Introduction

Presently agriculture in developed countries is dependent on crop protection chemicals as much as it is on the internal combustion engine. No one would want to return to the hunger, malnutrition disease and discomfort of a primitive existence. However, our goal must be to employ crop protection and pest control chemicals in agricultural, horticulture, public health and amenity and recreational areas as part of the total management of the environment for the long-term benefit and survival of mankind and to minimise any possible risks arising from their use (Green, Hartley & West, 1977).

Thus the present era of agriculture development and crop protection has led to an almost explosive expansion in the development of synthetic insecticides and pesticides. The green revolution undoubtly holds promise for increased crop protection but at the same time indiscriminate use of them is proving extremely hazardous to the innocent man.

Pesticides are inherently toxic not only to the pest against which they are used, but also to other organisms. Damage to non-target organisms, perturbation of structure and fuction of the ecosystem and general environmental contamination by pesticides have been the common consequence of pesticide use (Kalra & Chawla, 1981). A dramatic short term increase in crop yield could be upset by long-term deterioration in soil fertility due to resistance of nutrient cycling through the effects of pesticides on soil biota. Fungicides in particular, have been shown to adversely affect Rhizobia legume symbiosis even at the recommended dosages (Sarath Chandran, 1991). The pesticidal
contamination of aquatic environment could reduce the potential of such a system for fish production, an important source of proteins.

Synthetic pesticides have been a most powerful weapon for combating the menace of agricultural and public health pests during the 20th century. Their use has saved millions of lives and has improved the life standards of mankind by preventing hunger and eliminating outbreaks of pests and diseases of human, animal and plants. Simplicity, efficacy, and economic return have been the hallmarks of their wide use. The environment contamination, residues in food, feed and fibre, potential chronic toxicity disruption of non-target organisms pests resurgence and development of pest resistance are negative tracts that have brought continued use of pesticides as well as pesticide science as a whole, to crossroad, necessitating fresh appraisals and a search for new direction (Parmar, 1995).

Systematic crop protection with synthetic pesticide in India commenced with the use of DDT and HCH in 1947-48. A small manufacturing unit for HCH was established at Rishra by ICI in 1952. In 1954 Hindustan Insecticides Ltd. established a DDT factory of Delhi. HCH was also used for locust control in 1948 and in 1952 a Malaria control programme using DDT and HCH was launched. Pesticides also stayed on as an essential input for increased food production of organophosphorus compound and more sophisticated organochlorine molecules based on cyclodienes, and carbamates. Synthetic pyrethroids as well as the whole range of herbicides, fumigants, fungicides, chitin synthesis inhibitors and pheromones followed (Bami, 1995).

The use of insecticides for control of insect pests is an integral component of integrated pest management on cotton. The indiscriminate and over use of insecticides, however has created many

Synthetic pyrethroids recommended against bollworm have been reported to cause resurgence of whitefly (Dhawan & Saini, 1998). (Dominick & Mohanasundhram, 1992) reported cotton plants treated with cypermethrin, deltamethrin and fenvalerate registered higher sugar and protein contents in leaves and caused resurgence of whitefly.

Synthetic pyrethroids are synthesized derivatives of naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried *chrysanthemum* flowers. The insecticidal properties of pyrethrins are derived from ketoalchoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyze their nervous system (Reigart *et al.*, 1999). Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control pest insects in agriculture, homes, communities, restaurants, hospitals, schools, and as a tropical head lice treatment.

The natural pyrethrins have attracted attention because they are powerful insecticides but possess a very low mammalian toxicity. They have been used for many years mainly for the control of public health and veterinary vectors. The produce Inspection Division of the Ghana Cocoa Marketing Board routinely performs evening fogging of cocoa sheds with 0.5% natural pyrethrins to control insect pest, especially the tropical were house mouth *Ephestia cautella* (Kumar, 19).

However the present concern for contamination of the environment by the use of insecticides has resulted is search for alternative compounds and this includes some painstaking research on
pyrethrum extracts. Modern studies on extracts of *C. cinerariarefolium*, especially by (Elliot, 1978) co-worker at Rothamsted, have provided a considerable family of synthetic analogues, the modern pyrethroids, which constitute commercially important and useful materials. The initial aim of the synthetic studies was to produce non-persistent analogues which possessed low mammalian toxicity and were safe to use on food and edible crops.

