

Review

Mechanism of Insecticide Resistance in Insects/Pests

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Received: 11 December 2018

Accepted: 14 April 2019

Abstract

The main purpose of this study is to provide essential information regarding the molecular basis of insecticide resistance and to report candidate genes which are responsible for resistance in insects/pests. There are two basic resistance mechanisms existing in pests, i.e., target site resistance and metabolic resistance. During resistance of target site, the specific binding site of an insecticide is modified (mutated) and/or lost, which makes the target site incompatible for activation. Mutation occurs in most common pest (*Myzus persicae*, *Musca domestica* and *Drosophila melanogaster*) target regions, i.e., subunits like nicotinic acetylcholine receptors (nAChRs), knock-down resistance (KDR) etc. Due to these mutations, insecticides are unable to bind into the target region, resulting in loss of binding affinity. Furthermore, in metabolic resistance over production of enzymes occurs which break down (detoxify) insecticides and resulting resistance of pests. The amplification of metabolic enzymes, i.e., Cytochromes p450 monooxygenase, hydrolases, and Glutathione S-transferase play a central role in evolving metabolic resistance. Various successful approaches are used to combat pests resistance such as insecticides, bio-pesticides and biological control agents. However, some of these strategies have certain limitations such as contamination of the environment, while others possess a low capacity in management of pests. Recent studies have highlighted some novel mechanisms of insecticide resistance that are part of the ongoing efforts to define the molecular basis of insecticide resistance in insect species.

Keywords: resistance, pests, target-site, metabolic, insecticides

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Introduction

Insects/pests are very much hazardous for agriculture crops and forests and are highly engaged in a struggle with each other in order to obtain food. These pests interfere with production, processing, storage, transport, food marketing, agricultural commodities, wood products and animal feed stuffs, etc. They cause excessive damage to crops, fruits and retrograde quality of food products [1]. According to a current report, estimated destruction of crops is 7 to 50% annually [2, 3]. They have the capability to perform direct contamination and also act as vectors for various diseases caused by viruses, bacteria, etc., which intensively influence humans/animals and plants [4]. Therefore, it is essential to control and maintain harmful pests that have adversely affected our economy in the past. Approximately 40 billion dollars are lost due to various pest infestations annually [5]. The use of insecticides is one of the most efficient methods of pest management. These are chemical substances used for the destruction of pests and are immensely dangerous for humans/animals and plants. Therefore, a billion kilograms of insecticide are used every year in order to fight the infestation of crops [6]. The use of insecticides has increased in many countries, i.e., Germany (4800 tons), Poland (2400 tons), Britain (18000 tons), Italy (62000 tons) and 1.7 million tons in China [7].

The use of insecticides in agriculture is to control pests and vectors of various diseases. Insecticides such as Neonicotinoid, Thiamethoxam, Pyrethroids and Organophosphates are used as a primary control agent for several pests [8]. The excessive use of insecticides creates insecticide resistance in pests and is considered the greatest example of micro-evolution. Previous studies have shown more than 500 different types of pest species that have evolved insecticide resistance [9]. Various pests such as corn earworm and other species target numerous agricultural crops globally, i.e., tobacco, peanuts, cotton etc., and exhibit resistance to

novel insecticides. Several factors, including biological, genetic and operational involved in emerging of resistance, while genetic factors are considered the most advantageous [10]. However, controlling pests is incredibly challenging due to the constant spreading of insecticide resistance in future populations [11]. Different molecular mechanisms of resistance are indicated in pests, i.e., target site and metabolic.

Target-Site Resistance

During target-site resistance the binding site of an insecticide is modified (mutated) or lost and catalyzing the target-site is incompatible for activation [12]. A previous study on pests, i.e. *Anopheles albimanus*, *Culex quinquefasciatus*, Mosquitoes and *Culex pipiens pipiens* has suggested that alteration of target genes might lead to the reduction of binding affinity with insecticides, thereby overwhelming the effect of insecticides [13]. Four different types of target site resistance mechanisms exist in insects/pests.

