Effect of azadirachtin on insects

INTRODUCTION

Humans have always been in direct competition with a myriad of insects, pests from our ancestral beginning. This competition for food with insects intensified when humans began to cultivate plants converting the natural ecosystem to an agroecosystem. Also insects serve as vectors of various diseases caused by bacterial, filarial nematode, protozoans and viruses. Therefore control of insects posed a major concern for the development of the economy. In 1939, the discovery of insecticidal properties of DDT (Dichlorodiphenyl trichloroethane) by Paul H. Mueller changed the scenario of pest management. During World War 2 DDT was extensively used to prevent epidemics of several insect vectored diseases such as yellow fever, typhus elephantiasis and malaria. This drew attention to the possibilities of more synthetic insecticides and as a result the use of pesticides in various arenas soared from 1940-60, complete reliance on pesticides intensive pest management was leading agriculture on a “ pesticide treadmill”. The overreliance on synthetic pesticides from late 1940s to mid-1960 was referred to as “ Dark Ages” of pest control. The cheapness and effectiveness of synthetic insecticides threw natural compounds into shade. But very soon other shades also began to appear. In 1962, the appearance of book “ Silent Spring” (by Rachel Carison) showed that pesticide residues were building up in ecosystem with detrimental effects on wildlife and beneficial insects. Due to extensive and intensive use, misuse and abuse of insecticides the following problems were becoming prominent and intolerable (ecological backlashes):

1. Development of insecticide resistance- many insects started developing resistance against pesticides which increased the cost of management.
2. Due to killing of natural controlling agents, the phenomenon of pest resurgence became more evident.
3. Also minor pest were achieving the status of major pest i. e. secondary pest outbreak due to significant decline in its natural enemy (predators and parasites).
4. Ecological imbalance due to poisoning of all the realms of environment.
5. Increase in the concentration of hydrochlorinated insecticide in food chain.
6. Intolerable residues on the food made the food obtained after such treatment uneconomical as it became unfit for consumption and unfit for exports due to high toxic residues.
7. Killing and harmful effects on Non target organisms became more prominent (like birds, fishes and other wildlife).

Overviewing these effects, there was an utmost need for the development of environmentally sound management practices. This lead to the idea of Integrated Pest Management (IPM) . A panel of experts put the concept of IPM in 1968. IPM as defined by FAO is a system which in consideration with the present environment and pest population dynamics, integrates all the sustainable techniques of pest management as compatible a manner as possible and maintain the population of pest below the level which can cause economic damage (i. e. below economic injury level). The approach is to minimize the dependence on insecticides and maximize the use of ecofriendly methods so as to cause minimum damage to the environment. Botanical pesticides, thus is an very important component of IPM as

1. They are easily degradable.
2. Don't affect non target organisms, natural controlling agents such as predator, parasites.
3. Don't form residues
4. And has no harmful effect on humans as they are very specific in action.

Botanical pesticides refer to the use of chemical or organic compound produced by plants, plant products, which have harmful effects on the growth, development and survival of insect pests. Plants are a rich source of such organic compounds.

HISTORY

The practice of utilizing the derivatives of plant i. e. botanical pesticides in agriculture dates at least two millennia back in ancient China, Egypt, Greece and India. Even in North America and Europe, the documented use of botanicals extend back more than 150 years before the discovery of major class of synthetic chemical insecticides (OP, carbamates and pyretheroids) in mid 1930s to 1950. It is very clear from the recent history that the chemical insecticides have essentially relegated the botanical pesticides from an important role in agriculture to a trivial position in the market among various crop protection strategies.

The total number of 20 phylochemicals is estimated to be 500000, so far only 10000 of these have been isolated. At present four major types of botanicals are being used for the control of insects. These include:

1. Pyrethrum
2. Neem (Azadirachtin).
3. Rotenons.
4. Essential oils.

Others are in limited use like Ryania, Nicotine, Sabdella. Whereas Nicotine, Rotenene, Natural Pyrethrins constitute the outstanding example of older botanicals, extracts and compounds from the Neem tree (A. indica) have emerged as the most prominent phytochemical pesticides in recent years. Among the various biologically active compounds that can be extracted from the Neem tree like- triterpenoid, phenolic compounds, carotenoids, steroids, ketones; the tetranortriterpenoid azadirachtin has been the most extensively studied pesticide as 1) it is relatively abundant in Neem kernels. 2) has biological activity on a wide range of insects.

