INSECTICIDES AND THEIR APPLICATIONS

By:

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DEDICATION

This book is dedicated to the Almighty Allah Almighty whose blessings enable me to accomplish this wonderful task and Holy Prophet (SAW), whose life is a source of illumination and inspiration for the whole mankind. I would also like to pay words of appreciation to my dear students Dr. Naeem Abbas, Rizwan Mustafa Shah, Dr. Muhammad Baber Shahzad Afzal and Mamuna Ijaz whose team work enable me to present it in current form. No doubt, it was an effortless job and all of you make it an achievement. I cordially thanks to Higher Education Commission of Pakistan for giving me a chance to avail this gigantic opportunity through financial and logistic support.

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PREFACE

- Insecticides have become the most important part of the integrated pest management to minimize the insect pests born losses but the knowledge about this field is very rare. Therefore, the present book is an effort to convey the knowledge related to the insecticide use, their mode of action, classification and method of evaluation of their toxicity.
- Moreover, this book is also according to the HEC curriculum and addresses all topics of the course title under insecticides and their applications.
- Scarcity of the contender books on the same topic extremely require summing up the current knowledge for the students of plant protection specially entomology, for insecticides and field workers.
- The discussion of all the topics is very comprehensive that reader can get the enough background to start the future work.
- This book will also consider the comprehensive overview of the already published work in the same subject.
- I hope this book will prove a potential contending book for their targets in the market like students, reviewers and other organization that require this type of information at any level.

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LIST OF ABBREVIATIONS

ISO	International organization for standardization
ULVA	Ultra-low volume applicator
IUPAC	International Union of Pure and Applied Chemistry
SC	Suspension concentrate
SL	Soluble concentrate
EC	Emulsifiable concentrate
ULV	Ultra low volume
WP	Wettable powders
SP	Soluble powder
CRF	Controlled released formulation
OCs	Organochlorins
DDT	Dichlorodiphenyltrichloroethane
Ops	Organophosphates
AchE	Acetylcholinesterase
VSSC	Voltage sensitive sodium channel
nAChR	Nicotinic acetylcholine receptors
IPM	Integrated pest management
CNS	Central nervous system
GABA	Gamma amino butyric acid
RyRs	Ryanodine receptors
IRAC	Insecticide resistance action committee
MFO	Mixed function oxidase
Bt	*Bacillus thuringiensis*
IGRs	Insect growth regulators
JHAs	Juvenile hormone agonists or analogs
JH	Juvenile hormone
MSDS	Material safety data sheet
PPE	Personal protective equipment
LC_{50}	Lethal concentration 50
LD_{50}	Lethal dose 50
PCR	Polymerase chain reaction
FLs	Fiducial limits
D_{LC}	Degree of dominance
D_{ML}	Effective dominance
CI	Combination index
DNA	Deoxyribonucleic acid
PBO	Piperonyl butoxide
DEF	S,S,S-tri-n-butyl phosphorotrithioate
DEM	Glutathione S-Transferase
WHO	World health organization

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CHAPTER-1

1.0 INTRODUCTION AND NOMENCLATURE OF INSECTICIDES

The world population reached 7.5 billion in 2017 and is estimated to reach 9.8 billion by the year 2050. To properly feed and clothe additional people, food crop yields need to be increased and more natural fiber should be produced. It is projected that global food demand will double in the next 50 years. Agricultural experts believe that these food and fiber needs can be met by increasing the use of pesticides for the control of pests.

Pesticides are the most important chemicals used for the controlling pests. Pesticide can be define as a pesticide is any substance or mixtures of substances used for preventing, destroying, repelling, or mitigating any pest (Environmental Protection Agency). Pesticides include insecticides, herbicides, fungicides, nematicides, and rodenticides.

Insecticide
Chemicals used to kill the insect pests.

Herbicide
Chemicals used to kill the weeds.

Fungicide
Chemicals used to kill the fungus.

Nematicide
Chemicals used to kill the nematodes.

Rodenticide
Chemicals used to kill the rodents.

They are largest group of poisonous substances that are widely broadcast today. There are approximately 1200 active ingredients approved for use by the EPA from which over 20000 pesticide products are formulated and are being marketed in the United State (Simon, 2014). The use of pesticides in Pakistan was started in 1954 and is currently on the rise. In Pakistan, the purchase of pesticides was estimated at Rs. 19.612 billion during 2003 (Khooharo et al., 2008). Among the pesticides, the insecticides are the largest group of chemicals, followed by herbicides, fungicides, acaricides, and fumigants. The insecticide groups are organochlorins, organophosphates, carbamates, pyrethroids, insect growth regulators, neonicotinoids, microbial insecticides and other miscellaneous insecticides. The share of insecticides, herbicides, fungicides, acaricides and fumigants is 74%, 14%, 9%, 2%, and 1%, respectively (Khooharo et al., 2008). A large percentage of pesticides are being applied in the Punjab, followed by Sindh, Khyber Pakhtoonkhaw and Balochistan. Pesticide are mostly applied on cotton crop (70–85 %) and on other crops (15-30 %) such as sugarcane, wheat, rice, maize, tobacco, and fruits vegetables (Shahid et al., 2016).

Increased use of pesticides has threatened human and environmental health. Therefore, the selective compounds with minimum effect on natural enemies and the environment should be used (Ishaaya et al., 2005). Reducing the risks linked with insect pest management strategies by using the selective pesticides along with their resistance management to maintain their efficacy for a prolonged period is of utmost agricultural importance.

1.1 Nomenclature of Insecticide

The formal process by which insecticides are named is called insecticide nomenclature (Pedigo & Rice, 2014). Insecticides are known by three names:

1.1.1 Common names
1.1.2 Trade names
1.1.3 Chemical names

1.1.1 Common names

The common names are also based on active ingredients. A common name for the insecticide is usually adopted by the national standards body of the country where it is used, and these names sometimes vary widely; the name adopted in one country may be a proprietary name for the pesticide in another country. International standard common names for pesticides are adopted by the International Organization for Standardization (ISO) (Simon, 2014). During the process of selecting acceptable common names, a search for possible conflicts between proposed names and trademarks in the international register is carried out by the World Intellectual Property Organization, while national standards bodies that are members of ISO, carry out similar searches in the trademark registers of their own countries. A proposed common name must receive the votes of at least 75% of the ISO member bodies before it can be adopted as an international standard common name (Lowe & Stiles, 1973).

1.1.2 Trade names

The trade name is also called as the proprietary name or brand name. The trade name has a registered trademark superscript ® or ™, indicating the patent right of that name and is protected by law. More than one manufacturers may hold patent rights to one compound. The state cooperative extension service may publish either trade names or common names, usually depending on the name most frequently used for a compound. For example, the emamectin benzoate insecticide is sold under the trade name of Proclaim$^{(R)}$ by Syngenta Pak. Ltd.

1.1.3 Chemical names

The chemical name provides the description of the insecticide structure and is formed by following the agreements of the International Union of Pure and Applied Chemistry (IUPAC) or those of the 9th collective index period of the Chemical Abstracts Service (Simon, 2014). For example, the IUPAC name of fipronil is 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3carbonitri.

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Table 1.1 List of pesticides of Syngenta (Pakistan) Limited

Trade Name	Name of active Ingredient	Formulation	Recommended Dose	Target Pests
Weedicides				
Dual-Gold	S-Metolachlor	960 EC	800 ml / Acre	Itsit, Sawanki, Dela in Cotton
Gramoxone	Paraquat	200 SL	1-1.5 L / Acre / 100-150 L H2O	Post-Emerged Weeds of Cotton
Logran	Triasulfuron	85 WG	16 g/Acre	Piaz, Lehli, Mena, and Bathu
Axial	Pinoxaden	50 EC	330 ml/ Acre	Dumbi Sitti, Jangli Jai and Grassy Weeds
Rifit	Pretilachlor	500 EC	400 ml/ Acre	Mirch Boti, Sawanki, Ghoin, Bhoin
Prime Extra-Gold	S Metolachlor + Atrazine	720 SC	400 ml/Acre	Broad Leaf Weeds in Maize
Topic	Clodinafop	15 WG	120 g/ Acre	Jangli Jai, Dumbi Sitti and Grass like Weeds
Fungicides				
Score	Difenoconazole	250 EC	30 ml/ 100L H_2O	Anthracnose in Mango and Powdery Mildew In Vegetable
Revus	Mandipropamid	250 EC	240 ml/ Acre	Downy mildew of cauliflower, okra and onion, Early and late blight of potato.
Dividend Star	Difenoconazole	032 FC	1 ml/Kg seed	Loose Smut of Wheat
Ridomil-Gold	Mancozeb + Metalaxyl M	68 WP	200-250 g /100ml	Down Mildew Of Onion, Early Blight Of Potato
Topas	Penoconazole	100 EC	50 ml/100L H_2O	Powdery Mildew
Amistar Top	Azoxystrobin + Difenoconazole	325 SC	200 ml/Acre	Early and late blight, Powdery mildew
Thiovit	Sulphur	80 WG	1 kg/Acre	Brown leaf spot, Powdery mildew
Tilt	Propiconazole	250 EC	200 ml/Acre	Bunt, Kungi or Rith
Density	Azoxystrobin+Fludioxonil+Mefenoxam	125 FS	2-3 ml/Kg of Cotton Seed	Seed and Soil Borne Disease
Insecticides				
Polytrin C	Profenofos + Cypermethrin	440 EC	600 ml / Acre	Spotted bollworm, Pink bollworm,

				American bollworm eggs
Curacron	Profenofos	500 EC	800 ml / Acre	Chewing insects ,Thrips, Mealybug
Proclaim	Emamectin benzoate	019 EC	200 ml / Acre	American Bollworm and Army Worm
Karate	Lambda-cyhalothrin	2.5 EC	400 ml / Acre	Pink Boll Worm
Trigard	Cyromazine	75 WP	20-25 g / 100 L of H2O	Leaf miner of cucurbits
Polo	Diafenthiuron	500 SC	200 ml / Acre	Whitefly, Mite, Jassid
Virtako	Thiamethoxam+Chlorantraniliprole	0.6 GR	4 kg / acre	Stem borers of rice and maize
Actara	Thiamethoxam	25 WG	24 g / Acre	Jassid and Mango Hopper
Voliam Flexi	Thiamethoxam + Chlorantraniliprole	25 WG	80 ml / kg	Stem borer , leaf folder
Match	Lufenuron	050 EC	200 ml / Acre	Army Worm
Plenum	Pymetrozine	50 WG	80 g/100 L of H_2O	Aphid

*This information is collected from pamphlets published by the Syngenta

Table 1.2 List of pesticides of FMC Pakistan limited

Trade Name	Name of active Ingredient	Formulation	Recommended Dose	Target Pest
Weedicides				
Affinity	Carfentrazone-Ethyl +Isoproturon	50 WP	600 g/Acre	All Weeds in Wheat
Vitra	Bromoxynil + MCPA	60 EC	400 ml/Acre	Bathu, Lehli, Shahtera, Revari, Dhodhak, Maina
Platform	Clodinafop	15 WG	120 g/Acre	Narrow leaves in wheat
Starane-M	Fluroxypyr + MCPA	50 EC	300 ml/Acre	Broad leaves in wheat
Galaxy	Glyphosate	48 SL	100 ml/Acre	Non-selective
Metric	Metribuzin	25 ZC	500 ml/Acre	For potato weeds
Paraquat	Paraquat	20 SL	1 L/Acre	Non-selective
Volantis	Halosulfuron	75 WDG	20 g/Acre	Della in sugarcane
Aim	Carfentrazone-Ethyl	40 DF	16 g/Acre	Broad Leaves in Wheat/ Lehli
Stomp	Pendimethalin	455 CS	1 L/Acre	All weeds in cotton and other crops
Fungicides				
Acrobat MZ	Dimethomorph + Mancozeb	90/600 WP	500 g/Acre	Downy Mildew, Early and Late Blight of Vegetable
Rugby	Cadusafos	5 G, 10 G	10 kg	Nematodes in Orchards
Systhane	Myclobutanil	20 EW	100 ml/Acre	Powdery mildew in orchard
Shincar	Carbendazim	50 SC	150 ml/100 L water	Anthracnose in Orchards
Cabrio Top	Metiram + Pyraclostrobin	60 WDG (55%+5% w/w)	300 g/Acre	Downy Mildew, Early and Late Blight of Vegetables and fruits
Kumulus	Sulphur	80 DF	2 kg/Acre	Anthracnose in Orchards and Vegetables
Copride	Copper Oxychloride	50 WP	500 g/Acre	Downy Mildew of vegetables and orchard.
Pursue	Difenoconazole	25 EC	200 ml / Acre	Powdery and Downy mildew
Insecticides				
Talstar	Bifenthrin	10 EC	250 ml / Acre	Chewing insects
Novastar	Bifenthrin + Abamectin	56 EC	500 ml / Acre	Thrips, Jassid, Bollworms
Acelan	Acetamiprid	20 SL	75 ml / Acre	Whitefly
Corrado	Buprofezin	25 WP	600 g / Acre	Whitefly, Jassid, Aphid
Delphix	Diafenthiuron	50 SC	200 ml / Acre	Whitefly, Jassid, Aphid
Furadan	Carbofuran	3 G	8 kg / Acre	Borers
Squadron	Chlorfenapyr	360 SC	100 ml / Acre	Thrips, mites
Gravitan	Nitenpyram	10 WV	130 g / Acre	Jassid
Alcance	Imidacloprid	70 WS	50 g / 10 kg	Seed treatment
Paradigm	Pyriproxyfen	10 EC	400 ml/Acre	Whitefly
Advantage	Carbosulfan	20 ES	330 ml/Acre	Aphid
Cordelia	Chlorpyrifos	40 SC	250 ml/Acre	Cotton pest complex
Bestox	Alphamethrin	5 EC	250 ml/Acre	Bollworms
Telsta	Clothianidin	20 SC	200 ml/Acre	Dusky cotton bug
Palegane	Triazophos	40 SC	1000 ml/Acre	Pink bollworm
Arrivo	Cypermethrin	10 EC	330 ml/Acre	Spotted bollworm
Commando	Acephate	75 SP	300-400 g/Acre	Thrips, Jassid
Carvus	Novaluron	10 EC	300-400 ml/Acre	Army worm, Borers

*This information is collected from pamphlets published by the FMC

Table 1.3 List of pesticides of Bayer Crop Sciences Pakistan limited

Trade Name	Name of active Ingredient	Formulation	Recommended Dose	Target pest
Insecticides				
Belt	Flubendiamide	480 SC	25 ml / Acre	Army Worm
			50 ml / Acre	American bollworm
Confidor	Imidacloprid	20 SL	250 ml / Acre	Aphid, jassid, whitefly, mango midges
			100 ml/100 L of H_2O	galls, scales, hoppers on mango
Decis	Deltamethrin	10 EC	80 ml / Acre	American bollworm, Pink bollworm, Spotted bollworm
			240 ml / Acre	leaf folder in rice
Movento	Spirotetramat	240 SC	125-250 ml / Acre	Aphid, whitefly adults and nymphs
Oberon	Spiromesifen	240 SC	100 ml / Acre	Whitefly, Mites
Diptrex	Trichlorfon	80 SP	250 g/Acre	Fruit fly
Regent	Fipronil	80 WG	480 ml/Acre	Stem Borer
Lesenta	Imidacloprid and Fipronil	80 WG (40+40 w/w)	60 g / Acre	Jassid, thrips
Fungicides				
Alliett	Phosteyl-Aluminium	800 WP	250 g / Acre	Late blight, purple blough, gamoses
Antracol	Propineb	75 WP	500 g / Acre	Early blight
Bayton Foliar	Tridaminol	25 EC	50 ml / 100 L of H_2O	Powdery mildew
Melody Duo	Propineb + Iprovalicarb	66.75 WP (61.25%+5.5% w/w)	300 g / Acre	Early and late blight, downy mildew
Nativo	Tebuconazole + Trifloxystrobin	75 WG (50% +25% w/w)	65 g /100 L of H_2O	Broad spectrum fungicide, cover all fungal diseases
Weedicides				
Atlantis	Mesosulfuron-methyl + Iodosulfuron-methyl sodium	3.6 WDG (3% + 0.6 % w/w)	160 g / Acre	Dumbi siti, Jangli jai, Broad leaves of Wheat
Buctril Super	Bromoxynil + MCPA + Ethyl hexyl ester	60 EC	300 ml / Acre	All broad leaf weeds of wheat
Puma Super	Fenoxyprop-p-ehtyl	7.5 EW	500 ml / Acre	Dumbi siti, Jangli jai
Topstar	Oxidiargyl	80 WG	40 g / Acre	Sawanki
Sunstar Gold	Ethoxysulfuron ethyl	60 WG	20 g / Acre	Della
Sencor	Metribuzin	70 WP	250 g/Acre	All potato weeds itsit, gajar buti, karund, kulfa
Seed treatments				
Monceren	Pencycuron	25 FC	60 ml / Acre	*Rhizoctonia Solani*

Confidor	Imidacloprid	70 WS	5 g / Kg seed	Aphid, jassid, maize hopper
Hombre	Tebuconazole + Imidacloprid	18.625 FS	4 ml / Kg Seed	Aphid, bunt and smut
Raxil Ultra	Tebuconazole	120 FS	0.25 ml / kg seed	Bunt and smut in wheat

*This information is collected from pamphlets published by the Bayer Crop Sciences.

Table 1.4 List of pesticides of Arysta Life Science Pakistan limited

Trade Name	Name of active Ingredient	Formulation	Recommended Dose	Target pest
Insecticides				
Mospilan	Acetamiprid	20 SP	125 g/kg	Whitefly adult only
Radiant	Spinetoram	120 SC	120 g/L	Boll worm. DBM and leaf minor
Tracer	Spinosad	240 SC	240 g/L	Pink bollworm, leaf minor
Runner	Methoxyfenozide	240 SC	200 g/L	Army worm
Oshin	Dinotefuran	20 SG	100 g / Acre	Jassid
Lorsban	Chlorpyrifos	40 EC	750-1000 ml / Acre	American bollworm and army worm in cotton
			600-850 ml / Acre	Borers and bugs in sugarcane and maize
			200-250 ml / 100 L H_2O	Codling moth in fruits
			500-750 ml / Acre	Beetles in vegetables
Proaxis	Gamma-cyhalothrin	60 CS	500 ml / Acre	Pink, Spotted Bollworms, Army worm & Thrips
Nissorun	Hexythiazox	10 WP	100 g/kg	Mites
Matanza	Pyriproxyfen	10.8 EC	108 g/L	Whitefly nymph
Carbofuron	Carbofuron	3 G	30 g/kg	Borer, stem borer
Cartap	Cartap hydrochloride	4 G	40 g/kg	Stem borer, Shoot borer
Imidacloprid	Imidacloprid	20 SL	250 ml	Whitefly, thrips, jassid, plant hopper, aphid
Fungicides				
Success	Chlorothalonil + Metalaxyl	72WP	250 g/100 L H2O	Downy Mildew
Topsin-M	Thiophanate Methyl	70WP	100-150 g / 100 L H2O	Powdery mildew, Anthracnose of Mango, Water melon, Grapes,
			400-600 g / Acre or 2-2.5 g / kg seed	Rice blast
Kasumin	Kasugamycin	2 SL	600 ml/Acre	Blasts, Bacterial brown strip(Blight), Bacterial Brown Rot Rice, Leaf spots of sugar beet, Soft rot, Common Scab of potato
			2 ml / L H_2O	Citrus canker
Rally	Myclobutanil	40 WSP	50 g / Acre	Powdery mildew of mango, cucurbits, grapes, stone fruits, blackberry, strawberry, Rust and *Rhizoctonia* of cucurbits, black rot and anthracnose of grapes, scab of blackberry and strawberry

Curzate	Cymoxanil + Mancozeb	72 WP (8% + 64% w/w)	600-700 g / 100 L H_2O	Downy mildew of melon, Late blight of potato and tomato
Dithane M-45	Zinc 2%,w/w, Manganese 16% w/w, Ethylene Bis, Dithiocarbonate 62% w/w	80 WP	600-800 g / Acre	Early and late blight of potato, Downy mildew of water melon and melon, cucumber and other vegetables
Moncut	Flutolanil	400 SC	15 ml / 100 kg seed	Rhizoctonia of potato
Herbicides				
Cleanwave	Aminopyralid triisopropano Iammonium 1% w/w (10gm/L) + Fluroxypyr Meptyl 14% w/w (140 gm/L)	15 EO	320 ml/acre	Annual & perennial broad leaf weeds in wheat
Pallas	Pyroxsulam	45 OD	400 ml / Acre	Broad leaf weeds

*This information is collected from pamphlets published by the Arysta Life Sciences.

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CHAPTER-2

2.0 INSECTICIDE FORMULATIONS

For a few decades, growers have relied upon the chemicals to meet the increasing demands of feed and fiber of the human population (Knowles 2008). For this purpose, researchers have tried to develop more effective formulations that can be more efficiently applied to crops to optimize the crop production (Green, Hartley et al. 1987). However, different types of formulations are available in the market depending upon the consumer preference, application and their biological activity (Mulqueen 2003).

A formulation is generally referred to as homogenous mixture of an active ingredient/ technical grade material (responsible for killing the target pests) and inert ingredients that may enhance its physical and chemical properties (Burges 1998). Active ingredients cannot be applied directly in the field because they may be chemically unstable, difficult to handle and to apply in field and may not be mixed well in water directly (Rathburn Jr 1985). Therefore, chemicals are formulated with the objectives to apply them safely with higher shelf life and required biological performance (Seaman 1990; Mulqueen 2003).

A single technical grade material may be available in different formulations to control different stages or different types of insect pests. For example, a liquid formulation of spinosad (240SC) is used to control the armyworm, American bollworm, thrips and diamondback moth in different field crops while a solid formulation of spinosad is used as a bait to control the fruit fly in orchards and vegetables.

2.1 Types of Formulations

Three main types of formulation which are further subdivided:

2.1.1 Liquid formulation

2.1.1.1 Emulsifiable concentrate (EC)

This formulation shares the big volume of all the available formulations worldwide (Knowles 2008). EC is the suspension of oily liquid active ingredient mixed with organic/petroleum based inert solvents along with emulsifiable concentrate agent which allows the concentrate to be diluted in water to form emulsion. Percentage of emulsifying and other agents' represented as inactive ingredients. These are bipolar in nature with one end is hydrophilic and other is hydrophobic. So, careful consideration is required in selection of these agents to avoid creaming, flocculation and coalescence of oil droplets. Non-ionic surfactants usually block copolymers so other polymers are used to stabilize the emulsion (Tadros 1994; Knowles 2008). Emulsion is of milky in appearance (Sarwar 2015).

Target pests of EC formulation

These formulations are active against a wide range of pests (bollworms, whitefly, jassid, thrips, aphid, armyworm and mealybug) and are adaptable to various kinds of field application equipment like low volume ground sprayers, hydraulic sprayers, mist blowers etc.

Examples of EC formulation

Endosulfan (35EC), Fipronil (5EC), Deltamethrin (10EC), Cypermethrin (10EC), Bifenthrin (10EC), Chlorpyrifos (40EC), Esfenvalerate (110EC), Profenofos (500EC), Emamectin benzoate (019EC) and Lambda-cyhalothrin (2.5EC)

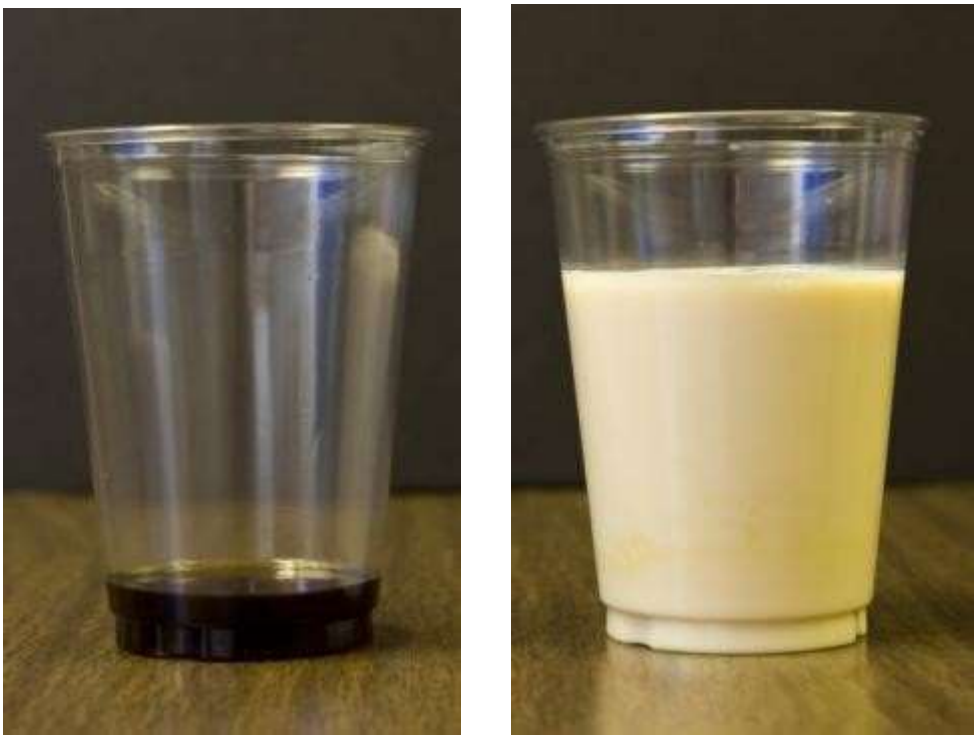
Advantages of EC formulation

EC formulations have many advantages (Fishel 2010).

- Require simple equipment for handling, storage and application.
- It needs little agitation because it does not separate out or settle down while spraying.

- It is not abrasive to the equipment used and does not clog the nozzle and pipes.
- It leaves little residues on treated surface like leaves.

Limitations of EC formulation
EC formulations have many disadvantages (Fishel 2010).
- It may cause damage to plants (phytotoxic) at high temperature.
- Accidental exposure of the poison may cause the skin irritation to animals (absorbed through skin).
- There may be calibration or mixing error due to high concentration of active ingredient that leads to the over or under dose problems.
- Some active ingredients are flammable so should be kept away from heat.
- Some solvents may be corrosive to the equipment.



Form of EC available at the shop Form of EC after mixing in water

Figure 2.1. Emulsifiable concentrate**Source Fishel, 2010**

2.1.1.2 Soluble liquid/ solutions/ water soluble concentrate (SL / S)

It is limited due its hydrolytic stability and solubility in water but simplest of all the marketed formulations (Knowles 2008). These concentrates constitute the liquid active ingredient and many other ingredients that can easily be dissolved in water to form a homogenous mixture or true solution in spray tank. In its concentrated form, it may be used directly or can be further diluted with carrier (Scott 2007; Sarwar 2015).

Target insect pests of SL formulation
Sucking insect pests of cotton like whitefly, thrips, jassid.

Example SL formulation
Imidacloprid (20SL)

Advantages of SL formulation

- Solution requires simple application equipment as they are easy to handle and measure.
- Not require additional ingredients and completely miscible in water.

Limitation of SL formulation

- Limited in number due to insolubility of active ingredients in water (Anonymous 2000).

Form of SL at the shop Form of SL after mixing in water

Figure 2.2 Soluble LiquidSource Fishel, 2010

2.1.1.3 Ultra low volume (ULV)

These are highly concentrated active ingredients (more than 8 pounds of active ingredient per gallon) that are dissolved in minute quantity of organic solvents (Sarwar 2015). These products are mostly used solely but can be diluted to some extent in specific carrier like crop or vegetable oil. It is formulated to be used in specific situations and can be sprayed in the form of mist by aerial equipment such as air crafts, helicopters or fixed-wing aircraft fitted with spray booms and also with ultra-low volume applicator (ULVA). ULV formulation requires much less field application rate about 2 to 4 L ha^{-1}.

Target insect pests of ULV formulation

Chewing insect pests

Advantages of ULV formulation

It shares all the advantages of emulsifiable concentrates except the followings

- It can be applied on large areas with less frequent refilling and refueling because of low application rates and little dilution.
- Reduced evaporation rate because oil is used as diluent rather than water.

Limitations of ULV formulation (Anonymous 2000)

- Require special equipment for field application.
- Difficult to confine the insecticide to target place due to high drift hazards.

- Some solvents may deteriorate the pumps, surface and other parts of the spray tank.
- May cause skin irritation because it can be easily absorbed by skin.
- May cause error while calibration and application to high concentration of technical grade material (Anonymous 2000).

2.1.1.4 Flowables / suspension concentrate (F / SC)

Flowables is solution of some insecticides that are not able to be dissolved with any carrier either oil or water. So, it is a suspension of solid active ingredient coated on clay or dust particles in small amount of solid diluent and wetting agent such as water. The wet blend can be further diluted by adding water to make the final volume for field application (Synek 1982; Sarwar 2015).

Target insect pests of F / SC formulation

Chewing insect pests especially American bollworm, armyworm.

