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SUBLETHAL EFFECTS OF SELECTED INSECTICIDES ON OXIDATIVE STATUS AND ANTIOXIDANTS IN COWPEA WEEVIL, *CALLOSOBRUCHUS MACULATUS* (F.) [COLEOPTERA: CHRYSOMELIDAE]

Ammar, Hager A.; El-Bermawy, Zeinab A.; Soliman, Zeinab A. and Abouelghar, G. E.*

Department of Pesticides, Faculty of Agriculture, Menoufia University, Shebin Elkom, Egypt

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ABSTRACT: The toxicity of pesticides is correlated to the generation of free radicals, oxidative damage induction, increased lipid peroxidation (LPO) and interruption of the total antioxidant potential. The present study was undertaken to characterize the role of sublethal concentration (LC_{50}) of five selected insecticides (abamectin, emamectin benzoate, imidacloprid, indoxacarb and spinosad) on the oxidative stress and antioxidants in *Callosobruchus maculatus* (F.). The 24 h- LC_{50} values of tested insecticides showed that emamectin benzoate was the most toxic insecticide (56.95 ppm) followed by indoxacarb (95.5 ppm) and spinosad (107.8 ppm) ; whereas abamectin and imidacloprid were the least toxic insecticides (148.2 and 209.8 ppm, respectively). For the oxidative status assays, the insect adults were exposed to cowpea seeds treated with LC_{50} of each tested insecticide for 24 h. The results showed a significant increase in the lipid peroxidation (LPO), expressed as malondialdehyde (MDA) content, after exposure to emamectin benzoate and imidacloprid than that of the control. Data of the antioxidants showed that both glutathione reduced (GSH) and glutathione peroxidase (GPx), significantly elevated in all tested insecticide treatments in comparison with the control. However, the activity of superoxide dismutase (SOD) was significantly decreased particularly by abamectin, indoxacarb and spinosad. Our study revealed that oxidative imbalance induced in *C. maculatus* adults after exposure to sublethal concentration (LC_{50}) of the tested insecticides may be helpful for molecular base study of the toxicity mechanism of these insecticides. Also, such biomarkers used in the present study represent good indicators of pesticide-induced oxidative stress.

Key words: Cowpea weevil, Novel insecticides, Sublethal effects, Oxidative stress, Antioxidants.

INTRODUCTION

The cowpea weevil (beetle), *Callosobruchus maculatus* (F.), is an important pest of bruchid seeds in the field and in storage as well. It is the most destructive on cowpeas, *Vigna unguiculata* (L.), causing over 90% yield reduction (Caswell 1981). The majority of the insect damage to the crops happens during storage, however the infestation begins in the field. The insect can grow exponentially, leading to significant loss in seed weight, germination viability, and the market value of the crop (Beck and Blumer 2014). Management of stored-cowpea pests is still being relied severely on the use of conventional chemical insecticides. Synthetic insecticides and fumigants like phosphine or methyl bromide act a crucial role in controlling this issue, however, they have been known to cause significant toxicological and environmental problems. Insect pests have developed resistance to the earlier generations of synthetic pesticides due to their similar mechanism of action and that leads to development of novel molecules with different target sites, including neonicotinoids, spinosyns, avermectins, oxadiazines, insect growth regulators, and pyrroles. These newer insecticides are characterized with their highly effectiveness against insect pests at low rates, highly specificity and selectivity against the target pests with lower toxicity to the non-target organisms and vertebrates. Reactive oxygen species (ROS) are produced when pesticides enter insect bodies. The insect body has a combination of detoxifying enzymes and

*Corresponding author: gamal.abouelghar@agr.menofia.edu.eg

antioxidants which work to eliminate the reactive oxygen species (Kodrík *et al.* 2015). Essential components of the antioxidant system include both the enzymatic and non-enzymatic antioxidants. (Tabrez and Ahmad 2011).

The glutathione reduced (GSH) is a crucial non-enzymatic antioxidant that offers both intracellular and extracellular protection (Zitka *et al.* 2012). One of the main and most powerful antioxidant enzymes is superoxide dismutase (SOD), which scavenges oxygen free radicals, as superoxide anion ($O_2^{\bullet-}$) and ultimately transforms them into hydrogen peroxide (H_2O_2). Subsequently, catalases reduce hydrogen peroxide to water and oxygen using the H_2O_2 molecule as their substrate; and this allows for a more comprehensive assessment of the tissue's overall antioxidant state (Khessiba *et al.* 2005).

