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Methylparathion- and carbofuran-induced mitochondrial dysfunction and oxidative stress in *Helicoverpa armigera* (Noctuidae: Lepidoptera)

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ABSTRACT

The cotton bollworm, *Helicoverpa armigera* is a polyphagous pest of several crops in Asia, Africa, and the Mediterranean Europe. Organophosphate and carbamate insecticides are used on a large-scale to control *Helicoverpa.* Therefore, we studied the effect of methylparathion and carbofuran, an organophosphate and carbamate insecticide, respectively, on oxidative phosphorylation and oxidative stress in *H. armigera* larvae to gain an understanding of the different target sites of these insecticides. It was observed that state III and state IV respiration, respiratory control index (RCI), and P/O ratios were inhibited in a dose-dependent manner by methylparathion and carbofuran under *in vitro* and *in vivo* conditions. Methylparathion and carbofuran inhibited complex II by ~ 45% and 30%, respectively. Lipid peroxidation, H_2O_2 content, and lactate dehydrogenase (LDH) activity increased and glutathione reductase (GR) activity decreased in a time- and dose-dependent manner in insecticide-fed larvae. However, catalase activity was not affected in insecticide-fed larvae. Larval growth decreased by ~ 64 and 67% in larvae fed on diets with sub-lethal doses of methylparathion and carbofuran. The results suggested that both the insecticides impede the mitochondrial respiratory functions and induced lipid peroxidation, H_2O_2, and LDH leak, leading to oxidative stress in cells, which contribute to deleterious effects of these insecticides on the growth of *H. armigera* larvae, along with their neurotoxic effects.

Keywords: *Helicoverpa armigera*; mitochondria; respiration; oxidative stress; methylparathion; carbofuran.

1. INTRODUCTION

Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Noctuidae: Lepidoptera), is one of the major constraints to crop production in Asia, Africa, Australia and the Mediterranean Europe. It is a polyphagous pest and has been reported to attack more than 200 different species of plants including cotton, pigeonpea, groundnut sorghum, maize, chickpea, vegetables, fruit and forest trees [1]. The Lepidopteran larvae are 'eating machines', and they grow much faster than young mammals and birds, with strong metabolic processes [2]. Fast growth of *H. armigera* larvae occurs due to large midgut epithelium, which digests and absorbs the nutrients from the food [3]. Because of its reliance on aerobic metabolism, it also requires the presence of an active mitochondrial system for oxidative phosphorylation to meet the energy demands of the insect during growth and metamorphosis through different stadia. The metabolic system conceptually divides oxidative phosphorylation into three blocks of reaction, the substrate oxidation system, the phosphorylation system, and the proton transport [3].

Insecticides exhibit a high level of pest control ability combined with a relatively low degree of environmental toxicity; hence, they are used widely around the world in agriculture and in households. Most classes of insecticides are neurotoxic in nature with their toxic effects employing on broad group from insects to mammals. There are a number of reports on the mode of action of insecticides on insects as well as on non-target groups with different target sites [4, 5]. Studies on the effects of insecticides have mainly been carried out under *in vivo* conditions in rodents [6], Pisces [7], and pigs [8]. However, there is little information on the effect of insecticides in insects under *in vivo* conditions, and hence, we evaluated the *in vivo* effects of methylparathion and carbofuran, organophosphorous and carbamate insecticides, respectively, on the larvae of cotton bollworm, *H. armigera*.

In addition to the inhibition of acetylcholinesterase, methylparathion also affects carbohydrate, nitrogen, lipid, and oxidative metabolism in crustaceans [9]. It also causes chromosomal aberrations, alterations in oxidative phosphorylation, and carbohydrate metabolism in fish [10]. Organophosphorous insecticides (OPI) induced oxidative stress, genotoxicity and DNA damage in human [11]. In addition to the inhibition of target enzyme, acetylcholinesterase, carbofuran has been reported to cause a number of other biochemical afflictions such as, altered energy metabolism, oxidative stress, mitochondrial respiratory chain dysfunction and DNA damage in different non-target organism including human [12, 13, 14, 15].

Several insecticides have been shown to affect mitochondrial bioenergetics. Pesticides adversely affect energy mitochondrial metabolism in fish [16] and mammals [17]. OPIs impaired mitochondrial energy metabolism, generated oxidative stress and caused neuronal apoptosis when exposed to rat brain [18]. Methylparathion results in deleterious effects on oxidative phosphorylation and membrane depolarization in rat liver mitochondria under *in vitro* conditions [19]; while carbofuran impairs mitochondrial functions in rat brain [20]. Toxicity of chlorpropham, a carbamate insecticide, is associated with the rapid depletion of ATP via impairment of mitochondrial function [21]. Carbaryl, another carbamate insecticide, has been shown to inhibit mitochondrial bioenergetics and succinate dehydrogenase in rats [22].

Induction of oxidative stress is also one of the main mechanisms of action of many insecticides. Exposure to insecticides induces superoxide, H_2O_2 and alters the levels of antioxidant enzymes in mice [23]. Phoxim and chlorfenvinphos, organophosphorous insecticides (OPI), induces oxidative stress in silkworm [24] and rat liver mitochondria [25], respectively. Carbofuran induced oxidative stress and impairment in mitochondrial functions has been clearly demonstrated in rat brain tissue [20]. Carbamates caused cytotoxicity and induced lipid peroxidation in Chinese hamster ovary cells [26]. Carbofuran-induced neurotoxicity has been correlated with the mitochondrial oxidative stress in rat [20]. Methylparathion and carbofuran are extensively used to control *H. armigera* [1]. The present studies were undertaken to evaluate the effect of methylparathion and carbofuran in inducing mitochondrial dysfunction and oxidative stress, which could affect the growth and development of *H. armigera*.

2. MATERIAL AND METHODS

2.1. Chemicals

NADH, bovine serum albumin, and ADP were purchased from Sigma Aldrich (Mumbai, India). Sucrose was purchased from Qualigens (Mumbai, India). Methylparathion (99.3%) and carbofuran (99%) were procured from Pesticide Analysis Laboratory, Gulbarga, India. The other chemicals used in these studies were of analytical grade.