Examples of F / SC formulation

Thiodicarb (80DF), Spirotetramat (240SC), Spiromesifen (24SC), Flubendiamide (48SC), Chlorfenapyr (360SC), Methoxyfenozide (24SC), Spinetoram (120SC), Indoxacarb (150SC), Spinosad (240SC), Difenthuran (500SC).

Advantages F / SC formulation

- Flowables are easy to handle and apply because of liquid nature.
- They require little agitation and leave little residues after application.

Limitations F / SC formulation

- It may be drain out during mixing and application.
- Flowables settle down in the containers so shake them thoroughly before mixing.
- Solid particles are abrasive to nozzle, pumps and pipes (Mulqueen 2003).

Form of SC at the shop Form of SC after mixing in water

Figure 2.3. Soluble concentrate

2.1.1.5 Aerosols (A)

Aerosol is the mixture of the low percentage of one or more active ingredients dissolved in inert liquefaction gas and solvent. Insecticides are mostly packed in pressurized containers. These can be ready to use or can be used with electric or gasoline powered aerosol generators. Fine droplets of solution released in the form of fog or mist under pressure.

Target insect pests of Aerosol formulation
These formulations are mostly used for domestic pests like mosquitoes and bugs (WHO 2006: Sarwar 2015). These can also be used in green houses and to limited extent in localized outdoor areas.

Example of Aerosol formulation
Baygon

Advantages of Aerosol formulation
- Aerosols are ready to use formulations.
- These can be easily and safely carried out from one to another place.
- Purchasers can buy small quantity of insecticide.
- Relatively more efficient and for longer period of time.
- Uniform coverage of the area to be treated.

Limitations of Aerosol formulation
- It can be applied to limited areas especially for indoor applications.
- Increased risk of inhalation and difficult to confine to target site due to high drift hazards.
- It is flammable so handle with care.
- May require extra cost for specialized equipment for artificial fog generation.

Figure 2.4 Aerosols (Pressurized form of insecticide, ready for spray)

2.1.1.6 Termiticide concentrate (TC)

It is one of the new formulations. This concentrate comprises of two components. One is the termiticide concentrate which contain one or more active termiticide (70% by weight) including phenylpyrazoles, neonicotinoids and many others while the other is the aqueous medium to which this composition is dispersed (0.01-2% by weight) (Nouvel and Boyd 2016).

Example: Biflex

Figure 2.5 Termiticide Concentrate

There are following methods for application

Broadcast spray

It is applied through low pressure broadcast sprayer.

Sub slab injection.

It involves the drilling of holes in the treated areas (walls, slabs) and injecting the effective amount of termiticide concentrate. Drilled holes should be filled properly after exposure.

Trenching / rodding

It involves the digging or rodding of narrow trenches that are flooded with termiticide. The excavated soil should also be treated with this concentrate before plugging the trench.

Target areas

This concentrate can be applied in pre and post construction areas including building, wood structures, crack, crevices, basements and other hard to reach areas. This can be used on crops by using common crop sprayer.

2.1.2 Solid/ Dry formulations

These are marketed as ready to use formulations (granules, pellets, dusts and baits) and concentrates (wettable powder, soluble powder) that needs further dilution for final application.

2.1.2.1 Wettable powders (WP/ W)

Dry formulation is mixture of finely ground concentrated powder (25-75 %) of active ingredient and solid diluent such as clay, wetting agent and disperser. Wetting agent helps to ties the active ingredient and diluent with water on mixing. It forms suspension when mixed with water in the spray tank because active ingredients are immiscible in water but these particles remain suspended or dispersed in the carrier. So these products must be added in the partially water filled spray tanks and should be constantly agitated to let the particles dispersed in the water. Wettable powders can be applied as spray as well as dust (Sarwar 2015).

Target insect pests of WP formulation

It is mostly used for sucking insect pests of different crops like whitefly, aphid, mites and leafminer of cucurbits.

Examples of WP formulation

Cyromazine (75WP), Pymetrazin (50WP), Hexithiazox (10WP), Buprofezin (25WP).

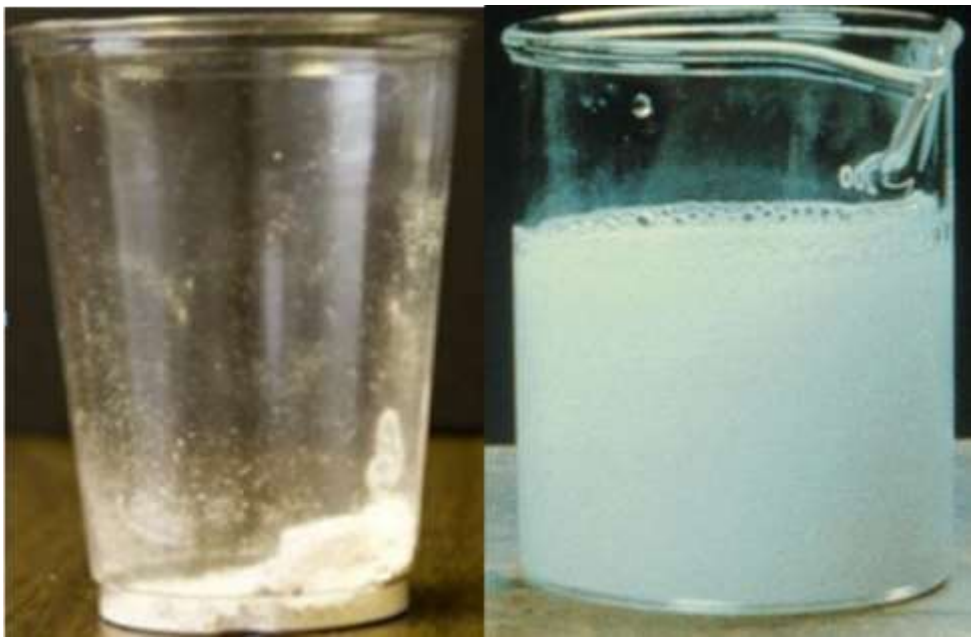
Advantages of WP formulation

- Easily and safely transported, stored and handled.
- Relatively less harmful to treated surfaces than emulsifibale concentrate.

- It can be easily calibrated and mixed.
- Less skin and eye absorption by animals than other liquid formulations.

Limitations of WP formulation

- Respiratory hazards by inhalation of powder while handling.
- Require constant agitation because it quickly settles down.
- Abrasive to application equipment.
- Block the pipes and nozzle of spray tank.
- Hard to mix in alkaline water.
- Leave visible residues.



Form of WP at shop Form of WP after mixing in water

Figure 2.6 Wettable Powder**Source http://edis.ifas.ufl.edu/pdffiles/PI/PI23100.pdf**

2.1.2.2. Soluble powder (SP/ WSP)

It is formulation of finely grounded soluble powder that dissolves readily in water and form true solution. The amount of active ingredient ranges from 15-95 %. These are limited in number because few active ingredients are water soluble (Scott 2007).

Target insect pests of SP formulation

Like wettable powders, it is also applicable against sucking insect pets as well as bollworms and armyworm.

Examples of SP formulation

Trichlorophone (80SP), Acephate (75SP), Acetamaprid (20SP), Methomyl (40SP).

Advantages of SP formulation

- Easy to handle during mixing and storage and safe to transport.
- Easily soluble in water which is the cheapest source for farmers.
- Relatively less harmful to plants (less phytotoxic) and other organisms (less absorption through skin) than petroleum based formulations.
- Formulations packages can be easily disposed of.

Limitations of SP formulation

- Limited in market because all active ingredients are not soluble in water.
- Inhalation hazards while mixing the formulation.

Form of SP at the shop Form of SP after mixing in water

Figure 2.7 Soluble Powder **Source Fishel, 2010**

2.1.2.3 Dusts (D)

Dust is the formulation of very fine particles mixed with inert carrier such as clay, chalk, silica, talc or volcanic ash. Some dust formulations are highly concentrated and may contain high percentage of technical grade material so these must be mixed with inert carrier while some products are ready to use and contain a small amount (< 10 %) of active ingredient (Scott 2007).

Target insect pests of Dust formulation

These products are mostly used for seed treatments, indoor (ants, termites, cockroaches) and other parasitic insect pests.

Example of Dust formulation

Coopex dust (permethrin)

Advantages of Dust formulation

- Ready to use formulation and need no further mixing.
- It can be efficiently applied in hard to reach areas by using simple equipment.
- It can be efficiently used where moisture of the spray may cause problem.

Limitations of Dust formulation

- Increased risk of inhalation and drift hazards.
- Residues may be easily moved off by wind and may irritate nose, throat, eyes and skin.
- Dampness may reduce the effectiveness by clogging and lumping of solid particles.
- May provide uneven exposure of the target area.

Figure 2.8 Dust
Source http://edis.ifas.ufl.edu/pdffiles/PI/PI23100.pdf

2.1.2.4 Granules (G)

The technical grade material is similar to dust (1-15 %) and coated on coarse sized and heavier sand or plant material to form granule. These products contain a low percentage of active ingredients (1-15 %) and defined by their particular size of granule (granules passed across 4-mesh sieve and retained through 80-mesh sieve). These are applied as dry and upon contact with water, they start to release toxin slowly (Anonymous 2000).

Target insect pests of Granule formulation

These are used against agricultural, soil (nematodes) as well as aquatic pests such as mosquitoes (Fishel 2010). For agricultural pests (borers of rice and sugarcane) they are being applied in the roots that transfer the toxin to upper parts by absorption.

Examples of Granule formulation

Carbofuran (3G), Cartap hydrochloride (4G), Thiomethoxam (25WG).

Advantages of Granule formulation

- Ready to use formulation without further dilution or mixing.
- Low drift hazards due to large particle size.
- Little risk of exposure to the applicator.
- Slow release rate than powder formulations.
- Can be applied by simple equipment.

Limitations of Granule formulation

- Non-target organisms like birds and other animals are at risk after its exposure.
- Unable to stick to foliage or may require water to release the active ingredient so not efficient in drought conditions.
- Cannot be dispersed uniformly.

Figure 2.9 Granules
Source http://edis.ifas.ufl.edu/pdffiles/PI/PI23100.pdf

2.1.2.5 Pellets (P/ PS)
Pellet formulations is resembling to the granules except a thick paste of liquid is coated on the solid carrier as like granule but under certain pressure it is extruded to cut into uniform shapes. Pellets are usually similar in shape and size and mostly applied in localized areas. Pellets are usually similar in shape and size and mostly applied in localized areas (Scott 2007).

Figure 2.10 Pellets**, Source http://edis.ifas.ufl.edu/pdffiles/PI/PI23100.pdf**

2.1.2.6 Baits (B)
Bait formulation is the combination of a toxic technical grade material (< 5%) mixed with any attractant or food based carrier like bran, orange pulp, corn cobs and sugar (Sarwar 2015). Pests are forced to being attracted by the bait and killed by consuming the toxic substance present in it.

Target insect pests of Bait
It can be effectively used for indoor and outdoor pests such as ants, roaches, flies (fruit fly) and even for vertebrate pests.

Example of Bait
Spinosad in Bait

Advantages of Bait

- Ready to use
- Not need to treat the entire area because bait attracts the insect pests.
- It is mostly used for mobile pests.

Limitations of Bait

- Other domestic animals, wild life and children can be attracted and killed by bait.
- Food or other crop may prefer by pests to bait.
- Dead insects may cause space or odor problems.
- It can serve as a food when active ingredient becomes ineffective.

Figure 2.11 Baits

2.1.3 Fumigants (F)

Fumigants are the solid or liquid active ingredients and become gaseous on application at room temperature. Liquid active ingredients may be volatile or may be formulated under high pressure. But some are solids in the volatile pellets or tablets and require humidity or small amount of water of being vaporized. These are active in gas tight atmosphere against all insects irrespective of their mouth parts (Anonymous 2000).

Target insect pests of Fumigants

They are non-selective and used against a wide range of pests.

Example of Fumigants

Methyl bromide, Aluminum phosphide, Dichlorvos, Naphthalene

Advantages of Fumigants

- Fumigants have broad spectrum activity to various insect pests.
- It easily and efficiently penetrates in hard to reach areas like grain bins, cracks, crevices and wood structures.
- Usually single treatment is enough to kill the target pest of that area.

Limitations of Fumigants

- Efficient in properly enclosed areas to prevent the escape of gaseous poison.
- Due to broad spectrum activity, it is also hazardous to non-target organisms.
- Fumigants require specialized training for application due inhalation hazards.
- Insecticidal exposure may require extra cost for specific equipment.

Figure 2.12 Fumigant

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CHAPTER-3

3.0 BIOCIDAL ACTIVITY OF INSECTICIDES

More than 70% population of world depends upon agriculture sector for their survival but unfortunately agricultural crops are facing huge reduction in their growth, production, and quality on regular basis (Oerke, 2006). In order to overcome the problem of yield reduction farmers started to use insecticides as one of the best control strategy (Martins et al., 2014). Protection of crops from injurious insect pests heavily relies on the use of various insecticides since many years (Hussain et al., 2012). Use of insecticides is dominant among all the pest management tactics due to their efficacy and quick action against different insect pests. Insecticide use also enhances the yield and quality of produce which give high returns to producer (Karar et al., 2012). Despite controlling crop pests, insecticides are also tool to control various vector borne diseases by killing the insect vectors and carriers such as houseflies, mosquitoes, sand flies and bedbugs (Kaur & Sandhu, 2008). There are two main categories of insecticides; natural and synthetic. Synthetic insecticides replaced the natural insecticides due to their quick killing action since World War II (Karar et al., 2013). The first used synthetic insecticide was DDT from organochlorins group (Abbas et al., 2014b). Later, the phenomenon of biomagnification and highly persistence character of organochlorins necessitated the development and launching of other insecticide groups including organophosphates (1960s), carbamates (1970s), and Pyrethroids (1980s) (Aktar et al., 2009). After the discovery of these conventional insecticides, new chemistry insecticides with novel mode of action were also discovered in 1990s (Ferré & Van Rie, 2002). The insecticide use in insects brings various morphological, physiological; biochemical and molecular changes in insect body that result in mortality in most of the cases (Ferré & Van Rie, 2002). The focus of this chapter is to highlight the various physiological, physical, biochemical and molecular pathways in insects that are responsible for the toxic effects of the insecticides.

3.1 Conventional Insecticides

3.1.1 Organochlorins

Organochlorins (OCs) are chlorinated hydrocarbons and contain the elements carbon and chlorine (Baird C, 2004). They have been used extensively from the 1940s through the 1960s. Some of the most widely used insecticides in this group are dichloro-diphenyl-trichloroethane (DDT),dieldrin, aldrin, heptachlor, chlordane, lindane, endosulfan, and toxaphene (Coats et al., 1990; Calle et al., 2002). DDT was first developed and most famous pesticide from this group and was used during World War II for control of lice and mosquitoes to eliminate typhus and malaria, respectively (Snedeker, 2001). Insecticides of this group target the peripheral nervous system. OCs are the axonic poisons, interrupt normal nerve impulse transmission along axon. These poisons after reaching the axon act on the sodium gates and keep them open for longer period of time. This results in constant leakage of sodium (Na) ions through the nerve membrane, creating a state of action potential continuously without any resting potential. This causes triggers (repetitive discharges) and insects show the symptoms of hyper excitability, paralysis, convulsions and finally death occurs (Hardman et al., 2007) (Figure 3.1). Death occurs only if doses are lethal. The use of OCs is banned now in majority of countries because of significant residual effects, high persistence in the environment and also due to phenomenon of biomagnification (decompose slowly in their environment and become part of animal's fatty tissues; they also stay longer in environment and food web after their application (Jayaraj et al., 2016).

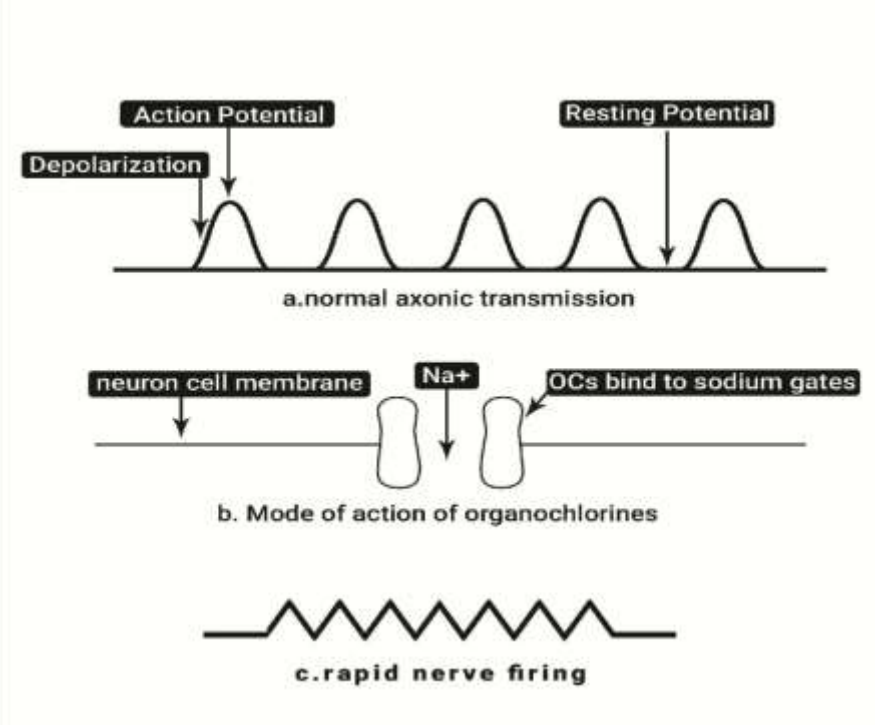

Figure 3.1 Interruption of axonic transmission by organochlorines

3.1.2 Organophosphates

Insecticides of this group are used most widely throughout the world. Above 100 compounds of this group are used commercially for the control of various insect pests although over one lac compounds have been tested for insecticidal properties. Some of the main insecticides of this group are triazophos, profenofos, chlorpyrifos, fenitrothion, quinalphos and malathion (Greene & Pohanish, 2005). Organophosphates (OPs) have a wide range of pest control applications as contact, systemic, stomach and fumigant insecticides. These insecticides show their toxic effect at insect synapse. The biocidal effects of the OPs are almost entirely due to the inhibition of acetylcholinesterase (AchE) in the nervous system at post-synaptic neuron, inhibiting the breakdown of neurotransmitter acetylcholine (Ach) resulting in respiratory, myocardial and neuromuscular transmission impairment (Smegal, 2000). Inhibition of AchE by OPs is irreversible and the process is called phosphorylation (Hwang, 2014) (Figure 3.2). The poisoned insect shows the symptoms of restlessness, hyper excitability, tremors, convulsion, paralysis and ultimately death occur due to rapid nerve firing (Costa et al., 2006).

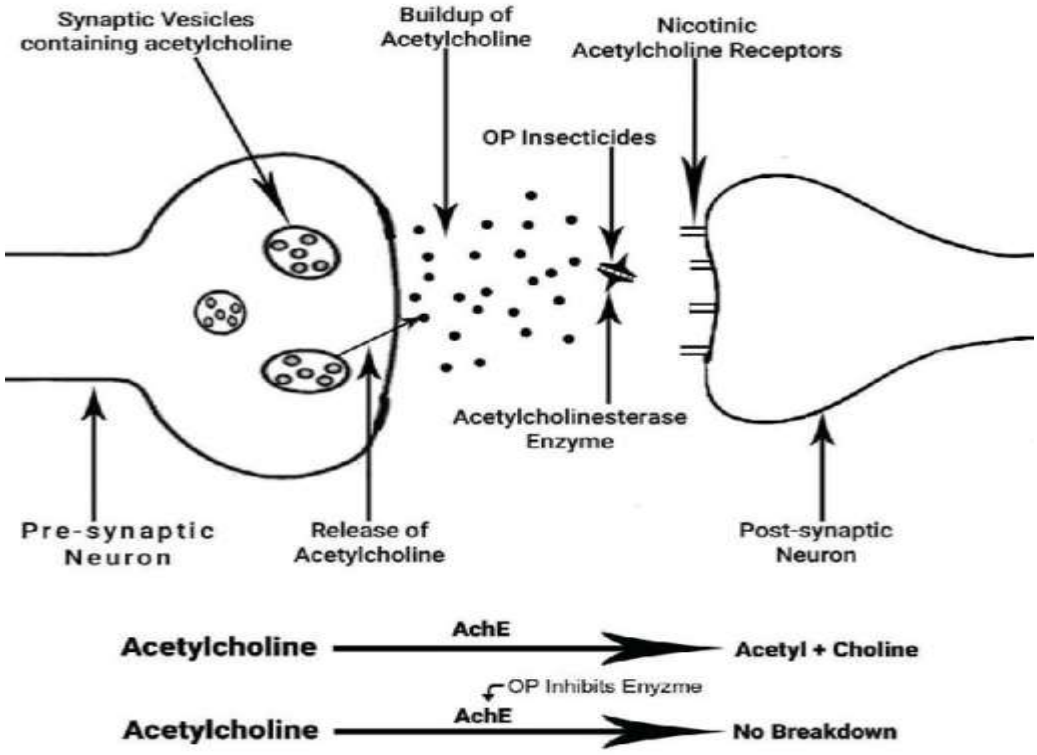

Figure 3.2 OP insecticides interrupt synaptic transmission

3.1.3 Carbamates

The derivatives of carbamic acid (NH_2COOH) are called carbamates. These are organic in nature. Chemicals from this group find applications in crop protection as insecticides, herbicides and fungicides. Some important representatives of carbamates are carbofuran, carbaryl, methomyl, oxamyl and propoxur. They act as stomach as well as contact poisons. Some carbamates are also used as fumigants. Insecticidal activity of carbamates is due to hydrogen (H) and methyl group (-CH3) in the place of R_2 and R_1, respectively (Figure 3.3). They are also called synaptic poisons. Their insect killing action is based on reversible AChE inactivation, the process is called carbamylation. Insect suffer same kind of symptoms as discussed for organophosphates (Colovic et al., 2013).

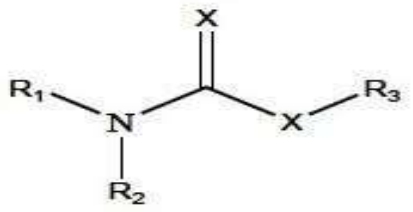

Figure 3.3 General chemical structure of biologically active carbamates

3.1.4 Pyrethroids

Pyrethroid insecticides interact with the insect voltage sensitive sodium channel (VSSC), causing death. Synthetic pyrethroids are derivatives of Pyrethrins. There are two types of

pyrethroids; Type I and Type II that differ in their structure and biocidal activity in insects. Allethrin, resmethrin, tetramethrin, bioresmethrin, and permethrin are Type I pyrethroids while deltamethrin, cypermethrin, fenvalerate, cyfluthrin, and lambda-cyhalothrin are Type II pyrethroids (Sumita & Aichi, 2016). Pyrethroids gain entry into insect body through contact and act as knockdown agents. Pyrethroids which are used as aerosols gain entry into insect body through spiracles and provide rapid knockdown action by acting on the nervous system. Similar to organochlorins, pyrethroids are also known as axonic poisons. In neurons, these insecticides bind to the voltage gated sodium channels (proteins) in nerve cell membrane, and delay the closing of sodium ion channels. It results in continuous nerve stimulation and multiple action potentials. Bursts of repetitive discharges and action potentials with increased amplitude are the characteristic effects of Type 1 pyrethroids. However, action potentials with lower amplitude are due to Type II pyrethroids. This significant depolarization of neuron membrane leads to total blocking of neural activity (Söderlund & Ihre, 1985) (Figure 3.4). Poisoned insects lose control over their nervous system, display lack of coordinated movement. Hyper-excited insects display the symptoms of frequent tremors, convulsions, paralysis and ultimately die. Many pyrethroid insecticides find applications as household products in the world due to their rapid knockdown effect and low toxicity profile to mammals.

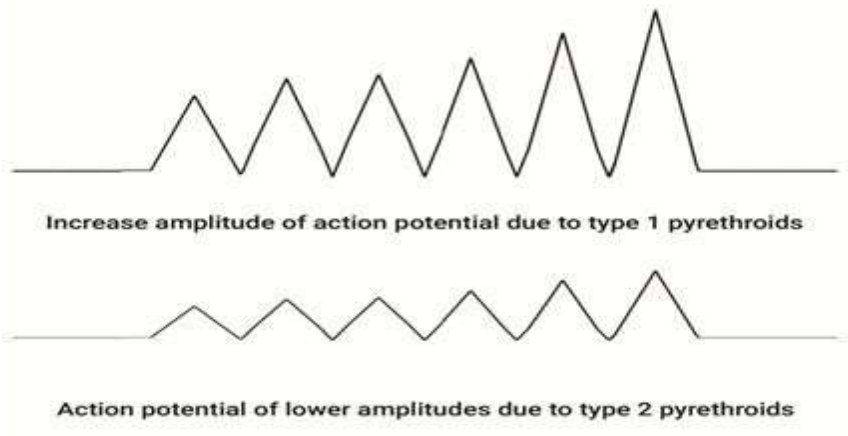

Figure 3.4 Effect of pyrethroid insecticides in insects

3.2 New Chemical Insecticides

3.2.1 Neonicotinoids

Efforts were made to synthesize the insecticides which have high binding affinity for insect nicotinic acetylcholine receptors (nAChR), and this ultimately resulted in the development of nicotinyl insecticides as one of a new group (Elbert et al., 1998). This is the group of insecticides with novel chemistries. Other names of this group are nicotinoids, neonicotinyls and chloronicotines. Insecticides of this group are imidacloprid, acetamiprid, thiamethoxam, clothianidin, nitenpyram, dinotefuran, thiacloprid, and nithiazine (Elbert et al., 2008). These insecticides are highly recommended to be used against various sucking insect pests such as thrips, jassids, mealybugs, dusky cotton bugs, aphids, planthoppers; and well fitted in multiple IPM programs due to their mild effect on some bio-control agents and honeybees (Ishaaya et al., 2007). These insecticides are systemic in nature and translocate throughout the plant tissues. These properties make these insecticides effective for many pests for longer period of time. These are also used in small quantities for seed treatment (Jeschke et al., 2011). These insecticides act in the insect central nervous system (CNS), bind to nicotinic acetylcholine receptors (nAChRs), agonizes their action and hinder nerve impulse transmission as a result of a depolarizing effect (Elbert et al., 2008). Irreversible blocking of nicotinergic acetylcholine receptors may also occur due to these insecticides (Figure 3.5).

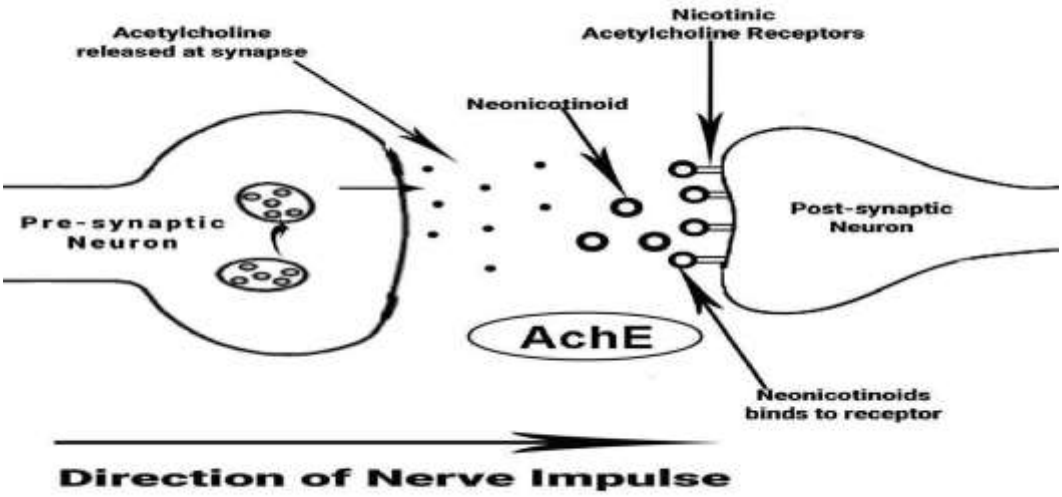

Figure 3.5 Biocidal mechanism of neonicotinoids

3.2.2 Oxadiazines

Oxadiazine contain single insecticide, indoxacarb that was discovered by E.I. DuPont Co. and is exploited for crop protection (McCann & Johnston, 1992). This insecticide gives promising control of different insects belonging to order Lepidoptera, Homoptera and Coleoptera and also possess low mammalian toxicity with reduced pesticide risk (Jeffery et al., 2000). Indoxacarb reaches the insect target site either through cuticle or via ingestion. It is a pro-insecticide and biologically activated during metabolism. The biocidal activity of indoxacarb in various insects is due to phenomenon of bio-activation. In this process, the indoxacarb act as pro-insecticides but after entering into insect body undergoes metabolism by esterase and amidase enzymes which ultimately produces an N-decarbomethoxylated metabolite. This active metabolite binds to the voltage gated sodium ions channels; blocks them and preventing sodium ions movement in to nerve cells, finally insect paralysis and death occur (Wing et al., 2000; Wing et al., 2010) (Figure 3.6). Potency of N-decarbomethoxylated metabolite is forty times higher as compared to pure indoxacarb in blocking the sodium ions channels (Silver et al., 2010; Wing et al., 2010). Feeding cessation, loss of nerve function, paralysis and death are the outcomes of indoxacarb poisoning. Feeding cessation occurs almost immediately after ingestion or absorption of indoxacarb even though it may take several days for insects to die (Wing et al., 2010). Indoxacarb was evaluated against the European earwig (*Forficula auricularia* Linnaeus), and was found to be an effective contact toxicant with residual activity on substrates commonly encountered in urban environments. Within 16 h of being directly sprayed with indoxacarb, ≥90% of earwigs were either ataxic, moribund, or dead, and 100% displayed these symptoms of severe intoxication at 1day. Brief exposure (5 min or 1 h) to dried residues on either a porous (pine wood) or non-porous (ceramic tile) substrate also was sufficient to cause severe intoxication of earwigs within 1 day. In all bioassays, indoxacarb-treated earwigs showed no signs of recovery during the 21 day observation period. In outdoor urban habitats, intoxicated earwigs would be more vulnerable to desiccation, predation, or pathogens leading to higher mortality than in a laboratory setting (Jones & Bryant, 2012).