Organophosphates have been shown in earlier studies to cause oxidative stress in non-mammalian systems, primarily in insects [Sule *et al.* 2022]. A variety of detoxifying enzymes are found in insects. For instance, catalase (CA), reduced glutathione (GR), glutathione peroxidase (GPX), and glutathione s transferase (GST) all play important roles in the reduction of dangerous chemicals. (Smirle and Winston 1988, Felton and Summers 1995). Nevertheless, not much research was done to assess how new pesticides affect the antioxidant system in insects, with bruchid beetles receiving particular attention. Poiani *et al.* (2023) have recently shown that workers of the leafcutter ant (*Atta sexdens*) exposed to fipronil increased oxidative stress, which is correlated with a significant increase in SOD activity. The study performed by Kolawole and Kolawole (2014) showed that *C. maculatus* exposed to the tested synthetic pyrethroids (λ-cyhalothrin and cypermethrin) had significantly higher levels of lipid peroxidation (LPO) and carbonyl protein, which indicated the extent of organ damage. In another study, Kolawole *et al.* (2014) found that adult *C. maculatus* exposed to certain pyrethroids (cypermethrin and λ-cyhalothrin) significantly altered their activities of SOD, CAT, polyphenol oxidase (PPO), and peroxidase (POX). According to their research, the oxidative imbalance might be the molecular cause of these substances' toxicity on insects.

Thus, our present study was undertaken to better understand how sublethal concentrations of some newer insecticides may contribute to oxidative damage and cellular antioxidant defense systems as well as how these processes may be related to toxicity mechanisms in the cowpea beetle, *Callosobruchus maculatus* (F.).

MATERIALS AND METHODS

Materials

Commercial formulations of selected insecticides, abamectin (VERTIMEC® 1.8% EC), emamectin benzoate (PROCLAIM® 5.7% SG), spinosad (TRACER® 48% SC), imidacloprid (COMMANDO® 35% SC) and indoxacarb (AVAUNT30% WG), were purchased from local market to use in this study. Commercial reagents Ready-to-use kits were purchased from BioDiagnostic **(**Dokki- Giza – Egypt) to determine biomarkers of oxidative stress and antioxidant defense.

Insects

A laboratory colony strain of the cowpea weevil (beetle), *Callosobruchus maculatus* (F.), utilized in the present study was kindly provided by Dr. Mahrous Gharib at Department of Stored-Product Insects, Plant Protection Research Institute, ARC, Giza, Egypt. The insect adults were maintained in plastic jars and fed on un-infested dried cowpeas (*Vigna unguiculata* (L.); the jars were covered with a black muslin fabric and kept at constant laboratory temperature of 27 ± 2°C, 60–80% ± 5°C, with a 12-hour light/dark cycle. For all bioassays, the newly emerging adults (1-3-days-old) were used.

Toxicity Bioassay

Bioassay tests were carried out under laboratory conditions to evaluate the insecticidal activity of the selected insecticides (abamectin, emamectin benzoate, imidacloprid, indoxacarb and spinosad) against *C. maculatus* adults. The concentration-mortality data were set to determine lethal concentration values (LCs) at exposure periods of tested insecticides. The

treatments were carried out by exposing the insect adults directly to cowpea seeds treated with an adequate solution of insecticide concentration and confined in plastic jars. Briefly, serial dilutions of each formulated insecticide, using distilled water was prepared, based on the active ingredient (A.I.) of insecticide. In preliminary tests, several concentrations were chosen for each test insecticide, ranged between the concentrations having no killing effect on the insects to the minimal one killing 100% of them. For each insecticide, six- to- seven diluted concentrations were selected. The insects exposed to cowpea seeds treated with only water served as the control. 100-gram seeds were mixed with the required quantity (0.15 mL) of insecticide solution until complete coverage of the seed surface. Then, twenty unsexed, newly emerged adults (1-2-day-old) were introduced into 400-mL plastic jar. The jars were covered with muslin cloth fixed with rubber band. The dead insects were counted after 24 h and removed from the jars. The insects that could not move or immobile were considered dead. Abbott formula was used to calculate the adjusted percent mortality (Abbott, 1925). The slope (b ± SE) and lethal concentration (LC_{50}, and LC_{90}) values with 95% Fiducially Limits (FL) and Chi-square values were determined after 24 hours of treatment using the Probit and Logit Analysis software program (Polo Plus, ver. 2.0, 2008), based on Finney analysis (Finney, 1971),

Sample Preparation for Biochemical Analysis

To determine the sublethal effects of tested insecticides on the oxidative stress indices in *C. maculatus*, newly emerged adults (1-3-old- days) were exposed for 24 h to cowpea seeds treated with LC_{50} of individual insecticide as summarized above. For each experimental group of insects, three replicate homogenates with ten insects for each replicate were prepared. By the end of the exposure time, the insects from both treated and untreated control were manually homogenized by using a Teflon pestle on 25 % (w/v basis) in 50 mM potassium phosphate buffer solution containing 1 mM EDTA at a pH of 7.4. Centrifuging the crude homogenates at 4,500 rpm and 4º C for 30 minutes. Subsequently, the supernatant was transferred into a new tube for biochemical analyses.