PLANT SECONDARY METABOLITES

Plants produce a large, diverse array of organic compounds that appears to have no function in growth and development. These substances are known as SECONDARY METABOLITES or secondary products or natural products. Secondary metabolites differ from Primary metabolites (amino acids, nucleotides, sugars, acyl lipids) as:

1. They have no direct roles in photosynthesis, respiration, protein synthesis etc
2. They have restricted distribution in plant kingdom.

In a seminal paper Fraenkel stressed the role of secondary metabolites as defense system against insects, pests and other natural enemies.

Though they play no role in growth and metabolism they play important ecological role in plants:

1. They protect plants against being eaten by herbivore and being infected by microbial pathogen.
2. They serve as attractants for pollinators and seed dispersing animals and as agents of plant-plant competition.

Because of their ecological role, plant secondary metabolites are classified as ALLELOCHEMICALS, a term coined by WHITTAKER. An allelochmical is defined as a non nutritional chemical produced by an individual of one species that affects growth, health, behavior, population ecology of another species. Plants produce an astonishing array of Secondary metabolites. Even a single plant species may produce an extensive pharmacopeia of recondite chemicals. Periwinkle for example contains about more than 100 monoterpenoid indole alkaloids. It has been estimated that plant kingdom synthesizes hundreds of thousands of different secondary metabolites. The no of identified compounds now exceeds 10000.

Secondary metabolites as plant defense is result of co evolution between plants and herbivores

Plant secondary metabolites can be divided into three chemically distinct groups:

1. TERPENES
2. PHENOLICS
3. NITROGEN CONTAINING COMPOUND

TERPENES

The terpenes constitute the largest class of secondary products . the diverse substances of this class are generally insoluble in water. They are biosynthesized from acetyl coA. Terpenes are classified by no of five carbon units they contain as:

1. Monoterpenes: Contain 2 five carbon skeleton
2. Sesquiterpenes: Contain 3 five carbon skeleton
3. Diterpenes: Contain 4 five carbon skeleton
4. Triterpenes: 30 carbons
5. Tetraterpenes: 40 carbons
6. Polyterpenoids: $(C5)n$, where $n> 8$

Some terpenes have role in growth and development

Terpenes defend against herbivore in many plants. Terpenes are toxins and feeding deterrentsto many plant feeding insects, thus they appear to play important defensive role in plant kingdom and protection of agricultural crops. Examples of important Terpenes:

1. PYRETHROIDS: These are monoterpenoid that occurs in leaves and flowers of Chrysanthemum species show very striking insecticidal activity. Both natural and synthetic pyrethroids are popular ingredients in commercial insecticide because of their low persistence in the environment. Pyrethrum is the predominant botanical in use accounting for 80% of global botanical insecticide.
2. ESSENTIAL OILS: These are the mixture of monoterpene and sesquiterpene that lends a characteristics odor to the foliage . e. g

Menttholin Peppermint oil and Limonenein lemon oil are monoterpenes. Essential oils have well known insect repellent properties. They are frequently found in glandular hairs and serve to advertize the toxicity of plant repelling potential. Phytophagus insects even they take a trial bite.

3. VOLATILE TERPENES: In corn & wild tobacco certain monoterpenes and sesquiterpenes are produced and emitted only after insect feeding has already begun. These substances prevent oviposition and kill plant feeding insects and so help in controlling further damage. These also attract natural enemies of plant feeding insects so promise a sound means of pest control.
4. LIMNOIDS: These are a group of nonvolatile Triterpene. Among these the most powerful deterrent to insects feeding known is Azadirachtin. It is a complex limnoid from Neem tree which is feeding deterrent to some insects at as low as 50ppm and it exerts a variety of toxic effect. It has considerable potential as a commercial insect control because of its low toxicity to mammals.

