CHAPTER-4

4.0 CLASSIFICATION OF INSECTICIDES ON THE BASIS OF THEIR MODE OF ACTION

Insecticides are the chemicals used to kill insects. They are classified into several groups depending upon their mode of action. The target site of the attachment for insecticides molecule is distinct for most of the groups. However, some insecticide groups may share common target site in the target insect species. Normally, insecticides work by disrupting the normal physiology of the insects either through neural or hormonal anomalies. In this chapter, we will discuss in detail both the normal functioning of the physiology of insect and modification in physiology after treatment with insecticides.

4.1 Acetylcholine Esterase Inhibitors

4.1.1 Background

Organophosphate and carbamate insecticides disrupt the nerve impulse transmission in the central nervous system of insects. Neuron is the basic functional unit of the nervous system of insects (Oland & Tolbert, 2003). The point of contact between two neurons or one neuron and the effector (muscle fiber or gland) is known as synapse (Toni et al., 2007). During the impulse transmission, the release of the neurotransmitter starts from the pre synaptic membrane as the impulse reaches at the termination point of the nerve fiber (axon) (Katz, 1959). Meanwhile, there is another belief that arrival of neural current at the end of axon triggers the flow of calcium ions into the pre synaptic membrane that ultimately leads to the release of neurotransmitter (Callec et al., 1971). Although, various neurotransmitters are involved in synaptic transmission, but we will discuss only the acetylcholine, being the major neurotransmitter involved in the synaptic transmission in insects (Bloomquist, 2009). After release, the acetylcholine passes on through synaptic cleft and binds to the highly specified receptor site on the post synaptic membrane known as acetylcholine receptor site (Barbara et al., 2008). The acetylcholine binding leads to depolarization of membrane and impulse is transmitted to the post synaptic cells which ultimately ends in contraction of muscle or release of secretion from gland (Nauen et al., 2001). The synapse containing acetylcholine as a neurotransmitter is regarded as a cholinergic synapse (Gu & O'Dowd, 2006). After the transmission of impulse, acetylcholinesterase binds to the acetylcholine and breaks it into the acetic acid and choline (Lunt, 1975). Acetylcholinesterase is the only enzyme present in cholinergic synapse in the insects (Toutant, 1989; Fournier, 2005). It is normally present in the synapse and has two active sites including the esteric site and anionic site. The binding and hydrolysis of the acetylcholine can be divided into three steps (Soreq & Seidman, 2001; Tougu, 2001).

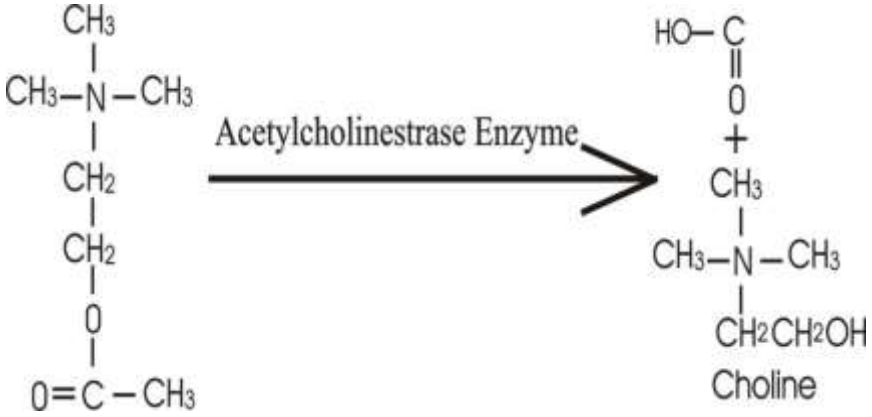

Figure 4.1 Diagrammatic representation of the acetylcholine binding and catalysis by the acetylcholinesterase.

In first step, positively charged nitrogen and electrophilic carbon of the acetylcholine binds to the negatively charged anionic site and serine hydroxyl group of the esteric site of the acetylcholinesterase (Nachmansohn & Wilson, 2009). In the second step, acylation of the acetylcholinesterase occurs. Acylation is a process in which hydrogen atom of the hydroxyl group is transferred to the choline moiety of the acetylcholine and choline is released (Sant'Anna et al., 2006). In third step, acetic acid and the active enzyme is produced by the hydrolysis of the acetylcholinesterase (Shi et al., 2004). After de-acylation, the enzyme become fully functional and is ready to process another acetylcholine.