Lipid Peroxidation (LPO)

The effects of tested insecticides on the lipid peroxidation (LPO) in *C. maculatus* were assessed using the thiobarbituric acid reactive substances (TBARS) assay. The degree of lipid peroxidation in the treated and control insect groups was estimated using the thiobarbituric acid test, which detects aldehydes like malondialdehyde (MDA) produced by the breakdown of peroxidized lipids. The assay was carried out based on the technique described by Yagi (1998). To achieve this, an equivalent volume of TBA reagent (BioDiagnostic kit, CAT. No. MD-2529) was combined with 500 µL of supernatant. After 60 minutes of incubation at 95 °C in a water bath, the reaction mixture was allowed to cool at room temperature in glass tubes. Lipid peroxidation is assessed by the interaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), which produces a pink-colored product. The amount of MDA present in the sample is directly correlated with the intensity of the pink colour, as measured at 525 nm. MDA concentration was expressed as nmol/mL or nmol/ g insect tissue used.

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) is a crucial enzyme involved in protecting the body from oxidative stress. Superoxide anion ($O_2^{\bullet -}$) dismutation into hydrogen peroxide and molecular oxygen (O_2) is catalyzed by SOD. The superoxide dismutase (SOD) activity was determined by following the technique of Nishikimi *et al.* (1972). The analysis was conducted using a commercial reagent kit (BioDiagnostic, CAT. No. SD-2521). This test is based on the enzyme's ability to stop phenazine methosulphate (PMS), which is detected at 560 nm, from reducing the nitro-blue tetrazolium (NBT) dye. One unit of SOD is the quantity of enzyme needed to demonstrate 50% of the

dismutation of the superoxide radical. The U/mL was used to express the SOD activity.

Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPx) enzyme is essential for shielding cells from the damaging effects of free radicals, especially lipid peroxidation. The Paglia and Valentine (1967)' technique was used to measure the GPx enzyme. The assay was conducted using a commercial reagent kit (BioDiagnostic, CAT. No. GP-2524). This assay depends on the capability of Glutathione peroxidase (GPX) to catalyze the reduction of an organic peroxide (ROOH), which oxidizes reduced glutathione (GSH) to generate disulfide glutathione (GSSG). The oxidized glutathione is then reduced by glutathione reductase (GR) and b-nicotinamide adenine dinucleotide phosphate (NADPH) to produce $NADP^+$, which lowers absorbance at 340 nm and recycles GSH. The reduction in absorbance at 340 nm is precisely proportional to the quantity of glutathione (GPx) since glutathione peroxidase (GPx) is limiting. U/g tissue is the unit used to assess enzyme activity.

Reduced Glutathione (GSH)

One of the most important antioxidants in biological systems is glutathione (GSH), a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine). Glutathione (GSH) is a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine), which is a key antioxidant in living systems. It appears that the level of GSH within a cell serves as a sensitive gauge of the cell's general health. The kit of commercial reagents (BioDiagnostic, CAT. No. GR-2511) was utilized in this experiment. The level of GSH was determined based on the technique of Beutler et al. (1963). The assay relies on the reaction of reduced GSH with 5,5′-dithiobis-2-nitrobenzoic acid (DNTB) producing glutathione disulfide (GSSH) and a yellow complex product, 2-nitro-5-thiobenzoate (TNB), which can be detected by colorimetric assay at 405 nm and calculate the reduced GSH content indirectly. After 5–10 minutes, the absorbance of the sample was measured at 405 nm in comparison to the blank. The unit of measurement for glutathione reduction is mmol/g insect tissue.

Statistical Analysis

All the experiments were carried out in three replicates. Using Tukey's test (mean comparison) and One-way ANOVA, the data were analyzed using Graph Pad software (v. 3.6, San Diego, CA). The mean ± standard error of the results is displayed. Plotting of the finished graphs was done with GraphPad Prism Software Inc. v. 5.03, 2009. The results of different treatments were deemed significant at $p < 0.05$.

RESULTS

Toxicity of Insecticides on *C. maculatus*

The mortality levels of tested insecticides against *C. maculatus* adults obtained in the concentration-mortality bioassay were satisfactorily described by using Probit analysis (Finney, 1971) exhibiting low Chi-square (χ^2) values (< 10). The 24 h-Lethal concentrations (LCs), slopes and 95 % fiducial limits of different tested insecticides are presented in Table (1). Based on the LC_{50} data, the results showed that emamectin benzoate was the most toxic insecticide against insect adults (LC_{50} = 56.95 ppm) following by indoxacarb (LC_{50}= 95.5 ppm) and spinosad (LC_{50} = 107.8 ppm). However, abamectin and imidacloprid were the least effective insecticides reporting LC_{50} values: 148.2 and 209.8 ppm, respectively. The same trend was observed at LC_{90} values, where emamectin benzoate was the most toxic compound (186.6 ppm) followed by abamectin and spinosad (427.3 and 408.7 ppm, respectively); whereas imidacloprid and indoxacrab were the least toxic (1019.7 and 889.4 ppm, respectively).

