Chapter: 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The toxicological features, systemic effects, safety and efficacy of pesticides on living system have been reviewed and presented in this chapter. The rapid industrialization phase of human civilization has lighted the thought of chemical application for human welfare. In industry, at home, in agriculture and in the control of vector born disease, man uses a great number of chemical substances, pesticide is one of them. Though these are abundantly used in environment but their impacts are insufficiently studied on human and on other beneficial organisms. Preliminary investigations on some environmental contaminants particularly pesticides claim their links with leading non-communicable diseases like cardiovascular diseases, metabolic disorders, neurodegenerative diseases and even cancer. This raises serious concerns regarding human health in our society.

2.1. Pesticide

Pesticides are any kind of chemical agents, used to either kill/repel or control certain form of living organism that damage crops and are directly associated with economic loss and thus are considered to be pests. Pesticides are used against vectors of several diseases and against wide range of pests that damage food products. Pesticides are commonly formulated as dusts, liquid or gels because of the wide spread use of pesticides in food production, human beings are at risk of exposure through diet. Humans are exposed to the hazards of several pesticides used in a variety of settings like home and work places. The estimated annual application is more than 4 million tons, of which only 1% of this reaches the target pests (Gavrilescu 2005). Since, scientists do not have any clear idea regarding the health hazards of several pesticides on animal physiology.

2.1.1. Toxicological classification of pesticides

Pesticide is a common term that characterizes several classes of insecticides, herbicides, fungicides, rodenticides, wood preservatives, garden chemicals and household disinfect-

ants that are used to either kill or protect from pests (Eldridge 2008). These pesticides differ in their physical, chemical and identical properties from one class to other. Therefore, it is worthy to classify them based on their properties and study under their respective groups. Synthetic pesticides are man-made chemicals and do not occur in nature. Depending upon the needs they are categorized into various classes. Presently, there are three most popular methods of pesticides classes includes (i) classification based on the mode of entry, (ii) classification based on pesticide function and the pest organism they kill and (iii) classification based on the chemical composition of the pesticide (Drum 1980).

A. Based on mode of entry in the target

I. Systemic pesticides

The systemic pesticides are absorbed by plants/animals and transfer to untreated tissues. Systemic pesticides move through the plant and can reach to untreated areas of leaves, stems and roots. They are capable for killing of weeds with partial spray coverage. They can effectively penetrate in the plant tissues and move through plant vascular system to kill specific pests. Due to control the pests some systemic insecticides are also applied and move through animals such as warble grubs, lice or fleas. The movement of pesticides in plant tissues may be either unidirectional or multidirectional. Some pesticides may only move in one direction either up or down within the plant while other pesticides may only move upwards in plants. If applied to the root zone, it will travel throughout the plant, but if applied to the leaves it will not move throughout the plant. Furthermore, few pesticides are considered locally systemic and move only to a short distance in a plant from the point of contact. e.g., 2,4-dichlorophenoxyacetic acid, glyphosate etc.

II. Contact/non-systemic pesticides

The contact pesticides are also called non-systemic pesticides as it acts on target pests when they come in contact. Physical contact of pest and pesticides is very necessary to be effective. The pesticide enters the body of pests via their epidermis upon contact and

causes death by poisoning. These pesticides do not necessarily penetrate the plant tissues and consequently not transported through the plant vascular system. e.g., paraquat, diquat, dibromide etc.

III. Stomach poisoning pesticides

The stomach poisoning pesticide enters the pest's body through their mouth and digestive system and causes death by poisoning. Stomach poisons are acquired during feeding of pests, at the time of their ingestion the insecticide applied in the leaves and other parts of the plant. Stomach toxicants may also enter the body of insects through the mouth and digestive tract, where they are absorbed into the insect's body. This is more appropriate especially in vector control including bacteria or their toxins, applied to the water where filter feeding mosquito or black fly larvae will consume the poison. These insecticides kill the vector by destroying the mid-gut/stomach of the larvae. e.g., malathion etc.

IV. Fumigants

Fumigants are such pesticides which act or kill the target pests by producing poisonous gases/vapour when applied. These pesticides in vapour form entered into the pests' body through their tracheal system/respiratory system through spiracles and causes death by poisoning. Some of their active ingredients are liquids when packaged under high pressure but change to gases when they are released. Other active ingredients are volatile liquids when it enclosed in an ordinary container and are not formulated under pressure. Fumigants are used to remove stored product pests from fruits, vegetables and grains. They are also very useful in controlling of pests in soil.

V. Repellents

Repellents do not kill but are unpleasant enough to keep pests away from treated areas/commodities. They also interfere with ability of pest to locate crop.

B. Based on pesticide function and pest organism they kill

I. Antimicrobials

These chemicals are used to control protozoa, bacteria and virus. e.g., antibiotics, sulphonamides, fluoroquinolones etc.

II. Fungicides

These chemicals are used to control moulds, mildew and rust. e.g., phosphide, methyl bromide, ethylene di-bromide etc.

III. Herbicides

These chemicals are involved in controlling unwanted plants (weeds). e.g., chlorophenoxy substances, acetanilides, bipyridyls, triazines etc.

IV. Insecticides

These chemicals are used to kill caterpillars, saw flies, fire ants, aphids etc. e.g., acephate, cryolite, DDT, chlorpyrifos, aldrin etc.

V. Molluscicides

These chemicals are designed to control slugs, snails and other molluscs. e.g., iron phosphate, aluminium sulphate, metaldehyde, methiocarb etc.

VI. Rodenticides

These chemicals are formulated to eradicate mice, rats etc. e.g., warfarin, bromodiolone, difenacoum etc.

C. Based on chemical composition of pesticides

I. Organochlorines

These are organic compounds containing at least one covalently bonded atom of chlorine. They kill their target pests by binding to sodium channels in neurons thereby increasing their permeability to sodium ions. This increased permeability facilitates uncoordinated discharge of neurons which damages central nervous system (CNS) of target pests. They react irreversibly with the acetyl-cholinesterase (AChE) which is responsible for deac-

tivating acetylcholine (ACh) at neuro-muscular junctions or at certain synapses in the nervous systems, e.g., DDT, methoxychlor, dieldrin, chlordane, toxaphene, dicofol and endosulfan etc. Dichlorodiphenyltrichloroethane (DDT) was the first of a long line of insecticides bases on hydrocarbons with chlorine atoms replacing some hydrogen atoms. Dichlorodiphenyltrichloroethane was introduced during World War-II and along with penicillin and the sulpha drugs was responsible for the fact that this was the World War-II in history where trauma killed more people.

II. Organophosphates

These are esters of phosphoric acids which are known to phosphorylate and inactivate AChE in synaptic sites. This causes accumulation of ACh at synapse resulting in hyper-excitability of nerve-fibres followed by paralysis and death of concerned pests. e.g., acephate, chlorpyrifos, parathion, malathion etc. Some organophosphates (OPs) are very toxic, like parathion is more toxic than DDT. Every year OPs poison causing hundreds of human deaths throughout the world. Unlike chlorinated hydrocarbons, OPs break down quickly in the environment and thus residues on crops are less likely to be a problem. They are not stored in animal tissue, so bio-magnifications have not been a problem either. Due to these reasons their use has greatly reduced the hazard to non-target species. Development of resistance is just as much a problem as it is within the chlorinated hydrocarbons.

III. Carbamates

These are the organic compounds derived from carbamic acid (NH_2COOH). Like OPs, they inhibit activities of AChE at synaptic region leading to paralysis and death of the target pests. e.g., aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb, methomyl etc. These compounds are detoxified very rapidly and excreted so their risk to warm blooded animals is less than the other agents. They are degraded rapidly in the environment, so

persistence is not a problem, they are however a danger too many useful insects. The diflubanzuran interferes with the synthesis of chitin the material that makes up the insects exoskeleton. It's had very low toxicity for vertebrates but is harmful to insects.

IV. Pyrethroids

These are organic compounds similar to the natural insecticide pyrethrin produced from the flowers of Pyrethrums (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids prevent closure of the voltage-gated sodium channels in the axonal membranes. This leads to permanent depolarization of axonal membrane and paralysis of animal. e.g., cypermethrin, permethrin, deltamethrin, bifenthrin etc. Pyrethrins break down rapidly in sunlight that they are of little use out doors on crops. However, a number of synthetic pyrethrins like substances called pyrethroies do not have this defect and are effective example.

