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CHEMICAL ANALYSIS OF THE REPELLENT SECRETION AND GLAND, FAT BODY AND HAEMOLYMPH OF ADULT VARIEGATED GRASSHOPPER *Zonocerus variegatus* (L) (ORTHOPTERA: PYRGOMORPHIDAE)

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Abstract
Chemical analysis of the repellent gland and secretion, fat body and haemolymph of adult variegated grasshopper *Zonocerus variegatus* (L) (Orthoptera: Pyrgomorphidae) reared on *Manihot esculenta* and those collected from the wild were analyzed using Gas Chromatography- Mass Spectrometry (GC-MS). The chemical compounds present in the repellent gland of the reared insects were twenty-two and were classified as esters, alkanes and alkenes while six compounds were present in those collected from the wild although with more varieties and were grouped into: heterocyclic aromatic hydrocarbon (pyrazine), amine, ether, ester and alcohol. Repellent secretion of *Z. variegatus* reared on *M. esculenta* had sixteen chemical compounds grouped into heterocyclic hydrocarbon, alkane, ester, alkene. However, that of the wild had five compounds classified as heterocyclic hydrocarbon and glycoside. Irrespective of the sources, pyrazine was present in the repellent secretion of *Z. variegatus,* though more abundant in the wild. Sixteen compounds were identified in the fat body of the reared insects and classified into alkanes, ester, alkene while those from the wild had thirteen compounds grouped into: alkane, alcohol, nitrobenzene and amine. The haemolymph of the reared insects had sixteen compounds classified as: alkane, alkene, acid and ester while that of the wild had seventeen compounds grouped as alkane, ester, nitrobenzene, aldehyde, heterocyclic hydrocarbon, acid, glycoside and alcohol. This study shows that the smell associated with *Zonocerus variegatus* is by the presence of pyrazine and esters containing sulphur present in the repellent gland and secretion.

Key words: *Zonocerus variegatus,* Repellent gland, Repellent secretion, Pyrazine, Chemical compounds

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INTRODUCTION

Variegated grasshopper *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae) is a polyphagus insect that feeds and defoliates a large number of farm crops (Youdeowei, 1974) such as citrus, cowpea, banana, cocoyam and cassava (Ademolu and Idowu, 2011). It is considered a serious pest of agriculture in sub–Saharan Africa (Chapman *et al.,* 1986). Only cassava and *Chromoleana odorata* have received much attention among the major food plants of *Z. variegatus* (Gall *et al.,* 2003). It was considered that *Z. variegatus* has great relationship with cassava (Bernays *et al.,*1977) and has been greatly damaged in the field due to group behavior with tolerance to hydrogen cyanide by the later instars (Chapman *et al.,*1986). *Chromolaena odorata* on the other hand; is known to produce pyrolizidine alkaloids, which could have attracted this insect since sequestration of alkaloids by grasshoppers has been recorded (Chapman *et al.,* 1986).

Zonocerus variegatus is easily recognized by the multi-coloured markings on its body with an unpleasant odour from its repellent gland (Idowu and Idowu, 1999). The repellent gland is present in all instars but functional and opens inside out from the 3rd instar till adult (Idowu, 2000). In adult *Z. variegatus*, the gland dimensions are: length 5.9mm; width 3.8mm; volume 225mm^3, orifice diameter 1.1mm . The gland is formed by the invagination of the body wall and lined with cuticle (Idowu and Modder 1998). Dettner (2015) identified that arthropods defensive glands are made up of sac-like cuticular reservoir of varying sizes and may be located in the head, thorax or abdomen. The repellent gland of *Z. variegatus* is situated between the 1st and 2nd abdominal segments having basement membrane with secretory and non-secretory cells. It is flattened when empty and ovoid when filled (Idowu, 1995). The gland stores secretion in its lumen (reservoir) and discharge of the secretion following any disturbance is a function of the size and volume of the gland lumen (Idowu, 1996). Changes in the pressure of the haemolymph cause the ejection of secretion from the repellent gland of *Z. variegatus* (Idowu, 2000). It is ejected in the form of a jet-like spray released to avoid both vertebrate and invertebrate predators (Idowu and Idowu, 1999).

The nature of chemicals present in defensive substances possesses multiple roles aside protection from attackers (Whitman *et al.,* 1992). Diverse chemicals have been found in the defensive secretion of arthropods such as benzoquinones, hydrogen cyanide, repellent alkaloids, quinones, phenols, aldehyde, ketones, tarpenoids and carboxylic acids but the biosynthesis have not been elucidated (Blum, 1981). Preliminary chemical analysis of *Z. variegatus* repellent secretion revealed that it contains chemical compounds such as alkaloids which are present irrespective of its food source. The mechanisms involved in its synthesis are unknown and the need to characterize the secretion has been stated (Idowu and Modder, 1998). Identification of the secondary chemical compounds present in repellent secretion and gland of *Z. variegatus* is crucial as these could be used to produce substances synthetically that would serve as effective lures with appropriate baits for the management of *Z. variegatus* as a pest.