4.1.2 Mode of action

There are two major groups of conventional insecticides i. e, organophosphates and carbamates that acts upon by inhibiting the normal activity of the acetylcholinesterase (Silman & Sussman, 2005). These insecticides interact with the acetylcholinesterase by forming the irreversible and reversible bonds for organophosphates and carbamates, respectively (Colovic et al., 2013). In case of organophosphates, acetylcholinesterase is phosphorylated resulting in inability of this enzyme to hydrolyze the acetylcholine (Simon, 2014). The process of dephosphorylation is very slow and may takes from days to weeks (Figure 2). Due to long-term inability of acetylcholinesterase, the acetylcholine is not hydrolyzed and remains attached to the postsynaptic receptor site leading to the excessive neuro-excitation (Bloomquist, 2009). The hyper excitability results in the restlessness, tremors, convulsion and paralysis. The carbamylated acetylcholinesterase has relatively weaker bond with the carbamates and decarbamylation occurs rapidly as compared with organophosphates (Figure 3) (Darvesh et al., 2008). The symptoms of intoxication with the carbamates are similar to the organophosphates.

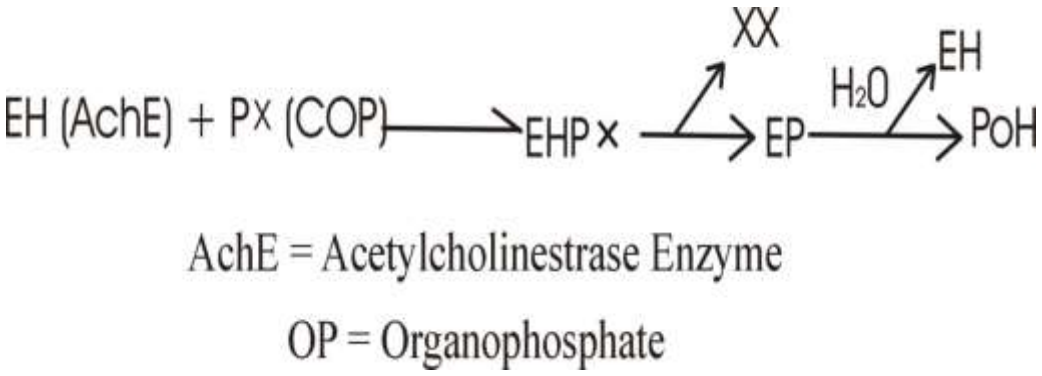

Figure 4.2 Binding of the Organophosphates to the acetylcholinesterase.

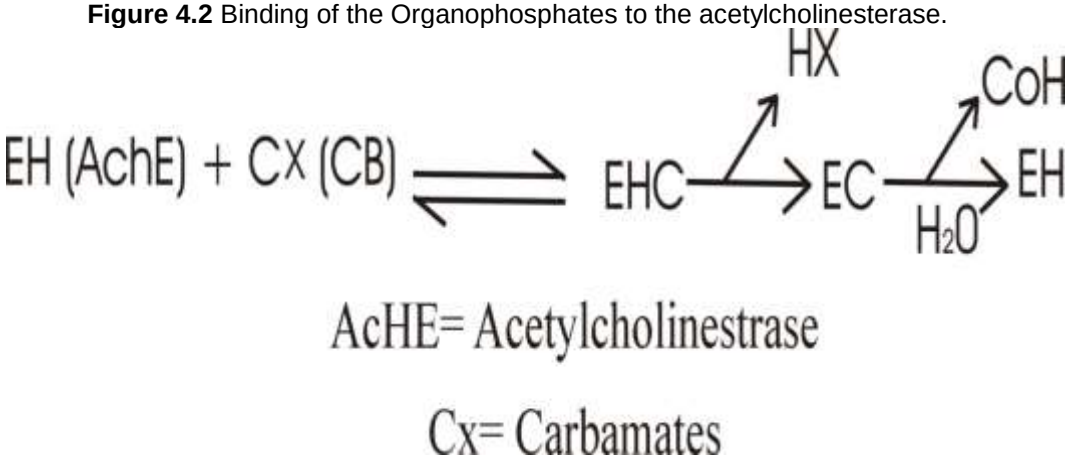

Figure 4.3 Binding of the Carbamates to the acetylcholinesterase

4.2 GABA-Gated Chloride Channel Antagonists

4.2.1 Backgrounds

GABA (Gama aminobutyric acid) is a neuro transmitter that is released from the presynaptic membrane as the action potential arrives at the terminal portion of the presynaptic membrane (Barbara et al., 2005). The binding of the GABA to the post synaptic receptor site (with an intrinsic provision of the chloride channels) results in the opening of the chloride channels and ultimately flow of the chloride ions. Resultantly, post synaptic membrane is hyperpolarized with an increased concentration of the anions and a resting potential is resumed to reduce the neural excitability.

