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Identification of Carboxylesterase genes and their expression profiles in conferring resistance to commonly used pesticides in *Zonocerus variegatus* (linneaus, 1758)

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Abstract

Identifying insecticide resistance mechanisms is paramount for pest insect control, as the understandings that underpin insect control strategies must provide ways of detecting and managing resistance, thus study identified the ability of Carboxylesterase (CarEs) genes in conferring resistance to commonly used pesticide by examining the expression of the gene in the susceptible and resistant strain of *Zonocerus variegatus*, after Cypermethrin bioassay. Adult *Z. variegatus* was sampled from Savannah area of Oyo State, Nigeria, samples were collected and preserved in "RNA Later" for RNA extraction. The two groups of *Z. variegatus* were later subjected to quantitative quantitative Polymerase Chain Reaction (qPCR) using two primers. CarEs gene expressions have both negative and positive ΔΔct value when LmcesA20 primer was used. However, no significant difference in expression level was observed between groups for both primers LmcesE9 ($F = 0.84$, $p > 0.05$) and LmcesA20 ($F = 0.499$, $p > 0.05$). The study concluded that since the expression level observed for CarEs gene was the same using LmcesE9 in both groups, LmcesE9 may not function for detoxifying Cypermethrin in *Z. variegatus*. LmcesA20 showed a little variation in expression level in both strains having higher fold change in resistant strain than the susceptible strain, thus these members of CarEs genes need to be further verified for their specific role in conferring resistance to use of pesticides.

Keywords: *Carboxylesterases, Gene, Cypermethrin, Pyrethroids, Susceptible, Resistant*

1. Introduction

Carboxylesterases (CarEs) play significant roles in metabolism of certain hormones and detoxification of dietary and environmental xenobiotics in insects. These classes of enzymes are responsible for hydrolysis of chemicals which contain a functional group of carboxylic acid ester, amide, and thioester (Wheelock *et al*., 2005; Yang, 2012). Many insect CarEs are associated with insecticide resistance or hormone and semiochemical metabolism (Oakeshott *et al*., 2010; Oakeshott *et al*., 2013). They are involved in the detoxification of many insecticides, including synthetic pyrethroids (SPs), carbamates (CBs) and organophosphates (OPs) which

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have been shown to be involved in development of resistance to insecticide groups SPs, CBs and OPs (Cui *et al*., 2011; and Wu *et al*., 2011).

Zonocerus variegatus, (Orthoptera: Pyrgomorphidae) genus of grasshoppers (Caelifera) in the family Pyrgomorphidae (Roskov *et al*., 2011). It has been regarded as the main pest of food crops such as citrus, banana, cassava, cocoa, coffee, cowpea and yams in West and Central Africa's moist lowland forest and savannas (Modder, 1994; and Kekeunou *et al*., 2006). Over the last 20 years, Z. *variegatus* outbreak have increased due to the high production rate of cassava and due to widespread or distribution of *Chromolaena odorata* (L.) (Bamidele and Muse, 2012). By the time the first, second and third instars live on weeds such as *C. odorata* and *Aspilia africana* (Asteraceae family), the fifth and sixth instars feeds on *M. esculenta* (Euphorbiaceae) (Chapman *et al*., 1986).

Cypermethrin belongs to a class of insecticide known as synthetic pyrethroids. It has a high level of insecticidal activity, low effect on class Aves and Mammalia. It is most preferable to other form of insecticides because it is less toxic to non target organisms and since others pesticides have been gradually phased out. It belongs to a class of insecticide that is widely used by farmers to control *Z. variegatus*, and it can also have effect on non-target organism like human being.

CarEs pyrethroid metabolism has been the subject of several studies due to its widespread usage in agriculture (Hodgson and Levi, 1996) and public health (Takken, 2002). CarEs plays an important role in detoxifying pyrethroids, which was demonstrated practically in recent rodent studies (Gaughan *et al*., 1980).

CarEs play a key role in the metabolism of xenobiotics including insecticides in insects. The understanding of expression patterns of such detoxifying gene and as well as it the effect of insecticides on its enzyme activities are necessary to clarify the function of this gene relevant to insecticides-detoxifying process. But little information is available in the variegated grasshopper *Zonocerus variegatus* (L.)