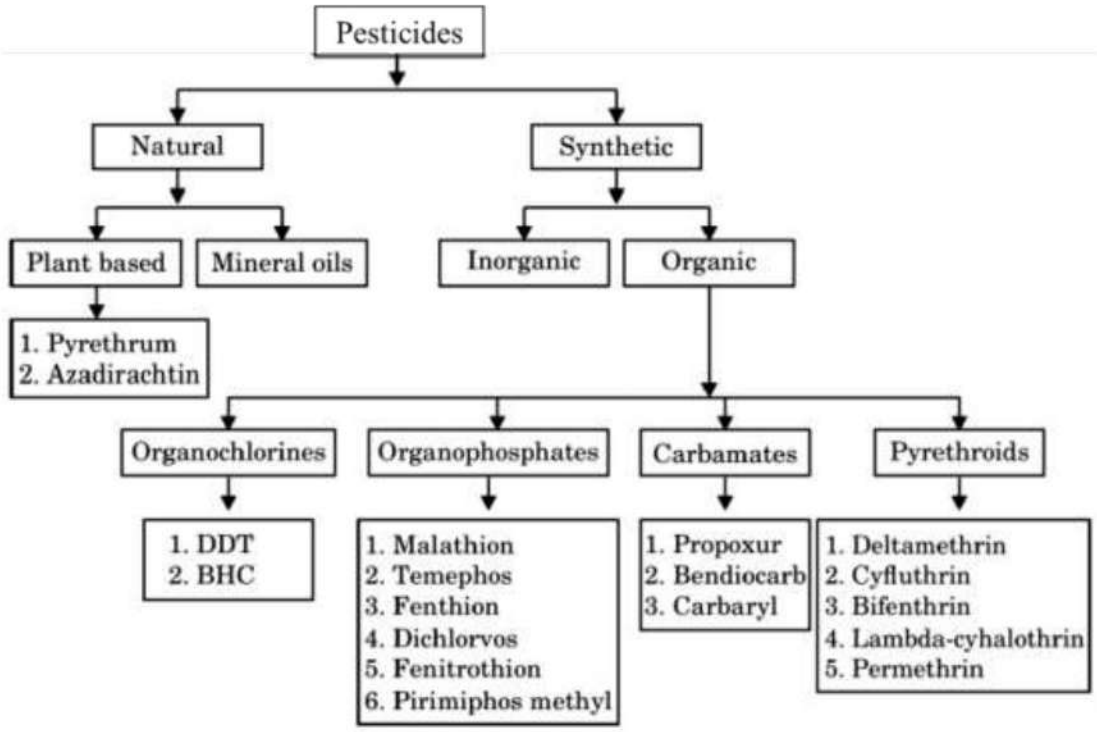

Plate 1. Classification of pesticides. (Yadav and Devi 2017)

D. Based on toxicity level of pesticides

Pesticides may be toxic to human and other beneficial organisms. Hence, the World Health Organization (WHO 2001) has developed a classification scheme that group pesticides based on their potential risks to human body. These are following categories:

Classes of pesticide	Toxicity level
Class-IA	Extremely hazardous
Class-IB	Highly hazardous
Class-II	Moderately hazardous
Class-III	Slightly hazardous
Class-IV	Products unlikely to existent acute hazard in common use

2.1.2. Toxicity mechanism of pesticide

Exposures to pesticides leave overwhelming effects on living creatures in the environment. Chemical pesticides have adverse effects on animal and human health (al-Saleh 1994). Pesticides and many other industrial chemicals are known to generate free radicals (FRs) in biological systems. Free radicals having unpaired electron, show various degrees of chemical configurations viz. superoxide anion radical (O_2•-), hydroxyl radical ($^{\cdot}$OH•), nitric oxide (NO•) and lipid peroxyl radicals (ROO•), which constitute reactive oxygen species (ROS). Seeking stability, FRs attack nearby molecules to occupy required electron and therefore induces oxidative stress (OS) by destroying proteins, carbohydrates, lipids and nucleic acids. Commonly used pesticides are organophosphates, organochlorines, carbamates etc. are being used in common agri-field (Pajoumand et al. 2002). These toxic chemicals mostly remain un-metabolized and non-biodegradable compounds in the ecosystem. The toxicity of pesticides is categorized in relation to their ability to produce lipid peroxidation (LPO), to impair antioxidant status, mitochondrial membrane potential/DNA stability. It is established that stimulation of FRs/ROS production, induction of LPO and disturbance of the total antioxidant defence capability of the living cells are the stepping-stone of the generation of toxicity by most pesticides. Reactive oxygen species are the main causative agents for OS which causes several non-communicable

diseases like cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer and amyotrophic lateral sclerosis (ALS), birth defects and reproductive disorders in human beings (Zarkovic 2003). In many cases, the FRs production is an integral part of the patho-physiological mechanism that helps in damaging major macromolecular and cytoskeletal structures. Moreover, mechanism of toxicity and its impact depend on the complexity of the exposed organisms. The complexity of the organism depends on its systematic position in the phylogenetic tree.

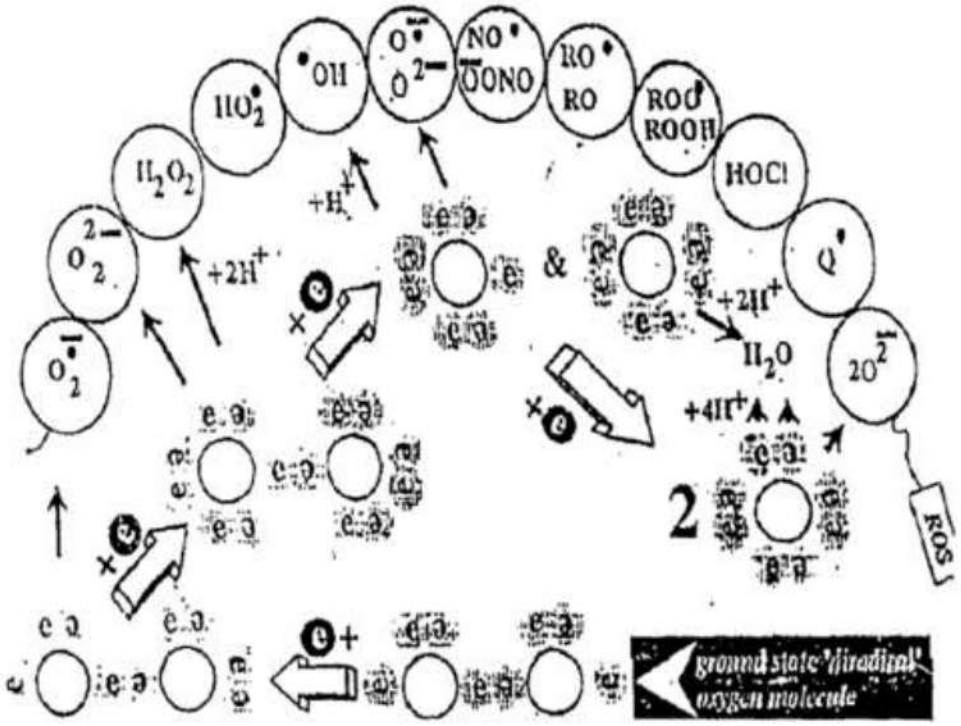

Plate 2. The four-step univalent reduction of 'di-radical' oxygen. Some commonly known members of the ROS family are: hydroxyl radical ($^{\cdot}OH\bullet$), semi-quinone ($\bullet Q^{-}$), superoxide anion radical ($O_2\bullet$-), singlet oxygen ($^1O_2$), nitric oxide (NO•), peroxynitrite anion ($ONOO^{-}$), peroxide ion (O_2^{2-}), alkoxy radical (RO•), peroxy radical (ROO•), alkyl hydroperoxide/organic peroxide (ROOH), hydrogen peroxide (H_2O_2), per-hydroxy radical (HO_2). (Kumar 2002)

A free radical defined as an atom/molecule containing one or more unpaired electrons in valence shell and is capable of independent existence. The odd number of electrons of a FR makes it unstable and highly reactive. Because of their high reactivity, they can abstract electrons from other compounds to attain stability. Thus, the attacked molecule loses its electron and becomes a FR itself, beginning a chain reaction cascade which finally damages a living cell (Mukherji and Singh 1986). Free radicals are derived from incompletely oxidated compounds that have undergone partial burning and that have oxygen groups capable of initiating, at the surface of the cell membranes or even within the cells, aggressive oxidation reactions. Free radical result from both processes occurring in the body i.e., incomplete catabolism, energy production, hepatic detoxification, etc. and the outer environment i.e., smoke, polluted air, ground water foods, medicines etc. (Pickrell et al. 2009). From an electric point of view, FR has a level-headed character and they generally have no charge (Rizzo et al. 2010). The forming of FR is complicated and it can initiate a series of unpredictable reactions in the body, damaging lipids, proteins and nucleic acid. Both ROS/RNS collectively constitute the FRs and other non-radical reactive species (Pham-Huy et al. 2008). The ROS/RNS play a dual job as both beneficial and toxic compounds to the living system. At moderate or low levels ROS/RNS have beneficial effects and involve in various physiological functions such as in immune function, in a number of cellular signalling pathways, in mutagenic response and in redox regulation (Valko et al. 2007). But at higher concentration, ROS/RNS generate OS and nitrosative stress, respectively, causing potential damage to the biomolecules. The OS and nitrosative stress are developed when there is an excess production of ROS/RNS on one side and a deficiency of enzymatic and non-enzymatic antioxidants on the other side. Most importantly, the excess ROS can damage the integrity of various biomolecules including lipids (Yla-Herttuala 1999), proteins (Stadtman and Levine 2000) and DNA (Marnett

2000) leading to increased OS. In living cells, the forming of FR takes place mainly through such processes as the homolysis of chemical bonds, photolysis, radiolysis and as a result of redox reactions. In the cells, FR is made up continuously as by-products of oxygen metabolism during the oxidative phosphorylation taking place in mitochondria.

