The major group of animal kingdom was dominated by insects, and it accounts around 75% of total animal population. Despite their small size, the sheer numbers or biomass of insects means that they have a significant impact on the environment and therefore upon our lives. Herbivores insects are the major threat in the continuous supply of food and fibers for human conception. Synthetic insecticides play a major role in insect pest control, with chemical, environmental and toxicological properties having been improved considerably over the last six decades. The world market for insecticides is still dominated by compounds irreversibly inhibiting acetylcholinesterases like organophosphates and carbamates. The insecticides acting on the voltage gated sodium channel like the pyrethroids, account for approximately 70% of the world market (Smagghe, 2007). These agents can exhibit considerable toxicity towards higher organisms and therefore, their non-selective mode of action may cause devastating environmental problems. The extensive use of such compounds has caused the development of resistance towards many pests. In order to control the pests with high resistance levels lead to the fact that effective concentrations must exceed the legally recommended concentrations, thus making the compounds useless.

A need to search for novel insecticides with a better efficacy or a new mode of action becomes more obvious and involves a race against time. An intense search for alternatives less harmful to the environment has been initiated in laboratories around the world. Since then, there is a steady progression towards the development of narrow
spectrum insecticides that act on insect specific targets. Insecticide that specifically interferes with insect metabolic pathways and endocrine mechanisms has been developed from the considerable knowledge on insect physiology and endocrinology. Agrochemical research over the last 30 years has resulted in the discovery of chemically novel insecticides that mimic the action of insect hormones. This concept of interfering with the insect hormones as a selective mechanism to control pest insects was introduced in 1967 by Carrol Williams as “third generation pesticides”, the insect growth regulator (IGR).

An IGRs may be defined in terms of its mechanism of action, as a substance which acts within an insect to accelerate or inhibit a physiological regulatory process essential to the normal development of the insect or its progeny, where the action of the substance is necessarily dependent on the life stage of the insect (Siddall, 1976). Insect growth regulators impair insect endocrine regulation of moulting and metamorphosis processes, such as juvenile hormone analogues (JHAs), ecdysteroid receptor agonists or moulting accelerating compounds (MACs), and chitin synthesis inhibitors (e.g., benzoylephyl urea, BPU). Additionally there are newer insecticides that act specifically on insect nerve, energy metabolism and muscle targets and the insect midgut structures (Dhadialla et al., 2005).

Insect hormones, especially ecdysteroids, are growth promoting at low concentrations and causes differentiation at higher (moulting inducing) levels (Smagghe, 2007). The chitin synthesis inhibitory benzoylephylurea (BPU) insecticides such as diflubenzuron and flucycloxuron prevent the molting process by inhibiting chitin synthesis, thereby causing abnormal endocuticular deposition and abortive molting (Dhadialla et al., 2005 and Ishaaya, 1990). In addition, the chitin synthesis
inhibitors (CSIs) like diflubenzuron and related compounds affect reproduction in several insect orders, primarily by causing a reduction in egg hatch (Soltani et al., 1987). Besides these chemistries, substituted dibenzoylhydrazine derivatives have been discovered in the past decade to act as non-steroidal agonists of the insect molting hormone 20-hydroxyecdysone, which later become a new class of IGRs (Dhandialla et al., 1998; Dhandialla et al., 2005).

Williams (1968) stated that the chemicals that mimic insect hormone actions or interact with their receptors can provide new possibilities to discover new insecticide activities and to overcome resistance problems with neurotoxins. Tebufenozide was the first commercialized example of such a highly target selective insecticide acting as a mimic of 20E in caterpillars, and currently, methoxyfenozide and halofenozide are two new structural analogues of tebufenozide both with a specific activity spectrum (Le et al., 1996; Dhandialla et al., 1998; Carlson, 2000). Methoxyfenozide is the most efficacious member of the dibenzoylhydrazine insecticides to reach the marketplace against Lepidoptera; halofenozide is found to possess a high toxicity against Coleoptera (Carlson, 2001). These first nonsteroidal ecdysone agonists reported to induce precocious and incomplete molting in several insect orders, particularly in Lepidoptera.

