International Journal of Energy and Sustainable Development
Vol. 4, No. 2, 2021, pp. 11-18
http://www.aiscience.org/journal/ijesd

Biocidal Effects of Anisaldehyde Compound on the Survival of Adult Sitophilus *Oryzae L.*

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Abstract

Anisaldehyde is an active compound found in the essential oil of *Clausena anisum-olens.* Contact toxicity of anisaldehyde on adult *S. oryzae* under laboratory conditions were evaluated in the Department of Crop, Soil and Pest Management, FUTA. The effects of the compound on the activities of the CAT, SOD, and GPx were reported vividly. The homogenized separation of the samples was prepared for the adult *S. oryzae* and exposed to 10, 20, 30, 40, and 50μl of the oil extract dosages of 2% concentration. Untreated rice served as a control. Results shows that anisaldehyde was effective against the survival of *S. oryzae.* High mortality of adult *S. oryzae*, percentage weight loss and high inhibition rate were recorded. The activities of the active dosage increases with an increase in the time of exposure. Lethal dose of 14.76 and 35.36μl of anisaldehyde is recommended to achieve 50 and 95% mortality of the insect within 24h post-treatment. All the treatments were significantly ($p<0.05$) different from the control irrespective of the period of exposure. The findings also revealed that activities of the antioxidant enzymes increase above that of the control treatment with increase in dosage of the compound but drastically reduced at higher dosages. Based on the results of this study, anisaldehyde is recommended as viable bioinsecticides in integrated pest management strategies for rice storage by farmer.

Keywords

Anisaldehyde, *S. oryzae,* Inhibition Rate, Antioxidant Enzymes

Received: September 28, 2021 / Accepted: Novermber 23, 2021 / Published online: December 6, 2021

1. Introduction

The adverse effects of chemical insecticides as a major approach in controlling insect pests of crop grains has led researchers to discover a new methods of preventing and controlling insect pests of stored products. The use of synthetic chemicals such as phosphine and methyl bromide in controlling the stored grains has led to lethal effects on non-target organisms, pest resurgence and resistance, food residues, and the effects on humans and their environmental health [27]. This has been a major drawback and are associated with many downsides frustrating their global applications [25, 42, 46]. The main concern among world entomologists, crop protection scientists, and farmers in several countries is the replacement of the danger imposed by the synthetic chemicals, due to environmental concerns and human health hazards. The adverse effects have led the researchers to investigate for an alternative to control insect pests. The problems of chemical insecticides have led to it been either banned or restricted [35, 47]. However, tropical regions are endowed with several plants with medicinal and insecticide properties [2, 17, 19].

In recent years, microbial pathogen-based insecticides also known as bioinsecticides have been reported as an alternative to synthetic insecticides in controlling the dreadful insect pest of cereal crops [23]. Meanwhile, the tropic regions across the globe are endowed with plants with medicinal and insecticide properties. These plants contain active compounds which can be highly specific for certain pests which may persist for a shorter time in the environment. The bioinsecticidal

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formulations are often simple, reduced risk, and inexpensive to the populace. Currently, bioinsecticides constitute only up to 2–3% of the insecticide market share because of their narrow spectrum of toxicity [41].

Active compound found in the essential oil of Clausena anisumolens, anisaldehyde, have been recently investigated for its effectiveness in preventing and controlling the insect pests against adult *S. oryzae*. The bioinsecticidal efficacy, antioxidant and detoxifying activities against any weevils of crops and grains is necessary. Clausena anisumolens, is a colorless liquid with a strong aroma, it provides a sweet, floral, and strong aniseed odor [40]. The compound is commercially produced by oxidation of anethole, a related fragrance that is found in some alcoholic beverages by oxidative cleavage of an alkene. It has been tested for its insecticidal activities against *Acanthoscelides obtectus* and *Callosobruchus maculatus*. [26, 33]. With all these findings, its insecticidal potential has not been fully investigated unlike other active compounds such as Neem products from *Azadirachta indica*., Pyrethrum from *Tanacetum cinerariifolium* and rotenone from *Derris* and *Lonchocarpus* sp. [16]. Therefore, this research investigates the bioinsecticidal effects of anisaldehyde on some enzymes in *S. oryzae* which are important insect pests of paddy rice in storage.