MATERIALS AND METHODS

Collection of *Zonocerus variegatus*

Dry season population of adults and 5th instars of *Z. variegatus* were collected using sweep net from an abandoned farmland at the Federal University of Agriculture, Abeokuta (FUNAAB). The insects collected were kept in the insectary of the Department of Pure and Applied Zoology, Federal University of Agriculture, Abeokuta (FUNAAB), in wire cages (30 ×30 × 45 cm). The nymphs of *Z. variegatus* were fed with *Manihot esculenta* until they metamorphosed into adults.

Tissue collection and preparation

Collection of Repellent secretion: Milking was done according to the method described by Idowu and Modder (1998). A pipette calibrated in microlitres was used to draw the secretion from the repellent gland, through the orifice on the dorsal surface of the insect.

Collection of Repellent glands: The repellent glands were removed using forceps according to the method of Idowu and Modder (1998).

Collection of Fat Body: The insects were dissected as described by Youdeowei (1974) and the fat body was collected following the method of Ademolu*et al.,* (2007). Forceps was used to remove the body wall and 5g of the sub-sample was weighed using sensitive balance into a test tube. And thereafter, homogenized in 5ml of distilled water. The homogenate was kept in the freezer for further analysis.

Collection of Haemolymph: The collection of haemolymph was in line with the method described by Ademolu *et al.,* (2007). A microneedle was inserted into the mid-ventral axis of the thorax for the collection of the haemolymph into a calibrated syringe, From each haemolymph sample, 100 µL was centrifuged at 1300 rpm for 15 min to spin down the debris and hemocytes. The supernatant was thereafter used for further analysis.

Determination of Secondary Compounds

The repellent secretion and tissues were analyzed to determine the presence of secondary compounds using Gas Chromatography – Mass Spectrometry (GC-MS). The carrier gas was Helium and the stationary phase was packed with silica column. The oven temperature was programmed from 80ºC to hold for 2 minutes at 10ºC per minute and to a final temperature of 240 ºC to hold for 10 minutes. The volume of sample injected was 1 microlitre, the scan range was from 50 to 550 amu.

Components extracted were identified by matching the peaks with computer Wiley MS libraries and was confirmed by matching spectra of peaks and using the database of National Institute of Science and Technology (NIST).

RESULTS

Chemical Analysis of Tissues Collected From *Zonocerus variegatus*

Repellent Gland

The Repellent Gland, upon analysis showed peaks with Twenty-Five (25) compounds. Including Esters, Alkanes and Alkenes. Hexadecanoic acid, methyl ester has the highest percentage Abundance (14.149%) as shown in Fig. 1 and Table 1 while the insects collected from the wild had Forty-one (41) peaks on the chromatograph. These compounds can be categorized as Heterocyclic aromatic hydrocarbon, Amine, Nitrobenzene, Ester and Alcohol. The highest percentage abundance was observed as Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)-(18.357) as shown in Fig. 2 and Table 1.

Repellent secretion

Sixteen (16) compounds were identified including Pyrazine –butyl- 3,5-dimethyl; Esters (including Esters bearing Sulphuric acid), Alkanes and Alkenes. Squalene has the highest Percentage Abundance of 15.119% as shown in Fig. 3 and Table 2. But for the wild, the repellent secretion, upon GC-MS analysis showed only Eight (8) peaks on the chromatograph. The compounds identified contains Heterocyclic aromatic hydrocarbon, glycoside and nitrobenzene compound. Octyl-.beta.-D-glucopyranoside has the highest percentage abundance (35.06%) as seen in Fig. 4 and Table 2.

Fat Body

There are seventeen (17) chemicals in the Fat Body of *Z. variegatus* reared on *M. esculenta*. The compositions of the compounds are hydrocarbon (Long chain, branched and unbranched), fatty acids and esters. Among these components, Hexadecanoic acid methyl esters have the highest percentage abundance of 28.744% as shown in Fig. 5 and Table 3 whilethirty-four (34) components were identified from the Fat body of *Z. variegatus* collected from the wild but only thirteen (13) was observed to have met the level of confidence. These compounds can be classified as Amines, Arenes, Halocarbon, Phenols and Alcohols. Of all, Oxime-, methoxy-phenyl- had the highest percentage as observed in Fig. 6 and Table 3.

Haemolymph

The number of peaks identified for haemolymph after subjection to GC-MS analysis was seventeen (17). Comprising of Esters, Alkanes, Alkenes. 10,13-Octadecanoic acid, methyl ester appeared most abundant (35.314%) as shown in Fig. 7 and Table 4. Comparatively, the haemolymph of insect collected from the wild had twenty-seven (27) components identified, but only seventeen (17) met the confidence level. These include: Alkanes, Nitrobenzene, Aldehyde, Nitrile, Heterocyclic hydrocarbon, Glycoside, Amine, Ester and Caboxylic acid. Octyl-beta-D-glycopyranosie (15.06%), of the compounds identified, appeared to have the highest percentage abundance as shown in Fig. 8 and Table 4.

Only compounds which meet the quality standards are presented in the tables.