Nicotinic Acetylcholine Receptor-Based (nAChRs) Target-Site Resistance

Pests are proficient in developing resistance to multiple classes of chemicals, i.e., organophosphates, carbamates and pyrethroids. Pests such as aphids (*Myzus persicae*) have developed resistance to 70 different synthetic compounds and have emerged as the first species in world with a high number of resistance mechanisms [14]. Neonicotinoid is of utmost significance and is the most widely used insecticide in the world for targeting the nervous system, resulting in pest paralysis. The neonicotinoid target site is nicotinic acetylcholine receptors of the central nervous system and induced target-site of resistance. Nicotinic receptors are ligand-gated ion channels consisting of five subunits and are arranged in combinations from a family of different subunit subtypes [15]. There are 10 nAChR

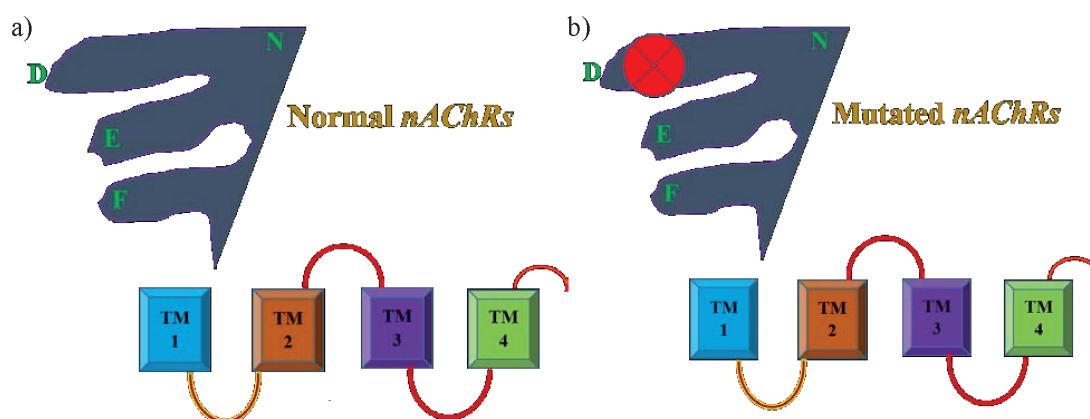


Fig. 1. Identifying target region (nAChRs) of insecticides with normal subunit a) while mutated region of pests indicate the insensitivity of the insecticide to the target region and hence develop resistance in future generations [32].

genes present in aphids, which consist of six subunits (*Mpa1* to 5 and *Mpβ1*). A single point mutation in the D-loop region of nAChRs “*Mpβ1*” subunit has been reported that developed resistance to Neonicotinoids [12]. The nAChRs of *Musca domestica* consist of subunits *Mda2*, *Mda5*, *Mda6*, and *Mdβ3* [16]. The neonicotinoid resistance mechanism has been identified in Danish house flies that depend on cytochrome P450 monooxygenase-mediated detoxification and the resulting reduced expression of nAChRs *Mda2* subunit [17]. The subunit *Mda2* has been isolated and characterized, which shows the homology to the *Da2* subunit of fly *Musca domestica*. The genomic sequence of *Mda2* consists of eight exons and is located on autosome 2. The current study was assumed to detect profile expressions and mutation in the subunit (*Mda2*) that introduced resistance in *Musca domestica* [18]. Spinosad insecticide has a distinct mechanism of action which primarily strikes the target site of nAChRs and GABA receptors. Therefore, alteration of such a target site is responsible for Spinosad resistance. Spinosad resistance of *Musca domestica* is associated with the recessive factor on autosome 1, and such autosomal resistance is controlled by more than one gene [19]. Ten nAChRs genes are found in *Drosophila melanogaster* (i.e., *Da1* to *Da7* and *Dβ1* to *Dβ3*) [19]. Subunit *Da6* is responsible for the high level of Spinosad resistance, while subunit *MDa6* is not related to Spinosad resistance [20, 21]. Mutation in *Da1* or *Dβ5* developed resistance to Neonicotinoids insecticides. According to Watson single ‘*Da6*’ is not responsible for resistance and hence has zero capacity to produce sensitive receptors against Spinosad, while the co-expression of *Da6*’ with ‘*Da5*’ ER evolved resistance to Spinosad Fig. 1 [32].