Lipid peroxidation (LPO)

The lipid peroxidation findings in adult insects subjected to the LC_{50} concentration of the corresponding pesticides are shown in Fig. (1) as malondialdehyde (MDA) content (nmol/g tissue used). Lipid peroxidation, which represents

stronger biomarker of oxidative stress, was significantly elevated (at $p < 0.05$) in insects exposed to seeds treated with LC_{50} values of emamectin benzoate (44.8 nmol/ g tissue), imidacloprid (35.9 nmol/ g tissue), and abamectin (28.6 nmol/ g tissue), compared to that of the untreated control (24.9 nmol/ g tissue). A slight reduction in MDA content was observed by indoxacarb (21.5 nmol/ g tissue) and spinosad (19.5 nmol/g tissue) than that of the control.

Table (1). Contact toxicity of selected insecticides against *Callosobruchus maculatus* adults exposed for 24 h to treated cowpea seeds

Insecticide	Slope ± SE	[a]LC_{50} (95% FL)	[a]LC_{90} (95% FL)	[b]Chi square (χ^2)
Abamectin (1.8% EC)	2.787±0.392	148.23a (126.2-174.9)	427.3ab (331.8-623.5)	4.459
Emamectin benzoate (5.7% SG)	2.486±0.387	56.95b (44.88-71.55)	186.66c (137.5-296.3)	3.082
Imidacloprid (35% SC)	1.886±0.32	209.82a (125.5-314.5)	1019.7a (601.7-3132.4)	5.45
Indoxacarb (30% WG)	1.32±0.260	95.51b (60.4-137.0)	889.4ab (509.3-2443.8)	3.968
Spinosad (48% SC)	2.214±0.266	107.8b (89.13-126.98)	408.7b (326.4-555.6)	1.116

[a]24 h-Lethal concentration expressed as ppm with 95% Fiducial Level (FL).

[b]Chi-square- difference between observed and expected values.

[c]LC_{50} and LC_{90} values followed by the same letter are not significantly different (95% confident limits, CL, do not overlap).

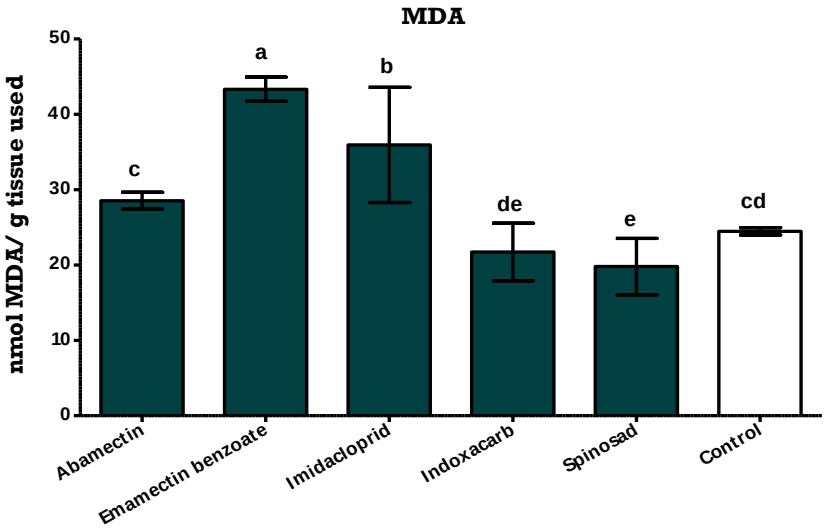

Fig. 1. Lipid peroxidation activity (expressed as malondialdehyde, MDA content) in the whole body homogenate of *C. maculatus* adults exposed to 24 h-LC_{50} of tested insecticides, compared to untreated control. Data shown are representive of three replicates. Means ± SEM are presented. MDA levels expressed as nmol/ g tissue used. Means followed by the same letter(s) are not statistically significant different using LSD (Least Significant Test) at $P \leq 0.05$.

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD), an enzymatic antioxidant, is thought to be the main detoxifying enzyme in a cell. It serves as the initial line of defense against ROS, or reactive oxygen species. In contrast to the LPO activity, the SOD activity decreased by indoxacarb (90.0 U/ mL), abamectin (165.0 U/ mL) and spinosad (176.3 U/ mL) when compared to that of the untreated control (322.5 U/ mL) (Fig. 2). While slight decrease in SOD activity was observed by emamectin benzoate (307.5 U/ mL) and imidacloprid (309.8 U/ mL) than that of the control.

Glutathione Peroxidase (GPx)

Glutathione Peroxidase (GPx) is an important enzyme that is mostly located in the mitochondria and sporadically present in the cytoplasm. It breaks down hydrogen peroxides (H_2O_2) into water and lipid peroxides into their corresponding alcohols. The enzyme is a key player in preventing lipid peroxidation, which protects cells from oxidative damage. The data in Fig. (3) represented the activity of GPx in insect adults exposed to LC_{50} of respective insecticides. It is obvious that GPx activity highly increased after exposure to the LC_{50} concentration of tested insecticides when compared to that of the control. Again, emamectin benzoate was the most effective in this respect indicating 77.8 U/g tissue, followed by indoxacarb (64.2 U/ g tissue), spinosad (59.2 U/ g tissue), abamectin (52.5 U/ g tissue) and imidacloprid (44.7 U/ g tissue), compared to 19.0 U/ g tissue in the untreated control.

