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PESTICIDES IN CROP PRODUCTION

PHYSIOLOGICAL AND BIOCHEMICAL ACTION

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Pesticides in Crop Production

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Physiological and Biochemical Action

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Preface

Pesticides have had a tremendous role in enhancing productivity and yield of crops prominently after the second half of the twentieth century. Most of the countries across the world are observing newer heights in total as well as specific crop production despite the fact that the agricultural fields are being used in non-agrarian tasks like the construction of roads, railways, industries and buildings for human settlements. A massive extent of credit goes to use of agrochemicals in general and pesticides in particular. Increasing human population and constricting agricultural lands do not permit us to give up the use of pesticides and to switch over completely towards organic farming. Additionally, development in industries and agriculture are taken as a general criterion for development of any country. This has resulted into imprudent and unlimited usage of agrochemicals in our farmlands leading to disturbance in abiotic as well as biotic components of soil and water ecosystem and culminating into ecological imbalance.

Pesticides are the only toxic chemicals deliberately released into the environment in large amounts. Some of the pesticides (organochlorines) are biomagnified in the terrestrial ecosystems, so they were banned worldwide. The organophosphorus pesticides were introduced in the 1970s as replacements for the persistent organochlorines. The increased use of organophosphorus pesticides originally seen as lesser threat to the environment but by the time organophosphorus pesticides have become a serious environmental concern due to their high acute toxicity despite their low persistence. Since most of the pesticides are non-biodegradable, they have long residence time in water and soil and thus may enter and magnify at various trophic levels. Excessive and imprudent usage of pesticides not only saturates the soil but also intoxicates the crops by harming their overall physiology and biochemistry. In addition to this, non-target organisms that are important components of the soil ecosystem like soil microbes, bacteria, fungi and blue green algae (privileged to be associated with atmospheric nitrogen fixation, fertility of the soil and nutrient recycling) may be harmed, which may indirectly affect the productivity and food security.

This book titled 'Pesticides in Crop Production: Physiological and Biochemical Action' is an important contribution towards understanding mode of pesticide action in plants, pesticide metabolism in soil microbes, plants and animals, bioaccumulation of pesticides, sensitiveness of microbiome towards pesticides and consequent risk assessment, development of pesticide resistance in pests, microbial remediation of pesticide intoxicated legumes, pesticide toxicity amelioration in plants by plant hormones. This book

also encompasses eco-friendly pest management, transgenic strategies to develop resistant plant against the pathogen and pest and impact of pesticide on food stuffs and human health. Analysis of pesticide by GC-MS/MS (Gas Chromatography tandem Mass Spectrometry) is a reliable method for the quantification and confirmation of multiclass pesticide residues in cabbage and cauliflower as case studies has well been included.

Writing an authoritative book that remains relevant over the coming years cannot easily be done by an individual, but rather requires the concerted effort of a team of expert scientists. This book is a concerted task of an assemblage of scholars working in different parts of India and the world along with all the six editors. All editors thankfully acknowledge their contributions. All editors also gratefully acknowledge the team at John Wiley & Sons Limited which made possible the proposed book in its present form.

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Development of Pesticide Resistance in Pests: A Key Challenge to the Crop Protection and Environmental Safety

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1.1 Resistance: The Introduction

Resistance is the micro-evolutionary course of action by which genetic adaptation through pesticide selection has resulted in increased arthropod populations for which management is more difficult (Whalon and McGaughey 1998). The outcome of resistance is the malfunction of plant protection tools, strategies to limit economic injury of pest populations where failure is due to a genetic adaptation in the pest.

Resistance to pesticides is a complicated and substantial problem in circumstances where chemicals are used to eradicate pest populations. On the other hand, against the economic, communal, and ecological costs linked with this problem, resistant insects are a physiological marvel. Certain populations have become highly resistant to a specified insecticide, which can survive exposure to almost any dose. More than 440 species of pest which have developed resistance to one or more pesticides have been documented. One of the most amazing things in evolutionary adaptation is pesticide resistance due to environmental changes, especially when this has occurred relatively quickly in terms of evolutionary time. Prevalent distribution of resistance in crops and livestock pests is the major threat to the agricultural productivity and many of the serious resistance problems are also documented.