PHENOLICS

Plants produce a variety of secondary products that contain a phenol group, these are called phenolic compounds. Plants phenolics are a chemically heterogeneous group of nearly 10000 compounds . many of these serve as defense compounds against herbivores.

The release of phenolics into soil limits the growth of other plants.

LIGNIN a highly branched polymer of phenylpropanoid group has significant protective function in plants. Its physical toughness deters feeding by insects and chemical durability makes it relatively indigestible.

The flavoids are one of the largest classes of plants phenolics e. g. anthocyanins, flavones etc.

Anthocyanins are colored flavonoids that attract insects to flower and fruits by providing visual and olfactory signal.

Flavonoids protect against damage by UV light.

Tannins deter feeding by herbivores and it also act as feeding repellents to a great diversity of insects

NITROGEN CONTAINING COMPOUND

A large variety of plant secondary metabolites have nitrogen in their structure. This category includes well known defense against phytophagus insects as alkaloids and cyanogenic glycosides, glucosinolates.

ALKALOIDS: These are a large family of more than 15000 nitrogen containing secondary metabolites with a heterocyclic ring. Several different types including nicotine and its relative are derived from ornithine . Most alkaloids now function as defenseagainst their predators because of their toxicity and deterrence capability. Alkaloids increase in response to initial damage fortifying against further damage e. g. wild tobacco produces higher level of nicotinefollowing damage by tobacco caterpillars.

CYANOGENIC GLYCOSIDES: These are not toxic themselves but are readily broken down to give off volatile poisons; well known poisonous gas Hydrogen cyanide. When the leaf is damaged due to insects feeding on it, the cell content of different tissue mix and HCN is formed. HCN is a fast acting toxin that inhibits metalloprotiens such as iron containing cytochrome oxidase; a key enzyme of mitochondrial respiration, thus affecting physiology of insects. Thus presence of cyanogenic glycosides deters feeding by insects.

GLUCOSINOLATES: A Class of plant glycosides that break down to release volatile defensive substances, also called Mustard oil glycosides. Found principally in the Brassicaceae and related plant families, where glucosinolates give off compounds responsible for smell and taste of vegetables like cabbage, cauliflower, mustards etc. These compounds function in DEFENCE as toxin and feeding repellent. But certain insects are adapted for feeding on glucosinolate containing plants without ill effects. For example glucosinolates serve as stimulant for Cabbage butterfly for feeding and egg laying and isothiocyanates serve as volatile attractants.

PLANT PROTIENS: Certain plant protein also interfere with insect digestion, for example plants produce LECTINS, defensive proteins that bind to epithelial cell lining digestive tract and interfere with nutrient absorption. The best known anti digestive proteins in plants are protein inhibitors found in legumes, tomatoes, and other plants. After entering herbivores digestive tract they interfere with protein digestion, as a result insects suffer reduced rates of growth and development.

THE NEEM TREE, PROPERTIES AND DISTRIBUTION

The Neem tree also known by names like Indian Lilac, Margosa tree is an evergreen fastgrowing tree belonging to the order “ Rutales” and family “ Meliaceae”. The genus Azadirachta indica was described by A. juss in 1830.

DISRIBUTION

Neem tree is indigenous to Indian Subcontinent from where is has spread to many Asian and African countries such as Pakistan, Bangladesh, Mynamar, Sri Lanka, Thailand, Indonesia, Malaysia, Singapore, Iran, Yemen, Australia, New Guinea, Nigeria, Fizi, Tanzania, Madagascar, USA, Latin America, Germany, France, Portugal, Spain and UK. It is now grown in most tropical and sub-tropical parts of the worls.

The origin of A. indica is not very clear. Some say that is has originated from Burma whereas others point it to south India. It is considered that it has originated from south-eastern and southern Asia.

