The insect *Helicoverpa* commonly known as cotton bollworm is an important polyphagous agricultural pest and has a worldwide distribution including Africa, Asia and some countries of Europe (Anonymous 2006). In India, the cotton bollworm complex comprises of the old-world bollworm, *Helicoverpa armigera* (Hübner) also called as American bollworm; spotted bollworm, *Earias vittella* (Fabricius); spiny bollworm, *E. insulana* (Boisduval) and the pink bollworm, *Pectinophora gossypiella* (Saunders). Among them, *H. armigera* is the most damaging and difficult to control bollworm pest. India accounts for approximately 25% of world's total cotton area and 13-16% of global cotton production and this lower production is mainly due to disease and pest attack (Anonymous 2006). Apart from cotton, the host species for *H. armigera* come from a broad spectrum of families and include important agricultural crops such as tomato, maize, chickpea, pigeonpea, sorghum, sunflower, soyabean and groundnut (Fitt 1989). The average crop losses in India due to this polyphagous pest are estimated to be US $350 million annually (Lammers 2007). Therefore it becomes important to study every aspect linked to this insect in order to understand it fully, so that a better control methodology can be devised against it in future. It is envisaged in the present investigation to study the insect by analyzing its gut bacterial diversity and to find out if any of the detected bacteria contributes towards the functional abilities of *Helicoverpa armigera*. The literature pertaining to the objectives enlisted is presented here.

1.1. **Taxonomy and Biology of *Helicoverpa armigera***

The taxonomic classification of this insect is as follows:

**Kingdom:** Animalia  
**Phylum:** Arthropoda  
**Class:** Insecta  
**Order:** Lepidoptera  
**Family:** Noctuidae  
**Genus:** Helicoverpa  
**Species:** armigera (Hübner, 1805)
Newly laid eggs of *H. armigera* are white and before hatching, their color turns yellowish to brownish. At optimal temperature, the larvae can hatch after less than three days. Young caterpillars are pale green, but the later instars can be of varied color (yellowish-green to dark brown) and markings (Rao and Abraham, 1956, Gopalan and Venugopal, 1972). The *H. armigera* larvae can grow up to 40 mm (1.57 inch) long in size (Uthamasamy *et al.*, 1988). Data on morphological characters of larvae, pupae and adults collected in different months have been well reported by Tripathy and Singh (1999). The larvae then pass through four or sometimes five instars over a period of three to four weeks. The caterpillars are voracious feeders, occasionally carnivorous on other smaller insects and, sometimes even cannibalistic. They feed on leaves, flower buds and flowers, developing pods, fruits and seeds. During the final instar the larvae crawl into the soil to pupate, usually not far from the base of the plant where they complete their development. These larvae dig a tunnel which is several centimeters deep and undergo pupation or 'chrysalis' stage. At this stage they undergo metamorphosis and finally emerge as adults. During summer pupal maturation takes about 16 days. However, as the temperature goes down and winter approaches, the pupae enter diapause and spend the winter as pupae (Tripathy and Singh, 1999). Although these pupae can overwinter if necessary, they cannot resist severe frost. Under favorable conditions, the life cycle can be completed in little more than a month, resulting in numerous generations per season especially in warmer areas. In the warmer areas like tropics, where the temperature shifts in winter are not very low, the reproduction continues throughout the year, as the pupae do not undergo diapause stage. The adult insects are good fliers and are mostly active at night (nocturnal). The wingspan of an adult moth is 30-45 mm. In females, the forewings are brownish or reddish-brown while in males they are dull greenish to yellow or light brown. Hind wings are usually pale with a broad, dark outer margin and consist of a pale patch near the centre of this dark region. Adult moths emerge from just after dark to midnight and crawl onto a plant or vertical substrate where their wings dry (King *et al.*, 1994). These moths feed on nectar and approximately 4 days after emergence, females release sex pheromones and mating occurs (Pearson 1958). Fecundity of *H. armigera* is as high as 3000 eggs (Shanower and Romeis
Eggs are laid in the dark, either singly, or in clusters, distributed on all parts of the plants, including developing flowers and fruits and sometimes on stems and growing points. The *H. armigera* females tend to lay eggs on the top third of the healthy plants and on vigorously growing terminals. Thus the cycle of egg laying, larval development and adult emergence continues.

