Chapter-I

Introduction

INTRODUCTION

A) Nicotinic acid derivatives

Nicotine an ingredient of the plant Nicotiana tabacum displays an antagonistic efficacy when applied to vertebrate and insect cholinergic neurons. Nicotine is one of the oldest known insecticides, which possesses remarkable insecticidal activity. Nicotine kills insects rapidly within an hour causing intensive tremors, convulsions and then paralysis. As early as 1746, the insecticidal activity of crude extracts of tobacco leaves was used to control the insects. Metcalf reported that 1.2 million pound of free nicotine was used in agriculture in the USA in the year 1944. The formerly used extracts of tobacco leaves have been replaced by technical nicotine with 95-98 % or by nicotine sulphate with 40% alkaloids. These are not persistent, non-systemic, contact insecticides with some ovicidal properties.

Nicotine has a fairly high mammalian toxicity and is toxic to man by inhalation and dermal contact (LD 50 for rat: 16mg/kg and acute, oral: 50,60 mg/kg). This is one of the reasons why nicotine has a narrow insecticidal spectrum of activity against aphids, thrips and certain species of mites. Very short life span and low residual activity under the field conditions. So the nicotine cannot compete with modern synthetic insecticides.

Nicotine represents the class of "nicotinoids" with a unique mechanism of action. Nicotine mimics Ach and some of the AchR types. A direct interaction with the AchR has been demonstrated and reported earlier.

Some work has been done to co-relate the chemical structure with biological activity of nicotine and related compounds in mammals. However much less attention has been devoted to their effects on insects. It has taken in to consideration, that the concentration required to kill houseflies is a lower than the concentration required for the inhibition of AchE. So the mechanism of action of nicotine and its derivatives is not primarily connected with antiacetylcholine esterase activity. Detailed requirements by (Yamamoto et.al, 1995) lead to understand the structure activity correlationship.

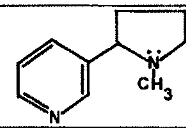
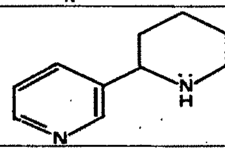
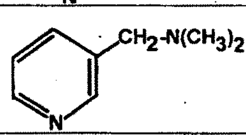
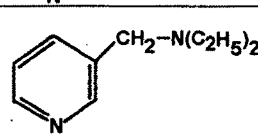
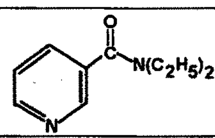
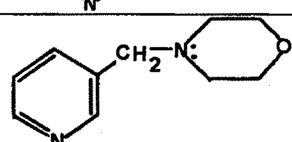
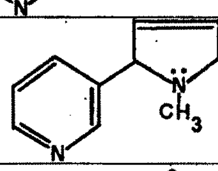
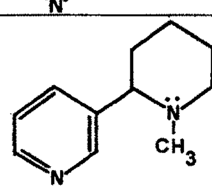
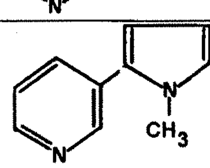
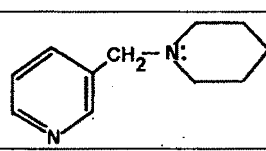
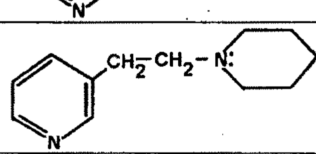
The distance between the two nitrogen's may be about 4.2 Å. So that the essential part of the molecule responsible for high the AchR affinity and high insecticide potency is far lower than the concentration required for inhibition of acetylcholine esterase.

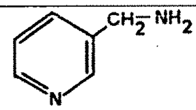
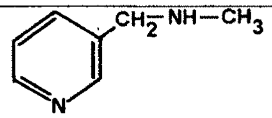
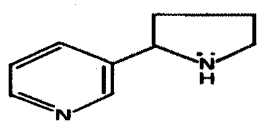
Altering the position of the side chain at the pyridine ring and the chain length between the 3–pyridyl and basic nitrogen had significant effect on toxicity. The secondary and tertiary amines were found to have insecticidal potency, where as the primary amino derivatives have not.

Because of the high basicity of the second nitrogen in comparison with the pyridine nitrogen, the pyrrolidine or related nitrogen becomes protonated in a physiological medium at pH 7, e.g. inside the insect body. In this way the molecule acquires the active form, which is able to compete with Ach on the receptor protein. From this point of view, nicotine or structurally related compound are only pre-insecticides, which enable the compound to penetrate easily into the body and the nervous tissue to the site of action. The protonoation converts this pre-insecticide to the active form with the quaternary nitrogen, which is essential for the binding of the molecule to the anionic site of the receptor.