Insects have developed resistance to almost all classes of chemical pesticides through detoxification mechanisms involving a number of detoxifying enzymes, including glutathione S-transferases (GSTs) (Ranson *et al*., 2001). B-esterases, including Cholinesterases and Carboxyesterases, are typically inhibited by OPs due to the extremely slow dephosphorylation of tetrahedral intermediates produced between OPs and aserine residue at their active sites (Fukuto, 1990). These three types of enzyme esterases (through ester hydrolysis), cytochrome P450 monoxygenases (through oxidation), and glutathione transfarases (through ester hydrolysis) are primarily used to turn insecticide into less harmful products (Hollingworth and Dong, 2008).

Pyrethroids and Carbamates are believed to be hydrolyzed by CarEs. The quantitative approach is determined by the overproduction of CarE proteins by gene amplification and transcriptional up-regulation.

2. Materials and methods

2.1. Insect collection

Z. variegatus were sampled in Savannah area of Oyo state using a sweep net (Kemp *et al*., 1990; and Cigliano *et al*., 2000). They were transferred to insect cage of dimension 29.5 cm × 27.5 cm × 40.5 cm for grouping. They were fed with cassava leaves for the period of collection before exposure to Cypermethrin.

The specimen collected were transported to the Entomology laboratory, where they were sorted in groups into hoarding plastic cage of dimension 10 cm × 12.5 cm × 6 cm for Cypermethrin bioassay.

2.2. Exposure to chemical and RNA extraction

Groups of adult *Z. variegatus* were tested for insecticide resistivity and some group without insecticide are used as the control. Z. *variegatus* and the *cassava* leaves were sprayed with different concentration of the recommended treatments (Cypermethrin) in different groups except for the control and observed for 1 h. The knockdown time of individual grasshopper were recorded in minutes, the dead ones are collected after 1 h of exposure and deemed insecticide susceptible if after mechanical stimulation, they remain motionless. The *Z. variegatus* alive after one (1) hour were scored as cypermethrin-resistant. The resistant and susceptible *Z. variegatus* are preserved in "RNAlater" (Qiagen) for RNA extraction.

Total RNA was extracted from three samples of susceptible and resistant strains of each concentration of chemicals used using Trizol reagent (Takara, Dalian, China) and treated with RNase-free DNase I (Promega

Corporation, Madison, WI), purity of the RNA was quantified by measuring UV absorption using a spectrophotometer, absorbance is measured at 260 nm (A260) and 280 nm. The first-strand cDNA was synthesized by reverse transcribing from 1.5 μg of total RNA using MLV reverse transcriptase (Takara). The relative expression levels of 25 CarE-like genes of the locust by real-time quantitative PCR (qPCR) with *β-actin* act as a reference gene.

2.3. Primer selection

Two primers were used for this study LmcesA20 and LmcesE9 based on specific band showed by gel electrophoresis.PCR amplicon electrophoresis was carried out by size fraction on 2.0% Agarose gels. Electrophoresis was used to determine the quality and integrity of the RNA and the binding of primers with cDNA by fractionation on 2.0% agarose gels. Agarose gels were prepared by dissolving and boiling 0.7 g agarose in 35 ml 0.5 × TBE buffer solutions. Electrophoresis was done for 75 ^{o}C for 30 min. The primer sequences and the expected size of each PCR product are shown in Table 1.

Table 1: Table of primer selected to test for Carboxylesterase gene in *Z. variegatus*

Gene name	Application of primers Sequences (59-39)	Product size (base point)
LmCesA3	F: GCGGAGCGACATGTCTTCC	164
	R:ATGCTGCTCTTTTTTAGTGAGCATT	
LmcesA20	F: GCCATGAATCCGTGCCTTCTCCA	
	R: GCGACCTCTTTAACGTACAG	75
LmCesD1	F: TGCTGGGATGTCACGGTCTC	
	R: GTAAAGCACTAATACTAATGAACCA	105
LmCesE1	F: GAAGATTTGGTGAGGTGAACAGTG	
	R:TTGTTAGGCATAATCCGTTTAGAGA	121
B-actin	F: CGAAGCACAGTCAAAGAGAGGTA	
	R: GCTTCAGTCAAGAGAACAGGATG	156
Lmces E9	F: CAGAACCTCCTGTTGGAACACA	
	R: CAGAGCATCTCTTACACCATTCCAT	77

2.4. Quantitative-PCR verification of CarEs genes associated with Cypermethrin resistance

The qPCR was performed by using three biological samples in each groups, Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Analysis of Variance was performed to determine significance of difference in the relative expression of the two (2) CarEs genes between the susceptible and resistant strain.

3. Results

3.1. Primers selection

Electrophoresis showed B-actin to be significantly expressed than the other primers and two primers LmcesA20 and LmcesE9 were chosen from five primers selected from *L. migratoria* CarEs genes based on specific band showed. The expression levels of the primers selected were very low in the selected sample.

