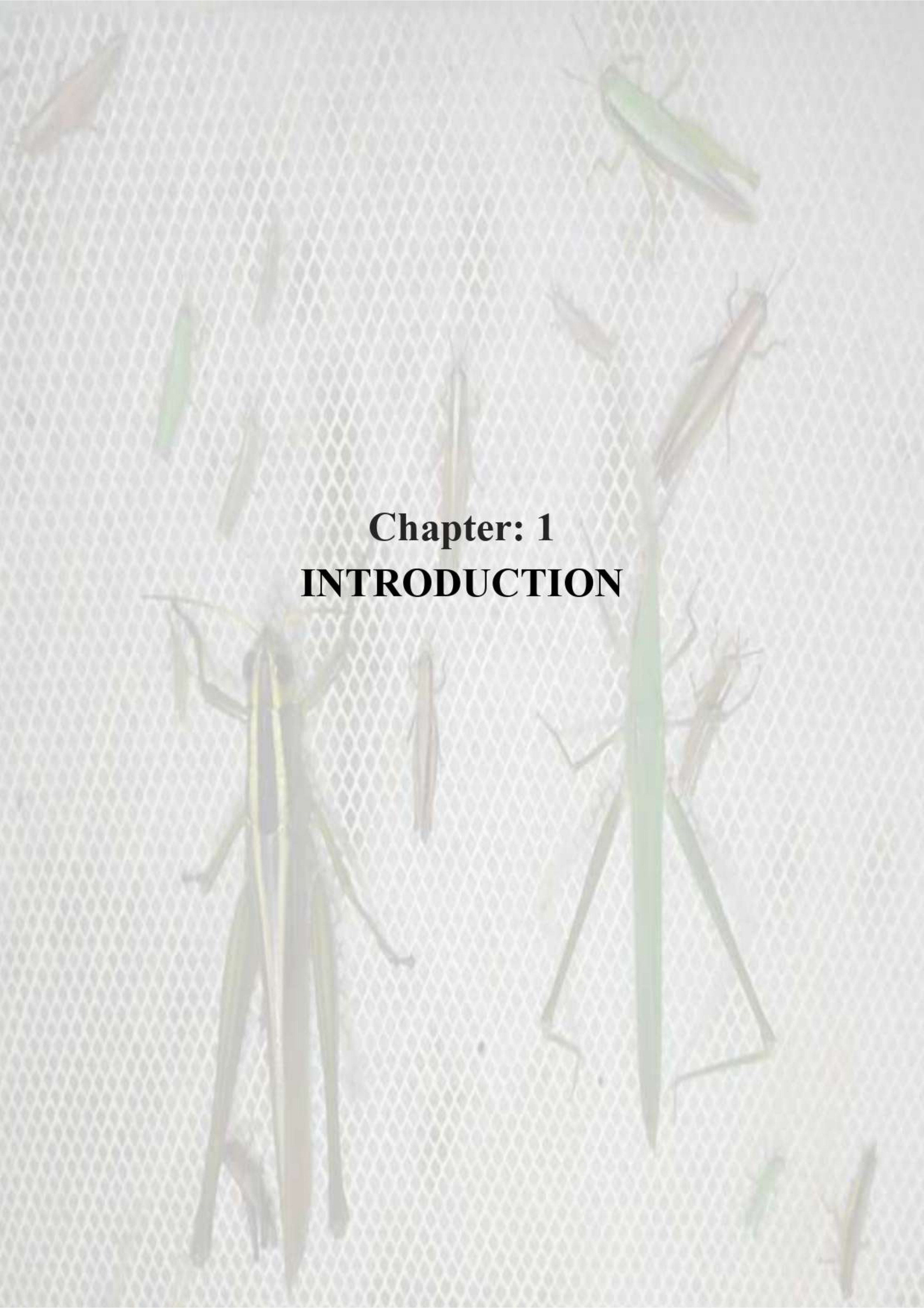

Chapter: 1

INTRODUCTION

1. INTRODUCTION

Toxicology is a branch of biological sciences, chemical sciences and medical sciences which concerned with the study of the harmful effects of biological, chemical and physical agents in any biological systems that cause significant damage in living cells/organisms. This also includes studies on the metabolism, action and excretion of toxins and the treatment of poisoning as well as the systematic chemical and physical analysis and diagnosis. Most of the chemicals are toxic to biological systems at very high doses. Evaluation of toxicity of a chemical could help in knowing its potentiality, so that new and more powerful formulations could be derived. It has now become a normal practice to test all new agro-chemicals for the toxicity before they reach to the consumers. The dosage of any compound is always a crucial factor in determining its effects (Hayes 1975). Hence, it is important to measure the toxicity i.e., the determination of the dose at which toxicant produces harmful response on a living organism. The toxicity of a pesticide to an organism is usually expressed in terms of LD_{50}, i.e., the amount of chemical/unit weight of an organism required to kill 50% of the test individual. The LD_{50} is usually expressed in mg/kg of body weight under certain conditions; the term μg/insect may be used when the chemical is applied topically to the insect. Value of the LD_{50} is generally estimated against time schedules, such as 24-hrs, 48-hrs, 72-hrs etc. Different investigators adopted different methods to assess the lethality/mortality of the pesticides. The LC_{50} is the concentration of the chemical required to kill 50% of the test individual in fixed time schedule. This is generally practiced for aquatic organisms. The term LT_{50} represents the time required for 50% mortality of the test organisms in a specified dose or concentration. This method is used when the number of test organisms is limited. In some instances KD_{50}, KT_{50}, ED_{50} and ET_{50} is used to assess the results of toxicity studies. The lethality of the pesticides is generally expressed as LD_{50}. There are several ways of direct-ing a pesticide to an insect. A common method employed is topical application. The toxicity of the animal is also influenced by biotic factors, nutritional status (Pal and Kushwah 1981), species specificity (Surendranath et al. 1987), sex (Klein et al. 1983) and also the developmental stage of the animal. Liberal work has been done on the ecotoxicological properties of organophosphates (OPs) and organochlorines (OCs) (Oliver et al. 2001) and the work on biological pesticides like azadirachtin (AZT) is almost lacking.