Several natural analogs of nicotine are found in tobacco or other plants, which have similar physiological effects in mammals. These include nor-nicotine and anabsin[2], which are toxic to insect's.

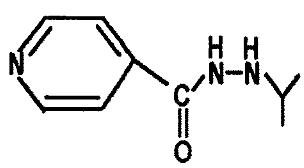
Table-1: Relative toxicity of natural occurring nicotine analogues(Ia-n).

Sr. No I	Structure of compound	Name of compound	Relative toxicity
Ia		Nicotine	1.00
Ib		Anabasine	0.75
Ic		N-(3-pyridyl methyl)dimethyl amine	0.46
Id		N-(3-pyridyl methyl)dimethyl amine	0.085
Ie		Coramine. N-(3-Nicotinoyl) diethyl amine	-
If		N-(3-Pyridyl ethyl) Morpholine.	0.038
Ig		1,2-dihydro nicotyrine	1.16
Ih		N-methyl Anabasin	1.00
Ii		Nicotyrine	0.18
Ij		3-(N-Piperidyl methyl) pyridine	0.79
Ik		3-(N-Piperidyl ethyl) pyridine	0.14

Il		3-Amino methyl pyridine	0.029
Im		3-Methyl amino methyl pyridine	0.300
In		Nor nicotine	-

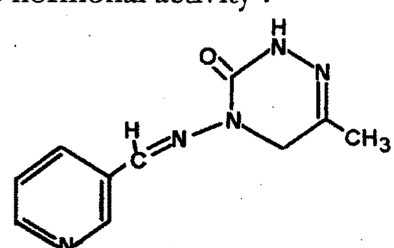
Yamamoto et.al, 1995 (2) and Yamamoto reported the detail investigations on the structure activity relationships of 26 synthetic nicotine analogues and to discover these compounds resembling acetylcholine in configuration and charge distribution toxic to several insect species[3].

Iproniazid, which has a week juvenile hormonal activity[4].



(I)

Pymetrozine



(II)

Iproniazide

Pymetrozine, a pyridine azomethrine, is an insecticide of novel structure and mode of action. It is effective on aphids, whitefly and hoppers. It is systemic and has long residual activity injurious to beneficial orthropods. It is unique in affecting nervous control of the salivary gland on which, it shows abrupt action and irreversible blocking of feeding, resulting in slow mortality due to starvation. It is considered safe to natural enemies and the environment (Kristinsson, 1994) [5].

Therefore, Eldetrawi (1985) thought of synthetic nicotinoids as effective future insecticides. Based on these lead around 1984 chemists at Nihon Bayer, Japan synthesized the nitroguanidine, which was later called imidacloprid. It's insecticidal activity was found (Yamamoto et.al, 1995) 10,000 fold higher than that of natural insecticide nicotine. (1):

Thus, the effectiveness of nitromethylenes depends on the configuration of the pyridyl moiety. However, this active principle was found to be naturally occurring in a compound named epibatidine found in the skin of poisonous frog (Spando et.al, 1992). Though, imidacloprid was developed following the lead of nitromethylene compounds, nicotine and epibatidine could also have served as the leading structures for these new insecticides (Leicht, 1996). Yamamoto et al, (1995) named this new group as neonicotinoids.

Further, the currently available insecticides acting on nicotinic acetylcholine receptors have no structural similarities with neonicotinoids. Hence, neonicotinoids constitute the compounds with a new mode of action (Leicht, 1996). These are acute, contact and stomach poisons with translaminar activity and systemic properties. At a lower concentration these compounds act as anti-feedant, a property they share with nicotine.

Lower efficacy of imidacloprid against lepidoptera pests opened the way for other neonicotinoids that can be utilized to complement each other. Half a dozen of neonicotinoids are available in the market however, the imidacloprid is an excellent insecticide against Homoptera, Coleoptera, Thysanoptera, Isoptera, Dipteras and Lepidoptera pests on rice, cotton, vegetables, fruit trees, sugarcane and sorghum.