The pyrethrins owe their importance due to their outstandingly rapid knock down action and due to the ease with which they are metabolized into non-toxic products. Unlike DDT, pyrethrum is not persistent and leaves no toxic residues which may be why this insecticide does not tend to induce the development of resistant insect populations. Pyrethrum is used to control pest in stored foods and against household and industrial pests. Pyrethrum aerosol sprays are excellent home insecticide because of their safety and rapid action (Cremlyn, 1974).

Today the main use of pyrethrum is as a constituent of space sprays against flying insects, e.g. flies and mosquitoes, in the form of oil solutions applied through a suitable spraying device or by aerosol dispensers. Pyrethrum has such a rapid action as a control insecticide that the question whether it also has stomach poison activity is irrelevant. The pyrethrins, rapidly lose activity in the presence of oxygen and light but despite this weakness, the use of pyrethrum, unlike that of derris and nicotine, has grown with the increase in the use of synthetic chemicals.

The pyrethroids also have the advantage of being cheap in terms of cost per hectare because they are so active and are applied at rates of around 50 grams/hectar.
The recent synthesis of biologically active, photostable pyrethroids has led the rapid development of the use of some of them as insecticides (Elliot et al., 1978). Cypermethrin, a recently introduced synthetic pyrethroid, has been gaining popularity as insecticide. Very little is known about the toxicity and cumulative effects of cypermethrin in higher animals including on man.

Cypermethrin, one of a handful of light stable synthetic pyrethroids, is registered to control cockroaches, fleas and other indoor pests in homes, restaurants, hospitals, school and food processing plants, and also in agriculture to control pest of cotton, fruits and vegetables. About 90% of the cypermethrin manufactured worldwide is used to combat pests feeding on cotton crops (WHO, 1989).

Cypermethrin a synthetic pyrethroid and ethion an organophosphorous insecticide have been recommended for the control of boll worms and sucking pest of cotton (PAU, 1998).

The term insect growth regulator (IGR) was used to describe a new class of biorational compounds by greater selectivity of action, which appear to satisfy the requirements for the 3rd generation pesticides. Such as absence of undesirable effects on man, wild life and environment and compatibility with mordern insect pest management principles (Stall, 1975).

Insect growth regulators (IGR) were believed to be on the safe regarding the evolution of resistance (Williams, 1967). However, various studies have shown that resistance can evolve in response to this chemical group (Cerf & Georghiow, 1972), more over if the selection pres-suries sufficiently strong.
Derivatives of benzoylphenyl ureas, diflubenzuron and penfluron represent one of the most important discoveries involving a new class of compounds affecting chitin biosynthesis in the growing larva, reproduction in adults and embryogenesis in egg of insects. The degree of insecticidal activity of these compounds usually depend on their ability of cuticular penetration and many (Bull, 1980; and Ivie, 1978) workers have shown the varying degrees of cuticular absorption of these compounds after different treatment. Chang & Borkovec (1980) evaluated the incorporation of these chemicals in embryos after houseflies were injected with the chemicals. Embryonic absorption after parental treatment has not been much looked into by many workers, although this aspect is important from the pest control point of view, since these chemicals, have also an additional ovidicial action and thus it enhances their utility in pest control programs (Satyanarayana & Sukumar, 1991).

The new group of insecticide, phenylurea compounds also attracted most of the biologists because of their novel mode of action, their specificity mainly to chitin and their lower ecological magnification (Post & Vincen, 1973; Wellinga et al., 1973; and Ishaaya & Casida, 1974). Tropical application of phenyl urea compounds are found to interfere with chitin biosynthesis by blocking polymerization of UDP-N-acetyl glucosamins units (Wellinga, et al., 1973; and Post & Mulder, 1974) and inhibited deposition of chitin in the cuticle (Grosscurt & Anderson, 1980; Kramer & McGregor, 1980; Saxena & Kumar, 1981; and Chockkalingam et al., 1982). Administrating of diflubenzuron influence food consumption (Sundermurthy, 1977) growth (Reed & Bass, 1979) as well as the rate and efficiency of food utilization (Chockalingam & Krishnan, 1984) in insects.
Benzoyl phenyl ureas are mostly effective against defoliators of Lepidoptera and Coleoptera, although some of the newer compounds of this series like flufenoxuron are even effective against hemipterons. These chemicals have a definite future in integrated pest management (Chen & Mayer, 1985; and Gujar, 1987).