GABA receptors are the trans-membrane proteins found both in the central and peripheral nervous system of the insects. Although, two types of GABA receptor including the metabotropic GABA receptors and ionoporic GABA receptors are linked with G-protein-coupled receptors and the chloride channels, respectively. But the ionoporic GABA receptors are the main target of the insecticides in insects. The ionoporic GABA receptors are the ligand gated ion channels (requires a chemical messenger like GABA to be attached for the channel opening) that belongs to a family of Cys-loop receptors. The Cys-loop find its name due to the presence of a disulfide bond between two cysteine residues that results in the formation of characteristic loop. However, ions channel in the ionoporic GABA is formed by the decentralization of the five protein subunits around a central pore. The GABA molecules bind at the interface between subunits in the extracellular domain. Each subunit of the receptor contains four membrane-spanning alpha helixes (M1, M2, M3, and M4). M2 helix is thought to line the channel pore, and the M3-M4 linker is the intracellular domain that binds the cytoskeleton.

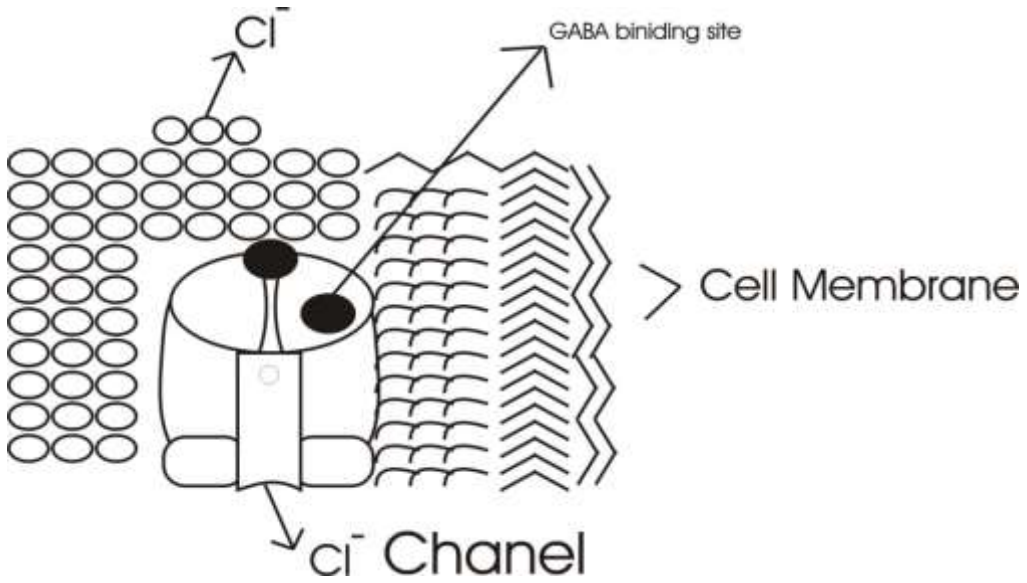

Figure 4.4 GABA-gated chloride channel antagonists

4.2.2 Mode of action

The insecticide groups having such type of mode of action are phenylpyrazoles and avermectins interfere with the GABA receptor to disrupt the normal nerve impulse transmission in the central nervous system (Casida & Quistad, 2004). The phenylpyrazoles (e.g. fipronil) binds to the chloride channels and inhibits the activation of these channels by GABA (Caboni et al., 2003). Blockage of the chloride channels results in reduction in the synaptic inhibition that causes an excessive excitability of the nervous system. A similar mechanism of action is evident in case of lindane and cyclodienes (Ratra et al., 2001).

On the other hand, avermectins (e.g. abamectin and emamectin benzoate) act as GABA agonists and causes the flux of chloride ions similar to GABA but in an irreversible fashion (Stock

et al., 2015). Resultantly, the agonists induced conductance increases due to continual flow of chloride ions in the post synaptic neuron. Finally, this condition results in the loss of sensitivity leading to paralysis in insect. However, avermectins may also cause the muscle paralysis by targeting the glutamate gated chloride channels (Wolstenholme & Rogers, 2005).