2. Materials and Methods

2.1. Insect Culture

The initial culture of *S. oryzae* was obtained from an already infested rice in the Pest Management Laboratory, Department of Crop, Soil and Pest Management at the Federal University of Technology, Akure. The insect was subcultured on clean uninfested local rice placed in a transparent jar covered with a muslin cloth to allow air movement to prevent moisture buildup and inhibit fungal growth [6, 33]. The cultured experiment was performed at ambient temperature of 28 ± 2 °C and relative humidity of 75 ± 5 %. The culture was maintained by replacing the devoured grain with a new uninfested grains.

2.2. Active Compound Preparation

The dried uninfested local rice used for this study was obtained from a farm at Ilesa Osun State, Nigeria. The uninfested rice was sterilized inside a freezer at 7°C for 4 weeks. The active compound, Anisaldehyde, used for this project was obtained from a Laboratory at the University of British Columbia, Canada. The compound was made into concentration by adding $9.8ml$ of Dimethyisulfoxide (Dmxo) to $0.2ml$ of the compound, this was used as a stock compound from which 10, 20, 30, 40 and 50 μl dosages of 2% concentration were separately made from it for the experiment.

2.3. Adult *S. oryzae* Survival

Twenty grams of rice were weighed into Petri dishes and different dosage which 10, 20, 30, 40 and 50 μl were typically applied to the grains. The mixtures were thoroughly mixed with the aid of a wooden stirrer to ensure even spread of the compound (Anisaldehyde). Ten unsexed *S. oryzae* were placed in each of the Petri dishes containing the treated local rice and each treatment was then replicated 3 times. The experiment was then set in Completely Randomized Design (CRD). Adult mortality was observed and noted by separating the live and dead *S. oryzae* after 24, 48, 72, and 96 hrs. The percentage mortality (M) was calculated using the formula:

$$\%M = \frac{ND}{TN} \times 100$$

where ND is the number of dead insect and TN is the total number of insect.

After the 4th day, the remains of the rice were then left for 30 days so as to see the percentage of the new emergence. The percentage of reduction in adult emergence or inhibition rate (%IR) was calculated with the formula used by Tapondjou *et al.* [43] as:

$$\%IR = \frac{C_n - T_n}{C_n} \times 100$$

C_n is the number of newly emerged insects in the untreated Petri dish and T_n is the number of insects in the treated Petri dish.

After the emergence of the new adults, the final weight of the treated grains were weighed. The percentage weight loss ($\%W_L$) was calculated using:

$$\%W_L = \frac{W_i - W_f}{W_i} \times 100$$

W_i is the initial weight and W_f is the final weight halves was recorded after 12h, 24h and 36h.

3. Enzyme Assays

3.1. Determination of Catalase Activity

Catalase, EC. 1.11.1.6 activity was assayed according to the method of Aebi [4] by mixing 2.4 ml phosphate buffer (50 mM , pH 7.0), 10 μl of 19 mM H_2O_2 and 50 μl enzyme source (supernatant). The decrease in absorbance was

measured at 240 nm over a 3-min period at 25°C against the blank on a spectrophotometer. Two readings were taking at 0 and 3 min. Catalase (CAT) activity was calculated with the equation below:

$$CAT = \frac{R_1 - R_2}{T}$$

where R_1 is the initial reading at 0 min, R_2 is the final reading after 3 min, and the T is the time intervals.