2.2. Insects

Larvae of *H. armigera* were obtained from the insect rearing laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The larvae were reared on a chickpea based semi-synthetic diet under laboratory conditions at 27 ± 1 ˚C, 65 ± 5% RH, and 12 h photoperiod [27].

2.3. Isolation of mitochondria

The fourth- and fifth-instar larvae were starved for 3 h, their midguts content removed, washed in cold distilled water, and then homogenized in dounce homogenizer under cold conditions in 0.25 M sucrose solution containing 0.1% defatted bovine serum albumin (BSA). The homogenate was filtered through a moist muslin cloth, the filtrate centrifuged at 800 xg for 10 min at 4˚C. The residue was re-suspended in extraction buffer, and centrifuged at 800 xg for 5 min. The supernatants from both the centrifugations were combined and centrifuged at 8,000 xg for 10 min. The mitochondrial pellet was re-suspended in the reaction mixture, and used immediately for measuring oxygen consumption [3].

2.4. Mitochondrial respiration

Polarographic determination of oxidative phosphorylation was made by using oxygraph (Hansatech Instruments Limited, Bachofer, Reutlingen 72734, Germany) fitted with a Clark type oxygen electrode. The reaction system contained 5 mM HEPES buffer, pH 7.2, 50 mM sucrose, 120 mM KCl, 5.55 mM $MgCl_2$ and freshly isolated mitochondria in a total reaction volume of 1.5 ml. After the addition of substrate (10 mM succinate), the rate of state III respiration was measured by the addition of 0.1 mM ADP and state IV respiration measured in absence of ADP [17]. Protein concentration was determined by Lowry's method [28] using BSA as a standard.

2.5. Enzyme assays

Complex I activity was measured using ferricyanide as electron acceptor. The reaction system contained 250 mM sucrose, 50 mM potassium phosphate buffer, pH 7.2, 1 mM KCN, 5 mM $MgCl_2$, 1 mM potassium ferricyanide and 200 mg mitochondrial protein in a total volume of 1 ml. The reaction was started with 0.4 mM NADH and the rate of disappearance of either NADH (340 nm) or potassium ferricyanide (420 nm) was measured spectrophotometrically [29]. Complex II activity was measured using phenazinemethosulphate (PMS) as electron acceptor. The reaction mixture was same as used for complex I assay, except that ferricyanide was substituted with 1mM PMS and 70 µM 2,6-

dichlorophenol indophenols (DCPIP). The rate of reduction of DCPIP was measured at 600 nm ($\epsilon_{\mu M}$ 16.2) [29]. Complex IV reaction was measured in 2 ml reaction mixture, containing 60 µM reduced cytochrome c in 50 mM phosphate buffer, pH 7.2. The reaction was initiated by adding mitochondrial protein, and oxidation of cytochrome c was measured at 550 nm [17]. F_0F_1 ATPase activity was determined by quantifying the release of inorganic phosphate from ATP in 50 mM Tris-HCl, pH 7.4. The released phosphate was measured calorimetrically at 660 nm [30].

2.6. In vivo effect of insecticides on the mitochondrial respiration and respiratory enzyme complexes

Fourth-instar larvae were fed on artificial diet containing 100 µM methylparathion and 100 µM carbofuran separately. After 24 h, the mitochondria were isolated from the insecticide-fed larvae, and the isolated mitochondria evaluated for mitochondrial respiration and enzyme activities as described above.

2.7. Effect of insecticides on the oxidative stress in H. armigera, in vivo

Fourth-instar larvae were fed with different concentrations of methylparathion and carbofuran (0 - 100 µM) and lipid peroxidation, lactate dehydrogenase leakage and H_2O_2 content were measured as oxidative stress markers as follows:

2.8. Lipid peroxidation

Lipid peroxidation was measured by quantifying malondialdehyde (MDA) levels in larval homogenates on the basis of reaction with thiobarbituric acid to form a pink colored complex. MDA produced was measured at 532 nm, and the nonspecific absorbance was subtracted by measuring the absorbance at 600 nm. Lipid peroxidation was calculated using

1.56 X 10^5 as extinction coefficient, and expressed as µmol of MDA/mg of protein extract [31].

2.9. Lactate dehydrogenase leakage

Lactate dehydrogenase (LDH) activity was determined in larval homogenates by measuring decrease in NADH content at 340 nm by using UV spectrophotometer (Hitachi, U-2900), and the enzyme activity was expressed as mmoles/min/mg protein [31].

2.10. Measurement of H_2O_2 content

H_2O_2 content was estimated in larval homogenates according to Noreen and Ashraf [32], and expressed as µmoles of H_2O_2/mg protein.

2.11. Assay of antioxidant enzymes

Catalase activity was determined by kinetic assay adapted from Olgun and Misra [23], in which the disappearance of peroxide is monitored spectrophotmetrically at 240 nm. One unit of catalase is equivalent to 1 µmol of H_2O_2 decomposed per minute per mg of protein using the extinction coefficient of 43.6 M^{-1} cm^{-1}. Glutathione reductase (GR) activity was determined in 1 ml reaction mixture, containing 1 ml 50 mM phosphate buffer, pH 7.2, 1 mM EDTA, 0.05% bovine serum albumin, 10 mM oxidized glutathione, and 10 mM NADPH. The rate of change in absorbance was measured at 340 nm. One unit of enzyme activity was expressed as 1 µmol of NADPH oxidized per minute per mg of protein [23].

2.12. Bioassay of insecticides

Methylparathion and carbofuran were incorporated into the artificial diet at different concentrations (0 to 100 µM). Third-instar larvae were released into the insecticide containing diets. The initial weights of *H. armigera* larvae were measured before releasing in the artificial diets. There were three replications for each treatment in completely randomized design, and there were 10 larvae in each replication. The larval weights were recorded 5 days after initiating the experiment.

2.13. Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) to judge the significance of differences between the treatments by using F-test, while the significance of differences between the treatment means was judged by least significant difference (LSD) at $p < 0.05$.