4.3 Nicotinic Acetylcholine Receptor Inhibitors

4.3.1 Background

The nicotinic acetylcholine receptors are the ionoporic ligand gated Cys-loop receptors having penta subunits (two *α* and three non *α*) (Colquhoun et al., 2003). They are present on the post synaptic sites sensory and interneurons in the nervous system of insects. The two major types of the nicotinic acetylcholine receptors including the muscle-type and neuronal-type nicotinic receptors are present in the invertebrates (Colquhoun et al., 2003; Dani & Bertrand, 2007). The binding of a chemical messenger, acetylcholine, results in opening of the ion channels and flow of sodium ions causing an action potential at post synaptic site (Tomizawa & Casida, 2001). After nerve impulse transmission, acetylcholinesterase hydrolyzes the neurotransmitter and terminates its synaptic action under normal physiological conditions in insects.

4.3.2 Mode of action

The two insecticides groups having such type of mode of action are neonicotinoids and spinosyns permanently attach to the nicotinic acetylcholine receptor site (Matsuda et al., 2001). The acetylcholinesterase is unable to hydrolyze these acetylcholine receptor agonists (Matsuda et al., 2001). The agonists binding permanently activate the nicotinic acetylcholine receptor that results in non-stop flux of sodium ions leading to generation of excessive action potentials (Colovic et al., 2013). The overstimulation of synapse causes hyper excitation, convulsion, paralysis and death.

4.4 Sodium Channel Modulators/Blockers

4.4.1 Background

Sodium channels are ion channels made up of the integral membrane proteins and are responsible for the conductance of sodium ions through plasma membrane of the cells (Frank & Catterall, 2003). The sodium channels are classified into two major types based on the triggers that binds and opens these channels. First type is known as the voltage sensitive or voltage gated and second one is the ligand gated ion channels that requires binding of a chemical messenger (Goldin, 2002; Sato et al., 2008). The sodium channel has three distinct states including deactivated,

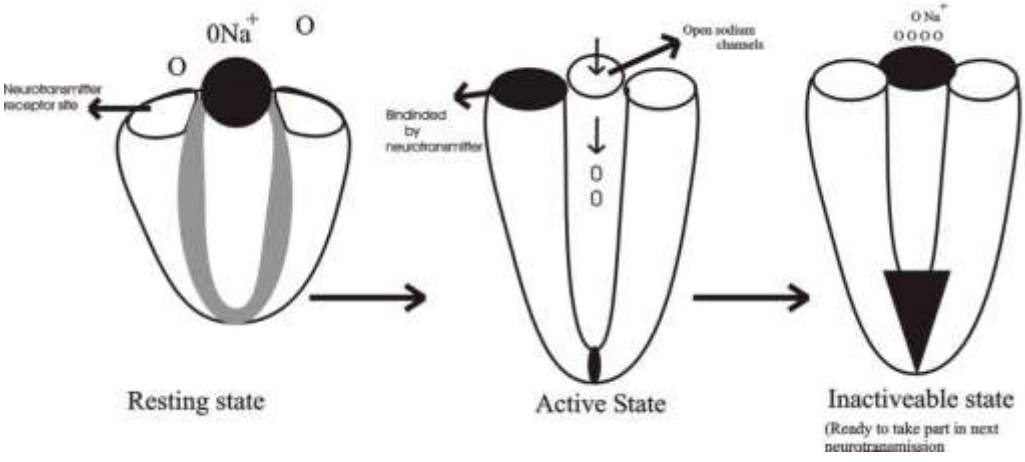

Figure 4.6 Phases of voltage gated sodium ion channel

activated or inactivated based on the conductance of Na^+ (Figure 6) (Greengard, 2001). The sodium channels are in deactivated form in the absence of action potential and axonal membrane is in its normal resting phase (Catterall, 2000). Arrival of an action potential stimulates the opening of sodium channels (activated); thereby allowing the inward flow of sodium ions and voltage across the neuronal membrane is on rise (Frank & Catterall, 2003). At depolarization stage, voltage across the membrane increased to zero which is initially negative. It is also named as the rising phase of the action potential. When the action potential reached to its peak, the inward flow of sodium ions is ceased due to inactivation of the sodium channels. The action potential starts falling due to stoppage in flow of sodium ions and neuron repolarizes and subsequently hyperpolarizes itself. This is also known as falling phase of the action potential. A very low membrane's voltage leads to opening of inactivation gates and closure of activation gates in sodium channels and sodium channels are ready to take part in next action potential (Goldin, 2003; Payandeh et al., 2012).

4.4.3 Mode of action

The toxins interfere with sodium channels by blocking or opening of sodium channels (O'Reilly et al., 2006). Pyrethroids and Organochlorins react with voltage-gated sodium channels on nerves, prolonging the time during which the channels are open (Davies et al., 2007). This results in altered nerve function, which manifests either as a series of short bursts or a prolonged burst, and is caused by repetitive discharge of nerve signals or stimulus-dependent nerve depolarization (Costa et al., 2008). In general, exposure to toxic doses of these compounds causes incoordination, convulsions, and paralysis. On the other hand, indoxacarb permanently blocks the sodium channels and stops the flow of sodium ions leading to hyperpolarization of the neuron and a permanent falling of action potential.