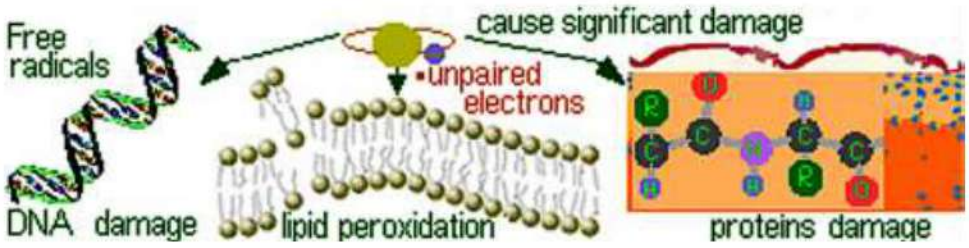

Plate 3. Reactive oxygen species damage proteins, lipids and DNA. (Butnariu and Samfira 2012)

Oxidative stress (OS) is defined as an overstated production of oxygenated FR, accompanied by a dislocation of antioxidation agents. Animals cannot live without oxygen, since it is essential in the functioning of energy producing cells (Gustafsson and Gottlieb 2008). A body transforms and eliminates carbon dioxide (CO_2) almost exclusively and the rest is at the origin of some hyper-reactive species called FR. These FRs are oxidated derivatives of the electron deficit, unstable oxygen molecule, that cause dysfunctions of all body cells. The main actions of FR in a living system are oxidation of polyunsaturated fatty acid (PUFA) in the cell membrane, oxidation of amino acids in proteins, depolymerisation of hyaluronic acid, oxidative-oxidation of DNA, modulation of the activity of nucleotide cyclase, modulation of prostaglandin activity and synthesis etc. The current concepts of ROS signalling can be grouped into two action mechanisms alterations of intra-cellular redox state and protein oxidative changes (Plate 3). Compared to the

extracellular environment, cytoplasm is normally maintained in reducing conditions (accompanied by the buffer redox capacity of intra-cell-thiol). These thiol-systems oppose intra-cell OS reducing H_2O_2, lipid-superoxides and peroxides.

Reactive oxygen species can alter the structure and normal function of proteins, changing the rest of amino acids, inducing protein dimerization and interacting with other metal complexes (Fe-S). Oxidative changes of amino acids in the protein functional domain can involve some pathways. The target cell components of FR action include lipids (LDL), macromolecules with complex structure, proteins and DNA. To minimize the negative effects of ROS, organisms are endowed with very efficient antioxidant defines system. Antioxidant enzymes (SOD/CAT) function as an antioxidant system by bonding potential damaging metal ions. In OS, the ratio between pro-oxidants and antioxidants is in favour of the former. The pro-oxidative status is stimulated by the presence of FRs, while the anti-oxidative one is due to free antiradicals; together, they make up the oxidative balance. In the presence of OS, the most vulnerable system of a body is the CNS. Health state analysis has thus shifted from cell level to molecular level and to atomic level. This is the biochemical and biophysical context in which the action of anti-oxidative compounds, taking into account that OS is responsible for chronic degenerative diseases (Akinci et al. 2008). Living organisms are very sensitive to the damage produced by FR due to some factors, viz. electron-rich neuronal biomolecules recognize the oxygen radical, an electrophil ROS, neuronal membranes are rich in PUFA, neuronal mitochondria are represented in large numbers, neurons are easily subjected to dedifferentiation as a result of the attack by FR on the DNA, neuronal oxygen is certainly present since the brain, unlike other organs and tissues, has an overall aerobic energetic metabolism. At brain level, there are small amounts of enzymatic and non-enzymatic antioxidants. Neurons are “perennial cells” and the cumulated lesions produced by FR on the different cell

structures could, in time, degrade quantitatively and qualitatively, the neurotransmission functions and result in behavioural changes. Cell lesions occur under conditions in which the rates of ROS are formed is high or when the activity of the defence system is low, when there is unbalance between the protection systems and the generation systems of ROS (Cynshi et al. 2010). Free radical can initiate reversible, small-size changes due not only to the low intensity of their formation, but also to the dissipation and decomposition reactions. The factor determining the increase of the strength of FR formation is oxygen activation. Due to the presence of this element not only in the atmosphere but also in almost all the substances in the body, the interaction of FR with oxygen is inevitable. They attack the existing pro-oxidants for which the FR has an affinity. The action of FR on pro-oxidants is neutralized by the antioxidants or by the reactions of FR. The potential of the process of peroxidation consists in the exceeding pro-oxidant concentration and is superior numerically to the pro-oxidant concentration (Molyneux 2004).

The most reactive FR is hydroxyl radical (·OH•), which reacts with deoxyribose and the bases of the DNA. Reactive oxygen species are measured factors that cause cell dedifferentiation since they react with chromatin, they change the bases of the DNA and they cause ruptures of the chain; they induce chromosomal aberrations and a long life in the species with a low rate of accumulation of chromosomal aberrations for many species. The oxidative changes of proteins under the action of ROS can result in inactivation of membrane enzymes and proteins and can induce structural changes resulting in destabilization of cell morphology when the target of the oxidative attack is cytoskeleton elements (Casado et al. 2007). In case of enzymes, the effect of ROS consists in a decrease of the catalytic capacity. The size of changes is determined by the relative location of the formation site of ROS of antioxidant systems and of target-protein. Reactive oxygen species play a role in mitochondrial damage and in permanent senescence. The results of ageing

show a decrease of the number of mitochondria and the fact that the organites in the aged cells undergo biochemical alterations. A target of the radicalic attack is membrane lipids because of the double bonds in the structure of PUFA. The most frequent PUFA in the structure of membrane phospholipids are linoleic, linolenic and arachidonic acids (Milne et al. 2005). The process of peroxidation of PUFA consists in the following steps: a FR extracts a hydrogen atom from the allylic position, with turning the PUFA into a lipid radical; then, there is intra-molecular rearrangement of the double bonds with formation of a conjugated-diene; as a result of the reaction between the conjugated-diene and the molecular oxygen, there occurs a peroxyl radical (ROO•) which can react with another molecule of PUFA forming lipid radicals, with the turning of the peroxyl radical (ROO•) into lipid hyper-oxide. Alternatively, the peroxyl radical (ROO•) can form cyclic peroxides. Lipid peroxides, once formed, undergo splitting resulting in the propagation of the reaction chain and in ramification and decomposition reactions (Kaynar et al. 2005). The decomposition of the lipid peroxides results in such compounds as alkanes (ethane, pentane), aldehydes (malonic-dialdehyde, hexanal, 4-hydroxinonenal), epoxy and hydroxy fatty acids. The peroxidation of membrane lipid affects the structure and functions of plasmatic and organite membranes. Thus, transmembrane potentials, ionic flows, and transmembrane transport are troubled, membrane receptors are inactivated and signalling paths are deregulated. Through the process of LPO, there occur membrane components changes of lipid and protein nature as a result of the reaction of some amino acids with aldehydes peroxidation products (Sowers 2002). Free radical and OS play a role in the decrease of dysfunctions at cell level and of the different diseases at body level. This review deals with the chemistry, formation, sources and molecular targets of FRs, it highlights the effect of FRs in pesticides stress conditions.

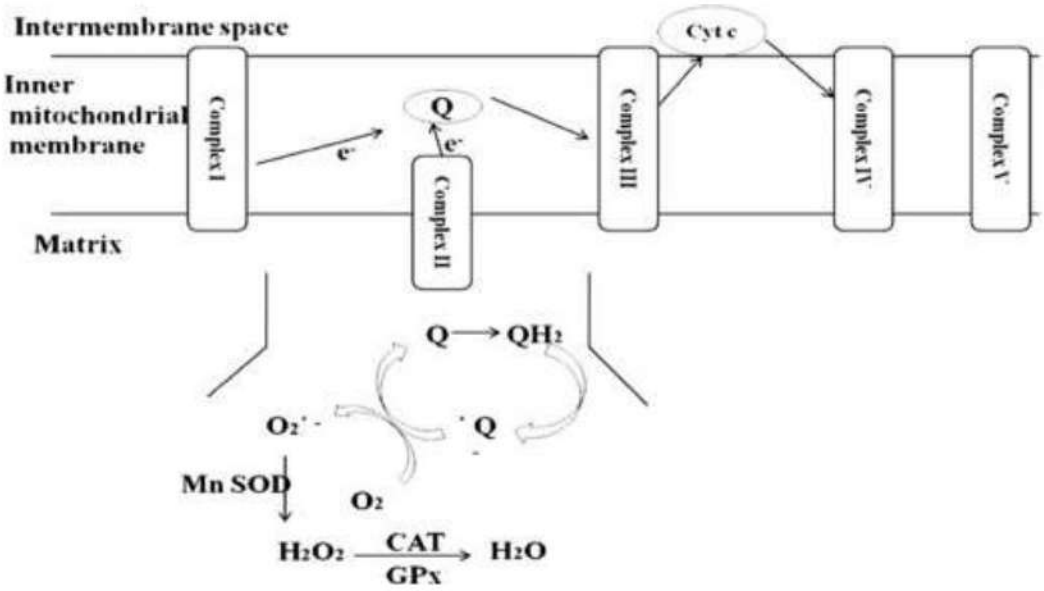

Plate 4. Mitochondrial ROS production in cell. (Phaniendra et al. 2015)

Several reports are available confirming pesticide-based environmental pollution and health hazards (Shukla et al. 2006). It has been indicated that, approximately 98% of sprayed insecticides and about 95% of herbicides reach sites like air, water, soil, food and non-target organisms other than their destinations (Miller 2004). As they are biocides and affect enzymes and different physiological systems in pests which may be identical or very similar to biological machinery in human beings, therefore they pose potential risks to human health.