### 1.1.1. Host plants and Distribution:

The host range of the insect, *Helicoverpa armigera* is very broad. It damages about 60 cultivated plant species and at least 67 other plant species in 39 families across Africa, Asia and Australasia (Reed and Pawar 1982). The most important crop hosts, of which *H. armigera* is a major pest, are tomato, cotton, pigeon pea, chickpea, sorghum and cowpea. Other hosts include *Dianthus*, *Rosa*, *Pelargonium*, *Chrysanthemum*, groundnut, okra, peas, field beans, soybeans, lucerne, *Phaseolus* spp., other leguminosae, tobacco, potatoes, maize, flax, a number of fruits (Prunus, Citrus), forest trees and a range of vegetable crops (Multani and Sohi, 2002; Chandra and Rai, 1974). Pearson (1958) and Roome (1975) observed that though *H. armigera* was polyphagous, the preference for a particular host depended heavily on the temporal and spatial availability of hosts at the feeding stage of development. Other host factors include plant species, plant height and plant physiological state (Zalucki et al., 1986; Firempong & Zalucki, 1990; Fitt, 1991; Jallow & Zalucki, 1996). The host selection behavior in the field can be inferred from observed patterns of egg distribution within and among host plant species. An aggressive oviposition response of the *H. armigera* was observed towards chickpea plants (Sequiera et al., 2001). In the past, studies have also been performed using laboratory reared insects wherein moths from six different sources were given a choice of host plants (Firempong & Zalucki, 1990b). It was found that a difference of choice existed between the larvae. The plants offered were ranked into the following categories depending on the preference of the larvae: most preferred-tobacco, maize, sunflower; least preferred-cabbage, pigweed and linseed; intermediate-soybean, cotton and lucerne. *Helicoverpa armigera* prefers particular host plants and appears to follow a hierarchy in food choice when a preferred host is unavailable (Gu et al., 2001; Jallow...
and Matsumara 2001). *H. armigera* can spread very rapidly with plants and plant products through trade from infested areas to non infested areas (Anonymous 2006). In nature, this species originates from tropical and subtropical regions, but it can migrate over long distances into areas with temperate climates each summer. The species is distributed in most of Asia, Australia, Africa and southern Mediterranean region, including 29 cotton producing countries such as India, China, Pakistan and Egypt (Anonymous 2005). Studies using mitochondrial DNA sequence data support the single species status of *H. armigera* across Africa, Asia and Australia (Behere et al., 2007). The currently reported global distribution of *H. armigera* suggests that the pest may be most closely associated with deserts and xeric shrublands; Mediterranean scrubs; temperate broadleaf and mixed forests; tropical and subtropical grasslands, savannas, and shrublands; and tropical and subtropical moist broadleaf (Venette et al., 2003). In the agriculturally diverse ecosystem like India, the adaptive advantage for better survival helps this insect species to thrive on diverse host plants and thereby emerging prevalent throughout the country. The natural spread of *H. armigera* in the past has been slow, but it may speed up in future as an effect of global warming (Lammers 2007).