4.5 Lipid Biosynthesis Inhibitors

4.5.1 Background: (Lipid biosynthesis)

Lipids are synthesized by the polymerization of fatty acids (Figure 7) (Boucher, 2007). The synthesis of fatty acids involves synthesis of fatty acids in presence of acetyl-CoA and then fatty acids are esterified to produce triacylglycerol, a process called lipogenesis (Giron & Casas, 2003). Moreover, a three-carbon intermediate, malonyl CoA participates in the biosynthesis of fatty acids that is formed from acetyl-CoA catalysis with acetyl-CoA carboxylase (Prentki et al., 2002).

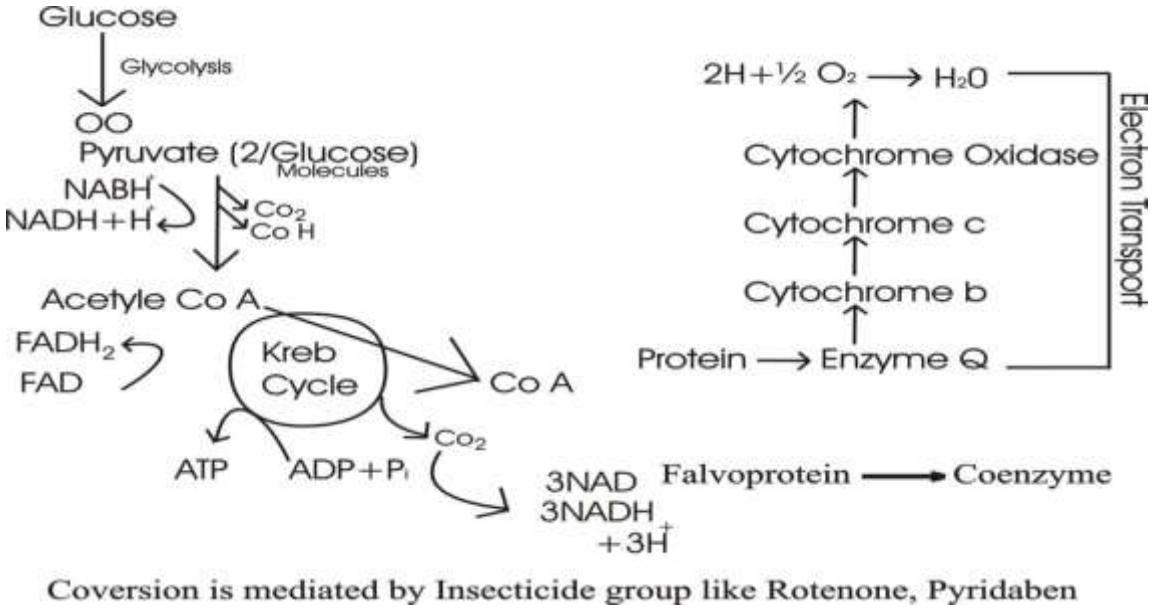

Figure 4.7 Biosynthesis of lipids

4.5.2 Lipid biosynthesis inhibitors

The keto-enols, a group of new chemistry insecticides act as an inhibitor of the acetyl-CoA-carboxylase an enzyme involved in the lipid metabolism (Lieb et al., 2000; Fischer et al., 2002)
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4.6 Insect Growth Regulators

4.6.1 Juvenile hormones mimics

4.6.1.1 Background

A pair of tiny glands located at the post brain position known as the *Corpora allata* is responsible to secret the juvenile hormone (Audsley et al., 2000). The higher concentration of juvenile hormone regulates the larval molts but its lower amount promotes pupation and complete absence leads to the adult formation (Zhou & Riddiford, 2002; Riddiford et al., 2003; Riddiford, 2012).

4.6.1.2 Mode of action

The juvenile hormone analogs (Figure 8) kept on the juvenility in the treated insects by mimicking the function of normal juvenile hormones (Flatt et al., 2005). The presence of higher concentrations of juvenile hormones at or near the pupal stage leads to abnormal larval molts or inhibition of adult emergence (Dubrovsky, 2005). The survivor adults are mostly less fecund and also has reduced life span as compared with the normal insect species (Shah et al., 2015).