2.1.3. Status of pesticides poisoning in World

Pesticide poisoning imposes significant morbidity and mortality world-wide (Kishi and Ladou 2001) but its reliable estimations of such poisoning are lacking. Low and middle-income countries, pesticide self-poisoning is comparatively more frequent (Gunnell and Eddleston 2003). Study on Sri Lanka estimated around 2,20,000 deaths due to pesticide toxicity (Jeyaratnam 1985) and on rural areas of China and South-East Asia claims that, pesticide ingestion accounted for over 60% of the suicides with as many as 3,00,000 deaths each year (Gunnell and Eddleston 2003). In Campinas, Brazil, 18% of male and

7% female hospital admission accounts for pesticide based self-harm (Fleischmann et al. 2005). In Southern Trinidad, over 80% of suicides in a rural area were due to pesticide poisoning (Hutchinson et al. 1999). Recent data from Malawi, indicated pesticide self-poisoning in almost 80% of suicides (Dzamalala et al. 2005). Therefore, these studies show that, pesticide poisoning is the most frequently used method of suicide and self-harm world-wide.

2.1.4. Status of pesticides poisoning in India

In agricultural sector, Indian economy is mostly dependent on application of pesticides. In India, use of pesticides was commenced in 1952 but now it becomes second largest manufacturer in Asia after China. India ranks 12th globally in production of pesticides (Mathur 1999). Among several types of pesticides used in India, approximately 76% belong to insecticidal category followed by 13% and 10% for fungicides and herbicides respectively. Despite of economic benefits, pesticide poisoning in India is also frequent. Organophosphate compounds in majority are used for self-poisoning in Southern and Central India (Batra et al. 2003). In Northern parts of India, most of the pesticide-based deaths are caused by fumigant aluminium phosphate (Singh and Tyagi 1999). Pesticides which are also used for self-poisoning include carbamates, organochlorines and pyrethroids (Eddleston 2000). Medical management is difficult due to lack of proper knowledge regarding mode of treatment and antidotes (Eddleston et al. 2003). The use of synthetic pesticide in agri-field comes with a cost for the environment as well as animals and humans health.

2.1.5. Routes of pesticides exposure for non-targets/beneficial animals

Non-target/beneficial animals are exposed to pesticides through contaminated air, water and soil. Application of pesticides in agri-fields facilitates contamination of crops, fruits and vegetables that affect herbivores and human. Pesticides contaminated water widely influences fishes and other non-target mammals. Livestock, birds and human undergo

pesticides exposure through inhalation of contaminated air through breathing. Moreover, human beings may acquire pesticide residues from meat of herbivores and dairy products.

2.1.6. Origin and properties of azadirachtin (AZT)

Over the years, extensive use of commercially available synthetic pesticides against phytophagous insects has led to their bioaccumulation in the environment causing increased resistance and reduction in soil biodiversity. Further, 90% of the applied pesticides enter the various environmental resources as a result of run-off, exposing the farmers as well as consumers of the agricultural produce to severe health issues. Therefore, growing attention has been given toward the development of alternate environmentally friendly pesticides/insecticides that would aid an efficient pest management system and also prevent chronic exposures leading to diseases. One such strategy is, the use of neem plant's (Binomial name: *Azadirachta indica*) active ingredients which exhibit agro-medicinal properties conferring insecticidal as well as immunomodulatory and anti-cancer properties. The most prominent constituent of neem is azadirachtin (AZT), which has been established as a pivotal insecticidal ingredient. It acts as an antifeedant, repellent and repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males. This review discusses, key neem pesticidal components, their active functional ingredients along with recent strategies on employing nanocarriers, to provide controlled release of the active ingredients and to improve their stability and sustainability.

In India, the neem tree is a member of the mahogany Family-Meliaceae and is known as margosa tree, *Azadirachta indica* A. Juss (Perry et al. 1998). Neem was adopted by several volumes dedicated to neem and neem insecticides have been published (Schmutterer 2002) but its commercial success has collapsed due to the relatively high cost of the refined product (Isman 2004) and the relatively slow action on pest insects.

Neem tree have been recognized world-wide for their unique properties that containing chemicals inhibits feeding capacity of grasshoppers in nature (Volkonsky 1937) and insecticidal properties have been observed on locusts (Pradhan et al. 1963) that was reviewed several times (Schmutterer 1981). Tetranortriterpenoids have been isolated from several parts of the neem tree and three compounds obtained from seeds are meliantriol (Lavie et al. 1967), salanin (Henderson et al. 1964) and azadirachtin (Butterworth and Morgan 1971) are found to be active as feeding deterrents, disruptants of growth and development against a variety of insect (Warthen 1979). Neem leaves the natural source of AZT at present depending on the origin of the seed material (Morgan 1981). The best yields were found from seeds by using ethanol extraction on thin layer chromatography (Ermel et al. 1984) and by using high performance liquid chromatography revealed that, AZT variation among individual trees by different factors like light, temperature and relative humidity and exposure to UV-radiation (Ermel et al. 1987). Crude biologically active component was first isolated in pure form of AZT (Butterworth and Morgan 1968) for insecticidal properties. Pure AZT is a clear, white, microcrystalline solid (mp 149°C) and demonstrated that several AZT having the basic triterpenoid structure. The influence of AZT on natural enemies is highly variable (Spollen and Isman 1996). Like the pyrethrins, AZT is rapidly degraded by sunlight. Azadirachtin has a half-life of approximately 20-hrs (Caboni et al. 2002). On the other hand, AZT has systemic action in certain crop plants, greatly increasing its efficacy and field persistence (Schmutterer 2002).

Biosynthesis of triterpenoids from *A. indica* initiates with azadirone and a C-ring opening, which culminates in AZT formation. Azadirachtin, along with other related triterpenoids such as azadirachtin-B, salannin and nimbin are the active ingredients in neem plant based bioinsecticides and they act by disrupting the growth and development of insects and by deterring their feeding. It is considered as a botanical pesticide with ex-

ceptional growth regulating and biocidal efficacy along with deterrent effects on the ovipositing and feeding of insects (Morgan 2009). An attempt to evaluate the exact molecular mechanism of insecticidal activity of AZT on *Monochamus alternatus*, a pine sawyer beetle, has indicated enrichment of differentially expressed genes (DEGs) in 50 pathways, where, 920 and 9984 unique genes were found to be up and down regulated significantly. Such detailed gene profiling to assess the AZT internalization with *M. alternatus*, can promote the development of efficient AZT derived herbal pesticides (Lin et al. 2016).

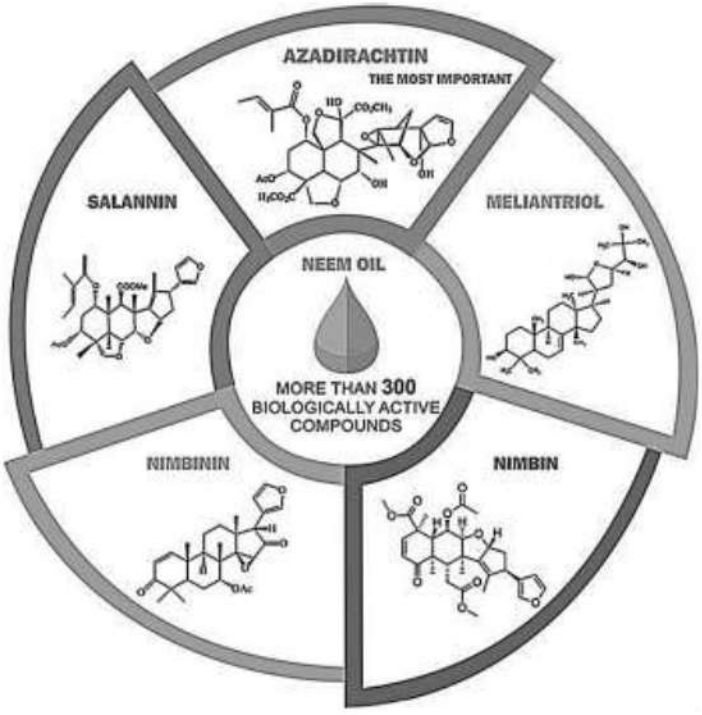

Plate 5. Secondary metabolites of neem tree, *Azadirachta indica* A. Juss. (Pascoli et al. 2019)

