Insecticide Resistance

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1. Introduction

Insecticide resistance is an increasing problem faced by those who need insecticides to efficiently control medical, veterinary and agricultural insect pests. In many insects, the problem extends to all major groups of insecticides. Since the first case of DDT resistance in 1947, the incidence of resistance has increased annually at an alarming rate. It has been estimated that there are at least 447 pesticide resistant arthropods species in the world today (Callaghan, 1991). Insecticide resistance has also been developed by many insects to new insecticides with different mode of action from the main four groups.

The development of resistance in the fields is influenced by various factors. These are biological, genetic and operational factors. Biological factors are generation time, number of offspring per generation and migration. Genetic factors are frequency and dominance of the resistance gene, fitness of resistance genotype and number of different resistance alleles. These factors cannot be influenced by man. However, such as treatment, persistence and insecticide chemistry, all of which may and therefore timing and dosage of insecticide application should be operational factors.

Pesticide resistance is the adaptation of pest population targeted by a pesticide resulting in decreased susceptibility to that chemical. In other words, pests develop a resistance to a chemical through natural selection: the most resistant organisms are the ones to survive and pass on their genetic traits to their offspring (PBS, 2001).

Pesticide resistance is increasing in occurrence. In the 1940s, farmers in the USA lost 7% of their crops to pests, while since the 1980s, the percentage lost has increased to 13, even though more pesticides are being used (PBS,2001). Over 500 species of pests have developed a resistance to a pesticide (Anonymous, 2007). Other sources estimate the number to be around 1000 species since 1945 (Miller, 2004).

Today, pests once major threats to human health and agriculture but that were brought under control by pesticides are on the rebound. Mosquitoes that are capable of transmitting malaria are now resistant to virtually all pesticides used against them. This problem is compounded because the organisms that cause malaria have also become resistant to drugs used to treat the disease in humans. Many populations of the corn earworm, which attacks many agricultural crops worldwide including cotton, tomatoes, tobacco and peanuts, are resistant to multiple pesticides (Berlinger, 1996).

Despite many years of research on alternative methods to control pests and diseases in crops, pesticides retain a vital role in securing global food production and this will remain the case for the foreseeable future if we wish to feed an ever growing population.
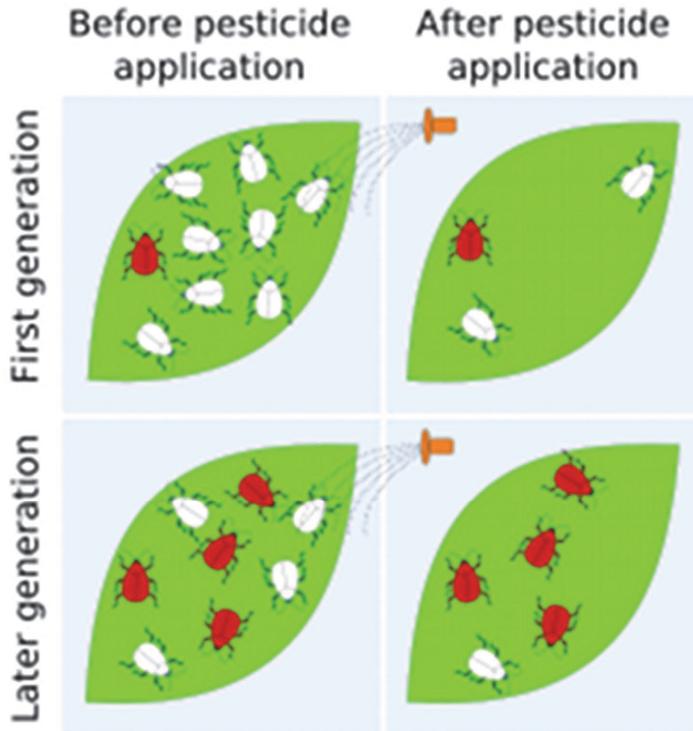
Fig. 1. Pesticide application can artificially select for resistant pests. In this figure, the first generation happens to have an insect with a heightened resistance to a pesticide (red). After pesticide application, its descendants represent a larger proportion of the population because sensitive pests (white) have been selectively killed. After repeated applications, resistant pests may comprise the majority of the population (PBS, 2001).

Insecticides are applied to reduce the number of insects that destroy crops or transmit disease in the field of agriculture, veterinary and public health. Insecticides are not always effective in controlling insects, since many populations have developed resistance to the toxic effects of the compounds. Resistance can be defined an inherited ability to tolerate a dosage of insecticide that would be lethal to the majority of individuals in a normal wild populations of the same species.