The foremost challenges in ecotoxicology are to create efficient ways to predict toxic power and exposure levels for pesticides that lack toxicological data in ecological settings. The demand is to assess large number of pesticides for hazard identification in a cost and time efficient manner. Therefore, the need is to generate high throughput toxicity assays coupled with ethical concerns over animal testing necessitated the pursuit of better tools for ecotoxicological studies. Hence, the development, validation and application of high throughput alternate animal models for ecotoxicity studies are high priority in ecotoxicology. The information on usage, exposure and effects obtained from quantitative structure activity relationships, read across methods, thresholds of toxicological concern and in-vitro tests prior to in-vivo testing are ideal routes for more rapid, efficient and cost effective risk assessment of pesticides. A major task is the development of diagnostic capabilities to specifically determine the cause effect relationships within impaired ecosystems. This will help in determining the extent to which existing remediation strategies are effective and the modifications needed in risk management.

The era of insecticides started with the invention of dichlorodiphenyltrichloroethane (DDT) in 1939. This marked the era of chlorinated-hydrocarbon insecticides with the subsequent synthesis of hexachlorocylclohexane and cyclodiene compounds. These chlorinated hydrocarbon insecticides were generally persistent and have the long residual properties. Their stability and hydrophobiaty resulted in the contamination of the environment and bio-accumulation in food chains. This resulted in the need for synthetic production of OP compounds. These compounds were less persistent but were highly toxic to mammals. To lower the mammalian toxicity and increase in the efficacy, carbamates made their entrance in 1940. The drawbacks such as persistency and mammalian toxicity of carbamates were overcome by pyrethroids. Pyrethroids have low mammalian toxicity, non persistency and high toxicity to insects coupled with remarkable effectiveness in causing insect loss of coordinate members (Casida and Quistad 1995). Conventional synthetic pesticides have been used world-wide in substantial amounts with high expectations. To a large extent the Green Revolution and multiplication of global food production can be traceable to these chemicals. However, their use and potential have both come under serious doubts with indisputable evidences of their less acceptable attributes surfacing dramatically over the last few decades. Apart from high general toxicity, these have included lesser bio-degradation, leading to high persistence and greater distribution, bio-accumulation, non-target beneficial parasites, predator's toxicity, leading to pest reappearance, high target-species eradication leading to secondary pest outbreaks and induction of resistance in the target pests, leading to synthesis and development of newer bio-poisons.