2.1.7. A brief review on AZT toxicity

Azadirachtin is a botanical origin, broad-spectrum pesticide used in agriculture to eradicate a wide range of insect pests. Some reports are available that document LD_{50} of AZT on other non-target/beneficial insect species. The effects of AZT on non-target/beneficial organisms have been studied in terrestrial ecosystems. In many pest systems, AZT is most

selective against the pest and has less effect on parasitoids or predators (Schmutterer 1992) but adverse effects of AZT against beneficial organisms have been reported (Price and Schuster 1991). Toxic effects of pure AZT on mosquito larvae have been demonstrated (Zebitz 1987). Azadirachtin is generally considered less harmful to the environment than other commonly used pesticides (Mordue and Blackwell 1993) that is highly oxidized with many reactive functional groups in proximity to each other (Ley et al. 1993). Neem is a natural insect growth regulator (Rembold 1989) as well as an antifeedant (Radcliffe et al. 1991). Neem has long been recognized for its natural insecticidal properties (Saxena et al. 1988). Azadirachtin is structurally somewhat similar to insect hormones, ecdysones and seems to be an ecdysones blocker, so that insect will not moult. It also interferes with the function of taste, so that many leaf-eating insects are deterred by plants to which even small amounts of AZT have been applied. The seed kernels, whole fruits or leaves of *Melia azedarach* contain meliatoxins including AZT are highly toxic to mammals (Schmutterer 1990).

The locusts preferred to starve to death rather than feed by neem extracts was sprayed on crops (Pradhan et al. 1962) and were most potent antifeedant activity belonging to other orders' viz. Coleoptera, Diptera, Heteroptera, Isoptera, Lepidoptera and Orthoptera (Jacobson 1986). Growth regulating effects were identified in crude extracts of neem leaves on *Antestiopsis orbitalis bechuana* without effects on fecundity but on *Epilachna varivestis* demonstrated the growth disruption and sterilizing effects of neem components (Schultz 1981). Methanolic neem seed extract disturbs the metamorphosis of *E. varivestis* (Ascher and Gseil 1981). Rational enriched neem seed extract resulted 100% larval mortality of *Piutella xylostella*, *Pieris brassicae* and *Leptinotarsa decernlineata* (Schmutterer 1984). Crude neem seed extracts on *Dysdercus fasciatus* resulted in mortality or deformity of body after moulting and reduced fecundity of the emerging adults.

Neem seeds extract and leaves extract with acetone on *Dysdercus cingulatus* led to substitute moults. Methanolic seed extracts to *Ceratitis capitata* larva led to prolongation of instars, low food intake, low rate of pupation and hatching, ultimately reduced adult population (Steffens and Schmutterer 1982). Aphids directly with neem extracts were not significantly affected (Schauer 1984). The fecundity of *Leptinotarsa decemlineata* was significantly reduced and some were absolutely sterile after consumption of formulated neem seed extract leaves (Schmutterer 1987). The aqueous neem extract had strong antifeedant activity than the formulated extracts. Both extracts of an azeotropic solvent mixture and of butylmethyl ether were evaluated against mosquito larvae depending on the dose, these extracts showed toxic, growth-regulating and sterilizing effects on *Anopheles* sp., *Cule*x sp. and *Aedes* sp. (Zebitz 1987). Neem oil showed antifeedant property against *Heliothis armigera* and has strong antifeedant against *Spodoptera litura* (Joshi et al. 1978). Neem oil had very poor antifeedant activity than crude extracts of neem against locusts and *S. litura*, but was effective on mosquito larva (Attri and Ravi Prasad 1980) and neem kernel suspension to *H. armigera* and *Chillo partellus* led to the developmental deformities (Jotwani and Srivastava 1984). The biological effects of various neem extracts and neem oil on several Indian insects were listed (Parmar 1987). These include synergistic, ovicidal and ovipositional deterrent effects besides the growth disrupting effects.

Azadirachtin is structurally similar to the insect hormones known as “ecdysones” which are responsible for metamorphosis in insects. The feeding behaviour in insects is dependent on the neural inputs received from the chemical sensors of the insects, for example, the taste receptors in the mouthparts, tarsi and oral cavity. These sensors integrate a “sensory code” that is delivered to the CNS. Manifestation of antifeedancy by AZT oc-

curs through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in insects by firing the sugar receptor cells (Mordue et al. 1998).

In addition to antifeedancy, AZT injection also leads to physiological effects in the insect's mid-gut, which causes a reduction in the post-ingestive-digestive efficiency. This reduction in efficiency is known as "secondary" antifeedancy and is due to disturbances in the hormonal as well as physiological systems. These disturbances include hindrance in the food movement through the insect's mid-gut and inhibition in production of digestive enzymes (Schmutterer 1985). An early study conducted by Nisbet et al. (1996) highlighted this antifeedant feature of AZT. It was established that a concentration of 50-100 ppm of AZT caused an insecticidal effect however, it has a high potential to harm beneficial insects as well. Therefore, a low concentration was tested which concluded that a concentration of only 5 ppm of AZT can dramatically decrease the fecundity in aphids within 48-hrs of feeding. Further, a diet containing more than 10 ppm AZT led to the production of non-viable nymphs.

Azadirachtin interferes with the growth and moulting process of insects. Its ingestion leads to abnormal moults, growth reduction and increased mortalities. Azadirachtin interferes with the synthesis of an "ecdysteroid" hormone, which is responsible for the moulting in insects. Indirectly, AZT affects the neurosecretory system in insects by blocking the release of morphogenetic peptide hormones such as prothoracicotropic hormone (PTTH) that control the prothoracic glands and allatostatins, which in turn control the corpora allata, that responsible for secreting juvenile hormone (JH). Moulting hormones from prothoracic glands are responsible for controlling the formation of new cuticle and play a central role in ecdysis. The formation of juvenile stages during each moult is controlled by the JH from the corpora allata (Nisbet 2000). Disruption in these events by AZT, leads to various sterility and moulting defects. Moreover, cellular uptake of AZT

inhibits both cell division as well as protein synthesis thus, causing mid-gut cell necrosis and flaccid paralysis of muscles (Nisbet 2000).

Neem products influence fecundity in female insects in a dose dependent manner. Azadirachtin prevents oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid. In males, AZT acts by interrupting the meiotic process responsible for sperm production (Linton et al. 1997).

2.1.7. Effects on insect

A. Physiological effect

The physiological effects of AZT are much more stable than the antifeedant effect which interferes with growth, moulting and other specific cellular processes. In some species tested, dose response effects can be observed as reduced growth, increased mortalities, abnormal and delayed moults which effects are related to disruption of endocrine system controlling growth and moulting. In all species investigated, physiological effects can be estimated as growth reduction, increased mortality and abnormal delayed moults. Such endocrine disruption effects can be demonstrated effectively in *Oncopeltus fasciates* (Mordue et al. 1995). The physiological effects of AZT can be categorised in two ways i.e., indirect and direct.

a. Indirect effect

This effect occurs through endocrine system. The neurosecretory systems of brain affected by AZT cause a block of the release of morphogenetic peptide hormones e.g., PTTH. These control the function of the prothoracic glands and corpora allata respectively. Moulting hormones from the prothoracic glands in turn controls new cuticle formation and ecdysis whereas JH from the corpora allata controls the formation of juvenile stages at each moult. In adult both hormones can be involved in the control of yolk deposition in

the eggs. Any disruption in these cascade events by AZT results in moult disruption, moulting defects and sterility effects (Bilton et al. 1987).

b. Direct effect

On cells and tissues, AZT is internalized by the cells and causes inhibition of both cell division and protein synthesis. Such effects are observed in flaccid paralysis of muscles, mid-gut cell necrosis and loss of regenerative cells of the gut and expresses lack of mid-gut enzyme production. The sum total of the physiological effects of AZT is consistent throughout species when compared to antifeedant effects (Mordue and Blackwell 1993).

