CHAPTER I

Introduction
Introduction

INTRODUCTION

Pesticides are agents used to kill or control undesired pests, such as insects, weeds, rodents, fungi, bacteria or other organisms. The term “pesticide” includes insecticides, herbicides, rodenticides, as well as disinfectants, fumigants and wood preservatives. These compounds have a vital role in controlling agricultural, industrial, home/garden and public health pests globally. Because they have the ability to reduce the level of vector born diseases and have offered lower cost, better quality goods and services to society, the public has been tolerant of their use. However, these economic and health benefits are not achieved without potential risk and possible adverse health effects to humans, domesticated animals and the environment. It has been estimated that 85-90% of the pesticides applied in agriculture never reach their target organisms, but instead are dispersed in the air, water and soil. Based upon such estimates, pesticide exposure is likely for non-target organisms. It is very likely that many non-target organisms are exposed to multiple pesticides throughout their lifetimes, either sequentially or concurrently (Repetto and Baliga 1996; Mumtaz, Poirier et al. 1997).

Pesticides are beneficial to humans in many ways; they are a group of chemicals of particular concern due to their deliberate introduction into the environment and their often inherently toxic nature that is required to exert their control over unwanted pests. Humans in the general population may incur long-term exposure to pesticides through the ingestion of residues in/on food and in drinking water. Individuals working with pest control products have the potential to be exposed to pesticides over an extended period, as do residents through the use of pesticides in and around the home. For these reasons, pesticides are highly regulated to ensure an appropriate level of human safety and to ensure that levels of pesticides to which humans may be exposed are within acceptable limits. Pesticides enter the human population primarily through consumption of food products (Gilden, Huffling et al. 2010; Repetto and Baliga 1996). Despite the tenfold increase in insecticide use from 1945-1989, total crop losses from insects have nearly doubled from 7% to 13%. This pesticide treadmill
effect is partly due to destruction of beneficial organisms by pesticides that otherwise would have contributed to pest biocontrol (Pimental and Greiner 1997).

From the health perspective, the potential for occupational and public exposure to multiple chemicals, either concurrently or sequentially, is very likely over the course of an organism’s lifetime. As awareness of chemical usage grows, so does interest in what type of effects these chemical exposures are having on an organism’s health. The health effects of many pesticides have recently begun to be examined and more investigations are needed.

The four primary factors determining the potential adverse effects of a pesticide are: inherent toxicity, stability, solubility, absorptivity, and characterization

Types of pesticides may be characterized by their "target" organism

a) herbicide - weeds, b) insecticide - insects, c) arachicides - spiders, and d) rodenticide – rodents.

Different classes of pesticides are

I. Inorganic compounds (e.g. lead)
II. Organochlorines (e.g. DDT and endosulphan)
III. Organophosphates (e.g. malathion, parathion)
IV. Carbamates (e.g. aldicarb and carbofuran)
V. Pyrethroids (e.g. allethrin and dimethrin)
VI. Biological insecticides (e.g. pyrethrins and azadirachtin)

Pesticides are biologically active compounds intended to target a narrow range of organisms; however these agents can affect a much broader range of targets and organisms, including humans. As a result, there exist ongoing concerns about the health effects of pesticide exposure in humans. These concerns have been heightened by pesticide-related poisoning episodes that have occurred during the past 60 years, such as those involving malathion (Baker, Warren et al. 1978), methylparathion (Rehner, Kolbo et al. 2000), and methamidophos (Sumi, Oode et al. 2008). Commonly used organophosphates have included parathion, malathion, methyl parathion, chlorpyrifos, diazinon, dichlorvos, phosmet, and fenitrothion. Malathion is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control
programs such as mosquito eradication. An organophosphate (sometimes abbreviated OP) is the general name for esters of phosphoric acid. Organophosphates are the basis of many insecticides. Some examples of the commonly used organophosphorous insecticides are given in table 1.

**Table 1: Commonly used organophosphorous insecticides**

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Common name</th>
<th>IUPAC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Parathion" /></td>
<td>Parathion</td>
<td>O,O-Diethyl O-(4-nitrophenyl) phosphorothioate</td>
</tr>
<tr>
<td></td>
<td>Methyl Parathion</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Malathion" /></td>
<td>Malathion</td>
<td>Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl] butanedioate</td>
</tr>
<tr>
<td><img src="image" alt="Chlorpyrifos" /></td>
<td>Chlorpyrifos</td>
<td>O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate</td>
</tr>
<tr>
<td><img src="image" alt="Diazinon" /></td>
<td>Diazinon</td>
<td>O,O-Diethyl O-[4-methyl-6-(propan-2-yl) pyrimidin-2-yl] phosphorothioate</td>
</tr>
<tr>
<td><img src="image" alt="Dichlorvos" /></td>
<td>Dichlorvos</td>
<td>2,2-dichlorovinyl dimethyl phosphate</td>
</tr>
<tr>
<td><img src="image" alt="Fenitrothion" /></td>
<td>Fenitrothion</td>
<td>O,O-Dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate</td>
</tr>
</tbody>
</table>
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EFFECT OF ORGANOPHOSPHATE INSECTICIDES ON HUMANS

Organophosphate insecticides are used extensively worldwide, and poisoning by these agents, particularly in developing nations, is a serious public health problem. Malathion is one of the most widely used organophosphate compounds (OPCs) applied in agriculture as a pesticide, in veterinary practice as an ectoparasiticide (Flessel, Quintana et al. 1993), in eradication of human body lice (Roberts 2002) and in food preparation and processing areas (Savage, Keefe et al. 1981). However, its widespread use in agriculture and household practices has raised concern over its potential to cause adverse health effects in humans, animals, wildlife and fish (Flessel, Quintana et al. 1993).

With an increasing world population, use of pesticides is unavoidable. However, indiscriminate use of these chemical agents has resulted in several incidents of human intoxication. Often exposure to pesticides is accidental and mild, yet in some cases severe pesticide poisoning occurs, an estimated 3 million cases annually, with 99% of those being in third world countries (Banerjee, Seth et al. 2001). Administration of 24 mg malathion/day for 56 days in volunteer male aged 23-36 years caused depression of plasma cholinesterase activity two weeks after the first administration of malathion, the maximum depression being 25%, seen three weeks after cessation of treatment; erythrocyte acetylcholinesterase activity was depressed to the same extent (Moeller and Rider 1962). During a malaria eradication program in Pakistan in 1976, out of 7,500 spray men, 2,800 became poisoned and 5 died (Baker, Warren et al. 1978).

Aerial application of malathion in an area containing approximately 1.5 million people generated 1874 reports of pesticide related illness. The majority of complaints dealt with respiratory tract irritation, headaches, and gastrointestinal tract symptoms. The other 299 complaints dealt with skin rashes (Schanker, Rachelefsky et al. 1992). Researchers conducted a study evaluating the health effects followed by aerial malathion treatments in an area containing approximately 132,000 people. There were 230 reports of
pesticide related illness, and researchers classified 123 of these as probable or possible cases (Fabricio, Ramiro et al. 2010).