3. Results

3.1. Oxygen consumption studies

Methylparathion and carbofuran inhibited both the state III and state IV respiration in a dose-dependent manner when succinate was used as the oxidisable substrate. State III and state IV respiration for the control mitochondria was 63.24 ± 5.23 and 18.12 ± 2.13 nmoles/min/mg protein, respectively. At 100 µM concentration, methylparathion and carbofuran inhibited 79.23 and 77.3% state III respiration, and 53.1 and 45.92% state IV respiration, respectively. Methylparathion and carbofuran also inhibited respiration control index (RCI) and P/O ratios in a dose-dependent manner, *in vitro*. The RCI and P/O ratio for the control mitochondria was 3.2 ± 0.41 and 2.21 ± 0.02, respectively, for succinate oxidation. The RCI was inhibited up to 55.32 and 58.44% and the P/O ratio was inhibited 45.46 and 49.1%, respectively, by 100 µM methylparathion and carbofuran (Fig. 1A, 1B, 1C, 1D).

3.2. Enzyme assays

Mitochondrial respiratory enzyme complexes were measured in the presence of different concentrations of methylparathion and carbofuran (0 to 100 µM). Among the enzyme complexes measured, both the insecticides inhibited respiratory complexes II in a dose-dependent manner. At 100 µM, methylparathion and carbofuran inhibited about 45 and 30% of the enzyme activities, respectively. And no significant effects of insecticides were observed on the activities of complex I, IV and F_0F_1 ATPase (Fig. 2, 3).

3.3. In vivo effect of insecticides on mitochondrial respiration

Mitochondria isolated from insecticide-fed larvae exhibited a significant inhibition in state III and state IV respiration. The RCI was severely inhibited in case of methylparathion- and carbofuran-fed larvae *in vivo*. State III respiration was inhibited up to 47.0 and 35.0%, state IV respiration by 30.81 and 20.17%, RCI by 23.74 and 18.76%, and P/O ratio by 35.33 and 43.96% in larvae fed on diets containing methylparathion and carbofuran, respectively (Fig. 4). None of the enzyme complexes were affected in insecticide-fed larvae, but a drop of about 42% and 38% activity was observed for F_0F_1 ATPase in methylparathion- and carbofuran-fed larvae, respectively, as compared to the control larvae.

3.4. Lipid peroxidation, H_2O_2 content and lactate dehydrogenase leak

In the control larvae fed on artificial diet without insecticides, MDA content was 0.5 µmoles/mg protein, H_2O_2 content 2.34 µmoles/mg protein, and LDH leak 0.116 mmoles/min/mg protein. There was proportional increase in lipid peroxidation (Fig. 5, 6), LDH leak (Fig. 7, 8) and H_2O_2 content (Fig. 9, 10) in insecticide fed larvae. There was a significant increase in these components in the larvae fed on diets containing insecticides after 18 h of feeding. Maximum concentration was recorded at 24 h after feeding. For dose response studies, lipid peroxidation, LDH leak and H_2O_2 content were estimated after 24 h with 100 µM of methylparathion and carbofuran. There was a proportional increase in lipid peroxidation, LDH leak and H_2O_2 content in a time- and dose-dependent manner in larvae fed on diet containing methylparathion (105, 69, 149%) and carbofuran (72, 64, 140%).

3.5. Antioxidant enzymes

The activities of catalase and GR in control larvae were 11.74 ± 1.23 and 40.57 ± 2.64 U/mg, respectively. The activity of GR was reduced in a dose- and time-dependent manner in insecticide-fed larvae, whereas, catalase was not affected in *H. armigera* larvae fed on diets amended with methylparathion and carbofuran (Tables 1, 2).

3.6. Bioassays of insecticides on H. armigera larvae

Both the insecticides inhibited the larval growth in a dose-dependent manner. At 100 µM, there was ~ 64% and 67% inhibition in larval growth in larvae fed on diets with methylparathion and carbofuran, respectively (Fig. 11).

4. DISCUSSION

Organophosphate and carbamate insecticides inactivate acetylcholine esterase, inhibiting the breakdown of acetylcholine, leading to accumulation of acetylcholine, which initially over-stimulates and then paralyzes the cholinergic transmission. Phosphorylation and carbamoyaltion of serine residue at the active site of acetylcholine esterase is the major difference in the mode of action of organophosphates and carbamates, respectively. In the present studies, effects of methylparathion and carbofuran on mitochondrial respiration and oxidative stress were studied to understand the effects of these insecticides on mitochondrial respiration. Both the insecticides have inhibited state III and state IV respiration *in vitro* when succinate was used as the oxidizing substrate. They also inhibited RCI and P/O ratios in a dose-dependent manner *in vitro*. A clear inhibitory effect on succinate dehydrogenase is induced by both the insecticides *in vitro*, suggesting the inhibition of electron flow through the electron transport chain, thus the insecticide-induced depression of phosphorylation efficiency of isolated mitochondria is mainly due to the inhibition of oxygen consumption and inhibition at complex II. Similar observations were reported for the insecticide-induced mitochondrial dysfunction *in vitro* in rats [19]. Under *in vivo* conditions, state III respiration and state IV respiration decreased, affecting RCI, suggesting absence of uncoupling mechanism of action in these insecticides. Mitochondria prepared from control larvae had a RCI of 3.01 whereas the mitochondria from methylparathion- and carbofuran-fed larvae had a RCI of about 1.66 and 1.67, respectively. The decrease in RCI further confirmed that these insecticides result in mitochondrial injury, *in vivo*. Phosphorylation efficiency (P/O) of the mitochondria was inhibited in larvae fed on diets containing insecticides, which is due to inhibition in the activity of F_0F_1 ATPase. Reduced rate of phosphorylation observed *in vitro* with methylparathion in rat liver mitochondria is due to inhibition of phosphate carrier and dislocation of F_0 and F_1 components of ATP synthase, which leads to less ATP content [19]. Chlorpropham, a carabamate insecticide, is also results in ATP depletion in mitochondria. Since mitochondrial respiratory chain produces the majority of ATP content of the cells, an impairment in the mitochondrial function could adversely affect the energetic state of the cell.