Insecticides are in common use in agriculture as well as in houseplant populations, gardens, and other living spaces in an attempt to control the invasion of a seemingly endless array of insects. Insecticides are used to keep populations under the control, but over time insects can build up a resistance to the chemicals used. This is called insecticide resistance. Insecticide resistance is apparent when a population stops responding or does not respond as well to applications of insecticides.

In recent years, many of the resistance mechanisms have been detected and resistance detection methods have been developed. These mechanisms have divided into four categories: a) increased metabolism to non-toxic products, b)decreased target site sensitivity, c)decreased rates of insecticide penetration, d) increased rates of insecticide excretion. There are different methods to determine that the mechanisms are available in any given population. We can see the structure of the resistance mechanisms from these assays.

There are several thousand species of insect in the world of particular nuisance to man, either as vectors of fatal and debilitating diseases or destroyers of crops. Insecticide resistance is an increasing problem faced by those who need insecticides to efficiently control medical, veterinary and agricultural insect pests.

2. History of insecticide resistance

In 1914 A. L. Melander reported the first case of insecticide resistance. He studied the effectiveness of lime sulphur, an inorganic insecticide, against an orchard pest, the San Jose scale (*Quadraspidiotus perniciousus*) in the state of Washington. A treatment with lime sulphur killed all scales in one week in typical orchards, but 90 percent survived after two weeks in an orchard with resistant scales. Although few cases of insecticide resistance were recorded before 1940, the number grew exponentially following widespread use of DDT and other synthetic organic insecticides (http://science.jrank.org)

Insects have evolved resistance to all types of insecticides including inorganics, DDT, cyclodienes, organophosphates, carbamates, pyrethroids, juvenile hormone analogs, chitin synthesis inhibitors, avermectins, neonicotinoids, and microbials.

In many insects, the problem extends to all major groups of insecticides. Since the first case of DDT resistance in 1947, the incidence of resistance has increased annually at an alarming rate. It has been estimated that there are at least 447 pesticide resistant arthropods species in the world today (Callaghan, 1991). Insecticide resistance has also been developed by many insects to new insecticides with different mode of action from the main four groups. For example, neoniconitoids.

Resistance occurs in thirteen orders of insects, yet more than 90 percent of the arthropod species with resistant populations are either Diptera (35 percent), Lepidoptera (15 percent), Coleoptera (14 percent), Hemiptera (in the broad sense, 14 percent), or mites (14 percent). The disproportionately high number of resistant Diptera reflects intense use of insecticides against mosquitoes that transmit disease. Agricultural pests account for 59 percent of harmful resistant species while medical and veterinary pests account for 41 percent. Many species have numerous resistant populations, each of which resists many insecticides. Statistical analyses suggest that for crop pests, resistance evolves most readily in those with an intermediate number of generations (four to ten) per year that feed either by chewing or by sucking on plant cell contents.

Resistant pest species outnumber resistant beneficial species such as predators and parasitoids by more than twenty to one. This pattern probably reflects limited attention devoted to resistance in beneficials as well as biological differences between beneficials and pests. Available evidence contradicts the hypothesis that natural enemies evolve resistance less readily because intrinsic levels of detoxification enzymes are lower in predators and parasitoids than in pests. An alternative hypothesis with more support is that natural enemies evolve resistance less readily because they suffer from food limitation following insecticide sprays that severely reduce abundance of their prey or hosts.

According to Georghiou (1986), pesticide resistance occurs in at least 100 species of plant pathogens, 55 species of weeds, 5 species of rodents, and 2 species of nematodes. This article focuses on resistance to insecticides in more than 500 species of insects and mites.

Sukhoruchenko and Dolzhenko (2008), presents the results of long-term monitoring of insecticide resistance in populations of agricultural pests in Russia. Over the last 45 years, resistance developments were recorded for 36 arthropod pest species in 11 agricultural crops and pastures in relation to nearly all commonly used plant protection products. Development of group, cross and multiple resistance has been revealed in populations of many economically important pests. Toxicological and phenotypical (for Colorado potato beetle) methods have been devised to monitor the development of pesticide resistance. Based on experience over the last century, systems aimed at preventing the development of pest resistance to insecticides and acaricides are elaborated. These systems are based on resistance monitoring and using plant protection measures which minimize the toxic pressure on agroecosystems.

3. Mechanisms of insecticide resistance in insects

There are several ways insects can become resistant to crop protection products, and pests often exhibit more than one of these mechanisms at the same time.