There are numerous case reports of individual poisoning. Dive et al. (1994), reported an instance in which an elderly woman consumed about 100 ml of a garden preparation containing 15% malathion in isopropyl alcohol. A typical cholinergic crisis was followed by cardiac, pulmonary, neurological, and renal manifestations. The cardiac manifestations included arrhythmia and conduction disturbances. Mild interstitial pulmonary fibrosis was observed in a lung biopsy sample. Matsushita, Aoyama et al. (1985) reported allergic contact dermatitis in people exposed to organophosphorous insecticides including malathion. An episode of epidemic hysteria was reported at an elementary school in Arizona, USA, in response to the smell of malathion (Baker and Selvey 1992).

Gupta, Dave et al. (1979), reported that a significant reduction in plasma and RBC cholinesterase activity was found in malathion exposed workers. Malathion significantly inhibited acetylcholinesterase activity in human lymphocytes and erythrocytes (Banerjee 1999; Datta, Gupta et al. 1994). Frequency of symptoms like dizziness, headache, lachrymation, burning sensation in eyes, nausea and anorexia, etc, was much more in the exposed workers. It has been established that in acute malathion insecticide poisoning, the CSF content of the stimulating mediator amino acids, aspartic and glutamic acids, rises within the early periods, whereas the concentration of the inhibitory mediator glycine decreases. The changes in protein fractions of the CSF are characterized by a fall of the content of globulins and a rise of albumins, thus attesting to the predominance of pathological processes in the brain, especially in the initial period of intoxication, and to the impairment of the blood-brain barrier. The development of intoxication is associated with activation of LDH isozymes which is viewed as the result of the membranotoxic effect of a malathion insecticide (Kolesnichenko, Dolgo-Saburova et al. 1992). In Ecuador, 10,000 out of 14,145 pesticide poisoning cases were reported due to the effects of the organophosphorous and carbamate insecticides (Fabricio, Ramiro et al. 2010).

Healthy agricultural sprayers, exposed to pesticides for 5 years, caused significant increase in lipid peroxide content. The concentration of
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Antioxidants such as glutathione (GSH) was significantly altered and the activities of antioxidant enzymes were remarkably elevated in sprayer populations, when compared to controls (Prakasam, Sethupathy et al. 2001). Furthermore, a study examined malathion exposure using human serum samples from individuals admitted for pesticide poisoning and found enhanced the levels of lipid peroxide content. Both, the level of blood and lymphocyte GSH and glutathione reductase (GR) activity were significantly decreased. Human blood sample obtained from malathion poisoning showed an increase in the level of lipid peroxide content and decreased level of GSH content (Banerjee, Seth et al. 1999).

Malathion under in vitro condition even at lower concentration (250 ppm) altered the level of enzymes associated with glutathione cycle and antioxidant defense system in human fetal brain and liver (Gupta, Datta et al. 1992). The organophosphate pesticides parathion and paraoxon at different concentrations increase the percentage of sperm with acrosome reaction and also increase the percentage of sperm with chromatin condensation in a dose-dependent manner in humans (Contreras and Bustos-Obregon 1999).

Many studies have addressed the association between cancer in humans and agricultural pesticide exposure. An association between pesticide exposure and cancer has been suspected following reports of the occurrence of cancer in European farmers using insecticides. In a few cases, the association between pesticide exposure and cancer has been confirmed (Blair and Zahm 1995; Zahm, Ward et al. 1997). Scientists have reported positive and negative mutagenicity for malathion. Epidemiological study indicated increased risk of lymphoma among grain mill workers using malathion (Alavanja, Rush et al. 1987). Buckley, Meadows et al. (2000) reported that a significantly increased risk of non-Hodgkin's lymphoma in children whose mothers had greater pesticide exposures during pregnancy, as well as in children who were exposed in the home. The chemicals most strongly associated with risk of non-Hodgkin's lymphoma were malathion, and dichlorvos (Cantor, Blair et al. 1992). Several reports indicated that organochlorine and organophosphorus compounds are associated with an increased risk of non-Hodgkin's lymphoma (Blair and Zahm 1995; Hoffman 1996; Rothman, Cantor et al. 1997). Viel and Richardson (1993)
reported that mortality was associated with increased pesticide use for leukemia and for myeloma.

Among the potential secondary biological consequences of pesticides, genotoxicity and carcinogenicity are of special importance. Clastogenetic effects of malathion were observed by Walter, Czajkowska et al. (1980) in human lymphocytes stimulated with phytohaemagglutinin. According to Walter, Czajkowska et al. (1980), malathion causes an increase in chromosomal aberrations. Chen, Hsueh et al. (1981) reported an increase in sister chromatid exchange frequency in cultured mammalian cells exposed to malathion. Malathion showed a dose dependent increase in the frequency of chromosomal aberration as well as sister chromatid exchanges in in vitro culture of human peripheral blood (Balaji and Sasikala 1993). Increased number of chromosomal aberrations, sister chromatid exchange frequency, micronucleus frequency, and values of comet assay parameters were observed in the blood samples of workers after they spent eight months in the production of malathion (Garaj-Vrhovac and Zeljezic 2002). Malathion alters the pattern of hypoxanthine-guanine phosphoribosyl transferase mutations in cultured human T-lymphocytes in an in vitro assay (Pluth, O'Neill et al. 1998). Significant increases in aneuploid sperm were seen in agricultural workers exposed to organophosphates in chinese factory workers (Padungtod, Hassold et al. 1999), and in indian applicators and sprayers exposed to a variety of pesticides (predominantly organophosphate insecticides). Moreover, increases in breakage/exchanges were also detected in the sperm of indian cotton field workers (Rupa, Hasegawa et al. 1997).

**EFFECT OF ORGANOPHOSPHATE INSECTICIDES ON ANIMALS**

The hematological parameters provide useful information on the balance between the production and destruction of cells of the circulatory system. Pesticides may cause increased destruction of red blood cells by directly damaging red blood cell membranes or by oxidizing hemoglobin. Increased immune responses brought about by exposure to a chemical may also result in the destruction of red blood cells (Bloom and Brandt 2001). Administration of
malathion residues (10 and 100 ppm) for 90 days showed changes in hemoglobin concentration in male rats (Neskovic, Karan et al. 1991). Bezencon, Durham et al. (1989) studied hematological changes in rats treated with technical grade malathion. Whole blood viscosity, plasma fibrinogen content and red blood cell (RBC) aggregation increased in treated animals compared to controls. The liver production of blood clotting factors was also damaged by trialkyl phosphorothioates, as evidenced by prolongation of blood clotting and increases in prothrombin and thrombin times (Keadtisuke, Dheranetra et al. 1990).

Acute toxic effects induced by malathion pesticides are mainly caused by inhibition of acetylcholinesterase (AChE) in the nervous tissue with a consequent increase in the levels of the neurotransmitter acetylcholine (Kwong 2002). The toxicity of organophosphorus compounds, such as paraoxon (POX), is due to their anticholinesterase action (Saleh, Vijayasarathy et al. 2003). Dermal application of malathion resulted 40% inhibition of acetylcholinesterase activity (Abou Zeid, el-Barouty et al. 1993). Feeding of bean-bound malathion residues to mice for 90 days resulted in inhibition of erythrocyte cholinesterase activity (Zayed, Farghaly et al. 1992).