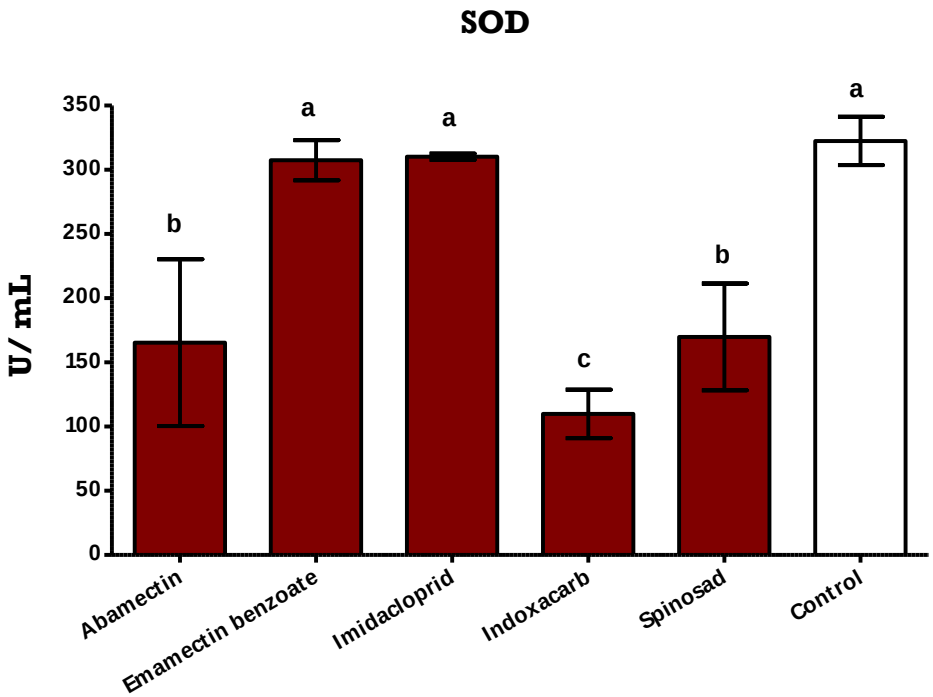

Fig. 2. Superoxide dismutase (SOD) activity (expressed as U/ mL) in the whole body homogenate of *C. maculatus* adults exposed to 24 h-LC_{50} of tested insecticides, compared to untreated control. Data shown are representive of three replicates. Means ± SEM are presented. Means followed by the same letter are not statistically significant different using LSD (Least Significant Test) at $P \leq 0.05$.

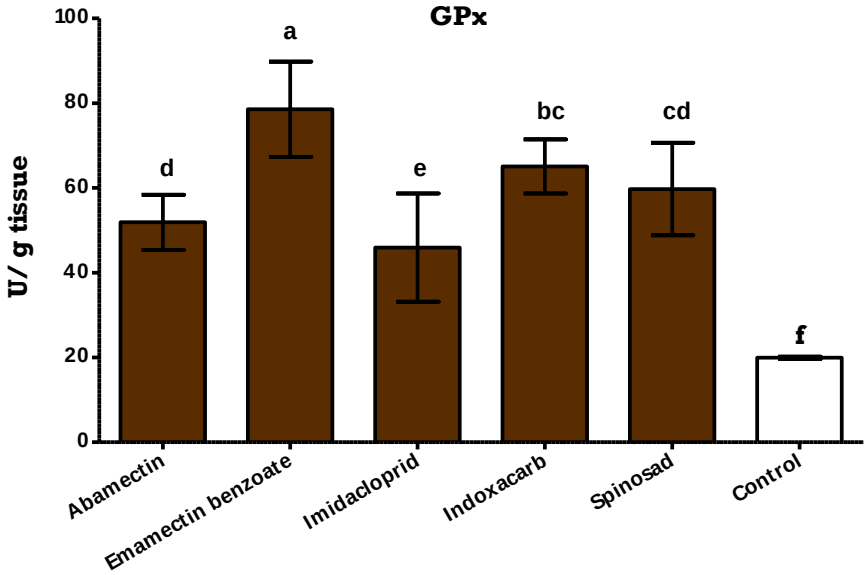

Fig. 3. Glutathione peroxidase (GPx) (expressed as U/ g tissue used) in the whole body homogenate of *C. maculatus* adults exposed to 24 h-LC_{50} of tested insecticides, compared to untreated control. Data shown are representive of three replicates. Means ± SEM are presented. Means followed by the same letter(s) are not statistically significant different using LSD (Least Significant Test) at $P \leq 0.05$.