Understanding the molecular mechanisms and resistance adaptations in pest populations is a significant problem. However, the molecular mechanisms of pesticide resistance have continued and the understanding of these resistance mechanisms plays an important role in improving the integrated management and in identifying new targets for the vaccine development which is useful for eradicating the pesticide-resistant pests on agriculture and for public health. Knowledge about resistance will pave the way for the fundamental perceptions into evolution, genetics, physiology, and ecology. Resistance can also make a severe economic loss with social disruption.

Over the past 15 years, the global area allotted to transgenic crops is more than 69 million hectares for reducing insecticidal toxins resultant of the bacterium *Bacillus thuringiensis* (Bt) which has emerged quickly (James 2008). Among these, Bt cotton and Bt maize were the most cultivated plants in this area (James 2008). Effective control of target pests, diminished use of conventional insecticides, and reduced harm to

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non-target organisms are the important benefits of the use of Bt crops (Huang et al. 2005; Cattaneo et al. 2006; Marvier et al. 2007; Hutchison et al. 2010). Another theme is, giving greater importance to field trials and assessment of resistance in field populations will improve resistance management from concept to practice. The final theme is the next generation methodology of pest control which may greatly depend on microbial toxins, mostly through the expression of *Bt* toxin genes in genetically engineered crop plants and microorganisms. The remarkable usefulness of *B. thuringiensis* in killing some pests but which are not applicable for all the species is one of the drawbacks of this technology.

1.2 Pesticide Resistance: A Global Analysis

The evolution of resistance against pesticides is a fundamental problem of modern agriculture (Takahashi et al. 2017). The Analysis of Global Pesticide Resistance arose because of the exponential increase in the cases of resistance worldwide during the second half of the twentieth century and also the recognition by industries of new chemistries ended up with novel modes of action which are a precious resource that should be conserved. International Insecticide Resistance Action Committee (IRAC) mainly worked on different aspects of resistance management, such as detection and monitoring programs, and even more helpful is IRAC's utmost development, which is the effort to develop resistance reporting by mode of action (MOA) classification of pesticides. Based on that, the agrochemical industries have often put the effort to understand, define, monitor, and manage pesticide resistance (www.irac-online.org). The pesticide industry formed IRAC and other resistance action committees after scientific, public, and new regulatory pressures.

1.3 Molecular Genetics and Biochemical Basis of Pesticide Resistance

For the last three decades, incredible advancements have been made in understanding resistance of pesticides in arthropods, initially biochemical and physiological mechanisms, and more recently at the level of molecular genetics and genomics. The greatest improvement in molecular genetic studies has exposed many details about the resistance mechanisms, both at individual and population levels. That improvement has provided new perceptions on the microevolutionary processes that have been produced by them; it has also revealed unforeseen complexities that are very complicated to unravel. There are several mechanisms available for pesticide resistance which has been discussed below (Figure 1.1).

1.4 Changes in Pesticide Binding Sites

Every potent pesticide has one or more specific binding sites on macromolecules within the insect except mitochondrial uncouplers. The malfunctioning of the macromolecular site of action results in the binding of insecticide, that initiates a cascade of events