Table 2: RNA concentration measured by spectrophotometer for each of the samples extracted

Sample ID	ng/ul	A260	A280	260/280	260/230	Const.	Cursor Pos.	Cursor abs.	340 raw
D2...2.9	99.20	2.48	1.19	2.09	2.30	40	230	1.08	0.05
B1...1.9	178.64	4.47	2.15	2.08	2.39	40	230	1.87	0.02
A2...2.9	189.64	4.74	2.29	2.07	2.38	40	230	1.99	–0.01
D...1.9	96.94	2.42	1.19	2.04	2.26	40	230	1.07	–0.01
C4	168.92	4.22	2.08	2.03	2.43	40	230	1.74	0.02
A3...1.9	31.40	0.79	0.42	1.89	1.99	40	230	0.40	0.01
B2...1.9	185.24	4.63	2.24	2.07	2.40	40	230	1.93	–0.01
D0.48	175.32	4.38	2.13	2.06	0.76	40	230	5.75	0.01
D0.9	174.12	4.35	2.11	2.06	0.77	40	230	5.64	0.03
A1....2.9	162.65	4.07	1.97	2.07	2.28	40	230	1.78	0.05
A2...1.9	19.05	0.48	0.23	2.04	1.63	40	230	0.29	0.03
C1	37.71	0.94	0.47	2.02	1.94	40	230	0.49	0.02
D2....1.9	106.74	2.68	1.28	2.09	2.24	40	230	1.19	0.06
D2...2.9	36.12	0.90	0.47	1.93	1.91	40	230	0.47	0.03
D2...2.9	35.68	0.89	0.47	1.90	1.85	40	230	0.48	0.03
A4 0.9	156.23	3.906	1.90	2.06	2.40	40	230	1.63	0.06
A30.48	67.26	1.68	0.83	2.03	2.30	40	230	0.73	0.03
A3 2.9	11.87	0.30	0.14	2.06	2.18	40	230	0.14	0.03
D12.9	34.22	0.86	0.39	2.21	2.46	40	230	0.35	0.02
D1.9	45.58	1.14	0.57	2.01	1.41	40	230	0.81	0.04
A2 0.48	23.52	0.59	0.28	2.13	2.08	40	230	0.28	0.02
C3	75.68	1.89	0.95	1.98	1.55	40	230	1.22	0.03
B3-19A	148.84	3.72	1.80	2.07	2.26	40	230	1.65	0.00
D2-2.9	32.6	0.82	0.40	2.02	2.48	40	230	0.33	0.01

3.2. Cycles threshold (Ct)

Cycle threshold (Ct) values for each of the samples were calculated according to the values generated from the analysis. All the Ct values were subjected to One Way Analysis of Variance (ANOVA), results showed that there was no significant difference between the susceptible strains and resistant strains for LmcesE9

($F = 0.734$, $p > 0.05$) and LmcesA20 ($F = 0.730$, $p > 0.05$). There was no significance difference between both strains using LmcesA20 ($F = 0.509$, $p > 0.05$) and LmcesE9 ($F = 0.281$, $p > 0.05$). Amplication curve for all the samples using the ct value for both primers were plotted and shown in Figures 1 and 2 respectively.

