ABSTRACT

Introduction: Tea, *Camellia sinensis* (L.) O. Kuntze, is an economic perennial monoculture crop in the sub-Himalayan region of West Bengal. This foliage crop is regularly infested by a large number of insect species. Of these, looper stage of black inch worm, *Hyposidra talaca* (Walker) (Lepidoptera: Geometridae), causes substantial crop loss in the Darjeeling Terai tea plantations and has emerged as a dominant tea pest in this region. The effective, eco-friendly pest management strategies against *Hyposidra talaca* demands the insight knowledge of the defense mechanisms of this lepidopteran pest against the chemical pesticides that are indiscriminately used in this region, as well as, against the entomopathogenic groups of organisms including viruses, fungi, bacteria, microsporidia, protists and nematodes, to provide a non-chemical alternative for insect pest management.

Background: Insect defense system against chemical pesticides as well as entomopathogens comprises cellular or haemocyte-associated molecules, humoral plasma borne factors and the defense enzyme systems. Cellular immune responses, including phagocytosis, nodulation and encapsulation are mediated by haemocytes against any type of foreign agents that enter hemocoel. The success of the cellular defense depends on the number and types of haemocytes involved in these processes. Melanin synthesis and antimicrobial peptide (AMP) production are two arms of humoral defense system. Melanization is caused by the activity of phenoloxidase (PO) as a response to infection or wounding. Melanin is deposited within nodules, composed of aggregated haemocytes and microorganisms that form in the heavily infected insects. AMPs are pattern-recognition molecules that recognize microbial cell wall components, called pathogen associated molecular patterns (PAMPs). The phytophagous pest can tolerate the toxic substances present in the host plant as well as the chemical pesticides through production of defense (detoxification) enzymes. Generally, three enzyme systems, general esterases, cytochrome P450-dependent monooxygenases and glutathione S-transferases are known to be involved in the detoxification of allelochemicals as well as insecticides.

Materials and methods: Considering the above facts, in this study the cellular immune system of *Hyposidra talaca* has been investigated by characterizing the haemocytes and determining the total and differential count of haemocytes in different ages of last two instars of naïve larvae, in different food regimes (natural and artificial diets) as well as under the challenge of two selected chemical pesticides, emamectin benzoate, cypermethrin and the entomopathogens, a nucleopolyhedrovirus and *Bacillus thuringiensis* infecting *H. talaca*. The activities of three defense enzymes, *viz.* general esterases, cytochrome P450 and glutathione s-transferase, have been determined under the challenge of two chemical pesticides, *viz.* emamectin benzoate and cypermethrin. The humoral defense system has been studied by gel electrophoresis of the cell free haemolymph taken from *Bacillus thuringiensis*-infected larvae of *Hyposidra talaca*.

Results:

Haemocyte count in respect to developmental ages and food regimes: In the life history of *H. talaca* five larval instars were recorded. From the haemolymph of 5^{th} instar larvae, five types of haemocytes *viz.* prohaemocyte (PR), plasmatocyte (PL), granulocyte (GR), spherulocyte (SP) and oenocytoid (OE) have been identified by light microscopic study. Irrespective of diet, total haemocyte count (THC) showed gradual increase along the development of last two instars with transient decrease just after ecdysis and finally declined steeply during the wandering and the pre-pupal stage in the last larval instar. However, the artificial diet (AD)-reared larvae showed higher haemocyte count in comparison to the larvae of the same age group, reared on natural diet. The proportion of the major haemocyte types, PLs, GRs and SPs showed fluctuation along the larval development with higher proportion of PLs and GRs (only immunoreactive haemocytes) in the haemolymph of the AD-reared larvae.

Haemocyte count in respect to the exposure of chemical pesticides and entomopathogens: A general reduction in THC was observed in the larvae exposed to sub-lethal concentrations of pesticides and entomopathogens used in this study; only a transient initial increase was recorded after 24 hrs exposure to

NPV. The differential count of haemocytes (DHC) showed an increase in the proportions of the PLs with a corresponding decrease of GRs in response to the treatment of the chemical pesticides and NPV, however, an opposite trend was recorded for GRs in *Bt*-infected larvae at 24 and 48 hrs post-infection.

Effects of the chemical pesticides on the activities of detoxifying enzymes: The activities of general esterases (GEs), cytochrome P450 (CYPs) and glutathione S-transferase (GST) in the larvae of *H. Talaca* were recorded to be changed in response to the treatment with the sub-lethal concentrations of emamectin benzoate and cypermethrin. GE activity decreased in comparison to control when exposed to pesticides, whereas the CYP activity increased after 24 hrs of exposure of pesticides but decreased only after long time exposure (72 hrs) which is just opposite to the effect of pesticides observed in GST.

Electrophoretic analysis of haemolymph proteins/peptides in relation to the microbial exposure: The SDS-PAGE analysis of cell free haemolymph of *H. talaca* larvae infected with sub-lethal concentration of *Bt*-based biopesticides revealed different proteins of varying molecular weights. The relative abundance of these proteins was found to vary in the infected larvae compared to the control. Hypoproteinemia of 65-77 kDa proteins (presumed to be storage proteins), after 24 hrs of post-infection and hyperproteinemia of 30 kDa protein (presumed to be 30K protein) and 18 kDa protein (presumed to be apolipophorin III) after 48 and 72 hrs of post-infection along with a general over expression of some low molecular weight proteins/peptides (11-13 kDa) have been detected in the infected larvae.

Conclusion: The results of this study showed the induction of cellular, humoral and metabolic defense of *Hyposidra talaca* larvae in response to chemical pesticides, emamectin benzoate and cypermethrin as well as entomopathogens, nucleopolyhedrovirus and *Bacillus thuringiensis*.