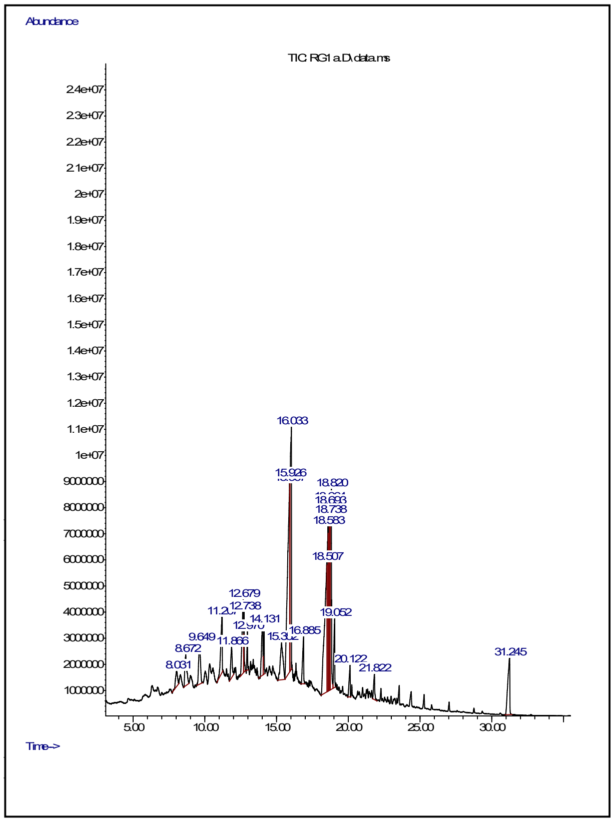


Fig. 1 Abundance and peak of components identified in the Repellent gland of *Z. variegatus* fed on *Manihot esculenta*

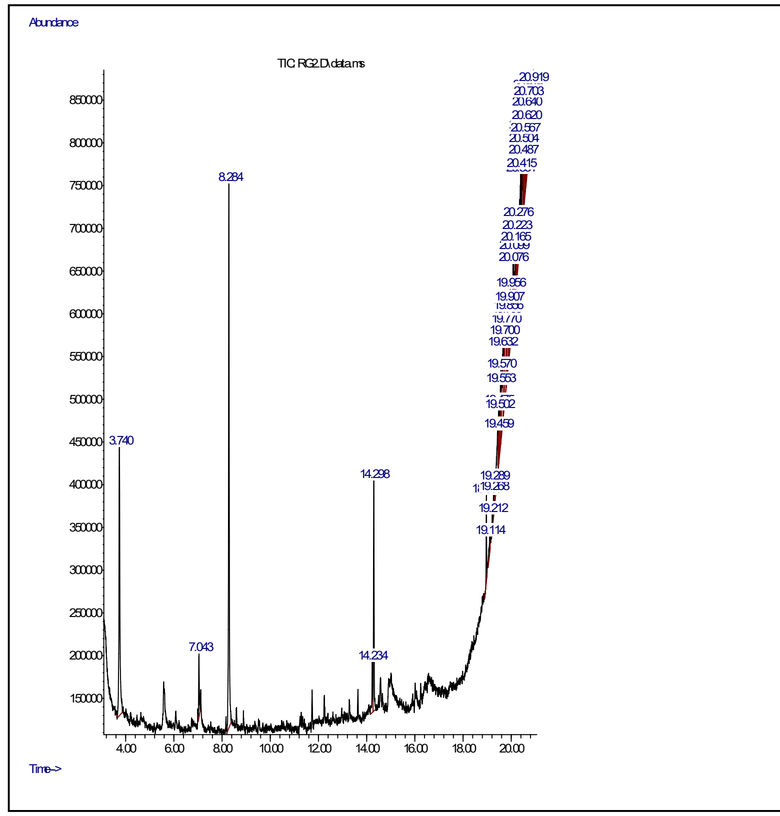


Fig. 2 Abundance and peak of components identified in the Repellent gland of *Z. variegatus* collected from the wild.

Table 1: Weight and Abundance of Repellent gland of *Zonocerus variegatus* (Reared and collected from the Wild) ran on GC-MS

Sn	Chemical compound	Molecular Weight	% Abundance (Reared)	% Abundance (Wild)
	Alkanes			
1	Docosane	310.6	1. 230	-
2	Dodecane, 2, 6,10-trimethyl-	212.41	1.399	-
3	Dodecane,2,6,11-trimethyl	212.41	3.744	-
4	Eicosane	282.55	1.969	-
5	Heptadecane	240.47	1.095	-
6	Hexadecane	226.44	3.148	-
7	Nonadecane	268.52	3.653	-
8	Octadecane	254.49	2.571	-
9	Pentadecane	212.41	2.798	-
10	Tetradecane	198.39	1.116	-
11	Tridecane, 5-propyl	226.44	1.342	-
	Alkene			
12	Squalene	410.72	4.264	-
	Total		28.329	
	Esters			
13	10,13-Octadecadienoic acid, methyl ester	294.47	2.461	-
14	11-Octadecenoic acid, methyl ester	296.49	1.838	-
15	9,12, octadecadienoic acid methyl ester	294.47	5.572	-
16	cis-13-Octadecenoic acid, methyl ester	296.49	8.452	-
17	Hexadecanoic acid, methyl ester	270.45	14.149	-
18	Methyl stearate	298.5	2.461	-
19	Nonanoic acid, 9- oxo-, methyl ester	186.25	2.164	-
20	n-propyl 11-octadecanoate	324.5	-	1.038
21	Pentadecanoic acid, methyl ester	256.42	1.762	-
22	Sulfuric acid, butyl octadecyl ester	406.66	1.696	-
23	Tridecanoic acid, 12-methyl-, methyl ester	242.4	0.867	-