Modified Acetylcholine Esterase-Based (MACE) Target-site Resistance

Acetyl-cholinesterase is a serine esterase belonging to the family of (α , β) hydrolase fold enzyme that has involved in resistance. An enzyme acetylcholine esterase produced by pests is used as a neurotransmitter of impulses, causing breakage of insecticides, resulting in paralysis and finally death [22]. Pirimicarb is an anticholinesterase insecticide whose primary function is inhibition of enzyme acetylcholine esterase [23]. Modified acetylcholine esterase (MACE) provides powerful resistance to the fundamental class of insecticides such as carbamate and organophosphate [14]. Thus pests

are categorized as MACE and non-MACE. Target-site resistance of pests occurred during the mutation of the gene, which encodes an enzyme acetylcholine esterase. This MACE causes insensitivity to dimethyl carbamates such as pirimicarb and organophosphate [24]. They are typically involved in the modification of AChE and specify slight sensitivity to inhibitors. The mutant form of AChE has been characterized biochemically, which indicates a broad spectra of insensitivity among species. Previously, four types of mutation are identified in the housefly gene that encodes AChE and is related to these phenotypes. Mutation of *G262V* in a strain reveals powerful resistance to carbamates and illuminates the significant role of five mutations that confer resistance to organophosphates and carbamate due to the expression of modified AChE [25]. *Musca domestica* is the first pest exploring the presence of five types of mutations in the AChE gene (Val-180!! Leu, Gly-262!! Ala, Gly-262!! Val, Phe-327!! Tyr and Gly-365!! Ala), and with different ranges of insecticide resistance [26]. Point mutation (SNP) occurred in the acetyl cholinesterase gene of *Drosophila melanogaster* and evolved resistance to organophosphates and carbamates (Table 1) [27].

Knock-down Resistance (KDR)

The voltage gated sodium channel of the central nervous system (CNS) consisting of 4 trans-membrane domains are recognized as a primary target-site for some insecticides such as pyrethroid and organochlorine. Any modification of leucine to phenylalanine in the voltage gated sodium channel protein of CNS causing KDR against insecticides. Insecticides (pyrethroid and organochlorine) causing KDR or super-KDR are conferred by modification in a voltage-gated sodium channel protein [28]. Resistance to pyrethroids has long been investigated, primarily depending on KDR factor [29], which is responsible for reducing sensitivity in the housefly’s central nervous system to DDT. The previous 15 years of research has provided substantial proof of KDR resistance in houseflies, which consists of *KDR*/super-*KDR* caused by point mutation in the (*vssc1*) gene. The most common type of L1014F mutation was found in KDR strains of housefly related to KDR resistance [30]. The substitution of leucine *Leu1014* to phenylalanine *L1014F* occurred in the 6th trans-membrane segment of domain II at position 1014, which is associated with resistance to Pyrethroids and DDT in various species of pests [31, 32].

Table 1. Classifying resistance of most pests against insecticides due to mutation in acetylcholine esterase enzymes; this type of modification changes AChE into modified acetylcholine esterase.

Species (pests)	Insecticides	Mutations	Results
<i>Myzus persicae</i>	Carbamates and organophosphate	AChE	MACE
<i>Musca domestica</i>	–	5- types of mutations in (ACHE)	–
<i>Drosophila melanogaster</i>	–	(SNP)-point mutation	–

Duplication of Resistance to Dieldrin (RDL) Gamma-Amino Butyric Acid (GABA) Receptor