3.2. Determination of Superoxide Dismutase Activity

The assayed of Superoxide dismutase (SOD) was carried out by Bamidele *et al*. [8]. The reaction mixture contained 1.17 µM riboflavin, 0.1 M methione, 0.2 µM potassiumcyanide (KCN), and 0.56 µM nitro blue tetrazolium salt (NBT) dissolved in 3 ml of 50 mM sodium phosphate buffer (pH 7.8). Three milliliter of the reaction medium was mixed with 1 ml of enzyme (supernatant) and the mixtures were illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes in a single row. Reaction was initiated at 30°C for 1 h by the illumination. Identical solutions that were kept under dark served as blanks while absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units (U mg^{-1} protein). One unit is defined as the amount of change in the absorbance by $0.1h^{-1}mg^{-1}$ protein. SOD activity was calculated with the equation below:

$$SOD = \frac{R_4}{A}$$

where $A = R_1\left(\frac{50}{100}\right)$ and $R_4 = R_3 \bullet R_2$, respectively. R_1 is the absorbance of the reference solution, R_2 is the absorbance of the blank, and R_3 is the absorbance of sample when enzyme has been added at a particular level.

3.3. Determination of Glutathione Peroxidase Activity

The GPx, EC 1.11.1.9 activity was assayed with H_2O_2 as substrate according to the method of Paglia and Valentine [36] as described by Bamidele *et al*. [8]. The reaction was monitored indirectly as the oxidation rate of NADPH at 340 nm for 3 min. Enzyme activity was expressed as micromoles of NADPH consumed per minute per milligram of protein, using an extinction coefficient of 6.220 M^{-1} cm^{-1} [37]. A blank without enzyme was used as a control for the non-enzymatic oxidation of NADPH upon addition of H_2O_2 in 0.1 M Tris buffer, pH 8.0. Glutathione peroxidase (GPx) activity was calculated with the equation below:

$$GPX = \frac{2(mRate_s - mRate_b) \times V_{R\times m}}{6.22 \times V_s} \times \frac{df}{1}$$

where $mRate_s = -1000 \times \Delta A_{340}$ / min of sample

$mRate_b = -1000 \times \Delta A_{340}$ / min of blank

6.22 = NADPH 340 nm millimolar absorption coefficient at 1 cm path length

$V_{R\times m}$ = Volume of reaction mixture

V_S = Volume of sample

2 = correction for 2 mol GSH oxidized to 1 mol GSSG per mole NADPH

df = Sample dilution factor

3.4. Data Analysis

The data obtained in this work were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20, where means existed, they were separated using Turkey's test at 5% level of probability.

4. Results

4.1. Effect of Anisaldehyde on Survival of Adult *S. oryzae* Infesting Stored Rice

The percentage mortality of adult *S. oryzae* exposed to different concentration of anisaldehyde was presented in Table 1. The mortality of the insect varied with the dosage of the compound and the period of exposure. At 24 h post treatment, all the dosages of the treatment recorded above 35% mortality of the insect except 10µl dosage of the compound that recorded 33.33% mortality of the insect. Only 50 µl dosage of the compound was able to cause 100% mortality of the insect at 48 hr post treatment and was significantly ($p<0.05$) different from 10 and 20 µl dosage and the control. All the treatments were able to achieve 100% mortality of the insect at 72hrs of exposure except 10 and 20 µl dosages that recorded 90 and 96.67% mortality respectively. All the treatments were significantly ($p<0.05$) different from the control irrespective of the period of exposure.

4.2. Amount of Anisaldehyde Required to Achieve 50 and 95 % Mortality of Adult *S. oryzae* Within 24 Hours Post Treatment

The lethal dosage of anisaldehyde required to achieve 50 and 95% mortality of the insect within 24 h post treatment (Table 2). The negative coefficient of the compound indicated that the higher the dosage of the compound, the higher the mortality of the insect. Also, Chi square values that are greater than zero indicated the high level of relationship between the

dosage of the compound and the mortality of the insect. There was a great significant relationship between the mortality of the insect and dosage of compound as the p-value of the calculated Chi square is lesser than 0.05.

Table 1. Mortality of *S. oryzae* exposed to Anisaldehyde at different concentration.