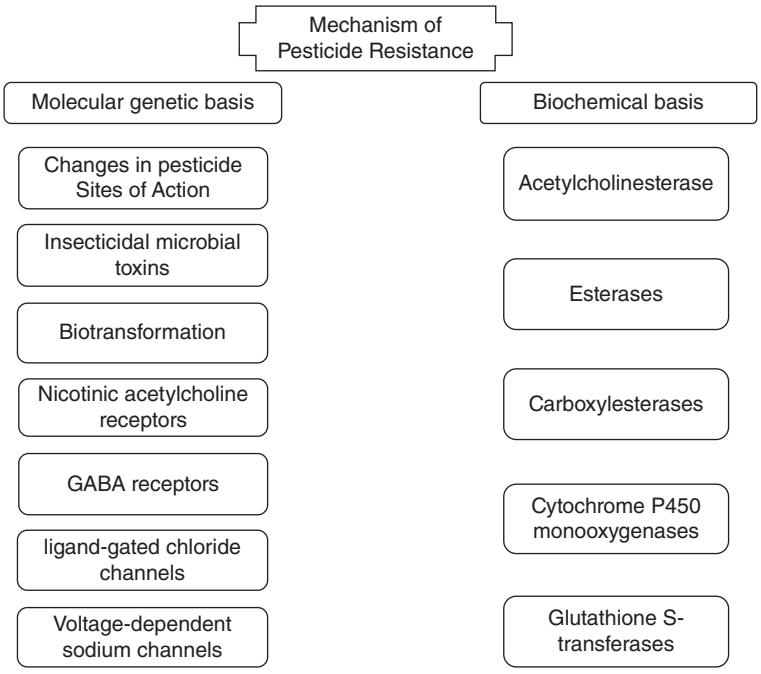

Figure 1.1 Schematic diagram for pesticide resistance mechanisms.

which leads to the death of the particular insect. Changes in insecticide binding to the site of action, or to disturb its functions after binding, must lead to major changes in the overall impact on the insect. There is plenty of evidence that changes are the initial cause of resistance to different types of pesticides. Mostly, the point mutations lead to critical changes in amino acid residues in the receptor molecule compared to changes in the expression level of existing receptors. However, in some cases it seems that a functional target site is not dangerous for the existence of the insect even though its interaction with the pesticide leads to death. Significant changes on sites, either through mutation or decreased expression, are not always disadvantageous, and sometimes the complete elimination of the gene product (null mutation) is a viable pathway to greater levels of resistance. There have been several researches which like, Gahan et al. (2001) confirmed the loss of a cadhedrin-binding protein for *B. thuringiensis* toxin in *Heliothis virescens*; the loss of the nicotinic acetylcholine receptor subunit that binds the spinosyns in resistant Drosophila (Orr et al. 2006); and the loss of a binding protein for juvenile hormone analogs in Drosophila (Wilson and Ashok 1998).

1.5 Nicotinic Acetylcholine Receptors

There are two different types of neurotransmitter ACh available in insects, including muscarinic receptors, which were linked to slower G-protein mediated postsynaptic actions, and a nicotinic receptor that open ion channels through the neuronal membrane prominent to a rapid but transient shift in membrane polarization. In resistant strains of *Drosophila melanogaster* formed by mutagenesis, resistance is attributable

to numerous different mutations in a gene encoding the Dα6nAChR subunit, which in some cases leads to the complete loss of this site of action for the insecticide (Orr et al. 2006). Resistance to neonicotinoids is increased with their extensive use and has been reviewed by Nauen and Denholm (2005). In a few cases, increased degradative metabolism, mainly by monooxygenases, is involved. However, in other cases, evidence for an alteration in the sensitivity of the nervous system to the effects of the insecticide has been reported (Liu et al. 2005; Mota-Sanchez et al. 2006). In the brown plant hopper, resistance to imidacloprid has been attributed to a mutation in AChR subunits that decreases specific binding to nervous system membrane preparations (Liu et al. 2005). The occurrence of a single mutation (Tyr151Ser) in a conserved region believed to be involved in ACh binding was found in two subunits, Nlα1 and Nlα3, and associated well with the existence of imidacloprid resistance. When these insects α-subunits were co-expressed in rat β2-subunits to form chimeric receptors, a virtually complete loss of imidacloprid binding was observed compared with the same subunits from susceptible insects.

1.6 GABA Receptors and Other Ligand-gated Chloride Channels

γ-Aminobutyric acid (GABA) is the main inhibitory transmitter in the insect nervous system, with GABA-ergic transmission occurring both within the central nervous system and at the neuromuscular junction (Casida 1993; Hawkinson and Casida 1993; Casida and Pulman 1994; Ozoe and Akamatsu 2001; Buckingham et al. 2005; Ozoe et al. 2009; Ozoe 2013). These fast-acting GABA receptors (GABARs) are linked to the channels that gate chloride ions and they lead to hyperpolarization. Another group of GABARs in insects' gate cations is having an electrifying effect, but very less is known about their functions and their insecticide action. Their MOA is distinct from that of the GABARs gating chloride channels (Gisselmann et al. 2004). The structures, functions, molecular genetics, and interactions of inhibitory GABARs with insecticides have been reviewed by Buckingham and Sattelle (2005) and Buckingham et al. (2005). The structure of inhibitory GABARs is pentamers with the subunits organized to form a central ion-conducting pore. Each subunit has four transmembrane domains and the receptor has abundant distinct binding sites for xenobiotics. There are several forms of the subunits which exist *in vivo* even though somewhat little is known about the number, nature, specific functions, or localization of the native receptors. Ionotropic receptors that are gate chloride ions occur in the insect nervous system having either glutamate or histamine as their activator. Particularly, glutamate-gated channels (glutamate H receptor) (GluHR) have a vital role in insecticide action. For certain compounds, including the avermectins, which act on both GluHR and GABAR are possibly involved in their complete toxicity to insects.