Humans are exposed to many pesticides through the environment. In order to understand the risk to human health of pesticides exposure, it is necessary to understand how these pesticides may affect normal cellular processes and so lead to toxicological consequences. The advent of high throughput genomic screens has led to the possibility of much greater extent of understanding of the effect of pesticides in biological systems. Moreover, there has been interested in the possibility of using the output of these assays as a signature of pesticides exposure, thus as a test procedure for the recognition of toxicological hazard. The living animal cell neutralized its oxidative stress (OS) by several mechanisms by producing antioxidants which are either naturally produced in body or externally supplied supplements. Cellular antioxidants act as free radical scavengers and therefore can enhance the molecular defense and lower the risk of regenerative and degenerative diseases. Reactive oxygen species (ROS) can be generated during UV-light irradiation, by x-rays and γ-rays, during metal catalyzed reactions, by neutrophils, eosinophils and macrophages during inflammatory cell activation, as by-products of mitochondrial catalyzed electron transport reactions, by cytochrome-P_{450} metabolism and the enzyme xanthine-oxidase, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid. Highly reactive molecules called free radicals (FRs) can cause tissue damage by reacting with polyunsaturated fatty acid (PUFA) in cellular membranes. There is some evidence that FR-damage contributes many chronic non-communicable health problems such as cardiovascular disease, inflammation, cancer etc. Free radicals and its adverse effects were discovered in the last decades. Pesticide, alcohol, UV-light, cigarette-smoke, pollution lead to toxicity due to their FR initiation capability. Antioxidants are substances capable to mop-up FRs and prevent them from causing cell damage. In the normal cell there is a balance between formation and removal of FRs. And there is also a mild balance exists between antioxidant defense systems and pro-oxidant mechanism of tissue destruction, which is tipped in favour of tissue damage, could lead to significant supplement loss. Oxidative stress is defined as an imbalance between productions of FRs and their elimination by defense mechanisms, referred as antioxidants. This imbalance leads to damage to important bio-molecules and cells, with potential impact on a whole living organism. The harmful effects of ROS are balanced by the action of antioxidants. Excessive uses of synthetic insecticides have negative effects on natural enemies and causes environmental pollution. Synthetic pesticides can be prohibited by using more biodegradable materials with greater discrimination (Raguraman and Singh 1999) and represents bio-control agents for the environment. For these reasons, the alternative and eco-friendly methods of pest control are encouraged (Hogsette 1999). One such alternative is the use of new botanical insecticides (Isman 1994) that are effective against target organisms and have shorter persistence in ecosystems (Stark and Walter 1995). Botanical insecticides like chemical insecticides have toxic, repellent and antecedent effects on their target pests (Dimetry and Schmidt 1992). Botanical insecticides should never be considered as the only means of plant protection but have negative impacts on the environment and human health, like synthetic insecticides. Evaluation of the side effects of insecticides on beneficial insects has attracted much research (Simmonds et al. 2002). Pesticides toxicity may produce an imbalance between the endogenous and exogenous ROS and can decrease in antioxidant defences and cause oxidative damage in living organisms (Valavanidis et al. 2006) specifically on lipids, proteins and DNA (Ballesteros et al. 2009). The endogenous enzymatic antioxidants for the change of ROS to harmless metabolites are protected normal or regular cellular functions (Bebe and Panemangalore 2003). Antioxidant defence systems such as superoxide-dismutase (SOD), catalase (CAT), glutathione-peroxidase (GPx) could inhibit ROS formation or clear ROS in-vivo, thereby preventing OS (Ahmad et al. 2000).