Methoxyfenozide [N-tert-butyl-N'-(3-methoxy-o-toluoyl)-3, 5-xylohydrazide; RH-2485] is the newest diacylhydrazine insecticide to reach the marketplace. It binds with very high affinity to the ecdysone receptor complex (EcR:USP) in lepidopteran insects, where it functions as a potent agonist, or mimic, of the insect molting hormone, 20-hydroxyecdysone (20E). Methoxyfenozide exhibits high insecticidal efficacy against a wide range of important caterpillar pests, including many members of the family Pyralidae, Pieridae, Tortricidae and Noctuidae. It is most effective when
ingested by the target caterpillar, but it also has some topical and ovicidal properties. It is modestly root systemic, but not significantly leaf-systemic. Evidence collected indicates that methoxyfenozide has an excellent margin of safety to non-target organisms, including a wide range of non-target and beneficial insects (Carlson et al., 2001).

The growth and development from one stage to another is regulated by two main hormones, the steroidal insect molting hormone, 20-hydroxyecdysone and the sesquiterpenoid (Juvenile hormone). While molting to accommodate growth is regulated by 20E, the development from an egg to a larva, a pupa and an adult is regulated by the timing and titers of juvenile hormone (Riddiford, 1996). In the adult stage, both these hormones, being pleiotropic, change their roles to regulating reproductive processes (Wyatt and Davey, 1996). These IGRs mimic the biological activity of the natural molting hormone (Wing et al., 1988; Retnakaran et al., 1995; Dhadialla et al., 2005).

Metamorphosis is one of the most fascinating phenomena in biology. During metamorphosis in holometabolous insects, reprogramming of cell fate takes place during the last instar and rearrangement of cells, tissues, and organs also take place (Beetz et al., 2008). During this process of metamorphosis, the morphological appearance of the individual insect changes dramatically. Growth and moulting in insects is initiated by a hormone produced in the neurosecretary cells of the brain (Wigglesworth, 1972). It was also reported by Wigglesworth (1955) that mucopolysaccharide secreted by the haemocytes is involved in the formation of basement membranes and connective tissue during metamorphosis. Insect’s blood or haemolymph circulates round the body cavity between the various organs, bathing them
directly. The haemolymph in general, serves the function of both blood and lymph of vertebrates. Thus the fluid fraction (plasma) is the transport system for nutrition, hormones and metabolic wastes and contains elements of the immune system while the cellular components are haemocytes (Gillot, 1995).

There is an inherent variability of haemocytes within a species as well as among closely related species (Arnold, 1974; Gupta, 1979). The number of cells present in the circulating blood of various adult species varied from 1000 per cubic mm to about 100 times of this number (Tauber and Yeager, 1936). The haemocytes present in the insect blood cells, change in number and morphology accompanying metamorphosis (Nittono, 1960; Chain and Anderson, 1983; Trenczek et al., 1997; Gardiner and Strand, 1999, 2000; Beetz et al., 2004). Several types of haemocytes have been described in insects by their morphological, cytochemical and functional characteristics. The most common types of haemocytes are prohemocytes, plasmatocytes, granulocytes, spherulocytes, adipocytes and oenocytoids. Their characteristics slightly differ in various insect species (Gupta, 1979; Ribeiro and Brehelin, 2006).

Marked changes in haemogram during the developmental stages of animals were reported in different insects. Arnold (1952) observed that in *Ephestia kuehniella* Zeller the haemocyte count increased during larval life but just prior to pupation it declined. Nittono (1960) reported that in *B.mori* the peak in number of haemocytes occurred prior to moult in the IV larval and pupal stages. Jones and Lin (1961) recorded that in *Rhodnius* prior to the moult the prohaemocytes decreased but adipohaemocytes increased. In *Spilostethus hospes* F. in relation to eclosion, sex and reproductive phases showed a remarkable variation among the same species within the different phase (Sanjayan et al., 1996). All changes in haemocyte counts and properties follow a
distinct time pattern that can be correlated to the precisely controlled insect’s hormone levels especially to ecdysone (Riddiford, 1991).