On of the number of chitin synthesis inhibitors, benzoyl phenyl ureas are the important group of chitin synthesis inhibitors. Their surprise discovery stems from the research work done on the synthesis of superherbicide from dichobenil and fenuron in the laboratories of Philips-Duphar, B.V. the Netherlands (Van Daalen et al., 1972). Diflubenzuron (Dimilin) is the first chitin synthesis inhibitors developed as an insecticide. Since than, a number of chitin synthesis inhibitors viz. Chlorfluazuron, flufenoxuron, teflubenzuron, triflumuron and many more have been developed as insecticides.

Since the discovery of benzoyl phenyl ureas as chitin synthesis have been carried out in vivo to study the effect of these inhibitors on the incorporation of radio labelled procursors like glucose, glucosamine or N-areyl glucosamine into acid - insoluble chitin component of cuticle. (Mulder & Gujswift, 1973) first demonstrated in vivo inhibition of chitin synthesis in fifth instar larval of Pieris by histological and autoradiographical methods. It was later shown that no radioactivity was found in endocuticle of DU-19111 treated larvae of Pieris brassica injected with radioactive glucose (Post & Wincent, 1973; and Post et al., 1974).

The role of benzoyl phenyl ureas in integrated pest management has been reviewed by Gujar (1992). Some results of practical significance have also been obtained by the foliar application of IGR
1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea which is a chitin synthesis inhibitor.

There was a disruption of endocuticle layer and also malformation of new cuticle in post moult period. Similar finding have also been reported in locusts, *Manduca sexta*, *Spodoptera littoralis*, *Tenebrio molitor* and many other insects. These findings were further confirmed with, in vitro studies using abdominal assays (Gijsswijt *et al.*, 1979; and Hajjar & Casida, 1979) and to some extent in tissue culture assays (Oberlander & Leach, 1974; and Sowo & Marks, 1975) and in cell-free chitin synthesizing preparation (Turnbull & Howells, 1983).

Hajjar (1985) extensively surveyed chitin synthesis inhibitor of different kinds and found them to be belonging to chlorinated hydrocarbons, phenyl carbamates, organophosphates, (Kitazin P), triazines, buprofezin, plumbagin, nikkomycins, polyoxins and tunicamycins. Antibiotics like polyoxins D from *Streptomyces cacoï, Var. asoensis*, nikkomycins from *Streptomyces tendae* were found very effective inhibitors of chitin synthetase under in vitro conditions, probably by inhibiting catalytic sites of chitin polymerizing enzyme. These were toxic to insect species viz., *Epilachna varivestis*, *Lucilia cuprina* and *Melanophus sanguinipes*. Nikkomycins were found to be effective against mites viz. *Tetranychus urticat Avermectins*, especially B<sub>12</sub> derived from *streptomyces avermitilis* was found an effective broad spectrum pesticide with chitin inhibitory activity to some extent.

Diflubenzuron (Marked as Dimilin 25 WP) is well known as a chitin synthesis inhibitor in insects. It is categorized as a fourth generation insecticide for its selective action. It is hoped that this chemical will occupy a status in future pest management programme. The chemical is also reported to have a fairly high degree of ovicidal,
larvicidal and pupicidal properties against various insect pests. It also causes morphogenetic deformities in egg and pupae (Rath et al., 1977).