Problems enumerated above has brought to fore the concept of integrated pest management (IPM) which includes the development and use of plant derived pest control agents as its integral and important feature. By its ubiquity, abundance and historical biomedical importance, the Indian neem tree has become the inauguration pack and sheet anchor of the sustainable low-input agriculture movement. Known since long for use as prophylactic against insects as well as specific sicknesses, traditionally the leaves of this tree have been most popularly used. Botanical insecticides are alternatives to synthetic-chemical insecticides for pest controlling because botanicals reputedly pose little threat to the ecosystem. Botanical insecticides have been considered for insect antifeedants or repellents but apart from, it modifies insect's behavior. Azadirachtin ($C_{35}H_{44}O_{16}$, AZT) is a tetranortriterpenoid obtained from most auspicious plants *Azadirachta indica* A. Juss having a varied spectrum of biological action (Mousa et al. 2008) with edible fruits and aromatic leaves (Talpur and Ikhwanuddin 2013). Parts of neem tree have been known to have a wide range of pharmacological, antifeedant, repellent, insecticidal, anti-ecdysis and sterilant properties (Biswas et al. 2002). It is the most important active component which shows variable effects on insect pest's oviposition, feeding deterrence, growth regulation, fecundity and fitness reduction (Schmutterer 1990). Although AZT is considered generally safe to beneficial, there are reports of its adverse effects on parasitoids (Condor Golec 2007). The bioactivity of AZT to acridids pests continues to increase, yet only a handful of botanicals is currently used in agri-farming in the industrialized world and there are few prospects for commercial development of novel botanical products has been documented. Azadirachtin are well established commercially available pesticides based on plant extracts have recently entered the market place and the use of rotenone appears to be declining. Several features appear to limit the success of botanicals, most notably regulatory barriers that are cost effective and relatively safe compared with their precursors. In the context of agri-field insect management, botanicals are best suited for use in organic food production in developed nations but can play a considerably greater role in the production and post-harvest protection of food for sustainable agriculture in modern countries. Botanical insecticides are reviewed as natural products which have received regulatory approval as antifeedants. Especially neem based products have made a relatively modest impact in the field. Essential oil products have recently emerged as the most important botanical insecticides.

Environmental changes, viz. fragmentation of habitat (Nufio et al. 2011), landscape changes (Steck 2007), industrialization (Jana et al. 2006), overgrazing (Branson and Sword 2010), invasion, over exploitation of exotic species, climate change and wildfire (Thomas et al. 2004) are the major threats to ecosystem health. Biological conservation typically involves either setting aside large tracts of land for nature or addressing and remediating specific processes that threaten large and charismatic invertebrates such as endangered insects or plant species or communities. The concept of protecting habitats for insects, thereof, seems of low priority on a threatened planet (Meffe and Carroll 1994). Various ecological studies strongly suggest that ecosystem drivers such as grazing, fragmentation and climate can adversely affect biota and ecosystem (Jonas and Joern 2007). Biodiversity study is now recognized increasingly as a vital parameter to assess global and local environmental changes and sustainable development (Jana et al. 2006). Indicator criteria for ecological changes are sensitive to changes, widely distributed, easy cost effective measurable, collectable, identifiable, ability to differentiate between natural and anthropogenic variations, relevant to ecological phenomena and economically important (Pearson 1994). There is a modern trend to use arthropod species especially grasshoppers are more appropriate indicator taxa (Kati et al. 2012). Grasshoppers constitutes the most abundant invertebrate groups and are leading consumers of plant biomass, hence played key role in ecosystem (Ingrisch and Köhler 1998). Grasshoppers especially belong to acridid family are major component in biodiversity model. The potential of grasshopper in relation to environmental changes has been documented by reviewing and compiling the scientific literature, which will clear the conservation strategy for grasshopper ecosystem management. Grasshopper is one of the most abundant insects in the agriculture field in India. As herbivores, grasshoppers might magnify heavy metals in their bodies and might transfer them to next tropic levels as found in case of aquatic insects (Jamil and