In Indonesia Neem exists in low lying Northern and Eastern parts of java. In Philippines it was introduced from India, Africa. Ketkar (1967) reported about 14 million trees in India. There are more than 20 million trees available in entire India. In Africa Neem was introduced from India and is concentrated in a belt stretching across the African continent from Somalia to Mauretania. In America Neem trees are prominent in Haiti, Surinam and propagation has started in Brazil, Puerto Rico, Cuba and Nicaragua. Neem trees also grow in our neighbouring countries, Middle East, Saudi Arabia and Yemen.

HABITAT

Neem tree is a fast growing sclerophyllous tree.

- It grows well in humid to semi-humid climate.
- It thrives well at altitudes upto 700-800m above the sea level.
- Neem trees are hardy and are able to grow in severe drought condition also. They thrive well in regions with less than 500 mm annual rainfall and upto 2500 mm annual rainfall.
- Neem tree exist in poor, shallow, sandy and stony soil. It also grows in black cotton soil in India.
- Neem tree can flourish in warm to very hot climates. It grows well between 21-320c temperatures but it can tolerate upto 500c during summer.
- Ph value between 6. 2-7 seems to the best for the growth of Neem tree.

CHARACTERISTIC FEATURES

- It is a fast-growing tree, reaching a height of 4-7 m during the first 3 years and 5-11m during the following 5 years.
- It begins to bear fruit within 3-5 years and becomes fully productive in the 10th year, when it may yield up to 50 kg fruit per tree per year.
- The Neem tree produces its fruits, which are the main source for its production of pesticides, on drooping panicles, usually about once a year, although two fruiting periods per year occur in certain areas (e. g. West Africa).
- A mature Neem tree produces annually 30-50 kg of fruit, but this may depend upon rainfall and soil conditions. More conservative estimates range around 20 kg per tree; 40 kg of fresh fruit yield about 24 kg of dry fruit.

- Neem has the reputation of possessing a large number of biological activities which include insecticidal, nematicidal, bactericidal, and anti-fungal. It has attracted world-wide attention due to its wide ranging capacity as a biocide.

PESTICIDAL CONSTITUENT OF NEEM

Neem tree is the only tree in which every part of tree produces biologically active products which has various properties such as antifeedant, deterrent, growth regulation, oviposition alteration, insecticidal properties, fungicidal properties, etc.

Though bark, heartwood, leaves, fruits of it produce these substances in various concentrations but it is the fruits specifically seeds which are of major importance. Neem seed kernels contain the highest amount of the active compound. 40-50 kg of fruit can yield about 5 kg of kernels (10% of fruit). Each seed contains about 1-3 kernels.

Till date more than 140 active principles have been identified in different parts of the tree. Insecticidal properties of Neem is due to the presence of a class of Limnoids which include compounds like Azadirachtin, Melantriol, Salanin, Mimbines, Salannol and various sulfur containing compounds. Among these Azadirachtin is the most active and predominant insecticidal compound concentrated mainly in the seed kernels. The Azadirachtin occurs in seeds at the concentration of about 0. 1-0. 9%. It is estimated that 20-30 kg of Neem seeds are required per hectare if 2g of Azadirachtin per kg of sed is obtained. The highest yield of Azadirachtin obtained till date was about 10g/kg of seed.

AZADIRACHTINAND ITS STRUCTURE

Azadirachtin is a highly oxidized limnoid chemically being a tetranortriterpenoid and is the main component responsible for both anti-feedant and toxic effects in Azadirachtin. Butterworth and Morgan were the first to isolate Azadirachtin in 1968 from Neem seed. Morgan established correct molecular formula of Azadirachitn (C55H44O16). In 1971 they developed a simplified method to isolate azadirachitn by doing solvent partitioning followed by column and preparative thin layer chromatography. However its structure was determined in 1975 by Nakanishi's team through the application of new NMR methods. There were some inaccuracies in the given model. Then again renewed efforts were made by the group of Ley, Kraus, Nakanishi and they gave the correct structure by using X-ray crystallography.