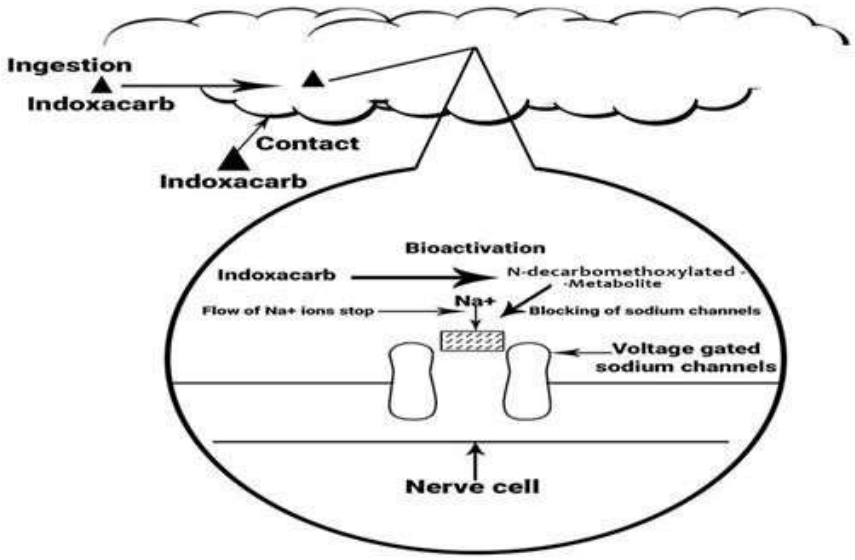

Figure 3.6 Mode of action of indoxacarb

3.2.3 Spinosyns

Spinosyns family contain two important insecticides spinosad and spinetoram with broad activity spectrum such as against crop pests that cause extensive damage and also kill many important external insect parasites of humans, livestock and companion animals. Spinosyns are macrocyclic lactones and were derived from actinomycete soil bacterium *Saccharopolyspora spinosa* Mertz & Yao (Sparks et al., 1998). Spinosad contains spinosyn A and D, with spinosyn A as a major component. Spinosad is toxic to insects both by contact and ingestion. Spinosad acts on insect nervous system and kills the insects in quite unique and different way when compared to other insecticides (Sparks et al., 1998), It has two different target sites, first one is the nicotinic acetylcholine receptor while gamma amino butyric acid (GABA) receptor the secondary site of attack (Salgado, 1997). Insects treated with spinosad result in excitation of the insect nervous system, which further causes involuntary muscle contractions, tremors, and paralysis and finally death (Duke et al., 2010). In the United States, spinosad has been registered for more than 180 crops and in over 35 countries for the control of leafminers, beetles, lepidopteran insects, and thrips (Zhao et al., 2002). The effects of spinosad on tobacco beetles at different life stages were investigated by feeding tobacco beetles with tobacco leaves and flours containing different doses of spinosad and the larvae of tobacco beetles were treated with spinosad solution of different concentrations. The results indicated that the contact and stomach toxicities of spinosad to 5-instar larvae were weak, however at the concentrations of 5 and 10 mg/kg, spinosad could completely inhibit the hatching of tobacco beetle eggs and reproduction of adult tobacco beetle (Mei & Ling, 2009).

3.2.4 Avermectins

Avermectins are compounds of biological origin and are obtained during fermentation process by soil actinomycete bacterium, *Streptomyces avermitilis* (Burg et al., 1979). The important representatives of this class are abamectin and emamectin benzoate and are known for insecticidal and miticidal activity, and are known as macrocyclic lactones (Dybas, 1989). These compounds act on the GABA and/or glutamate receptors as chloride channel agonist at neuromuscular junction and cause the permanent activation (opening) of chloride channels. These actions stimulate an influx of chloride ions into neurons which results in disruption of nerve impulses and complete loss of cell function (Figure 3.7). Insects are paralyzed and stop their feeding activity, which leads to death. These compounds cause maximum mortality in mites and insects in approximately 4 days. Although, avermectins are not known for quick knock down effect but shortly after their ingestion feeding is ceased due to rapid paralysis and feeding damage to crops is reduced significantly.

Arthropods intake avermectins both by ingestion and contact, however, primary route of uptake is ingestion whereby arthropods accumulate a lethal dose (Turner & Schaeffer, 1989). Avermectins also possess translaminar activity thus providing a relatively prolonged residual activity (MacConnell et al., 1989). Abamectin has biocidal activity against some mite species such as eriophyid mites (citrus rust mite) and tetranychid mites (two spotted spider mite) (Dybas, 1989). Among insects, the abamectin has been proved highly toxic to diamondback moth *Plutella xylostella* (Linnaeus), tobacco budworm *Heliothis virescens* (Fabricius), tomato pinworm *Keiferia lycopersicella* (Walsingham), tobacco hornworm *Manduca sexta* (Linnaeus), serpentine Leafminer *Liriomyza trifolii* (Burgess) and Colorado potato beetle *Leptinotarsa decemlineata* Say (Dybas, 1989). It is also effective in controlling the vegetable Leafminer *Liriomyza sativae* Blanchard (Reitz, 2013). Insecticidal activity spectrum of emamectin benzoate is much broader as compared to abamectin. Emamectin benzoate is recommended at low use rates in field crops and vegetables for the control of different lepidopteran pests for instance *H. virescens*, beet armyworm *Spodoptera exigua* (Hübner), *P. xylostella*, and cabbage looper *Trichoplusia ni* (Hübner) (Ishaaya et al., 2002). It has been studied that spray of emamectin with concentration of 25 mg a. i /liter in a cotton field resulted more than 90% suppression in larval population of *H. armigera* up to 28 days after treatment. It is also effective insecticide against the western flower thrips, *Frankliniella occidentalis* Pergande both under laboratory and under both field and laboratory conditions. Its activity for adult insect stages was greater than 10-fold as compared to abamectin. Emamectin also exhibits a significant activity against whitefly *Bemisia tabaci* (Gennadius) under laboratory conditions (Ishaaya et al., 2002).

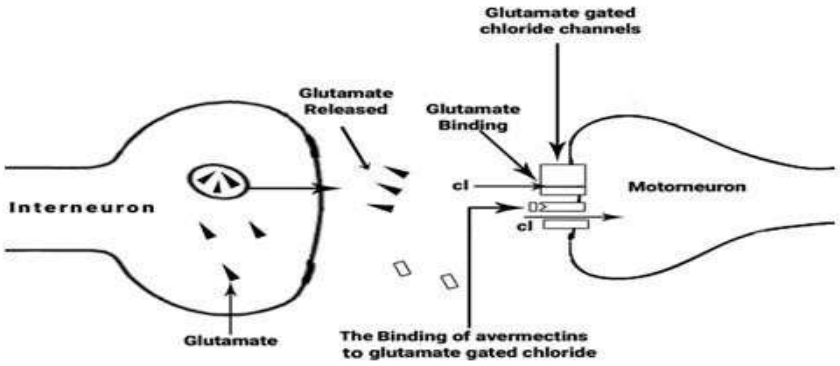

Figure 3.7 Mode of action of avermectins

3.2.5 Anthranilic diamides

Anthranilic diamides are insecticides with unique modes of action from IRAC group 28 and contain chlorantraniliprole and cyantraniliprole as major active ingredients (Lai & Su, 2011; Wang & Wu, 2012). These insecticides bind to the ryanodine receptors (RyRs) at insect muscles and cause the continuous opening of calcium ion (Ca^{2+}) channels, releasing stored calcium from the sarcoendoplasmic reticulum (Figure 3.8). The uncontrolled release and depletion of calcium from muscle cells stops further muscle contraction and insect dies in 72 hours due to feeding cessation, regurgitation, general lethargy, and muscle paralysis (Wang & Wu, 2012) Chlorantraniliprole (®) has excellent insecticidal activity against many pests of Hemiptera, Isoptera, Coleoptera, Diptera and Lepidoptera and it is the first generation ryanodine receptor insecticide from this novel class (Lahm et al., 2005; Sattelle et al., 2008). Cyantraniliprole is a second-generation insecticide of this class with systemic and translaminar action against wide range of chewing and sucking insect pests (Sattelle et al., 2008). Chlorantraniliprole has been shown to cease feeding in *S. exigua*, corn earworm, *Helicoverpa zea* (Boddie), *T. ni* and *P. xylostella* within 25.3, 20.3, 23.4 and 15.4 min, respectively, after larval exposure to chlorantraniliprole treated at the rate of 167 mg a.i/L (Hannig et al., 2009). Exposure of tobacco thrips, *Frankliniella fusca* (Hinds) and *F. occidentalis* to cyantraniliprole treated plants at the rate of 4.41 mg a.i per plant has also reduced feeding (Asner et al., 2011). Up to a 50% reduction in Asian citrus psyllids *Diaphorina citri* Kuwayama feeding was also recorded following cyantraniliprole treatment (Tiwari & Stelinski, 2013).

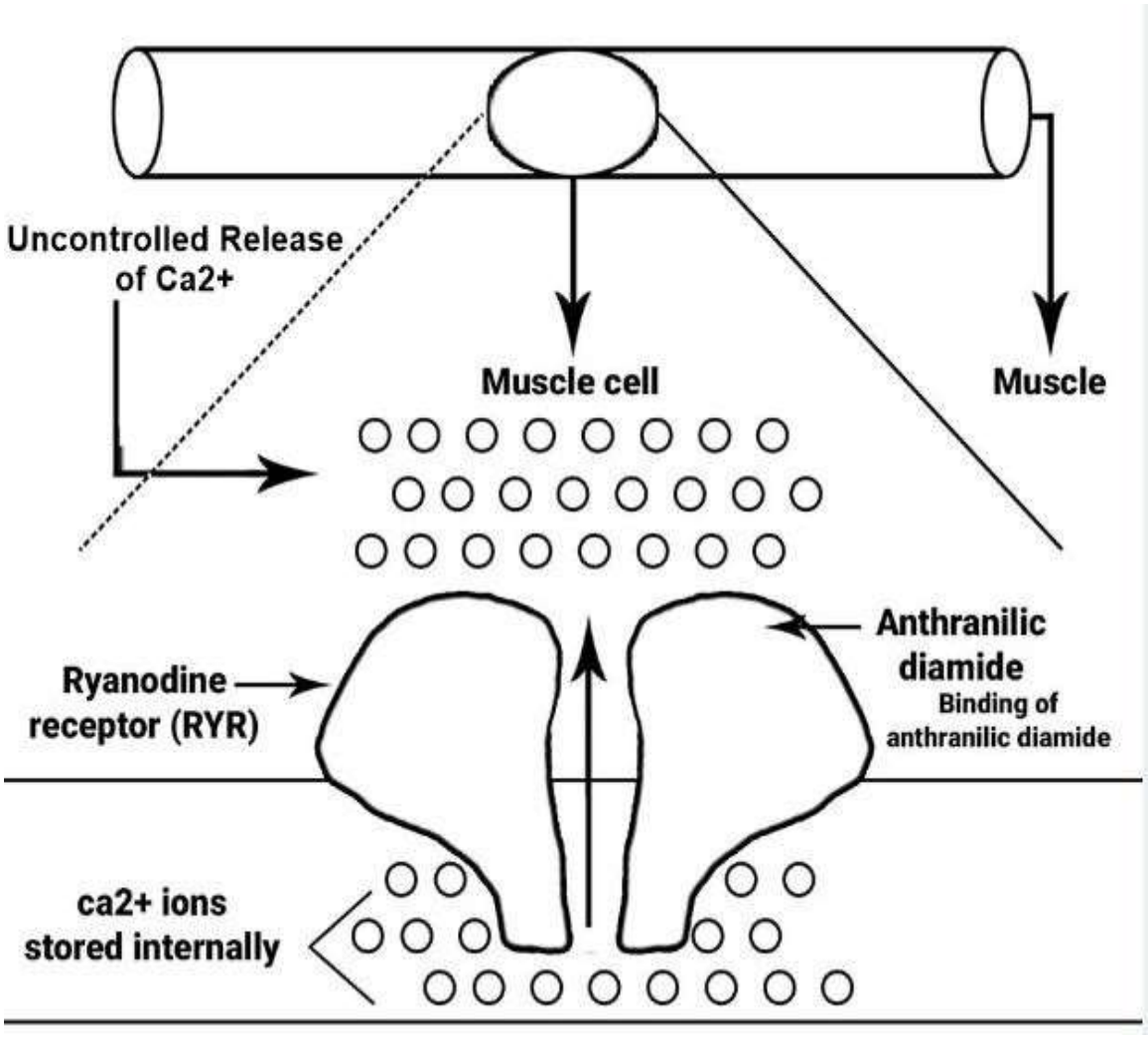

Figure 3.8 Biocidal mechanism of chlorantraniliprole (Rynaxypyr®)

3.2.6 Phenylpyrazoles

Phenylpyrazoles have been developed to manage insect pests of field crops and public health importance. This group contains two insecticides Ethiprol and Fipronil, the latter is widely used. Fipronil has biocidal activity against insects of the order Hemiptera, Lepidoptera, Thysanoptera, Coleoptera, Diptera and other pests especially rice borers (Abbas et al., 2014a; Abbas et al., 2014b). For toxicity, the main function of this product is to cause stomach poisoning as well as contact and systemic poisoning. Fipronil is noncompetitive blocker and inhibits the chloride ion flow by targeting the gamma-amino butyric acid receptor (GABAR), primary site of action, in the central nervous system. It is antagonistic in action and prevents the inhibitory effect of neurotransmitter, the GABA, and ultimately causes the hyper excitation of central nervous system (CNS) (Cole & Engeström, 1993). In cockroach neurons, recently fipronil has been found to potently block glutamate-activated chloride channels (Zhao et al., 2004). Being systemic, fipronil translocate throughout all plant tissues making it toxic to any insects (and potentially other organisms) that feed upon the plant. This protects the plant from direct damage by herbivorous (mainly sap feeding) insects and indirectly from damage by plant viruses that are transmitted by insects (Simon-Delso et al., 2015).

3.2.7 Pyrroles

Pyrroles are broad-spectrum insecticides, which show contact and stomach toxicity (N'Guessan et al., 2007). Pyrroles are pro-insecticides that are transformed into the toxic form by mixed function oxidases (MFOs) within the body of an insect. The activated Pyrroles after reaching mitochondria express their biochemical action by uncoupling oxidative phosphorylation. These insecticides impair the mitochondrial ability to produce ATP by disrupting the proton gradient across mitochondrial membranes. This causes the disruption of respiratory pathways, cell death and ultimately affected pest dies (Hunt & Treacy, 1998) (Figure 3.9). The important active ingredients of this group are cyanopyrrole and chlorfenapyr. Laboratory assays have shown that cyanopyrrole and chlorfenapyr possess potent, broad-spectrum biocidal activity against many species of Lepidoptera, Coleoptera, Thysanoptera and Acarina. Field trials conducted with cyanopyrrole at the dose 60-400 g/ha have been proved very effective for the control of more than 70 species of phytophagous insects and mites in different crops (Miller & Borden, 1990).

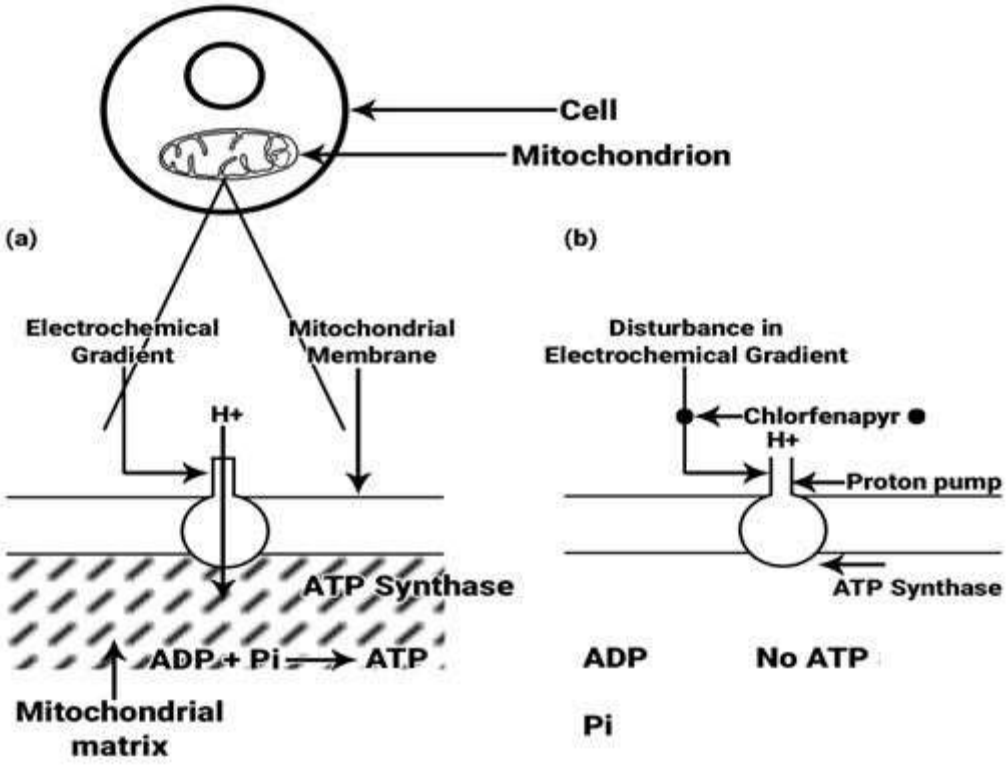

Figure 3.9 (a) Normal ATP synthesis in insects (b) ATP synthesis inhibited due to chlorfenapyr poisoning in insects

3.2.8 Thiourea insecticides

This group has single active ingredient called diafenthiuron. Diafenthiuron is broad-spectrum insecticide and is reported to be effective against all sucking pests in cotton against whiteflies, aphids and thrips in tomato, whiteflies and leaf hoppers in brinjal, and is also effective against diamondback moth (DBM) in vegetables. It also acts as acaricide. Contact or stomach action of this insecticide kills the larvae, nymphs and adults. It also possesses ovicidal properties. Pests hidden in the plant canopy or below the leaves surfaces are also controlled by this insecticide due to its trans-laminar action. Its vapor phase action enables the farmers to apply this insecticide in larger fields as well as in dense cropping system. Diafenthiuron is pro-insecticide and after entering the insect body or by sunlight converted to more toxic form called carbodiimide (Farlow et al., 1991). Hence biocidal activity of thiourea insecticides is due to carbodiimide. This active compound targets the insect mitochondria and inhibits the process of oxidative phosphorylation and mitochondrial ATP synthesis by binding with ATPase, which ultimately disturbs the insect

respiratory system (von Ruden & Neher, 1993) (Figure 3.10). Insects become immobile and paralyzed, death occurs in 3-4 days after initial exposure.

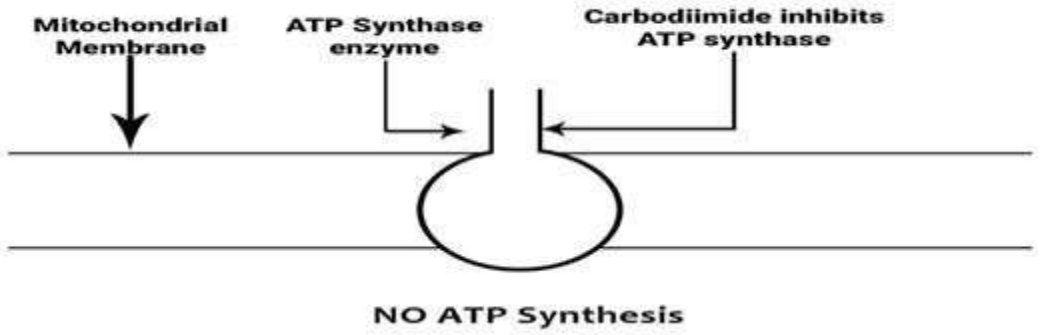

Figure 3.10 Biocidal activity of thiourea insecticide

3.2.9 Ketoenoles

This is the novel group of insecticides. It has two important active ingredients spiromesifen and spirotetramat. Spiromesifen is a derivative of spirocyclic tetronic acid, acts as non-systemic insecticide/ acaricide. It is mainly active against whiteflies following foliar applications, and as an acaricide possess highly active against *Tetranychus spp*. in many cropping systems such as cotton, vegetables, and ornamentals (Nauen & Lauder, 2002). This insecticide enters the arthropod body by direct feeding or as a result of contact and inhibits lipid biosynthesis in insects and mites by preventing the formation of fatty acids and their derivatives. Ultimately, it inhibits development in egg and immature stages and also reduces fecundity of adult female (Nauen & Lauder, 2002). Spirotetramat is a derivative of spirocyclictetramic acid. This is also lipid biosynthesis inhibitor. It is chiefly effective against immature stages of sucking pests such as aphids, scales, whiteflies, psyllids, and mealy bugs. The compound reduces the fecundity and fertility in adult female significantly and in consequence reduces insect populations. It is a fully systemic insecticide with distinguishing translocation properties. Following foliar application and uptake the insecticidal activity is trans-located throughout the vascular system, i.e. through its translocation in the xylem and phloem it moves upwards and downwards, respectively. Hence it is called two-way systemic (ambimobile) insecticide. These unique characteristics of spirotetramat allow the control of concealed pests for instance root aphids and the protection of new shoots or leaves appearing after foliar application (Nauen et al., 2008).

3.2.10 Bt toxins

Bacillus thuringiensis Berliner (*Bt*) is rod-shaped, gram-positive, and spore forming bacterium which has been isolated worldwide from different sources such as dead insects, soil, water, silos and insectivorous mammals (Palma et al., 2014). Bt strains are sources of different insecticidal proteins (crystal proteins) that are useful in killing the larvae of different insects. Toxins produced by *Bt* strains are called endotoxins which are actually inactive proteins. For biocidal activity, insect (larvae) must ingest these toxins. After ingestion, these toxins reach the insect midgut where they are dissolved in midgut lumen having alkaline pH. These toxins are activated in midgut due to proteases (Proteolytic enzymes includetrypsin-like serin-proteases, elastase-like and chymotrypsin-like proteases). In the midgut, these active toxins bind to specific receptors of midgut epithelial cells. At this stage insect stops feeding. The active toxins cause perforation and lyses of midgut epithelial cells and enter to insect haemolymph where they germinate and spread. Finally, insect dies due to septicemia (Palma et al., 2014).

3.2.11 Insect growth regulators (IGRs)

These are the novel insecticides, also called third generation insecticides; kill the insects by interfering with the normal process of growth and development in insects (Keeley, 1990).

3.2.11.1 Juvenile hormone agonists or analogs (JHAs)

Active ingredients of this group are fenoxycarb, hydroprene, kinoprene, methoprene, and pyriproxyfen. In immature stages of insects, these toxic compounds mimic juvenile hormone (JH), which is important in controlling development and growth in insects and also helps in normal maturation process. The high quantity of JH in haemolymph blood of immatures prevents them from becoming adult. During the course of development, the level of JH reduces in the immature insects due to degradation of this hormone by enzymes.(DeFur, 1999). The insect can proceed naturally toward adulthood with less JH. Reproductive maturation such as spermatogenesis and oogensis in adult males and females respectively is also considered to be due to JH. Differentiation of casts in social insects for example termites also depends upon presence of JH for instance; worker termites with high JH levels develop into soldiers.

Studies suggest that Biocidal activity of Juvenile hormone analogs (JAHs) is due to binding of these chemicals with JH-degrading enzymes of JH receptors. JHAs help in maintaining JH at high levels within the insect body at a time when it should not be present naturally. This condition effects the reproduction and survival in insects significantly, severely altering its reproductive physiology and/or disrupting the insect's development. Exposure to JHAs often results in death or sterilization. For example, egg production in queens of fire ants was stopped after exposure to JHA-based baits (Nijhout et al., 2014).

Physiological processes related to development in immature fleas and mosquitoes are negatively affected when exposed to methoprene, which results in severe developmental abnormalities that finally lead to death. In a study, exposure of German cockroaches to JHAs converted the last instar adult males or females with physical inability to mate and deformed ovaries, respectively. This resulted in the production of sterile adults in the population of cockroaches. This ultimately resulted in the decline of population due to death of sterile adults. Interestingly, sterile adults have curled, crinkled or twisted wings, which is the only apparent sign of JHA exposure.

3.2.11.2 Ecdysone agonists

The compounds halofenozide, tebufenozide, and methoxyfenozide have been developed as insecticides and act as ecdysone agonists. These compounds are called diacylhydrazines. All these compounds are safe for mammals and environment but are very toxic to insects. These insecticides are selective in action. Methoxyfenozide and tebufenozide are effective against larvae of Lepidoptera that ingest this material but weakly active or inactive on Diptera and Coleoptera. However, halofenozide is effective on Coleoptera but mildly active on Lepidoptera. These compounds mimic the action of 20-hydroxyecdysone (20E), the insect molting hormone. They bind directly to the binding sites of 20-hydroxyecdysone and act as complete agonist at that site. This binding induces premature apolysis in the larvae and they stop feeding. Intoxicated larvae after 24 h start abnormal molting process characterized by premature removal of old head capsule, abnormal cuticle deposition and lack of sclerotization and tanning of the new cuticle, loss of hemolymph and molting fluid. This incomplete molting results in desiccation and death of larva (Smagghe & Degheele, 1998).

3.2.11.3 Chitin synthesis inhibitors:

This group includes the compounds like buprofezin, cyromazine, diflubenzuron, leufenuron, novaluron and noviflumuron. Like the JHAs, these insecticides have no action on nervous system of insects. They interrupt the normal biochemical pathway essential for chitin synthesis. Chitin is dominating and most critical component of insect exoskeleton. During molting process chitin is synthesized and deposited into insect newly generated exoskeleton. Scientific studies reveal that biocidal action of chitin synthesis inhibitors is due to blocking of enzyme designated as chitin synthase responsible for its synthesis. In the absence of this enzyme, chitin cannot be synthesized. The prevention of chitin synthesis is fatal for the affected insect.

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CHAPTER-4

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

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

4.1 Acetylcholine Esterase Inhibitors

4.1.1 Background

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

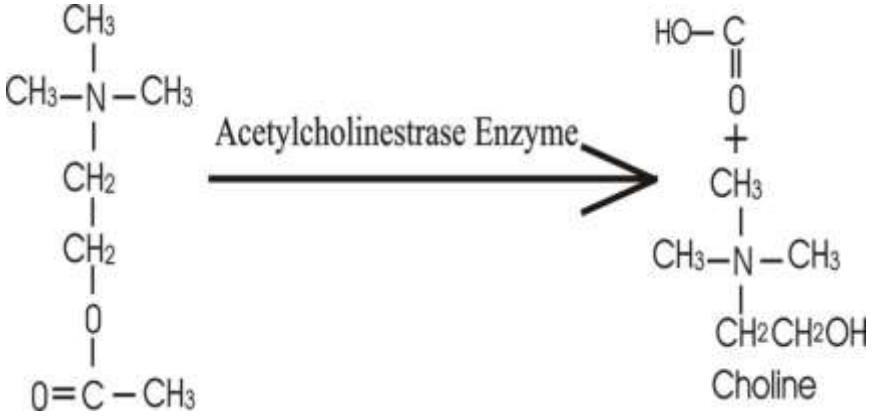

Figure 4.1 Diagrammatic representation of the acetylcholine binding and catalysis by the acetylcholinesterase.