Dosage (µl)	% mortality in hours			
	24	48	72	96
10	33.33±3.33b	63.33±3.33b	90.00±0.00b	100.00±0.00b
20	36.67±3.33bc	73.33±8.81bc	96.67±3.33bc	100.00±0.00b
30	50.00±5.77bcd	86.67±3.33cd	100.00±0.00c	100.00±0.00b
40	56.67±3.33cd	93.33±3.33cd	100.00±0.00c	100.00±0.00b
50	63.33±6.666d	100.00±0.00d	100.00±0.00c	100.00±0.00b
Control	0.00±0.00a	0.00±0.00a	6.67±3.33a	6.67±3.33a

Each value is mean ± standard error of three replicates. Values followed by the same alphabet are not significantly ($p>0.05$) different from each other using New Duncan's Multiple Range Test.

Table 2. Lethal dosage of anisaldehyde required to achieve 50 and 95% mortality of *S. oryzae* within 24 h of application.

Slope ± S.E	Intercept ± S.E	X^2	LC50 (95% FL)	LC95 (95% FL)	Sig.
1.141±0.135	-0.528±0.066	34.926	14.76 (12.13-16.94)	35.32 (32.91-53.52)	0.001

Note: S. E: standard error; X^2: Chi square; LC: lethal concentration; FL: Fidiciual limit.

Only 14.76 and 35.36 µl of the compound is required to achieve 50 and 95% mortality of the insect within 24 hrs post treatment.

4.3. Adult Emergence of Adult *S. oryzae*, Percentage Seed Weight Loss and % Inhibition Rate of Different Dosages of Anisaldehyde

Figure 1 presented the mean value of adult insects emerged from -treated rice and the weight loss caused by the insects as well as the percentage inhibition rate caused by the compound. The dosage of the compound at 10, 20, and 30 µl significantly reduced the emergence of the insect, and their ability to cause seed weight loss while the compound at 40 and 50 µl prevented the emergence of the adult insect and seed weight loss. Only 40 and 50 µl dosage of the compound effected 100% inhibition of the adult insect. Regardless of the dosage used, the compound is significantly ($p<0.05$) different from the control.

4.4. Correlation Between Mortality, Adult Emergence and Weight Loss

The correlation between adult emergence and weight loss of the protected rice grains is presented in Table 3. The R-value of 0.879 that tends to 1 reflected high correlation between adult emergence and seed weight loss. The R^2 value showed that only 87.9% of the seed weight loss can be explained by the adult emergence. However, after the adjustment of the R^2 value, only 87.2% of the seed weight loss can be determined by the adult emergence. The t-value of 10.799 that is greater than -1.98 indicated that there was a great statistically significant relationship between the adult emergence seed weight.