	Total		41.422	1.038
	Ether			
24	Oxime-, methoxy-phenyl-	151.16	-	12.357
	Amine			
25	Octodrine	129.24	-	1.834
26	1,2-Benzisothiazol-3-amine, TBDMS derivative	264.46	-	3.498
	Total			5.332
	Alcohol			
27	N-Desmethyltapentadol	207.31	-	7.78
	Heterocyclic hydrocarbon			
28	Pyrazine, 2,5-dimethyl-3-(3-methylbutyl)-	164.25	-	18.357

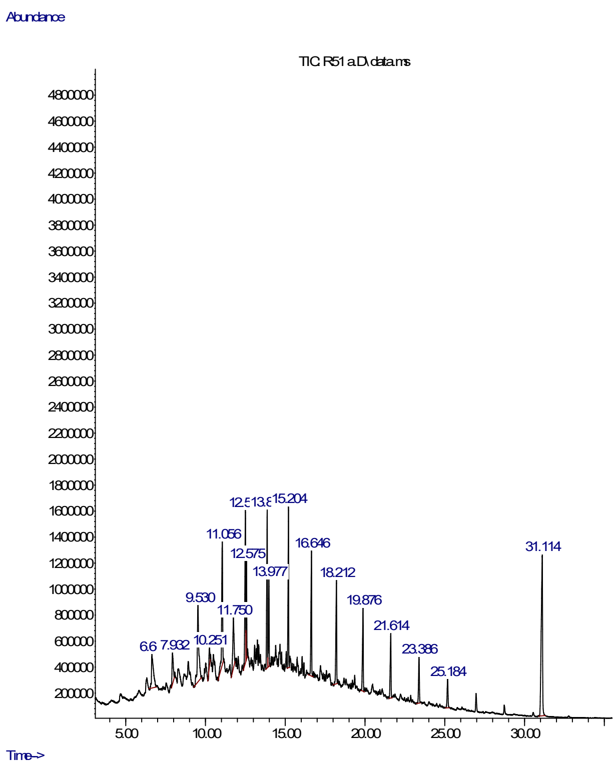


Fig.3: Abundance and peak of components identified in the Repellent secretion of *Z. variegatus* fed on *Manihot*

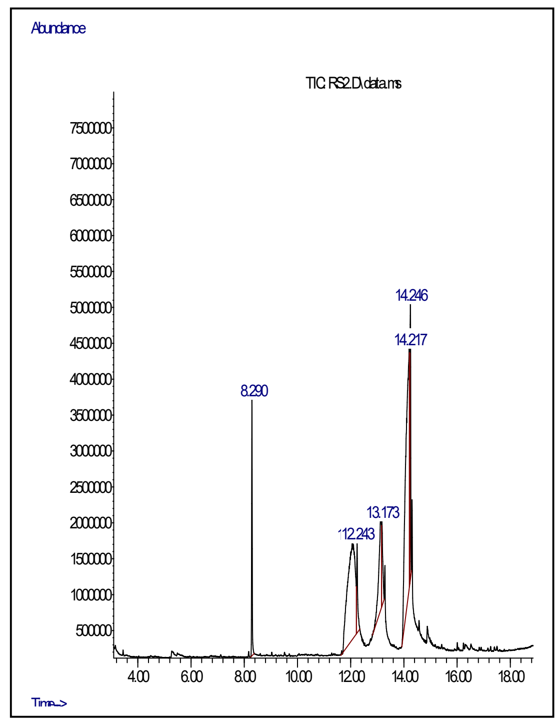


Fig.4: Abundance and peak of components identified in the Repellent secretion of *Z. variegatus* collected from the wild.