Reduced Glutathione (GSH)

The cellular systems contain large amounts of reduced glutathione, which is essential for the detoxification of many electrophilic substances. In addition to scavenging free radicals and providing antioxidant protection, glutathione also helps to regenerate vital antioxidants like vitamins C and E. The data in Fig. (4) showed that the levels of reduced glutathione (GSH) had similar trend like the activity of glutathione peroxidase (GPx) where remarkable increase in GSH levels were reported in all insecticide-treatments when compared with that of the untreated control. Indoxacarb was the most effective causing highly significant increase in GSH level (54.6 mmol/ g tissue), followed by abamectin (47.1 mmol/ g tissue), imidacloprid (42.3 mmol/ g tissue), emamectin benzoate (37.6 mmol/ g tissue) and spinosad (26.1 mmol/ g tissue), compared to 10.2 mmol/ g tissue) in the control.

DISCUSSION

At present, there is more attention has been considered on the effects of pesticides at sublethal dosages that don't directly affect insects, but induce some alterations in biochemical, behavioral, and physiological indices (Jankowska *et al.* 2023). In insects, physiological systems may be altered as a result of exposure to sublethal pesticide dosages. It is recognized that a variety of synthetic and natural insecticides, with different mechanisms of action, may produce free radicals that lead to lipid peroxidation, which upsets cellular homeostasis (e.g. Leelaja and Rajini 2012; Prakash 2015). Overproduction of reactive oxygen species (ROS) can also be detrimental to cells since it can cause oxidation of proteins, lipids, DNA damage, inhibition of enzymes, activation of the apoptotic pathway, and death of the cells. (Melusova *et al.* 2014). Insects have developed an antioxidant defense mechanism to counteract the effects of reactive oxygen species (ROS). Signals from the insect's endocrine and neurological systems are released throughout this process. The stressed insect secretes antioxidant enzymes such glutathione S-transferase (GST), CAT, SOD, GP, and GR as a result of these signals. (Felton and Summers 1995).

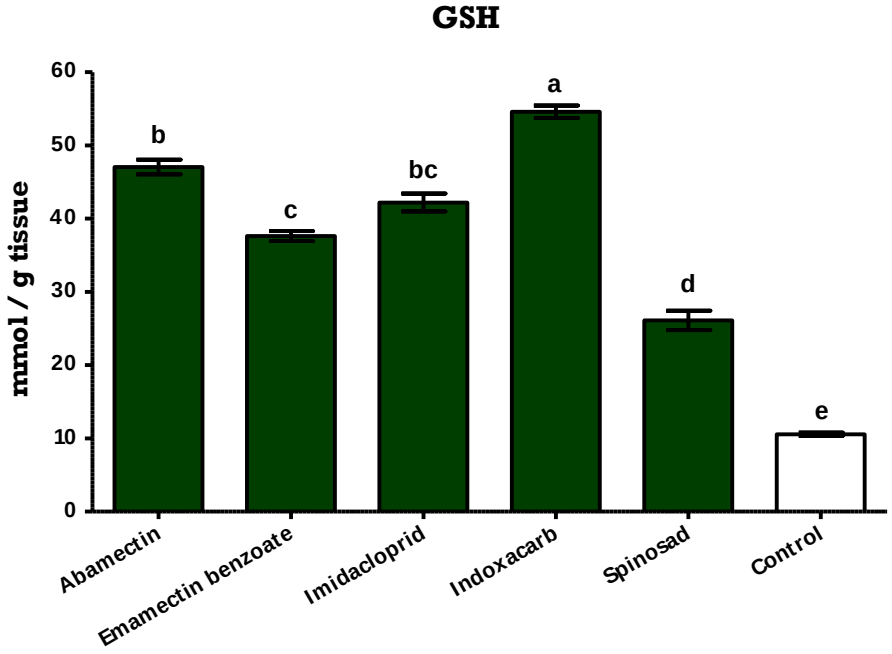

Fig. 4. Glutathione reduced (GSH) levels (expressed as mmol/ g tissue used) in the whole body homogenate of *C. maculatus* adults exposed to 24 h-LC_{50} of tested insecticides, compared to untreated control. Data shown are representive of three replicates. Means ± SEM are presented. Means followed by the same letter(s) are not statistically significant different using LSD (Least Significant Test) at $P \leq 0.05$.

Very little information has been published on the sublethal effects of newer non-conventional insecticides in insects, with emphasis on stored-product insect, e.g. *C. maculatus*. The current study's results unequivocally showed that, at LC_{50}, the majority of the tested pesticides caused oxidative damage to adult *C. maculatus*. The adult insect's lipid peroxidation (LPO) activity, measured as the amount of malondialdehyde (MDA), dramatically increased following exposure to imidacloprid and emamectin benzoate. Malondialdehyde (MDA) is a known biomarker of oxidative stress because of its important involvement in lipid peroxidation by reactive oxygen species (ROS) and because its value increased following pesticide treatment. These findings are consistent with the results of Kolawole and Kolawole (2014) who reported that in *C. maculatus* subjected to varying doses of tested pyrethroid insecticides, cypermethrin and cyhalothrin, showed a significant increase in the lipid peroxidation (LPO) levels than that of the control. Likewise, Lu *et al.* (2021) found that silkworms fed a continuous diet containing a low concentration of the neonicotinoid acetamiprid (0.15 mg/L) had higher contents of both H_2O_2 and MDA in addition to significantly higher levels of enzymatic antioxidants, such as superoxide dismutase (SOD) and catalase (CAT) activities. Our findings also corroborated those of Lalouette *et al.* (2011), who reported that elevated MDA indicates oxidative stress in cells and that ROS induces LPO, which impairs the fluidity of the cell membrane and results in lesions. Likewise, Aslanturk *et al.* (2011) found that the Gypsy moth's midgut tissues showed an increase in MDA levels following a 48-hour exposure to the LC50 of organophosphate, methidation. Recently, Jankowska *et al.* (2023) demonstrated that exposure of the cockroach, *Periplaneta americana*, for only one hour to sublethal dosages of the carbamate pesticide bendiocarb significantly elevated the MDA levels compared to the untreated insects; this