4.6.2 Chitin biosynthesis inhibitor

4.6.2.1 Background

Chitin is a naturally abundant polymer which is present in the cuticle, peritrophic metrics and extracellular linings of insects that protect insects' body layers present at the exterior and interior surface (Merzendorfer & Zimoch, 2003). Chitin synthase plays a key role to transfer the sugar moiety at the non-reducing end of the growing sugar chain from UDP-GlcNAc (Merzendorfer, 2006). However, translocation of the chitin is believed to be carried out by the chitin synthase by the involvement of the transmembrane segments present in the C-terminal domain. The hydrogen bonding between the chitin chains leads to formation of the microfibril, a process known as the fibrillogenesis (Mao & Schwarzbauer, 2005). Finally, the association of the chitin with the insect cuticle occurs (Rudall, 2011).

4.6.2.2 Mode of action

The benzoylureas are the group of insecticides that functions as the inhibitors of the chitin biosynthesis (Matsumura, 2010; Merzendorfer, 2013). Although, the complete understanding of the mechanism of action of the chitin synthesis inhibitors is still elusive but two types of speculation are present in the literature (Sun et al., 2015). First, these insecticide bind to the chitin synthase and restricts the catalytic activity, thereby inhibiting the chitin formation. Second, the insecticide molecules disrupt the post catalytic activity of the chitin biosynthesis leading to reduction in chitin synthesis. However, defects in peritrophic metrics of treated insects may result in the enhanced susceptibility of insects towards pathogenic microbes. In addition to this, the malformed cuticle is also evident in the chitin biosynthesis inhibitor applications on insects.

It is noteworthy that cyromazine does not inhibit the chitin biosynthesis but it disrupts the molting by increasing the cuticle stiffness in insects (Bel et al., 2000; Nauen & Bretschneider, 2002). Due to improper sclerotization, the cuticle becomes less extensible and is unable to achieve the normal expansion level (Mun et al., 2015). It is believed to be due to increased interaction among the cuticle component. The cyromazine treatment results in the formation of lesions on the cuticle, impaired growth and death of insects (Kamaruzzaman et al., 2006).

4.6.3 Ecdysone agonists

4.6.3.1 Background

The 20-hydroxyecdysone is a molting hormone that is released as a pulse during each instar. The 20-hydroxyecdysone controls the expression of cascade of genes that leads to the molting in insects. This molting hormone not only controls the expression of up regulatory genes

but also that of the down regulatory genes as it is necessary for cuticle elaboration, sclerotization, and ecdysis (Jindra et al., 2013).

4.6.3.2 Mode of action

Benzoyl hydrazine, insect growth regulator, works as the non-steroidal ecdysone receptor agonist and cause premature larval molting (Retnakaran et al., 2003). The principle target site for benzoyl hydrazine is the steroid insect molting hormone 20-hydroxyecdysone receptor site (Riddiford et al., 2000). Although, these chemicals have no structural resemblance to the 20-hydroxyecdysone but they are capable to attach to the 20-hydroxyecdysone receptor site and triggers the same response as that of natural hormone (Restifo & Wilson, 1998). After agonist binding at receptor site, the expression of up-regulated genes is extended and down-regulatory genes is inhibited (Jindra et al., 2013). In this condition, the larva undergoes apolysis and head capsule slippage and takes on the appearance of the pharate larva. Moreover, these synthetic analogs bind strongly to the receptors and remain in place and repress all the down-regulatory genes such as ones necessary for cuticle elaboration, sclerotization, and ecdysis resulting in a developmental arrest in this state. As a result, the treated larva goes into a precocious incomplete molt that is lethal.

4.6.4 Microbial disruptors of insect midgut membranes

4.6.4.1 Background

In the midgut of insects especially the lepidopteran, two major types of the cells prevails including the columnar cells and goblet cells (Takeda, 2012). It is evident that the concentration of K^+ ions is relatively higher in the midgut lumen as compared to that of the cells or hemolymph (Klein et al., 1996). This increased amount of the cation has pivotal role to carry out numerous cellular processes in the midgut. For example, the higher K^+ concentration helps the amino acid symporter (on the apical membrane of the columnar cells) to transport the amino acids from the lumen of gut to the cells (Sacchi & Wolfersberger, 1996). Due to amino acids and other nutrients transport, the concentration of K^+ rises up in the columnar cells. This extended amount of K^+ ions in the columnar cells is transported potentially through the K^+ channels or flow via intercellular space junctions of the goblet cells. Likewise, the K^+ concentration is uplifted in the hemolymph due to nutrients assimilation. The reduction in the K^+ concentration in the hemolymph is necessary for the normal physiology of insects. In case of lepidopteran, K^+ concentration is reduced by goblet cells. The V-type ATPs is responsible to move protons from the goblet cells cytoplasm to goblet cells cavity. Moreover, the goblet cells' apical membrane contains a K^+/nH^+ antiport protein that facilitates the exchange of the K^+ of the goblet cells with the proton. Additionally, the goblet cells also extrude the K^+ by pumping out it with the carbonates and bicarbonates. The interaction of these ions may result in the formation of the potassium carbonate and bicarbonate that keeps the midgut alkaline. It is obvious that there is an osmotic balance and ion regulation in the insects' gut.