Table 2: Weight and Abundance of Repellent secretion of *Zonocerus variegatus* (Reared and collected from the Wild) ran on GC-MS

Sn	Chemical compound	Molecular Weight	% Abundance (Reared)	% Abundance (Wild)
	Alkane			
1	Eicosane	282.55	6.427	-
2	Heneicosane	296.57	5.796	-
3	Heptadecane	240.47	8.316	-
4	Hexacosane		3.717	
5	Hexadecane	226.44	8.453	-
6	Hexadecane, 2, 6,10,14-tetramethyl	282.55	4.105	-
7	Nonadecane	268.52	6.983	-
8	Octadecane	254.49	7.606	-
9	Pentacosane	352.68	1.843	-
10	Pentadecane	212.41	8.241	-
11	Pentadecane, 2, 6, 10, 14-tetramethyl	268.52	3.844	-
12	Tetracosane	338.65	2.702	-
13	Tetradecane	198.39	2.597	-
	Total		70.63	
	Alkene			
14	Squalene	410.72	15.119	-
	Ester			

15	Sulphurous acid, butyltridecyl ester	286.45	4.223	-
	Heterocyclic hydrocarbon			
16	Pyrazine,2-butyl-3,5-dimethyl	164.25	4.497	6.287
	Glycoside			
17	Ethyl .alpha.-d-glucopyranoside	208.1	-	13.429
18	Ethyl-alpha-d-glucopyroside	208. 21	-	3.461
19	Methyl-.beta.-D-thiogalactoside	210.25	-	29.423
20	Octyl-.beta.-D-glucopyranoside	292.37	-	35.060
	Total			81.373

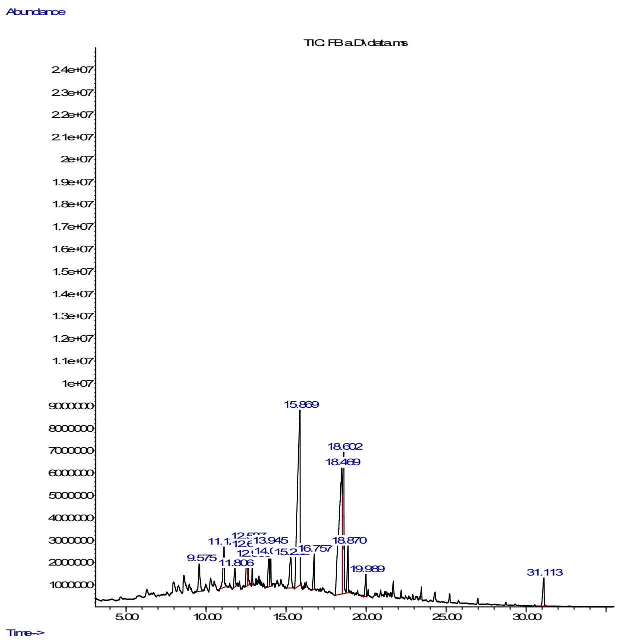


Fig.5 Abundance and peak of components identified in the Fat body of *Z. variegatus* fed on *Manihot esculenta*

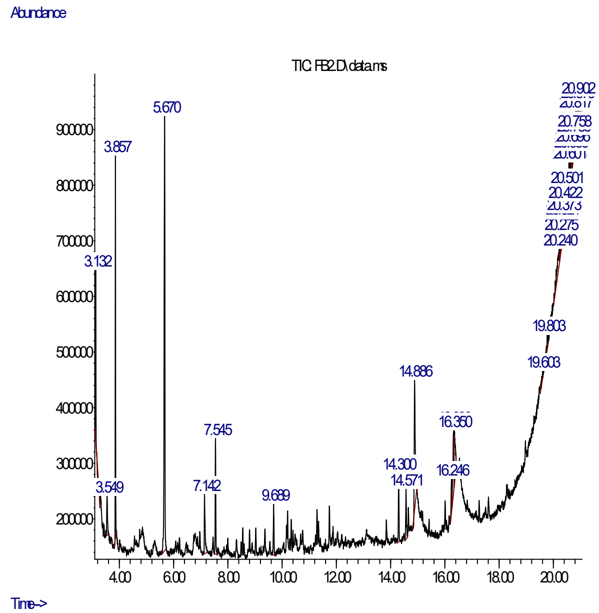
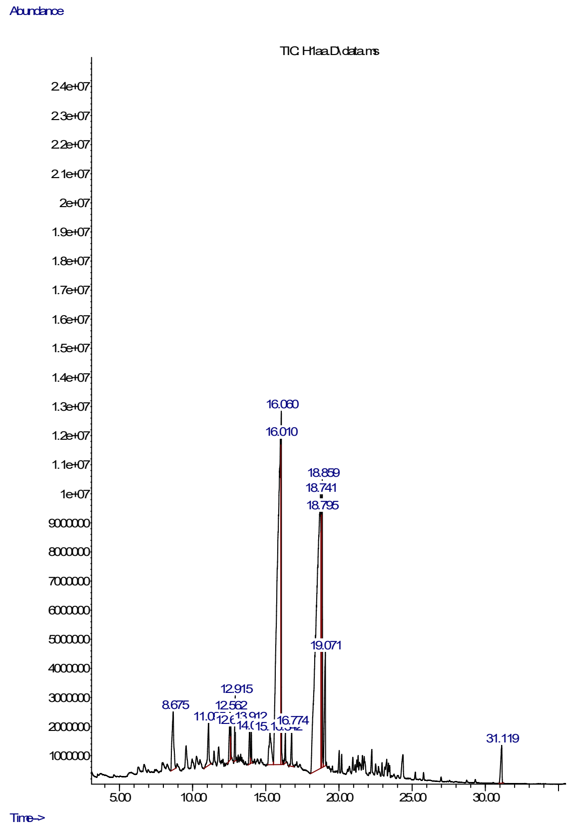