Table 1. Behavioural sensitivity of insects to AZT: (ED_{50})-50% inhibition of feeding. (Mordue and Nisbet 2000)

Insect's Order	ED_{50} (ppm)
Lepidoptera	<0.001-50
Coleoptera	100-500
Hemiptera	100-500
Hymenoptera	100-500
Orthoptera	0.001->1000

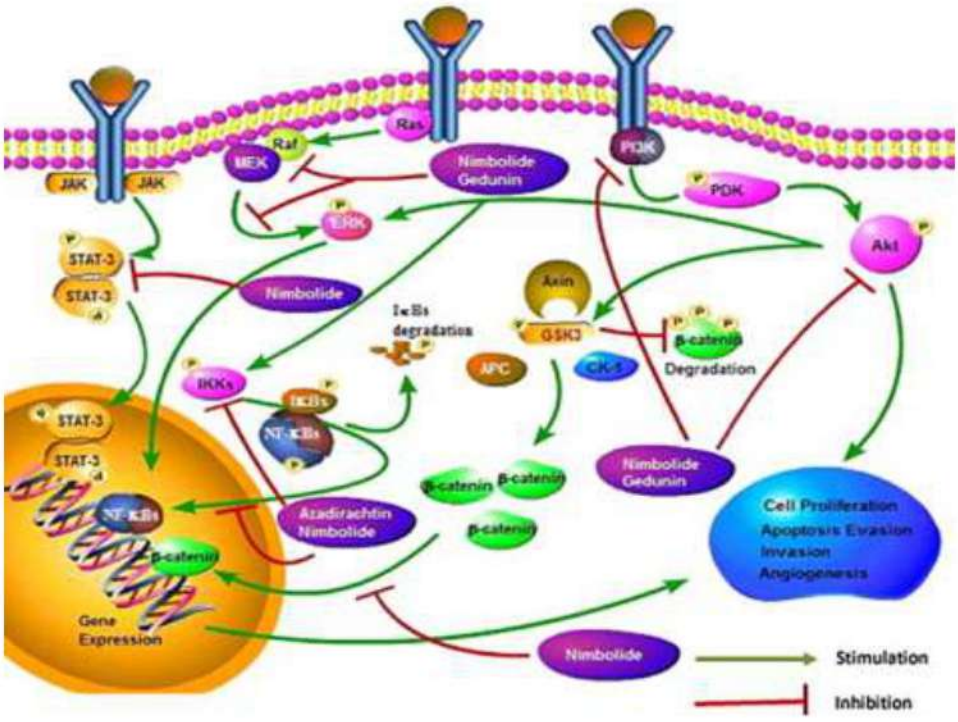

Plate 6. Mechanism of AZT toxicity in a living cell. (Nagini 2014)

B. Effects on endocrine system

The haemolymph ecdysteroid titre pattern is modified and the peaks suppressed instar *Locusta migratoria* nymphs by AZT injection at the beginning of the instars (Sieber and Remboid 1983). Such modification of ecdysteroid titre is closely correlated with morphogenetic effects. The ecdysteroid levels can be drastically reduced, delayed and extended or not significantly affected by time dependent AZT treatment on final instar nymphs of *L. migratoria* (Mordue et al. 1986). Two types of effects were found at the time dependent injection of AZT into fifth instar larvae of *Bombyx mori*. When injected prior to release of PTTH by larvae, defective pupae were formed, whereas injection into matured larvae led to complete failure of pupation (Koul et al. 1987). By tissue culture experiments demonstrated that AZT has no direct effect on PTTH and prothoracic gland secretion. Azadirachtin decreased the elevation of JH titre in *Galleria mellonella* larvae but had no effect on the allatotropic activity of the brain (Malczewska and Gelman 1988). Ecdysteroid titres were too low for induction of ecdysis in the AZT treated nymphs of *Rhodnius prolixus* (Garcia et al. 1986). Ecdysones given orally and JH analogue counteracted the ecdysis inhibition by AZT induction (Garcia and Rembold 1984). *Oncopeltus fasciatus* larvae induced by AZT show neither ecdysis nor apolysis and have a delayed and distinctly lower ecdysteroid peak (Dorn et al. 1986). The effect of AZT on JH and ecdysones might be interpreted as an interference with neuroendocrine system which controls the ecdysones and JH synthesis. The tropic hormone (PTTH) and allatotropic hormone (ATTH) secreted from brain are involved in such a regulation. Histological studies have been made to understand the neuroendocrine control mechanism in AZT treated insects (Rao and Subrahmanyam 1986). *Locusta migratoria* nymphs fail to moult after treatment as well as the female adults whose gonadotropic cycle was suppressed due to AZT toxicity, that show remarkable accumulation of stainable neurosecretory product in

the corpus cardiacum which plays in the storage and release of neuro-hormones. Therefore, this is considered as the leading step in the mode of action of AZT. However, these studies do not give a complete answer to detailed mode of action without fate of AZT in the insect body.

C. Effects on reproductive system

The disruption of insect reproduction is also an important feature of AZT because ecdysteroid is one of the hormones regulating vitellogenesis. Azadirachtin can modify ecdysteroid by inhibiting the release of PTTH and ATTH from the brain corpus cardiacum complex and have adverse effects on ovarian development, fecundity and fertility of Orthoptera, Hemiptera, Heteroptera, Coleoptera, Lepidoptera, Diptera and Hymenoptera (Mordue and Blackwell 1993). The sex behaviour of the females and males in mating in response to sexual pheromones (Dorn et al. 1987) and spermatogenesis in males (Shimizu 1988) are affected by AZT treatment. When oviposition sites are treated with AZT or other neem products, oviposition repellence, deterrence and inhibition occurred in Coleoptera, Lepidoptera and Diptera (Dhar et al. 1996). Azadirachtin has a profound influence on the growth and maturation of oocytes in several insect orders. Ecdysteroid synthesized in cells of follicular epithelium at the end of oocyte maturation. Finally, all the ovarian ecdysteroid are contained in the newly laid eggs and control cuticulogenesis during early embryonic development. After a single injection of AZT into mature female locusts, follicle growth is inhibited. The ovarian ecdysteroid levels of *L. migratoria* showed that the ovarian ecdysteroid level in control increased near the end of vitellogenesis and reached a maximum within hours (Rembold and Sieber 1981). Whereas AZT injection into females at the end of vitellogenesis resulted only in very small amounts of moulting hormone in the ovaries and the ovaries were smaller and weighed become half that of controls where the number of mature oocytes was less, probably due to resorption. Juvenile hormone

plays a vital role in vitellogenesis (Rembold et al. 1987) as well as ecdysones levels of AZT injected female of *L. migratoria* show that AZT significantly affects vitellogenin synthesis. These observations clearly demonstrate the delay and derangement of the gonadotropic cycle. Azadirachtin inhibits in a dose dependent fashion, the cytochrome P_{450} dependent ecdysone-20 monooxygenase activity that converts ecdysones to its active metabolite 20-hydroxy ecdysones in homogenates of *Drosophila melanogaster* larvae, *Aedes aegypti* adult female abdomens or body or mid-gut of *Manduca sexta* (Smith and Mitchell 1988). Azadirachtin has no direct influence on prothoracic gland activity (Koul et al. 1987).

D. Effects on excretory system

Constant quantity of biologically active dihydroazadirachtin was recovered unchanged after injection of any physiologically effective doses in female *L. migratoria* (Rembold et al. 1988). It was notable to observe that malpighian tubules retained dihydroazadirachtin in the whole body. The site of its metabolism is not yet known but it is not degraded significantly by fat bodies and it was localised in the basal and inner regions of malpighian tubules (Garcia et al. 1989) hence AZT acts in its unchanged form through high affinity binding to organ specific membrane receptors.

E. Effects on nervous system

Nervous system is at a high risk to FR-induced injury because it is rich in PUFA. Moreover, high oxygen tension and relatively poor antioxidant capacity impair stability of the macromolecular structure in brain (Halliwell 1992). Azadirachtin concentrates more in the corpus cardiacum than in the brains and that while it does not penetrate the blood brain barrier, which completely covers the corpus cardiacum gland structure (Subrahmanyam and Rembold 1989). This advocates that though AZT does not directly interfere with the neurosecretory activity of brain but may act on the functioning of the corpus car-

diacum. Turnover of the neurosecretion in the corpus cardiacum of AZT injected *L. migratoria* was very poor, leading to accumulation of the stainable neurosecretory material. Hence, AZT may influence the release mechanism of tropic hormones leading to disruption of hormonally controlled moulting and vitellogenesis processes.

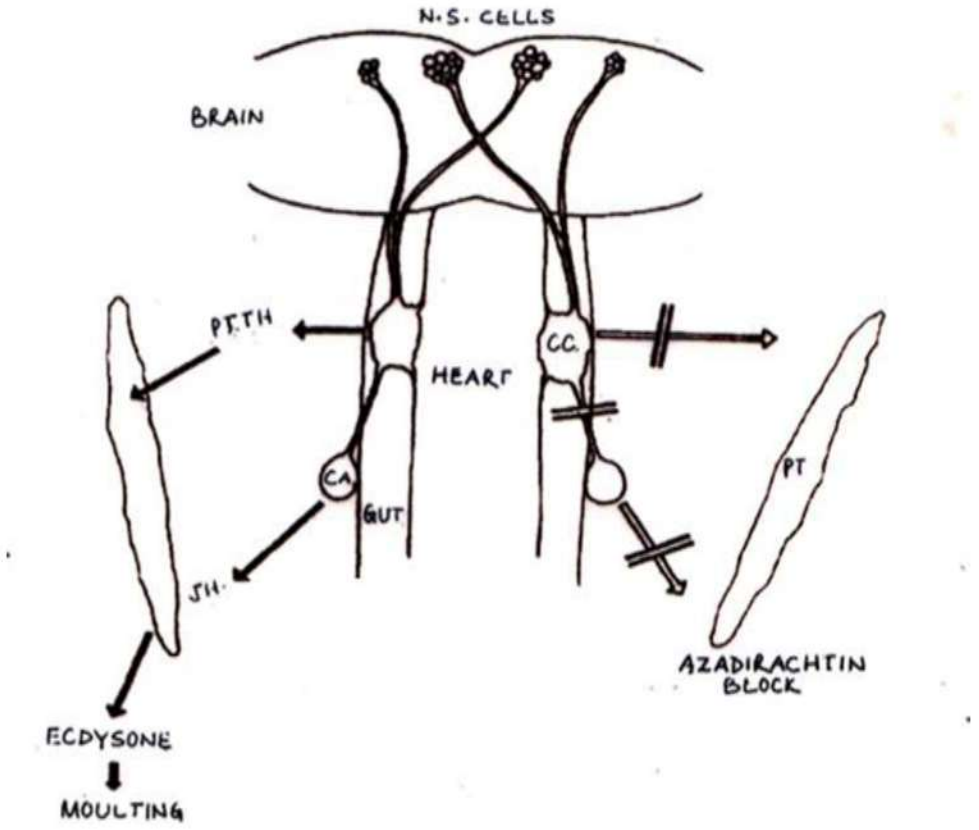

Plate 7. Acetylcholine, its regulation by AChE and inhibition of AChE activity by AZT. (Manikanta and Dokuparthi 2014)