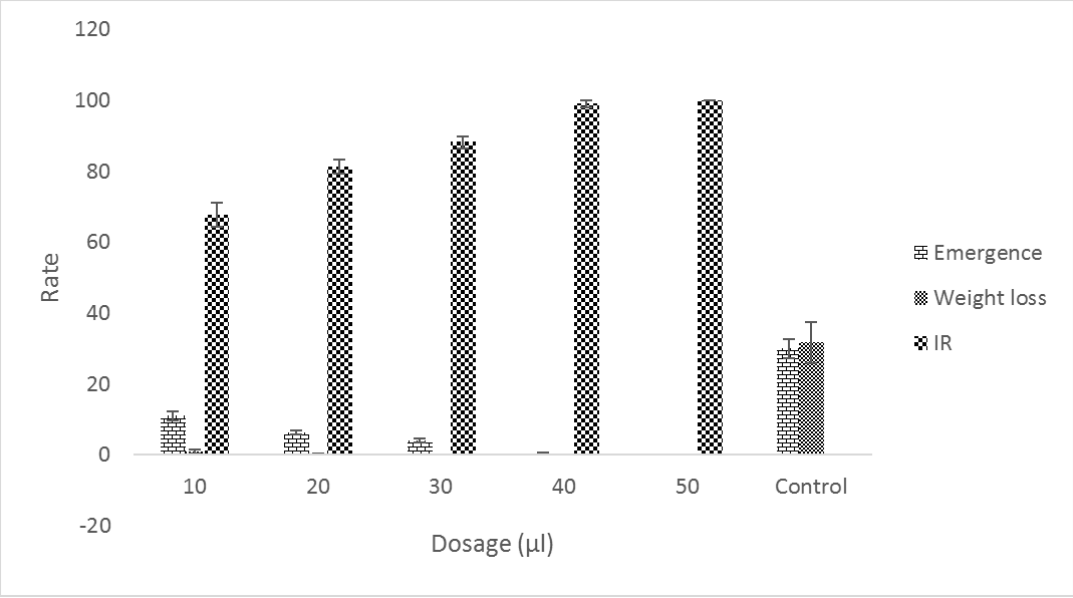

Figure 1. Adult emergence of *S. oryzea*, seed weight lost and inhibition.

Table 3. Correlation between adult emergence and seed weight loss.

R	R^2	AD(R^2)	K±S.E	(Rc)±S.E	(RE.)	t-value	Sig.
0.938	0.879	0.872	-3.70±1.33	1.07±1.10	W= -3.70+1.07 (A)+E	10.799	0.0001

Note: Where AD) = adjusted R square; K = constant; RC = regression coefficient; RE = regression equation; E= error; and W = weight loss.

4.5. Effects of Anisaldehyde on the Activities of Antioxidant Enzymes in *S. oryzae*

The effects of anisaldehyde compound on the activities of SOD, CAT, and GPx were presented in Figure 2. The effects of the compound was directly proportional to the dosage of the compound. Statistically significant differences existed between the treatments. The highest SOD activity was recorded at 20 µl (52.3 µmol/min/ml) of the compound while the lowest activity of 31.17 µmol/min/ml was recorded at 50 µl dosage of the compound they were not significantly ($p > 0.05$) different from all other dosages. The lowest CAT activity (8.05 µmol/min/ml) was recorded at 50 µl of the compound while the highest activity (3.28 µmol/min/ml) was recorded at 40 µl of the compound and was significantly different from others. At 50 µl, the highest activity of GPx (38.04 µmol/min/ml) was recorded while the lowest activity (20.06 µmol/min/ ml) of the enzyme was recorded by the control. Generally, the activities of the three enzymes increased above that of the control treatment with increase in dosage of the compound but drastically reduced at higher dosages.