- **Behavioral resistance:** Resistant insects may detect or recognize a danger and avoid the toxin. This mechanism of resistance has been reported for several classes of insecticides, including organochlorines, organophosphates, carbamates and pyrethroids. Insects may simply stop feeding if they come across certain insecticides, or leave the area where spraying occurred (for instance, they may move to the underside of a sprayed leaf, move deeper in the crop canopy or fly away from the target area) (www.irac-online)

- **Penetration resistance:** Resistant insects may absorb the toxin more slowly than susceptible insects. Penetration resistance occurs when the insect's outer cuticle develops barriers which can slow absorption of the chemicals into their bodies. This can protect insects from a wide range of insecticides. Penetration resistance is frequently present along with other forms of resistance, and reduced penetration intensifies the effects of those other mechanisms.

- **Metabolic resistance:** Resistant insects may detoxify or destroy the toxin faster than susceptible insects, or quickly rid their bodies of the toxic molecules. Metabolic resistance is the most common mechanism and often presents the greatest challenge. Insects use their internal enzyme systems to break down insecticides. Resistant strains may possess higher levels or more efficient forms of these enzymes. In addition to being more efficient, these enzyme systems also may have a broad spectrum of activity (i.e., they can degrade many different insecticides).

- **Altered target-site resistance:** The site where the toxin usually binds in the insect becomes modified to reduce the insecticide's effects. This is the second most common mechanism of resistance.

There are four major mechanisms of resistance in insects. These are:

1. Increased metabolism to non-toxic products
2. Decreased target site sensitivity
3. Decreased rates of insecticide penetration
4. Increased rates of insecticide excretion

Of these four categories the first two are by far the most important.

Metabolic resistance: The normal enzymatic metabolism of insect is modified to increase insecticide detoxification or prevent activation of insecticides.

The enzymes responsible for detoxification of xenobiotics in living organisms are transcribed by members of large multigene families of esterases, oxidases, and GST. Glutathione transferases (GSTs) are a diverse family of enzymes found ubiquitously in aerobic organisms. They play a central role in the detoxification of both endogenous and xenobiotic compounds and are also involved in intracellular transport, biosynthesis of hormones and protection against oxidative stress. Interest in insect GSTs has primarily focused on their role in insecticide resistance. GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione, to produce water-soluble metabolites that are more readily excreted. In addition, they contribute to the removal of toxic oxygen free radical species produced through the action of pesticides. Annotation of the *Anopheles gambiae* and *Drosophila melanogaster* genomes has revealed the full extent of this enzyme family in insects (Enayati et al,2005). Perhaps the most common resistance mechanisms in insects are modified levels or activities of esterase detoxification enzymes that metabolize (hydrolyze ester linkages) a wide range of insecticides. These esterases comprise six families of proteins belonging to the α/ß hydrolase fold superfamily. In Diptera, they occur as a gene cluster on the same chromosome. Individual members of the gene cluster may be modified in instances of insecticide resistance, for example, by changing a single amino acid that converts the specificity of an esterase to an insecticide hydrolase or by existing as multiple-gene copies that are amplified in resistant insects (the best studied examples are the B1 and A2-B2 amplicons in *Culex pipiens* and *C. quinquefasciatus* (Brogdon and McAllister,1998).

The cytochrome P450 oxidases (also termed oxygenases) metabolize insecticides through O-, S-, and N-alkyl hydroxylation, aliphatic hydroxylation and epoxidation, aromatic hydroxylation, ester oxidation, and nitrogen and thioether oxidation. The cytochrome P450s belong to a vast superfamily. Of the 62 families of P450s recognized in animals and plants, at least four (families 4,6,9,18) have been isolated from insects. The insect P450 oxidases responsible for resistance have belonged to family 6, which, like the esterases, occur in Diptera as a cluster of genes. Members of the cluster may be expressed as multiple (up to five) alleles. Enhanced levels of oxidases in resistant insects result from constitutive overexpression rather than amplification. The mechanisms of oxidase overproduction in resistance are under extensive investigation and appear to result from both cis- and trans-acting factors, perhaps associated with the phenomenon of induction ((Brogdon and McAllister,1998).