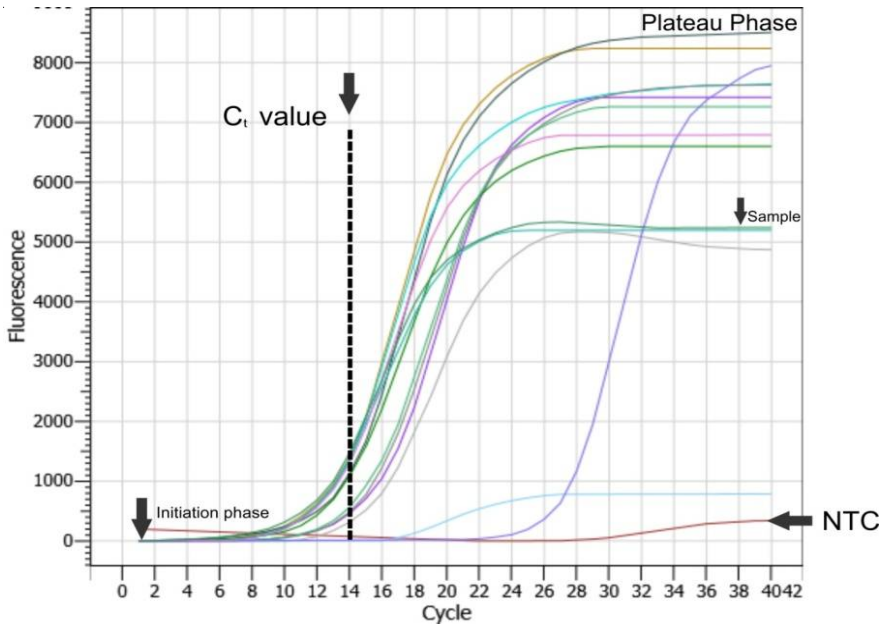

Figure 1: Amplification curves for LmcesA20. This graph shows the fluorescence increase for different samples with the number of cycles. The threshold was set above the limit of detection, but well below the stage where the amplification rate slows down

Note: NTC means Null Template Control

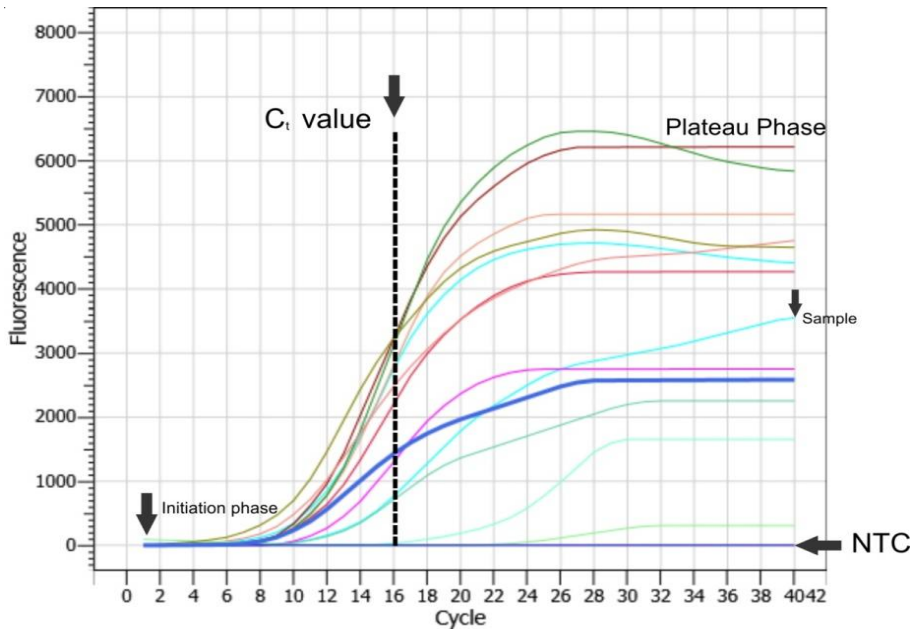

Figure 2: Amplification curves for LmcesE9. This graph shows the increase of fluorescence with the number of cycles for different samples. The threshold was set above the detection limit but well below the plateau phase where the amplification rate slows down

Note: NTC means Null Template Control

3.3. Comparative quantification using ΔΔCt ($2^{-\Delta\Delta Ct}$)

In using LmcesE9 the highest ΔΔct value was obtained from susceptible strain D0.9 (-22.30) and lowest value from A0.48 (-14.18) from resistant strain, likewise using LmcesE9 highest ΔΔct value was from A2.9 (-6.21) from resistant strain and lowest ΔΔct value was from D 0.9 (-22.3) from resistant strain as well. Ct value varied significantly in both the primers, meanwhile ΔΔct value of 0.25 indicated 4X decrease in transcript level compared to housekeeping gene. One way ANOVA was used to compared significance difference between the ΔΔct value in susceptible strain and resistant strain using both primers, values gotten showed no significant difference in susceptible strain and resistant strain for both primers LmcesE9 ($F = 0.84$, $p > 0.05$) and LmcesA20 ($F = 0.499$, $p > 0.05$). Fold change expression using LmcesE9 range from 5163793-33689 for susceptible strains and range was 1100707-18561 for resistant strains. For LmcesA20 fold change range from 0.54-1.71 for susceptible strains and 1.69E-7 – 74.03 for susceptible strains.