### 1.1.2. Mode of Damage

This polyphagous pest prefers the reproductive and vegetative tissues (Zalucki et al., 1986) of agricultural and horticultural crops. Its preference for flowering and fruiting parts of high-value crops, results in a high socio-economic cost to agriculture in tropics. The first-instar *H. armigera* consume food at five times the rate of third-instar larvae (Johnson & Zalucki 2007). On whole plants, *H. armigera* will initially start feeding on tender leaves, but eventually their movements on plants will primarily take them to the reproductive organs (Liu et al., 2004). The neonates clear a patch of hair by grazing before they begin to establish a feeding site on the leaf surface (Johnson and Zalucki 2005). In chickpea and a few other legumes the larvae initially feed on the foliage (young leaves) but in cotton, pigeon pea, etc. they feed mostly on flowers and flower buds. The young seed pods in chickpea are destroyed completely when the larvae bore into the pods and consume the developing peas inside the pod. This is
prevalent particularly under tropical climates in southern India (Nagaraja et al., 1956). In cotton, the bored holes are visible at the base of flower buds giving them a hollowed out appearance. Bracteoles become spread out and curled downwards. Larger larvae bore into maturing green bolls and these young bolls fall due to excess larval damage. Leaves and shoots of the plant are also consumed by larvae. When feeding on vegetative part of plants like soybean, the *H. armigera* larvae may completely consume small pods, but do not totally remove fully-expanded pods (Rowden and Rowden 1987). Apart from eating out the developing seeds and leaving the empty pod on the plant, these larvae consume leaf veins, petioles, apical growing points and axillary buds, and in doing so compromise the plant's ability to compensate for the damage. The flower buds and flowers bored by small larvae may drop. In case of fruits, the *H. armigera* completes its larval development inside it (Torres Vila et al., 2000). The larvae enter fruit at the stem end when it is between 0.75 to 2 inches in diameter at early stages. During development, larvae may emerge from one fruit and enter another to continue feeding. Due to their feeding a messy, watery, internal cavity filled with cast skins and feces is formed. This damaged fruit ripens prematurely. In plants producing noxious secondary metabolites, larvae reduce toxin exposure, undertake large inter-meal bouts of resting for toxin processing, and/or due to the rapid onset of the effects of the toxins adapt by feeding on it in short bouts; (Bernays et al., 1994). Adult *H. armigera* females lay eggs on the flowering and fruiting structures of most crops, thereby making them easily accessible to larva which feed voraciously leading to substantial economic loss (Reed & Pawar, 1982). The innate ability of oviposting females to locate and utilize a wide range of hosts from a number of families is one of the major factors contributing to the pest status of this moth (Zalucki et al., 1986; Fitt, 1989).

### 1.2. History of economic impact

As discussed earlier, the preference of *H. armigera* towards the harvestable flowering parts of high-value crops results in both high economic cost, and a high socio-economic impact in subsistence agriculture. Severe crop losses have been reported due to severity of *H. armigera* attack but cost of controlling this pest sometimes
exceeds value of crop production (Mathews, 1997). Monetary losses result from cost of monitoring and control, particularly cost of insecticides, and direct reduction of yield. In countries like India and China, 50% of all insecticides used are directed to control this pest (Anonymous 2006). Farmers spend up to 40% of their annual income to buy chemicals to curb *H. armigera*. In Australia, the total losses by this pest were estimated as $A 23.5 million (Wilson 1982). The increase in the prices of insecticides and the replacement of the cheaper pyrethroids with more expensive alternatives to counter pyrethroid resistance also increased the cost to millions of farmers on annual basis (Karim 2000).

In Côte d'Ivoire, Africa, between 1978 and 1983, cotton crop loss was ca 60%, primarily due to *H. armigera* (Moyal, 1988). In Zimbabwe, the potential cotton losses due to *H. armigera* were 1175 kg/ha while in Tanzania the economic loss of cotton was estimated at around $US 20 million (Reed and Pawar, 1982). In Thailand, *H. armigera* has been the principal cotton pest since the mid-1960s and the losses due to this pest was at least 31% in 1975-79 (Mabbett et al., 1980). In China, cotton yield losses due to *H. armigera* larvae increased with plant age. The damage threshold, 7.5 kg/ha, was reached at 35 egg clusters/100 plants. Introduction of integrated pest management reduced *H. armigera* infestations from 1.6 to 0.1% in Jiangsu between 1976 and 1982 (Liu et al., 2004).

