Abstract

Pesticide is an inevitable part of modern agricultural practice and during the past several decades the use of pesticides is tremendously increasing in global scenario. In this study, the effect of commonly used pesticides chlorpyrifos and cypermethrin on development and silk production of *Philosamia ricini* (eri silkworm) have been evaluated. This domesticated and commercially beneficial insect feeds primarily on castor leaves. In North East region, castor grows as stray plant nearby vicinity of agricultural land and tea gardens. Therefore, these plants are likely to be exposed to pesticide contamination by spray drift or residual effect and thereby affecting the silkworm rearing upon these plants. The LC$_{50}$ values of chlorpyrifos and cypermethrin were determined and further experiments were carried out with sub lethal concentrations of the pesticides. Herein, the LC$_{50}$ values at 96 h were recorded as 2.35 mg/L and 58.41 µg/L for chlorpyrifos and cypermethrin respectively. Two sub lethal concentrations of both pesticides were selected for further study. For chlorpyrifos the sub lethal concentrations were 1.5 mg/L and 2.0 mg/L. For cypermethrin the sub lethal concentrations were 10 µg/L and 20 µg/L. Intoxication symptoms like body blackening, less feeding and vomiting were observed in pesticide exposed larvae of eri silkworm. Toxic effects of a commercial formulation of both pesticides were evaluated in terms of alteration in metabolites, digestive physiology, enzyme activity, economic characteristics and induced genotoxicity. Results showed significant alteration in morphometric characteristics such as larval growth, duration, pupa formation, moth emergence etc. in both chlorpyrifos and cypermethrin exposed groups of eri silkworm. Findings showed a significant decrease in biochemical constituents (protein, trehalose and lipid) in the larvae after exposed to sub lethal concentrations of chlorpyrifos and cypermethrin. The larvae exposed to pesticide contaminated leaves showed less feeding behavior and thereby their nutritional requirements were not fulfilled properly. The antifeedant characteristics and starvation of the larvae can induce alteration in biochemical constituents. Impairment of digestive physiology was observed in pesticide exposed groups in terms of alteration in amylase, cellulase, protease, lipase activity. An increasing trend in ALT activity was observed in pesticides treated groups at 24 – 72 h time intervals which indicated tissue injury. Degenerative changes in gut structure were observed in pesticide exposed groups. Chlorpyrifos exposed groups showed thin mucus...
layer which was followed by ruptured microvilli and vacuole formation. Fused microvilli and vacuole formation was also prominent in cypermethrin exposed groups. Additionally, significant alteration in phenoloxidase and lysozyme activity was observed in pesticide exposed groups. Pesticide induced immunotoxicity was also obvious from changes in proportional counts of hemocytes. Results showed that sub lethal concentrations of pesticides exposure caused an increase of granulocytes and plasmatocytes in a concentration-dependant manner. However, prohematocytes, spehrulocytes and oenocytes abundance was decreased significantly in pesticides exposed groups. Further, genotoxicity study revealed higher tail percentage, tail length and tail moment of the damage DNA in pesticide exposed groups. Caspase activation (procaspase to active caspase) elucidates the mechanism of cell death. Results also showed activation of caspase activity in pesticide exposed groups in a dose-dependent manner. Moreover, in order to confirm apoptosis in hemocytes exposed to pesticides annexin V assay was performed. In both pesticides exposed group numbers of apoptotic cells were significantly higher which clearly indicated the link between apoptosis and DNA damage. Additionally, effect of chlorpyrifos and cypermethrin on acetylcholinesterase enzyme system was taken into consideration. Initially IC$_{50}$ values (inhibition concentration) of chlorpyrifos and cypermethrin were calculated. For chlorpyrifos, IC$_{50}$ value was recorded as 2.15 mg/L whereas for cypermethrin the value was 8.84 µg/L. This indicated more inhibitory effect of cypermethrin than chlorpyrifos. 

*In vitro* results showed inhibition in brain and body tissue acetylcholinesterase enzyme activity in pesticide exposed groups. Further enzyme kinetics parameters such as Km and Vmax were also calculated. Increase in Km values were observed in chlorpyrifos and cypermethrin exposed group in both brain and body tissue. However, Vmax value alteration was not significant except higher concentration of cypermethrin which predicted competitive mode of inhibition. *In vivo* study results showed inhibition of acetylcholinesterase activity. Further, to validate the mode of inhibition *in silico* assay was performed. The interaction analysis revealed that the best fitted pose of chlorpyrifos interacted with Tyrosine (TYR 130,116), Proline (PRO 86), Serine (SER 122), Glycine (GLY 118,123,117), Glutamic acid (GLU 199) and Tryptophan (TRP 84) residues of the acetylcholinesterase enzyme receptor. Cypermethrin formed a pi-pi interaction with Phenylalanine (PHE 290), Tryptophan (TRP 84) and Tyrosine (TYR 442) residues of
the target molecule and formed a hydrogen bonding with the Glutamic acid (GLU 199). Increase in acetylcholinesterase (AChE) gene expression was also observed in both chlorpyrifos and cypermethrin exposed groups which might be due to the autologous feedback response of transcription. In present study, the economic traits such as cocoon length, breadth, weight, shell weight, shell ratio etc. were found to be decreased in almost all concentrations of chlorpyrifos and cypermethrin treated groups. Since the pesticide treated leaves fed larvae showed alteration in development and different metabolic process, therefore it might be correlated with altered cocoon characters. Reduction of tensile strength was observed which was followed by decrease in fiber diameter. However no alteration in surface morphology, thermal property and secondary structure of silk fiber was observed. Taken together, this study suggested that chlorpyrifos and cypermethrin pollution might have adverse effect on overall development and silk quality of eri silkworm that could lead to reduction in survivability of this economically beneficial insect. Thus the findings will help to manipulate the safe and judicious uses of pesticides in the field to avoid the hazardous effect on non targeted organisms as well as human beings.