implies a rise in oxidative stress, particularly when contrasted with variations in the levels of antioxidants, glutathione and catalase.

Based on our best knowledge, very little published papers have focused on the oxidative stress and activity of antioxidant defense systems in cowpea beetles in response to exposure to sublethal doses of novel insecticides. The present study showed that both antioxidant biomarkers, reduced glutathione (GSH) and glutathione peroxidase (GPx), significantly elevated in all tested insecticide-treatments, in comparison with that of the control. However, SOD activity was significantly decreased particularly by in treatments of abamectin, indoxacarb and spinosad. These results are in line with Kolawole and Kolawole (2014) who reported a significant increase in the GPx activity in the cowpea weevil exposed to different concentrations of either cypermethrin or cyhalothrin. Similar trend was reported by Chintalchere *et al*. (2021) who found that the activity of both GPx and glutathione reductase (GR) were significantly increased in *Musca domestica* larvae exposed to LC_{50} concentrations of tested essential oils.

It has been demonstrated that glutathione (GSH), which co-factors with glutathione peroxidase (GPx) and glutathione-dependent enzymes, is essential for the defense and detoxification of peroxides, hydroperoxides, and xenobiotics (Li *et al.* 2010). Using GSH and/or other reducing equivalents, glutathione peroxidase is known to detoxify a range of organic hydroperoxides generated during lipid peroxidation (LPO) to the corresponding hydroxyl molecules. The cell is shielded from mild oxidative stress by glutathione peroxidase (Yan *et al.* 1997). The consequent oxidative stress indicates that a rise in H_2O_2 and organic hydroperoxides (ROOH) concentration may be the cause of the increase in glutathione peroxidase activity. According to Kolawole and Kolawole (2014), it seems that exposure to the tested pesticides quickly caused oxidative stress to start. This demonstrates unequivocally the connection between lipid peroxidation, cellular reduction-oxidation equilibrium, and glutathione peroxidase. It seems that the stimulation of glutathione antioxidant defense is insufficient to prevent oxidative damage. Kolawole and Kolawole (2014) investigated the potential relationship between the glutathione antioxidant defense system in *C. maculatus* and the pesticidal effectiveness of several documented bio-insecticides and commercially synthetic insecticides. On contrary, Jankowska *et al.* (2023) reported a considerable decrease in GSH concentration in the American cockroach, *P. americana*, after exposure to low dose (0.1 nM) of carbamate insecticide, bendiocarb. This might be attributed to GSH's direct consumption as an antioxidant in the removal of ROS, which was heightened by the bendiocarb exposure. Catalase (CAT) activity increased in tandem with the drop in GSH levels, suggesting an adaptive response that may withstand pesticide overdose. Even at very low dosages, GSH and CAT work together as efficient reactive oxygen species scavengers and contribute to protection against lipid peroxidation brought on by pesticide exposure (Jankowska *et al.,* 2023).

For the antioxidant enzyme, superoxide dismutase (SOD), our present study revealed a significant decrease in the SOD activity in *C. maculatus* after treatment with LC_{50} of abamectin, indoxacarb and spinosad, compared to the control. Our results are consistent with those of Kolawole *et al.* (2014), who found that when *C. maculatus* groups were treated with different bioinsecticides, their SOD activities significantly decreased when compared to the control; however, when all insect groups were treated with pyrethroid insecticides, such as cypermethrin and cyhalothrin, their enzyme activity increased as the concentrations of all the treatments increased. Recently, Poiani *et al.* (2023) demonstrated that exposure of the leafcutter ant, *Atta sexdens*, workers to sublethal concentrations of fibronil for 24 h induced significant increase in SOD activity. SOD may act as an adaptive reaction to unbalanced level of free radicals within the insect body (Akhgari *et al.* 2003). In this regard, Kolawole *et al.* (2014) suggested that when *C. maculatus* was exposed to higher concentrations of the tested insecticides, more $O_2^{\bullet -}$ free radicals may be

generated. These radicals may build up to a point where they are unable to be scavenged by the SOD enzyme, which would reduce the enzyme's activity and cause ROS to accumulate. This, in turn, would cause oxidative damage in the insecticide-stressed beetles. A reduction in SOD activity may also affect the cells' capacity to scavenge O_2^-, which would encourage the buildup of $O_2^{\bullet -}$ and H_2O_2.