A. indica produces a plethora of triterpenoids, the biosynthesis of which culminates in azadirachtin. The biosynthesis of azadirachtin starts with a steroid precursor – tetracyclic triterpene " tirucallol". Opening of C-ring followed by processing via two main levels of structural complexity i. e. furan ring formation leads to Azadirachtin.

VARIOUS PESTICIDAL FORMULATIONS

Neem insecticides which are obtained from Neem seeds contain various arelated triterpenoids in addition to the Azadirachtin. However their efficacy is related directly to the content of Azadirachtin. These compounds do possess biological activity and they add to its effects. Pure Azadirachtin was shown to be effective in the fields (Mordue et al, 1997) but the natural mixtures of azadirachtin in Neem insecticides may usefully mitigate against the development of resistance compared to azadirachtin alone (Feng and Isman, 1995).

The complex nature of azadirachtin and other sophisticated Neem constituents prevent their mass production by synthesis in the foreseeable future. The pesticidal Neem products used in practice include dried leaves, whole seed, decorticated seed, seed kernels, Neem oil, and Neem cake, remaining after extraction or extrusion of the oil from the seeds. Several Indian companies or institutions produce commercially Neem-based insecticidal formulations, such as " RD-9 Repelin" and " Wellgro", for spraying against cutworms and other insect pests in tobacco growing areas; " Nimbosol" and " Biosol" for control of whiteflies; and the products " Neemrich" and " Neemark, the latter also as an azadirachtin-enriched granular Neem formulation. In the U. S. A. , the EPA hasgranted registration to " Margosan-O", an azadirachtin-enriched, concentrated Neem seed kernel extract formulation, for use on non food crops and ornamentals. Margosan-0 was developed by R. Larson of Vikwood Botanicals Inc. at Sheboygan, WI, in collaboration with the USDA Agricultural Research Center at Beltsville, MD. The rights to this product, which contains 0. 3% azadirachtin and 14%Neem oil (the 0 in the name of the product stands for oil), and has an oral toxicity in excess of 5, 000 mg/kg in rats. Margosan-0 has been evaluated successfully against an extensive series of insects in the U. S. A. and Canada, Lyriornizu leafminers on ornamentals and tomatoes, cotton bugs, cockroaches and mosquitoes. Margosan-0 demonstrated highest activity against Ostriniu nubilalis , and against leafhoppers, against two species of local cotton pests, Enrias insulana and Spodoptera littoralis. Recently in the

U. S. A. a further Neem formulation, developed. under the auspices of the Natural Products Institute, Salt Lake City, UT is ” Azatin”(Agridyne Technologies, Salt Lake City, UT). Also, Safer Ltd. , a Canadian manufacturer specializing in environmentally safe pest control formulations, developed insecticides based on Neem. Safer, however, has been acquired recently by Ringer Corp. , Minneapolis, MN, which distributes Margosan-0 in the home garden market under the tradenames of “ Bioneem” and “ Neemesis”.

Contrary to registration practices in use until now, no precise chemical descriptions of all the ingredients of Margosan-0 were required, but rather, demonstration of the biological activity and innocuousness of the whole mixture to no target organisms was used in the registration process. Hopefully such specially tailored toxicity studies will be used to judge and register Neem and similar natural products in the future. A recent report claims that the EPA has approved a Neem-based biological pesticide developed by an Indian company for use on a wide range of food crops.

MODES OF ACTION

Major modes of action of azadirachtin are:

1. Powerful IGR.
2. Feeding Deterrant.
3. Oviposition Deterrant.

These are the three modes of action of azadirachtin which make azadirachtin much sought after biopesticide in today’s agriculture industry.

IGR: Azadirachtin acts as a powerful growth regulator for insects and this IGR effect is the most pronounced mode of action of Azadirachtin.

Normally IGR effect the hormonal system of insects, preventing the insects from developing into normal mature insects. This IGR property of Azadirachtin doesnot leads to immediate death of insects, pests.

Azadirachtin as an IGR:

The IGR property of Azadirachtin arise due to the fact that:

Azadirachtin is structurally analogous to natural hormone Ecdysone. As Ecdysone regulates the development of insect, any disruption in its balance leads to improper development.