Oral administration of malathion significantly decreased the activity of acetylcholinesterase in rats (Mathews and Devi 1994). It was observed that chlorpyrifos, an OPI (13.5 mg/kg body weight) treatment resulted in significant inhibition of serum and hepatic acetylcholinesterase (AChE) activities after 8 weeks (Goel, Chauhan et al. 2000). Daily dermal spray of malathion for four weeks in recommended (0.5 and 1.0 per cent) and higher (5.0 per cent) in Bubalis bubalis (Buffallo) species showed significant inhibition in the activity of cholinesterase in both RBC and plasma (Gupta and Paul 1978).

While acute organophosphorous compound poisoning due to inhibition of AChE is a well-established clinical entity, the existence of chronic poisoning due to exposure to low levels of organophosphorous compounds (below the threshold required for cholinergic clinical symptoms) is a hotly debated issue (Masoud, Vijayasarathy et al. 2003). Studies on organophosphate insecticide toxicity have focused on chronic intoxication, environmental contamination and diseases not immediately related to their toxic potential on
AChE such as Parkinson’s disease, skin, lung and immune diseases. Most of these diseases appear as long term and delayed health effects in agricultural workers and in populations exposed to environmental sources (Blasiak, Jaloszynski et al. 1999). In an effort to understand the pathophysiology of these non-cholinergic effects, both in vitro and in vivo investigations have been carried out, using concentrations of organophosphate insecticide comparable to their chronic exposure levels (Samimi and Last 2001; Saleh, Vijayasarathy et al. 2003). These studies have provided insights into the mechanisms of action of OPIs that are independent of AChE inhibition. Subchronic malathion exposure would affect gastrointestinal system in test animals. After 45 days of exposure, the animals showed a significant decrease in sodium and potassium ATPase activity which suggests the disruption of ion transport processes in intestine after pesticide exposure (Wali, Singh et al. 1984).

The toxicological literature is replete with studies attempting to explain the mechanism of action of organophosphorous insecticides to their anticholinesterase activities, but not much is known about the metabolism and detoxification of these compounds. Three microsomal enzymes; cytochrome P$_{450}$ dependent monoxygenase, carboxylesterase, and glutathione-S-transferase (GST) are responsible for the metabolism of malathion. In the enzymatic defense reaction, the chemical is first functionalized by phase I enzymes, usually by the cytochrome P$_{450}$ enzyme system, and then conjugated to a more soluble and excretable form by other enzyme systems, like glutathione S-transferases (Guengerich and Shimada 1991; Pelkonen and Raunio 1997). Sometimes, however, these enzymes transform an otherwise harmless substance into a reactive form (Guengerich 2000). Cytochrome P$_{450}$ constitute a superfamily of enzymes crucial for the oxidative, peroxidative, and reductive metabolism of a diverse group of compounds, including pesticides (Nelson, Koymans et al. 1996). The cytochrome P$_{450}$ enzymes are involved in the oxidation of xenobiotic chemicals including drugs, pesticides, and carcinogens (Sheweita, El-Gabar et al. 2001). Malathion treatment in rats significantly depleted liver glutathione (35%) content with stimulation of glutathione-S-transferase (50%) and inhibited the activity of mixed-function oxidases (Srikanth
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and Seth 1990). Fenitrothion, an OPI (100 µM) decreased the activities of GST (El-Shenawy 2010). Technical grade malathion contains approximately 92 to 98% parent compound and is responsible for direct inhibitor of cholinesterase requiring no metabolic activation to exert its toxic action (Clothier, Johnson et al. 1981; Thompson, Frick et al. 1989). Several co-products found in the malathion technical mixture are carboxylesterase inhibitors and may contribute to the potentiation of malathion toxicity in mammals. The mechanism of this potentiation is thought to be the inhibition of carboxylesterases, the enzyme responsible for the metabolism of malathion into the relatively non-toxic alpha and beta monoacids of malathion and malaoxon. This relationship has been demonstrated by a number of investigators (Mallipudi, Talcott et al. 1980; Malik and Summer 1982). Nemery (1987) reported metabolic alkalosis shortly after exposure to technical grade malathion at a dose of 10 mg/kg. Kidney damage induced by technical grade malathion was studied microscopically by Keadtisuke, Dheranetra et al. (1990).

There are numerous cellular defenses, which under normal metabolic conditions, regulate the level of reactive oxygen species (ROS) and protect against the ill-effects of free radicals (figure 1). Glutathione (GSH) is a hydrophilic tripeptide and is critical to the detoxification processes of xenobiotic metabolism. Antioxidant scavenging enzymes include superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Superoxide dismutase (SOD) dismutates superoxide radicals to hydrogen peroxide (H₂O₂). Catalase decomposes H₂O₂ to water. Glutathione peroxidase (GPx) decomposes organic hydroperoxides and this enzyme utilizes GSH, which is oxidized to GSSG, as a second substrate. Glutathione reductase (GR) catalyzes the reduction of GSSG to GSH using NADPH as an electron source (Yu 1994). Many compounds have been found to result in free radical generation and have the potential to promote cell injuries. Pesticides may induce oxidative stress leading to generation of free radicals and alterations in antioxidants or free radical scavenging enzymes.
Nowadays, people's exposure to chemical compounds such as organophosphorous insecticides is continuously on the rise. These compounds have induced an excessive production of free radicals which are responsible for several cell alterations in the organism. Oral administration of dimethoate, an OPI in rats caused significant increase in hepatic malondialdehyde content and increase in the activities of SOD and GPx while catalase (CAT) activity was significantly reduced (Saafi, Louedi et al. 2010). Toxicity of OPI is mainly due to inhibition of acetylcholinesterase (AChE), but many authors postulate that OPI in acute as well as in chronic intoxication disturb the redox processes, changing the activities of antioxidative enzymes and causing enhancement of lipid peroxidation in many organs. In the rat brain, the activities of antioxidant enzymes such as SOD, catalase, GPx and GR were found to increase, while GSH level was decreased in chlorfenvinphos (an OPI) intoxication at a dose of 0.3 mg/kg/day (Lukaszewicz-Hussain 2008). Fenitrothion, an OPI (100 µM) increased the cellular lipid peroxidation (LPO) levels and decreased the activities of the antioxidant enzymes like SOD, GPx and GST indicating an oxidative stress (El-Shenawy 2010). Malathion is a pesticide with high potential for human exposure. However, it is possible that during the malathion metabolism, there is generation of ROS and malathion may produce oxidative stress in intoxicated rats. Malathion administration induced oxidative stress and modulated SOD and...
CAT activity in selective brain regions in rats (Fortunato, Feier et al. 2006). The organophosphorous insecticide phosphamidon and malathion were found to elevate the level of lipid peroxidation (Datta, Gupta et al. 1994).

Malathion like other organophosphates, is detoxified via conjugation reactions with glutathione: In a study on rat hepatocytes, increasing concentrations of malathion (0.25 mM to 30 mM) depleted GSH in a dose-dependent manner and carboxylesterase activity was inhibited (Malik and Summer 1982). In vitro administration of malathion (2 and 4 mM) on rat blood, liver and kidney homogenates caused a depletion of liver GSH content during the entire exposure period (Lechner and Abdel-Rahman 1986).

In rats, administration of malathion (20 ppm) for 4 weeks increased the malondialdehyde (MDA) levels in serum, and decreased the GSH level in whole blood (Ahmed, Seth et al. 2000). Rate of lipid peroxidation was found to be increased in all parts of the brain following intraperitoneal injections of malathion (150 mg/kg body weight for 7 consecutive days) to albino rats (Haque, Rizvi et al. 1987). Treatment of OPIs, methyl parathion, methyl paraxon, and fenitrothion in mice markedly depleted GSH levels and potentiated the acute toxicity (Sultatos, Huang et al. 1991). Malathion and isomalathion caused a time and dose dependent depletion of hepatocellular GSH levels in rats (Malik and Summer 1982).