Fig.6 Abundance and peak of components identified in the Fat body of *Z. variegatus* collected from the wild

Table 3: Weight and Abundance of Fat Body of *Zonocerus variegatus* (Reared and collected from the Wild) ran on GC-MS

Sn	Chemical compound	Molecular Weight	% Abundance (Reared)	% Abundance (Wild)
	Alkanes			
1	Dodecane	170.33	3.029	-
2	Dodecane2, 6,10-trimethyl	212.41	1.515	-
3	Eicosane	282.55	2.58	-
4	Ethane, 1-(4,4,4-trifluoro-1,3-dithiobutyl)-2-(3,3,3-trifluoro-1,2-dithiopropyl)-	356.39	-	0.628
5.	Heptadecane	240.47	4.035	-
6	Hexadecane	226.44	3.662	-
7	Nonadecane	268.52	4.018	-
8	Octadecane	254.49	2.82	-
9	Tetratetracontane	619. 2	1.468	-
10	Tridecane, 6-propyl	226.44	1.69	-
11	Tritetracontane	605.16	1.532	-
	Total		26.349	0.628
	Alkene			
12	Squalene	410.72	2.607	-
	Esters			
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	294.47	25.605	-
14	Cis-13-Octadecanoic acid, methyl ester	296.49	13.707	-
15	Hexadecanoic acid, methyl ester	270.45	28.744	-
16	Methyl Stearate	298.5	2.856	-

17	Tridecanoic acid, 12- methyl- methyl ester	242.4	1.021	-
	Total		71.933	
	Alcohol			
18	2,3-Butanediol [R-(R*,R*)]-	90.12	-	2.151
19	2-Propyl-tetrahydropyran-3-ol	144.21	-	9.516
	Total			11.667
	Nitrobenzene			
20	Cyclotrisiloxane, hexamethyl-	222.46	-	0.896
21	Cyclotrisiloxaneoctametyl	296.62	-	5. 251
22	Oxime-, methoxy-phenyl-	151.16	-	9.913
	Total			16.06
	Amine			
23	1,2-Benzisothiazol-3-amine, derivative	150.2	-	0.600
24	Acetamide, N-(aminocarbonyl)-2-chloro	136.54	-	1.946
25	N-Methyl-2-phenyl-1-propylamine	149.23	-	2.886
26	N-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine	255.35	-	5.328
27	Nortriptyline	263.38	-	3.304
28	Phenylephrine	167.205	-	4.509
29	Propanamide	73.09	-	0.46
	Total			19.033



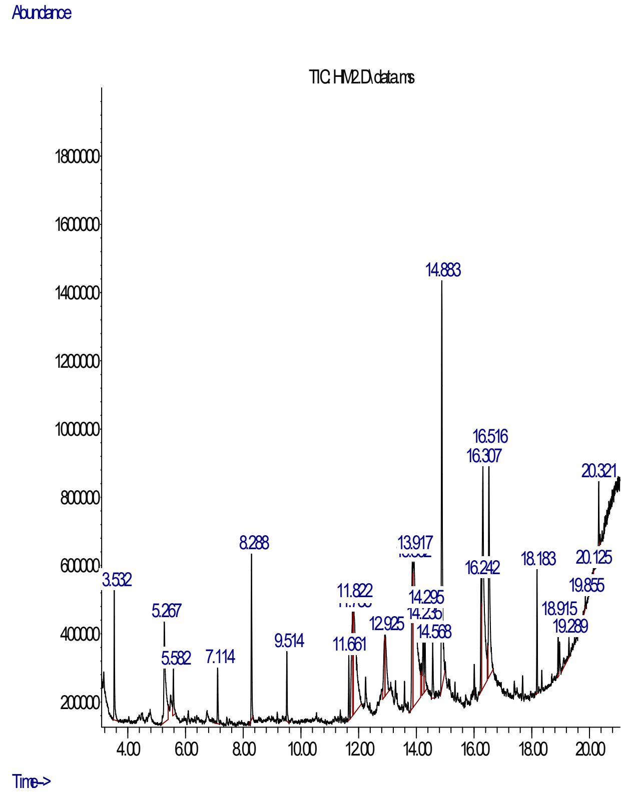


Fig.7 Abundance and peak of components identified in the Haemolymph of *Z. variegatus* fed on *Manihot esculenta*

Fig. 8 Abundance and peak of components identified in the Haemolymph of *Z. variegatus* collected from the wild

Table 4: Weight and Abundance of Haemolymph of *Zonocerus variegatus* (Reared and collected from the Wild) ran on GC-MS

