INTRODUCTION
"Struggle is the father of all things. It is not by the principles of humanity that man lives or is able to preserve himself above the animal world, but solely by means of the most brutal struggle. If you do not fight, life will never be won."

-Adolf Hitler

INTRODUCTION

Crop pests have been a major threat to the crop productivity and to the economy of any country. The main brunt of crop loss has been faced by the farmers (1). The continuous fear of crop loss and virtually no profit in farming, has forced farmers to migrate and explore other options of earning a living. The situation is so grim that on one hand we have huge population to feed and on the other fewer people are opting for farming profession. The demand of time is that we should have high crop productivity with our existing and limited resources with a minimal crop loss. Chemical insecticides are being used efficiently to avoid crop losses but over the last few decades, chemical insecticides are more a cause of concern than solution. Development of resistance in the pests against the used chemicals, non-specific targeting of insects affecting the beneficial insects, accumulation of chemicals in various trophic levels of our food web (bio-magnification) and contamination of environment by polluting the ground water are some of major problems created by the overuse of insecticides. The present need is to find an effective biological solution as alternative to chemical insecticides that would target the pest specifically and would be biodegradable. The Cry toxins from Bacillus species have marginally solved the problem and are used as a sprayable insecticide. Over the years, it is learnt that application of cry toxins as sprayable insecticides has certain limitation like; poor shelf life, heat instability and irrational use may lead to resistance development in pests. These limitations made researchers to look for novel biological molecules and alternate usage of deploying the proteins.
Introduction

Introduction of crystal toxin encoding, *cry* genes and their expression in plants has been an effective method of pest management with considerably less risk. These transgenic plants are resistant to the pest and have been effective in its field application. The *cry* proteins can be expressed in targeted tissue by using tissues specific promoters which make them applicable to a range of crops, fruits and vegetables. Globally, products from transgenic crops expressing these toxins have been increasing steadily (2).

In the last few years, there have been reports of resistance development against Cry toxin expressing transgenic plants in different pests like; Black diamond moths and *Helicoverpa armigera* (Cotton Ball Worm) from the different continents (3, 4). These reports project a scenario that in a similar situations the transgenic crops can be ineffective to combat the pest threat in coming years. So new strategies like, expression of multiple of *cry* toxins in one plant and crop rotation etc. are adopted to avoid the problem of resistance development in the field against these Cry toxins. This has directed our attention toward identifying novel molecule which can be used as a substitute or in combination with Cry toxins.

Alternative source of insecticidal factors have been explored in different systems; bacteria nematode association, viruses like *Autographa californica*, Nuclear Polyhedrosis Virus (NPV) (5) and Baculovirus (6), fungi like *Beauveria sphaericus* (7, 8, 9), and bacteria like *Bacillus sphaericus* (10, 11, 12), *Photorhabdus luminescence* and *Xenorhabdus nematophila* (13, 14) are some of the example documented in the literature. *X. nematophila*, is a gram-negative bacterium that resides as a symbiont in the gut of a soil nematode of the genus *steinernema* (15, 16, 17). The bacteria-nematode association is highly toxic to many insect species as bacterium secretes a number of proteins in the insect hemocoel to kill the prey. The bacterium has a complex life cycle, encompassing symbiotic and pathogenic stages. The symbiotic phase is spent in the nematode gut while pathogenicity is manifested in the insect larval body. The bacterium is released in insect hemocoel (15), or gut (18), where it produces a variety of effector molecules including toxic proteins to kill the prey. The larval carcass provides a nutrient rich environment for growth and development of both the nematode and the bacteria. *X. nematophila* produces outer membrane vesicle (OMV) during growth in the broth culture (19), showing oral
toxicity to neonatal larvae of Helicoverpa armigera (20). The proteins associated with the OMVs provided a pool of potential insecticidal molecules for investigation.

In this study, we have identified a ~60 kDa protein in the OMV as GroEL homolog (XnGroEL) secreted by X. nematophila. The GroEL protein belongs to a highly conserved family of molecular chaperones which facilitate folding of nascent non-native proteins (21). The GroEL protein exist in two heptameric rings of identical subunits which are stacked back to back and make a large double ring complex of ~800 kDa enclosing a central cavity (22). It uses a 10 kDa co-chaperone GroES, Mg^{2+} and ATP to carry out the chaperoning activity (23, 24).

Though the primary function of GroEL protein is to carry out chaperoning activity in the cytosol but several pathogenic bacteria strongly express the protein on the cell surface and secrete it in the culture medium under stressful conditions (25). The GroEL protein is expressed constitutively at a high level in endo-symbiotic bacteria compared to free living bacteria. It has been reported to protect the endosymbiont against accumulation of deleterious mutation and preserve fitness of the species (26). GroEL from Enterobacter acrogenus was shown to be paralytic to cockroaches when injected in hemocoel (27). GroEL of various bacterial pathogens are shown to activate both innate and acquired immunity of the host, producing T-cell and antibody mediated response. GroEL protein of Mycobacterium tuberculosis, Chlamydia and E. coli all induce production of pro-inflammatory cytokines by monocytes (28).

In this work, we focused on ~60 kDa GroEL homolog of X. nematophila (XnGroEL) which was secreted in outer membrane enclosed vesicles (OMVs). The present study deals with identification, isolation and characterization of an insecticidal GroEL of X. nematophila.