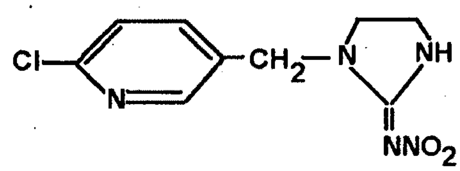
Nitenpyram is less persistent and effective against Homoptera and Thysanoptera on rice, vegetables, fruit trees and tea. Acetamiprid is a broad-spectrum insecticide effective against aphids, Coleoptera, Thysanoptera and Lepidoptera on vegetables, fruit trees and tea. Thiamethoxam is a effective against Homoptera and Thysanoptera on cotton, vegetables and fruit trees.

Thiacloprid, a new neonicotinoids is effective against Homoptera, Lepidoptera and Coleoptera on rice, vegetables, fruit trees, tea and stored products and safe to pollinators (Yagach and Sato, 2003). Further, imidacloprid is also found to be effective for the control of cockroaches and veternary pests[6].

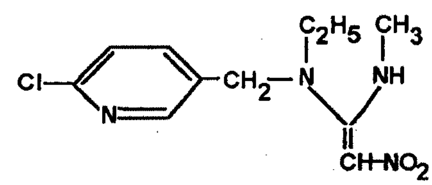
Imidacloprid: 1-(6-Chloro-3-pyridyl methyl)-N-nitro-imidazolidine-2-ylideneamine.

Acetamiprid: (E)-N[1]-(6-Chloro-3-pyridyl methyl)-N[2]-cyano-N[1]-methyl acetamidine.

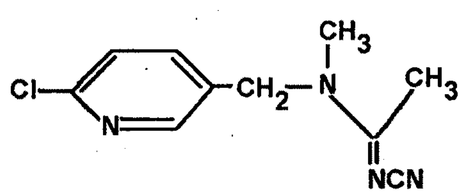
Nitenpyram: (E)-N-(6-Chloro-3-pyridyl methyl)-N-ethyl-N[1]-methyl-2-nitro-vinylidene-diamine[5].



Imidacloprid



Nitenepyram

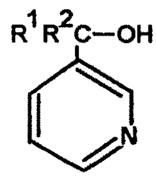


Acetamiprid

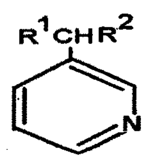
Fungicidal Property:

Structure activity relationships of a new group of pyridine alkanes and carbinols (I), several of which show an excellent systemic fungicidal activity, reported recently (Brown et.al, 1967). The pathogens which have been controlled in green house tests, include *Erysiphe polygoni* and causing dollar spot, brown patch and helminthosporium leaf spot in turf grasses. Maximum activity was shown when the pyridine ring substituted in the 3-position and similar substitution to the position 2 or 4 led to reduced activity against *E. polygoni*. The substituted methanol's and ethanol's (I) were more active than the alkanes (II) and this activity was not altered by the substitution of amino, chloro, cyano or methoxy groups on the β-carbon atom, although such changes did cause a reduction in the control of the turf pathogens. Excellent control of *E. polygoni* was also obtained with analogues having two aryl substituents on the α-carbon atom and this activity was further increased by the order being; diphenyl<4-chlorodiphenyl<bis (2,3-or 4-chlorophenyl). The activity of this type of compound against turf

pathogens in turf grasses could be increased by replacement of aryl by cycloalkyl groups, and maximum activity was observed when one or two cyclohexyl groups were present.



(I)



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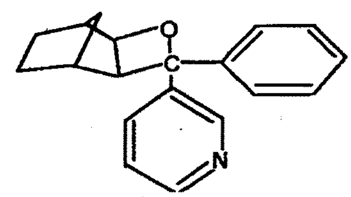
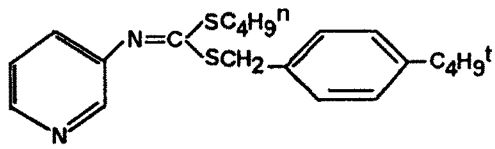


(III)

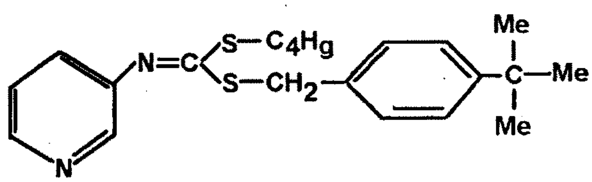
Bis-(p-Chlorophenyl)-3-pyridine methanol (I:R^1=R^2=P-chlorophenyl) or parinol was in fact introduced as a new fungicide for the control of powdery mildews, foliar application at 10 ppm giving> 95 percent control at *Erysiphe cichoracearum* on squash, canteloupe and cucumber (Thayer et.al., 1967) and >90 percent control of *Podosphaera leucofricha* on apple, *S. pannosa* on roses and *Uncinula necator* on vines was obtained using 25 ppm. Arinol is of the considerable interest because it exhibits translaminar activity and a valuable attribute for the control of pathogens such as powdery mildew when treated with conventional protectant sprays which tend to proliferate unhindered on the under surfaces of leaves.