Altered target site: The site of action has been altered to decrease sensitivity to toxic attack. Alterations of amino acids responsible for insecticide binding at its site of action cause the insecticide to be less effective or even ineffective. The target of organophosphorus (OPs) (e.g., malathion, fenitrothion) and carbamate (e.g., propoxur, sevin) insecticides is

acetylcholinesterase in nerve synapses, and the target of organochlorines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath. DDT-pyrethroid cross-resistance may be produced by single amino acid changes (one or both of two known sites) in the axonal sodium channel insecticide-binding site. This cross-resistance appears to produce a shift in the sodium current activation curve and cause low sensitivity to pyrethroids. Similarly, cyclodiene (dieldrin) resistance is conferred by single nucleotide changes within the same codon of a gene for a γ-aminobutyric acid (GABA) receptor. At least five point mutations in the acetylcholinesterase insecticide-binding site have been identified that singly or in concert cause varying degrees of reduced sensitivity to OPs and carbamate insecticides.

Physical resistance mechanisms: The pickup or intake of toxic agent is slowed or reduced by modification to the insect skeleton, or the rate of excretion of the toxic compound is increased.

4. Insecticide resistance detection techniques

The mode of action of the insecticides, duration life cycle, clutch size and availability of host determine rate of evolution of resistance. Documenting the dynamics of resistance plays another important role in the approach of its mitigation. Reliable, quick and effective techniques to distinguish between susceptible and resistant individuals are necessary (Gunning,1993 and Brown,1981).

There are several phenogenetic methods available to diagnose resistance in populations of pest species which enable the assessment of how shifts in composition and structure of a population caused by pesticides, may affect its development geographically and over time. Among these, easy-to-use toxicological methods have gained the most recognition worldwide. They enable the determination of levels of population susceptibility to pesticides used, in relation to the ratio of resistant and susceptible genotypes. In 2004 under the aegis of the Commission on resistance, a method manual was published: 'Monitoring the resistance to pesticides in populations of arthropod pests'. Methods included in this manual enable scientists to evaluate development of resistance in populations of 37 species of insects and mites of great practical importance for agricultural practice and medicine. At present, researchers are trying to identify easy-to-see visual morphological characters which could be used for the diagnosis of resistance. In order to achieve this, adults from populations under investigation are sampled and fractions of different morphotypes (morphs) are determined. Each morphotype recognized is then tested from the viewpoint of its susceptibility to toxicants used (Benkovskaya et al., 2000; Vasilyeva et al., 2004, 2005; Fasulati, 2005). The frequency of occurrence of different morphs in the Colorado potato beetle has been shown to be related to their susceptibility to pyrethroids. This has enabled a rapid method to be devised for revealing the resistance to pyrthroids in populations of the pest immediately after appearance of overwintered adults in potato crops (Sukhoruchenko et al., 2006). The above method allows potato growers to rationally schedule the use of these pesticides in seasonal application charts.

5. Insecticide resistance detection methods

The primary mechanisms of resistance are decreased target site sensitivity and increased detoxification through metabolism or sequestration. Target sites are the molecules in insects

that are attacked by insecticides. Decreased target site sensitivity is caused by changes in target sites that reduce binding of insecticides, or that lessen the damage done should binding occur. Metabolism involves enzymes that rapidly bind and convert insecticides to nontoxic compounds. Sequestration is rapid binding by enzymes or other substances with very slow or no processing. Reduced insecticide penetration through the cuticle, and behavioral changes that reduce exposure to insecticide are also mechanisms of resistance. Different mechanisms can occur within an individual insect, sometimes interacting to provide extremely high levels of resistance.

Resistance can be determined by using conventional standard bioassay methods published by International Resistance Action Committee (IRAC) and biochemical, immunological and molecular methods.

1) Conventional Detection Methods

The standard method of detection in to take sample of insects from the field and rear them through to the next generations. Larvae or adults are tested for resistance by assessing their mortality after exposure to a range of doses of an insecticide. For susceptible and field populations, LD_{50} or LC_{50} values were calculated by using probit analysis

The results are compared with those from standard susceptible populations. These method includes some differences for the different pest species. These methods are published by Insecticide Resistance Action Committee (IRAC).

The other traditional method of detecting insecticide resistance is to expose individual insects to a diagnostic single dose for a set time period in a chamber impregnated with the insecticide or on a filter paper impregnated with the insecticide. These tests only give an indication of the presence and frequency of resistance and limited information can be gained as to the resistance mechanism.

Evolution of resistance is most often based on one or a few genes with major effect. Before a susceptible population is exposed to an insecticide, resistance genes are usually rare because they typically reduce fitness in the absence of the insecticide. When an insecticide is used repeatedly, strong selection for resistance overcomes the normally relatively minor fitness costs associated with resistance when the population is not exposed to insecticide.