1.7 Voltage-Dependent Sodium Channels

Sodium ions often move across the axonal membrane is an important factor in the enhancement of a nerve action potential. The opening of voltage-dependent

sodium-specific channels produced as the wave of depolarization induced by an approaching action potential reaches a critical value. The sodium channels are quickly inactivated, after the opening, which switch over the inward flow of sodium current and consequently limits the depolarization. Three different groups of insecticides influence this process by their actions on voltage-dependent sodium channels (VDSCs). Even though having different structures, its relatives and the pyrethroids have their MOA as a common feature. They slow channel deactivation and trap sodium channels in the open configuration, to modify the sodium channels, resulting in elongated channel opening evidenced by a large tail current associated with repolarization (Vijverberg et al. 1982; Narahashi 1988). This may lead to the repetitive discharge of action potentials, or, if depolarization is in an elevation state, a complete block on axonal transmission will formed (Narahashi 2002). Both of the actions have greater effects on the nervous system. Indoxacarb is an oxadiazine insecticide that acts on VDSCs by a different mechanism of actions. Indoxacarb and its N-decarbomethoxylated metabolite block the sodium channels of various insects and mammalian neurons by maintaining VDSCs in an inactivated form (Wing et al. 2010). These studies proposed that the actions of indoxacarb and DCJW (N-decarbomethoxylated metabolite) share some similarities with that of local anesthetics, which impede sodium currents by binding selectively to the inactivated state of the sodium channel (Hille 1977). Changes in target site mediated resistance to indoxacarb have not yet been reported, however, mutations in the VDSC are a general cause of resistance to Dichloro diphenyl trichloroethanes (DDT) and the pyrethroids (Soderlund et al. 2002; Soderlund and Knipple 2003; Khambay and Jewess 2005; Soderlund 2005; Dong 2007).

1.8 Insecticidal Microbial Toxins

Currently different types of proteinaceous bacterial toxins were utilized for the insect control. Among which, *B. thuringiensis* (*Bt*) and *Bacillus sphaericus* (*Bs*) are the two major sources. *Bt* strains produce arrays of crystal protein δ-endotoxins which are availed either in prevalent spray-on applications or are genetically engineered into plants. More than 200 disparate toxin-producing *Cry* genes have been established. The collection, nomenclature, and uses of *Bt* toxins have been summarized by Bravo et al. (2005). Certain groups of endotoxins are produced which are intended to be profoundly specific for different orders of insects, including lepidopterans, coleopterans, and mosquitoes. Behind the solubilization and proteolytic activation into the insect gut, it is considered that *Bt* endotoxins apply their toxicity by binding to receptors on the mid gut epithelial cells of sensitive insects. The group of several toxin molecules then directs their insertion into the luminal membrane to form a pore. This comes up with the loss of ionic control which leads to the osmotic disruption of the mid gut cells causes swelling and lysis, which is lethal. Some types of receptor, including glycosyl phophatidylinositol-anchored aminopeptidase-*N* (APN), a digestive enzyme and cadherins for the *Bt* toxins, which are toxic to lepidopterans have been identified on the mid gut epithelial cell surface, known to act as intercellular adhesion molecules. Various receptor types are visibly involved in the actions of different *Bt* toxins, while binding site-based resistance to Cry1A toxins does not direct to cross-resistance to Cry1C toxins (Ferré and Van Rie 2002). The different mechanisms of resistance to *Bt* genes