In conclusion, our data indicate that such newer insecticides have the ability to induce an oxidative damage to the cowpea beetle, *C. maculatus*, even when applied at LC_{50} over short periods of time (≤ 24 h). The insect had elevated levels of malondialdehyde (MDA) in response to treatment with various insecticides, particularly abamectin, emamectin benzoate, and imidacloprid, indicating enhanced lipid peroxidation as a result of pesticide exposure. All the studied insecticides examined at LC_{50} enhance the production of antioxidants, which prevents cellular damage by significantly increasing reduced glutathione (GSH) and glutathione peroxidase (GPx). The reactive oxygen species (ROS) could be considered as essential targets for such insecticides.

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التأثيرات تحت المميتة لمبيدات حشرية مختارة على الحالة التأكسدية ومضادات الأكسدة في حشرة خنفساء اللوبيا

Callosobruchus maculatus (F.) [Coleoptera: Chrysomelidae]

هاجر علي عمار ، زينب عبد الغني البرماوي ، زينب الشحات سليمان ، جمال السيد أبو الغار*

قسم مبيدات الآفات - بكلية الزراعة، جامعة المنوفية - شبين الكوم

*gamal.abouelghar@agr.menofia.edu.eg

الملخص العربى

تسبب الجرعات تحت المميتة من المبيدات الحشرية العديد من التأثيرات التي يجب أخذها بعين الاعتبار عند مكافحة الآفات. فمن المعلوم وجود إرتباط بين آليات سمية معظم المبيدات الحشرية والزيادة الحادثة في إنتاج الشوارد الحرة، والإجهاد التأكسدي، وأكسدة الليبيدات (LPO) علاوة علي إحداث خلل في مقدرة مضادات الأكسدة كنظام دفاعي بجسم الحشرات. ولذلك، أجريت هذه الدراسة لتوضيح دور التركيز تحت القاتل (LC50) لمبيدات حشرية مختارة في زيادة معدل الإجهاد التأكسدي وإحداث خلل وظيفي في مستويات مضادات الأكسدة مما يفسر سميتها المحتملة في حشرة خنفساء اللوبيا المعرضة للجرعة النصفية القاتلة للمبيدات المختبرة. وفي اختبار التقييم الحيوي للمبيدات المنتخبة، تم تقدير قيمة LC_{50} لكل مبيد بعد ٢٤ ساعة من تعرض الحشرات البالغة لبذور اللوبيا المعاملة بتركيزات مختلفة. وقد أوضحت نتائج التقييم الحيوي للمبيدات، بناء علي قيم LC_{50}، أن مبيد إيمامكتين بنزوات هو المبيد الحشري الأكثر سمية (٥٦.٩٥ جزء في المليون) يليه الإندوكساكارب (٩٥.٥ جزء في المليون) والسبينوساد (١٠٧.٨ جزء في المليون)؛ في حين كان الأبامكتين والإيميداكلوبريد أقل المبيدات الحشرية سمية (١٤٨.٢ و ٢٠٩.٨ جزء في المليون على التوالي). أما بالنسبة لاختبار تقدير معدل الاجهاد التأكسدي، فقد تم تعريض الحشرات البالغة لمدة ٤٨ ساعة للوبيا المعاملة بتركيز LC_{50} لكل مبيد حشري تم اختباره. وقد أظهرت النتائج بوضوح أن معدل أكسدة الليبيدات (LPO)، والذي يعبر عنه بقيمة المحتوى من مركب مالونديالدهيد (MDA) في الحشرات، قد زاد بشكل ملحوظ بعد التعرض لمبيد إيمامكتين بنزوات ، ومبيد إيميداكلوبريد، مقارنةً بالكنترول غير المعامل. كذلك أظهرت نتائج نشاط مضادات الأكسدة ، ارتفاع معنوي في قيم كل من الجلوتاثيون المختزل (GSH) والجلوتاثيون بيروكسيديز (GPx) في جميع معاملات المبيدات الحشرية المختبرة، مقارنة بالكنترول. في حين، انخفض نشاط إنزيم سوبرأكسيد ديسميوتيز (SOD) بشكل ملحوظ خاصة مع الأبامكتين والإندوكساكارب، والسبينوساد. وتخلص نتائج هذه الدراسة إلي أن عدم التوازن التأكسدي الناجم عن الحشرات بعد التعرض للتركيز تحت المميت (LC_{50}) يمكن أن يكون الأساس الجزيئي الذي يفسر سمية هذه المبيدات الحشرية. أيضًا، تمثل هذه الدلائل الحيوية المستخدمة في هذه الدراسة مؤشرات حيوية جيدة لحدوث مستويات مرتفعه من الإجهاد التأكسدي الناجم عن التعرض للمبيدات الحشرية.

الكلمات المفتاحية: حشرة خنفساء اللوبيا- مبيدات حشرية حديثة – التركيز النصفي القاتل – الإجهاد التأكسدي – مضادات الأكسدة.