Treatment of formulation grade malathion produced significant decrease in dam weight gain during pregnancy and a slight decrease in the number of implantations and number of live fetuses. The treated groups showed a significant reduction in placenta weight. Significant increase in external hemorrhagic spots was observed with malathion at 50 mg/kg and the high-dosage mixture group (Lechner and Abdel-Rahman 1984). Administration of malathion in pregnant rats significantly decreased the activity of antioxidant enzymes and GSH content and significant elevation of thiobarbituric acid reacting substances (TBARS) content in dams and pups (Mathews 1994).

Malathion has a teratogenic effect on mice spermatid differentiation, which compromises mostly the flagella, perhaps due to an alkylating effect that disturbs the normal assembling of tail structural protein.
components (Contreras and Bustos-Obregon 1999). Single intraperitoneal injection of malathion at a dose of 900 mg/kg bw affected the body weight of the dams (Kimbrough and Gaines 1968). Rats given malathion in the diet at about 240 mg/kg bw, a higher incidence of ring-tail disease was seen in treated than in control (Kalow and Marton 1961). Koizumi, Montalbo et al. (1988) found that oral administration of single doses of technical grade malathion to pregnant rats resulted in increased fetal deaths compared to controls.

There are several parameters which indicate hepatic toxicity. These include a variety of enzymes (e.g., alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH)) that can be released from hepatocytes into circulation following membrane damage. ALT is considered to be the most specific of these enzymes to indicate hepatocellular injury, whereas increased circulating levels of AST or LDH also may reflect muscle injury. Biliary toxicity is evaluated through the measurement of other enzymes, such as alkaline phosphatase (ALP). Normally, reduced activities of hepatic enzymes are not considered adverse toxicological outcomes.

Daily dermal spray of malathion for four weeks (0.5, 1 and 5%) showed significant elevation in the activities of serum AST, ALT and ALP with 1.0 and 5.0 per cent spray and enzyme activities remained altered even during post-medication. The extent of various biochemical changes were dose and time dependent (Gupta and Paul 1978). A single oral dose of malathion (687.5 mg/kg) to adult male albino rats, resulted in an increase in intestinal ALP activity (Saigal, Bhatnagar et al. 1982). The aspartate and alanine aminotransferases in the tissues of the snail, Pila globosa showed high catalytic potentials during malathion exposure in vivo (Sahib and Rao 1988). White leghorn cockerels were fed a diet containing 800 and 1600 ppm of malathion for 90 days caused a significant decrease in body weights and significant increase in liver/body weight ratio (Varshneya, Bahga et al. 1986). Feeding of bean-bound malathion residues to mice for 90 days resulted in a reduction in body weight and increased activities of serum AST and ALP were also observed (Neskovic, Karan et al. 1991; Zayed, Farghaly et al. 1992). Oral administration of dimethoate, an organophosphorous insecticide, caused hepatotoxicity as monitored by the increase in the levels of
hepatic markers enzymes like transaminases, alkaline phosphatase, and lactate dehydrogenase. These biochemical alterations were accompanied by histological changes marked by appearance of vacuolization, necrosis, congestion, inflammation, and enlargement of sinusoids in liver section (Saafi, Louedi et al. 2010). Increased leakage percentage of LDH, ALT and AST were detected in isolated rat hepatocytes treated with 100 µM fenitrothion, an OPI (El-Shenawy 2010).

Intravenous administration of sub-lethal doses of malathion and methyl-parathion at weekly interval for four weeks resulted in increase in heart and spleen weight. Short term (24 hr) and long term (4 weeks) treatment resulted in increased specific activities of liver enzymes like ALP, AST and ALT. Malathion had greater effect than methyl-parathion on the biochemical parameters studied (Jabbar, Khawaja et al. 1990). A significant increase in the activities of various serum and liver marker enzymes (ALP, AST, and ALT) were observed following 8 weeks treatment with chlorpyrifos, an OPI at a dose of 13.5 mg/kg body weight (Goel, Chauhan et al. 2000). Animals kept on low protein diets (5% and 10%) when exposed to 400 mg malathion showed significant increase in the activities of AST, ALT and ALP in liver, kidney, brain, lungs and spleen, while a marked inhibition in the activity of AChE was observed under similar treatment. (Vaishwanar and Mallik 1984). Malathion caused significant alterations in ALT, AST, and beta-glucuronidase activities in rats maintained on 16, 6, and 1% protein diets for a period of 3 weeks (Bulusu and Chakravarty 1984).

Both under in vitro and in vivo conditions, malathion has been shown to induce DNA damage, chromosomal aberrations (Blasiak, Jaloszynski et al. 1999) and malignant transformation (Cabello, Valenzuela et al. 2001). Flessel, Quintana et al. (1993) reviewed five in vivo animal studies and observed that exposure to technical grade malathion gave positive results for chromosomal damage. Salvadori, Ribeiro et al. (1988) reported that malathion induce mutations in somatic and germ cells in mice. Studies have demonstrated the ability of malathion to act as a strong positive alkylating and cause genotoxic effects through degradation of DNA. Technical grade malathion is a potent genotoxic
agent and may be regarded as a potential germ cell mutagen also. All the three acute doses (2.5, 5 and 10mg/kg) of malathion tested in the mice, induced significant dose-dependent increase in the frequency of chromosome aberrations and sperm abnormalities (Giri, Prasad et al. 2002). Organophosphorous pesticides induce changes in the epithelium of mammary gland influencing the process of carcinogenesis, and such alterations occur at the level of nervous system by increasing the cholinergic stimulation in rats (Cabello, Valenzuela et al. 2001).

In the present study, a commonly used organophosphorous insecticide malathion was used for the toxicity comparison. Malathion is a non-systemic, wide spectrum organophosphate insecticide (OPI). Malathion, in its pure form, is a clear, amber coloured liquid with skunk like odour. Its molecular formula is C_{10}H_{19}O_{6}PS_{2} and melting point is 330.4. It is slightly soluble in water (145mg/L at 20°C) and is miscible in organic solvents. Malathion is applied in million pound quantities worldwide because of its potent insecticide activity and relatively low mammalian toxicity compared with other organophosphate insecticides. It is considered a general use pesticide and is often utilized in situations where large urban populations or domesticated animals may be exposed (Rodgers and Ellefson 1992).

NEEM BASED FORMULATION

Compared to chemical pesticide, biopesticides are safe, but still it affects non target organisms including humans. Pyrethroids, a biopesticide, at high micromolar concentrations, target the glutamatergic system in mouse cortical and spinal cord tissues (Shafer, Rijal et al. 2008), and they could be implicated in some neurotoxic effects in mammals (Soderlund, Clark et al. 2002; Ray and Fry 2006). In the search for environmentally safe pesticides, much research has been done on the use of plants for the protection of crops in the field or in storage. Especially in tropical regions, the application of botanical material to protect a crop against insects is often traditional and centuries old. The one plant species that is probably best investigated for its effects against insects is the neem tree, *Azadirachta indica* A. Juss. (Meliaceae). All parts of this tropical tree
contain bitter compounds (van der Nat, van der Sluis et al. 1991) that often have an antifeedant effect and can interfere with hormonal processes in insects (Ascher 1993). The pesticidal nature of neem compounds has been well known since at least the 5th century BC. The most frequently reported indications in ancient ayurvedic writings are skin diseases, inflammations and fevers, and more recently rheumatic disorders, insect repellent and insecticide effects. (van der Nat, van der Sluis et al. 1991). In India, neem is extensively used in the traditional system of medicine. Aqueous neem kernel extracts were used as insecticides. (Gandhi, Lal et al. 1988).