Chemical toxic pollutants are important sources of ROS in biological systems [33]. A time- and dose-dependent increase in lipid peroxidation, H_2O_2 content and LDH leak was recorded in insecticide-fed larvae. There was a little increase in oxidative stress markers after 12 h of feeding on diets containing the insecticides, but a significant increase was recorded after 18 h, reaching the maximum level at 24 h. Inhibition of electron transport chain at any site could lead to generation of H_2O_2 and ROS, which in turn peroxidise membrane lipids, as evidenced by high levels of MDA in insecticide-fed larvae. These findings are similar to earlier reports, wherein, toxicity of many xenobiotics, including pesticides has been found to be associated with the generation of ROS [34]. The LDH activity is the most sensitive parameter for evaluation of tissue damage and toxicity. Significant increase in LDH activity in insecticide-fed larvae indicated the higher rates of glycolysis, indicating that aerobic oxidation was adversely affected in insecticide-fed larvae, as confirmed by inhibition in oxygen uptake *in vivo*. Elevated levels of LDH activity have been associated with inhibition of aerobic oxidation in pesticide exposed fish [35]. Bidrin, an organophosphate insecticide, induced lipid peroxidation, H_2O_2 and LDH levels in cultured renal tubular cells [31], while carbofuran-induced mitochondrial dysfunction and lipid peroxidation in rat brain [20].

Pesticides are known to alter the level of antioxidant enzymes. In insecticide-fed *H. armigera* larvae, catalase activity was unaffected whereas glutathione reductase was inhibited in a dose- and time-dependent manner. Similar observations have earlier been made by which Olgun and Misra [23]. However, lindane, an organochlorine insecticide, reduced the activity of liver catalase, but did not affect glutathione reductase [36]. Because the K_m value for the catalysis of H_2O_2 by catalase is in the range of 1.1 M [23], and the increased levels of H_2O_2 produced during exposure to insecticides never exceeded this level, no change in levels of catalase are not surprising. Reduction in glutathione reductase levels may be because of a direct effect of these insecticides and their metabolites on this enzyme. Reduction in larval growth in larvae fed on diets with these insecticides may be due to impairment in the mitochondrial function as evidenced by depression in mitochondrial respiration, respiratory control index (RCI), P/O ratio and increase in oxidative stress as evidenced by high levels of lipid peroxidation, H_2O_2 content, and LDH leak, under *in vivo* conditions. OPI-induced oxidative stress is associated with the degeneration of neurons and apoptosis [18].

In conclusion, methylparathion and carbofuran exposure impedes mitochondrial respiratory functions and induced lipid peroxidation, H_2O_2 content and LDH leak in a time- and dose-dependent manner, leading to oxidative stress in cells, resulting in deleterious effects on the growth of *H. armigera* larvae, along with the neurotoxic effects. There were some differences in mode of action of these chemicals in mitochondrial oxidation, and hence, these can be used alternatively for the control of *H. armigera*.

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Figure legends:

Fig. 1. Effect of methylparathion (●) and carbofuran (▲) on mitochondrial respiration when succinate was the oxidisable substrate. Conditions for oxygen uptake measurements are describes in text. Insecticide was incubated for 2 min with mitochondria, prior to addition of succinate. (A) Mitochondrial oxygen uptake in the presence of ADP (state III respiration), (B) mitochondrial oxygen uptake in absence of ADP (state IV respiration), (C) RCI and (D) P/O ratio for the isolated mitochondria. The traces are the representative of three individual experiments.

Fig. 2. Effect of methylparathion on NADH dehydrogenase (♦), succinate dehydrogenase (●), cytochrome oxidase (▲) and F_0F_1 ATPase (■) of isolated mitochondria. Enzyme activities were assessed as described in text. The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 3. Effect of carbofuran on NADH dehydrogenase (♦), succinate dehydrogenase (●), cytochrome oxidase (▲) and F_0F_1 ATPase (■) of isolated mitochondria. Enzyme activities were assessed as described in text. The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 4. *In vivo* effect of methylparathion and carbofuran on mitochondrial respiration for the oxidation of succinate. Oxygen consumption in presence of ADP (state III) (■), in absence of ADP (state IV) (■); RCI (■), P/O ratios (▲) and F_0F_1 ATPase activity (●) was measured in insecticide-fed larvae as described in text. The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 5. Dose-dependent response for lipid peroxidation in the larvae fed on diet containing methylparathion (●) and carbofuran (▲). The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 6. Time-dependent response for lipid peroxidation in the larvae fed on diet containing methylparathion (■) and carbofuran (■). The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 7. Dose-dependent response for H_2O_2 production in the larvae fed on diet containing methylparathion (●) and carbofuran (▲). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.01$).

Fig. 8. Time-dependent response for H_2O_2 production in the larvae fed on diet containing methylparathion (■) and carbofuran (■). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.01$).

Fig. 9. Dose-dependent response for LDH leak in the larvae fed on diet containing methylparathion (●) and carbofuran (▲). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 10. Time-dependent response for LDH leak in the larvae fed on diet containing methylparathion (■) and carbofuran (■). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

Fig. 11. Bioassay for methylparathion and carbofuran. Neonates were fed on artificial diet containing varying concentrations of methylparathion (■) and carbofuran (■). The data represents the Mean ± S.D. (n = 3) (Significantly different from control at * $p < 0.05$).

Table 1
Dose-dependent response of antioxidant enzymes in *H. armigera* fed on diet containing insecticide.

	0 µM	20 µM	40 µM	60 µM	80 µM	100 µM
MP-fed larvae						
Catalase (U/mg)	11.74 ± 1.23	11.82 ± 0.13	11.81 ± 1.45	11.91 ± 1.22	11.99 ± 0.22	12.24 ± 1.22
Glutathione reductase (U/mg)	40.57 ± 2.64	34.48 ± 2.63*	28.39 ± 3.22*	27.38 ± 1.73*	23.53 ± 2.73*	16.83 ± 1.82*
Carbofuran-fed larvae						
Catalase (U/mg)	11.74 ± 1.23	11.99 ± 0.23	11.98 ± 0.24	12.34 ± 0.34	12.44 ± 0.89	12.54 ± 1.11
Glutathione reductase (U/mg)	40.57 ± 2.64	32.45 ± 2.73*	26.37 ± 3.62*	23.12 ± 3.22*	20.28 ± 1.73*	19.87 ± 1.22*

Values in the Table represents Mean ± S.D. of at least three determinants (significantly different from control at * $p < 0.05$).

