cells earlier recorded in *Z. variegatus* by Idowu and Sonde (2004). Gouli *et al.* (2000) earlier observed that Oenocyloids were extremely rare and few numbers were also seen in *Adelges tsugae*, likely due to their insignificant role in fighting foreign agents. Similarly, Jone (1962) pointed out that Oenocytoids were either absent or questionably present in same orthopterans as they rapidly disintegrate outside the host. On the other hand, granulocytes were the most abundant, followed by plasmatocytes and prohematocytes. This might be connected to their functions. Both granulocytes and plasmatocytes are involved in phagocytosis of the foreign agents and also important in wound healing (Gupta, 1985).

One essential role of microbes in the gut of *Zonocerus* is to synthesize enzymes needed to break down food plants consumed by insects. Idowu *et al* (2009) reported that microbes inhabiting the gut of *Zonocerus* secrete cellulolytic enzymes used by the insects. The source of these microbes has been linked to the food plants (Ademolu and Idowu, 2011) and their removal by sterilization shown to influence the production of these enzymes. This explains the reduced carbohydrases activities in the midgut of *Zonocerus* fed sterilized leaves.

It is worthy to note that the two groups had the highest activities of α – glucosidase and lowest activities of amylase in the midgut. The main diet of the insect is cassava leaves which is a form of polysaccharide and requires high activity of amylase to break it down to disaccharides. However, digestion of polysaccharide begins at the foregut as the enzymes move backward from the site of secretion (midgut) to the fore gut (Hill and Orchard, 2015). Hence, higher α – glucosidase activities in the midgut is needed to hydrolyse the inflow of disacharides from the foregut. Detection of cellulase in the midgut of the insects suggests the presence of microbes as animals lack the ability to synthesize cellulase. Microorganisms live symbiotically in the gut of phytophagous insects and assist by synthesizing the enzymes required for the hydrolysis of cellulose portion of their diet.

Conclusion

This study suggests that food microbes present on the food plants of *Z. variegatus* aid digestion through enzymes secretion and elicit the insect's defense system.

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