which have been discovered until now, have been reviewed by Ferré and Van Rie (2002), Bravo *et al*. (2005), and Griffitts and Aroian (2005). The diamond-back moth (*Plutella xylostella*), is the only one insect species known to have resistance to *Bt* toxins during field exposure and the complete mechanism for this resistance is not identified. Yet, it is monogenic and partially recessive and is characterized by a remarkable decrease in the specific binding of radiolabelled Cry1A toxins to encounter the border membrane vesicles isolated from the resistant mid guts (Ferré and Van Rie 2002; Sayyed et al. 2004, 2005). The exposure of a premature stop codon into the cadherin coding sequence has also been directly linked to Cry1Ac resistance in *Helicoverpa armigera* (Xu et al. 2005). In this perspective, it is fascinating that three different recessive cadherin mutations were recognized in the field-identified pink bollworms, *Pectinophera gossypiella* (Morin et al. 2003). Respective genetic changes lead to the deletion of at least eight amino acids upstream of the presumed binding zone. Even though, resistance only arose from a combination of any two of the deletion-bearing alleles were closely linked.

1.9 Biotransformation

The metabolic changes of an insecticide within the target organism are a frequent defensive mechanism that directed toward a decrease in the period and intensity of the exposure to the target site, which minimize the probability of lethal condition. Several insects have developed wide and rapidly inducible defenses against pivotal toxic xenobiotics that are initially taken in during the diet; therefore, these defenses may be modified to behave as the path of resistance. Three key mechanisms of metabolic transformation of insecticides cause the huge number of examples of biotransformation-based resistance: (i) oxidation; (ii) ester hydrolysis; and (iii) glutathione conjugation. Although, the products of these reactions are most frequently less toxic than the parent, there are numerous examples of an increase in toxicity as a result of a biotransformation reaction, in which the insecticide is applied as a pro-pesticide. Apparently, an increase in the rate of metabolic conversion in this case should result in being more toxic to the insect, and a decrease in the rate of activation is one of the routes to resistance. By comparing to the resistance evolving from site-of-action changes in which mutations in the structural genes are most predominate as a fundamental mechanism, biotransformation-based resistance frequently involves the overexpression of prevailing metabolic enzymes by modifications in their regulatory systems and by gene duplication. Esterases, cytochrome P450 monooxygenases, and glutathione transferases are the most significant factors in specific cases of resistance. Further conjugation reactions, including glucose and sulfate conjugations likewise appear to involve little role in well-known cases of resistance.

1.10 Acetylcholinesterase

Acetyl cholinesterase (AChE) play a primary role in the removal of excitatory neurotransmitters and the acetylcholine (ACh) from cholinergic synapses, which is the target region for carbamate and organophosphate inhibitors in both insects and vertebrates (Giacobini 2000). The inhibition of AChE through accumulation of ACh leads

to the continuous stimulation and the desensitization of the ACh receptors (AChR), severe neurological disruption, and eventually to death. The molecular architecture of the homodimeric AChE enzyme and its catalytic site were initially described based on crystallographic analysis in Torpedo (Sussman et al. 1991) and the parallel majority of structural features in the Drosophila enzyme were described by Harel et al. (2000) and revised by Oakeshott et al. (2005). Resistance to the insecticides like organophosphorus and carbamate in most of the arthropod pests were conferred by a series of common/shared point mutations in acetylcholinesterase (AChE). However, the mutations linked with the insecticide sensitivity often results in reduced catalytic efficiency and leads to a fitness disadvantage (Lee et al. 2015).

AChE has different allosteric sites that modify the activity of the enzyme, but these are not usually considered as the primary site of action of pesticides. Diptera family insects have only one gene for AChE whereas other insects, including mites and mosquitoes, have two, while possibly only one (ACE-1) is expressed in, and is active in eradicating ACh from the central nervous system (Russell et al. 2004). Interestingly, this gene is not the orthologous or even the single enzyme in the higher Diptera, so it seems that two somewhat distinct genes encode for the neuronal AChE even in different members of the same order, Diptera (Weill et al. 2002). Recently the third ACE gene has identified by Fournier (2005) and the functions of the other ACE genes are unknown, but they do not appear to be related to resistance.