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

4.1.2 Mode of action

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

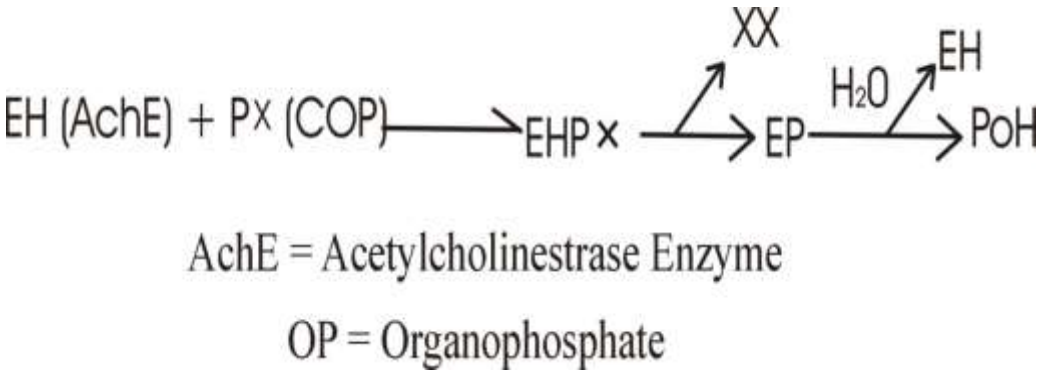

Figure 4.2 Binding of the Organophosphates to the acetylcholinesterase.

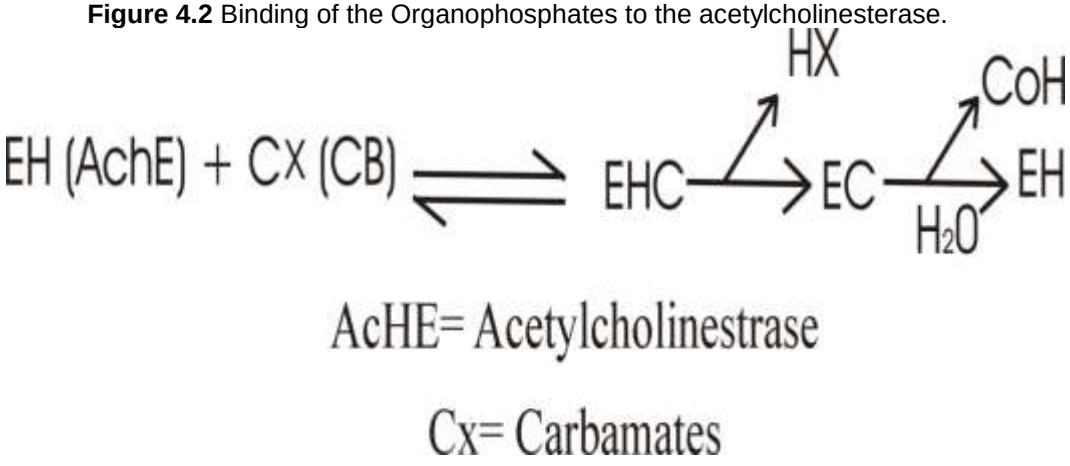

Figure 4.3 Binding of the Carbamates to the acetylcholinesterase

4.2 GABA-Gated Chloride Channel Antagonists

4.2.1 Backgrounds

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

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

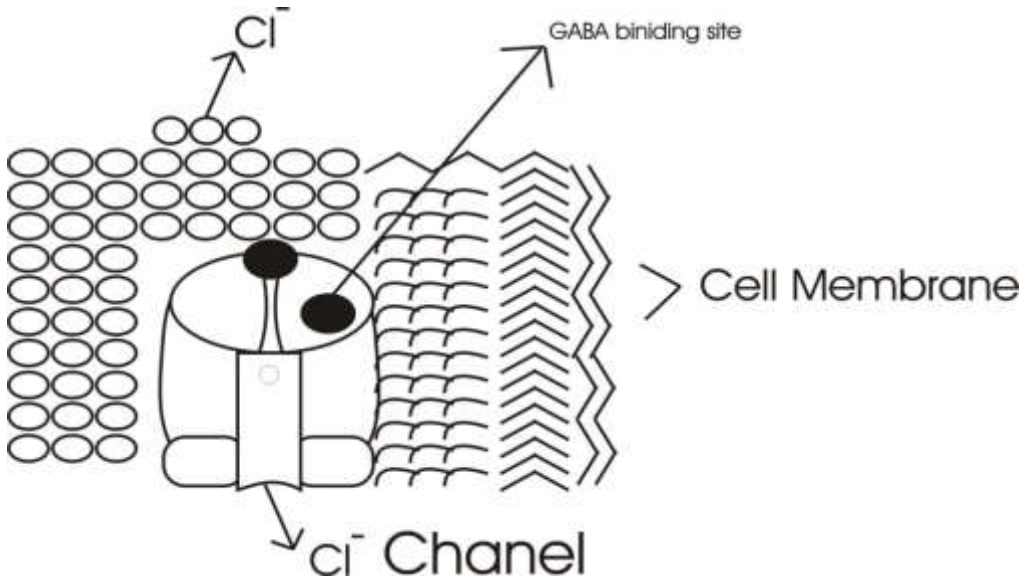

Figure 4.4 GABA-gated chloride channel antagonists

4.2.2 Mode of action

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

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

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

4.3 Nicotinic Acetylcholine Receptor Inhibitors

4.3.1 Background

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

4.3.2 Mode of action

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

4.4 Sodium Channel Modulators/Blockers

4.4.1 Background

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

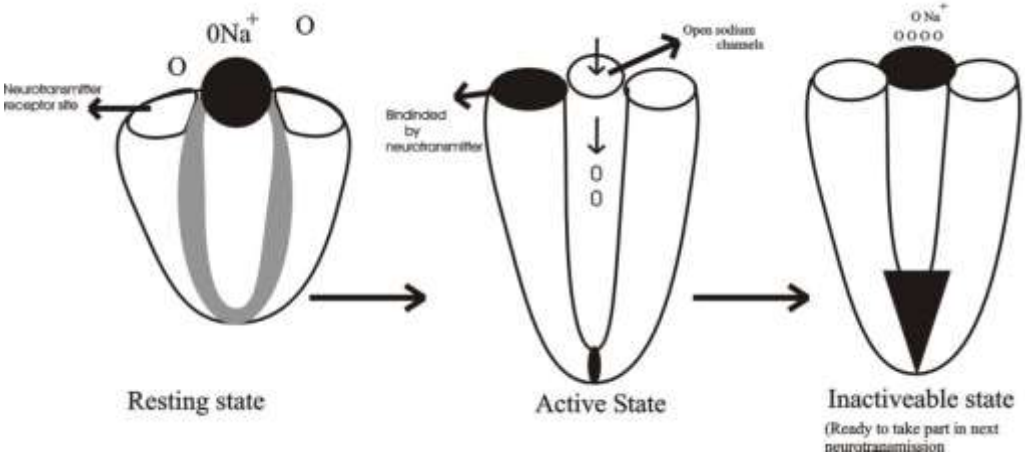

Figure 4.6 Phases of voltage gated sodium ion channel

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

4.4.3 Mode of action

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

4.5 Lipid Biosynthesis Inhibitors

4.5.1 Background: (Lipid biosynthesis)

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

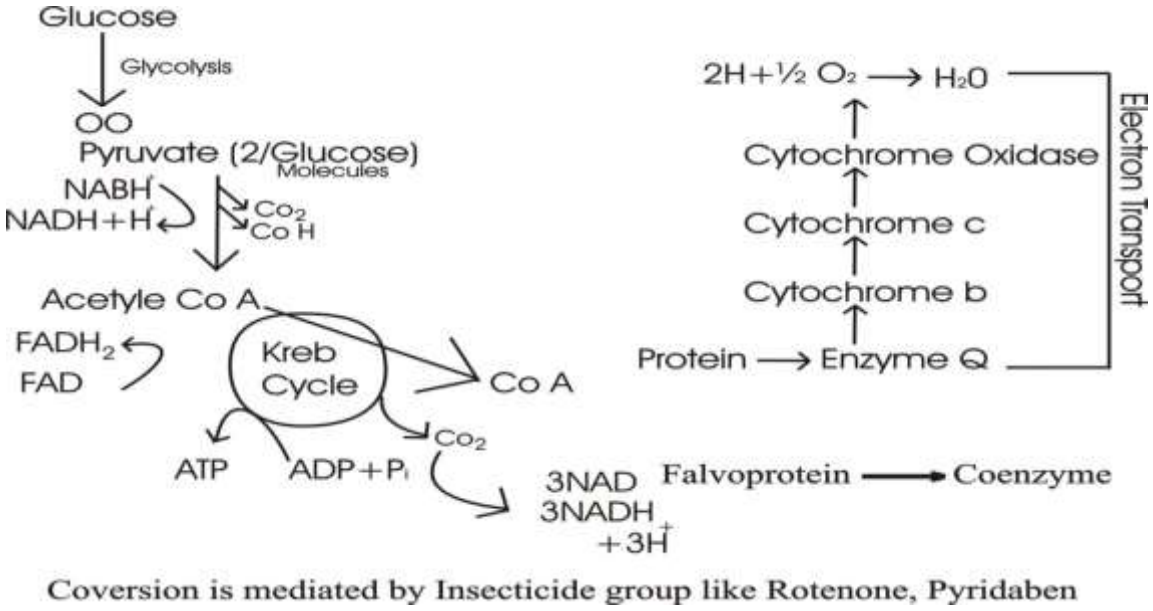

Figure 4.7 Biosynthesis of lipids

4.5.2 Lipid biosynthesis inhibitors

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

4.6 Insect Growth Regulators

4.6.1 Juvenile hormones mimics

4.6.1.1 Background

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

4.6.1.2 Mode of action

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

4.6.2 Chitin biosynthesis inhibitor

4.6.2.1 Background

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

4.6.2.2 Mode of action

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

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

4.6.3 Ecdysone agonists

4.6.3.1 Background

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

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

4.6.3.2 Mode of action

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

4.6.4 Microbial disruptors of insect midgut membranes

4.6.4.1 Background

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

4.6.4.2 Mode of action

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

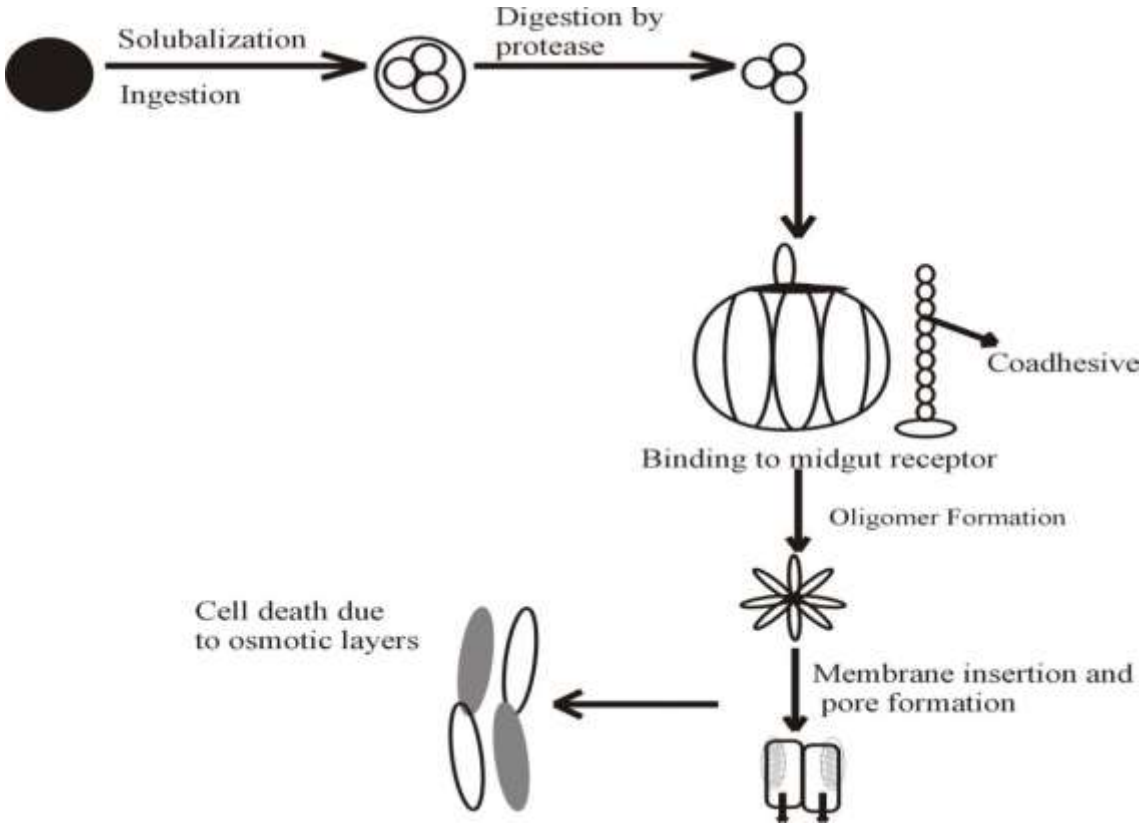

Figure 4.10 Schematic representation of the disruption of midgut microbial insecticides

4.6.5 Inhibitors of oxidative phosphorylation, disruptors of ATP formation

4.6.5.1 Background

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

4.6.5.2 Mode of action

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

4.6.6 Uncouplers of oxidative phosphorylation via disruption of proton gradient

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

4.6.7 Octopaminergic agonists

4.6.7.1 Background

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

4.6.7.2 Mode of action

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

4.6.8 Aconitase inhibitors

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

4.6.9 Ryanodine receptor modulators

4.6.9.1 Background

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

4.6.9.2 Mode of action

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

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CHAPTER-5

5.0 CLASSIFICATION OF INSECTICIDES ON THE BASIS OF CHEMISTRY

5.1 Organochlorins (OCs)

The insecticides which contain carbon, hydrogen and chlorine are known as organochlorins. These are chlorinated hydrocarbon, chlorinated cyclic and cyclodiene insecticides. These all organochlorine insecticides are banned in Pakistan due to long residual life which causes environmental hazard problems.

Examples of OCs

The typical examples of this class are DDT and their analogs, benzene hexachloride (BHC), lindane, dieldrin, endrin, heptachlor, toxaphene and endosulfon.

5.2 Organophosphates (OPs)

Organophosphates are byproduct of nerve gases (e.g. sarin, soman and tabun), developed during World War II in Germany. These are highly toxic and large class of insecticides. All organophosphate insecticides are derivatives of phosphoric acid. When H atoms of phosphoric acid are changed with organic radicals such as ethyl, methyl, or phenyl, the resulting compounds are named as organophosphates. Oxygen atom can be replaced with carbon, sulfur or nitrogen to produce different derivatives. Organophosphates inhibit acetylcholinesterase in the nervous system of pests and are divided into six subclasses such as:

1. Phosphates
2. Phosphorothioates
3. Phosphorodithioates
4. Phosphorothiolates
5. Phosphonates
6. Phosphoramidates

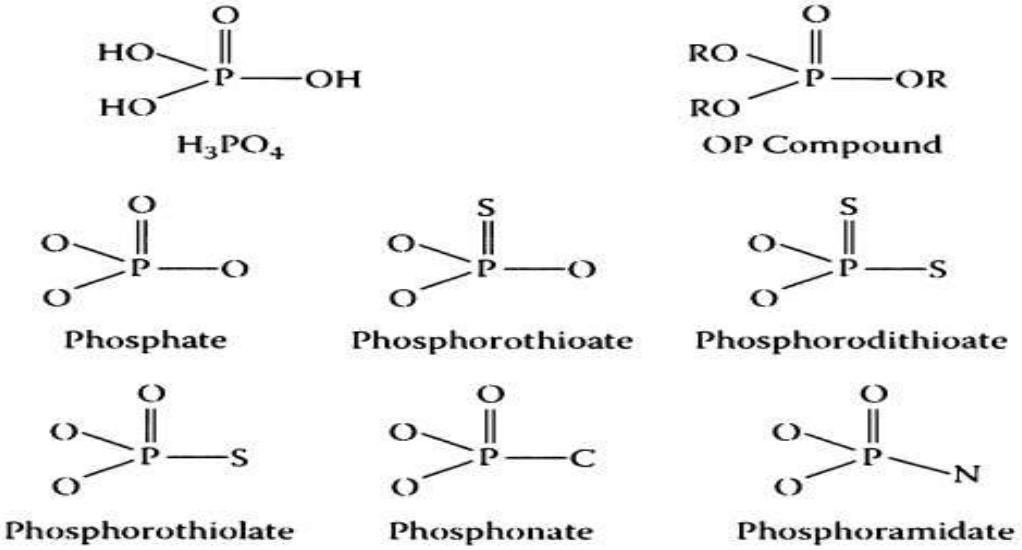

Figure 5.1 Chemical structures of Organophosphates

(Source: Simon, J. Y. (2014). The toxicology and biochemistry of insecticides: CRC press, Taylor & Francis Group, Boca Raton, London, New York)

5.2.1 Phosphates

This subclass of organophosphates has few insecticides such as mevinphos, monocrotophos and dicrotophos. These insecticides have been banned by Ministry of National Food Security and Research, Department of Plant Protection, Government of Punjab, Pakistan due to toxic effects on natural enemies and health hazards in human beings.

5.2.2 Phosphorothioates

The sulfur atom is double bonded to the phosphorus atom in this subclass of organophosphates. Chlorpyrifos is a typical example of phosphorothioates. It is used as an acaricide and insecticide having contact and stomach poison activity against various insect pests on cotton, corn, sugarcane, fruits and vegetables. The trade name of chlorpyrifos is Lorsban® (40EC, Arysta Life Sciences, Pakistan). Chlorpyrifos has 95-270 $mgKg^{-1}$ oral LD_{50} and > 2000 $mgKg^{-1}$ dermal LD_{50} in rats.

Triazophos is another phosphorothioates having insecticide, acaricide and nematicide activity. It is used for the control of bollworms, beetles, soil insects, mealybugs and nematodes in cotton, fruits and vegetables (Afzal et al., 2015). Triazophos has 57-59 $mgKg^{-1}$ oral LD_{50} in rats (Simon, 2014).

5.2.3 Phosphorodithioates

The subclass containing two sulfur atoms in phosphoric acid molecule are known as phosphorodithioate insecticides. The examples of this class of insecticides are malathion and dimethoate. Malathion is a phosphorodithioate insecticide that is used for the control of household, greenhouse and garden pests (Selvi et al., 2010). Malathion has 5400 $mgkg^{-1}$ oral LD_{50} in male rats and 5700 $mgkg^{-1}$ in female rats, while dermal LD_{50} is > 2000 $mgkg^{-1}$.

Dimethoate is another phosphorodithioate insecticide. It has systemic, contact and stomach poison activity. It is used for the control of aphids, whiteflies, mites, thrips and leafhoppers on various crops. Dimethoate has 235 $mgkg^{-1}$ oral LD_{50} in rats (Simon, 2014).

5.2.4 Phosphorothiolates

The subclass which contains a single bond, sulfur to phosphorus atom is known as phosphorothiolate insecticides. Profenofos is a typical example of this class. It is used for the control of lepidopterans pests, mealybug and thrips on cotton and vegetables (Abbas et al., 2014b). The oral LD_{50} of profenofos is 358-1178 $mgkg^{-1}$ and dermal LD_{50} is > 2000 $mgkg^{-1}$ in rats.

5.2.5 Phosphonates

The subclass in which the phosphorus atom bonds directly to carbon atom is known as phosphonate. Trichlorfon is an example of this subclass. It is a contact and stomach poison and used for the control of fruit fly on fruits and vegetables. The trade name of trichlorfon is Diptrex® (80SP, Bayer Crop Sciences). The oral LD_{50} of trichlorfon is 450 $mgKg^{-1}$ and dermal LD_{50} is > 2000 $mgKg^{-1}$ in rats.

5.2.6 Phosphoramidates

The subclass in which the phosphorus atom bonds to nitrogen atom is known as phosphoramidates. Methamidophos and acephate are typical examples of phosphoramidates. **Methamidophos** is used as an insecticide and acaricide for the control of thrips, aphids, whiteflies, cutworms, armyworm and mites on cotton, fruits and vegetables. Methamidophos has 21 and 16 $mgkg^{-1}$ oral LD_{50} in male and female rats, respectively and 50 mgkg-1 dermal LD_{50}. It is banned in Pakistan due to effects on human and natural enemies.

Acephate is a systemic insecticide having stomach and contact poison activity. It is used for the control of aphids, jassid and thrips on cotton. The trade name of acephate is Commando® (75SP, FMC, Pakistan). The oral LD_{50} of acephate is 866$mgkg^{-1}$ and 945$mgkg^{-1}$ in female and male rats, respectively.

5.3 Carbamates

Carbamate insecticides are derivatives of carbamic acid (NH_2COOH). Three H atoms can be replaced with aromatic or aliphatic radicals to formulate the carbamate insecticides. However, second H atom on N atom cannot be replaced because the mono alkyl structure is more toxic compared to the N distributed molecule. Typical examples of carbamates are carbaryl, methomyl, carbofuron, carbosulfon, and thiodicarb.

Carbaryl is a broad spectrum carbamate insecticide which is used for the control of aphids, leafhoppers, bollworms, boll weevils and armyworms on cotton, apple, pear and vegetables. The trade name of carbaryl is Sevin® (Bayer Crop Sciences). The oral LD_{50} of carbaryl is 250-850 $mgkg^{-1}$ and dermal LD_{50} is > 2000 $mgkg^{-1}$ in rats.

Methomyl is used for the control of aphid, fruit fly and armyworm on cotton, vegetables and ornamentals (Byrne & Toscano, 2001). The trade name of methomyl is Lannate® (40SP, Arysta Life Sciences). Methomyl has 17-26 $mgkg^{-1}$ oral LD_{50} and > 1000 $mgkg^{-1}$ dermal LD_{50} in rats (Simon, 2014).

Carbofuron is a systemic carbamate insecticide used for the control of borers on rice, sugarcane and maize. The trade name of carbofuron is Furadan® (3G, FMC). The oral LD_{50} of carbofuron is 5-13 $mgkg^{-1}$ in rats and > 1000 $mgkg^{-1}$ dermal LD_{50} in rabbits.

Carbosulfon is a carbamate insecticide that is used for the control of sucking pests and soil pests such as nematodes on various crops. This insecticide is marketed under the name of Advantage® (25DS, FMC). The oral LD_{50} of carbosulfon is 250 and 185 $mgkg^{-1}$ for male and female rats, respectively (Simon, 2014).

5.4 Pyrethroids

Pyrethroids are derivatives of pyrethrum which is extracted from a flower, *Chrysanthemum cinerariaefolium* and their active ingredients are pyrethrins. Pyrethrins contain four esters, two different alcohols and two different acids as follows:

1) Pyrethrins I (pyrethrolone + chrysanthemic acid)
2) Pyrethrins II (pyrethrolone + pyrethric acid)
3) Cinerins I (cinerolone + chrysanthemic acid)
4) Cinerins II (cinerolone + pyrethric acid)

Examples of pyrethroid insecticides are permethrin, bifenthrin, cypermethrin, fenvalerate, esfenvalerate, deltamethrin and lambda-cyhalothrin.

Permethrin is used for the control of mosquitoes, house flies, ticks and various pests on cotton, maize, wheat and alfalfa. The oral LD_{50} of permethrin ranges from 430-4000 $mgkg^{-1}$ in rats and dermal LD_{50} is > 2000 $mgkg^{-1}$ in rabbits.

Bifenthrin is a pyrethroid insecticide which is used for the control of sucking and chewing pests on cotton, mango and rice (Jan et al., 2015). It is also used for the control of household and livestock pests (Abbas et al., 2015a). The trade name of bifenthrin is Talstar® (10EC, FMC). The oral LD_{50} of bifenthrin ranges from 53.4-210.4 $mgkg^{-1}$ in rats (Tomlin, 2000). The dermal LD_{50} of this insecticide is >2000 $mgkg^{-1}$ in rats and rabbits (FAO, 2010).

Cypermethrin is used for the management of chewing pests of cotton such as spotted bollworms and American bollworms (Jan et al., 2015). The trade name of cypermethrin is Arrivo® (10EC, FMC). The oral LD_{50} of cypermethrin ranges from 187-326 $mgkg^{-1}$ in male rats and 150-500 $mgkg^{-1}$ in female rats. The dermal LD_{50} of cypermethrin is 1600 $mgkg^{-1}$ and > 2000 $mgkg^{-1}$ in rats and rabbits, respectively (Simon, 2014).

Esfenvalerate is a synthetic pyrethroid used for the control of lepidopteran pests such as spotted bollworms, American bollworms and armyworms on cotton, alfalfa and vegetables (Shad et al., 2012). The trade name of esfenvalerate is Sumi-Alpha® (110EC, Arysta Life Sciences). Esfenvalerate has 458 $mgkg^{-1}$ oral LD_{50} in rats and has 2000 $mgkg^{-1}$ dermal LD_{50} in rabbits.

Deltamethrin is a broad spectrum pyrethroid insecticide which is used for the control of lepidopteran pests, mites, weevils and beetles on various crops (Jan et al., 2015). The trade name of deltamethrin is Decis Super® (100EC, Bayer Crop Science). The oral LD_{50} of deltamethrin in rats ranges from 30-140 $mgkg^{-1}$. The dermal LD_{50} of deltamethrin is >2000 $mgkg^{-1}$ in rabbits (Rehman et al., 2014).

Lambda-cyhalothrin is used for the control of lepidopteran pests such as American bollworms, spotted bollworms, armyworms and leaf folders on cotton and rice (Shad et al., 2012). The trade name of lambda-cyhalothrin is Karate® (2.5EC, Syngenta). The oral LD_{50} of lambda-cyhalothrin is 79 $mgkg^{-1}$ and 56 mgkg-1 in male and female rats, respectively. The dermal LD_{50} of lambda-cyhalothrin is 632 $mgkg^{-1}$ and 696 $mgkg^{-1}$ in male and female rats, respectively (EPA, 2007).

5.5 Novel Chemistry Insecticides

5.5.1 Neonicotinoids

Neonicotinoids are new group of insecticides that are analogs of nicotine and also known as chloronicotinyls. Neonicotinoids affect the central nervous system of insects. These insecticides have low mammalian toxicity than nicotine. The examples of this group are imidacloprid, acetamiprid, thiamethoxam and nitenpyram.

Imidacloprid is a systemic insecticide used for the control of sucking insect pests on cotton, sugarcane and tobacco (Saeed et al., 2017). The trade name of imidacloprid is Confidor® (Bayer Crop Science, Pakistan). The oral LD_{50} of imidacloprid is 450 $mgKg^{-1}$ and dermal LD_{50} is >5000 $mgKg^{-1}$ in rats (Simon, 2014).

Acetamiprid is a systemic neonicotinoid insecticide used for the effective control of whitefly, jassid, thrips, mealy bug and leaf minor on cotton, tobacco and melon (Saeed et al., 2017). In Pakistan, acetamiprid is sold under different trade names such as Mospilan® (Arysta Life Sciences) and Acelan® (FMC). The oral LD_{50} of acetamiprid in male and female rat is 217 and 146 $mgKg^{-1}$, respectively. The dermal LD_{50} of acetamiprid is >2000 $mgKg^{-1}$ in rats (Paranjape et al., 2015).

Thiamethoxam is a systemic neonicotinoid insecticide having broad spectrum activity against various pests. It is used for the control of whitefly, aphid, jassid and others on various crops such as cotton, rice, potato, vegetables and ornamentals (Saeed et al., 2017). The trade name of thiamethoxam is Actara® (Syngenta, Pakistan). The oral LD_{50} of thiamethoxam is > 5000 $mgKg^{-1}$ and dermal LD_{50} is 2000 $mgKg^{-1}$ in rats (Simon, 2014).

Nitenpyram is used for the control of whiteflies, thrips, jassid, and aphids on various crops. The trade name of nitenpyram is Paranol® (10EC, Kanzo Agrochemicals). The oral LD_{50} of nitenpyram is 1575 $mgKg^{-1}$ in female rats and 1680 $mgkg^{-1}$ in male rats (Simon, 2014). The dermal LD_{50} of nitenpyram is > 2000 $mgKg^{-1}$ in rats.

5.5.2 Insect growth regulators (IGRs)

IGRs are novel chemistry insecticides that disrupt growth and development of insects, resulting eventually death. IGRs are divided into five types, namely juvenile hormone mimics, benzoylphenylureas, diacylhydrazines, triazines, and thiadiazines. These insecticides have very low mammalian toxicity.

5.5.2.1 Juvenile hormone mimics

Pyriproxyfen, a juvenile hormone mimic insecticide, have been used for the control of house fly, whitefly, boll weevil, mosquito and cockroach (Crowder et al., 2007; Abbas et al., 2015b). The trade name of pyriproxyfen is Admiral® (FMC, Pakistan). Pyriproxyfen is highly toxic to target pests and low toxic to mammals. It is used in integrated management strategies for the control of different pests due to reduced risks. The oral LD_{50} of pyriproxyfen is > 5000 $mgkg^{-1}$ and dermal LD_{50} is > 2000 $mgkg^{-1}$ in rats (Simon, 2014).