Table 2
Time-dependent response of antioxidant enzymes in *H. armigera* fed on diet containing insecticide.

	Control	**12 h**	**18 h**	**24 h**	**48 h**
MP-fed larvae					
Catalase (U/mg)	11.74 ± 1.23	11.76 ± 0.93	11.92 ± 0.64	12.24 ± 1.22	12.28 ± 0.83
Glutathione reductase (U/mg)	40.57 ± 2.64	32.88 ± 2.28*	26.64 ± 3.22*	16.83 ± 1.82*	17.11 ± 2.63*
Carbofuran-fed larvae					
Catalase (U/mg)	11.74 ± 1.23	11.82 ± 1.02	12.11 ± 1.45	12.54 ± 1.11	12.66 ± 1.54
Glutathione reductase (U/mg)	40.57 ± 2.64	34.98 ± 1.98*	27.44 ± 2.43*	19.87 ± 1.22*	19.88 ± 3.74*

Values in the Table represents Mean ± S.D. of at least three determinants (significantly different from control at * $p < 0.05$).

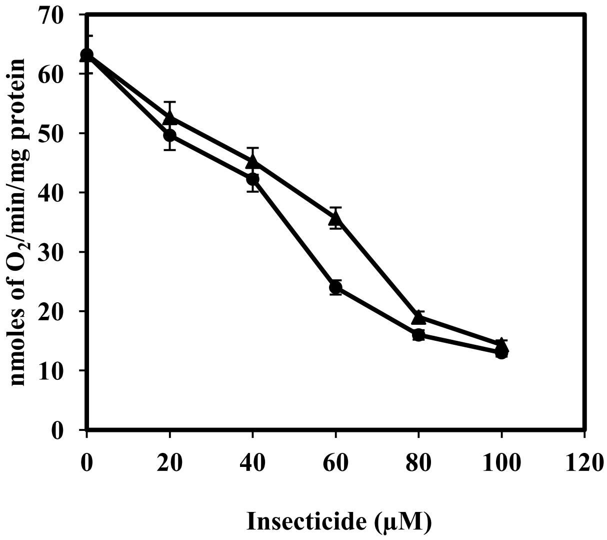
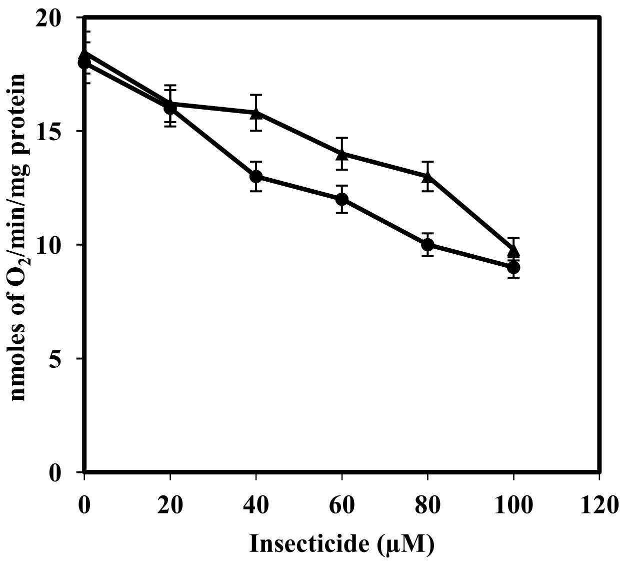

70
60
50
40
30
20
10
0
nmoles of O_2/min/mg protein
0
20
40
60
80
100
120
Insecticide (µM)

Fig. 1A.



20
15
10
5
0
0
20
40
60
80
100
120
nmoles of O2/min/mg protein
Insecticide (µM)

Fig. 1B.

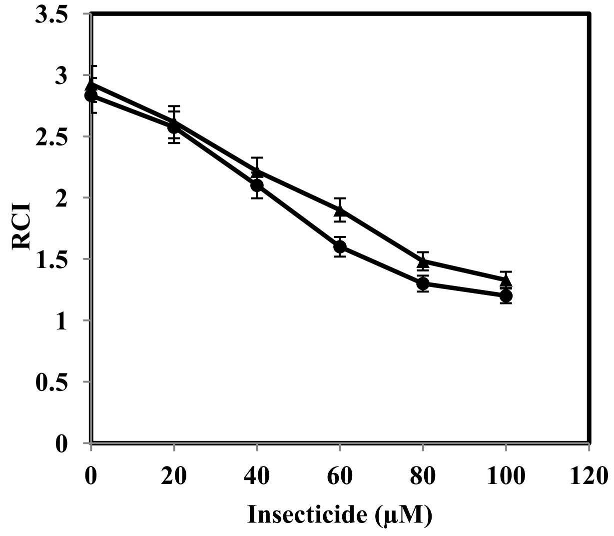


Fig. 1C.

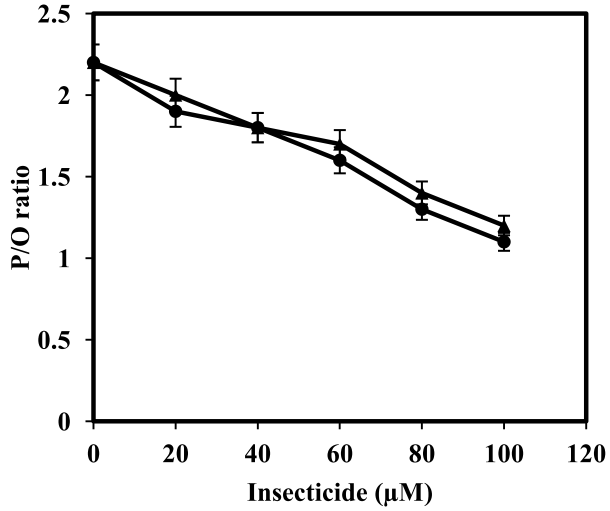


Fig. 1D.