With the related experimental fungicide 4-phenyl-4-(3-pyridyl)-3-oxatricyclo[4.2.1.0,2,5] nonane (III), no systemic movement of the chemical was observed following soil, stem or leaf applications, but fumigant activity from 100 mg topically applied completely eradicated powdery mildew from the leaf surface of bean plants (Spurr and Chancey 1970). This is not the first case of fungicide redistribution in the vapour phase, as these authors seem to claim, but a rediscovery of the phenomenon reported first by Yarwood (1950) and more recently by Bent (1967), Hislop (1967) and by Clifford et.al, (1970).

The fungicide S-1358 from sumimoto chemical Co-Ltd, which is a derivative of 3-aminopyridine, has also been shown to interfere with sterol biosynthesis, in this case in *Monilinia fructigena* (Kato et.al, 1974 ab). It is particularly active against powdery mildews.



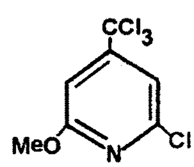
S-1358 a new fungicide active against powdery mildews; of a series of fungi studied in vitro, the Pycomycetes , Basidiomycetes and a variety of Ascomycetes and fungi Imperfecti were, however, insensitive (Kato et.al, 1975 b)



S-1358

Pyroxychlor this experimental fungicide is very active mainly against Phytophthora diseases (Hoitink and Schmittbrenner, 1975).

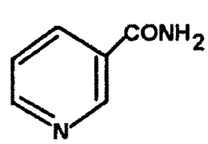
In vitro, it inhibits *Phytophthera parasitica* and *Pithium ultimum* at about 10 ppm, but the compounds appear to be for more effective when applied to plants or to soil than invitro. It is there fore suggested that a metabolite might be the actual active fungicide (Noveroske, 1975 a,b).and no further information available so for[7].



Pyroxychlor

Pharmacological Property:

The antituberculous activity of nicotinamide (1950), was first reported.



The nicotinic acid was inactive and the tuberculostatic effect was not related to vitamin activity. Mckenzie, et.al, also observed the effect of nicotinamide on the experimental tuberculosis independently and an extensive series of structural modifications were as made.

B] Status of the major sucking and nonsucking insect pest:

i) *Myzus persicae.* (Tobacco aphid)

Tobacco, an important commercial crop of India is damaged by a number of insect pests. The green peach aphid, *Myzus persicae* sulzer is known to be an important pest causing significant losses on tobacco directly by their feeding and honeydew deposition (Feinstein and Hannan, 1951). It occurs in all tobacco growing states across the country with incidence level as high as 65 percent (Patel and Patel, 1985). It has wide distribution, infests plants belonging to more than 30 families and transmits about 200 viral diseases (Jayaraj, 1985).

It was reported that aphids on tobacco could be effectively controlled by spraying of methyl demeton (0.025%), dimethoate (0.02%) and phosphomidon (0.02%) (Joshi and Rao, 1971). Recent findings indicate that many of the recommended insecticides were becoming ineffective against this pest in tobacco growing areas of Karnataka (Patil et al, 1999)[9].

The arriving alaram of most aphid species select plant in a particular stage of growth and leave of an age characteristic for the species. They avoid mature tissue, which is least active physiologically in terms of growth and sencence [Kennedy 1985]. On crucifers and potatoes for example, *M. persicae* mainly colonies ageing and senescing leaves. *M. persicae* has been controlled by pinching out the top leaves three times a year. The leaf age selection of a phids has been interpreted as a response to high levels of organic soluble nitrogen in the phloem associated with plant growth and senescence though secondary substances may also be involved, and there is evidence that some selection for young and aging leaves occurs as an optomotor response by the aphids to yellow before alightment [Kennedy and Stroyan (1959)].