5.5.2.2 Benzoylphenylureas

Benzoylphenylurea insecticides are derivatives of urea (H_2NCONH_2). Lufenuron is a benzoylurea insecticide that inhibits the production of chitin in insects. Due to inhibition of chitin synthesis, insects never develop a hard exoskeleton, and ultimately die after hatching or molting. Lufenuron have been effectively used against different insect pests such as lepidopteran and coleopteran pests (Abbas et al., 2015b; Nascimento et al., 2015). It is also used for the control of citrus leafminer and mites on citrus. The trade name of lufenuron is Match® (Syngenta, Pakistan). Its oral and dermal LD_{50} in rats is >2000 and >4000 $mgKg^{-1}$, respectively (Simon, 2014).

Diafenthiuron is a thiourea insecticide. It is used for the control of whiteflies, aphids, jassids and mites on cotton and vegetables. It kills both nymph and adult stages of pests by contact and

stomach poison activity. Trade name of diafenthiuron is Polo® (Syngenta, Pakistan). The oral LD_{50} of diafenthiuron is 2068 $mgKg^{-1}$ and dermal LD_{50} is >2000 $mgKg^{-1}$ in rats (Simon, 2014).

5.5.2.3 Diacylhydrazines

Diacylhydrazine insecticides are derivatives of hydrazine (H_2N-NH_2). These insecticides mimic the action of molting harmone, ecdysone in larvae of lepidopteran pests. The examples of this group are tebufenozide and methoxyfenozide.

Tebufenozide is used for the control of lepidopteran pests on vegetables. The trade name of tebufenozide is Topgun® (Jaffer Brothers, Pakistan). Its oral and dermal LD_{50} is > 5000 $mgKg^{-1}$ in rats (Simon, 2014).

Methoxyfenozide is a highly selective insecticide for the control of armyworm on cotton, vegetables and fruit trees. The trade name of methoxyfenozide is Runner® (Arysta Life Sciences, Pakistan). The oral LD_{50} of methoxyfenozide is >5000 $mgKg^{-1}$ and dermal LD_{50} is >2000 $mgKg^{-1}$ in rats (Simon, 2014).

5.5.2.4 Triazines

Triazine insecticides are derivatives of triazine. Currently, only example of this group is cyromazine which is a cyclopropyl derivative of melamine. It affects the nervous system of immature larval stages of certain insects. It is used for the control of dipterous pests, including leaf-miners on ornamental and vegetable crops. The trade name of cyromazine is Trigard® (Syngenta, Pakistan). The oral LD_{50} of cyromazine is 3387 $mgKg^{-1}$ and dermal LD_{50} is > 3100 $mgKg^{-1}$ in rats (Simon, 2014).

5.5.2.5 Thiadiazines

Thiadiazine insecticides are derivatives of thiadiazine. Buprofezin is the only present example of thiadiazines. It is used for the control of whiteflies, aphids, jassids and thrips on cotton and vegetables (Basit et al., 2012). The oral LD_{50} of buprofezin is 2198 $mgKg^{-1}$ and dermal LD_{50} is > 5000 $mgKg^{-1}$ in rats (Simon, 2014).

5.5.3 Avermectins

Avermectins are macrocyclic lactones and extracted from soil microorganism, *Sepreptomyces avermitilis* Kim and Goodfellow (actinomycete). The typical examples of avermectins are abamectin and emamectin benzoate.

Abamectin is derived from naturally occurring avermectins and contains a combination of avermectins B1a and avermectins B1b as active ingredients. It is used as insecticide and acaricide for the control of insect and mite pests on various field crops, fruits, vegetables and ornamentals (Simon, 2014). The trade name of abamectin is Alarm® (1.8EC, DJC, Pakistan). The oral LD_{50} of abamectin is 300 $mgkg^{-1}$ in rats and dermal LD_{50} is >2000 $mgkg^{-1}$ in rabbits (Simon, 2014).

Emamectin benzoate is a semisynthetic bio-insecticide derived from a naturally occurring avermectin compound and contains B1a and B1b as active ingredients. Emamectin benzoate is used for the control of lepidopteran pests on various crops at low rates (Shad et al., 2012). It is a chloride channel activator causing prevention of muscle contraction, cessation of feeding and finally death. The trade name of emamectin benzoate is Proclaim® (019EC, Syngenta). The oral LD_{50} of emamectin benzoate is 1516 $mgkg^{-1}$ in rats and dermal LD_{50} is > 2000 $mgkg^{-1}$ in rabbits (Simon, 2014).

5.5.4 Spinosyns

Spinosyns are derived from the soil microbe, *Saccharopolyspora spinosa* Mertz & Yao. Spinosyns affect the gamma aminobutyric acid and acetylcholine transmission by depolarizing neurons in the insect nervous system. These are reduced risk insecticides for example, less toxic to mammals, birds, fish and human beings and highly effective against targeted pests. The typical examples of Spinosyns are spinosad and spinetoram.

Spinosad is derived from Spinosyns and contains spinosyn A and spinosyn D as active ingredients. It is used for the control of armyworms, American bollworms, diamondback moths, thrips, leaf miners, fruit flies, house flies and mosquitoes (Zhao et al., 2006; Shad et al., 2012). The

trade name of spinosad is Tracer® (240SC, Arysta Life Sciences). The oral and dermal LD_{50} of spinosad is > 5000 $mgkg^{-1}$ and > 2800 $mgkg^{-1}$ in rats (Simon, 2014).

Spinetoram has mixture of spinetoram J and spinetoram L as active ingredients. It is used for the control of lepidopteran pests, leafminers, thrips and certain psyllids on various crops (Li et al., 2015). The trade name of spinetoram is Radiant® (120SC, Arysta Life Sciences). The oral and dermal LD_{50} of spinetoram is more than 5000 $mgkg^{-1}$ in rats (Kagaku, 2012).

5.5.5 Phenylpyrazoles

Phenylpyrazole insecticides are derivatives of phenylpyrazole that disrupt GABA-gated and glutamate-gated chloride channels in the insect central nervous system. Fipronil is the only member of this class that is marketed in Pakistan. The trade name of fipronil is Regent® (Bayer Crop Science, Pakistan). Fipronil is a systemic insecticide and is used for the control of rice and sugarcane borers and house flies (Abbas et al., 2014a). The oral and dermal LD_{50} of fipronil is 97 $mgKg^{-1}$ and > 2000 $mgKg^{-1}$, respectively in rats (Simon, 2014).

5.5.6 Pyrroles

Pyrrole insecticides are derived from pyrrole. Chlorfenapyr is the only example of this class. It is metabolized into an active insecticide after entering into host. Chlorfenapyr is used for the control of whiteflies, bugs, thrips, jassid, armyworm and mites on cotton, vegetables and ornamentals (Ullah et al., 2016). Its oral LD_{50} in rats is 441 $mgKg^{-1}$ (Simon, 2014).

5.5.7 Oxadiazines

Oxadiazine insecticides are derivatives of oxadiazine. These insecticides are voltage dependent sodium channel blockers. Indoxacarb is the only example of this class. It is used for the management of lepidopteran pests on corn, vegetables and fruits (Sayyed et al., 2008). The trade name of indoxacarb is Steward® (DuPont, Pakistan). The oral and dermal LD_{50} of indoxacarb in rats is 1732 $mgKg^{-1}$ and > 5000 $mgKg_1$, respectively (Simon, 2014).

5.5.8 Pyridinecarboxamide

Pyridinecarboxamide insecticides are modulators of chordotonal organs. Flonicamid is the only example of this class. It is a novel systemic insecticide and used against aphids, whiteflies, thrips, leafhoppers and plant bugs on wheat, cotton, potatoes, pome fruit and vegetables. Flonicamid has an excellent safe profile against beneficial insects and will provide a new option in integrated pest management programs. It is highly effective for the control of all aphid species on various crops and fruits. It inhibits the aphid feeding by inhibition of stylet penetration to plant tissues, resulting death due to neurological effects (Morita et al., 2007). Flonicamid had 884 $mgkg^{-1}$ and 1768 $mgKg^{-1}$ oral LD_{50} in male and female rats, respectively (Simon, 2014). The dermal LD_{50} of Flonicamid is >5000 $mgKg^{-1}$ in rats.

5.5.9 Diamides

Diamides are a new group of insecticides that have been classified as ryanodine receptor modulators. Ryanodine receptors are calcium channels located in the sarcoplasmic reticulum. Diamides cause continuous opening of calcium channels and uncoordinated muscle contraction, subsequently resulting death of insects. Diamide insecticides are particularly active against lepidopteran pests at lower dose rates and have an excellent safety profile. Diamide insecticides comprise 7 % of the global insecticide market after eight years of market launch, which highlights the importance of this chemistry. To date, two representatives of the diamide insecticides are registered on the basis of chemistry as shown follows:

5.5.9.1 Phthalic Diamides

Phthalic diamides are also known as benzenedicarboxamides. Flubendiamide is an only example of this class, registered with the trade name of Belt® (Bayer Crop Science, Pakistan). It is effectively used for the control of lepidopteran pests (Tohnishi et al., 2005). The oral and dermal LD_{50} of flubendiamide is >2000 mg/Kg, respectively in rats (Singh & Mandal, 2013).

5.5.9.2 Anthranilic Diamides

Anthranilic diamide insecticides are derivatives of anthranil. Chlorantraniliprole is a first generation of anthranilic insecticides. This insecticide is currently registered under the trade name of Coragen® (DuPont). It is effectively used for the control of lepidopteran hemipteran and coleopteran pests on various crops (Sial & Brunner, 2012; Su et al., 2012). The oral and dermal LD_{50} of chlorantraniliprole is >5000 $mgKg^{-1}$ in rats (Simon, 2014).

Cyantraniliprole is a second generation anthranilic diamide insecticide, currently registered under the trade name of Cyazypyr® (DuPont). It has very low toxicity to natural enemies and vertebrates. It has systemic and translaminar activity against various sucking and lepidopteran insect pests. The oral and dermal LD_{50} of cyantraniliprole is > 5000 $mgKg^{-1}$ in rats (Simon, 2014).

5.5.10 Tetronic acids

These insecticides are derivatives of spirocyclic tetronic acids that inhibit the lipid metabolism enzyme, acetyl-CoA-carboxylase. The trade name of spiromesifen is Oberon® (Bayer Crop Science). It is a novel insecticide/acaricide, used effectively against whiteflies and mites on cotton, fruits, vegetables and ornamentals. Its oral and dermal LD_{50} is > 2500- and > 4000 $mgKg^{-1}$, respectively (Simon, 2014).

5.5.11 Tetramic acids

Tetramic acid insecticides are derivatives of tetramic acid. The example of this class is spirotetramat, registered under the trade name of Movento® (240SC, Bayer Crop Science). It is used for the control of whiteflies, aphids, thrips and scale insects (Peng et al., 2017). The oral and dermal LD_{50} of spirotetramat is > 2000 $mgKg^{-1}$ in rats (Shimokawatoko et al., 2012).

5.5.12 Nereistoxin analogs

The insecticides of this class are analogs of nereistoxin, a natural toxin of marine worm. Cartap hydrochloride is the member of this class that is marketed under the trade name of Padan® (FMC). It is systemic insecticide and effectively used for the control of rice and sugarcane borers. The oral LD_{50} of cartap is 345 $mgKg^{-1}$ in rats (Simon, 2014).

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CHAPTER-6

6.0 TOXICITY AND ITS METHODS OF EVALUATION

6.1 Toxicity

It is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity) (Stellman, 1998). By extension, the word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large. Sometimes the word is more or less synonymous with poisoning in everyday usage.

6.2 Bioassays

It is an experiment in which a living organism is used as a test subject with the intention to estimate the relationship between the response and quantity or intensity of the stimulus (toxicant) under standard set of conditions (Agatonovic-Kustrin et al., 2015).

6.2.1 Prerequisites of bioassay

6.2.1.1 Insects

Mass capturing of the insects should be done using lures/pheromone-baited traps, sweep nets, light traps and aspirators etc. at adult stage (Levi-Zada et al., 2017). Immatures like larvae or pupae may also be collected manually. After collection, the insects should be reared in the laboratory under standard conditions of temperature and humidity on a suitable source of food to obtain a uniform population which is most important prerequisites of bioassay. However, field collected insect population of same stage can also be exposed directly after observing its natural mortality due to damages during collection by maintaining its population for 8-24h. It is very important to expose healthy and active insects in the bioassay. Extent of robustness of data set for a bioassay depends upon the numbers of insects exposed. It is mandatory to expose more number of insects in a concentration is necessary to achieve more dynamic results. Ten-twenty five insects should be exposed in one concentration but the numbers could be reduced depending upon the availability of insects. However, the number of replicates should not be less than three per concentration. Random selection of insects for each concentration of a bioassay is of paramount importance to obtain valid results. For example, never use all healthy individuals in one concentration or all weaker individuals in one concentration. Instead assure the exposure of blend of both weaker and healthier individuals by random selection from the pool to get reliable results.

6.2.1.2 Determining a range of concentrations

Establishing a range of concentrations causing <100% and >0% response is difficult task. Normally 5-8 different levels of a toxicant are made by serial dilution method like 10, 20, 40, 80 and 160µg/mL etc. Dilution is normally made in water but other diluents may be used depending upon availability or suitability of diluents with toxicant. Several repetitions are done to select a desired range of concentration on hit and trial basis. This range may vary over time with changes in the level of susceptibility. Several factors such as species, stage, previous exposure of insects, and size of insect as well as the class of insecticide also influence the range of concentrations for a bioassay. Previously published bioassay results assist in developing the desired range of concentration at initial stage (Sayyed & Crickmore, 2007; Shah et al., 2007; Shah et al., 2015a; Shah et al., 2015b; Shah et al., 2016).

Bioassays have been published for families like Brentidae (Smith & Hammond, 2006), Miridae (López et al., 2008), Thripidae (López et al., 2008), Pentatomidae (Nielsen et al., 2008), Aphididae (Foster et al., 2002), Curculionidae (James, 2003), Coccinellidae (Smith & Cave, 2006), Cybocephalidae (Smith & Cave, 2006); Encyrtidae (Smith & Cave, 2006), Culicidae (Shah et al.,

2016), Tortricidae (Sial et al., 2010), Noctuidae (Ahmad et al., 2008; Lai & Su, 2011), Braconidae (Shi et al., 2004), Ichneumonidae (Cordero et al., 2007), and Aleyrodidae (Bi & Toscano, 2007).

6.2.1.3 Stock solution preparation

In serial dilutions, highest concentration of any testing material or chemical is known as Stock Solution. Stock solution is prepared by mixing insecticide in a solvent like water or alcohol. Quantity of insecticide is determined by following formulae (Kranthi, 2005).

$$\text{Dose (µl or mg)} = \frac{\text{PPM required} \times \text{Water/solvent required}}{\%\text{F} \times 10}$$

Generally, (%F indicates the percentage of active ingredient in the formulated product) formulations are either solid or liquid and measured in mg/µl. Parts per million (PPM) tells about the quantity/strength of insecticide in stock solution. Water is most easily available and inexpensive solvent that is capable to dissolve most of the insecticide formulations. Quantity of the solvent is calibrated prior to performing bioassay e.g. amount of solvent needed to dip a filter paper is different from that required for a leaf. In case of diet incorporation bioassays, amount of the solvent required is always higher as compared to the other types of bioassays because the paste is prepared in such cases.

6.2.3.3 Safety Statement

Using proper safety precautions is important when dealing with insecticides. Consult the material safety data sheet (MSDS) i.e. Gain an understanding of the hazards and precautions necessary for the safe use of chemical products (WP, 2015). An MSDS is normally a prescription regarding identification (name, composition of ingredients, supplier), recommended usage, possible hazards, first aid measures, fire fighting measures, accidental release measures, handling and storage, personal protection, physical and chemical properties, stability and reactivity of product, toxicological information and transport measures (Fait et al., 2001). It is mandatory to wear the personal protective equipment (PPE) before handling pesticides (Perry et al., 2002). PPE refers to protective clothing, helmets, goggles, or other garments or equipment designed to protect the wearer's body from injury or infection. Specific laboratory training on handling pesticides should be required by personnel before attempting any bioassays (Paramasivam & Selvi, 2017).

6.2.2 Types of bioassay

6.2.2.1 Topical application method

It is one of the most commonly employed method in which insecticides are directly applied to the body surface of the insects (Galdino et al., 2011). The insecticide is mixed in a volatile solvent like acetone and applied to insect using a micro syringe. Although, it is one of the most effective method of direct application of insecticides but has some serious flaws including intensive labor, size and behavior of the insects (Matthews, 2008).

6.2.2.2 Injection method

Although the topical application method is useful to assess the effects of contact toxicity but could not determine the actual amount of the toxicant entering into the insect body. Actual amount of the insecticides entering the insect body could be accurately assessed by injection method (Nuringtyas et al., 2014). First of all, insecticide is dissolved in a carrier solvent such as acetone, propylene glycol and injected into the body cavity. Inter segmental region or the abdominal sterna is usually selected for injection. Insects should make unconscious. The needle should not be injected into the body of insects in longitudinal position so that the nerve card should be protected from harm. Furthermore, needle should be held in a position for a while and pulled away to avoid bleeding. There are some serious limitations of the injection method including the finding of a solvent that is non-toxic as well as vigorously dissolve the insecticide (Paramasivam & Selvi, 2017). Sometime insect behavior or size is also an important factor in limiting the applicability of this method.

6.2.2.3 Dipping method

In insecticide dipping or immersion method of bioassay, whole insect or its desired life stage is dipped into the prepared insecticide solution (Miller et al., 2010). Normally forceps, screened containers or dipping nets are used for the purpose of dipping and holding the insects for a few seconds in toxic solutions (Chandrasena et al., 2011). Different concentrations of the insecticide solutions could be prepared by serial dilution method (Shah et al., 2015b). Insects treated with the insecticides are placed in clean containers. Data of mortality is assessed after the specified intervals (Shah et al., 2016; Shah et al., 2017).

6.2.2.4 Contact method

In this method, insecticide is mixed with a volatile solvent and applied to the surface of a glass jar (Snodgrass, 1996). The solvent evaporates and the insecticide residues film will remain on the surface of the glass jar. Insecticides solution could also be applied to the filter paper by dipping it in the toxic solution which is placed in the petri dish (Ullah et al., 2015).

6.2.2.5 Fumigation method

This is an efficient method employed to evaluate the toxic vapors of a particular insecticide against the stored product pests. The insecticide is introduced into a sealed container containing the insect pest infested stored grain (Kim et al., 2003). The mortality is recorded at different intervals.

6.2.2.6 Feeding method

It is a bioassay method in which insecticide is mixed in the diet. Insects being exposed are starved for few hours before exposure. Mostly immatures are exposed in this method to check the susceptibility to a particular insecticide (Abbas et al., 2012; Shah et al., 2015a). For phytophagous insects, leaf disc is treated with the toxic solution (Afzal et al., 2015). The mortality data is assessed after different intervals of post treatment.

6.2.2.7 Toxicity testing for higher animals

It is almost impossible to perform the traditional toxicity tests as used for insects in case of higher animals. The availability of hundreds of higher animals for using in a single bioassay is impractical. Therefore, several adjustments are done to evaluate the toxicity of pesticides with higher animals. There are three different methods including acute, sub-acute and chronic used which are mandatorily required in the pesticide industry (Simon, 2014).

6.2.2.8 Acute toxicity bioassay

In this type of bioassay, oral feeding, dermal injection or inhalation intake methods are employed to determine the oral, dermal and inhalation LC_{50} or LD_{50} (Pandey et al., 2009).
The acute oral toxicity is determined by administering single dose by normal feeding or forced feeding i.e. stomach tube or capsule (White & Bradnam, 2015). Mortality data is assessed after 24h of post treatment. To assess the safety of insecticide to workers acute dermal toxicity tests are performed. For this purpose, an albino rabbit is shaved and painted with the chemical in question. The median lethal dose 50 is determined after 24 h of post treatment. For the determination of acute inhalation toxicity, tests are performed in an exposure chamber to assure the nose or head only exposure and minimize the oral entry of chemicals by licking of fur by the animals. Insecticides to be tested are employed in the form of aerosol, dust or mist formulations. In acute inhalation testing, individuals are exposed for 4 h and then shifted to other container for next 14 days period (Matsumura, 2012).

6.2.2.9 Sub-acute toxicity and chronic toxicity tests

To evaluate the primary chronic toxic effect on the tissues and organs as well as the secondary toxic effect such as carcinogenicity, teratogenicity, mutagenicity and no effect levels. These tests are performed to assess the response of the animals for prolonged time periods as compared to the acute toxicity tests. Data is observed for 90 d for sub-acute toxicity tests while for whole life time in chronic toxicity testing. The amount of toxicant received by an animal varies

depending upon its species, age and size. No effect level of the compound is determined by conducting these tests.

6.2.2.10 Probit Analysis

Probit analyzes the relationship between the stimulus i.e. dose of a toxicant and quantitative response. In an insecticide bioassay, mortality is recorded at different levels of the toxicant. This data is then subjected to probit analysis to estimate the lethal concentration/lethal dose at which 50 percent population (LC_{50}) is killed, x^2 (for testing heterogeneity), 95% fiducial limit of the LC_{50}(FL) and slope value (±SE).

Steps for manual calculation of LC_{50} values

Normally five concentrations of a toxicant excluding control (untreated insects group) can be chosen for determination n of LC_{50} starting from no death to 100% mortality. At least thirty individuals should be exposed at each concentration and the number may vary depending upon the availability of the specimen. The mortality data is assessed after 24h, 48h or 72h depending upon the method of bioassay, type of chemical used and purpose of study.

Step1.

First of all range of exposure concentrations is decided based on the hit and trial basis or consulting the literature or both.

Example:

Let us consider that we have conducted the bioassay of spinosad on fourth instar *Culex quinquefaciatus* Say larvae. The 8mg/L was used as highest dosage, 30 individuals were exposed at each concentration and mortality data was assessed after 24h.

Table 6.1. Dose response or mortality data (Shah et al., 2015a)

Conc.(mg/L)	Total Number exposed	Number dead (24 hours)
0.0 (control)	30	1
8.0	30	25
4.0	30	20
2.0	30	15
1.0	30	11
0.5	30	7

Step2

Abbot's correction:

The control mortality is corrected by using the following formulae:

$$\text{Corrected Mortality (\%)} = \frac{\text{M(obs)} - \text{M(control)}}{100 - \text{M(control)}} \times 100$$

Where "M (Obs)" represents percent mortality in response to a concentration and "M (Control)" is used for percent mortality in control groups.

Example

If control mortality is 1 out of 30 i. e, 3.3% and observed treatment mortality is 15 out 30 (50%).the Abbott correction would be

$$\text{Corrected} = (\frac{50 - 3.3}{100 - 3.3}) \times 100$$
$$= 48\%$$

Table 6.2. The corrected mortality % by using Abbots formula

Conc.(mg/L)	Total No.	No dead (24 hours)	Corrected mortality %
0.0(control)	30	1	-
8.0	30	25	83
4.0	30	20	66
2.0	30	15	48
1.0	30	11	35
0.5	30	7	21

Step3

Data is transformed by consulting the Probit values table for %corrected mortality. In case of 1st treatment where corrected mortality (%) is 83, we can see it from transformation table, vertically at 80 and moving up to 3 horizontally ahead of 80 i. e, 5.95. The arrow below 80 shows the method to see the Probit value for 83% mortality.

Table 6.3. Transformation of the percentage mortalities to probits

Transformation of percentage mortalities to probits

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

Table 6.4. Probit values for our data are as follows

Conc.(mg/L)	Total number exposed	Number dead (24h)	Corrected mortality %	Probit values
0.0(control)	30	1	-	
8.0	30	25	83	5.95
4.0	30	20	66	5.41

2.0	30	15	48	4.95
1.0	30	11	35	4.61
0.5	30	7	21	4.19

Table 6.5. The Log_{10} values of concentrations

Conc.(mg/L)	Total No.	No dead (24h)	Corrected mortality %	Probit values	Log10 concentration
0.0 (control)	30	1	-		
8.0	30	25	83	5.95	0.90309
4.0	30	20	66	5.41	0.60206
2.0	30	15	48	4.95	0.30103
1.0	30	11	35	4.61	0
0.5	30	7	21	4.19	-0.30103

Why Probit transformation?

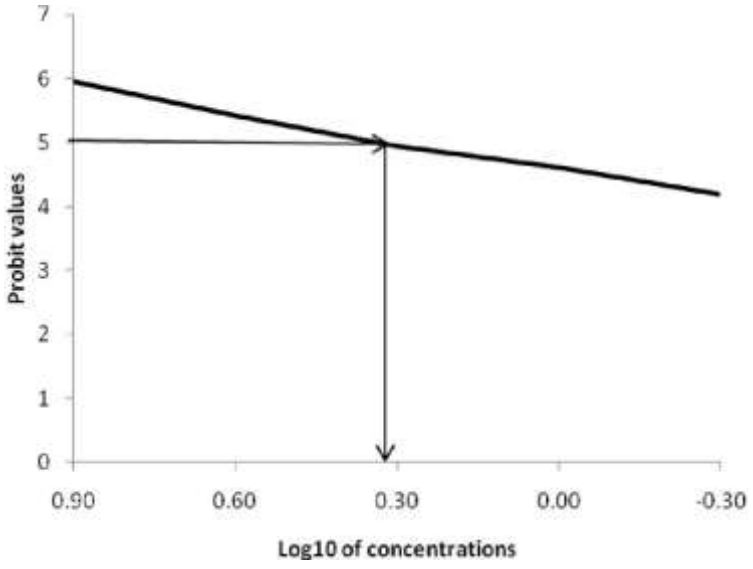
If we plot a graph between the corrected percent mortality and log10of concentration we would not get a straight line. For the purpose to get the straight line we plot a graph between the Probit value and log10 of concentration and estimation of LC_{50} would be easier.

Step4

Graphical Method

A graph between the Probit value and log10 of concentration (See Table 5) is plotted to assess the LC_{50}.

Graphical method



X =0.32
LC_{50} =antilog (0.32)
=2.1mg/L

Figure 6.1 Plot of log_{10} concentrations versus Probit values

Calculation of standard error of LC_{50}

Following formula is used to assess the SE of the LC_{50}that is needed for the estimation of the FLs of the LC_{50}

$$\text{Approx. SE of } LC_{50} = \frac{(\text{Log}LC_{84} - \text{Log}LC_{16})}{\sqrt{2N}}$$

Where N is the total number of subjects in each treatment

Consult the Probit value table to see the probit value of the 84 and 16.The Probit values for 84 and 16 are 6 and 4, respectively after rounding off to single significant digit.

$$\text{Approx. SE of } LC_{50} = \frac{[(\text{Log}(6) - \text{Log}(4)]}{\sqrt{2N}}$$

While the log-concentration values corresponding to these Probit units are 0.9 and -0.3, respectively (Figure 6.1). The antilog values for 0.9 and -0.3 are 7.94 (LC_{84}) and 0.50 (LC_{16}), respectively.

$$\text{Approx. SE of } LC_{50} = \frac{(7.94 - 0.5)}{\sqrt{2(30)}}$$

$$= \frac{7.44}{7.74}$$

=0.9612

Hence the SE is 0.9612.

Estimation of 95% FL of LC_{50}

The 95% FL of the LC_{50} can be calculated by using SE = 2.1±0.9612=2.1 (1.138-3.06)

Step7

Estimation of standard deviation (σ)

$$\sigma = \frac{1}{2}\left(\frac{LC84}{LC50} + \frac{LC50}{LC16}\right)$$

$$\sigma = \frac{1}{2}\left(\frac{7.94}{2.1} + \frac{2.1}{0.5}\right)$$

=3.99

Step8

Estimation of slope (β)

$$\beta = \frac{1}{\sigma}$$

$$\beta = \frac{1}{3.99}$$

=0.250

Step9

Estimation of chi-square (χ^2)

$$\chi^2 = \sum \frac{(E - O)^2}{E}$$

Where **E** is the expected mortality and **O** is the observed mortality.

The expected mortality for each treatment is calculated by following formula

Expected mortality =Sum of mortalities of the all the treatments of bioassay/Number of treatments

Table 6.6 Chi-square values of the bioassay

Conc.(mg/L)	Total Number	Number dead (24h)	Corrected mortality %	Observed Mortality (corrected)	Expected mortality	Chi-square
0.0(control)	30	1				
8.0	30	25	83	24.9	15.18	6.22

4.0	30	20	66	19.8	15.18	1.40
2.0	30	15	48	14.4	15.18	0.04
1.0	30	11	35	10.5	15.18	1.44
0.5	30	7	21	6.3	15.18	5.19 Sum=14.29

χ^2=14.29

Step10

Goodness of fit test was used to determine probability

P = CHIDST (df, χ^2)(performed using Microsoft X – cel)

P = 0.120

6.2.2.11 Comparison of Toxicity

Generally, toxicities of different insecticides are compared based on their respective values of the LC_{50}. Generally, lower the LC_{50} value and higher will be the toxicity of the considered insecticide. However, CI of the LC_{50} are the considered the base to compare the toxicity of the different insecticides.

Example:

Below is given the LC_{50} values of insecticides with their respective 95% CI in "()"

1. Clothianidin 0.31 (0.19–0.45) vs Emamectin benzoate 0.27 (0.17–0.42)
   In above mentioned example, toxicities are similar because of the overlapping 95% FL.
2. Fipronil 0.92 (0.72–1.18) vs Clothianidin 0.31(0.19–0.45)
   In second example, LC_{50s} of the mentioned insecticides are similar because of overlapping 95% FL.