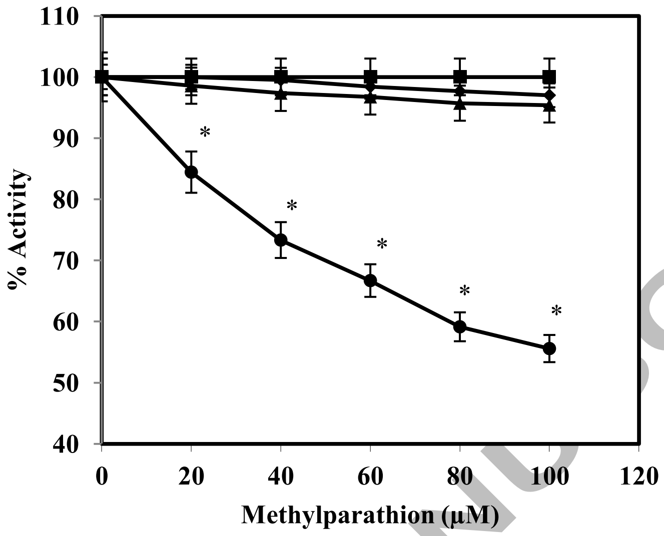

110
100
90
80
70
60
50
40
% Activity
0
20
40
60
80
100
120
Methylparathion (µM)
*
*
*
*
*

Fig. 2.

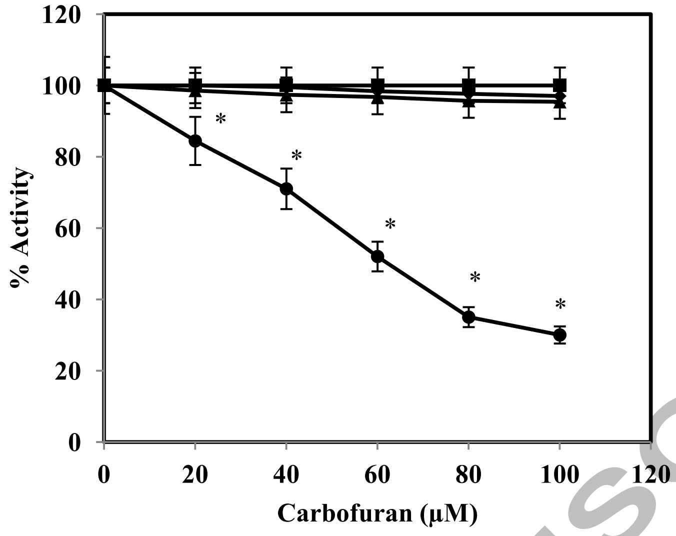
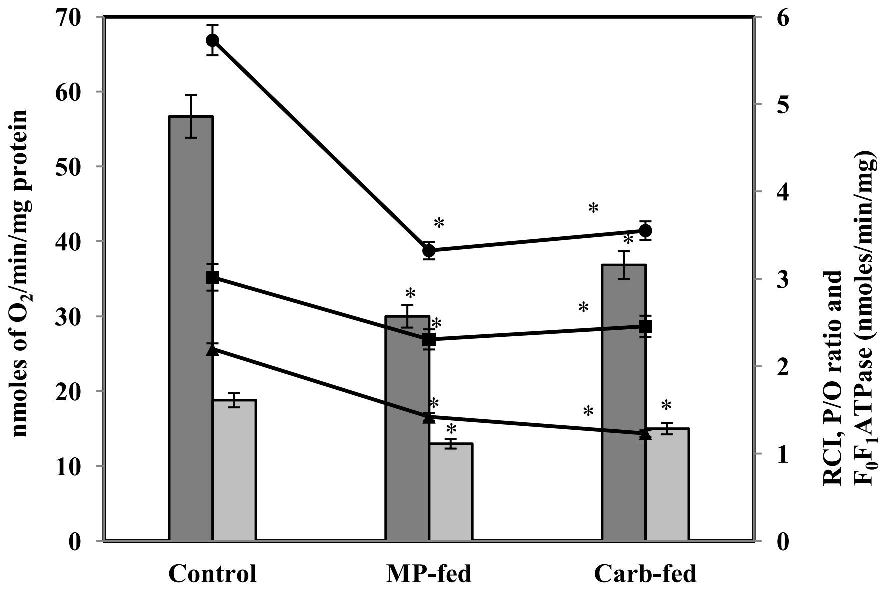

120
100
80
60
40
20
0
% Activity
*
0
20
40
60
80
100
120
Carbofuran (µM)

Fig. 3.



70
60
50
40
30
20
10
0
nmoles of O2/min/mg protein
6
5
4
3
2
1
0
RCI, P/O ratio and
F0F1ATPase (nmoles/min/mg)
Control
MP-fed
Carb-fed
*

Fig. 4.

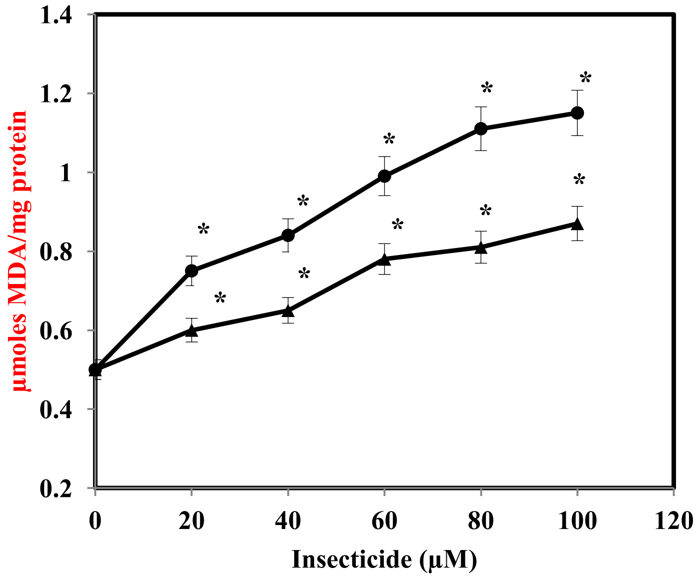

1.4
1.2
1
0.8
0.6
0.4
0.2
µmoles MDA/mg protein
0
20
40
60
80
100
120
Insecticide (µM)

Fig.5.