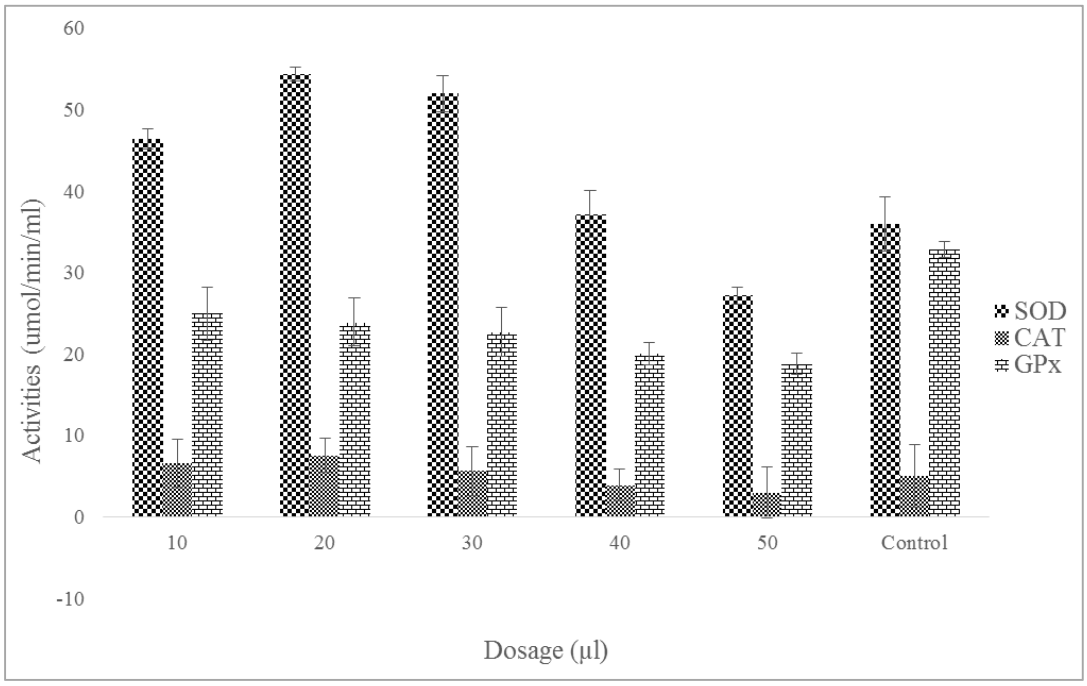

Figure 2. Effect of anisaldehyde on antioxidant enzymes in adult *S. oryze.*

5. Discussion

Plants and their derivatives have been proven to be effective as bioinsecticides against wide range of insect pests range from moths to beetles to weevils [5]. Despite the effectiveness of these plant-based insecticides either in powdered or liquid form, only few are still found on the global insecticides market as chemical insecticides that have been tagged with many adverse effects are still dominant up till now [9, 13, 18, 33]. Probably because they are readily available and easy to use. Isman and Grieneisen [19] as well as Ogungbite [29] reported that despite many botanical powders and extracts have shown insecticidal potential, the active compounds in such botanicals have not been individually tested for their efficacy as botanicals are known to contain myriads of active compounds [47]. Testing these active compounds for their insecticidal potential is key to successful large production of botanical based insecticides that could be readily available to both large scale and poor resource farmers.

5.1. Effect of Anisaldehyde on Survival of *S. oryzae*

Anisaldehyde is a botanical active compound that has not been fully tested for its insecticidal activities. The result of this research showed that this compound is very effective against the survival of *S. oryzae* as it effect high mortality against the adult insect. The effectiveness of the compound varied with the time of exposure and the dosage of the compound. As

botanical base insecticides have been noted to have negative effect on respiratory organ of insects; thereby, leading to hyperactivity and convulsion and total knockdown of insects [38, 44, 47]. It was noted that the compound was required at low dosage to achieve 50 and 95% death of the insect within short period of time as reflected by the probity analysis.. The result obtained showed that the anisaldehyde significantly affected the emergence of the insect as reflected by the percentage inhibition rate. The low rate of adult emergence recorded in this work could reflect that the insects did not lay much eggs that could emerge to adult as botanical insecticides are known to cause sterility of adult insect [3, 30]. Also, the low adult emergence recorded by the insects could be due to high mortality rate recorded by the compound by causing reduce period of mating. Digestion of food plays an important role in the existence of living organisms and it involves assistance of many enzymes. The disruption of the normal activities of these digestive enzymes cause inability of insects and other living organisms to provide their nutrients for biological requirements [47]. Several botanical extracts have been reported of inhibiting the a-amylase, glycosidases, lipases and proteases [47]. Therefore, no or low adult emergence of *S. oryzae* exposed to different dosages of anisaldehyde could be due to inability of the insect larvae to digest the treated rice grains which in turn lead to their death. The result of this work acquiesced with the findings of Epidi and Udo [12] in which different extracts of *Ricinodendron heudelotii* reduced the emergence of *C. maculatus*. The result of this research also acquiesced with the findings of Ileke and Olotuah [6, 17, 32] in which botanical oils and powders were found to significantly reduced or prevented the emergence of adult *C. maculatus*. The low seed weight loss recorded in this work implies low feeding habit of the insect larvae, which could have caused high seed weight loss. The linear regression analysis showed that more than 80% of the seed weight loss was determined by the adult emergence. Consequently, no or low weight loss recorded by the seeds reflected no or low adult emergence of the weevil.