Differential haemocyte counts by various authors have indicated that some of the haemocytes appear in the haemolymph in greater numbers at certain times and under certain physiological conditions. For example, cystocytes (Wheeler, 1963) and adipohaemocytes (Jones and Lin, 1961) increase in numbers prior to ecdysis; adipohaemocytes are also most numerous in prepupal stages of Prodenia eridania Cram (Yeager, 1945) and in Galleria mellonella (L.) (Shapiro, 1966), spherule cells (Jones, 1956) and oenocytoids (Yeager, 1945) are likewise most numerous prior to pupation in Sarcophaga bullata (Parker) and P.eridenia respectively, and podocytes abruptly increase during the initial stages of puparium formation in Drosophilla melanogaster Meigen (Rizki, 1962). In lepidoptera the oenocytoid disappear during the last larval stages, the granular cells and plasmatocytes were found to be predominant cell type at the end of larval life (Nittono, 1960; Gardiner and Strand, 2000).

Hence the knowledge of normal haemocytes of an insect is necessary to physiologist, toxicologist and biochemist that any alterations in structure, types and number of cells reflect changes in physiological and biochemical processes. Insect haemocytes respond to internal changes during development and to conditions such as starvation, diseases and chemicals including insecticides (Qamar and Jamal, 2009).

Metamorphosis also involves histolysis, histogenesis and differentiation (Argell, 1953). These events are accompanied by physiologically conditioned metabolic events that are associated with the remodeling and disintegration of fat body and other tissues (Archana et al., 2006). A recent report indicated that prevalence of a dynamic biochemical relationship between the disintegrating gut and haemolymph on one hand
and between the haemolymph and fat body on the other (Sivaprasad and Sailaja, 2010). Such events are obviously associated with the turnover of proteins, enzymes and many other biochemical constituents (Seong et al., 2005; Yaginuma and Ushizima, 2005; Sarangi and Anitha, 2007). Most of the haemolymph proteins arise from the fat body at regular intervals during insect metamorphosis (Dean et al., 1985; Keeley, 1985). They accumulate in the fat body in the form of dense granules during pupal stage but sharply decreased during the adult stage (Kim et al., 2005). Storage proteins such as SP-1 and SP-2 are mobilized from haemolymph to fat body of insects during larval pupal transformation in a sex specific manner that differs in their levels in males and females (Tojo et al., 1980). The metamorphic events of histolysis and histogenesis are accompanied by great turnover of proteins and assisted by a number of proteases during larval to pupal and pupal to adult transformations (Bharathi and Sucharitha, 2006; Wang et al., 2008).

Haemolymph proteins play a vital role in transport and metabolism in insects. The synthesis and utilization of haemolymph proteins are controlled by genetic and hormonal factors. It brings tissue degraded proteins to the fat body and also for taking away the newly synthesized or processed proteins to the growing tissues such as those in gonads and other organs (Harliman and Chen, 1974). The trends in protease activity indicate that the silkworm fat body undergoes a dramatic remodeling process that involves an early phase of consolidation and a later phase of disruption with regard to its biochemical composition (Hemalatha et al., 2013).

In insects the principal forms of storage of energy are represented by carbohydrates and lipids and are closely related to the physiological events such as the flight, the moult and the reproduction. This phenomenon takes place however with the
detriment of their structural or functional role since these biomolecules are not synthesised and are not stored with an air of providing energy (Ramdev and Rao, 1986). Proteins are the first biological factors making their manifestation during development. During metamorphosis of an insect, processes like destruction of certain larval tissue and rejuvenation and remoulting of various tissues into adult take place. They are also involved in the synthesis and consumption of the macro molecules as well (Venugopal and Dinesh, 1997). The fat body tissue plays a key role in storage proteins increased during successive stages of development (Kanost et al., 1990; Rajathi et al., 2010).