F. Effects on growth

Azadirachtin affects growth, development and metamorphosis in diverse insect taxa, which effects on insect's muscles, gut, CNS, immune system and finally results in death of insects (Mordue et al. 2010). Lepidopteran insects demonstrated enhanced antifeedant sensitivity against AZT exposure (Sami and Shakoori 2014) but Coleoptera, Hemiptera

and Homoptera are less sensitive to AZT (Mordue et al. 2010). Report reveals that *Schistocerca gregaria* are very sensitive to AZT (Mordue et al. 1996). Significant sensory response of chemoreceptor on the insect's mouthparts to antifeedant activity and stimulates specific deterrent cells in chemoreceptor has correlated (Mordue et al. 1998). This recommends that AZT has direct and/indirect effects on dose dependent neural toxicity. Free radicals promote LPO with loss of membrane integrity. In addition, degeneration of mitochondrial membrane can initiate a cascade of FR-reactions. It is currently recognized as a mechanism of cell injury (Tappel 1973). Though a strong antifeedant to locusts (Butterworth and Morgan 1971) and to other insects of several taxa, it also acts as a potent growth inhibitor (Mordue et al. 1986). The most defined effects are delay and/or inhibition of moult into the successive instar, disturbance of the moulting process and delay, disturbance or inhibition of ovarian development. Treatment of insects by injection, oral ingestion or topical application of AZT causes larval growth inhibition, malformation, mortality leading ultimately death. This activity has been proved in Orthoptera, Hemiptera, Lepidoptera and Diptera which alter ecdysteroid and JH with significant reduction by inhibiting the release of morphogenetic peptide, prothoracicotropic hormone and allatotropins from the brain corpus cardiacum complex (Ascher 1993). Extracts from various parts of the *A. indica* have different degrees of insect's growth regulator activity. The effects of AZT on growth and moulting have been explained somewhere else (Mordue and Blackwell 1993) comprise growth reduction, abnormal or delayed moults and increased mortalities in insects. Cellular site of action of AZT binding on insect tissues (Nisbet et al. 1995) shows a high level of specific binding of tritiated dihydroazadirachtin to cell membranes. Cellular binding is time dependent and dissociation of the AZT membrane complex is incomplete showing very tight binding is not affected by AZT except at very high doses (Nisbet et al. 1996). Azadirachtin binding complex integ-

rity within membranes is essential for its activity (Mordue et al. 1999). It appears from current knowledge that AZT has more than one mode of action and alters or prevents the formation of new assemblages of organelles as well as cytoskeleton resulting in disruption of cell division, blocked transport, release of neurosecretory peptides and inhibits protein synthesis in cells which are metabolically active and have been switched on to produce large amounts of protein such as mid-gut cells for digestion and fat body cell production of mixed function oxidases for detoxification processes. Thus, at the molecular level AZT may act by altering or preventing transcription and translation of proteins express during certain stages of the cell cycle. Azadirachtin injection caused dose dependent reduction in body weight of final instars nymphs of *S. gregaria* and even the highest dose did not cause absolute feeding inhibition (Rao and Subrahmanyam 1986). Such treatments reduced the feeding rate, growth and utilization of food to body mass. Similarly, a physiological dose injected into female *L. migratoria* did not cause starvation, though food consumption was reduced without significant loss or addition to body weight (Subrahmanyam et al. 1989). Besides antifeedant effect, AZT causes disorders in metamorphosis (Ruscoe 1972) and these effects were observed in several orders on *L. decemlineata* and *E. varivestis* (Steets and Schmutterer 1975), *Dysdercus koenigii* (Koul 1984), *B. mori* (Koul et al. 1987), *L. migratoria* (Sieber and Rembold 1983) and *R. prolixus* (Garcia et al. 1986).

G. Effects on feeding behaviour

In most phytophagous insects, antifeedant together with physiological effects can be found against botanical pesticides. Hemipteran insects are less susceptible to antifeedant properties of AZT than Lepidoptera. As a result, hemipteran insects feed sufficient quantities of the compound to produce profound physiological effects. After being systemically treated with AZT, aphids fed normal initially. Then, the next feed is significantly de-

layed and following feeding activity is suppressed (Nisbet et al. 1993). Most of the active principles of terpenoids found in AZT and other related analogues are responsible for insect antifeedant and insecticidal properties (Isman 1997) by its biological activity (Mordue and Nisbet 2000). Insect's antifeedancy is the incapability to ingest food, resulting from the sensitivity of the antifeedant (Schmutterer 1985) is important in some selected lepidopteran and orthopteran species. The desert locust is very sensitive to AZT dependent upon species. It is highly interrelated with the sensory response of chemoreceptor on the insect's mouthparts (Mordue et al. 1998). Feeding behaviour depends upon both neural input from the insect's living senses and neural integration of this sensory code. Azadirachtin stimulates specific deterrent cells in chemoreceptor and blocks the firing of sugar receptor cells, which stimulate feeding (Blaney et al. 1990) results in inhibition of feeding, terminating in starvation and ultimately death. The antifeedant effect reliable efficacy is linked to the physiological action of AZT as an insect growth regulator, which is remarkable in the locust, that is highly variable among pest species and even those species initially discouraged are often able of rapid desensitization to AZT (Bomford and Isman 1996). The component responsible for the antifeedant activity of neem extracts to the desert locust was identified as AZT (Butterworth and Morgan 1968) and its activity was demonstrated on diverse groups of insects, particularly lepidopteran larvae (Warthen et al. 1978). Newly hatched house crickets *Acheta domesticus*, when fed, had less weight and development than controls and the effect was proportional to the concentration of the antifeedant (Warthen and Uebel 1981). Contrary to the observation on *S. frugiperda*, the nymphs of the house cricket that were fed on treated diet, when transferred to normal diet, developed normally. Antifeedant activity of AZT is not a universal phenomenon in insects and exceptions do occur. The webworm larvae treated with low concentrations of AZT showed that on the first day consumption index, larval body weight gain and growth

index decreased with increasing concentrations, whereas the digestibility and efficiency of consumption of food to body mass were highest at highest concentration (Fagoone 1984). Subsequently, feeding on untreated food led to a rapid decline in food utilization efficiencies and also reported in case of tobacco budworm *Heliothis virescens* (Barnby and Klocke 1987). The antifeedant effects of AZT are partly due to sensory detection and avoidance by insects (Schoonhoven and Jermy 1977) and partly due to feeding controlling centres (Sieber and Rembold 1983).

H. Effects on biological fitness

Azadirachtin changes the biological fitness with reduced lifespan, low absorption of nutrients (Wilps 1989), high mortality (Dorn et al. 1987), loss of flying ability, immune depression (Azambuja and Garcia 1992), enzyme inhibition (Naqvi 1987) and disruption of biological rhythms (Smietanko and Engelmann 1989) in insects. This means that AZT has direct biological effects on different insect's tissues and cells, especially on those with rapid mitosis in gonads.

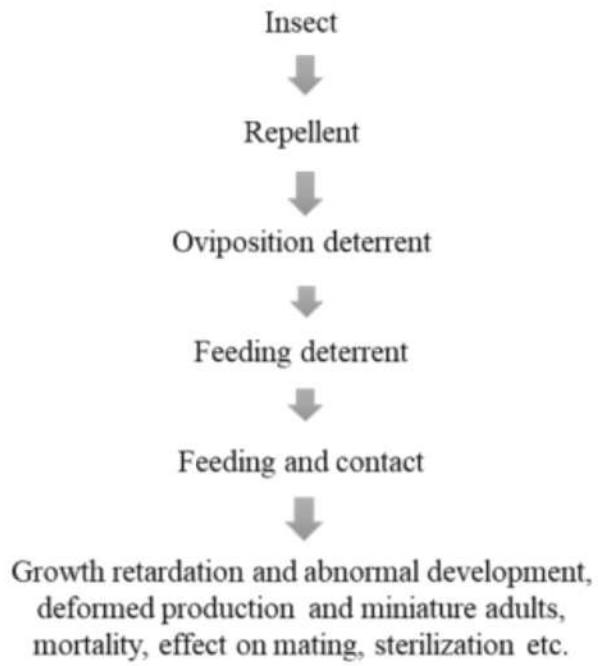

Plate 8. Biological effects of AZT on insect.