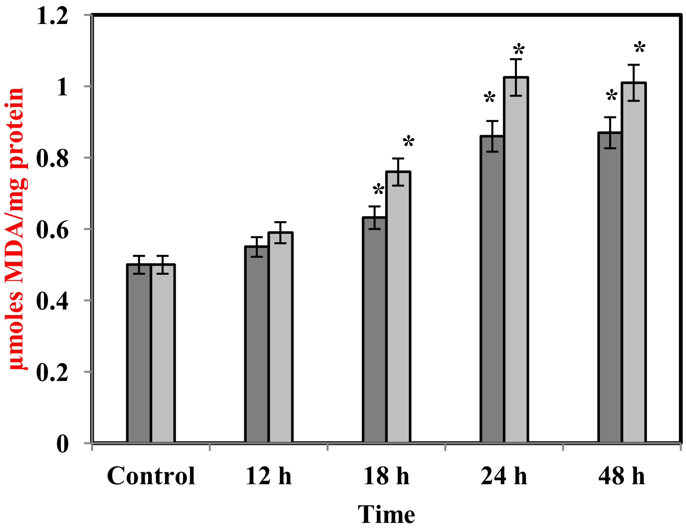

µmoles MDA/mg protein
1.2
1
0.8
0.6
0.4
0.2
0
*
*
*
*
*
*
Control
12 h
18 h
24 h
48 h
Time

Fig.6.

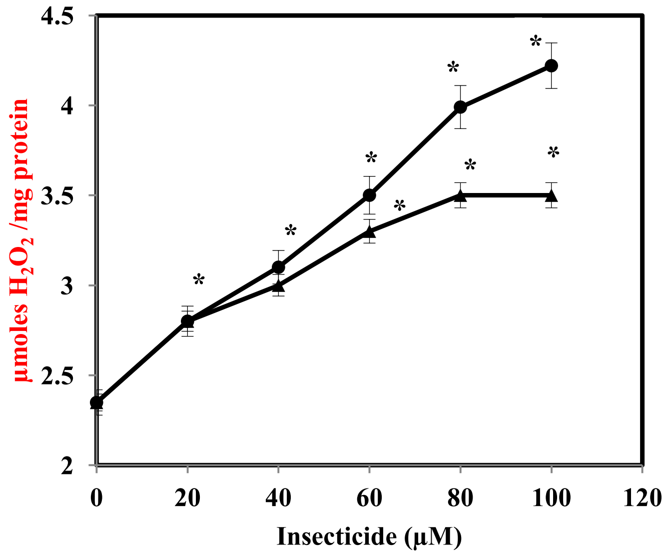
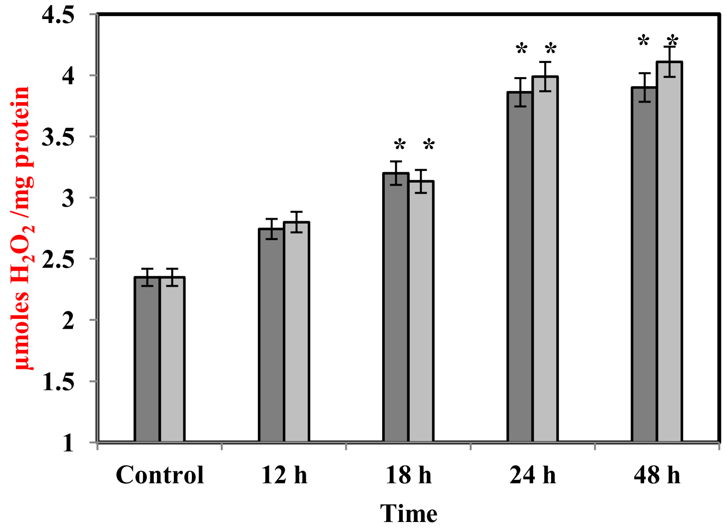
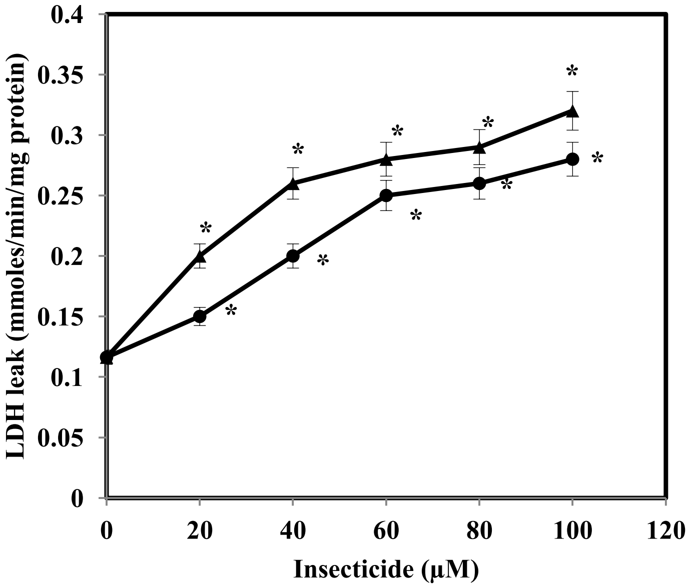

4.5
4
3.5
3
2.5
2
0
20
40
60
80
100
120
Insecticide (µM)
µmoles H2O2 /mg protein
*

Fig.7.



4.5
4
3.5
3
2.5
2
1.5
1
µmoles H2O2 /mg protein
*
Control
12 h
18 h
24 h
48 h
Time

Fig.8.