It has long been proposed (Busgen, M. 1954) that the high rate of food ingestion by aphids and the production of honeydews are connected with a food source rich in carbohydrates but relatively poor in nitrogenous compounds and vitamins. It is interesting that *M. Persicae* can synthesize a number of fatty acids from artificial diets (F.E.Strong, 1963) and that the honeydew of the same species feeding on young plants contains free fatty acids and free sterols (F.E. strong, 1965). Damaged leaves may surprisingly have higher moisture content and root reduction is perhaps be the main cause of the early wilting of plants attacked by aphids (A.L. Carter) through the drain of liquid imposed by the aphids (Kloft, W. 1954).

The production of honeydew by aphids is a further source of damage to plant *M. persicae* and noticeable honeydew deposits are usually only found in the glasshouse though, they have been reported out of doors on tobacco (Dominick, 1949). As honeydew evaporates get concentrated to a sugar concentration which plasmolyses the leaf or many form a medium for the growth of sooty mould [(Dominick, (1949) Ponnuswami (1964)]. Infested plant showed a decrease in photosynthesis and an increase in respiration which would further lead to depletion of carbohydrate in plant associated with an increase in phosphatase activity[10].

ii) ***Helicoverpa armigera*:**

H.armigera (Noctuidae; Lepidoptera) is a thread for successful cultivation of several economically important crops. This notorious pest has a very high migration capacity coupled with enormous fecundity. Being polyphagous in nature *H.armigera* has a wide host range, which includes 181 cultivated as well as wild host plants (Manjunath et.al, 1985)[11]. These include cereals, vegetables, pulses, oilseeds, millets, plantation crop, ornamental crops and several other weeds[12]. It was a pest of minor importance 50 years ago, which has now, come in a greater prominence with chemical agricultural practices and now it has acquired status of national agricultural pest. The status of national agricultural pest all over the globe indicates that it is largely a man made problem. Amongst several reasons that gave a big name to this pest "the big boss"[13].

Helicoverpa armigera (Hubner) is a major pest of cotton (*Gossypium sp.*), chickpea (*Cicer arietinum*) and pigeonpea (*Cajanus cajan*),[14] and tomato (*Lycopersicon esculentum.* mill).

American bollworm, *H.armigera* is the most dreaded pest of cotton that causes yield losses up to 60 percent (Mukherjee, 1982). The pest is highly mobile with high fecundity (in mouth stage), polyphagous, voracious in their feeding habits coupled with innate ability to develop resistance to different groups of insecticides. The ravages of cotton bollworm are known to cause total cotton crop failures in various regions, specifically in parts of Andhra pradesh and Maharastra were farmers are victims of pest menace resulting in socio-economic calamities like suicides[15]. For instance more than 55 percent of total insecticides used in the country are for cotton pest management with around 75 percent being targeted against *H.armigera*, ironically the crop occupies just about 5 percent of total cultivable area in India (Puri, 1995) [11]. The defection of resistance to synthetic pyrethroids in *H. armigera*, recorded in 1987-88, cotton season in Andhra pradesh brought in to focus, for the first time the problem of insecticide resistance in agriculture in India [14].

Chickpea (*Cicer areitinum* L.) is a major commercially cultivated pulse crop of Rajasthan, particularly in Sriganganagar area of Rajasthan however, successful cultivation of the crop is often hampered due to gram pod borer (*Helicoverpa armigera* Hub.) causing huge economic losses[16]. *H.armigera* is a primary and key pest of chickpea (Shengel & Vjagir, 1990) during rabi and summer season. This host crop is seriously affected due to the pod boring nature of damage due to the fruit boring nature of damage the depletes the crop yield up to 38-47% (Sachan and Katti, 1994)[13] .In chickpea alone, the pest caused pod damage up to 90% (Shengal and Vjagir, 1990). Powar (1998) reported the annual crop losses due to Helicoverpa in India are about Rs. 2000 crores in spite of use of insecticide chemicals worth about Rs 500 crores and the annual losses of pigeonpea and chickpea were estimated to exceed US $600 million[12].

Pigeonpea (*Cajanus cajan* L.) is the second most important pulse crop of India after chickpea. It is grown mainly in the states of Uttarpradesh, Madhya pradesh, Maharashtra, Karnataka, Bihar, Gujarat, Tamilnadue and Andhra Pradesh. The losses due to insect pests are much higher in the case of pulses than those in the cereal grains. Pod borer, *Helicoverpa armigera* (Hb) Noctuidae (Lepidoptera) is one of the major constrain to pigeonpea production (Shanower et.al, 1999)[17]. Gulbarga district of Karnataka is known as dhal bowl of South India and has highest area under pigeonpea in the whole country, several species of pod borers are attacking. Among these species, the pod borer *H.armigera* is a major impediment in enhancing the production and productivity of pigeonpea. In the last decade three out breaks of this pest were noticed and the latest being 1997. On an average the pod borer incidence caused 90 to 100 percent yield loss in 1992-93 and 1997-98 in Gulbarga district[16].