4.6.4.2 Mode of action

Following ingestion of the Bt. molecules, the crystals are solubilized and activated to give rise to the monomeric molecules (δ-endotoxin) (Pigott & Ellar, 2007). The toxin monomer binds to the cadherin receptors on the microvillar membrane of the midgut epithelial cells after proteolytically converted to the small toxin molecules (Figure 10). After binding, toxin molecules cause the pores in the epithelial microvillar membrane, thereby disrupting the ionic regulation and osmotic balance and causing the cells to lyse and swell ultimately destruction of the midgut (Peyronnet et al., 1997). This results in the an extreme rise in the alkalinity of the gut pH up to 9-10.5, this also changes the blood pH from the 6.8 to greater than 8 that causes the generalized paralysis and death of insects. The time course of poisoning of a toxin varies depending upon the insect species. Usually, the feeding is stopped after 1h of toxin ingestion, reduction in normal activities after 2h and paralysis and death may occur after 6h.

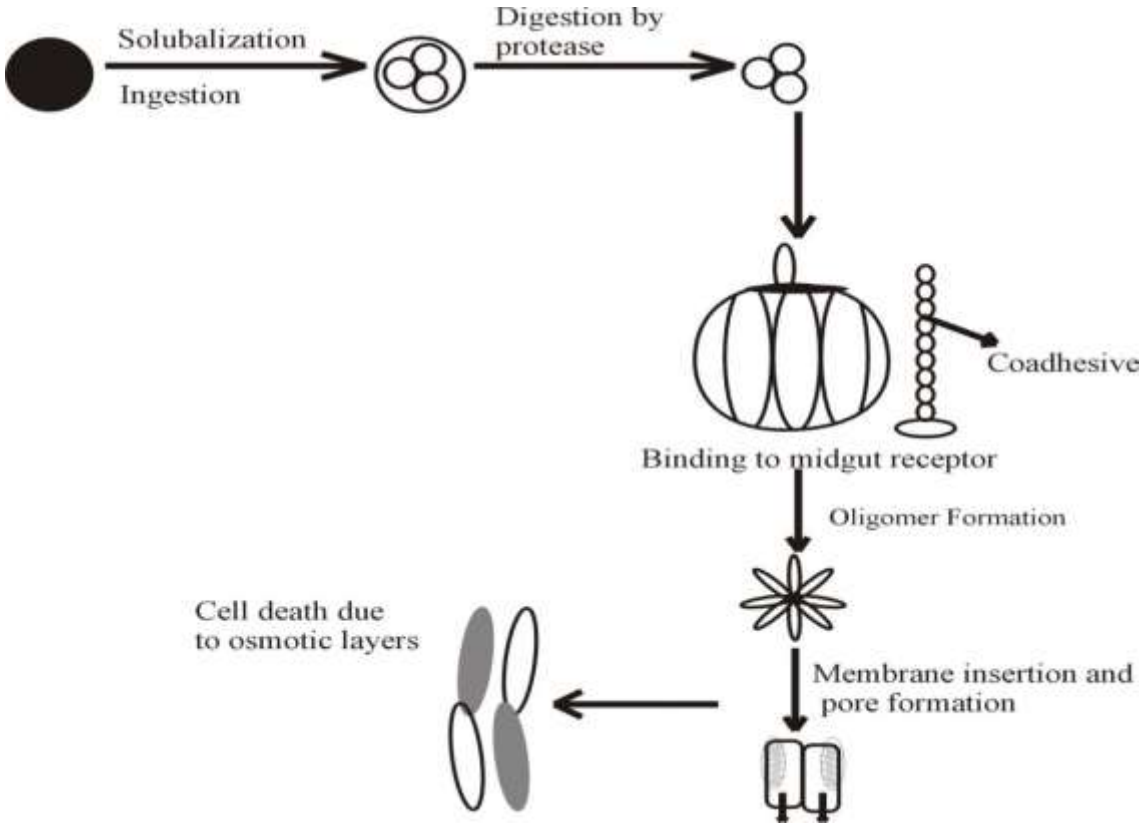

Figure 4.10 Schematic representation of the disruption of midgut microbial insecticides