LDH leak (mmoles/min/mg protein)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
20
40
60
80
100
120
Insecticide (µM)

Fig.9.

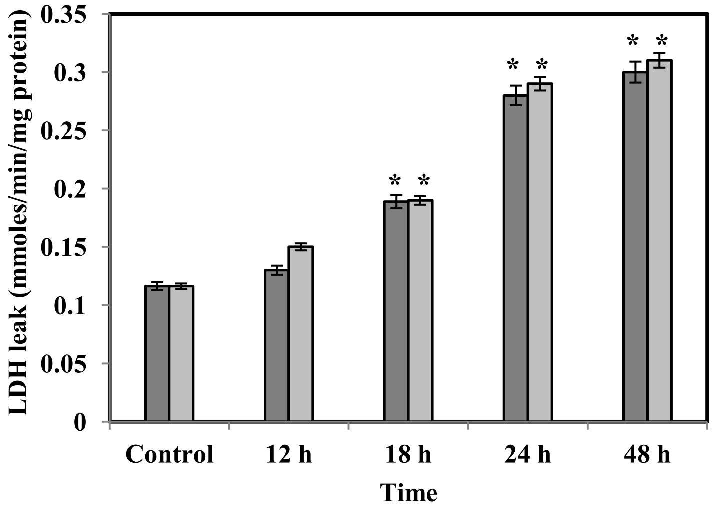
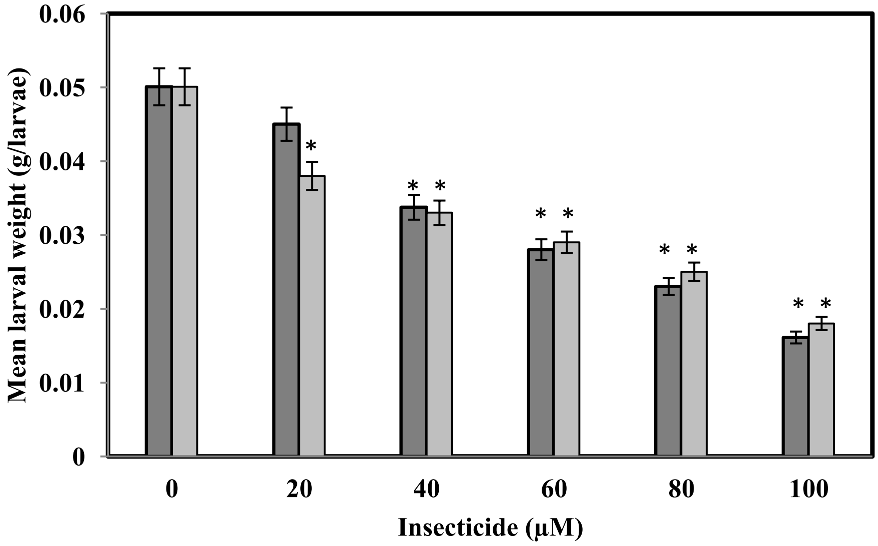

0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
LDH leak (mmoles/min/mg protein)
Control
12 h
18 h
24 h
48 h
Time

Fig.10.

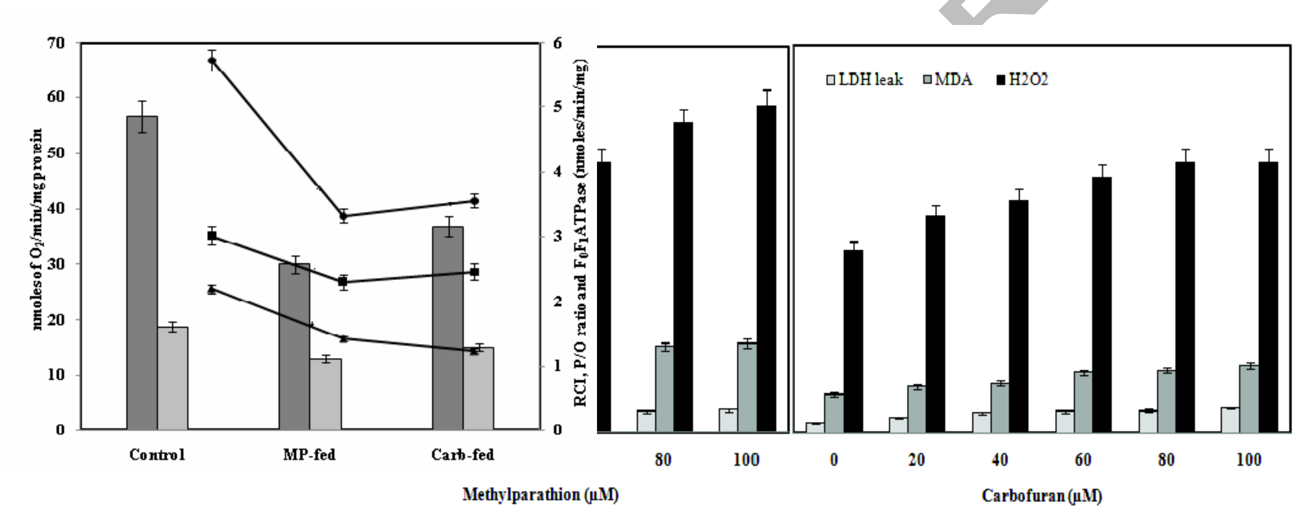


0.06
0.05
0.04
0.03
0.02
0.01
0
Mean larval weight (g/larvae)
*
* *
* *
* *
* *
0
20
40
60
80
100
Insecticide (µM)

Fig. 11.

Graphical abstract

In vivo effect of methylparathion and carvofuran was investigated on the respiratory parameters and oxidative stress markers in *Heloicoverpa armigera*.



Research highlights

- ► Methylparathion and carbofuran inhibited state III and state IV respiration *in vivo*.
- ► Both the insecticides also inhibited P/O ratio, RCI and F_0F_1 ATPase *in vivo*.
- ► The insecticides induced LDH leak, MDA, H_2O_2 content *in vivo*.
- ► The insecticides inhibited larval growth in dose- and time-dependent manner.