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CHAPTER-7

7.0 INSECTICIDE RESISTANCE AND MANAGEMENT

7.1 Insecticide Resistance

The insecticides resistance may be defined as: “The inherent ability of individuals in a normal population to survive against lethal doses of certain insecticide” (FAO, 1979).

------OR-----

“The ability of an organism to survive a dose of toxin which is lethal to a susceptible one” (Georghiou & Saito, 1983).

------OR-----

“Any heritable decrease in sensitivity to a chemical within a pest population” (Brent, 1986).

------OR-----

“Any heritable change that reduces susceptibility of pests relative to conspecifics and do not include economic impact as a criterion for resistance” (NRC, 1986).

------OR-----

“Genetically based decrease in the susceptibility to a pesticide” (Tabashnik et al., 2014).

------OR-----

“A heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest specie” (IRAC, 2016).

In 1914, resistance to lime sulfur was first time reported in San Jose Scale. Resistance to hydrogen cyanide was observed in red scale in California during 1916. In 1946, house flies were discovered resistance to DDT in Sweden. The insecticide resistance was reported in a total of 11 arthropod species such as: citrus thrips to potassium antimonyl tartrate, codling moth to lead arsenate, walnut husk fly to cryolite, since 1946. After this, resistance to every class of commonly available compound was reported in 428 species of arthropods (Georghiou & Mellon, 1983). In the early 1990s, approximately 504 arthropod species were proven resistance to one or more insecticides of the major insecticide groups (Georghiou & Lagunes-Tejeda, 1991). A typical example is Colorado potato beetle in which resistance to 52 chemicals belonging to different insecticide classes has been observed. Thereafter, the cases of resistance development to organochlorine, organophosphate, carbamate, pyrethroid and novel chemistry (neonicotinoids, *B. thuringiensis*, spinosyns, avermectins, diamides and insect growth regulators) insecticides have been increasing day after day in many insect pests. This phenomenon is termed as the ‘pesticide treadmill, and the sequence is familiar (IRAC, 2016). The continued application of insecticides has led the development of resistance and the strains which become resistant are increasingly challenging to control at the label recommended rates. This led the farmers to use high doses of the insecticide that is very dangerous. Both the genetics of resistance and the extensive application of insecticides are responsible for the rapid increase of resistance in insect pests (Abbas et al., 2014b, a; Khan et al., 2014a; Abbas & Shad, 2015; Afzal et al., 2015a). Globally pesticide resistance is documented in 954 pest species with 546 arthropods, 190 plant pathogens, and 218 weeds (Tabashnik et al., 2014). Overall, 14644 cases of resistance to 336 compounds in 597 arthropods species have been reported since 1914 to 2015. Highest cases of resistance to different insecticides against different insect pests have been reported in European Union (3520) following United State of America (2621), China (1923) and Pakistan (1693). Pakistan is the fourth country out of top 20 countries in resistance development to different insecticides (IRAC, 2017).

7.1.1 Field evolved resistance

Genetically based decline in the susceptibility to an insecticide in a field population is called field evolved resistance (Tabashnik et al., 2014). The effect of field evolved resistance might vary from no to very high on pest control. None to very high levels of field-evolved resistance to various insecticides have been documented by many authors in cotton leafworm *Spodoptera litura*

(Fabricius) (Ahmad et al., 2007a; Ahmad et al., 2008; Saleem et al., 2008; Shad et al., 2012; Ahmad & Mehmood, 2015), house fly *Musca domestica* Linnaeus (Khan et al., 2013a; Khan et al., 2013c; Abbas et al., 2015b; Abbas et al., 2015d; Abbas et al., 2015e), beet armyworm *Spodoptera exigua* (Hübner) (Ishtiaq et al., 2012), cotton whitefly *Bemisia tabaci* (Gennadius) (Basit et al., 2013a), cotton mealybug *Phenacoccus solenopsis* Tinsley (Saddiq et al., 2014; Saddiq et al., 2015), citrus psylla *Diaphorina citri* Kuwayama (Naeem et al., 2016) and cotton jassid *Amrasca devastans* (Distant) (Saeed et al., 2017).

7.1.2 Laboratory selected resistance

Genetically based decline in the susceptibility to an insecticide in a population due to continuous exposure in the laboratory is called laboratory selected resistance (Tabashnik et al., 2014). Laboratory-selected resistant strains are used to study the risk assessment, cross-resistance, stability, fitness costs, genetics and biochemical and molecular mechanism of resistance. A lot of laboratory-selected resistance reports to different insecticides in insect pests are available in Pakistan (Basit et al., 2012a; Abbas et al., 2014a, b; Abbas et al., 2014d; Zaka et al., 2014; Afzal et al., 2015a; Afzal et al., 2015b; Shah et al., 2015a; Shah et al., 2015b; Abbas et al., 2016a; Abbas et al., 2016b; Ullah et al., 2016).

7.1.3 Practical resistance

A field evolved resistance which decreases insecticide efficacy and has practical concerns for the control of pests is called practical resistance (Tabashnik et al., 2014). The efficacy of an insecticide can be assessed as a percent decline in the pest density due to exposure of an insecticide (Burkness et al., 2001; Tabashnik et al., 2014), and calculated as:

$$\text{Efficacy} = \frac{\text{Density of pest in control} - \text{density after exposure to pesticide}}{\text{density of pest in control}}$$

If the pest density is same in the treatment and control, the efficacy of that insecticide is 0% and if the insecticide reduces the pest population to zero, the efficacy of that insecticide is 100%.

7.1.4 Sequential resistance

The growth of resistance to different insecticides at different times in the same population is called sequential resistance (Tabashnik et al., 2014).

Sequestration

The resistance occurred due to increase in the extent to which an insecticide that enters an organism is kept away from the target sites, yet remains inside the organism is known as sequestration (Tabashnik et al., 2014).

7.1.5 Cross resistance

Cross resistance can be defined as:

A population or strain which exhibit resistance due to a single mechanism of one molecule which offers resistance to the other molecules. This phenomenon is known as Cross Resistance (Sparks et al., 2012).

Resistance to an insecticide caused by the exposure of a population to a different insecticide with same mode of action is called cross resistance (Tabashnik et al., 2014).

Cross resistance is assumed a useful tool in assessing the mechanisms of insecticide resistance (Abbas et al., 2014a; Khan et al., 2014b). It is more common for insects that show resistance to an insecticide to be resistant to other insecticide with the same mode of action (Sparks et al., 2012). For example, a acetamiprid selected strain of *P. solenopsis* confirmed high cross-resistance to imidacloprid (62-fold), which have same mode of action (Afzal et al., 2015d). However, no cross-resistance to bifenthrin in the lambda-cyhalothrin selected population of *M. domestica* (114-fold) was also observed (Abbas et al., 2014b). The result of no cross-resistance between lambda-cyhalothrin and bifenthrin having same mode of action is may be due to independent mechanism (Khan et al., 2014b). Such results are interesting in term of control strategy, as the same mode of action insecticides can be used in rotation that reduce the selection pressure of a specific

insecticide and ultimately delay the resistance rise to both insecticides but such cases are rare. Cross resistance may occur due to: (1) non-specific enzyme (microsomal oxidases) that attack functional groups of insecticides rather than specific molecule; (2) mutations at target sites that can lower sensitivity to insecticides; (3) delayed cuticular penetration that can affect chemically different insecticides.

7.1.6 Multiple cross resistance

Resistance to an insecticide caused by the exposure of a population to a different insecticide with unlike modes of action is known as multiple cross resistance (Tabashnik et al., 2014). Insects develop this type of resistance by expressing multiple resistance mechanisms (Qian et al., 2008; Afzal et al., 2015d). With multiple resistances, two resistance mechanisms are acquired independently through exposure to two different pesticides. Multiple cross resistance is not very common than cross resistance but has a big concern if occurred because it significantly reduces the number of insecticides that can be used for the control of pests. Cross resistance among insecticides from different group could also be possible when an iso-enzyme from insects act on different insecticides. For example, the MFO iso-enzymes selected by tebufenozide could well detoxify abamectin in *Plutella xylostella* (Linnaeus) (Qian et al., 2008). A acetamiprid selected strain of *P. solenopsis* showed multiple cross-resistance to deltamethrin (30-fold) (Afzal et al., 2015d).

7.1.7 Negative cross resistance

A situation in which an insect strain resistant to one insecticide is hyper-sensitive to other insecticide is known as negative cross resistance. It is a phenomenon in which increasing resistance to one insecticide lead to decrease in resistance to another insecticide (Gorman et al., 2010; Abbas et al., 2012). Negative cross resistance plays an important role in developing insecticide rotation strategies. Negative cross resistance has been reported in many resistant strains. For example, the imidacloprid-selected strain of *S. litura* demonstrated negative cross-resistance to methomyl (Abbas et al., 2012). Another example is the spinosad selected strain of *M. domestica* which showed negative cross-resistance to imidacloprid (Khan et al., 2014b). Negative cross resistance may occur due to fitness costs of resistant strains, allosteric effects at the target site, and increased metabolic processes.

7.2 Resistance Mechanisms

The genetically based alteration in physiology, morphology, or behavior that declines susceptibility to an insecticide is called resistance mechanism (Tabashnik et al., 2014).

7.2.1 Types of resistance mechanisms

7.2.1.1 Behavioral resistance

Resistance occurred due to changes in behavior that reduce exposure to an insecticide is known as behavioral resistance (Tabashnik et al., 2014). Avoidance of chemical baits is an example of behavioral resistance. When an insect no longer response to insecticide bait, it will not come in contact with that insecticide, ultimately limiting the exposure.

Example:
The avoidance of German cockroach *Blattella germanica* Linnaeus resistant strain from harborages treated with cypermethrin and chlorpyrifos and survived is the example of behavioral resistance (Hostetler & Brenner, 1994).

7.2.1.2 Physiological resistance

Reduced cuticular penetration

The resistance occurred due to reduced entry of an insecticide into cuticle of an insect is known as reduced cuticle penetration (Tabashnik et al., 2014). A change in integument structure of insect might affect the amount of chemical that reaches the target site.

Example
A change in cuticle has been documented in a common bed bug, *Cimex lectularius* Linnaeus strain resistant to pyrethroid insecticide (Lilly et al., 2016).

Increased metabolic detoxification

The resistance occurred due to enhanced enzymatic activity of an insecticide to make it less toxic is known as increased metabolic detoxification (Tabashnik et al., 2014). The enzymes such as acetylcholinesterase, cytochrome-P450, glutathione-S-transferase, and carboxylesterase are involved. It is very common resistance mechanism and has been reported in many insect pests (Kristensen, 2005; Abbas et al., 2014a; Afzal et al., 2015b; Afzal et al., 2015c).

Target site resistance

Target site is a part of an insect at which the insecticide interacts to kill the insect. It can be a specific molecule or portion of a molecule that interact with target site. Resistance occurred due to changes in the target site that reduce the toxicity of insecticide is known as target site resistance (Tabashnik et al., 2014). Small changes in the interaction of insecticide with the target site can have dramatic effects on the susceptibility which lead the development of resistance. Such mechanisms have been commonly shown for every class of neurotoxic insecticides. Some examples are acetylcholinesterase insensitivity for organophosphates and carbamates, acetylcholine receptors for neonicotinoids and spinosyns, sodium channel sensitivity for pyrethroids and point mutations for fipronil (Matsuda & Sattelle, 2005; Millar & Denholm, 2007; Tian et al., 2011; Zhang et al., 2016).

Example

Mosquito resistance to pyrethroid is due to insensitive of sodium channel.

7.3 Factors for resistance development

- Extensive rise of pesticide use for the control of different pests.
- Resistance to an insecticide occurs when susceptibility of a pest population changes.
- Extensive applications of insecticides and genetics basis of resistance are accountable for the evolution of resistance in insect pests. Due to selection pressure with insecticides, the insects having resistant genes survive and transfer these resistant traits into their progeny. In this way, the quantity of resistant insects increased in a population due to elimination of susceptible ones. Ultimately, resistant ones outnumber susceptible ones and the particular insecticide is no longer effective.
- More persistency of poison and more rapid life cycle of insects are the greater risk for the rapid development of resistance.
- The influx of unexposed migrants dilutes the inbreeding of resistant insects in the field conditions, so that the heterozygotes are often found to develop resistance more rapidly.
- Selection pressure of an insecticide in the laboratory can lead rapid development of resistance.

7.4 Monitoring of Resistance

The development of resistance to insecticides is an expected consequence of insecticide usage for the control of insect pests. The efficacy of chemical control is economically unacceptable when the frequencies of resistant phenotypes increase at certain level in the field populations. However, poor efficacy of insecticides is not always due to resistance under the field conditions. Other factors such as quality of technical grade materials used, formulations, doses of application and method of application may also play a significant role in harming field control. But if resistance is major factor, the field control failure is expected, regardless of above mentioned factors and a major threat to sustainable pest management. Therefore, it is most important to monitor the resistance in field populations so that appropriate measures can be implemented for effective pest management (Abbas et al., 2015d; Jan et al., 2015; Saddiq et al., 2015; Saeed et al., 2017).

Applications of resistance monitoring are as follows:

1. Monitoring of resistance to insecticides helps to know the temporal and geographical changeability in a population response to selection pressure of particular insecticide.
2. Resistance detection helps us to avoid ineffective insecticides and to make proper recommendation of alternate effective insecticides.
3. Resistance monitoring helps to prevents wastage of pesticide applications that would otherwise pollute the environment.

4. Resistance detection confirms the reasons of pest control failure by particular insecticide under the field conditions.
5. Monitoring of resistance helps to assess the influence of implemented resistance management strategies.

7.5 Genetics of Resistance

7.5.1 Gene frequency

In generally, frequency of resistant alleles (R) in natural/field strains would be expected to be low ranged from 0.0001-0.01. For example, the estimated frequency of resistant alleles to Cry1Ab was 0.0023 in sugarcane borer field populations (Huang et al., 2007). However, the frequencies of resistant alleles in some field populations are surprisingly high. For example, a bedbug population contained 0.5% DDT-R genes prior to use of DDT in Taiwan.

In resistant populations, the frequency of resistant alleles can be very high. For example, the frequency of pyrethroid resistance allele ranged from 0.25-0.966 using the real time PCR amplifications of specific allele (rtPASA) method in eleven field population of malarial mosquito *Anopheles sinensis* Wiedemann in Korea (Kim et al., 2007).

7.5.2 Autosomal or sexed linked

1. **Sex linked resistance**
   Alleles controlling resistance to any insecticides are present on sex chromosome is called sex linked resistance.
2. **Autosomal resistance**
   Alleles controlling resistance to any insecticides are not present on sex chromosome is called autosomal resistance.

It can be evaluated by reciprocal crosses between susceptible and resistant homozygotes insects. By comparing the LC_{50} values of reciprocal crosses, if the CI of LC_{50} values are overlapping suggests autosomal inheritance of resistance to insecticides. If CI not overlapped, then there is sex linked inheritance. In mostly studies, the autosomal resistance is reported in many insect pests in *M. domestica* to pyriproxyfen, and *P. solenopsis* to chlorpyrifos (Abbas et al., 2014b; Khan et al., 2014a; Afzal et al., 2015b; Afzal et al., 2015c; Shah et al., 2015c). However, few cases of sexed linked inheritance are documented in insects, for example *P. xylostella* resistance to tebufenozide (Cao & Han, 2013).

7.5.3 Dominance of resistance

The inheritance of resistance in which insects have a resistant phenotype only if they have two resistant alleles at a genetic locus that controls susceptibility is called **recessive resistance** (Tabashnik et al., 2014).

The inheritance of resistance in which insects have a resistant phenotype only if they have either one or two resistant alleles at a genetic locus that controls susceptibility is called **dominant resistance** (Tabashnik et al., 2014).

The degree of dominance can play an important role in expression of the resistance genes (Abbas et al., 2014b). Resistant genes may be completely recessive, incompletely recessive, incompletely dominant, or dominant. Chemical control is very difficult if genes controlling resistance are completely dominant to insecticides. Resistance due to dominant genes can increase quicker than the resistance due to recessive genes, because resistance genotypes increase as R:S = 1:3 for recessive and R:S = 3:1 for dominant (Wang et al., 2009; Khan et al., 2014a). In toxicology, dominance levels were firstly assessed by the comparison of mortality curves of the susceptible, resistant, and hybrid insects. Resistance was categorized as recessive, incompletely recessive, dominant, and incompletely dominant based on whether the mortality curve of hybrids was closer to mortality curve of susceptible or resistant insects, respectively.

There are two formulae used for determination of value of dominance.

(i) A formula was introduced by Stone (1968) to calculate the value of dominance level as:

$$D = \frac{2\log LCRS - \log LCR - \log LCS}{\log LCR - \log LCS}$$

Where, LC_R, LC_{RS}, and LC_S are the median lethal concentrations for the resistant, hybrid and susceptible insects, respectively. D value varies from -1 (completely recessive) to +1 (completely dominance).
Stone's formula is the most extensively used method to determine the dominance values of resistance to insecticides.

(ii) There is another formulae which was introduced by Bourguet & Raymond (1998) which is given as follows:

$$D_{LC} = (\log LC_{RS} - \log LC_S) / (\log LC_R - \log LC_S)$$

Its value ranged from 0 to 1. Zero means completely recessive and one means completely dominant.

7.5.3.1 Completely dominant resistance

The distribution and expression of dominant resistant genes in the heterozygotes is called completely dominant resistance to insecticides. If resistance is completely dominant, only one parent has to possess the trait for it to be fully expressed in the offspring.

Example
Completely dominant resistance has been reported in house fly resistant to pyriproxyfen (Shah et al., 2015c).

Remedy
The dominant resistance can rapidly become established within the populations and very difficult to manage therefore those insecticides should be withdrawn from resistance management strategies.

7.5.3.2 Incompletely dominant resistance

The distribution and expression of partially dominant resistant genes in the heterozygotes is called incompletely dominant resistance to insecticides. If resistance is incompletely dominant, only one parent has to possess the trait for it to be partially expressed in the offspring.

Example
Incompletely dominant resistance has been reported in house fly resistant to fipronil and lambda-cyhalothrin and cotton mealybug resistant to spinosad (Abbas et al., 2014b, a; Afzal et al., 2015c).

Remedy
This type of resistance can be managed by cautioned and rotational use of insecticides with different mode of action.

7.5.3.3 Completely recessive resistance

The distribution and expression of recessive resistant genes in the heterozygotes is called completely recessive resistance to insecticides. If the resistance is completely recessive, both parents must possess the trait for it to be fully expressed in the offspring.

Example
Completely recessive resistance has been reported in diamondback moth resistant to *Bt* toxin Cry1Ac.

Remedy
The recessive resistance cannot rapidly become established within the populations. It is considered an advantage for resistance management because heterozygotes should be easily killed under field conditions.

7.5.3.4 Incompletely recessive resistance

The distribution and expression of partially recessive resistant genes in the heterozygotes is called incompletely recessive resistance to insecticides. If the resistance is incompletely recessive, both parents must possess the trait for it to be partially expressed in the offspring.

Example

Incompletely recessive resistance has been reported in house fly resistant to imidacloprid and cotton mealybug resistant to chlorpyrifos (Khan et al., 2014a; Afzal et al., 2015b).

Remedy

If the heterozygotes tolerate a higher dose of insecticides compared to the susceptible strain, therefore those insecticides should be used cautiously for the management of pests to retain long term efficacy.

7.5.4 Effective dominance (D_{ML})

Effective dominance measures the relative mortality level for a given insecticide concentration on different concentrations of bioassay. D_{ML} can be quantified as follows according to Bourguet & Raymond (1998).

$$D_{ML} = (ML_{RS} - ML_{SS}) / (ML_{RR} - ML_{SS})$$

Where, ML_{RR}, ML_{RS}, and ML_{SS} are the mortalities on given concentrations for the resistant, hybrid and susceptible insects, respectively. D_{ML} varies between 0 and 1 (0 recessive and 1 dominant). The extent of resistance dominance depends upon the doses of specific insecticide used and is reported in many insect pests (Abbas et al., 2014a, b; Khan et al., 2014a; Afzal et al., 2015c). ~~The recessive resistance gives an advantage for the management of resistance because heterozygotes can be easily killed in the field. However, if the heterozygote insects tolerate a higher dose of insecticide compared with the susceptible insects, then~~ particular insecticide should be used sensibly for the management of insect pests to retain long te efficacy.

7.5.5 Monogenic or polygenic resistance

On the basis of number of genes

1. Monogenic resistance
   Resistance controlled by one gene (major gene effect) is known as monogenic resistance.
2. Polygenic resistance
   Resistance controlled by more than one genes (minor gene effect) is known as polygenic resistance.

To know the resistance is monogenic or polygenic, the reciprocal crosses (F1) of the susceptible and resistant strains are backcrossed with parent strains (either susceptible, resistant or both).

Monogenic or polygenic resistance can be estimated by using a chi-square goodness of fit test according to Sokal & Rohlf (1981) as follows:

$$\chi 2 = (Ni - pni)2 / pqni$$

Here, *Ni* is the observed mortality in backcross strain to a particular dose, *ni* is the number of insects exposed to a particular dose, *p* is the expected mortality calculated following Georghiou (1969) and q is calculated as 1-p. The expected mortality is calculated as 0.5 (number of cross (F1) insects killed + number of resistant insects killed)/number of insects exposed in concentration. If there are significant differences between observed and expected mortalities at more than half of total concentrations (e.i. $P < 0.05$ in 4 concentrations out of 6 concentrations), the null hypothesis of monogenic resistance is rejected.

Polygenic and monogenic resistance to insecticides can happen in the natural strains (Ahmad et al., 2007b; Abbas et al., 2014b). Polygenic resistance is more likely under laboratory selections, due to lack of rare variants in the laboratory selected strains than natural strains

(McKenzie et al., 1992; Abbas et al., 2014b). Polygenic resistance is observed in many laboratory selected insect species to different insecticides; for example, *M. domestica* resistant to imidacloprid, lambda-cyhalothrin, fipronil, pyriproxyfen, and spinosad (Abbas et al., 2014a, b; Khan et al., 2014a; Khan et al., 2014b; Shah et al., 2015c), *P. solenopsis* resistant to chlorpyrifos, spinosad, and acetamiprid (Afzal et al., 2015a; Afzal et al., 2015b; Afzal et al., 2015c). Monogenic resistance is also observed in *M. domestica* resistant to beta-cypermethrin (Zhang et al., 2008). The change in resistance type depends upon the selection history, number of insect exposed and geographical origin of the pest species.

7.6 Fitness Costs

The ability of a certain individual in a population to survive and reproduce compared to other individuals of the same species is called fitness. The development of resistance to an insecticide is complemented with high energetic cost or significant disadvantage that weakens the fitness of insects compared with its susceptible counterparts in the population is called fitness cost (Kliot & Ghanim, 2012).

Relative fitness is a significant tool for evaluating the biological changes in insecticide resistant strains and the adaptability of insects to insecticides. Relative fitness is measured as Ro of resistant strain/Ro of susceptible counterpart strain, where Ro is net reproductive rate calculated as N_{n+1}/N_n, where N_n is the parental population quantity, and N_{n+1} is the number of larvae produced in next generation (Abbas et al., 2012). Moreover, biological parameters such as survival rates, larval durations, pupal durations, pupal weights, development times, growth rates, fecundity, hatchability, female ratio and biotic potential are studied to see the fate of fitness. Fitness costs in resistant insects may delay the development of resistance under certain conditions.

7.6.1 Types of fitness costs

7.6.1.1 Increased fitness costs

When the resistant individuals of a strain showed disadvantageous biological parameters compared with counterpart susceptible / unselected strain are called increased fitness costs. These fitness costs have been observed in pests and chance of the resistance development is low. These traits could increase the impact of insecticide rotations and sustainability of the resistance management strategy for insecticides. In this, value of relative fitness is less than one.

Example

Increased fitness costs have been reported in house fly resistant to fipronil, imidacloprid, methoxyfenozide, pyriproxyfen and lambda-cyhalothrin (Abbas et al., 2015c; Shah et al., 2015d; Abbas et al., 2016a; Abbas et al., 2016b; Shah et al., 2017).

7.6.1.2 Decreased fitness costs

When the resistant individuals of a strain showed advantageous biological parameters compared with counterpart susceptible strain are called decreased fitness costs. Such fitness costs have been observed in natural enemies and chance of the resistance development to insecticides is high which show compatibility of natural enemies with chemical control. In this, value of relative fitness is more than one.

Example

Increased fitness has been observed in spinosad and emamectin benzoate resistant strain of *C. carnea*.(Mansoor et al., 2013; Abbas et al., 2014c) If increased fitness of resistance to insecticides is observed in pests, then those insecticides should be withdrawn from resistance management strategies.

7.6.1.3 No fitness costs

When the resistant individuals of a strain showed neither disadvantageous nor advantageous biological parameters compared with counterpart susceptible strain are called no fitness costs. If there are no fitness costs of resistance, these traits could decrease the impact of insecticide rotations ~~and~~ threaten sustainability of the resistance management strategy for insecticides. In this, value of relative fitness is equal to one.

Example
Lack of fitness costs have been reported in whitefly resistant to pyriproxyfen and acetamiprid (Crowder et al., 2009; Basit et al., 2012b).

7.7 Stability of Resistance

Determination of insecticide resistance stability has practical implication in making effective resistance management strategies (Abbas et al., 2015b; Afzal et al., 2015d). A decline of resistance to insecticides in the resistant strains is determined as R = log final LC_{50}-log initial LC_{50}/number of generations selected with a particular insecticide. If R value comes in negative, the resistance is unstable. The stability of insecticide resistance is also assessed by comparing the LC_{50} values of field strain and laboratory selected strain. If the fiducial limits of LC_{50} of both strains did not overlap, the resistance is unstable. The removal of an insecticide from spray program for some time may bring the resistance level lower if resistance is unstable, and therefore, prolong the efficacy of that particular insecticide. The decline in resistance levels is observed in many insecticides selected populations of different species in the laboratory and might be due to high fitness cost of resistance to insecticides (Abbas et al., 2015c; Afzal et al., 2015d; Shah et al., 2015d; Abbas et al., 2016a; Abbas et al., 2016b; Shah et al., 2017).

7.8 Insecticide Mixtures

Insecticide mixtures work as an important tool for resistance management in many insect pests and are used to delay the development of resistance to insecticides or to increase the efficacy of control (Abbas et al., 2015a). Insecticides mixtures are assessed by using ratios of 1:1, 1:10, 1:20 and LC_{50}:LC_{50} (Khan et al., 2013b; Abbas et al., 2015a). A combination index (CI) is calculated to assess the mixture effects according to Chou & Talalay (1984) as:

$$\mathrm{CIx} = \frac{\mathrm{LCx\ (1m)}}{\mathrm{LCx1}} + \frac{\mathrm{LCx\ (2m)}}{\mathrm{LCx2}} + \left(\frac{\mathrm{LCx\ (1m)}}{\mathrm{LCx1}} \times \frac{\mathrm{LCx\ (2m)}}{\mathrm{LCx2}}\right)$$

LC_x $^{(1m)}$ and LC_x $^{(2m)}$ are the ~~median lethal concentrations of insecticide mixtures~~ and LC_x^1 and LC_x^2.are the median lethal concentration of insecticides alone~~, giving mortality x~~. Combination index values are categorize as a synergistic effect (CI <1), an additive effect (CI = 1), and an antagonistic effect (CI >1).

The insecticide mixtures with different modes of action indicating synergistic effects should be used in insecticide resistance management strategies. For example, synergistic effects of pyrethroid and organophosphate/new chemical and neonicotinoid and insect growth regulator mixtures are observed in *B. tabaci* (Basit et al., 2013b), *S. litura* (Ahmad, 2009), and *M. domestica* (Khan et al., 2013b). Furthermore, the evaluation of newly introduced insecticide mixtures is needed in future for effective resistance management

7.9 Detoxification Mechanisms of Insecticides

Detoxification is the biotransformation of complex/toxic compounds into simple/less toxic compounds is known as detoxification. Detoxification mechanisms occur by the following type of chemical changes:

7.9.1 Phase 1 reactions

Phase 1 reactions are also known as primary reactions. These reactions are:

7.9.1.1 Oxidation

The removal of hydrogen atom or addition of oxygen atom is called oxidation. This detoxification reaction is carried out by the enzyme, cytochrome P450 monooxygenases, microsomal oxidase or mixed function oxidase (MFO). The overall reaction is as follows:

$RH+NADPH+H^+ +O_2 \rightarrow ROH+NADP+H_2O$

Oxidation reactions are hydroxylation, de-alkylation, dearylation, epoxidation, sulfoxidation and desulforation.

Example
Carbamate insecticide carbaryl (1-naphthyl methylcarbamate) converted into 1-naphthol which is less toxic compound.

7.9.1.2 Reduction
The removal of oxygen atom or addition of hydrogen atom is called reduction. The reductive detoxification of nitro group to amino group is occurred by reductase enzyme.