The neem tree, *Azadirachta indica* (Meliaceae), also known as “margosa,” is indigenous to the arid parts of India. Azadirachtin was isolated as one of the major bioactive limonoids from the seeds of *A. indica*. Dried seed kernels may contain up to 0.9% azadirachtin. Its molecular formula is C$_{35}$H$_{44}$O$_{16}$. Melting point of pure azadirachtin is 160°C; at ambient temperature, azadirachtin is slightly soluble in water (1–3 g/l), readily soluble in polar organic solvents but insoluble in hexane. Azadirachtin is relatively stable in crystalline form if stored in the dark. Its laboratory half-life in mildly acidic solutions (pH 4–6) is 50–100 days at room temperature, but rapid decomposition occurs at higher temperatures, in alkaline and strongly acidic media, and especially in the light (Barrek, Paisse et al. 2004). Neem formulations typically retain over 59% of their azadirachtin content for about a year when stored at 10–15°C in the dark (Kumar and Parmar 2000).

The most important substance in neem seed oil in terms of insect phagorepellent and insect growth inhibition properties is azadirachtin, a C-26 triterpenoid compound. Other compounds include meliantriol, salannin, 7-desacetyl-7-benzyolazadirone, 7-desacetylbenzoylgedunin, cis-(beta-epoxy) azadiradione, 17-beta-hydroxyazadiradione, salannin and nimbin (Ascher 1993). Neem seed oil has a bitter taste due to the presence of several bitter principles: nimbidin (1.4%), nimbinin (0.01%), nimbin (0.12%) and nimidiol (0.5%) (Chinnasamy, Harishankar et al. 1993). The commercial and partially standardized formulations are based on refined extracts of neem seed kernel extracts or neem oil and sold as powder or emulsifiable liquid concentrates with
specified (typically 0.1–25%) azadirachtin content. The biological activity, including toxicity to nontarget organisms, of a formulation varies according to its azadirachtin content, the nature and the relative amount of other neem constituents might be different even for batches from the same manufacturer (Kumar and Parmar 2000; Goktepe and Phak 2002). The active agents for insecticidal and insect repellent effects attributed to neem are primarily azadirachtin and a number of less active limonoids and protolimonoids (van der Nat, van der Sluis et al. 1991).

Preparations rich in azadirachtin may disturb soil microflora if applied at higher concentrations. Physiologically, they exhibit strong behavioral, growth regulatory and reproductive activities and the subject has been reviewed extensively. In spite of considerable research efforts, the mode of action of neem formulations has not been clarified at the cellular or biochemical level. Commercial neem formulations contain other bioactive but less studied limonoids, the mode of toxic action of such preparations is obviously more complex than that observed for pure azadirachtin (Ascher 1993; Gopal, Gupta et al. 2007). Compounds obtained from neem, either in pure form or in the form of extracts obtained from different plant parts, are claimed to display a wide range of biological activities, from antimalarial to spermicidal to insecticidal (Akhila and Rani 1999).

**EFFECT OF NEEM BASED FORMULATION ON HUMANS**

Several reports indicate that neem and neem based formulations are toxic to humans. It is hardly possible to remove the very bitter oil from treated seeds, and that the germination of treated seeds is negatively influenced and is easily contaminated with aflatoxins (Sinniah, Baskaran et al. 1982). Neem seed oil produced occasional diarrhea, nausea, and general discomfort when given orally. Neem leaf extract-poisonings with ventricular fibrillation and cardiac arrest have been reported (Balakrishnan, Pillai et al. 1986). Dhongade, Kavade et al. (2008) have described a severe poisoning case of a 5-year-old boy who presented refractory epileptic seizures, dilated pupils, tachycardia, and
dyspnea 1 h after accidental ingestion of neem oil; metabolic acidosis was also found. Although neem preparations have been used safely against a variety of skin diseases, occasional dermatitis in sensitive individuals have been reported (Reutemann and Ehrlich 2008). Kadiri, Das et al. (1999) reported that traditional neem leaf-based medicines, taken to treat febrile illness, abdominal upset or to induce abortion or infertility had acute toxic effects. The major features observed were oliguria or anuria, jaundice and anemia. The picture was consistent with acute tubular necrosis in all the cases and the mechanisms causing the effects were intravascular haemolysis, hepatotoxicity, nephrotoxicity and three out of 53 patients died.

Two cases were described where oral administration to young children resulted in acute toxic effects. The oil, even in small amounts was reported to cause toxic encephalopathy. Features were vomiting, drowsiness, tachypnoea, and recurrent generalized seizures. Laboratory tests showed that the oil causes leukocytosis and metabolic acidosis (Lai, Lim et al. 1990). Sinniah, Baskaran et al. (1982) reported the case of a child, who died after administration of the oil as treatment for a cough.

Sinniah and Baskaran (1981) reported thirteen cases of infant and child poisoning after an oral intake of neem oil. The dose ranged from 5 to 30 mL in patients aged 21 days to 48 months. With a latency of 0.5 to 4.5 h all patients showed symptoms of poisoning, which included diarrhoea, vomiting, drowsiness, tachypnoea with acidotic respiration followed by recurrent generalised seizure (associated with loss of consciousness and coma), lasting from a few minutes to several hours. Hematological and clinical chemistry evaluations showed significant anemia (hemoglobin (Hb) <10 g/100 mL) in six patients and metabolic acidosis in 6/8 investigated cases. Liver biopsy one week after admission showed marked fatty infiltration and mitochondrial damage typical of that seen in Reye’s syndrome. Most children recovered, but two died, although in one case (21 day old baby) death was probably due to meningitis. The other death occurred ten days after admission after consumption of 24 mL of neem oil by a four months old child. Kroes, Van den Berg et al. (1993) reported that aqueous neem extract inhibited the human complement system and the activity of
polymorphonuclear leukocytes from healthy volunteers. A skin prick test on human volunteers revealed several major allergens in neem pollen extract (Karmakar and Chatterjee 1994).