The insecticides Dimilin (Diflubenzuron PH-6040 from philips-Duphar) is now generally considered to act as an inhibitor of chitin synthesis (Verloop, 1976; and Ker, 1976). This renders the untanned pharate cuticle weak so that it fails mechanically during ecysis of the insect, either of muscle insertions or the general body wall or both. Dimilin seems not to affect the synthesis and transport of protein (Post et al., 1974; and Hunter & Vincent, 1974; Anderson so pers. omm.).

These chemicals have been found to affect almost all stages of insect development. Feild studies have shown that acly ureas are highly effective stomach poisons for defoliators belonging to Lepidoptera, Coleoptera and Diptera.

The reason for diflubenzuron interest is that it disrupts the metabolism of chitin, a polysaccharide of particular importance to arthropods. It thus appears to alter the prospect of a broad spectrum of insect control, with minimal environmental damage in most habitats. Moreover so long as no untoward side effects appear it could be expected to have a high safety margin for vertebrates.

The first histological observation that diflubenzuron probably interfered with the deposition of cuticle was made by (Mulder & Gijswijk, 1973) and it was later demonstrated that this interference resulted from inhibition of the deposition of chitin within the integument. It now seems likely that the inhibition is a consequence of a rapid and complete blocking of chitin synthetase rather than to an activation of chitinase, an enzyme that breaks chitin down. Much of the work was done using larvae of Pieris brassica (Deul et al., 1978) but (Hajjar & Casido, 1979) obtained similar results using nymphs of the
dilubenzuron on different tissues of the red cotton bug *Dysdercus similis*. These chemicals were chosen due to their low mammalian toxicity. Furthermore, the histopathological observations of dilubenzuron treatment in *Dysdercus similis* have been confirmed by morphogenetic effects.
Materials and Methods

Collection and Rearing of Insect:

*Dysdercus similis* (Freeman) were collected from Bhindi (*Abelmoscus esculentus*) and Cotton (*Gossypium spp.*) plants in the fields around Sagar town during the months of May to November. The nymphs and adult insects were found on fruits and under surface of leaves. Insects were also collected from hedge plants and field cracks.

Insect were reared throughout the year in round glass jars. They were fed on Bhindi and moist cotton seeds, depending upon the availability of food in different seasons. The soil inside the glass jar bottles was moistened with few drops of water daily. Freshly moulted 5th instar nymphs and adult insects were selected and used for the experiments.

Chemical used and the method of treatment:

The chemical used were of technical grades. The synthetic pyrethroid (*α*-cypamethrin) was supplied by Meghmani Organics Ltd. (Ahmedabad) and insect growth regulator (*Diflubenzuron*) was supplied by Sigma-Aldrich Laborchemikalien GmbH.

Chemicals:

Pyrethroid - *α*-Cypermethrin

IUPAC Name:

A racemate of (s)-alpha cyno-3-phenoxbenzyl (1R)-cis-3-(2,2,dichlorovinyl)-2,2 dimethyl-cyclopropanecarboxylate and (R)-alpha-cylo-3-phenoxybenzyl (1s)-cis-3 (2,2-dichloroinyl)-2,2-dimethyl-cyclo propanecarboxylate.
History:

Cypermethrin was initially synthesized in 1974 and first marketed in 1977 (IPCS, 1989C). Alpha-cypermethrin contains more than 90 percent of the insecticidally most active pair of the four cis isomers of cypermethrin as a recemic mixture (IPCS 1992).

Chemical group: Typer II Synthetic Pyrethroid
Physical Properties:

$\alpha$-cypermethrin of molecular weight in 416.3. Physical states at room temperature: solid (crystal); technical grade is a powder, colour: colourless; technical grade is white to pale.

Solubility - low solubility in water to Acetone 620 g/l at 25°C.

Boiling Point - 200°C at 9.3 pa.

Properties:

$\alpha$-Cypermethrin controls sucking insects in fruit, vegetables, vines, cereals, cotton, soybeans, and other crops. It also controls cockroaches, mosquitoes, flies and other insect pest and is used as an animal electro parasiticide.

Insect growth regulator - Diflubenzuron

![Diflubenzuron Structure]

Diflubenzuron

History:

The insecticidal properties of the first member of this class of compounds were first described by (Van Daalen et al., 1992).
Diflubenzuron was introduced by Philips - Duphar B.V. under the code name pH 6040 and the protection of B.P. 1324293.