The Indian Council of Agricultural Research (ICAR) has identified *H. armigera* as the single most damaging insect on cotton and legumes in Indian subcontinent and the major biotic constraint to increased crop production. In India, where *H. armigera* commonly destroys over half the yield of pulse crops, pigeon pea and chickpea, losses were estimated at over $US 300 million per annum (Reed and Pawar, 1982), however by late 1980s losses of both pulses and cotton went up to as high as $US 500 million, where an additional $US 127 million spent on insecticides to treat these two crops annually (Dhingra et al., 1988). However these figures will certainly need to be revised after the rapid increase of pyrethroid resistance and reduced effectiveness of other insecticide groups in *H. armigera*. The problems in *H. armigera* control in India were first encountered in Andhra Pradesh in the year 1987. The average yield of
cotton fell by up to 61% from 436 kg/ha in 1986/87 to 186 kg/ha in 1987/88 in spite of the application of more than 30 rounds of insecticides (Armes *et al.*, 1992). For potatoes, crop losses of 10-100% have been estimated in India. The resulting loss was 2.1%, based on the average yield for India of 15.8 t/ha for potatoes, (Parihar and Singh, 1988). Another important crop of India; chickpea is grown on 7.3 million hectares in various agro-climatic conditions. Although the yield potential of this crop is 2.5-3 t/ha, but the average yield is only ca 0.8 t/ha. The extent of losses caused by *H. armigera* differs between various regions and depends upon the climate and crop intensity of that region. A total monetary loss of Rs 203cr is estimated annually due to the reduced yield (Yadava *et al.*, 1991). Changes in sowing date have had a considerable influence on pod damage and seed yield of chickpea. Pod damage due to *H. armigera* increased with delay in sowing dates. It was found that the co-efficients of correlation between sowing date and pod damage and between pod damage and seed yield were significant (Srinivasan *et al.*, 1998). The population of *H. armigera* larva varied between dense spacing (33 plants/m²) and wide spacing (3 plants/m²) (Yadava *et al.*, 1998). The application of nuclear polyhedrosis virus reduced larval populations by 26.8% and pod damage by 36.6% and increased yields by 72% compared with untreated plots (Cowgill and Bhagwat 1996). And in spite of the control measures employed, the production still is not adequate to meet the domestic demand. As a result, India has to spend over Rs. 1,000cr on pulse imports every year. It has been estimated that the annual loss of different crops caused by *Helicoverpa armigera* is approximately US$5 billion worldwide (Sharma *et al.*, 2007).

1.3. **Factors influencing *H. armigera* pest status**

The pest status of this species is derived from wide range of physiological, ethological and ecological characteristics namely, polyphagy, high mobility and migration, high fecundity and facultative diapause. These enable them to survive in unstable habitats and adapt to seasonal changes (Fitt, 1989). Other important biological factors contributing to *H. armigera* pest status are its short development time, wherein the
insect completes development in a short span of 30 days, and its large body size, due to which the food consumption/plant damage rate is high (Karim 2000).

1.3.1. Polyphagy

The host plant type influences the development, reproduction, survival and life table constraints in the insects feeding on them (Tsai and Wang, 2001; Kim and Lee, 2002; Li et al., 2004). For establishment of pest population on a crop plant, it is important that the plant aids in the growth and development of the polyphagous insect. The plant emits physical and volatile signals and attracts the insect to its surface where the food substrate for consumption is available, thus helping in development and survival in the larval stages and egg production of subsequent adults (Singh and Mullick, 1997). Therefore a shorter developmental time and greater total reproduction of insects on a host plant indicate greater suitability of that plant (Van Lenteren and Noldus, 1990).

When the larvae emerge out of the eggs, their activities are limited to finding food (crawling and head waving that is associated with searching for and locating a suitable site to feed), feeding, resting and dropping off. The resting phase could be linked to the avoidance of predation or parasitism, by staying still, as well as digestion of food (Johnson and Zaluki 2007). In phytophagous insects having wide host range, the time spent in foraging is linked to the time spent in vigilance. These insects may take longer time to make decisions during feeding and have shorter feeding bout length because they have potentially greater ranges of cues to evaluate (like predation), or have divided attention between alternative foods (Bernays et al., 1994).