Tomato (*Lycopersicon esculentum.* mill) is an important vegetable crop grown around the world occupying the daily food regime of a majority of people[15]. Among the other pests *Helicoverpa armigera* (Hubner) has been reported to cause maximum economic damage to the Tomato crop in Udaipur region (Srinivasan, 1959 and Vevai, 1971)[13]. They cause direct marketable loss upto 60 percent (Tewari and Krishnamoorthy, 1984; Lal and Lal, 1996). For managing the pests the farmers rely mostly on the chemical pesticides because of quick knock down effect. As fruits are harvested frequently and used for consumption immediately, the dependence on chemical pesticides for managing the pests will be the result in several problems[17].

iii] Maize weevil:

A complex of weevils, the rice weevil (*Sitophilus oryza*), granary (*Sitophilus grenarius*), and maize weevil (*Sitophilus zeamais*). Weevils are among the most destructive pest of grains, seeds and grain products. Stored in elevators and bins. They probably are not native to North America, but entered in the seeds carried by settler's thought Ports. These weevils are pests of grain throughout the world.

The maize weevil is small snout beetle, which varies in size, averaging about three thirty seconds inch in length. It varies from dull red brown to nearly black and marked on the back with four light reddish or yellowish spots. The maize weevil has fully developed wings beneath its wing covers and can fly readily. The thorax is densely pitted with some what irregularly shaped punctures, except for a smooth narrow strip extending down the middle of the dorsal (top) side. An egg hatches in a few days into a soft white, legless, fleshy grub, which feeds on the interior of the grain kernel. The grub changes to a naked white pupa and later emerges as an adult beetle. The rate of the development is slightly slower for the Maize weevil than for the rice weevil. A minimum of thirty days is required for passing through the egg larval and pupal stages.

Life history of maize weevil for a long time was referred to as a large strain or race of the rice weevil, the degree of variation within each species. The maize weevil is slightly larger strain or race of the rice weevil, the degree of variation within each species make them difficult to tell apart. The thorax of maize weevil is densely and uniformly pitted with round punctures. An egg hatches in a few days in to a soft, white, legless, fleshy grub[18].

The protection of grain from destruction by insects has for many years depended largely on the chemical treatments to eradicate or repel the pests. However, the non-directional and indiscriminate use has resulted into several problems like increasing insecticide resistance in insects and pesticide residues in foodstuffs. Although the control of stored grain pests have been dominated by chemical insecticides are not found entirely satisfactory because of development of the resistance in insects, persistence in toxicity and residual toxicity to consumers. Therefore, there is need for research of an alternate and suitable insecticide with low mammalian toxicity for use in an efficient way[19].

C] Bio-assay:

Biological assays are methods for the estimation of the nature, constitution, or potency of a material by means of the relation that follows its application to living matter[20]. Bioassay is made up of two words vit, Bioas-life, and assay-determination. In a simple word bioassay is the determination of response of the chemical on living organisms. Finney (1952) defined the bioassay as "the measurement of the potency of any stimulus which may be physical, chemical, biological, physiological or psychological etc. by means of the reactions which it produces in a living organism."[21]

i) Insecticidal bioassay

In bioassay the ultimate effect i.e. death is measured not the symptoms so that dose response relation ship could be worked out. Insects are small in size, there fore the dose of the insecticide is not taken on weight basis, that is not on the basis of milligram per kilogram body weight as in case of LD_{50}, but the dose of Insecticide either in concentration (%) or in microgram in/on insect is taken in to account while bioassaying. In such cases the response is measured in terms of mortality after a definite exposure period. Further the dose/ concentration versus mortality data are subjected to work out LC_{50}. Thus, LC_{50}. may there fore, be define as the lethal dose or concentration which is given as 50% kill of the test insect population, which is not expressed as milligram per kilogram body weight, instead in dose (μg) or concentration (%)[21].

ii] Anti-microbial bioassay

Microbioassay is a type of biological assay specially performed with microorganisms like bacteria and fungi. In a typical microbiological assay, evaluation is performed on the various cultures of microorganisms and the activity is represented on the basis of average response of a large population of microorganism. The micro bioassay procedures are used for the evaluation of the potency of chemicals as on antibiotics[22].