The relative expression of CarEs genes measured by qPCR was represented by diagram of fold gene expression. It was plotted using the descriptive statistics calculated, the value of calibrator used was obtained by taking the average of all control values, for LmcesE9 it was 12.68. The average value of $2^{-\Delta\Delta Ct}$ for all the samples in both treated (susceptible and resistant strains) and control group were calculated for LmcesE9, it was 498.60 and 0.45 respectively, while the standard deviation in the treated was 345.17 and control was 0.77. The standard error calculated was 199.28 in the treated group and 0.45 in the control group. The plot of the fold gene expression for LmcesE9 shows high fold gene expression level in the treated compared to the control group (Figure 3). The descriptive statistics calculated for LmcesA20 showed the value of calibrator used and it

Table 3: Calculated $2^{-\Delta\Delta CT}$ for LmcesE9 primers with respect to the gene of interest and housekeeping gene (B-actin1)

Sample	E9 CT1	E9 CT2	GOI Avg. Ct	HKG Ct1	HKG Ct2	HKG Avg. Ct	ΔCt	ΔΔCt	2^-(ΔΔCt)
Control 1	10.59	10.59	10.59	11.01	11.01	11.01	–0.42	–13.10	8779.97
Control 2	25.78	25.78	25.78	0.00	0.00	0.00	25.78	13.10	0.00
Control 3	18.19	18.19	18.19	5.51	5.51	5.51	12.68	0.00	1.00
D / 0.48	11.23	11.23	11.23	18.04	18.04	18.04	–6.81	–19.49	736333.60
D / 0.9	8.37	8.37	8.37	17.99	17.99	17.99	–9.62	–22.30	5163793.94
D / 1.9	8.60	8.60	8.60	17.90	17.90	17.90	–9.30	–21.98	4136559.78
D2.9	8.79	8.79	8.79	11.15	11.15	11.15	–2.36	–15.04	33689.23
A / 0.48	11.49	11.49	11.49	12.99	12.99	12.99	–1.5	–14.18	18561.17
A / 0.9	6.80	6.89	6.85	9.38	9.38	9.38	–2.54	–15.22	38033.95
B1 / 1.9	5.50	5.50	5.50	7.68	7.68	7.68	–2.18	–14.86	29737.59
B2 / 1.9	8.73	8.73	8.73	10.50	10.50	10.50	–1.77	–14.45	22381.20
B3 / 1.9	11.23	11.23	11.23	18.62	18.62	18.62	–7.39	–20.07	1100707.72
A1 / 2.9	7.64	7.64	7.64	9.88	9.88	9.88	–2.24	–14.92	31000.42
A2 / 2.9	8.73	8.73	8.73	11.43	11.43	11.43	–2.70	–15.38	42642.37
NTC	0.00	0.00	0.00	28.50	28.50	28.50	–28.50	–41.18	2.49124E+12

Note: GOI means gene of interest, HKG means house keeping gene.

Table 4: Descriptive analysis for ΔΔct values of LMCESE9 primers in each sample and average value of calibrator generated from control group (normalizer)

Calibrator (average ΔCt control group)	**12.68**		
Descriptive statistics			
Group	**Average**	**SD**	**SE**
Control	0.45	0.77	0.45
Treated	498.60	345.17	199.28

Note: SD mean Standard Deviation; and SE means Standard Error.

Table 5: Calculated $2^{-\Delta\Delta}$CT for LmcesA20 primers with respect to the gene of interest and housekeeping gene (B-actin2)

Sample	**A20 CT1**	**A20 CT2**	**A20 Avg. Ct**	**HKG Ct1**	**HKG Ct2**	**HKG Avg. Ct**	**ΔCt**	**ΔΔCt**	**2^-(ΔΔCt)**
Control 1	9.13	9.13	9.13	20.55	20.55	20.55	–11.42	–7.38	166.57
Control 2	17.79	17.79	17.79	14.45	14.45	14.45	3.34	7.38	0.01
Control 3	13.46	13.46	13.46	17.5	17.5	17.50	–4.04	0.00	1.00
D/0.48	11.01	11.01	11.01	14.29	14.29	14.29	–3.28	0.76	0.59
D/0.9	8.31	8.31	8.31	13.12	13.12	13.12	–4.81	–0.77	1.71
D/1.9	7.75	7.75	7.75	12.17	12.17	12.17	–4.42	–0.38	1.30
D2.9	11.18	11.18	11.18	14.33	14.33	14.33	–3.15	0.89	0.54
A/0.48	10.87	10.87	10.87	13.30	13.30	13.30	–2.43	1.61	0.33
A/0.9	9.01	9.01	9.01	9.14	9.14	9.14	–0.13	3.91	0.07
B1/1.9	8.94	8.94	8.94	12.85	12.85	12.85	–3.91	0.13	0.91
B2/1.9	7.35	7.35	7.35	11.72	11.72	11.72	–4.37	–0.33	1.26
B3/1.9	31.36	31.56	31.46	13.00	13.00	13.00	18.46	22.5	1.69E–07
A1/2.9	12.44	12.44	12.44	15.41	15.41	15.41	–2.97	1.07	0.48
A2/2.9	8.70	8.70	8.70	18.95	18.95	18.95	–10.25	–6.21	74.03
NTC	24.04	24.04	24.04	25.46	25.46	25.46	–1.42	2.62	0.16