One of the important functions of fat body tissue is synthesis and storage of proteins. These proteins are secreted in large amounts by fat body cells during the final instar stage and selectively sequestered during pupal stages that are also found to be incorporated later into various organ including ovaries (Vallae, 1993; Vanishree et al., 1999). Any change occurring in the body of the test animal will be reflected in the haemolymph protein. The major haemolymph proteins are synthesized by the larval fat body and play an important role as reservoirs of amino acids utilized during pupal development of B. mori (Tojo et al., 1980; Levenbook, 1985). These storage proteins are highly sensitive to nutrition, hormones, and temperature (Janarthanan et al., 1998). It is known that stress induces many biochemical changes in the hemolymph of insects (Salama et al., 1999; Tripathi and Singh, 2000).

Quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as maintenance, growth and reproduction, and this balance is disturbed by any toxic product (Canavoso et al., 2001). Lipid turnover in insects is regulated by
neuroendocrine controlled feedback loops (Downer, 1985). Although the first site of action of JH in particular and IGRs in general is the endocrine system, many biochemical and physiological changes have been reported to occur in different metabolism pathways (Leonardi et al., 2001; Kim et al., 2002; Etebari et al., 2007).

Protein degradation is essential physiological process and in insects, considerable effort has been made by many authors to elucidate which types of peptidolytic enzymes are involved in this process. Moreover there are also other reasons for characterization of these enzymes in insects. Larval peptidolytic enzymes and their inhibitors are now involved in the development of new strategies and approaches for enhancing of resistance of agricultural crops and forest trees to insect herbivores (Terra and Ferriera, 1994). Protein digesting enzymes may also be involved in activation and degradation of some insecticidal proteins of bacterial origin (de Maagd et al., 2001) and are presumed to participate in major part as events joined with the use of larvae in clinical practice (Sherman, 2002; Blahovoc et al., 2005).

The IGRs were found to influence the biochemicals in insects. The biochemical effect of IGRs on the carbohydrate (Bouaziz et al., 2011), glucose, protein (Zibaee et al., 2008; Rharrabe et al., 2009), lipids (Hamadah et al., 2012), amino acids (Assar et al., 2012), phosphatase, transaminase and phenol oxidase enzymes on insects was studied by several authors (Kishori and Vrushali, 2011). Some of the insecticidal effects have been found to influence the enzyme activity in insects (Abdel-Hafez et al., 1988, Martinez and van Emden, 1999; Mamatha et al., 2008). The influence of IGRs on biochemicals were experimented in various animals like silkworm (Etebari et al., 2007), Periplanata americana (L.) (Michitsch and Steele 2008), Plodia interpunctella Hubner (Rharrabe et al., 2008; Ghasemi et al., 2010) Rhynchophorus ferrugineus (Olivier) (Ghomeim et al., 2003).
One of the strategies for controlling pest insects involve the use of naturally occurring substances that enter the insect by penetrating the cuticle of the insect or by accompanying the food ingested by the insect. The complexity of the digestive system has often deterred scientists from considering the food stream as a way to deliver a substance to control an insect (Habibi et al., 2008). Digestive system is an important organ in insects especially for voracious feeders like worms, and it is the organ of extreme importance in ecotoxicological studies of insects which is the gut. The digestive system of insects does functions like absorption, secretion, role in nutrient storage and in fluid and ion transport (Rocha et al., 2010).

The alimentary canal in larvae is a straight tube divided into foregut, midgut, and hindgut (Lucarotti, 2011). The histology of digestive system of midgut is divided into three main regions: anterior, middle and posterior midgut (Dimitriadis, 1991). The midgut is the largest portion of the digestive tract with three distinct regions: the proximal, medial and distal. Its wall is formed by pseudostratified columnar epithelial tissue having four cell types as columnar, goblet, regenerative, and endocrine cells of Anticarsia gemmatalis Hubner (Levy, 2004). The midgut of insects comprises the longest and functionally most important part of the digestive tract, dealing primarily with the digestion of food stuffs and the absorption of nutrients (Ranjini and Mohamed, 1999) and the most important site for secretory granules accumulation and secretion (Dimitriadis, 1991).