5.2. Biochemical Study of the Anisaldehyde Against *S. oryzae*

The result of this work showed that the activity of the antioxidant and detoxifying enzymes in adult *S. oryzae* varied with the dosages of the dosage of anisaldehyde. SOD is the first line of defense against toxic substance in insects, thus it prevents accumulation of oxygen free radicals [22]. The dismutation ability of the enzyme enables it to remove superoxide radicals (O^{2-}) to oxygen and hydrogen peroxide (H_2O_2) [15, 31]. The variations in the activity of SOD at different dosages of the compound revealed that the compound had induced some level of toxicity to the enzyme [10]. Reactive oxygen species (ROS) are the contributors of oxidative stress that cause different diseases and disorders in insects [11]. John et al. [20] reported that increase in SOD activity is an indicator for increase in ROS accumulation in insects. The overproduction of ROS leads to inability of the cell endogenous systems to neutralize them causing damage to proteins, lipids, mitochondria, and DNA of insects [22, 39]. Therefore, the decrease in the activity of SOD at higher dosage of anisaldehyde implied that more ROS had accumulated in the cell of the insect due to inability of the enzyme to scavenge them. The decrease in the activity of SOD at higher dosages of the compound showed that O^{2-} and H_2O_2 had accumulated in the cell of the insect causing some levels of oxidative damages to the anisaldehyde-stressed *S. oryzae* [1]. Wang et al. [45] opined that scavenging ability of SOD is temporary and limited. Thus, this supported the reason why the activity of the enzyme increased with increase in the dosage of the compound and drastically decreased with further increase in dosage of the compound. Similar result was obtained by Kolawole et al. [22] research in which SOD in C. maculatus exposed to different dosages of some botanical insecticides was first increased and decreased with further increase in concentration of the biopesticides. Aslanturk et al. [7] also reported decrease in SOD activity in mid-gut tissues of Lymantria dispar exposed to methidathion, an organophosphate insecticide. The generation of SOD activity leads to conversion of superoxide radicals to less H_2O_2 which in turn induces CAT activity that perfectly reduces accumulation of H_2O_2 to water. He also opined that increase in activity of SOD would lead to increased H_2O_2 thus increase the activity of CAT. The increase in CAT activity in C. maculatus stressed with anisaldehyde could be due to increased SOD activities. Thus, this must have caused conversion of hydrogen peroxide to water and prevention of oxidative damage. Hence, it lowers the risk of hydroxyl radicals' formation through Fenton reaction [22]. This finding supported the reports of Orr and Sohal [34] suggested that CAT protects cells against oxidative stress and extends lifespan of insects. However, it was noted in this work that further increase in the dosage of anisaldehyde has not significantly reduced the activity of CAT. This could be due to ability of the enzyme to catalyze the accumulation of H_2O_2 that resulted from SOD activity. This result acquiesced with the findings of Kaur et al. [21] and Łukasik [24]. The result of this work revealed that activity of GPx increased at lower dosages of the compound and significantly reduced at higher dosages. The increase in the GPx activities could be associated to the increased CAT activity because it is that when CAT is saturated that GPx, the second line of defense regulated by Selenium availability, is activated [14]. GPx catalyzes the glutathionedependent reduction of lipid peroxides and hydrogen peroxide for detoxification at the membrane level into less reactive

species by using GSH as substrate [39]. Thus, it prevents the progressive formation of free radicals and protects the cell against oxidative stress and lipid peroxidation [39]. Hence, the decrease in the activity of GPx indicates that there could be accumulation of lipid peroxides and hydrogen peroxides. This result agreed with the findings of Aslanturk et al. [7] in which methidathion caused an increase in oxidative stress of L. dispar larvae.

6. Conclusion

In conclusion, the oil extract of *Clausena anisum-olens* (anisaldehyde) is highly effective against the activities of all the antioxidant enzymes in rice weevils. The inhibitory effects of the compound on treated rice grains caused a high mortality rate of the insect pest, the inability of the insect to cause weight loss and reduced adult emergence (*S. oryzae*). Based on these findings, the extract could be introduced as a pure potential biopesticide for the control of *S. oryzae* infection.

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