was obtained by taking the average of all control values as well, for LmcesA20 it was -4.04, the average value of $2^{-\Delta\Delta Ct}$ for all the samples in both treated (susceptible and resistant strains) and control group was observed to be 19.72 and 918.87 respectively, while the standard deviation in the treated was 9.28 and control was 1577.23. The standard error calculated was 5.36 in the treated group and 910.61 in the control group. The plot of the fold gene expression for LmcesA20 shows high fold gene expression level in the control group compared to the treated group (Figure 4).

Table 6: Descriptive analysis for ΔΔct value of LmcesA20 in each sample average value of calibrator generated from control group (normalizer)

Calibrator (average ΔCt control group)	**-4.04**		
Descriptive statistics			
Group	**Average**	**SD**	**SE**
Control	918.87	1577.23	910.61
Treated	19.72	9.28	5.36

Note: SD mean Standard Deviation; and SE means Standard Error.

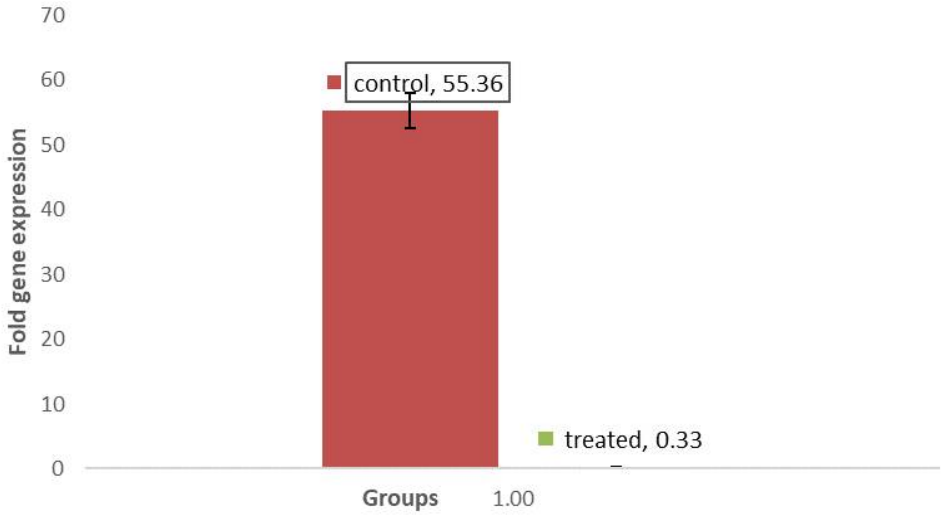

Figure 3: Fold gene expression level for control and treated group using LmcesA20

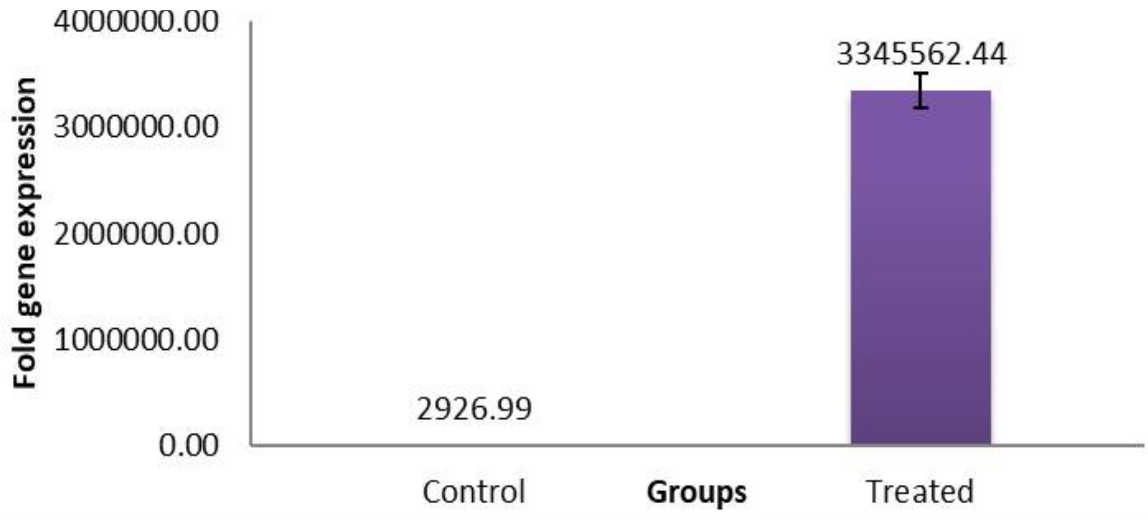

Figure 4: Fold gene expression level for control and treated groups using LmcesE9

