INTRODUCTION
1. Introduction

Agriculture is responsible for providing food and livelihood to 6 billion of human population everyday and is expected to increase to 9 billion within 50 years (UN forecast). It is estimated that one third of world’s agricultural production is lost to pests, pathogens and weeds. Insects are considered to be one of the major agricultural pests as they consume a large share of food and fibre, both during both pre and post harvest, which is destined for human use.

Among the insect pests, the order Lepidoptera represents a diverse and important group of agricultural pests. Lepidoptera is represented by more than 160,000 species and is the most biodiverse group of animals after Coleoptera. Among Lepidopterans, world’s major crop pests belong to the Heliothines and include *Helicoverpa armigera* in Asia, Australia, Southern Europe, Africa and the Pacific and *Helicoverpa zea* and *Heliothis virescens* in the Americas. *Spodoptera litura* in Asia and *Spodoptera frugiperda* in the Americas are also responsible for major agricultural losses. The larval stages of these pests infest the crops and feed on the foliage and the fruits, considerably reducing the crop yield. These pests infect maize, sorghum, soybean, cotton, sunflower, pulses and many other horticultural crops. They are characterized by their high polyphagy, high mobility and high fecundity. These traits contribute to their status as major agricultural pests.

The control of agricultural pest populations is achieved mainly by the application of chemical insecticides that represent a global cost of $12 billion yearly. However, the long term use of chemical insecticides results in threats to human health and the environment. Biological control of pests is an attractive alternative to the use of hazardous chemicals. Entomopathogenic bacteria such as *Bacillus thuringiensis*, fungi such as *Metarhizium anisopliae* and baculoviruses like nucleopolyhedrosis viruses are being used as biocontrol agents in integrated pest management programmes. However, resistance towards these pathogens is commonly observed and efforts are being directed towards increasing the insecticidal property of these pathogens.
Chitinases, enzymes that hydrolyze chitin, have been reported to increase the insecticidal activity of many of these entomopathogens. A synergistic effect between *Bacillus thuringiensis* insecticidal crystal proteins and chitinase is well established (Regev et al, 1996). Chitinases are also involved in the fungal and viral pathogenesis of insects. A recombinant baculovirus expressing an insect chitinase gene exhibited increased toxicity towards *Spodoptera frugiperda* larvae as compared to the wild type virus. In addition, chitinases by themselves also have insecticidal and antifungal properties. Chitinase from *Manduca sexta* has been demonstrated to be toxic to the mercant grain beetle, *Oryzaephilus mercator* (Wang et al, 1996).

Physiologically, chitinases in insects hydrolyze chitin, which is found as a major component of the insect cuticle and also occurs in the midgut as a constituent of the peritrophic membrane. Insects undergo repeated cycles of moltings and metamorphose completely to attain the adult stage. These moltings are brought about by the action of chitinases and N-acetylglucosaminidases on the cuticular chitin. Chitinase expression therefore coincides with the molting process, and is developmentally regulated in insects. Chitinase expression is under hormonal control and is induced by the molting hormone, ecdysone and repressed by the juvenile hormone, fenoxycarb (Kramer et al, 1993). It is believed that interfering with the tightly regulated chitin metabolism by introducing exogenous chitinases may destabilize the insect physiology. Therefore, chitin synthesis and hydrolysis are considered to be potential targets for pest management. It is believed that insect chitinases are more potent towards insects as compared to chitinases from other sources (Kramer and Muthukrishnan, 1997). No insecticidal activity was observed when a bacterial and a plant chitinase were fed to first instar larvae of *O. mercator* (Ding et al, 1998).

A detailed understanding of chitin metabolism in target pests is necessary for the development of chitinase-based insecticides. Chitinases have been studied in lepidopteran insects like *Manduca sexta* (Kramer et al, 1993), *Bombyx mori* and the genes encoding chitinases have been cloned from *Manduca sexta* (Kramer et al, 1993), *Bombyx mori*, *Hyphantria cunea* (Kim et al, 1998) and *Choristoneura*
fumiferana (Zheng et al, 2002). The genomic structure of chitinase genes has been described in M. sexta (Choi et al, 1997) and B. mori (Abdel-Banat and Koga, 2001). The chitinase cDNA from M. sexta and C. fumiferana have been expressed in insect cells and the recombinant chitinases have been biochemically characterized. M. sexta chitinase was also introduced in tobacco plants and the resulting transgenic plants blocked the development of the tobacco hornworm, H. virescens (Ding et al, 1998). M. sexta chitinase also enhanced the insecticidal activity of a recombinant AcMNPV against S. frugiperda larvae (Gopalakrishnan et al, 1995).

The present work was undertaken in two polyphagous pests, H. armigera and S. litura with the following objectives:

1) To clone and characterize the chitinase genes from the polyphagous pests, H. armigera and S. litura.
2) To study the genomic organization of the chitinase genes.
3) To study the tissue specific developmental expression of the chitinase transcript and chitinase protein.
4) To express chitinase in E. coli and insect cells and to characterize the recombinant protein.