4.6.5 Inhibitors of oxidative phosphorylation, disruptors of ATP formation

4.6.5.1 Background

Adenosine triphosphate is a primary source of energy synthesized from the organic molecules in the food supply of animals (Jurgens, 2002). The raw food supplements are firstly metabolized to their simpler units in the cytoplasm and then converted to acetyl-coenzyme A in the mitochondria (Focke et al., 2003). A number of metabolic processes including Krebs cycle and electron transport with coupled oxidative phosphorylation are carried out in the mitochondria for ATP synthesis (Figure 11) (Senior et al., 2002). Finally, the ATP is synthesized as the end product of these metabolic pathways due to involvement of the ATP synthase.

4.6.5.2 Mode of action

A number of synthetic insecticides belonging to different chemical groups block the ATP production by inhibiting the electron transport system and reducing the mitochondrial oxygen consumption (Bloomquist, 2009). The chemicals belonging to rotenone, hydramethylnon and aluminum phosphide are the inhibitor of the electron transport of the mitochondrial complex I, mitochondrial complex III and Mitochondrial complex IV, respectively.

4.6.6 Uncouplers of oxidative phosphorylation via disruption of proton gradient

Some insecticides affect oxidative phosphorylation by disrupting the tight coupling between electron transport and oxidative phosphorylation due to dissipation of the proton gradient (Hunt & Treacy, 1998; Ware & Whitacre, 2004). Chlorfenapyr and sulfluramid are oxidatively metabolized (by removal of the N-ethyl and N-ethoxymethyl group, respectively) to CL 303,268 and perfluorooctane sulfonamide, respectively, by the cytochrome P450 mono-oxygenases (Figure 12). These oxidative metabolites act as the un-coupler of the oxidative phosphorylation by disrupting the proton gradient, ultimately inhibiting the production of ATP in the mitochondria (Hunt & Treacy, 1998).

4.6.7 Octopaminergic agonists

4.6.7.1 Background

Octopamine is a neurotransmitter at the octopaminergic synapses. octopamine receptor site is present at the octopaminergic synapses (Roeder, 1999; Evans & Maqueira, 2005). The binding of octopamine to the receptor site increased the amounts of the secondary messenger like cyclic adenosine monophosphate that stimulates the neuro-excitation (Leitch et al., 2003). Normally, the octapamine carries out the regulation of the insects' behaviors including feeding, mating and flight activity.

4.6.7.2 Mode of action

Amitraz functioned as the agonist of the octopamine and permanently binds to the receptor site and results in hyper neuro-excitation (Figure 13) (Prullage et al., 2011). Amitraz in the body of treated insects is hydrolyzed to a secondary metabolite that mimics the octapamines and attaches to the receptor site and induce the behavioral modifications including reduced feeding, inability of the coupled mates to separate, tremors and continuous flight behavior (Bloomquist, 2009; Marrs, 2012).

4.6.8 Aconitase inhibitors

Aconitase is an enzyme that catalyses the isomerization of citrate to isocitrate via cis-aconitate in the tricarboxylic acid cycle, a non-redox-active process. It is evident that the fluoroacetate acts as the competitive inhibitory substrates for the aconitase (Beinert et al., 1996). Moreover, the actual inhibitory substance is fluorocitrate, formed metabolically by the condensation of fluoroacetate and oxaloacetate in the citrate synthase reaction of the tricarboxylic acid cycle (Cronan Jr & Laporte, 2006) .

4.6.9 Ryanodine receptor modulators

4.6.9.1 Background

Ryanodine receptors (RyR) form the calcium channels intracellularly in the muscles and neurons of animals (Lanner et al., 2010). The release of calcium ions from the intracellular organelles, that take part in the signaling of the several metabolic pathways, is controlled by the three major isofroms of the ryanodine receptors present in the different tissues (Fill & Copello, 2002). The RyR1, RyR2 and RyR3 are primarily expressed in the skeletal, heart and brain muscles (Mori et al., 2000). The RyR mediated release of calcium ions plays an important role in the muscle contraction and also regulate ATP production in the heart and pancreas cells (Berridge et al., 2000).

4.6.9.2 Mode of action

Diamides binds to the ryanodine receptor site permanently and cause an uncontrolled release of the calcium ions within insect muscle cells (Troczka, 2013). This abnormally abundant amount of ions disrupts the calcium homeostasis of the muscles leading to symptoms of poisoning like no feeding, paralysis of muscles and death. The commercially available diamides are flubendiamide, chlorantraniliprole and cyantraniliprole.

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