3.4. Expression profiles of CarEs genes

The expression profiles of CarEs genes measured by qPCR in all the evaluated samples were represented by a hierarchical clustering in both primers. It showed the relationship between expression of CarEs genes in both susceptible and resistant strains for all the evaluated samples. Using LmcesE9 primers the expression level for CarEs genes were viewed to be at the same level for both the susceptible stains and resistant strains, as well as the control group but the only out group which the expression level was different was said to be NTC (No 15) in which the template cDNA sample is absent, but only provides a measure of reaction set up contamination (Figure 6). Using LmcesA20 primers expression level of CarEs genes were also observed to be at the same level for both susceptible and resistant strains but differences were observed in the control group (No 1) and susceptible strain A 2.9(No14), Figure 5.

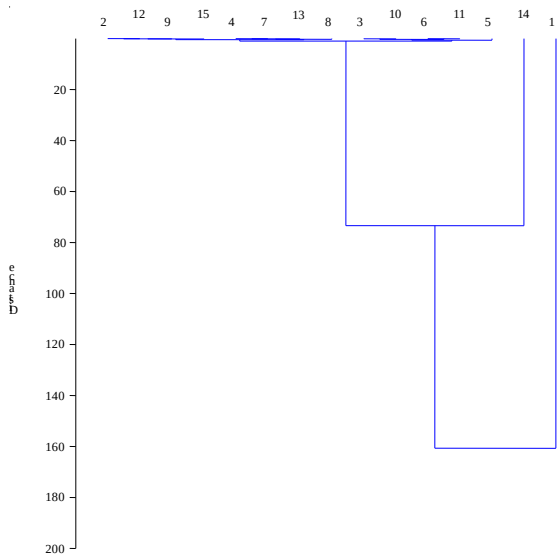

Figure 5: Dendrogram representing expression profiles that shows the relationship of CarE genes observed in both susceptible and resistant strains using LmcesA20 primers measured by qPCR

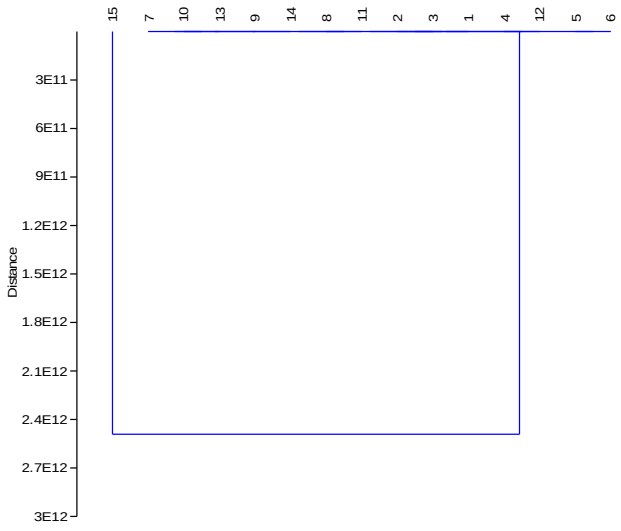

Figure 6: Dendrogram representing expression profiles that shows the relationship of CarE genes observed in both susceptible and resistant strains in *Z. variegatus* using LmcesE9 primers measured by qPCR

4. Discussion

4.1. Identification of CarEs genes

The cDNA of *Z. variegatus* subjected to qPCR using primers from *L. migratoria* indicated expression of two CarEs alone, though CarEs gene maybe more than two as it was revealed from literature review of other organism like *L. migartoria* in which about twenty five CarEs genes were being identified (Zhang *et al*., 2013).

Negative value for ΔΔct shows there is an up-regulation of genes, all samples with negative ΔΔct values have an up-regulation of CarEs genes in them indicating the CarEs genes is well expressed / amplified in LmcesE9 primers, but results showed using LmcesA20 that some samples have the CarEs genes being up-regulated while some being down-regulated. Negative sign for ΔΔct give value greater than 1 for fold change expression which automatically indicate up-regulation of the gene, while positive sign for ΔΔct give a fraction, i.e., a value less than 1 for fold change expression which indicate down-regulation.