Sn	Chemical compound	Molecular weight	% Abundance (Reared)	% Abundance (Wild)
	Alkanes			
1	Dodecane, 2, 6,11 –trimethyl	170.33	0.503	-
2	Eicosane	282.55	0.964	0.88
3	Hexadecane	226.44	1.595	-
4	Hexadecane	226.44	-	1.14
5	Hexadecane, 2,6,10,14-tetramethyl-	282.55	0.591	-
6	Octadecane	254.49	1.041	-
7	Tetracosane	338.65	-	2.02
8	Tetradecane	198.39	-	1.33
9	Tetratetracontane	619.19	1.322	-
	Total		6.016	5.37
	Alkenes			
10	Cyclohexene, 3-(2-methylpropyl)-	138.25	0.601	-
11	Squalene	410.72	1.377	-
	Total		1.978	
	Acid			
12	10, 13-Octadecadienoic acid	280.45	35.314	-
13	9-Octadecenoic acid, (E)-	282.46	**-**	12.61
14	Octadecanoic acid	284.48	**-**	7.97
15	Propanedioic acid, propyl-	146.14	-	10.83
	Total		35.314	31.41

	Esters			
16	9-Octadecanoic acid, methyl ester	298.49	2.874	
17	cis,13-Octadecanoic aci, methyl ester	296.49	7.081	
18	Hexadecanoic acid, methyl ester	270.45	30.974	1.335
19	Methyl hexadec-9-enoate	268.43	2.182	-
20	Methyl stearate	298.29	3.272	-
21	Methyl tetradecanoate	242.2	1.331	-
22	Nonanoic acid, 9-oxo-, methyl ester	186.25	2.947	
23	Pentadecanoic acid, 14- methyl, methyl ester	270.5	6.033	-
	Total		56.694	1.335
	Nitrobenzene			
25	Benzonitrile, 2-methyl-	117.1479	-	5.06
26	Cyclotrisiloxane, hexamethyl-	222.4618	-	1.06
24	Oxime-, methoxy-phenyl-_	151.16	-	2.144
27	Tetraacetyl-d-xylonic nitrile	343.29	-	3.511
	Total			11.775
	Aldehyde			
28	Pentanal	86.13	-	1.23
	Heterocyclic hydrocarbon			
29	Pyrazine, 2,5-dimethyl-3-(3-methyl	136.19	-	3.04
	Glycoside			
30	Octyl-.beta.-D-glucopyranoside	292.37	-	15.06
	Alcohol			
31	Z,E-2,13-Octadecadien-1-ol	266.5	-	2.51

DISCUSSION

This study reveals that the repellent gland, repellent secretion, fat body and haemolymph of *Zonocerus variegatus* are rich in organic compounds. The chemical nature present in the secretion and other tissues has been further elucidated. Ranging from alkanes, alkenes, acids, ether, amine, alcohol, glycosides, nitrobenzene and heterocyclic hydrocarbon: pyrazine. Bowers and Larin (1989) identified some compounds been sequestered by insects: cardenolides, iridoid glycosides, sesquiterpenes, pyrollizidine alkaloids, salicylaldehyde, and hypericin. Also, the results of this findings is also similar to the work of Idowu and Modder (1998) who recorded the presence of alkaloid and glucose in the preliminary chemical analyses conducted on the repellent secretion of *Z. variegatus*. The haemolymph and fat body possess the same number of compounds though slightly different. But similar pattern was observed in the chemical groups present in the repellent gland and secretion of the grasshopper collected from the wild and those reared. For instance, alkanes, alkenes are present in the repellent gland and repellent secretion of the reared insect. Pyrazine was present in the repellent secretion of both the reared grasshopper and those collected from the wild. But only present in the repellent gland of insects gotten from the wild.

Eisner and Meinwald (1966) stated that the defensive secretion of arthropods contain different compounds of low molecular weight belonging to common chemical classes, including acids, aldehydes, ketones, esters, hydrocarbons lactones and phenols. These compounds include simple straight chain, cyclic and acyclic chemical substance As shown in this study, hydrocarbons are identified along with other constituents / chemical group. Alkanes function as a cuticular wetting and spreading agent which can promote the penetration of more polar compounds in the defensive/repellent secretion (Blum, 1981). In *Philanthus triangulum (*Female beewolves), long chain saturated and unsaturated hydrocarbons protects its symbionts from a lethal compound, Nitric oxide. This compound produced by the female beewolves, is intended to protect its brood cell from antagonistic soil dwelling microbes (Chantal, *et al.,*2023). Squalene, an alkene was identified in all tissues and secretion of reared insects. It has been recorded to be widely distributed in nature and abundant in shark liver and plant seeds. It is an important metabolic intermediate involved in the secretion of animal cholesterol and plant steroid. Physiologically, it is an anti inflammatory and antioxidant substance. It is normally a colourless and odourless, greese. Squalene extracted from shark has been stated to aid cardiovascular health and relieves skin damage caused by dryness and eczema (Du *et al.,* 2024).