The foraging behavior of the larvae and adults of *H. armigera* have been extensively studied in many aspects. Zalucki et al., (1986) has described this species as having a ‘mixed’ feeding strategy and that the feeding and foraging behavior of this insect was found to vary at different larval instars (Johnson and Zalucki, 2007). Host plant nutrition plays a very important role during selection of the host by the insect. The *H. armigera* adults feeding on nectar however have different foraging strategies and host preference. Moths that experience feeding on a particular host species as larvae, preferred to forage for nectar on that host species (Cunningham et al., 1998). The host selection behavior of *H. armigera* is subject to considerable variation which arises...
through both non-genetic and genetic factors. Very recently, one particular study has linked the polyphagy of *H. armigera* to the highly developed, enzyme regulation mechanism within its gut (Sarate *et al.*, 2012). Other important factors such as surface attributes of the host plant, host age and host abundance also determine the feeding behavior in this polyphagous insect (Shelomi *et al.*, 2010, Cunningham *et al.*, 1999, Johnson and Zalucki 2005).

### 1.3.2. Migration and Mobility

The migration of an insect is one of a behaviorally distinct form of movement that serves as an adaptation to its temporary habitats and is usually promoted by a series of behavioral and physiological traits (Rochester 1999). Interaction between the environment and the species migration syndrome influences the pattern and role of migration in a particular population. Relative distribution of the migratory moth species of *H. armigera* varies not only between crops and regions, but also on a temporal scale (Cherry *et al.*, 2003). This is because the insect adopts spatial redistribution by migration in order to cope with the seasonality of its habitat, and it is due to its dispersive and migrational attributes, the incidence of this species is unpredictable (Karim 2000). The evidence supporting migration in *Helicoverpa* spp. was well reviewed by Farrow & Daly in the year 1987. In the year 1999 Rochester described the development and application of conceptual models of the migration systems of *H. armigera* in Australia. Recent studies have identified characteristic patterns of migratory flight that are largely associated with vertical profiles of temperature and wind speed (Westbrook 2008). Local movement within crops and between nearby alternative crops and other wild hosts form another aspect of *H. armigera*’s mobility. Hence, when eggs are laid away from the host plant or on parts of the host plant unsuitable for larval establishment, the movement to locate a feeding site becomes critical. Long distance travel in neonate caterpillars is commonly accomplished by ballooning, a procedure in which the larva lowers itself on a strand of silk to be carried away by the wind (Anonymous 1990) In addition to ballooning, the neonates move within a host plant by lowering themselves on silk threads. The migration ability of this pest allows it to quickly disperse across a broad geographic
range, exploiting various host plants. This results in increased gene flow and may lead to the spread of resistance of these pests to insecticidal compounds in pesticides and transgenic crops (Pietrantonio et al., 2007).

1.3.3. Fecundity
A chief factor responsible for the pest status of *H. armigera* is its high fecundity, which is dependent on the temperature, humidity and nutrient availability (Nadganda and Pitre 1983). The fecundity increases with short generation time resulting in an impetus for population outbreaks. Many investigations have been performed in order to study the reasons associated with the high fecundity of this noctuid plant pest. Life and fecundity values are important for examining and understanding the impact of an external factor on the insect (Bellows et al., 1992). Under laboratory conditions, Liu et al., (2004) and Jallow et al., (2001) determined the effect of different host plants and of the artificial diet on the fecundity of *H. armigera* females. The preferred ovipositional site for this insect on tomato, maize, okra and pepper was on leaves, and on eggplant, flowers and fruits were more preferred. When given a choice of different soyabean varieties, the female moths preferred to lay eggs on only a certain varieties than the rest. The effect of egg load on the host selection behavior of *H. armigera* has been studied under laboratory conditions (Jallow and Zalucki, 1998). It was shown that increased egg load in females led to a greater propensity to oviposit on both cowpea and maize. The distribution of oviposition with age of mated females peaked shortly after mating and declined steadily thereafter until death. Adult feeding directly affects the fecundity of female moths and enhances the fitness of individual cotton bollworm offspring (Song et al., 2007). Another study linked the effects of multiple mating on the fecundity and longevity of female moths. A positive relationship between the number of spermatophores received by a female and fecundity, and a negative relationship between the number of spermatophores and longevity was established (Hou et al., 1999). Effect of temperatures on the fecundity revealed that the females reared under all alternating temperature regimes laid more eggs than those reared at any constant temperature treatment (Mironidis et al., 2008). The females of this species readily oviposit on flowering non-host plants in comparison to host plants.
that are not in the flowering stage, thereby adding onto the damage resulting from their high fecundity (Firempong and Zalucki 1990).