Example
Parathion is reduced to aminoparathion which is less toxic to parathion.

7.9.1.3 Hydrolysis
Addition of water molecule to esters or amides resulting in the cleavage of ester or amide linkage is called hydrolysis.
$R^1COOR+H2O \rightarrow R^1COOH+ROH$
Organophosphates, carbamates, pyrethroids and juvenoids that contain ester linkages are hydrolyzed by the enzymes, carboxylesterase, phosphatase and amidase etc.

Example
Malathion hydrolyzed into α and β-monoacids and ethanol.

7.9.1.4 Dehydrochlorination
Removal of HCL from the insecticide to convert the toxic substance to none toxic metabolites is known as dehydrochlorination. The enzyme involved is called dehydrochlorinase.

Example
DDT is converted into DDE, resulting in detoxification.

7.9.2. Phase II reactions
7.9.2.1 Conjugation
Conjugation reaction is biosynthesis pathway (production of chemical substance by living organisms) by which foreign compounds and their metabolites containing certain functional groups are linked to endogenous substrate and convert them generally to less toxic compounds.

Example
O-Alkyl conjugation of methyl parathion with GSH produces desmethyl parathion and methyl glutathione.

7.10 Genotoxicity
The property of a chemical that damages the genetic makeup within a cell causing mutations, which may lead to cancer, is known as genotoxicity. Genotoxicity is often confused with mutagenicity; all mutagens are genotoxic but all genotoxic substances are not mutagenic. Pesticides are considered likely chemical mutagens because various agro-chemical ingredients contain mutagenic properties such as chromosomal alterations mutations, or DNA damage (Bolognesi, 2003). For example, cytotoxic and genotoxic effects on human intestinal cells by acetamiprid, DNA damage by low dosage of avermectin in silkworm hemocytes, and cytotoxic, genotoxic, and aneugenic effects by pyrethroid insecticides on human blood lymphocyte are observed (Shen et al., 2011; Çavaş et al., 2012; Muranli, 2013). Genotoxic effects of insecticides can be tested in both in vitro and in vivo systems by Micronucleus Assay, Ames Test, Pig-a Assay, Reconstructed Skin Micromolecule Assay, Comet Assay/Single Gene Electrophoresis.

7.11 Resistance Management
1. Resistance gene frequency can be reduced by using low doses of insecticides, using insecticides with short environmental persistence, providing refugia where susceptible insects reproduce and treating more damaging life stage.

2. Resistance can be delayed by using insecticide mixtures having synergistic interactions and no cross resistance between them.
3. Rotations of insecticides with different mode of action having no cross resistance/multiple cross resistance to each other and unstable resistance should be implemented to delay the development of resistance.
4. By using insecticide synergists such as PBO, DEF and DEM, the efficacy of insecticides can be increased, ultimately delay the resistance.
5. New bio-rational insecticides and bio-pesticides should be integrated for the control of insect pests.
6. By using resistant predators and parasites, the selection pressure of insecticides can be reduced.
7. Monitoring of resistance to insecticides should be done on regularly basis that reduce the frequent use of insecticides in the field.
8. Cultivation of transgenic crops (Bt cotton) may reduce the number of sprays especially for lepidopteran pests which may delay the development of resistance.

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CHAPTER-8

8.0 INSECTICIDE APPLICATION

8.1 Application Equipment

A number of application equipment is available for applying insecticides. But the type of application equipment used depends upon the following factors (Edward, 1975).

- Size of the area (mechanical equipment for large area while hand equipment for small area)
- Availability and the type of carrier (oil, water etc.)
- Availability of the workers
- Cost, availability and durability of equipment
- Type of insect and insecticide formulation
- Time, speed and accuracy of application equipment

8.1.1 Boom sprayer

8.1.1.1 Parts of Boom Sprayer

A number of insecticide formulations are sprayed by the sprayers so the material of sprayers should be such that it can withstand the effects of spraying material. Some of the insecticide formulations like wettable powders exhibited abrasive activity while other may have corrosive effects in contact with the sprayer material. Boom sprayer has following parts (FAO, 1994; Malik, 2012).

8.1.1.2 Pump

Pump produces the necessary pressure to facilitate the flow and atomization of spray material towards the nozzle. Different types of pumps are available in the market like roller, piston, diaphragm and centrifugal pumps. Piston and diaphragm pumps are more suitable to develop required amount of pressure for thorough plant coverage. Two things must be considered for choosing a good quality pump, one is the gallons per minute supplied by the pump and the other is the pressure range that can handle by it.

Figure 8.1 Pump of boom sprayer

8.1.1.3 Tank

Sprayers used water and other materials as diluents in addition to the insecticides. Spray tank is a necessary component to carry the spray material. The size of the tank should be large enough to avoid the frequent refilling. The size of the tank also depends upon the rate of application

and space available for its mounting. Tanks must be equipped with large upper and bottom opening. The large top opening with strainer helps in easy filling, inspecting and cleaning while the lower opening is used for draining. Both openings must be provided with water tight cover to avoid spillage. A good tank must have gauge to indicate the water level of the tank.

Spray tank can be made of different materials including steel, fiberglass, aluminum and polyethylene. Tanks made of fiberglass, polyethylene or stainless steel is more preferred because of their resistance towards corrosion and abrasion. In case of other materials tank should be coated with a protective lining to resistant the above activities.

Figure 8.2 Tank of boom sprayer

8.1.1.4 Spray Lance of boom

It is a horizontal pipe of varies length (1-15m) with two or several nozzles separated by 50cm apart. Usually long boom is used for tractor sprayers. It is more beneficial than spray lance because of its wide swath (Area covered by a single nozzle during spray) it covers in each trip. Width of swath can be adjusted to obtain three types of sprays.

- Directed spray
- Band spray
- Uniform spray

It contains 28-36 nozzles.

Figure 8.3 Spray Lance of Boom

8.1.1.5 Power source

It is the main requirement to operate the sprayer. Different power sources are operated now days including the manual, tractor or tractor with air craft engines, traction and motor.

8.1.1.6 Control Valve and gauge

It is valve which control flow of pesticide solution and gauge depicts pressure of solution which is being flowing.

8.1.1.7 Nozzles

Its main function is to convert the pressurized liquid into small droplets or mist for thorough application on the target site. It also controls the droplet size, amount of liquid and distribution pattern. Size of the droplet and flow rate is also dependent upon the pressure in addition to the design of the nozzle. Under high pressure and with small nozzle tip droplets of smaller size are produced and vice versa. Small droplet size provides thorough and even coverage while bigger size results in the reduction of off-target drift.

Nozzles can be available in different materials including brass, aluminum, stainless steel, ceramic, and plastic. Selection of nozzle material is quite dependent upon the type of formulation. Aluminum or brass can't be used for abrasive formulation (wettable powders and dry flowables). These materials wear down quickly. Selection of nozzle type consider many factor like coverage desired, target pest or area, method of application and potential for drift. A nozzle consists of nozzle body, a strainer, replaceable nozzle tip and a cap to hold it. Nozzles can be classified on the basis of droplet size and spray pattern (UK, 2016). A nozzle performs three important functions.

- Covert the spray able liquid into smaller droplets
- Spray and spread these droplets in a specific pattern
- Nozzles regulate the rate of release of the sprayer

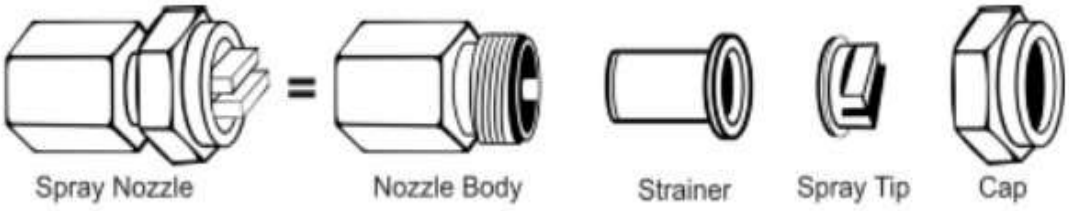

Figure 8.4 Parts of nozzle

8.1.1.7.1 Fan nozzle / Tee jet

These are used for banding sprays and produce a narrow, oval pattern with a sharp cutoff at the edge. These are available in different types like even flat fan, standard flat fan, low pressure, off center, and twin orifice flat fan nozzles. These are mostly used for applying herbicides. Nozzle spray angle and boom height effect the width of the spray. These are available in different colors depending upon the size of the orifice.

Figure 8.5 Fan nozzle

8.1.1.7.2 Hollow cone nozzle

These nozzles release a more uniform and fine spray particles than the solid cone nozzles. These are used for spraying agricultural crops with formulation of wettable powders, suspensions and flowables at higher pressure. These are usually used to apply fungicides or insecticides when complete coverage and foliage penetration is the priority. Spray drift is more than other nozzles. These are available in varying colors with different hole sizes.

Figure 8.6 Hollow cone nozzle

8.1.2 Knapsack sprayer

It is commonly used and small unit of sprayer which is operated by hand or engine. These are also manually operated and carry by operator on its back by a pair of mounting straps during application of insecticides. It is suitable for small lands up to some acres. The lever is used to push the liquid from spray tank to the air cylinder with the help of a piston. Air present in the cylinder creates pressure that releases the water through via cut-off valve. A plastic tank of 14-16 liters capacity is used with the sprayer. Hand level is usually operated at 15-20 strokes/ minute at the pressure of 40psi. However, applicator requires constant pumping to develop pressure. It requires good practice for thorough coverage of the area to be treated (McAuliffe & Gray, 2002).

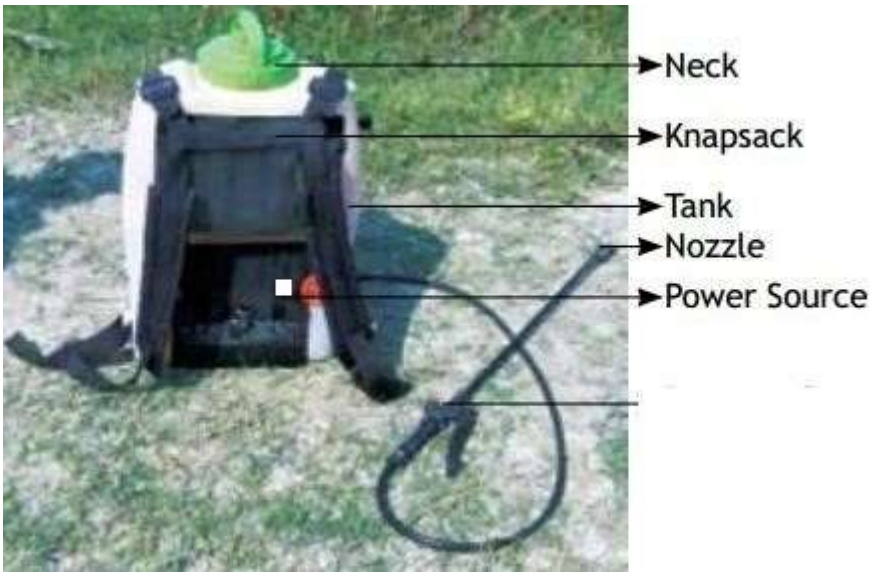

Figure 8.7 A Knapack sprayer

8.1.2.1 Parts of Knapsack Sprayer

8.1.2.1.1 Spray tank

This is main part of sprayer which is filled with pesticide solution being sprayed for control of different pests.

Figure 8.8 Spray tank

8.1.2.2 Spray lance

It is a long rod of 90cm in length made up of steel or brass. On one side it is attached to the delivery pipe and provided with a replaceable nozzle at the other end. At the hose side it is equipped with a trigger mechanism to regulate the flow of liquid and it bents at its nozzle end forming a goose neck. Sometimes a plastic shield is used to fix the spray lance to prevent chemical drifting.

Figure 8.9 Spray Lance

8.1.2.3 Control valves

Additional cutoff valves are provided between the pressure regulators and nozzle to provide the on/off function. These should be easily reachable by the spray person and should large and tight enough that does not hamper (when open) and release the liquid flow (when closed).

Figure 8.10 Control valve

8.1.2.4 Pressure regulators

Pressure regulators and gauges are provided to control and check the rate of pressure, respectively. It helps to maintain the operation at constant pressure. These also protect the sprayer parts to wear down due to excessive pressure. The type of regulators depends upon the type of pump. Likewise the type of pressure gauge depends upon the type of sprayer. High pressure gauge not provides the accurate readings for low pressure sprayer. These functions are sometimes performed by the cut-off valves of the sprayer.

Figure 8.11 Pressure regulator

8.1.2.5 Hose

These are used to deliver insecticides out of tank. These should be strong enough to resist the pressure and the effect of different formulations of insecticides. The inlet diameter of hoses should be greater or at least equal to the diameter of inside parts of the pump. Undersized hoses can reduce the pump capacity that can alter the pump pressure and ultimately results in uneven flow rate. The most effective materials used for the hoses are neoprene, plastic and rubber.

8.1.2.6 Pump

Pump is provided in a automatic sprayer which provide source to throw pesticide solution outside sprayer.

Figure 8.12 Pump of knapsack sprayer

8.1.2.7 Strainers

These are also called filters and are in the form of small mesh screen. These present at different places in the sprayers and help to filter the small particles present in the spray solution that may clog or damage the distribution system. Strainers of different sizes are present in the following places

- In the nozzle body to screen out the small particles to avoid the clogging of nozzle
- At the entrance of pump intake pipe (25-50 mesh size)
- In the way between the pressure regulator and boom (50-100 mesh size)

- For wettable powders all screens should be of 50 mesh size or coarser

Figure 8.13 Both are Strainer

8.1.2.8 Tank agitator

Spray material of an insecticide is a mixture different component (See chapter Formulations) including the insecticide and carrier. Agitator devices help to maintain the homogeneity of the spray material. Constant mixing is necessary for some of the formulations like dry flowables and wettable powders (suspension, emulsion). It will produces the uniform spraying material for even application. Agitation can be achieved by using paddles provided in the tank while jet agitation is another method for constant stirring the spray mixture.

In jet agitation, a nozzle is present inside the tank that continuously throws the stream of spray within the tank to keep it agitated constantly. Jet agitation is controlled by different ways. The amount of stream released depends upon the type of formulation and size of the tank. For the foam forming formulations, flow rate of liquid or agitation is reduced by using the control valve of the agitator.

8.1.3 Granule spreaders

These spreaders are used to apply uniformly coarse sized, dry particles of insecticides to water, soil and foliage. These are mostly used in broadcast and band applications. These are less likely used as a single unit because they are mostly attached with cultivating equipment (planters). It is usually operated by gravity (gravity feed) and has an adjustable opening to control the release of granules.

Two types of spreaders are commonly used including the rotary and drop spreader. Rotary spreader distributes the granules at the sides and front by using the rotating fan or disk while the drop spreaders differs by an opening at the bottom side which opens by means of a sliding gate that controls the flow of granules by gravity feed. Moat often drop spreaders are preferred over rotary spreaders when precise application is required (Roberson, 2017).

Advantages

These are simple, light weight and easy to calibrate and require no carrier.

Fig 8.14 Granular applicator

8.1.4 Pressurized cans (Aerosols)

These are in the form of pressurized disposable packaging or cans with the capacity of ≤ 1L. These are manually operated sprayers and mostly used for small land holdings such as green houses or for small land holdings. It consists of an air pump that develops pressure in the spray tank that slowly release the liquid droplets form nozzle. It provides less uniform coverage of the treated area.

Figure 8.15 Pressurized cans

8.1.5 Trigger pump sprayers (Gun)

These are non-pressurized sprayers because they do not use separate pressurized air source. The insecticide force through the nozzle under pressure created by the squeezing the trigger. These sprayers give an even application. They are mostly used for small areas.

Figure 8.16 Triger pump sprayer

8.1.6 Motorized / Power operated / Mechanical sprayers

Power driven sprayers have many advantages over conventional sprayers. These provide power and high pressure to operate the different parts of the sprayer. Power is provided by electric motors or engines. The sprayer under this heading may require high to low pressure depending upon the requirements of the pump or other components of the sprayers. These can be mounted on trucks, tractors and aircrafts. These are good for the large scale application in orchards and other crops (Edward, 1975; Malik, 2012; Anonymous, 2017).

8.1.6.1 Boom Sprayer

These are also mounted on trucks, trailers or tractors. These are also designed to apply the insecticides over large field areas in swaths. The application rate may vary from 50-500L/ha at the pressure of 50-500Kpa. The length of boom may be 6-10 meter long with the nozzle distance of 50100cm. these sprayers provide uniform and full coverage of large areas but they may not penetrate the dense foliage. These sprayers mostly use hydraulic sprayers that may cause problem with the wettable formulations.

Figure 8.17 Boom sprayer front and rear view

8.1.6.2 High-pressure / Hydraulic Sprayers

These sprayers are used for thick foliage, to the top of the trees and for the areas where high pressure is a necessary requirement for complete and uniform coverage. The sprayers work at a pressure of 7000Kpa. The design of the high-pressure sprayers is similar with the low pressure sprayers except the components are designed to withstand high pressure. The sprayer may be equipped with a boom, a hose, multiple nozzles or a handgun nozzle for spraying, shade trees,

orchards, building, ornamentals, livestock and commercial crops. They are also used mechanical agitators that well mix the wettable powders.

8.1.6.3 Air blast sprayers

These sprayers use a combination of water and air to deliver the insecticide to the target site. Insecticide is pushed through a nozzle or series of nozzle. A high speed fan or blower blows away the insecticide through nozzle in the form of an air blast. The high pressure air converts the insecticide solution into small droplets that moved away by an air blast. The pressure and volume of these sprayers can be adjustable according to the needs. The air blast may be carried 10-40 feet away from the sprayers. Capacity of tank is 500Ltr. These sprayers are used for tree spraying and spraying of agricultural crops (Malik, 2012).

Figure 8.18 Air blast sprayer front and rear view

8.1.6.4 Jeeto sprayer

It is of aluminum three-way valve enabled high efficiency sprayer. Its tank is made up of fiber glass with the capacity of 400 Liters. Its each disc of three stage turbine atomizes the chemical in small droplets (mist). It is mostly used for spraying orchard trees. Flow rate varies from 0.3 to 1.6L/ Min with the speed of 3-45KM/hr.

Figure 8.19 Jeeto Sprayer

8.1.7 Aerosol generators/ Foggers

These have a metallic container to withstand the pressure of liquefied propellant. The droplet size is very small (1-50µm) and these are suspended in the air for long period of time. These are mostly used to control the flying insects like mosquitoes and flies. Dispenser is fitted with a delivery tube and a propeller. The propeller forces the spray to leave through the delivery tube in the form of fine droplets. These are mostly available with the capacity of 300-400g of insecticide while 2-5kg sizes are also available. These are used at the rate of 7-14g per $100m^3$ of the area to be sprayed. On the other way compressed air, centrifugal energy and hot velocity air is also a source of energy (Malik, 2012; UK, 2016).

Figure 8.20 Aerosol generator

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CHAPTER-9

9.0 INSECTICIDE LEGISLATIONS IN PAKISTAN

AGRICULTURAL PESTICIDE ORDINANCE 1971 AMENDED 1997 AND AGRICULTURAL PESTICIDES RULES 1973 PAKISTAN

Main points of pesticide ordinance are

1. Short title, range and commencing.

(1) This Ordinance should be pronounced as the Agricultural Pesticides Ordinance, 1971.

(2). It is applicable to whole of Pakistan.

(3). This ordinance will be effective immediately.

2. Other laws for the time being in force will not be interrupted by this ordinance.

3. Definitions– In this Ordinance include different definitions of terminology which is used in rest of ordinance.

(a) "Adulterated" is a pesticide that is not of the standard quality or fulfills the values written on the label. It is either mixed with a material and is not effective against the target pests];

(b) "Advertise" referred to awareness of the community through publication, notice or circular.

(d) "Committee" is meant for Agriculture Pesticide Technical Advisory Committee

(e) "Formulation" is a preparation of a pesticide by mixing an active ingredient with an inert material to make it readily usable to overcome the pest problem.

(f) "Fungi" indicates all kinds of diseases causing mildews, moulds, yeasts, rusts, smuts, and similar other fungi effecting plant life.

(g) "Government analyst" is person who works as a Government Analyst in the Pesticide laboratory under.

(h) "Guarantee" means written statement by an applicant about the quality of pesticide required to submit under the rules when applying for registration of the pesticide.

(i) "inspector" is any Government officer exercising powers under this Ordinance;

(j) "ingredient" means chemicals used to prepare pesticides;

(k) "insect" indicates invertebrate animals

(l) "label" is the wrapper of the pesticide container containing the information regarding its usage, handling, quantity, and retail price etc.;

(m) "package" is a container of pesticide;

(n) "pesticide" is any material or mixture of matter (not a drug according to the definition of drug in the [(Drugs Act 1976 (XXXI of 1976)] applied to repel, mitigate, prevent, destroy, or control an insect, weed, virus, bacterial organisms, fungus, nematodes, rodent, or other plant or animal pests; but does not under comes within the meaning of drug.

(o) "prescribed" according to the Ordinance;

(p) "registered" according to the Ordinance;

(q) "registration number" is a code assigned to each registered pesticide product

(r) "rules" are made under this ordinance (rr) "sub-standard" pesticide does not fulfill the criteria of purity or strength mentioned on the label of the container. Its ingredients are partially or wholly destroyed]

(s) "weed" a plant grown on an unwanted place.

4. **Pesticides to be registered**– No one is allowed to manufacture, formulate, sell, distribute or stock any pesticide until unless it is registered according to this ordinance. A pesticide, notified by the Government in an official gazette that a pesticide without trade will be imported by particular firms. It is also possible that a registration process but notification by the Government for that particular pesticide will be issued.

5. **Application for registration of Pesticide.**

(1) Registration of the name of the pesticide is required by the person (by submitting application to the Government), who is intended to import, manufacture, import, sale or stock for sale.

(2) Following sub-section (1) of the ordinance an application for the registration along with the fee should be submitted.

(3) A person who is not Pakistani and is intended to apply for the registration of pesticide under subsection (1) must have a representative in Pakistan to sign the application.

(4) A pesticide should be registered with name mentioned in the application submitted for this purpose.

(a) A name of pesticide should be chosen keeping in mind that it will not deceive or mislead the public about its quality, ingredients or other criteria written on its label.

(b) The guarantee of one pesticide is such that it will never confuse with the similar product launched or registered by another firm.

(c) A pesticide shall be effective against the pest for which it is meant.

(d) A pesticide applied at label recommended dose is not detrimental to non-target organisms including vegetation except weeds, human beings and wild life etc.

(5) After the successful registration of a pesticide, Government shall issue a certificate of registration to a person who will apply for a particular pesticide.

6. Registration shall be effective for three years and needs to be renewed before the fifteenth day of June of the third year.

7. Cancellation of registration shall be done by Government for a pesticide that violates any provisions of the ordinance or rules or observed to have negative effect on the non-targets like humans, animals or plants other than weeds. However, the Government shall provide an opportunity of clearance to the person who owned its registration by hearing his opinion.

8. (1) Registration of a registered pesticide is renewed for the period of three years on the application to the Government. Meanwhile, the applicant has to assure the guarantee that ingredients are same as that to date of its registration.

(2) The application for the renewal of a registration of shall be submitted under sub-section (1) of this ordinance.

9. Importation of a pesticide that is found adulterated, ineffective or contravenes any rule of ordinance may be prohibited into Pakistan by Government through notification in the official Gazette.

10. **(**1) Proper labelling of packages (pesticide containers) is required in the form of a printed material before it is presented for sale or stocked for such purpose. Advertisements should also follow this format.

(2) Black-listing of a dealer, wholesaler, retailer or an agent who has been convicted two offences under this ordinance shall be done for a specific pesticide.

11. A person can only store or use the pesticide as per requirements of this ordinance or its rules specified.

12. The Agriculture Pesticide Technical Advisory Committee.

(1) The committee should be devised on first priority basis after the commencement of this ordinance. The advice and guidance about the technical matters and hindrances arisen as a result of the administration of this ordinance should be provided to the Government by the Agriculture Pesticide Technical Advisory Committee.

(2) Government may appoint the officers (Government servants) as the chairman, vice chairman and members of the committee and persons indulged in the pesticide business could also be selected as its members.

(3) The names of the Chairman, the Vice-Chairman and the other members of the Committee shall be published in the official Gazette.

(4) The Government should select one of the members of this committee who is officer as the secretary of the committee for the period he owns the membership.

(5) The non-official members of the Committee shall hold office for a term of three years and shall be eligible for re-appointment.

(6) A member of this committee reserves the right to resign from the membership of the committee by writing his resignation to the chairman of the committee. But the seat of that member would be considered vacant only at the conditions that resign is accepted.

(7) An appointment made on a post due to death of or resign of member will remain effective only for residue period of that specified post.

(8) The functions of the Committee may be exercised notwithstanding any vacancy in the membership thereof.

(9) The procedures of working of committee are regulateable by the prior approval of the Government.

(10) The committee has power to appoint the sub-committee consists of specialists to perform the special tasks.

13. Pesticide Laboratory

(1) The Government should set up a pesticide laboratory at provincial level to fulfill the tasks assigned by this ordinance.

(2) Submission of the sample for analysis to the pesticide laboratory and functions of the laboratory may be as such prescribed.

(3) The information regarding the formula or other aspects of the pesticides should be duly safeguarded as may be prescribed.

14. Government Analyst. – The Government shall appoint one or more than one persons as the Government analysts of pesticides by a notification that may also describe the local limits to perform their duties.

15. Inspectors. – The Government notify the officers working related to plant protection as the inspectors in their local limits.

16. Powers exercisable by the Inspectors. – An Inspector may enter into the premises holding the pesticide in bulk or storage, could also took samples and no compensation will be paid for this purpose.

17. Procedure of Sampling. –

(1) An inspector taking sample under section 16 shall intimate in writing to the owner (Unless he is willfully absent), properly seal the sample, mark it and divide it into three portions. The owner should also be allowed to add his signature and stamp on the challan forms. Provided that the containers are of small size, chances of the deterioration of the pesticides by disturbing its container, three containers shall be marked with the same sign and sealed if necessary.

(2) Out of three portions of the pesticide samples drawn by an inspector, one portion should be given to the dealer or the person from whom sample of pesticide is drawn and remaining should be disposed of as follows:

(i) Second portion of the sample should be sent to the Govt Analyst for analysis.

(ii) Third portion of the sample should be sent to the Reference Laboratory (One at Provincial laboratory).

18. Report of Government Analyst. –

(1) After the analysis of the pesticide samples received from the inspector under subsection 2 of the section 17. The Government Analyst delivers his signed report to the inspector in triplicate form. (If the sample is fit the analysis report will be in duplicate form which include one copy for whom from where the sample is taken and second for the office record of inspector. While in case of unfit sample, analysis report is in triplicate form to deliver one copy to whom from where the sample is taken, second to the Director, Reference Laboratory and third to be submitted as evidence in police station to lodge FIR against the accused).

(2) The Inspector shall forward one copy to whom from where the sample was taken and other copy to the Director of reference laboratory.

(3) Anyone who wants to challenge the result of the Government Analyst shall put an application to the Government (Competent authority) with solid evidence that is assumed to be enough to contravene the correctness of results.

(4) If the filer of the applicant under sub-section (3) has evidence of strong nature and convincing, 2nd portion of the sample sent to the reference laboratory shall be analyzed to confirm the results.

(5) After the receipt of the sample in a pesticide laboratory (Reference laboratory), results of analysis are recorded and forwarded to the Government.

(6) The report of the results of the analysis shall be considered as the final evidence.

19. The Government Analyst or pesticide laboratory shall publish the results of the analysis and related information thereto.

20. Purchaser of Pesticide may have it tested or analyzed. –

(1) The purchaser of the pesticide could apply for analysis of the pesticide to the Government analyst or pesticide laboratory.

(2) Fees should also be paid along with the sample submitted for the analysis under sub-section 1 of section 20.

(3) The Government Analyst should issue a report to the applicant duly signed by him after the analysis of the sample received under sub-section-1.

21. Offences and Penalties.

(1) It is an offence to deal an adulterated or sub-standard pesticide in any way the for import, manufacture, formulate, sell, or advertises for sale.

(2) Offences made under sub-section (1) are punishable

(a) In the case of an adulterated pesticide, accused shall be punished for imprisonment not less than one year or more than two years and with fine that may extend to five hundred thousand rupees. In all consequent offenses, punishment of two years with a fine that may extend to one million rupees and punishment shall not be less than charged in first offence.

(b) For a substandard pesticide, accused shall be punished for imprisonment not less than six months or more than two years and with fine that may extend to five hundred thousand rupees. In all consequent offenses, punishment shall extend for three years with a fine not less than charged in previous conviction.

(c) 21A. All the offences under this ordinance for whom the punishment is not defined are punishable with a fine that may extend to the one hundred thousand rupees.

22. Manufacturer's Warranty to dealers. –

Warranty by the manufacturer to the purchaser about the fitness of a substandard or adulterated pesticide is same offence and deserves the same punishment as described under section 21.