Esters are another group identified in the repellent secretion, repellent gland and fat body of the reared *Z. variegatus* and the haemolymph of both the reared and insects collected from the wild. However, Hexadecanoid acid, methyl ester was identified in the repellent gland of reared insect, fat body of reared insect and also present in haemolymph of the reared and the insects obtained from the wild. This compound has been identified in the haemolymph of *Archachatina maginata* and is known to function as antioxidant, hypocholesterolemic, nematicide, hemolytic and 5- alpha reductase inhibitor (Lawal *et al.,* 2015). The importance of esters in defensive glands and secretion is that it is accompanied by alcoholic and acidic moieties to which may be probably related metabolically (Blum, 1981). More so, esters containing sulphur was commonly identified in the repellent secretion and gland of *Z. variegatus* of the reared insects.

Amines, nitrobenzene and alcohols were identified in the repellent gland, fat body and haemolymph of *Z. variegatus* of grasshoppers collected from the wild. The polyphagus nature of the insect could be responsible for the presence of these chemical groups as they are absent in the grasshopper whose diet was *Manihot*. In addition, the capability of the insect to sequester chemicals for defense may be responsible for this trend (Bernays et al. 1977).

Glycosides were also identified in the Repellent secretion and the Haemolymph of *Z. variegatus* collected from the wild. This secretion is rich in glucose compared to lipid and protein content as recorded by Ajao *et al.,* (2021 Unpublished). Idowu and Modder, (1998) identified glucose in the repellent secretion of *Z. variegatus* and stated that glucose in the secretion could be a by- product of some reactions in the secretory cells of the gland. The presence of glucoside in the defensive arsenal of insects could be by *de novo* synthesis or sequestration. *Phaedon cochleariae* from *de novo* synthesis have been recorded to produce 8-OH-Geranoil-O-glucoside while sequestration of plant-derived glycosides by the leaf beetles have also been recorded (Boland, 2015). Glycosides have been found to be precursors for the enzymatic production of deterrents in *Chrysomelina* larvae where all compounds reaching the glandular reservoir via the haemolymph are glucosides that are enzymatically converted into biologically active form within the reservoir (Boland, 2015). Octyl beta -d-glucopyranoside which is commonly identified in the secretion and haemolymph of *Z. variegatus* in this study could be as a result of enzymatic reactions in the haemolymph of the insect which diffused into the defensive gland of the insect. This substance is a non ionic surfactant used to solubilise integral membrane proteins. Pharmacologically, the repellent secretion has been reported to induce oedema in the hind paw of rat (Idowu and Idowu, 1999). Oedema formation in man has been linked to high level of glucose in the body due to low level of insulin. Thus, increased amount of glucose in the defensive secretion of *Z. variegatus* could be responsible for the formation of oedema in rats.

Bernays *et al.,* (1977) identified the ability of *Z. variegatus* to sequester and store pyrrolizidine alkaloids (PA's) after been reared on *Crotalaria retusa.* The function of PA's was stated to be probably defensive. Furthermore, Idowu and Modder (1998) recorded the presence of alkaloid in their study with no specificity on the exact type but emphasis was made that even insects reared on Non-alkaloid plant had alkaloid. This study based on qualitative and quantitative analysis revealed the presence of pyrazine in the repellent secretion of insects reared on *Manihot* and those collected from the wild. The pyrazine identified could be the alkaloid present in the defense secretion. Although the repellent gland of grasshopper raised on *Manihot* lacks pyrazine but its presence in the repellent gland and haemolymph of grasshoppers collected from the wild could be due to the polyphagous nature of the insect (Chapman et al., 1986) and the ability to sequester compounds from plants consumed on the field. The biochemistry of sequestration was reported to start with the absorption by the gut, transport from the gut to the haemolymph, transport in the haemolymph, then deposition in organs and other sides of storage (Bowers and Larin, 1989).

Pyrazine, has been recorded to be produced by animals, plants as well as microorganisms (Ona *et al.,* 2017). In plants and animals, it functions as warning odour (Eduardo *et al.,* 2018). Many primitive ponerine ants produce prodigious quantities of pyrazine which appear to function primarily as allomone (Evans and Schmidt, 1990). This compound is considered to also serve as aposematic signals, for amplification of the visual aposematism provided by the body colouration (Nickle *et al.,*1996). Furthermore, Nishida (2002) reported that pyrazine as an olfactory cue, have a low olfactory threshold causing a sharp learning potential in vertebrates. The avoidance of *Z. variegatus* by both vertebrate and invertebrate predators (Idowu and Modder, 1998) could be as a result of pyrazine. The presence of esters containing sulphur as an accompanying agent might be to aid the pungent smell which is a characteristic of the secretion. More so, pyrazines are used as flavouring agents. They occur naturally in heat treated food products. 2, 5-Dimethylpyrazine have been found as a compound present in roasted *Tenebrio molitor* and *Zophovasmorio* larvae having an odour description of cocoa, roast beef and roasted nut (Zolneierczyk and Summy, 2021). The edibility of *Z. variegatus* using different preparation methods has also been confirmed (Idowu *et al.,* 2004).

CONCLUSION

This study shows that the smell associated with *Zonocerus variegatus* as recorded by Bernays *et al*. (1977) and Idowu and Modder (1998) has been confirmed by the presence of pyrazine and esters containing sulphur.

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