1.3.4. Facultative Diapause

In many insects diapause is a fixed trait but in others the diapause is facultative as it allows them to attain development when the conditions are favorable or to enter diapause when they are not. Facultative diapause has a profound effect on the ecology and evolution of the species. In *Helicoverpa armigera* facultative diapause is reached at the pupal stage primarily in response to decreasing photoperiod and temperature (Wilson *et al.*, 1979; Mironidis *et al.*, 2010), which provides cues of impending winter conditions unfavorable for reproduction. High temperatures can also induce diapause in *H. armigera* (Liu *et al.*, 2006). It enters a true summer diapause when the larvae are exposed to very high temperatures (43°C for 8 hours daily), although the proportion of females entering diapause is nearly half compared to that of males. A recent study also shows that the varying nutritional level of host plants affect the diapause induction, in *H. armigera* larva, independent of abiotic factors such as temperature and photoperiod (Liu *et al.*, 2010). Additionally the study also shows that in order to reach diapause, individuals must attain sufficient weight. Therefore diapause explains the seasonal occurrence of *H. armigera* in many areas by maintaining local population during periods when hosts are not available or when the conditions are not conducive to development, reproduction and to initiate additional generations.

1.4. Insecticide resistance in *Helicoverpa armigera*

Among various methods of management of *H. armigera*, the use of insecticides is prevalent in farming communities. However, the indiscriminate use of insecticides has subjected this pest to heavy selection pressure. Resistance towards major group of insecticides such as cyclodienes, organochlorines, organophosphates, synthetic pyrethroids and carbamates (Gunning *et al.*, 1989, Daly *et al.*, 1986, Gunning *et al.*,...
1992) has been documented in Australia, Asia and to a lesser extent in Africa (McCaffery 1998).

In India, insecticide resistance was first reported in 1987 in the cotton growing fields of Andhra Pradesh (Reddy 1988). Other areas in India include Maharashtra, Tamil Nadu, Uttar Pradesh, Gujarat, and Punjab where the resistance varies between low, moderate and high (Kranthi 2005). Due to this, frequent outbreaks of *H. armigera* were witnessed in the cotton growing areas of India. The suicide of several farmers 1992-1997, due to crop failure in many states of the South Indian cotton ecosystem, particularly Andhra Pradesh and Karnataka, has been traced to insecticide resistance in *H. armigera* (Kranthi *et al.*, 1997).

### 1.4.1. Resistance to different classes of pesticides

The continuous application of pesticide in the past has resulted in outbreak of resistant *H. armigera* individuals in unsprayed fields. Resistance to pyrethroids first appeared in Australia in the year 1983 (Gunning and Easton 1989), in New Zealand from 1992 (Cameron *et al.*, 1995), in Thailand in 1985 (Ahmad and McCaffery 1988), in Indonesia in the early 1988 (McCaffery *et al.*, 1991) and in China around 1989 (Wu *et al.*, 1997). Other affected countries included Russia, Pakistan, Turkey, Israel and Africa (McCaffery 1998). In the Indian subcontinent, pyrethroid resistance in *H. armigera* was found to be ubiquitous. Here, the resistance to pyrethroids was found to be frequently accompanied by resistance to endosulfan, to OPs such as quinalphos and monocrotophos, and to the oxime carbamate methomyl (Armes *et al.*, 1992, 1996).