EFFECT OF NEEM BASED FORMULATION ON ANIMALS

Sub acute exposure of neem leaf powder caused a decrease in the weight of the seminal vesicle and the ventral prostate (Kasturi, Ahamed et al. 1997), a reduction in the sperm count and sperm motility as well as an increased percentage of malformed sperm (Parveen, Manivannan et al. 1993). Biochemically, the leaf powder caused increases in activities of alkaline phosphatase and lactate dehydrogenase (Kasturi, Ahamed et al. 1997). The results of an oral toxicity study performed by Gandhi, Lal et al. (1988) suggested that neem oil is acutely toxic to rats and rabbits, with mortality observed within 24 h and 72 hours. The clinical signs of hypoactivity were observed at all dose levels. The target organs of toxicity are the lungs and liver, and death resulted probably from respiratory arrest. Various fractions of neem seed oil extract showed in vitro cytotoxicity (Cohen, Quistad et al. 1996). The fraction content of nimbolide generally correlated with the observed cytotoxicity. Nimbidine through an IP route, both mice and rat showed mild CNS sedation at doses >250 mg/kg body weight (Pillai and Santhakumari 1984). Male Sprague Dawley rats were given two IP doses of Margosa oil showed tachypnoea, lethargy and histopathological changes in the liver (Sinniah, Schwartz et al. 1985). In model studies with rat liver, Trost and Lemasters (1996) proposed that the pathogenesis of Reye’s syndrome, caused by neem oil, is associated with the induction of mitochondrial permeability.

Rats treated with neem leaf extract showed decreased appetite, body weight and pupillary reflex. Their total erythrocyte count (TEC) and blood glucose level were reduced. Histopathological studies revealed congestion in the liver, kidneys, lungs and brain (Hore, Maiti et al. 1999). Administration of neem leaves in goats and guinea pigs has decreased body weight. Acute and chronic toxicity were evident through signs of weakness, decreases in heart pulse and
respiratory rates, loss of condition and depression. Liver and kidneys were most affected and diarrhoea, tremors and ataxia were occurred in some animals. TEC, packed cell volume (PCV) and haemoglobin (Hb) decreased slightly, whereas the activity of serum AST was increased. (Ali 1987).

Aqueous neem extract had toxic effects, as reflected by body weight loss and high percentage mortality in rats (El Hawary and Kholief 1990). Dose-dependent subacute effects were observed in mice, where aqueous leaf extract reduced tri-iodothyronine (T3) and increased serum thyroxine (T4) concentrations and hepatic lipid peroxidation and decreased glucose-6-phosphatase activity while enhancing the activities of superoxide dismutase and catalase (Panda and Kar 2000). Two fractions of an acetone leaf extract showed central nervous system (CNS) depressant activity in mice as evidenced by a reduction in locomotor activity. Both fractions caused reductions in blood pressure and heart rate in rats without showing diuretic activity (Singh, Junnarkar et al. 1987).

When rats were treated with azadirachtin, increased serum AST and ALT activities and increased bilirubin content were observed. Histopathological studies showed pathological changes in the liver in terms of congestion, hydropic degeneration, necrosis and lymphocytic infiltration (Abdel Megeed, Radwan et al. 2001). When rats were administered azadirachtin at high dose, a decrease in body weight gain and relative liver weights of rats was observed. There were decreases in Hb, erythrocyte sedimentation rate (ESR), and PCV. Serum protein albumin and creatinine were lowered, AST increased, but no effect was found on blood urea nitrogen and ALT. Histopathologically non-specific generalized degenerative changes were found. Thus, the formulation led to adverse effects on the haemopoietic system (Gupta, Gupta et al. 1998). High doses of Ectozee, a neem based formulation in rats led to anorexia, enlargement of the abdomen, drowsiness, tetanic spasms and haemorrhagic diarrhoea mostly resulting in death (Das 1999).

In rats treated with Tric Vet Care, a neem based formulation, catalase activity of the red blood cells increased. At high doses, lipid peroxidation increased in the brain and total ATPases decreased in both brain and liver.
Acetylcholinesterase activity in the brain it decreased. The product affected liver and brain functions, possibly through membrane alteration and it could influence the oxidant defense mechanism of red blood cells and brain (Kataria, Gupta et al. 1998).

Gandhi, Lal et al. (1988) reported acute toxicity after ingestion of the oil by rats and rabbits. The oil-induced dose and time-dependent effects on motor activity, respiration and on the orientation within the cage and the animals had diarrhoea, tremors and convulsions. The oil was not toxic to mice at lower doses, but at high dose, treated animals showed hyper-excitability to sound and touch, convulsive jerks, laboured respiration, and some animals died (Tandan, Gupta et al. 1995). In rats, administration of neem oil during the first few days of pregnancy had a higher abortive effect than later administration. At a dose of 6 ml/kg bw, even 3 out of 13 adult animals died (Lal, Gandhi et al. 1987). Administration of oil increased tail flick reaction time and reduced induced writhing (Khosla, Sangeeta et al. 2000).

Male and female Wistar rats treated at 160, or 320 mg/kg body wt/day of vepacide (12% azadiracthin), a neem based formulation for 90 days showed dullness, irritation, diarrhoea, weakness and, mortality in females. Feed intake and body weight gains were reduced and haematological parameters were changed at all doses in both sexes. After 90 days of treatment RBC acetylcholinesterase activity were decreased in a dose related manner in both sexes (Rahman, Siddiqui et al. 1996). In a similar treatment pattern significant inhibition of brain AChE activity was seen in both sexes reaching maximum at high dose (Rahman, Siddiqui et al. 1999). Rats treated with vepacide showed a significant increase in the activity of ALT and AST in serum, lungs and kidneys, generally in a dose and time related manner (Rahman, Siddiqui et al. 2001). Prolonged oral administration of vepacide (80, 160 and 320 mg/kg) caused a significant increase of LDH activity in serum and lung tissues and a decrease in liver and kidney in both male and female rats when measured after 45 and 90 days of daily treatment. Necrosis of the liver and kidney tissues was also observed after this treatment (Rahman, Siddiqui et al. 2002). Long term administration of Vepacide resulted in a significant increase in acid phosphatase
and alkaline phosphatase activity in serum, kidney, lung, and liver tissue, whereas a significant decrease of acid phosphatase in liver was observed in male and female rats after 45 and 90 days of treatment with moderate (160 mg/kg/bw) and high doses (320 mg/kg/bw). The alterations in these enzymes indicated that lung tissue was the most susceptible, followed by liver and kidney (Rahman and Siddiqui 2004).

In the subchronic study, rats treated with vepacide for 90 days 10% of animals at the high dose died, reduced feed intake and loss of body weight occurred and clinical signs consisted of dullness and irritation. GSH levels and activity of GST were significantly reduced in the lung, liver, kidney and brain after 45 and 90 days. Activity of UDP-Glucuronyl transferase (UDPGT) was significantly inhibited in liver, brain and lung at days 45 and 90 but in the kidney inhibition occurred only after 90 days of treatment. In an acute toxicity study rats treated with vepacide (12% azadirachtin) at 1000, 1500 and 2000 mg/kg bw/day showed mortality rate of 10, 40 and 80 % at the low to high dose respectively, yielding an LD$_{50}$ of 1570 mg/kg bw (Mahboob, Siddiqui et al. 1995). In the LD$_{50}$ study one rat died at 1000 mg/kg bw, 4 died at 1500 mg/kg bw and 8 died at 2000 mg of vepacide/kg bw/day. Neem based formulation vepacide administered rats at a concentration 80, 160 or 320 mg/kg bw/day for 90 days showed significant decrease in drug metabolizing enzymes (Mahboob, Siddiqui et al. 1998).