1.11 Esterases

The majority of Esters are the most widely used insecticides. Those are almost all carbamates and OPs, maximum of pyrethroids and other compounds such as indoxacarb, methoprene and similar juvenoids, fluacrypyrim, and bifenazate. Most of the cases, the hydrolysis of the ester group lead to a significant reduction in, or total removal of toxicity. Only in a few cases does ester or amide hydrolysis act as an activation reaction; for example, indoxacarb, acequinocyl, or dinitrophenol esters such as dinocap are all influenced by ester hydrolysis for their toxicity. Subsequently, esterase activity frequently plays an important role in determining the comparative responses and resistance to present insecticides. In insects, esterases hydrolyse the esters of carboxylic acids so therefore they are termed as carboxylesterases. The nature and consequence of esterases in insecticide toxicology and resistance have been reviewed by Oakeshott et al. (2005) and Wheelock et al. (2005). The different types of structural features of the substrate also changes the rates of ester hydrolysis.

1.12 Carboxylesterases (B-Esterases)

More than thirty genes of insects have been involved in the production of esterases that hydrolyse the carboxylic acid esters. They are members of the large and versatile family of enzymes that contain the α/β hydrolase fold with a nucleophile–acid–histidine catalytic triad (Oakeshott et al. 1999), and which was distributed into several subgroups (Oakeshott et al. 2005; Wheelock et al. 2005). One subgroup which is inhibited by OPs and most importantly, which hydrolyses aliphatic substrates is generally

termed as carboxylesterases. These include phosphotriesterases. Calcium-dependent phosphotriesterases promptly hydrolyse many insecticidal Ops; to be precise the more labile phosphate esters, mostly cleaving the ester at the most anhydride bonds (Vilanova and Sogorb 1999). Mostly, in mammals, it presents predominantly but much less in fish, birds, and several insects OP metabolism (Dauterman 1983; Vilanova and Sogorb 1999).

1.13 Cytochrome P450 Monooxygenases

The cytochrome P450 catalyzes the multifunctional monoxygensases, which encompasses the highly versatile system for the metabolism of insecticides. These enzymes play a major role in the toxicity of many pesticides and are a key player in the development of resistance in insects. The cytochrome P450-dependent monooxygenases mediated resistance has been reviewed by Bergé et al. (1998), Scott (1999), and Feyereisen (1999, 2005). The monooxygenase was regulated by NADPH through a flavoprotein, NADPH: cytochrome P450 oxidoreductase. Cytochrome b5 will possibly involve electron transfers with some forms of cytochrome P450. A huge number of the cytochrome P450 (CYP) superfamily genes is present in insects, with nearly 100 so far characterized in some insect genomes. Some of the genes possess known physiological functions in intermediary metabolism, but others play a role in defenses against the many xenobiotic chemicals. Previously in several studies P450 inhibitors have been used, mainly piperonyl butoxide (PBO), that synergize compounds degraded by P450. The role of P450 in resistance was indicated by a decline in the resistance level in synergized insects. The PBO is not completely specific for oxygenase reactions; besides it may decrease the proportions of insecticide penetration (Sanchez-Arroyo et al. 2001) and may inhibit certain esterases by synergistic activities (Young et al. 2005). In order to conclude the resistant strains has greater level of P450 catalyzed reactions compared to susceptible strains; model P450 substrates are used widely. This has been produced useful information and caution is necessary, although meanwhile several P450 isoforms are available which varied extensively in substrate specifications.

1.14 Glutathione S-Transferases

The Glutathione S-transferases (GST) are a huge group of enzymes that enrich the reaction of the cysteine sulphydryl group of the tripeptide glutathione (GSH) with xenobiotics. In xenobiotics, the sulphydryl group of GSH a nucleophile which reacts with the electrophilic sites that leads to the GSH conjugate formation. The less toxic conjugates are most freely evacuated than the parental insecticide. The overall properties and toxicological significance of GSTs have been reviewed by Eaton and Bammler (1999), Sheehan et al. (2001), and Hayes et al. (2005). Even though a cluster of microsomal GSTs exists in insects, the transferases of high toxicological interest is soluble, which are relatively small (50–55 kDa) proteins with a dimeric structure. Certain GSTs are very quickly inducible by improved transcription after exposure of the insect to xenobiotics, such as pesticides and phytochemicals however GST activity in resistance systems is unknown (Yu 1996).