Benha University
Faculty of Science
Entomology department
Entomology & Chemistry stu.

Time: 2 hrs.
first semester 2016/2017
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Insect resistance 461E; for 4th year students

Answer the questions:

AQ1- Write briefly on Insect Metabolic resistance (15 marks)

a- One Metabolic enzyme

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. 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. Perhaps the most common resistance mechanisms in insects are modified levels or **activities of esterase detoxification enzymes that metabolize (hydrolyze ester linkages) wide range of insecticides**. These esterases comprise six families of proteins belonging to the hydrolase fold superfamily. 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*.

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. 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. 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

b- Sodium channel 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 crossresistance 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 an aminobutyric

acid (GABA) receptor. At least five point mutations in the acetylcholinesterase insecticide-binding site have been identified that singly or in concert because varying degrees of reduced sensitivity to OPs and carbamate insecticides.

c- Penetration resistance

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.

AQ2- Define the following (12 marks)

a- Susceptibility strain

Pesticide application can artificially select for resistant pests. In this diagram, 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.

b- Cross resistance

When an insect population develops resistance to one pesticide, it may also prove to be resistant to similar compounds that have the same mode of action. So, insects have abilities to developed resistance to all classes of insecticides causing cross-resistance

c- Alternative pesticides

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).

3- Give short notes on (10 marks)

a- 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. 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.

b- Management of insect resistance

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. 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. Pest resistance to a pesticide can be managed by reducing selection pressure by this pesticide on the pest population. This can be achieved by avoiding unnecessary pesticide applications, using non-chemical control techniques, and leaving untreated refuges where susceptible pests can survive. Also, include non-chemical pest control (IPM), Keep records and watch for resistance, Alternate pesticides from different groups and Mix pesticides from different groups

AQ4- Comment on the table with your recommendations (**11 marks**)

Resistance to insecticides by insects is considered to be a recent evolutionary adaptation to environmental changes, occurring in less than one century in response to sequential applications of chemical insecticides, organochlorines, organophosphates (OPs), carbamates and pyrethroids, and even biological insecticides. The emergence and increase of resistance problems not only shortens the lifespan of currently available insecticides but also undermines the efficacy of newly discovered or developed insecticides owing to cross-resistance and multiple resistance mechanisms. **For A. sinensis**, only resistance monitoring to malathion and fenitrothion have been reported. Obvious resistance to malathion was found in Hubei province. In Yunnan province, mosquitoes in most areas were sensitive to malathion and fenitrothion.

Resistance is commonly monitored by bioassay, by determining LC50 (50% lethal concentration) or by using uniform diagnostic doses. When the LC50 is measured for populations, usually a high resistance level (H) is defined as a resistance ratio (LC50 of field population divided by LC50 of susceptible strain) greater than 20, a moderate resistance level (M) is defined as a resistance ratio between 10 and 20 and a low resistance level (L) is defined as a resistance ratio between 2 and 10, and less than twice that of the susceptible strain is defined as susceptible (S). Standardized by the WHO resistance levels OF the LC50 system. Obvious resistance (R*) is when death is less than 80% of that under the diagnosis dose, elementary resistance (M*) is when death is between 80% and 99% and susceptibility (S*) is when death is greater than 99%.

With best wishes
Dr/ Mohamed M. Baz