A range of moderate to low resistance has been recorded in *H. armigera* towards cyclodyne group of chemicals all over the world. The levels of resistance to endosulfan in Australia were moderate. In later years Armes *et al.* (1996) suggested that incipient resistance to endosulfan was present in this species in India, Nepal and Pakistan. *H. armigera* individuals collected from Indonesia in 1987 and 1988 showed medium resistance (McCaffery *et al.*, 1991).
Among carbamates, thiodicarb and methomyl were the most widely used chemicals against *H. armigera* in Australia. Budding resistance to thiodicarb resulted in cross resistance to methomyl (Gunning *et al.*, 1996) in fields of New South Wales in 1993. From then on, the carbamate resistance increased to moderate levels. *H. armigera* from Pakistan displayed low-level of resistance to thiodicarb (Ahmad *et al.*, 1995). In China, significant resistance to methomyl was recorded in strains of *H. armigera* from Shandong province (Wu *et al.*, 1997), while in India the resistance to methomyl was substantial in populations from Andhra Pradesh (Armes *et al.*, 1992, 1996), with lower levels being more typical of other locations including Gujarat and Maharashtra. *H. armigera* resistance to organophosphates is widely monitored and it has been found that the comparative resistance levels appear to vary among chemicals as well as in different locations. Low resistance to chlorpyriphos has been documented in various parts of the world including India (Patel *et al.*, 2000), Pakistan (Ahmad *et al.*, 1995) and Egypt (Abdallah *et al.*, 1982). Significant resistance to monocrotophos has been widely reported in China, India and Pakistan (Pan *et al.*, 1997, Kranthi *et al.*, 2001, Ahmad *et al.*, 1999). In India, resistance to acephate was found to be very low. Tolerance to methamidophos was recorded in China and Pakistan (Pan *et al.*, 1997, Ahmad *et al.*, 1999). In 1990-91 low levels of tolerance to quinalphos was recorded in India at certain regions of Andhra Pradesh and Tamil Nadu. Moderate level of resistance to this chemical was observed later in fields of Maharashtra, India (Nimbalkar *et al.*, 2009). The variation in the resistance of these insecticides observed in different regions could be a result of the way farmers use these chemicals in pest management. When used extensively and continuously, these chemicals result in resistance and thus resurgence of pest.

### 1.4.2. Factors responsible for Resistance

The most important spot of action of pyrethroids is the voltage-gated sodium channel of nerve cells (Bloomquist 1996). The kinetics of the sodium channel is altered which leads to prolonged sodium currents and accounts for the delay in depolarization after potential. This action causes the continuous firing of neurons which is typically found in pyrethroid-poisoned insects. Pyrethroids also cause membrane depolarization due
to the prolonged opening of sodium channels which results in massive release of neurotransmitter, leading to severe disturbance in synaptic transmission. Indirect evidence of target site resistance (Armes et al., 1996) and direct evidence of nerve insensitivity (West and McCaffery 1992) have been attributed to pyrethroid resistance in *H. armigera*. Metabolic mechanisms involve systems of enzymic detoxication: oxidation by the microsomal P450-dependent monooxygenases (or mixed-function oxidases) and hydrolysis by esterases. Initially, three mechanisms of resistance were thought to function in *H. armigera* insecticide metabolism; strong nerve insensitivity (super-*kdr*) and penetration resistance (*Pen*), together with a third factor PBO-synergizable monooxygenase (*Pbo*) (Gunning et al. 1991). But together they conferred only low order resistance when compared to P450-mediated metabolic resistance (Forrester et al., 1993). Similar facts were shown for increased monooxygenase activity being a factor of resistance towards pyrethroids in *H. armigera* in India (Kranthi et al., 1997) and China (Wu et al., 1997a). Kennaugh et al., (1993) demonstrated reduced penetration as an important mechanism of esfenvalerate resistance in this insect. Another important study by Gunning et al., (1996) suggested that in pyrethroid-resistant *H. armigera* of Australia, an enhanced esterase activity is seen which is due to the increased production of enzymes. Cyclodiene compounds act as GABA antagonists and because of their down regulation of the inhibitory transmitter action of GABA, postsynaptic neuronal activity is augmented. Target-site insensitivity to cyclodiene action has been inferred in resistant insects (Kanga and Plapp 1995). The organophosphate and carbamate chemicals prevent the breakdown of acetylcholine, by keeping a check on inhibition of its activity. This causes increase in the retention time of acetylcholine in the synapse leading to continuous stimulation of the postsynaptic neuron and thus neuronal hyperactivity. Target site resistance involving decreased sensitivity to acetylcholinesterase to inhibition is seen in resistant individuals (Chaturvedi 2007). Other metabolic mechanisms like higher esterase and monooxygenase activity are important mechanisms of resistance to carbamates (Goh et al., 1995). The metabolic resistance to organophosphate insecticides in heliothine insects is due to enhanced activity of number of detoxification methods (Martin et al., 2003). Very often,
resistance to these insecticides has been associated to elevated esterase activity (Gunning et al., 1996). Glutathione S-transferases have also been frequently linked with organophosphate resistance and thought to be accountable for metabolism of these compounds (Whitten and Bull 1978).