Husain 1992). Arsenic contamination, a magnifying problem in the Gangetic plains of India and use of contaminated water deteriorating soil fertility, can be taken up by plants and thereby enter the food chain (Meharg and Rahman 2003). Being a primary consumer and also preyed upon by other insectivores, grasshopper may transfer arsenic to higher tropic levels in the nature (Nath et al. 2008). Terrestrial birds were found to be highly adapted to arsenic compound and that was due to elevated concentration of these heavy metals (Koch et al. 2005). An orthopteran insect was founded flourished with increasing doses of arsenic, confirming not only eco-toxicological effects as found in *Aiolopus thalassinus* exposed to cadmium (Schmidt et al. 1991) but it may also influence bio-transfer of these toxic metals up to the highest tropic level in a food chain. Short-horned-grasshoppers are one of the most dominant and important herbivorous insects plays an important role in the functioning of agriculture ecosystems world-wide and potentially useful bio-indicators (Branson and Sword 2010) due to their abundance species composition (Branson et al. 2006) and their significance as a food resource for a variety of predators especially for reptiles and birds (Andersen et al. 2001). The structure of communities is sensitive to environmental changes, habit specificity, simple sweep net sampling methodology, identification is relatively easy due to low number of species and their taxonomic stability, their pronounced functionality in food web, attributed to their ability to recycle ground biomass to generate nutrients for other taxa (Samways 1994). Many studies imply effects of ecological changes of grasshoppers, among other things they have been used to assess the effects of forestfire land, overgrazing (Paschetta et al. 2012), habit reduction (Nufio et al. 2011), environmental factors (Kati et al. 2012), Ski run management (KeBler et al. 2012), edge effect (Bieringer and Zulka 2003), forest ecosystem management (Schmitz 2005), grassland managements (Marini et al. 2008), radiation (Møller and Mousseau 2011), pollution (Devkota and Schmidt 2000), urbanization (Cherrill 2010) and predation (Joern and Pruess 1986). Grazing also has the potential to harm grassland orthopteran insect's population, depending upon grazing intensity (Poyry et al. 2005) and through competition or indirectly via changes in plant community composition (Branson and Sword 2010). Grasshoppers were increasingly investigated locally for habit conservation, monitoring and restoration. Grasshopper communities clearly show that where anthropogenic disturbance is greater, the biodiversity of this group tends to decline. The sedentary nature of orthopteran insects gives rapid response to habitat disturbance, due in part to their high agility and rapid rate increase. Orthopteran insects can also be used to evaluate the short-term impact of environmental changes on a variety of habitats have resulted in the development of national and global models. Orthopteran insects are widely distributed in all agricultural ecosystems with significant economic importance due to their destructive role to almost all type of green vegetation. Among Class-Insecta, the Order-Orthoptera is one of the largest having over 20,000 species world-wide with about 10% of the total world species has been recorded from India (Tandon and Hazra 1998). Acridoidea is a superfamily of insects in the Order-Orthoptera. They are commonly known as the short-horned-grasshopper and placed in the Suborder-Caelifera. Grasshoppers have antennae that are more or less always shorter than the body and short ovipositors. Several species of short-horned-grasshoppers of the Family-Acrididae have solitary and swarm phases and can cause considerable damage to crops. Since the civilization, insects have been plaguing human societies. Various botanical, chemical and mechanical means to control insect pests have been employed to protect the food supply from time to time. In an unremitting struggle against insect, chemical control has come to play the most important role. The history of insecticides may go back as far as 2500 BC when Sumerians already knew sulfur like powders to have insecticidal properties (Perry et al. 1998). The era of insecticides started with the discovery of insecticidal properties of DDT by Paul Muller in 1939. Since that day, more than 1,00,000 organic chemicals have been evaluated for potential insecticidal action and more than 1000s are in commercial use. Pesticides remain in many instances our sole weapons of defense to combat insect pests and diseases. In a country like India, growing population is imposing increasing demand on agricultural productivity, in order to feed the teeming millions. On the otherhand, insects in the form of pests are causing alarming damage to the food crops. To combat this adverse situation there are many pesticides available. Pesticides are widely used for the control of agricultural and household pests (Kyvic and Bente 1995). The pesticides were successful in controlling pests during the past five decades, thereby minimizing losses in agricultural yields. Unfortunately, many of these chemicals are harmful to human and other beneficial organisms, thereby causing ecological disturbances. The drawbacks of pesticides fall under four main categories, viz. environmental contamination, human health effect, insect resistance, resurgence and higher farming cost.