2) Biochemical detection of insecticide resistance

Biocahemical assays/techniques may be used to establish the mechanism involved in resistance. When a population is well characterised some of the biochemical assays can be used to measure changes in resistance gene frequencies in field populations under different selection pressure.

3) Immunological Detection Methods:

This method is available only for specific elevated esterases in collaboration with laboratories that have access to the antiserum. There are no monoclonal antibodies, as yet , available for this purpose.

An antiserum has been prepared against E4 carboxylesterase in the aphid *Myzus persicae* . An affinity purified 1gG fraction from this antiserum has been used in a simple immunoassay to discriminate between the three common resistant variants of *M. persicae* found in the UK field populations (Devonshire et al, 1996).

4) Detection of monooxygenase (cytocrome P450) based insecticide resistance.

The levels of oxidase activity in individual pests are relatively low and no reliable micrtitre plate or dot-blot assay has been developed to measure p450 activity in single insects. The

p450s are also a complex family of enzymes, and it appears that different cytocromes p450s produce resistance to different insecticides.

6. Management of insecticide resistance

Resistance monitoring programme should no longer rely on testing the response to one insecticide, with the intention of switching to another chemical when resistance levels rise above the threshold which affects disease control. Effective resistance management depends on early detection of the problem and rapid assimilation of information on the resistant insect population so that rational pesticide choices can be made.

After a pest species develops resistance to a particular pesticide, how do you control it? One method is to use a different pesticide, especially one in a different chemical class or family of pesticides that has a different mode of action against the pest. Of course, the ability to use other pesticides in order to avoid or delay the development of resistance in pest populations hinges on the availability of an adequate supply of pesticides with differing modes of action. This method is perhaps not the best solution, but it allows a pest to be controlled until other management strategies can be developed and brought to bear against the pest. These strategies often include the use of pesticides, but used less often and sometimes at reduced application rates.

The goal of resistance management is to delay evolution of resistance in pests. The best way to achieve this is to minimize insecticide use. Thus, resistance management is a component of integrated pest management, which combines chemical and non chemical controls to seek safe, economical, and sustainable suppression of pest populations. Alternatives to insecticides include biological control by predators, parasitoids, and pathogens. Also valuable are cultural controls (crop rotation, manipulation of planting dates to limit exposure to pests, and use of cultivars that tolerate pest damage) and mechanical controls (exclusion by barriers and trapping).

Because large-scale resistance experiments are expensive, time consuming, and might worsen resistance problems, modeling has played a prominent role in devising tactics for resistance management. Although models have identified various strategies with the potential to delay resistance, practical successes in resistance management have relied primarily on reducing the number of insecticide treatments and diversifying the types of insecticide used. For example, programs in Australia, Israel, and the United States have limited the number of times and periods during which any particular insecticide is used against cotton pests.

Resistance management requires more effective techniques for detecting resistance in its early stages of development.

Pest resistance to a pesticide can be managed by reducing selection pressure by this pesticide on the pest population. In other words, the situation when all the pests except the most resistant ones are killed by a given chemical should be avoided. This can be achieved by avoiding unnecessary pesticide applications, using non-chemical control techniques, and leaving untreated refuges where susceptible pests can survive.[17][18] Adopting the integrated pest management (IPM) approach usually helps with resistance management.

When pesticides are the sole or predominant method of pest control, resistance is commonly managed through pesticide rotation. This involves alternating among pesticide classes with different modes of action to delay the onset of or mitigate existing pest resistance.[19]

Different pesticide classes may have different effects on a pest. The U.S. Environmental Protection Agency (EPA or USEPA) designates different classes of fungicides, herbicides and insecticides. Pesticide manufacturers may, on product labeling, require that no more than a specified number of consecutive applications of a pesticide class be made before alternating to a different pesticide class.

Tank mixing pesticides is the combination of two or more pesticides with different modes of action in order to improve individual pesticide application results and delay the onset of or mitigate existing pest resistance.

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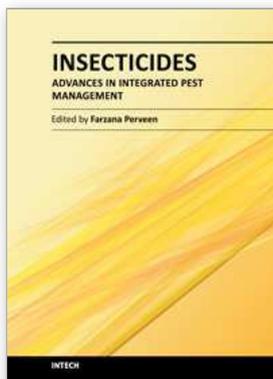
Insecticides - Advances in Integrated Pest Management
Edited by Dr. Farzana Perveen

This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through Bacillus sphaericus and B. thuringiensis, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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