Expression level of LmceE9 was revealed to be high in both susceptible and resistant strains of *L. migratoria* in their response to malathion, (Zhang *et al*., 2010). This study also revealed the same expression pattern using LmcesE9 in their response to Cypermethrin. The expression level observed in LmcesE9 was said to be high in the susceptible strains than the resistant strains. For expression level using LmcesA20 it was observed higher expression was obtained in the resistant strains than the susceptible strains. LmcesE9 was found to be highly expressed in both the susceptible and resistant strain and susceptible strain was significantly up-regulated with about 5163793 fold, but in LmcesA20 CarEs identified was relatively low in expression though the strains differ in their level of expression, highest fold was obtained in the resistant strain with 74.3 fold. Among the two CarEs genes LmcesA20 tends to show variation between the two strains and will be good for further studies on *Z. variegatus* resistance to pesticides.

Increased production of CarEs due to gene amplification or gene transcription up-regulation has been found to confer insecticide resistance through increased hydrolysis and / or insecticide sequestration in resistant insects (Small and Hemingway, 2000; and Cui *et al*., 2007). This study observed expression level of CarEs gene but not over expressed as compare to other studies. Most of the previous work compared changes in enzyme activity between susceptible and resistant strains. Reyes *et al*. (2011) demonstrated that higher activity in the responsive strain was observed than in the codling moth's OP-resistant strains which support that same degree of expression may not be significantly different in susceptible and resistant strains.

In this study, both the CarEs gene identified had almost the same level of expression in both the susceptible and resistant strains though the gene was highly expressed using LmcesE9 primers, which signifies variation in the CarEs genes of insects and pointing to other functions than resistivity to Cypermethrin. The relatively expression of CarEs gene using LmcesE9 and same level of expression in both strains may function in resistance to other pesticides other than Cypermethrin (cross resistance).

4.2. Expression profiles of CarEs genes in Z. variegatus

This study verified the activities of CarEs genes that may be associated with Cypermethrin resistance and this was as a result of recent reports on the resistivity of some *Z. variegatus* to insecticide, and the fact that these genes have been earlier reported in other insects that they confer resistance. Wherefore it is often not clear whether these genes is expressed in *Z. variegatus* and the role it played in conferring resistance to use of pesticides, thus making the selectivity and sensitivity of our qPCR method, and Cypermethrin as pesticide as it is being commonly used, 'especially owing to the fact that these organisms are causing destruction to farm produces which its effects need to be curtailed.

Expression level for CarEs gene was observed in Z. *variegatus* for both susceptible and resistant strains. The expression of CarEs genes in *Z. variegatus* suggested some potential roles it is playing in insects. Therefore, the expression pattern in both susceptible and resistant strains and its distribution give a basic knowledge that all the CarEs have different expression pattern and the role each is playing differ from each other in *Z. variegatus*. Expression profiles are important for the purpose of transcription profiling and Class discovery of CarEs. The two CarEs gene expressed was probably not of the same class because different expression level.

4.3. Comparing expression profiles in both susceptible strain and resistant strain

This present study focus on the quantitative expression profiles of CarEs genes in susceptible strain and resistant strain of *Z. variegatus;* the first finding, examined the expression of CarEs using different primers which indeed varied significantly while the second finding examined the expression profiles for both the susceptible strain and the resistant strain in all the Cypermethrin concentration used, which indicate no significantly difference. Relative higher expression level was obtained using LmcesE9 primers but lowly expressed using LmcesA20 this indicate specificity in the use of primers. The tissue in which the analysis compared the expression pattern may seem to contribute to the expression pattern in the strains because of assumed diverse functions of CarEs genes.

5. Conclusion

Thus, the study concluded only LmcesA20 have potential role in conferring resistance to use of Cypermethrin in *Z. variegatus* because of different expression level in susceptible and resistant strains which can possibly be a good candidate of the detoxification genes. LmcesE9 have same level of expression in both the resistant and susceptible strains and so it point to other biological roles performed by CarEs genes, e.g., production of hormones and degradation of pheromones.

6. Recommendations

From this study, the following are therefore recommended in order to obtain data that can be used to verify the clades of genes involved in conferring resistance:

1. Sequencing of CarE genes in *Z. variegatus,*Study should compare different expression level of CarEs genes in other tissues other than the Limb.
2. Clarify the CarE activity variations correlated with gene transcript variations, more CarE genes should be further investigated.

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