Also Azadirachtin interferes with the production and reception of Ecdysone at the time of insects' growth and moulting. Thus Azadirachtin in this manner block the moulting cycle resulting in the death of the insect, pest.

The main action of Azadirachtin appears to be at the release site of PTTH. The mode of action of Azadirachtin as IGR is thus an Indirect Physiological Effect. It is exerted via the endocrine system. The copora cardiaca is supposed to be the target for the Azadirachtin as is affects the PTTH, Eclosin Hormone, Bursicin Hormone release. PTTH release is inhibited rather than Ecdysine from Prothorasic gland. Thus the Azadirachtin affects the neurosecretory cells of Brain. Various experiments show that Azadirachtin doesn't directly act on Prothorasic Glands.

1. In the in vitro culture of Prothorasic (H. virescens) gland showed that the PTTH induced release of the Ecdysine was medium (Bidmon et al, 1987, Barnby and Klocke, 1990). Also it was not blocked in PTTH simulated cultured glands from M. sexta pupa penetrated with

Azadirachtin in last larval instar (Pener et al, 1988). However receptivity of Prothorasic gland to PTTH was affected in H. virescens.

2. Neurosecretory proteins stained with paraldehyde in L. migratolia females when was compared with similar aged azadirachtin treated females there was an accumaulation of stainable material in corpora cardiaca of brain neurosecretory system in treated insects. Thus is appears that azadirachtin blocks release of neurosecretory material from corpora cardiac.

It can thus be concluded that Azadirachtin does block the release of peptide hormones from brain neurosecretory cell corpora cardiac complex.

Azadirachtin also exhibit IGR effect by altering the titre of Juvenile Hormone (JH). Azadirachtin affects the release of allotropins into corpora dillata hence block the synthesis and release of the Juvenile Hormone. This block leads to a rapid decrease in whole body JH titres, which is maintained for several days. Experiments prove that in M. sexta larvae, azadirachtin infection on day 0 (1. 0-10 μg/ larva) results in induction of supernumerary moults (Sch et al, 1985; Beckage et al, 1988) presumably due to an inhibition and subsequent delay in JH titre. In adult female L. migratolia also azadirachtin treatment causes a rapid decrease in juvenile hormone titres with associated disturbances in oogenesis (Rembold, 1984; Rembold et al, 1987).

Thus, on a conclusionary note, the effect of azadirachtin is both dose and time dependent. It prevents both apolysis and ecdysis and thus can cause death before the moults, during the moults or delays of moult to form permanent larvae.

Feeding Deterrance: Feeding behavior is both dependent on chemical senses stimulated due to contact chemoreceptors on trasi, mouthparts and oral activity and integration of the sensory code with the CNS. Azadirachtin acts as feeding deterrant. Inhibition of the feeding behavior occurs:

There are receptors present on and around mouthparts of insects which normally respond to Phagostimulants. So azadirachtin may act by blocking the input from these receptors.

Also there are present specific “ deterrent cells” in insects which prevent insect from feeding. Azadirachtin acts to stimulate these “ deterrent cells” leading to feeding deterrence. Many experiments were done in this regard.

Using different concentration of sucrose and azadirachtin, either singly or together, the neurophysiological responses from medial and lateral sensillia styloconica of maxillae showed different group of receptors are receptive to sucrose (sugar cells) or azadirachtin (deterrent cell) in S. exempta and M. brassicae in most of the cases, the rate of firing of sugar sensitive cells were reduced in presence of both chemicals (Simmonds and Blaney, 1984). Such an interaction was also found in P. brassicae. This leads to a reduced or complete inhibition of feeding.