**Physical Properties:**

Diflubenzuron is a white to yellowish brown crystalline material, M.P. 210-230°C (230-232°C for the pure compound). It is almost insoluble in water (about 0.2 ppm at 20°C) and polar solvents. In polar to very polar solvents, the solubility is moderate to good e.g. in acetone 0.65 gm/100 ml at 20°C, the partition coefficient in dichlacomethene - water (pH 5.6), in 1-Octanal-water about 5000. The volatility is very low; 0.5 mg of the compound spread over 50 cm² aluminium foil less than 4% in weight during 48 hours at 20°C.

**Empharical formula -**

\[ \text{C}_{14}\text{HgClF}_{2}\text{N}_{2}\text{O}_{2} \]

**Mol. wt. - 3107**

**Biological Properties:**

Diflubenzuron belongs to a new group of insecticide (Wellinga *et al.*, 1873). It is a mainly a stomach poison that acts by interfering with the deposition of insect chitin (Mulder & Gijswijt, 1973). Thus all stages of insect known to form new cuticle are in principle susceptible to this compound. They include larvae of many economically important insect species.

A species - dependent ovicidal effect by contact activity has also been demonstrated (e.g. cotton, leaf worm, *Spodoptera litoralis*) as has prevention of egg enclosure after uptake by females (e.g. cotton boll weevil, *Anthonomas grandis*). Both these effect on eggs are also based on interference with chitin deposition.
The synthetic pyrethroid and insect growth regulator were dissolved in acetone and diluted further with distilled water prior to the experiment. LC$_{100}$, LC$_{50}$ and LC$_{0}$ (experimental concentration) values were calculated by the method of (Finney, 1971) LC$_{100}$ 0.00002 ppm, LC$_{50}$ 0.00001 ppm and LC$_{0}$ 0.000005 ppm for cypermethrin and LC$_{100}$ 0.01 ppm, LC$_{50}$ 0.005 ppm and LC$_{0}$ 0.0025 ppm for disflubenzuron. These doses were calculated as the experimental concentration for cypermethrin and was used as the experimental concentration for disflubenzuron. The experimental concentration was used throughout the experiment.

The insects were topically applied between the abdominal segments with the use of microinjector holding a 1.0 ml calibrated syringe fitted with 25 gauge needle. Comparable 1% acetone injected and untreated individuals constituted the controls.

The adult insects which were treated with the synthetic pyrethroid and insect growth regulator, were vivisected after 4, 8 and 12 days of the treatment. The insects did not survive after 12 days of the treatment. The nymphs were treated only with disflubenzuron were also vivisected after 4, 8 and 11 days of the treatment. The treated nymphs did not moult into normal adults.

The experimental insects were divided into three groups. 10 pair of insects (nymphs and adults) were used in the experiment. The experiments were carried out in the following combinations.

(i) The group of insects which was neither treated with the drug nor their solvents, comparised the normal group.

(ii) The group of insects which was treated with same volume of the solvent acetone comparised the control group.
(iii) The group of insects which were treated with the drugs comprised the experimental group.

**Histological Studies:**

The tissues selected for the present study are the adipose tissue and the gonads.

To study the affected histopathology and histochemistry the tissues from the control as well as treated insects were fixed in aqueous Bouin's fluid and Carnoy's fluid. The paraffin in blocks of the tissues were cut at 6µ thickness. This was followed by staining techniques.

**Staining Techniques:**

1. **Histopathological Studies:**

   Delafield's Hemotoxylin and Eosin method was applied from Pearse (1960).

2. **Histochemical Studies:**

   **For Protein** - For the detection of protein Mercuric Bromophenol Blue staining technique from Pearse (1960) was used.

   **For Carbohydrate** - Periodic Acid Schiff's Reaction (PAS) method of McManus and Cason (1950) from Davenport (1966) was applied for the detection of carbohydrates.

   **For Lipid** - Calcium Formal fixative Pearse (1960) and Sudan black B method was used for detection of lipid in the tissue.