The toxicity of vepacide, an enriched neem based formulation containing 12% azadirachtin, upon oral administration of 80, 160, and 320 mg/kg daily doses for 90 days, was studied in male rats (Mahboob, Siddiqui et al. 1998). On the 90th day, the high and medium doses caused significant decreases in cytochrome P$_{450}$ concentration in the liver, lungs, and kidneys. The highest dose caused 10% mortality; the medium dose elicited toxic signs, including behavioral abnormalities, lacrimation, reduced feeding, and loss in body weight. Azadiracthin was cytotoxic to several human glioblastoma cell lines by reducing cell survival and preventing mitosis (Akudugu, Gade et al. 2001).

Male and female rats were administered azadirachtin technical (12% azadirachtin) in peanut oil, by gavage, at doses of 0, 500, 1000, 1500 mg/kg
Introduction

bw/day for 90 days caused reductions in RBC and Hb values in males at 1000 and 1500 mg/kg bw/day and an increase in serum bilirubin levels was observed in both sexes at these doses (Raizada, Srivastava et al. 2001). Rats fed a diet containing 10% debitterised-neem oil showed significantly lowered cholesterol and triglyceride levels in the serum and liver. Both the leaf extract and seed oil produced an approximately 35% reduction in blood glucose levels in normal and diabetic rabbits (Khosla, Bhanwra et al. 2000). Neem seed-based animal feed supplement was reported to be toxic to sheep, goats and guinea pigs (Ali 1987).

Several scientists investigated reproductive toxicity of neem oil in rats (Lal, Sankaranarayanan et al. 1986; Prakash, Tewari et al. 1988; Riar, Bardhan et al. 1988; Upadhyay, Kaushic et al. 1990). Neem oil was shown to exert a potent contraceptive effect, particularly when applied directly in utero. Treatment of 10% debitterized neem oil in rats showed an overall increase in the mean relative liver weight (10% below the control group) in every generation, except for third generation females and reduced body weight gains(15% below the control group) (Chinnasamy, Harishankar et al. 1993). Possible adverse effects of neem used as a pesticide is the reproduction disturbance in both male and female mammals upon subacute and chronic exposure (Boeke, Boersma et al. 2004; Brahmachari 2004). Oral administration of neem seed extract to female rats from days 8 to 10 of pregnancy caused complete resorption of embryos by day 15 of pregnancy (Mukherjee and Talwar 1996). Single intrauterine administration of 100µl neem oil caused lasting infertility by apparent induction of leukocytic infiltration in the uterine epithelium during the preimplantation period (Upadhyay, Kaushic et al. 1990). Daily intramuscular injection of 250 and 500 mg/kg doses of neem oil for 8 days to male rats caused significant decreases in sperm counts and epididymal weight. Marked structural changes in the testes and impaired spermatogenesis were also observed. It was suggested that neem oil impaired the androgen supply to the testicular and epididymal tissues (Ghodesawar, Nazeer et al. 2004; Aladakatti and Nazeer 2005). In a semi-chronic study, neem leaf powder in rats caused a decrease in total sperm-count and in sperm motility (Aladakatti, Nazeer et al. 2001). The relative percentage of abnormal sperm increased. Since the effects of the powder were annihilated when
testosterone was administered simultaneously, the authors suggested that the effects were due to an androgen deficiency, thereby affecting the physiological maturation of sperm. Dose-related decrease in serum testosterone levels across all doses and a reduction in relative seminal vesicle weights, prostate weights, a significant increase in adrenal weights and decreased potassium and bilirubin levels were observed in male rats administered crude aqueous neem extract at a dose level of 125, 500 or 2000 mg/kg bw/day (Parshad, Singh et al. 1994). In powdered neem leaf treated rats, the height of the epithelium and the diameter of the nuclei of the epididymis were reduced. The sperm concentration in the lumen of the caput region was lower and these were packed with lymphocytes (Kasturi, Manivannan et al. 1995).

Histologically, complete arrest of spermatogenesis, mass atrophy of spermatogenic cells, atrophy of the Leydig cells, and reduced diameter of seminiferous tubules were observed after 24 days of administration of aqueous extract of powdered neem leaf treated rats. The nuclei of spermatogonia, spermatocytes, spermatids and Leydig cells were significantly reduced in diameter. Testicular protein and acid phosphatase were reduced and total free sugar, glycogen, cholesterol, LDH and alkaline phosphatase were increased (Joshi, Ahamed et al. 1996).

Neem seed oil caused complete resorption of embryos, in all treated pregnant rats. Administration of the serum from neem oil treated animals to other, via intraperitoneal injection on days 8, 9 and 10 of pregnancy, produced complete termination of pregnancy (Mukherjee and Talwar 1997). Pregnant rats were given neem seed fractions orally on days 8, 9 & 10 of pregnancy. Litter size as a percentage of implantation sites at day 7 ranged from 100% at a 3% concentration through 55% at 10% concentration to 4% at a concentration of 75% and 0 % with the pure fraction. Tumor necrosis factor (TNF) and gamma-interferon in lymph nodes and placental tissue peaked at day 13 and spleen weights were increased by approximately 50% at the end of treatment (Mukherjee, Garg et al. 1999). A dose and time dependent inhibition of the formation of total and hatching blastocysts was observed in two-cell mouse embryos in culture exposed to neem oil. A dose dependent inhibition of
blastocyst attachment to human endometrial stromal cell monolayers or extracellular matrix (ECMs) was observed with 0% attachment at 0.1% neem oil and 45.2 and 83.9% attachment at 0.075 and 0.05% respectively (Juneja, Pfeifer et al. 1994).

In normal females mated with treated males (aqueous ethanol extract of powdered neem leaf), the number of implantation sites was markedly reduced. Mating behavior and spermatogenesis were apparently unaffected but a slight reduction in testicular tubule diameter was observed. Spermatozoa were of normal number and morphology but only 50-60% was motile compared with 85-90% in controls (Choudhary, Singh et al. 1990). Aqueous extract of powdered neem leaf treated rats showed dose related decreases in protein and increases in the activities of alkaline phosphatase and LDH in both the caput and caudal regions of the epididymis. Investigations of the antifertility property of neem oil in rodents and humans affected the commercialization of human contraceptive formulations in India (Singh and Singh 2002; Subapriya and Nagini 2005).

In mice, crude ethanol extract of neem showed dose-dependent increases in both individual (breaks and gaps) and gross (aneuploidy and polyploidy) types of abnormalities (Awasthy, Churasia et al. 1995). Akudugu, Gade et al. (2001) reported that 28 μM azadirachtin reduced the proportion of dividing cells and induced formation of micronuclei in TP53 mutant, but not in TP53 wild-type, cell lines. Oral administration of a crude ethanol extract of the leaves of neem (Azadirachta indica) to adult Swiss albino mice for 7 days at 5 mg, 10 mg or 20 mg/10 g bw/day significantly increased the incidence of structural and mitosis disruptive changes in metaphase chromosomes of bone marrow cells on days 8, 15 and 35th of observation (Awasthy, Chaurasia et al. 1999). These results suggest that neem leaf extract and azadirachtin can be genotoxic to mammalian cells. Daily oral doses of 0.5–2.0 g/kg of an ethanolic neem leaf extract of unknown composition to male mice showed cytotoxicity and caused chromosomal abnormalities in spermatocytes after 7 days of treatment (Khan and Awasthy 2003).
PLANT BASED PESTICIDES

Natural products have been used to control animal pests, plant diseases, and weeds since ancient times. Plants have been the most important sources of natural pesticides for centuries and preparations standardized for the active ingredients, making possible the manufacture of reliable products. Combined effect of the constituents in a crude preparation of plant extracts often complicates the evaluation of the mixture. Minor components can have unique, either favorable or unfavorable, biological properties that are unveiled only after separation of the ingredients. Although crude preparations are still continue to be used in practice (Copping and Duke 2007).