The anterior midgut composed of thick epithelial cells surrounding a large lumen, well developed microvilli occurs at the basal region of the epithelium (Zhang et al., 2012). One of the characteristics of many insects is the presence of the peritrophic membrane in the midgut which has a fundamental role of protection of the
midgut. It works in protecting this epithelium from mechanical and chemical damages against toxins, harmful chemicals (Terra, 2001) and protect from invasive pathogens that are ingested with food (Levy, 2004).

The environment of insect plays an important role in determining the quality and quantity of internal microbiota. The micro organisms in the digestive tract are generally bacteria or bacteria like organisms and occasionally yeasts. The anatomy of the insects may also govern the types and quantities of microorganisms (Tannada, 1993). Microbes were found to play an important role in nutrition digestion (Bignell and Eggleton, 1995), growth and development, host defense, insecticide degradation and antagonism to entomopathogens (Ramesh et al., 2009).

This intact architecture of the gut is found to be affected by various agents like synthetic organic insecticides (Toppozeda et al., 1968; Rizvi and Khan, 1973), bacterial toxins (Kinsinger and McGaughey, 1979), plant extracts (Emara and Sser, 2001; Rawi et al., 2011) and IGRs (Ghoneim et al., 2008; Rharrabe et al., 2009). The ecdysone agonist tebufenozide exerted several histopathological effects on the midgut of last instar hoppers (Ghoneim et al., 2008). 20-hydroxyecdysone on the midgut on larvae of *P. interpunctella* caused disorganization and vacuolization of the cytoplasm, disorganization of plasma membrane and cellular organelles (Rharrabe et al., 2009). IGRs reduced digestive enzyme activity and caused imbalance in enzyme substrate complex and inhibition of peristaltic movement of the gut (Hori, 1969) might have inhibited the enzyme activity in the treated insects.

Silk worm *Bombyx mori* is not only one of the most economically important insect, but also a best characterized model for lepidopteran insects. It would be used as a reference for functional studies of other lepidopteran insects. The silk worm *B. mori*
has been used in silk production for more than 5000 years. There are millions of farms raising silk worm in countries such as China, India, and Thailand. So in addition to its commercial value, it is also a useful model system for pest control (Nagaraju and Goldsmith, 2002).

The vast amount of literature reviewed has revealed comparatively less work done on IGR methoxyfenozide in relation to the beneficial insect *Bombyx mori* L. The different sources used to study the effect of IGR are

- The V instar larva of *Bombyx mori* was selected so that maximum development and peaked metabolic changes will take place during this stage.

- The IGR Methoxyfenozide (RH-2485) have been classified by the US Environmental Agency (EPA) as reduced risk pesticides because of their low acute and chronic mammalian toxicity, low avian toxicity, and their safety to most beneficial arthropods and compatibility with integrated pest management programs (Dhadialla *et al*., 2005).

- Haemocytes were readily obtained and are the primary target of cellular defense system of insects. They are biochemically sensitive to changes (Pandi and Tiwari, 2012).

- Any change occurring in the body of the test animal will be reflected in the haemolymph protein (Janarthanan *et al*., 1998).

- Larval fat body plays an important role as proteins that are synthesized and released into haemolymph; it acts as a reservoirs of amino acids utilized during pupal development (Tojo *et al*., 1980; Levenbook, 1985).
Silk gland is an important organ in silkworm not only responsible for silk production but synthesis of certain protein which involve in protecting functions (Bradfield, 1946; 1950).

Mid gut is a very important tissue and relevant for pest control and pathology (Roel, 2010)

The objective of this study was to find the effect of methoxyfenozide on V instar larva of *Bombyx Mori* (L) on various parameters like

- Cellular and numerical analysis of haemocytes.
- Haemolymph, fat body, silk gland and gut tissues biochemistry.
- Histological analysis of mid gut and gut microflora.