The indiscriminate and ill planned use of pesticides is also posing a serious threat to human health due to residues in food (Turi et al. 2000). Alarming rise in the number of deaths from pesticides is attributed to the increasing number of toxic chemicals and their large-scale use without proper testing and knowledge. Lack of community awareness about the hazards of the chemicals is also a cause of death in children and in families using pesticide containers. The food poisoning in Basti district of U.P. (1996), taking a toll of 150 lives and the incident in August 2001 at Nulvadi district in Assam (India) are best examples of pesticide contamination. Pesticides also cause occupational hazards for the people who work in manufacturing plants (Oliver-Silva et al. 2001). In 1984 disaster at the Union Carbide pesticide plant at Bhopal, took a human toll of 3800, leaving thousands of others with lingering disability. A survey conducted in Costa Rica during 1982, 1987, 1992 showed thousands of human killed and others with disabling skin and eye injuries

(Wesseling et al. 2001). In the summer of 1995, 30 tonnes of *Anguilla anguilla* died in Lake Balaton, Hungary. An investigation revealed that the eel devastation was due to residues of pyrethroid, deltamethrin (Nemcsok et al. 1999). Pest control operators must use pesticides in a most safe and judicious manner, so as to minimize these health hazards and environmental pollution. Thus we should attempt towards achieving balance between protecting our health and producing sufficient food (Hadwinger and Miller 1979). Our failure to recognize the damaging consequences of new pesticide, has resulted in well known undesirable side effects such as resistance, pest resurgence and increased pest population, hence the term pesticide is an all inclusive word meaning the killer of pests. Pesticides are legally labelled or classified as poisons and are defined as substances used for controlling, preventing, destroying, repelling or mitigating any pest. Depending on the nature of the pest intended to be controlled, pesticides are classified as insecticides, fungicides, rodenticides etc. Insecticides represent a group of pesticides that are used against insect pests. Sometimes, however they not only kill a pest, but also affect beneficial organisms whose control is not intended. Hence a general term 'biocide' has been preferred by many authors. Pesticides are classified into two main groups, based on their mode of entry and their chemical nature. Based on their mode of entry into the body, the pesticides are classified into three types (Perry et al. 1998). These are contact poisons (pyrethrum, azadirachtin etc.), stomach poisons (arsenic radicals, flurosilicate, salts of lead and sodium etc.), inhalation poisons (rotenone, phosphine, methyl-bromide etc.). Based on their chemical nature, they are classified into three types, i.e., inorganic compounds (arsenicals, mercurials, borates etc.), organic compounds (organochlorides, organophosphorous compounds, carbamates etc.), natural organic compounds derived from plants (pyrethrum, azadirachtin, rotenone, derris etc.). After the initial elucidation of neem's biological activities and major chemical constituents, there have been commendable works on exploring the range of activities as well as target species of neem extractives. As mentioned earlier, AZT itself has been shown to exert antifeedant as well as insect growth regulatory effects, though it is not often realized the effects obtained in total or fractional neem extractives need have a direct quantitative, correlation with AZT content. Azadirachtin is quite patently the single most potent neem constituent with pronounced and multi-pronged biological activities.