On account of ecohazardous nature, nontarget specificity of chemical insecticides and evidences of developing resistance against them in the exposed species, currently, importance of secondary plant metabolites has been acknowledged. Insecticides of plant origin are environmentally safe, degradable, and target specific. Extracts of *Amaranthus oleracea* and *Euphorbia hirta* showed larvicidal property against the third instar larvae of *Anopheles stephensi*, the urban malaria vector (Sharma, Mohan et al. 2009). Glucosinolate and flavonoid isolated from *Brassica rapa* ssp. can be used as insecticides (Mucha-Pelzer, Mewis et al. 2010). Saponins, a group of steroidal or triterpenoidal secondary plant metabolites have insecticidal activity against living caterpillars (*Spodoptera littoralis*) and aphids (*Acyrthosiphon pisum*) via treatment on artificial diets containing different concentrations of saponins (De Geyter, Geelen et al. 2007). Usnic acid, a commonly encountered lichen secondary metabolites, showed strong larvicidal activity against the larvae of *Culex pipiens* L. (Diptera: Culicidae) under laboratory conditions and caused 100% mortality on third-fourth larval stages of the species at 24 h at the doses of 5 and 10 ppm (Cetin, Tufan-Cetin et al. 2008). The insecticidal properties of the crude extracts of the leaves and flowers of *Anemone pavonina* were evaluated on *Pheidole pallidula* ants and showed significant levels of activity (Varitimidis, Petrakis et al. 2006).
REVIEW OF PLANT: *STREBLUS ASPER*

*Streblus asper*, Lour. (Family- Urticaceae, Sub family- Moraceae) is a traditionally used Medicinal Plant in India (English- Siamese rough-bush, Malayalam- paruva, Sakhotavriksam, Parakam, Sanskrit- Sakhotah, Patrollekhataruh). It is a small, rigid evergreen tree, up to 15m in height with light grey or greenish rough bark and it is distributed throughout India (figure 2). The bark is vulnerary, anti inflammatory and constipating and is useful in foul ulcers, diarrhea, dysentery, inflammations and fever (Varier 1996). The seeds are reported to be beneficial in epistaxis, and diarrhoea. Root has been found useful in epilepsy and inflammation (Nadkarni and Nadkarni 2000; Nadkarni and Nadkarni 1976). The root extract of *S.asper* has been recommended in the treatment of cardiac disorders and oedema (Gaitonde, Vaz et al. 1964), and applied to ulcers, sinuses and a local antidote to snake bite (Chopra 1956). They are useful in vitiated conditions of kapha, ulcers, dysentery, and syphilis. The leaves are useful in agalactia, swellings and hyperhidrosis (Varier 1996). The petroleum ether extract of leaves of *Streblus asper* exhibited antioxidant and hypoglycemic property in streptozotocin induced diabetic rats (Monjoy, Venkatraman et al. 2011). Stem bark is reported to be effective against lymphoderma, chyluria and other manifestations of filariasis (Singh and Singh 1987; Singh 1988). *Streblus asper* is a medicinal plant used in folk medicine for the treatment of several inflammatory diseases (Sripanidkulchai, Junlatat et al. 2009). Preleminary organic analysis of the root stem bark of *Streblus asper* contains carbohydrates, glycosides, phytosterols, phenolic compounds, tannins and saponins (Madhavan, Parveen et al. 2008).

Insecticidal effects have been shown in extracts of the *S. asper* stem (Atal, Srivastava et al. 1978) and leaves (Kritsaneepaiboon 1989). Two cytotoxic cardiac glycosides- namely strebloside and mansonin, have been found in the chloroform extracts of *S. asper* root (Gaitonde, Vaz et al. 1964) and stem (Fiebig, Duh et al. 1985).Two glycosides namely asperoside and strebloside isolated from the bark of *Streblus asper* were found to possess promising microfilaricidal activity (Chatterjee, Fatma et al. 1992). *Streblus asper* leaf extract were shown considerable antimicrobial activity (Taweechaisupapong,
Wongkham et al. 2000; Wongkham, Laupattarakasaem et al. 2001). Ethanol extracts from the sticks and leaves of \textit{S.asper} have been shown to inhibit the growth of \textit{Streptococcus mutans} (Triratana and Thaweboon 1987). \textit{S. asper} has a specific effect on the pathogenic bacteria; although no mutagenicity or toxicity of \textit{S. asper} leaf extract up to 50 mg was observed (Wongkham 1996). Sripanidkulchai, Junlatat et al. (2009) reported the molecular effects of ethanolic extract of \textit{Streblus asper} leaf as a potential anti-inflammatory agent, which supports the fact that the plant is employed in traditional remedies.

\textit{Streblus asper} (Malayalam: Sakhota vriksham)
IMPORTANCE OF COTTON

Cotton is the world’s most important non-food agricultural commodity, yet it is responsible for the release of US$ 2 billion of chemical pesticides each year, within which at least US$ 819 million are considered toxic enough to be classified as hazardous by the World Health Organisation. In total almost one kilogram of hazardous pesticides is applied per hectare under cotton, and cotton is responsible for 16% of global insecticide usage – a figure higher than any other single crop. Between 1 and 3% of agricultural workers worldwide suffer from acute pesticide poisoning with at least 1 million requiring hospitalization each year. The victims of cotton pesticide poisoning, experience a broad spectrum of negative health impacts ranging from headaches, to seizures, loss of consciousness, and death. While developing countries account for less than 30% of global pesticide consumption, the bulk of pesticide poisonings occur in a developing world scenario; including an estimated 99% of pesticide induced deaths (Dinham and Malik 2003).

Globally, India is the third largest cotton producers. Cotton yield in India is mainly affected by the problems due to insects and pests. Cotton is a crop to which 45% of the pesticides and 58% of insecticides used in India are applied. Of all the communities adversely affected by hazardous cotton pesticides, substantial proportions are located in India: home to more cotton farmers than any other country in the world. Indian cotton production is heavily associated with the intensive use of hazardous pesticides, and is responsible for over half of all agricultural pesticides applied nationally. Within this figure Indian cotton is associated with some of the most hazardous pesticides used anywhere on earth. Observational studies reveal a heavy toll exerted on the health of those who work with cotton pesticides and chemical analysis has revealed traces of pesticide residues in blood samples taken from Indian cotton laborers. Cotton undoubtedly represents one of India’s most important economic, nutritive and cultural commodities, but its conventional cultivation has become deeply problematic, both for those who grow it and because of the external costs of its impact on health and the environment. The red cotton bug, *Dysdercus cingulatus*,
an important polyphagous pest, causes heavy loss to cotton crops which badly affects the economy of poor farmers (Bhagirath and Gaurav 2001; Mancini, Van Bruggen et al. 2005).

**Red cotton bug (Dysdercus cingulatus)**

Thus keeping the above facts in view, the present study has been planned to prepare various solvent extracts from the stem bark of *Streblus asper*, to assess its insecticidal activity and its mode of action, and its toxicological evaluation in comparison with a synthetic insecticide malathion and a plant based insecticide formulation vepacide.