Direct mode of action: Incorporation of azadirachtin results in direct toxic effect after ingestion. Azadirachtin prevents the secretion of Proteolytic enzymes and thus significantly impair ability of insects to digest and absorb nitrogenous food. When azadirachtin is ingested it can result in the disfunctioning of gut, as a result of which midgut epithelial cells become round. Swelling of cells and organells occur with some vacuolization and cell burst resulting in necrosis (as observed in S. gregarea and L. migratolia Naseruddin and Mordue (Luntz), 1993a; Cottee, 1984). There is also reduction in the regenerative cells and increase in the connective tissue layer with some invading heomocytes. This would lead to disruption of enzyme secretion and nutrient absorption.

Also the antifeedant effect can be attributed to the action of azadirachtin on the peristaltic movement of gut wall. The gut of treated insects lack tone, midgut to hindgut junction becomes flaccid and co-ordinated peristalsis is lacking which leads to antifeeding behavior.

EFFECT OF AZADIRACHTIN ON INSECTS

Effects on Feeding

Azadirachtin is a classical example of a natural plant defence chemical affecting feeding. Antifeedancy is the major insecticidal effect of Azadirachtin. Antifeedant effect in insect pest on application of Azadirachtin is divided into two main categories:

1. Primary Antifeedancy: It refers to the deterrence of feeding in insects. Primary Antifeedancy is also called Gustatory antifeedancy. It can be defined as the inability to ingest resulting from the perception of antifeedant at a sensory level (Schmutterer 1985). Insects fail to eat treated crops and as starvation ensued results in the death of insects.
2. Secondary Antifeedant effect: It refers to the non-feeding after the ingestion of treated plant. Secondary antifeedancy is also called Non-Gustatory antifeedancy. It can be defined as the reduction in food consumption and digestive efficiency subsequent to and as a

consequence of ingestion, application or injection of antifeedant (Schmutterer, 1985).

Experiments conducted in the past in this regard by various persons:

The first detailed experiment was conducted in S. gregaria (desert locusts) in India. Insects from different orders show marked difference in their response to azadirachtin. (Table 1)

1. Lepidopteras showed extreme sensitivity to azadirachtin and depending upon species, effective anti-feedance was observed from less than 1 to 50 ppm.
2. Hemiptera (Homoptera), Coleoptera are less sensitive to azadirachtin with 100 % antifeedancy observed at 100-600 ppm.
3. However, in Orthoptera wide range of sensitivity has been observed.

Reed and Pierce in 1981 tested the repellant effect of Neem extract to striped cucumber beetle (A. vittateim), by cutting leaves and dipping them in extract solution and placing them in a dish with untreated leaf pieces. When 5 fasting beetles were placed in a dish, 0. 1 % azadirachtin gave protection for atleast three days.

The intake of food by various homopteran insects Nilaparvata lugens, Nephotettix virescens was significantly reduced on rice plants sprayed with 1-50% emulsion of Neem oil. ( ). In green rice leafhopper, N. virescens feeding on the phloem of neem oil treated plants (1. 25-10%) was significantly less than of solvent treated control plants, whereas xylem feeding increased. Hemipteran insects feeding on tobacco seedlings which had been systemically treated with 500 ppm azadirachtin, were shown initially to feed normally but, after termination of the initial feed, the interval prior to the next subsequent feed was significantly increased and feeding activity thereafter was suppressed (Nisbetet al. 1993). When azadirachtun was impregnated on discs at a concentration of 0. 1-10 ppm, S. littoralis(African cotton leafworm), Spodoptera frugiperda(J. E. Smith) (fall armyworm), Heliothis virescens(F.) (Tobacco budworm) andHelicoverpa armigera(Hüb.) (Old world bollworm) showed significant behavior response and are prevented from feeding on the discs dependent on species (Blaney et al. 1990, Simmonds et al. 1990, Mordue (Luntz) et al. 1998)

Insects from different Orders differ markedly in their behavior responses to azadirachtin (Table 1). Lepidoptera are extremely sensitive to azadirachtin and show effective antifeedancies from <1-50 ppm, depending upon species. Coleoptera, Hemiptera and Homoptera are less sensitive to azadirachtin behaviorally with up to 100% antifeedancy being achieved at 100-600 ppm although there are some aphid species which also show behavioral sensitivity