23. Any person who–

(a) Unlawful use of registration number either not assigned by or assigned by this Ordinance

(b) Intentionally alters the mix something or alters the composition of the pesticides after it is offered in market by a formulator or distributor.

(c) Intentional interference in performing the duties of Inspectors is offence

(i) A person accused of the sub-section (a) or (b) shall be punished with imprisonment of two or more years along with fine that is extendable to one hundred thousand rupees.

(ii) A person accused of the sub-section (c) with imprisonment for a term which may extend to six months and with fine which may extend to one hundred thousand rupees.

24. Entry and seizure. –

(1) An Inspector may enter a place where any provision of this ordinance is believed to be violated and seize an article or pesticide that believes to commit an offence according to this ordinance.

(2) Material seized under sub-section (1) shall be handled following the decision of the court.

(3) An Inspector may take the assistance of the police party in pesticide sampling by applying to the magistrate who can direct an executive magistrate to accompany the inspector.

25. Court has power to order for penalty. – if a person is convicted under this ordinance, court may further order the person to forfeiture the thing, pesticide or article.

26. Cognizance of Offence etc.–

(1) Offences made under this ordinance shall not be punished in a court inferior to that of the first-class magistrate.

(2) The first-class magistrate can pass any sentence of this ordinance following section 32 of the Code of Criminal Procedure, 1898 (V of 1898).

26A. Cognizance and prosecution of offences. – Offences under this Ordinance are cognizance and non-bail able, only be registered on the complaint by the Inspector. Prosecution of offences under this Ordinance is the responsibility of Public Prosecutor.

27. Power to try offences summarily– Any Magistrate of the first class or any bench of Magistrates has power to try any offence summarily that is punishable under section 21 of this ordinance.

28. Indemnity. –Under this ordinance or rules any suit, complaint or prosecution shall lie against anything or person done or intended to be done in good faith (Inspector will not be punished by the court in case of weak/incomplete evidence for a case put by him against an accused).

29. Power to make rules. –

(1) The Government makes rules to make the provisions of this ordinance into effect.

(2) Rules provide guidance for all or any of the below mentioned matters and will not prejudice to any of the foregoing power.

- (a) Names of the animals and plants. (effects of pesticide to be registered on target and non- target organisms)
- (b) Form in which an application for the renewal or registration of pesticide should be made containing the necessary information and fee.
- (c) The procedure for the grant of certificate of registration or renewal of registration of a pesticide not having a trade name.
- (d) The language of tags/labels and character and location of printing.
- (e) Following are the functions of the Pesticides Laboratory including
  - (i) To make sure the secrecy of the formulae of a pesticide known to him.
  - (ii) Collection of pesticide samples for test or analysis; and
  - (iii) the form to write down the reports of analysis
- (f) Criteria of variability for a tested pesticide product keeping in view the label information and method to be followed for analysis.
- (g) Nature of Job and academic requirement for the Government Analyst;
- (h) Form that contains information like intimation to the whom from where sample is to be taken and other relevant information including batch no, quantity, manner of preservation and way of sending to the Government Analyst.
  - (i) Form used to file an application by the purchaser of a pesticide for the analysis of a pesticide that shall send to the Government Analyst. Complete Application along with the necessary fees should be sent.
- (j) Generally detrimental poisons (pesticides) which are deleterious to human health even when applied according to the recommended dose.
- (k) Labeled contains the words the poison and their antidotes should be mentioned
- (l) Storage requirements
- (m) The requirements and conditions for the premises where the pesticide is stored and quantity of the pesticide that a person can stock.

(n) Precautionary measures regarding pesticide poisoning of the agriculture workers.
    (i) Spraying pesticides in agriculture
    (ii) on health of agricultural land being sprayed with pesticides
(o) The restrictions, purpose, terms and conditions and circumstances in which the pesticides are being used.
(p) Prohibition of the use of a pesticide in agriculture due to some reasons.
(q) The availability of the materials for cleaning and washing of the body parts or clothes impregnated with the pesticides.
(r) The observation of the precaution measures to avoid the pesticide poisoning that is defined in the rules and abstentions form eating, circumstances and drinking.
(s) Limitations/interval between the consequent exposures of the workers to the pesticide poisoning.
(t) Prohibitions or restrictions on employment of workers to the person who is poisoned by the pesticide
(u) The measure about the exploration of the pesticide poisoning cases by investigation or detection
(v) The provision of the first aid in case of pesticide poisoning and also taking effective efforts to avoid such disturbances
(w) Impart training related to the use of the instruments provided.

30. Delegation of powers. – The Government may delegate power to any of its sub-ordinate officer on its own terms and conditions.

CHAPTER-10

10.0 INSECTICIDE DISPOSAL AND ENVIRONMENTAL SAFETY

The use of insecticide is unavoidable by farming community in the whole world for better crop production to satisfy the human food needs. The agricultural production consumes large amount of pesticides which are not free from many hazards such as development of resistance in insects, accumulation of insecticide residues in food, animal tissues and environment (water bodies, air and soil) (Aktar et al., 2009). Proper training regarding safe handling and use is pre-requisite for the people who work with pesticides. Therefore, safe use of insecticides is necessary at every step such as insecticide formulation, storage, distribution in market, application in field by farmers and disposal of empty bottles (Ogg et al., 2013).

10.1 Pesticide Safety Measures

10.1.1 Safety measures during insecticide formulation

Labeling and packaging of pesticides should be done according to recommendations of World Health Organization (WHO). All the information i.e. active and inert ingredients, instructions for safe use of pesticide and first-aid measures in cases of contamination or swallowing of pesticide must be given on the label in English and/or local language. Pesticides should always be kept in their original containers. Persons working in the pesticide formulation plant must wear the protective clothing and adopt safety measures at every step (WHO, 1990).

10.1.2 Safety measure during insecticide transportation

Transporting pesticides safely is a key responsibility of all workers who supervise, transport, use or apply pesticides in any agriculture related business. Certain safety measures should be taken during transport of pesticides to avoid accidents and any damage. We must also make sure to have the technical data sheets, proper labels and material safety data sheets (MSDS) or safety data sheets for the pesticides being transported in the vehicle. The pesticide label and MSDS contain important information about storage and handling. Transport vehicles should be in good mechanical condition. During transport, improper loading of pesticides may lead to falling and leakage of containers in vehicles and significant loses occur. Avoid transportation of pesticide with minerals, any food item for human consumption, grains, seeds and livestock feed. Pesticides should be transported in trucks or back of pickup and never in passenger vehicles. It should be ensured that vehicles must be in good operating condition. While loading pesticide on the vehicle, always use the protective clothing and equipment. Inspect all the pesticide containers for possible leakages before loading. See if the labels are firmly attached to the containers. Leaked containers must be rejected for loading. Load all containers in a manner that will minimize the risk of puncturing or tearing the containers. Protect containers made of paper, card-board, or similar materials from rain or moisture. After reaching the storage site, unload the pesticide containers with great care. Inspect the vehicle completely to determine the possibility of any pesticide leakage from containers (Ross & Bartok, 1995).

10.1.3 Safety measures at insecticide storage

Storage area for Pesticide products should be secured one and only trained people such as dealers, distributors and applicators should be given permission to enter there. They must not be placed with drink or food as they may be consumed by anyone mistakenly. Awareness on pesticide storage practices among homeowners is important to create. Pesticides should always be kept dry and must be protected from fire and direct sunlight. A vehicle carrying food should not be used to carry and transport pesticides. Humans especially children may get harm due to accidental exposure to pesticides in storage area therefore safe storage should be ensured. Safe storage prevents environment and property from any damage and also increases the shelf-life of chemicals. It is also important to display warning signs on windows and doors of the pesticide

storage area to keep everyone alert and cautious. The storage area should be well-ventilated and well insulated against the extremes in temperatures. The storage structure should be fire-resistant. Floor of storage should be non-permeable such as sealed concrete that will not allow fluids pass through it. Always consult the pesticide product label and MSDS for specific storage information. Storage facilities for fertilizers, pesticides and other similar products should be separate one although existing buildings are often used for pesticide storage. Pesticides should always be stored in their original containers along with label. The labels must contain information on ingredients, directions for use and first-aid measures for accidental poisoning. Pesticides should not be stored with/or near animal feed, human food and medicines. Containers should not be placed directly on the floor. In order to avoid caking of different formulations such as granules, dusts and wettable powders, these must be placed inside the cartons while concentrated formulations in the glass bottles must also be stored inside the cartons to prevent breakage. The height of storage shelves must not be more than 2m; this will prevent the use of ladders. At every pellet, the height of pesticide containers must not be greater than 107 cm. Stability of cartons and containers during stacking can be ensured by placing them at safe heights. The safe height depends on container material. There should be a proper space called aisle space between rows of pesticide stacks for the movement of free air (Dean & Bucklin, 1995).

10.1.4. Safety measures during pesticide application

As pesticides are toxic materials, therefore too much care and caution is needed regarding the indoor or outdoor use of these chemicals. Following safety measures should be adopted for safe application of pesticides (Momanyi, 2017):

- Prior to opening a pesticide container always read the label.
- All directions and precautions as well as requirements for protective equipment should be followed according to label.
- The crops recommended on pesticide label should be sprayed only.

Apply pesticides in the situations listed on the label.

- Dose of pesticide application must be used according to label recommendations.
- During insecticide application, it must be ensured that non-target organisms will remain protected and contamination due to runoff, residues or drift will not occur. The animals that eat the poisoned rodents may experience special hazard due to application of rodenticides.
- For the protection of field workers, *restricted entry intervals* are established after the application of some materials. After application workers are kept out of the field for the specific time and display the signs of safe re-entry date as required by regulations in treated areas.
- Certain chemicals under certain conditions may cause injury to crop (phytotoxicity). Therefore, always see the label for limitations.
- Before the application of pesticides, it is important to consider developmental stage of plant, the soil condition and type, moisture status, temperature and wind. Incompatible materials must not be used as it may result in injury.
- Abstain from smoking, eating and drinking during use of insecticides.
- Proper equipment should be used for calibration, transfer and mixing of insecticide.
- Avoid stirring or scooping insecticides while you are bare-handed.
- For effective removal of blockages in nozzle, prefer using pressure release valve or a soft probe.
- After re-filling the pump, rinse the hands and face with soap and water.
- Make sure to wash the hands and face before eating.
- At the end of the day, take a shower.
- Pesticide applicator must wear shirts with long sleeves or overalls and trousers and sturdy shoes or boots.
- Facial area must be completely covered with washable mask or disposable paper mask and eye protection or goggles be worn.
- It is important not to touch any area of the body with gloves while using pesticides.
- Equipment and protective clothing must be washed everyday but separately from other clothing.
- Replace the gloves with any sign of wear and tear.

- The discharge from the sprayer should be directed away from the body.

In case of any accidental contamination, wash the skin and consult doctor if problem is serious.

- Equipment must be monitored time to time for possible leakage and repair if necessary.
- Pesticide ingestion or extensive skin contact may lead to acute poisoning. First aid is the immediate solution for pesticide poisoning and medical advice and help must be sought at the earliest opportunity. Patient should be taken to the nearest hospital if possible.

10.1.5 Safe disposal of used pesticide bottles or containers and excess waste

It is the sole responsibility of all pesticide users to dispose of pesticide wastes properly. These wastes include pesticide containers and unused chemicals. Pesticide wastes that are not disposed in recommended ways can be a source of serious hazards for environment, the humans and animals (GFIAP, 1987; Ross & Bartok, 1995).

Pesticide wastes that are necessary to dispose of properly come under three types:

1. Empty pesticide containers
2. Unused pesticides that remain in the original container
3. Pesticide mixtures that are left unused after an application
4. Disposal of pesticides should be done according to the directions of label.
5. Statement related to "Storage and Disposal" should be read from pesticide label.
6. Left over pesticide in the container should be disposed of as household hazardous waste.
7. Rinsing of empty pesticide container is essential for recycling and disposal.
8. Triple-rinsing of empty pesticide container, bottles, drums and/or cans should be practiced after complete use of pesticide. Duration between each rinse should be 30 seconds.
9. Don’t forget to make holes in the pesticide containers/bottles to make sure that they cannot be reused for any other purpose.
10. To be recycled, containers must be free of any pesticide residue inside and outside.
11. Pesticide container must never be re-used for any purpose.
12. Children must not be allowed to play with empty containers.
13. Break the containers if possible, before disposal.
14. Paper containers must not be burnt.
15. Protective clothing (apron, gloves, and goggles) are also important to wear while rinsing pesticide containers.
16. Water for rinsing should also be applied according to instructions on the label.
17. Rinsing water should not be drained into any site not mentioned on the label, as it can contaminate environment.
18. Excessive insecticide suspensions should be disposed of either into a specifically dug hole. Hole for disposing off this suspension must be away from homes, streams and wells, by 100 meters minimum. The hole should be dug in a low-lying area if the area is hilly. This hole should also be utilized to bury the empty bottles, boxes and containers. The hole must be closed as soon as the suspension and containers are buried into it. If legally permitted, paper, cardboards or simple plastics should be burnt at a place that is away from residential area and drinking water. Pyrethroids suspensions can be disposed of by pouring them on dry ground because they are promptly absorbed by the ground, degraded and pose no environmental hazards.

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CHAPTER-11

11.0 SUITABILITY OF CHEMICAL CONTROL IN INSECT PEST MANAGEMENT

Insects are the largest and more diverse group of animals and found almost everywhere in the environment ranging from high tropical forests to pools, glaciers and below the soil surface. The number of insect species are greatest than all other known species of organisms. These are adapted to all environments (except marine) but the maximum numbers are found in the warm climate due to the suitable environmental conditions and high availability of food sources. These insects exhibit the phenomena of varying life span, high fertility, long diet breadth (from wood to blood) and efficient body constriction. Various developmental stages like larvae and adults of insect pests attack economically important plant parts resulting in the death or lower yield of the plant.

Control of insect pest and other arthropods is a complex phenomenon and involves a number of insect control tactics including chemical control. Now day's pest control emphasis on the most potential pest control tactics which consider both the ecological and economic consequences. The decisions based on the economic injury level and economic threshold level. Chemical control should become the most important component for the insect management. Despite of the high overall cost, use of insecticides is increasing day by day for intensive agriculture production and vector control. Use and commercialization of synthetic insecticides is due to their high efficacy, ease of use, low cost relative to their benefits and widespread adaptation.

Insecticides are being the most power tool for the insect management. These are adoptable to almost all situations, highly effective exhibited knock down effective and flexible in relation to various ecological and agronomic measures. Moreover, these are the economical, most reliable and dependable tool when pest populations exceeding form the economic threshold and emergency control is needed. There are many pest problems when chemical control is the only solution e.g. secondary pest problems and resurgence of primary insect pests.

11.1 Integrated Pest Management

The implementation and growth of the word IPM in the agricultural system is a new phenomenon with the expression of numerous concerns about the over reliance on the chemical control and its negative impact on agro-ecosystem. The term IPM is synthesized and published by Stern et al. (1959) which was previously used by a number of authors who were gaining the awareness about the negative role of insecticides. So, the new strategy was originated a formalized strategy to address the consequences of excessive insecticidal use and a plan to integrated the all the possible pest control methods and use of insecticides when needed.

The increasing population of Asia and increasing demands for food and for other agricultural products forced the farmers for the increased use of insecticides. Some famers are adapted to use the available control methods in combination. It involves the number of compatible pest management tactics to overcome the pest out break and potential damage to the environment.

11.1.1 Why to use IPM?

The question arises that why to practice IPM when chemical control often succeeded to control the insect pests. There are various reasons to broaden the pest management tactics instead of using the only one.

1. It helps to balance and sustain the ecosystem
2. Insecticides can be ineffective
3. It promotes a healthy environment
4. It can save money

11.1.2. Components of IPM

Planning is the key component of the IPM. Every crop has its own pest which should be considered accordingly. The following components help to set up a successful IPM plan.

11.1.2.1Monitoring and identify the pest problems

Knowing the field condition is the key component for taking the best management decisions. For this purpose, crops should be regularly monitored to identify the pest problem and their damage pattern, potential for the future damage, crop growth, field and environmental conditions. Scouting helps to take the early actions for the management of the problem and provide a chance to avoid the potential economic losses. Models for different pests have been developed to early forecast the need of the pest management tactics. Scouting enables to know and identify the problem and allow determining and mapping the problematic areas by using the right scouting method.

Identification is the most important for applying the better suited management tactics. Identification also involves the correlation of the damage symptoms or signs that either the observed injury is due to the identified pest or not. Lack of information and improper identification of the pests results in the wrong choice of control method or application time that lead to the pest control failure. Identification can be done by different types of reference materials like books, field guides and keys having pictures and other biological information about pests. Another way is to send the specimen to professionals.

11.1.2.2Selecting the best and compatible management tactics

Correct identification of a pest aids to select the suitable control methods that are effective, economically, environmental friendly and practical for the particular pest situations. The choice of the control method depends upon the understanding the life cycle and damaging stage of the pest, economic levels of the infestation and comparisons of the cost/benefit ratio of the control methods applied. Various economic concepts are useful in determining the point at which control measures should be triggered.

11.1.2.3Record keeping and evaluation

It is very essential to evaluate the results of the control methods regularly. Keeping records is easier with different booklets, factsheets and software programs. This will help to determine the impact of management strategies on environment, their success to IPM before implementing them again.

11.2 Goals of IPM

A number of pest control methods that are effective in controlling pest are adopted for achieving the specific goal. Prevention, suppression and eradication are the approaches to maintain the pest status below the economic threshold.

11.2.1 Prevention

This strategy prevents the infestation of pest to the crops. A number of pest management tactics such as resistant varieties, sanitation, cultural control, sowing and planting times are useful for reducing the pest problem.

11.2.2 Suppression

These are used to control the pest population levels below the economic threshold. These methods not completely eliminate the pest infestation but reduce the infestation to tolerable levels. Additional suppressive measures may also require to if the first treatment does not achievement the goal.

11.2.3 Eradication

Eradication aimed at the total elimination of the pest population form the designated area. It is quite expensive for the large area and success is limited. Eradication programs are adopted over wide areas posing economic or health threats of introduced or exotic pests.

11.3 Tools of IPM

11.3.1 Natural control

These are the factors that destroy or check the pest population continuously irrespective of the human activities. It involves the climatic factors like temperature, rain, wind, sunshine etc., topographical features like lakes, mountains, rivers etc. and naturally occurring beneficial insects for the control of insect pests like predators, parasitoids and pathogens. Other control measures

should be applied in the field when natural control is not operative or unable to check the pest population.
Consideration of natural measures should be the part of IPM programs.

11.3.2 Biological control

Biological control includes the use of 3P (predator, parasitoids and pathogens) for the control of insect pest population. It provides partial solution to insect pest problem and integrated with other insect control methods. Use of natural enemies provides long term control for many of the key pests.

Predator: an organism which feed upon on another organism having body size or equal to the predator e.g. ladybird beetle, green lacewing.
Parasitoid: an organism which feed internally or externally upon the body of another insects e.g. egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), larval parasitoid (Bracon spp.), pupal parasitoid, adult parasitoid *Epiricania melanoleuca* (Fletcher) (Lepidoptera: Epipyropidae).
Pathogen: organisms that develop diseases in insects to complete their development and kill the insects e.g. bacteria (*Bacillus thuringiensis* Berliner), fungi *Beauveria bassiana* (Bals.) Vuill. (Hypocreales Clavicipitaceae), virus (nuclear polyhedrosis virus), protozoa *Nosema bombysis* Nägeli (Dissociodihaplophasida: Nosematidae) and nematodes.
Biological control if often used for the insect pests that is not native to the specific geographical area. Newly introduced pests always cause problem because they lack natural enemies. It also includes the mass rearing and release of natural enemies into fields, greenhouse and orchards to control the pest population but in many cases their failure is also reported.

Conservation practices

- Provide nectar source and flowering plants
- Provide shelter in landscape
- Protection of habitat
- Reduce the use of insecticides

11.3.3 Cultural control

These control methods make the environment and conditions less conducive for the pest feeding, reproduction and survival or sometimes more favorable for the natural enemies of the pests. This technique includes the cultivation, crop rotation, sanitation, selection of varieties, time of planting and harvesting, irrigation and fertilizer management, use of trap crops etc. Cultivation reduces the pest population by starvation, desiccation, injury and exposure. Sanitation involves the clean cultivation and removal of breeding and overwintering resources. Crop rotation involves the sowing of non-host crop for the particular insect pest.

11.3.4 Mechanical and physical control

This control method uses the physical techniques such as use of machines, devices or other manual techniques to control the insect pest species or to alter their environment. These methods are used to exclude or to trap the particular pest specie. Some of the examples include the use of cultivation devices like disks, mowers and cultivators for the destruction of soil inhabiting stages/pests. Some barriers like screens and patching or cracks or crevices is also another phenomenon to prevent the entry of flying insect pest species into an area like mosquitoes, aphid, flies etc. use of sticky materials or wires around the tree trunk is also a physical control method (e.g. mealybug). Manual picking of insect pest larvae is also practiced under physical control methods.

11.3.5 Genetic control

Sometimes plants and insects are genetically modified to reduce the pest problem in an area. Many host plants are naturally resistant form the attack of insect pests. Researchers also take advantage of this character by selecting or hybridizing the more resistant plants. Control of European corn borer, wheat stem saw fly and alfalfa aphid is well reported. Sometimes the genetic material from a source is transferred to a certain plant species to make it repellent or toxic e.g. Bt. containing varieties.

11.3.6 Chemical control

Chemical control is an important part of IPM despite of the availability of the other effective control methods. The reason may be the other control methods are quite expensive or not very effective for particular conditions and all these control methods cannot assess by every farmer. In fact, it is not possible to obtain the potential yield without prescribed use of chemicals. However, use of the chemicals with particular characteristics is recommended; selective toxicity to the target pest, high efficacy at low application rates, less persistency and good biodegradability, low toxicity to non-target organisms, less mobility to the ground and safe for the environment. Furthermore, treatments should be made at proper timing (when and where they needed) following the label directions.

11.3.6.1Particular of effective chemical control

Insecticides are one of the most important tools in the IPM programs. This control method can be integrated with other control methods but sometimes it cannot provide the control as expected. So, the following points should be kept in mind to attain an effective treatment.

11.3.6.2Pest identification

Wrong identification of a pest can fail the insecticide treatment. Correct identification of a pest requires both experience and practice. For example, knowing the difference between a sawfly larvae and caterpillar is necessary for the success of Bt. insecticides because it is recommended for the caterpillars but not for sawfly. Moreover, misidentification may fail the non-chemical tactics if susceptible cannot be identified.

11.3.6.3Dosage

Make sure to apply the correct insecticide at proper dosage according to the label directions and pest status.

11.3.6.4 Correct use

Always apply the insecticides for the pests for which they are recommended. For example, some insecticides are only recommended for the particular sucking or chewing insects and not for others.
Always follow the label directions for this purpose.

11.3.6.5 Application timing

Improper application time sometimes leads to the failure of the pest control. The pest may not be in the area to be treated or it may not in its susceptible stage. Insects are mostly vulnerable to insecticides when they are immature. Already present insects are also a source of infestation that developed to pest status long after the chemical application.

11.3.6.6 Application equipment

Knowing the right habitat is essential for an effective treatment. Sometimes concealed pests are difficult to detect. For this using, the best equipment is very necessary. For example, air blast sprayer should be used for the insects hiding under tree leaves but a granular applicator should be used for subterranean insects.

11.3.6.7 Environmental conditions

Application of an insecticide must consider the environmental conditions. Mostly treatments are not made just before a rainstorm, at temperature extremes and in windy conditions. All these conditions may wash off the plants or move away the insecticide from the treated area.

11.3.6.8 Insecticide degradation

In storage conditions insecticides may degrade or change into an ineffective form. This may also due to the age of the product. For example, most of the solid formulation can become ineffective under high moisture conditions due to formation of clumps.

11.3.6.9 Insecticide resistance

Knowing the resistance level of the insects is very necessary for reducing the pest population below the injurious levels. Resistant insects initially require frequent applications and higher application rates of specified insecticides. This will sometimes result in the complete failure

of the control methods or development of resistance in other insects. So, choosing the better suited form and mode of action is very important for successful control.

11.3.6.10 Insecticides should be the part of IPM programs in the following ways

1. Consider the socioeconomic effects and use of selective insecticides
2. Insecticides should be used to avoid the predictable damage losses using most effective techniques at optimum dosage and appropriate time.
3. Insecticides should be integrated to other control methods and applied when other control methods fail to check the pest population.

11.3.7 Other techniques supporting the use of insecticides in IPM

11.3.7.1 Precision and application

New application equipment and methods increased the efficacy and reduces the potential for environmental contamination. These equipment releases precise application rates regardless of the speed and environmental conditions. Conventional equipment changes their release rate as the speed decreases thus more quantity is being applied than needed. Modern equipment also reduces the extra amount deposited at various areas (end row) by the shut off valve. Such precise application rates enhance the effective use of insecticides in IPM.

11.3.7.2 Remote sensing

It is a satellite or aircraft based system that capture high resolution images to assess the crop health and to take future decisions. This system has the potential to improve the precisions of the damage assessment methods and to transfer the information to the farmers and extension workers in a useable manner within short period of time. These images combined with geographic information system exhibiting many benefits in IPM programs.

11.3.7.3 Controlled released formulations

Newer insecticide formulation also supports the use of insecticides in the IPM programs. Use of controlled released formulation has many benefits. The controlled release rate increased the stability of active ingredients, exhibited prolonged residual activity and improved penetration power. Moreover, lower application rates reduced the losses of insecticides to the environment. However, this area is under progress and need more attentions but it would be a preferred option for an IPM developer.

11.3.8 Changing role of chemical control in IPM

Chemical control is now considered as a part of solutions for pest control but not the only solution for it. However, it becomes the only vital part when all other methods fail to control the pest population. According to a study complete elimination of chemicals from the IPM would not allow the presence levels of productivity of many crops. Changing approach for the chemical control is rather than excluding chemicals, it is better to increase the beneficial use by lowering the risk to environment and non-target organisms. The chemicals should be used to enhance the efficiency of other control methods whenever they are required.

Other advances include the development newer and selective mode of actions with increased efficacy at lower rates. High biodegradable and non-persistence chemicals along with lower application rates also improve the environmental situations. Alternate use of newer mode of actions now becomes a source to break the evolution of resistance. Another important point is the consideration of realistic threshold.

The main goal of the insecticide research is to discover and develop new products and application methods for safer and effective pest management in order to maximize the crop production and to reduce the public health concerns. Although, insecticide research has made significant victories but insects always remain a competitor for food and shelter.

11.3.9 Insecticides and resistant cultivars

Some rice varieties are resistant to some rice insect pests while other insects may require compensatory insecticidal treatments. For example, Philippines IR26 is resistant to brown plant hopper (BPH) but insecticides protect the rice varieties against whorl maggot. Moderately resistant varieties usually support small and less vigorously with low fecundity and may withstand more insecticidal treatments. For example, stem borer infestations received one or two treatments of insecticides are similar to the double received on susceptible varieties. Even the use of moderately

resistant varieties along with insecticidal treatments can be an important strategy for the management of many insect pest species.

11.3.10 Insecticides and biological control

Biological control involves the use of biological organisms like predators, parasitoids and parasites to lowers the pest status. The success of biological agents is not well reported and many failures have been reported. However, the integration of chemical control along with biological control agents revolutionized the thoughts of pest management. For example, the use of *T. chilonis* and green lacewing *Chrysoperla carnea* (Stephens) for the control of various sucking insect pests of cotton and bollworm proved more economical than the insecticides alone. This integration also reduced the application of chemicals from eight to two only. Similarly, use of pink bollworm ropes in combination with *T. chilonis* proved quite effective to check the bollworm population. In another study use of insecticides like chlorpyriphos, amitraz and chlorfamaniphos along with *C. carnea* exhibited significant results for the reduction of whitefly. Similar integration was also devised and reported by many authors. Integration of biological and chemical control is possible in the following conditions

- Suitable low density natural enemy is available
- Selective insecticides available which control the insect pests but not lowers the density dependent population of natural enemies
- Presence of maximum insect pest density which do not produce economic damage is not lower than the population needed to retain the population of natural enemies
- Relative stability of the ecosystem that favors the relationship of host and natural enemy.

11.3.11 Insecticides and cultural methods

Integration of cultural and chemical control is an important for the management of various pests. It has been used successful for the control of white stem borer in Java and yellow stem borer in Japan in rice. Management of cultural practices like planting distance, use of fertilizers and drainage of water proved quite effective for the control of rice insect pests like close planting not only increase the hopper population but also prevents the penetration of foliar sprays to lower canopy. Similarly, high use of nitrogenous fertilizer also increases the pest population.

Successful complementation of cultural and chemical control in rice ecosystem can play significant role in IPM. For example, application of insecticides enhanced the effect of water drainage on brown plant hopper population. For the control of other insects spraying of susceptible trap crop has also proved beneficial. These cases indicated that cultural practices that increase in pest population could be integrated with chemical control. In such circumstances, cultural practices could be used for the control of key pest while insecticides could be applied to control the economically less important insect pests.