Table 2. The overall effects of AZT against insect. (Mordue and Nisbet 2000)

Effects	Target	Mode of action
Primary antifeedancy	Mouthpart & other chemoreceptors	Deterrent cell stimulation Sugar cell inhibition
Secondary antifeedancy	Gut	Peristalsis inhibited Enzyme production reduced
		Midgut cells not replaced
Insect growth regulation	Cuticle	Alterations to ecdysteroid and JH titres by blockage of release of morphogenetic peptides leading to moulting defects
Sterility	Reproductive organs	Alterations to ecdysteroid and JH titres leading to reduction in number of viable eggs and live progeny
Cellular processes	Dividing cells	Blockage of cell division post metaphase in meiosis and mitosis
	Muscles	Loss of muscle tone
	Cell synthetic machinery	Blockage of digestive enzyme production in gut Inhibition of protein synthesis in various tissues

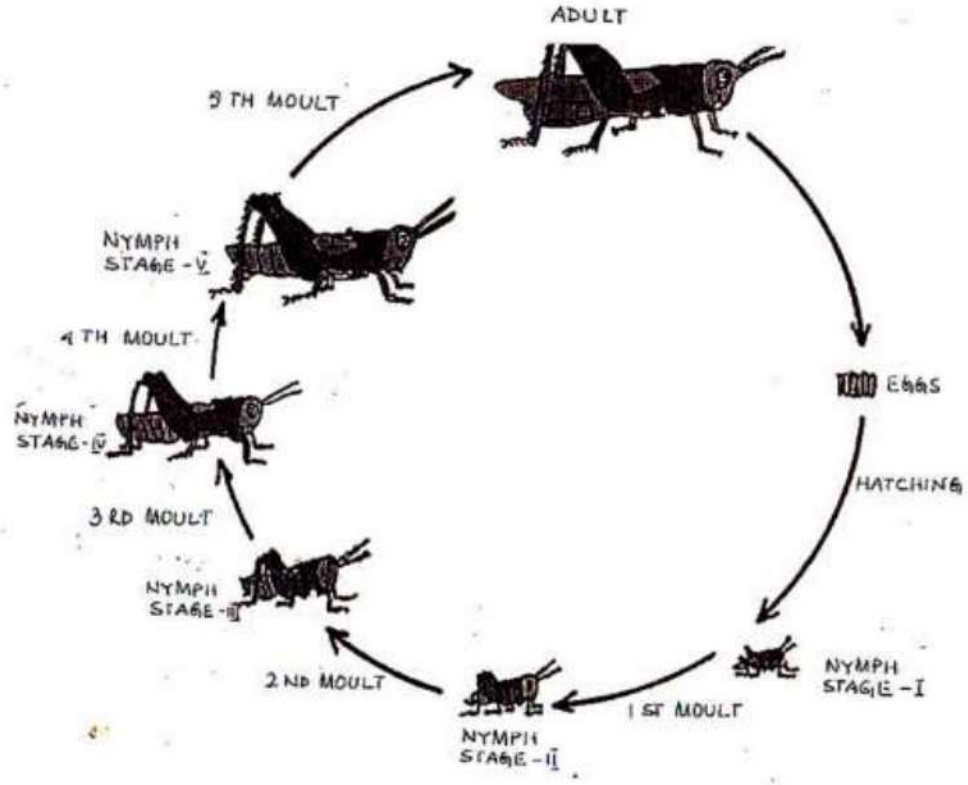

Plate 9. Life cycle of *Spathosternum prasiniferum prasiniferum*.

2.2. Order: Orthoptera

The Order-Orthoptera is one of the largest order of insects having over 17,250 species known to world-wide, of which more than 900 species recorded in India, which are dreadfully destructive locusts. The orthopteran insects are distributed largely depend upon the vegetation's prevailing in grasslands, forests and agri-fields. Temperature, rainfall and soil conditions are some important factors that determine the distribution of grasshoppers. India provides a unique habitat for this group of insects, for there exist humid grasslands in East and North-East India, semi-arid grasslands in North-West and Southern parts of India, vast agricultural fields, sub-mountainous forests and scrub jungles, vegetation adjoining lakes, river basins and numerous water bodies scattered throughout the country. The order is divided into two suborders viz. Ensifera and Caelifera (Ander 1939). Ensifera has two superfamilies' viz. Tettigonoidea and Grylloidea. Caelifera is divided into four superfamilies' viz. Acridoidea, Tridactyloidea, Eumastacoidea and Tetrigoidea (Dirsh 1961). Superfamily-Acridoidea displays maximum diversity and is divided into five families of which families Acrididae and Pyrgomorpbidae are widely distributed in India. Acrididae is divided into 17 subfamilies and altogether over 6000 species and 1000 genera are known from the world-wide, of which 310 species under 138 genera and 14 subfamilies are known from India. From West Bengal 69 species under 49 genera have been recorded (Hazra et al. 1992). Family-Pyrgomorphidae divided into some subfamilies. The Subfamily-Pyrgomorphinae includes 440 species under 148 genera reported from all over the world, of which 40 species under 19 genera are known from India, while 8 species under 5 genera are known so far from West Bengal.

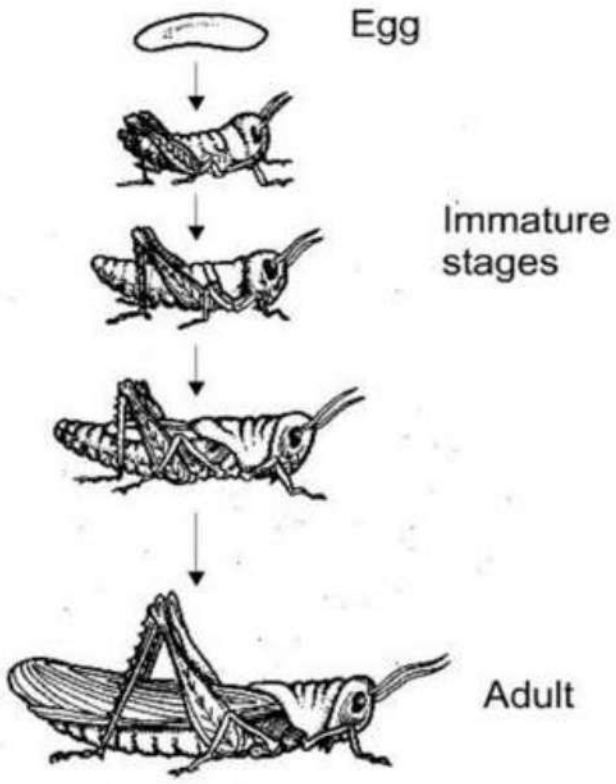

Plate 10. Life table of Class-Insecta. (hps://allyouneedisbiology.files. wordpress.com/2015/07/heterome tabolo.jpg)

Azadirachtin has appealed world-wide consideration not only as the most popular deterrent to insects but also a favourable growth regulator. The rising interest on AZT is specific to detail physiological and biochemical investigations on the activity of this compound. Here, I am reviewing the literature of toxicological effects of AZT on insects.

The primary goal of this work is to highlight on the potential of grasshopper as multidisciplinary species model in the field of environmental changes. Grasshoppers are effective indicator because of their high mobility, wide variety of microhabitats and sensitivity to anthropogenic activities. The present study will provide initial references as an avenue in the literature and it lends support to the suggestion that grasshopper are potentially useful bio-indicator for ecological disturbances. Therefore, *S. pr. prasiniferum* is a promising model for toxicological assessments of chemicals and environmental contaminants.

2.3. Scope of the present investigation

Phylum-Arthropoda is considered as the largest invertebrate phylum of the global biodiversity. Family-Acrididae under Order-Orthoptera is a short-horned-grasshopper with ecological, economical, nutritional and medicinal importance. Natural habitat of this species often faces the risk of contamination by diverse xenobiotics including AZT based pesticide. Azadirachtin is a newly introduced neem derived botanical pesticide, which adversely affects the physiology of this species.

Present study would provide baseline information of toxicity of AZT based pesticide in this species. Generated data would provide important entomological information in reference to toxicity of AZT and related antioxidant response of a specific sex of this species, an important bio-indicator species of agro-ecosystem.

The review of literature presented reveals that although information is available on the effect of AZT on the feeding, biology, survival and biochemical profiles of insects. Here, the effect of AZA on *Spathosternum prasiniferum prasiniferum* (Orthoptera: Acridoidea) needs in depth investigation. Hence, the present investigation was carried out to examine the dose dependent effect of AZA on OS and antioxidant defence status in relation to both sexes of *S. pr. prasiniferum*.