So far, the increased level of esterase enzyme is a uniformly observed phenomenon to be present in all the resistant insects. The exact mechanism by which the increase occurs is still a question that is hanging in the air. Therefore, with the ever increasing use of diverse insecticides and propensity of this pest to develop resistance easily, it becomes important for us to delve deeper to look for more promising answers.

After giving a brief overview about the physiology, ecology and the ethology of the plant pest Helicoverpa armigera, this review will now proceed by highlighting the various roles of gut bacteria within an insect, since this study will be aiming to link these two aspects with each other.

1.5. Intestinal bacteria and their contributions towards an insect host’s biology

Bacteria associated with insects are usually of a wide host range. Within an insect gut, the microbial consortium is so rich that it outnumbers the total number of cells in the insect body (O’Hara et al., 2006). In return for the warm, protected and nutrient rich habitat, these intestinal residents contribute towards various aspects of the host’s functional abilities. They help the host by aiding in food digestion, producing essential vitamins for the host and keeping out potentially harmful microbes.

1.5.1. Dietary contribution

The nutritional contributions of gut bacteria include: enhanced survival on suboptimal diets, improved digestion efficiency, acquisition of digestive enzymes, and provision of vitamins. Insects like Bemisia tabaci that feed upon unbalanced diets that are poor in essential nutrients such as amino acids, sterols or vitamins, are
compensated in nutrition by their endosymbiotic bacteria (Douglas, 2006). In other insects like crickets and cockroaches the digestion of food by gut associated bacteria has been demonstrated (Santo Domingo et al., 1998). Bacteria also contribute to fatty acid metabolism like *Wigglesworthia* of tsetse flies and *Blochmannia* of ants (Gil et al., 2003). Micro-organisms are also involved in the production of detoxification enzymes that degrade a variety of compounds such as plant allelochemicals, mycotoxins, insecticides and herbicides (Bhatt 1998). In termite gut microbial degradation of plant aromatic compounds can occur and this contributes to the carbon and energy requirement of the host (Brune et al., 1995).

1.5.2. **Contribution to pathogen resistance**

Symbiotic microbes protect their hosts from invading pathogenic micro-organisms. Evidences for symbiont protection have been shown in diverse arthropods including shrimp, lobster, aphids and mosquitoes. In *Drosophila melanogaster*, the gut bacterial natives delayed the colonization of the bacterial pathogen *Serratia marcescens* and increased the lifespan of the host (Nehme et al., 2007). In addition to protecting their hosts from other microorganisms, these bacteria also protect their hosts from parasitic invertebrates. Research in mosquitoes suggests that the natural gut bacterial flora activates the immune response against *Plasmodium* (Warr et al., 2007). Finally, studies have also suggested that symbionts can produce toxic compounds and thereby protect their hosts from predators. In *Paederus* beetle larvae, the gut bacteria aid in production of toxins known as ‘pederins’ that protects the hosts from wolf spider attack (Kellner & Dettner 1996).

1.5.3. **Other important contributions**

Indigenous bacteria are also involved in modulating the expression of genes involved in several important structural functions including mucosal barrier fortification and tightening of tight junctions (O’Hara et al., 2006). Immune system development by gut microbes via the induction of IgA is seen in higher vertebrates. Functional relationships along the length of the mammalian gastrointestinal tract were defined by
analyzing expression profiles of large numbers of genes, showing that anatomically defined regions could be distinguished on the basis of gene expression profiles (Bates et al., 2002).

From above studies it can be concluded that even though much work has been done on the agricultural pest *H. armigera* and field of insect gut bacteria separately, no study has to date tried to explore the possibility of gut bacterial involvement in the biology of this insect. Therefore the functional role of gut bacteria of *H. armigera* in imparting resistance to its host has been dealt in Section A of this thesis.