Most of the pests revealed resistance to the dieldrin (*Rdl*) gene with primarily functions of encoding GABA receptors consisting of five subunits around a central gated ion channel and exhibited insecticide resistance (cyclodiene) in fly (*D. melanogaster*) and other species [33]. The number of *Rdl* genes is one in most pests, while those found in different allelic forms are in some insects/pests. A single nucleotide polymorphism (SNP) mutation in (*Rdl*) affects the normal function. Resistant phenotype is related to a single mutation of alanine to serine at position 302 in the second transmembrane region of the *Rdl* subunit [34]. Four types of alleles are present in *Myzus persicae* in which wild type of allele (called allele A) encode 'Ala302,' while the other three alleles encode 'Gly302,' also known as allele 'G', TCG codon encodes 'Ser302' and 'AGT' codon encodes 'Ser302' (known as allele 'S'). Alanine and glycine are the central cause of resistance, while the other locus of two serine-containing "s" alleles are not responsible for resistance [34]. The locus S/S is responsible for resistance, while A/G has a mechanism of resistance to GABA receptors and is insensitive to dieldrin [24].

Metabolic Resistance

Metabolic resistance is the detoxification of chemicals/insecticides that occurs in insects/pests. The over-production of some enzymes breaks down insecticides before reaching and binding the target sites. The over-produced enzymes in pests have the capacity to develop protection against insecticides [35-37]. Metabolic resistance is a common mechanism of defense that depends supremely on various enzymes such as monooxygenases (e.g., cytochrome P-450 monooxygenases), hydrolases (e.g., esterases), and transferases (glutathione-S- transferase) detoxicate [38]. The significance of these enzymes is that they detoxify xenobiotics into non-toxic compounds. There are two stages of detoxification: primary phase I comprising of hydrolysis or oxidation and secondary phase II consisting of conjugation of phase I products (Fig. 2) [39].

Over-production of Esterases

Esterase is a large group of phase I metabolic enzymes with the capacity to metabolize different endogenous and exogenous substrates. The esterases such as E4 and FE4 are over-produced in response to some classes of insecticides by degrading ester bond of pyrethroids, organophosphates, carbamates and neonicotinoids before reaching the target-site and the resulting insecticide resistance [40]. The over-expression of esterases is due to gene amplification, up-regulation and/or a combination of both. The over-production of carboxyl-esterase was observed in green peach aphid.

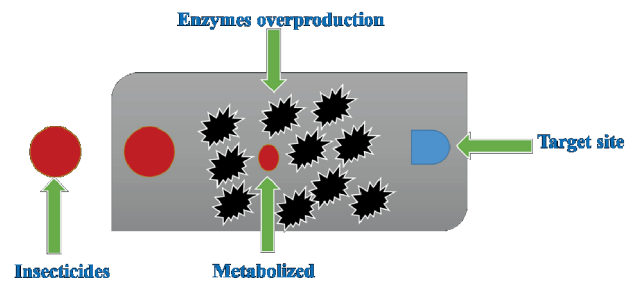


Fig. 2. Signifying that amplification of metabolic enzymes detoxify the insecticides before reaching their target region and causing resistance in pests.

E4 and FE4 esterases are produced by *E4* (19 kb) and *FE4* (5 kb) genes respectively. Fluorescence in situ hybridization (FISH) shows that resistant aphids have amplification in their *E4* gene (three in copy number) situated in a single heterozygous site on autosome at position 3T. The amplified *FE4* genes are found in aphids with normal karyotype and are widely distributed around the genome from three to eight sites. The resistant aphids consist of 5 to 11 times higher esterase gene sequences [41].

Over-production of Cytochrome P450s Monooxygenases

Cytochrome P-450s are unique enzymes with a significant role in breakage of endogenous compounds, xenobiotics, chemical carcinogen and insecticides such as neonicotinoid and organophosphates. They have the ability to catalyze different reactions, i.e., N-dealkylation, epoxidation, hydroxylation, desulphurization or O-dealkylation. P450s have a substantial role in plant host interactions and the metabolism of different insecticides [42, 43]. The over-production of cytochrome p450 monooxygenase enzymes occurred in response to neonicotinoid insecticides and conferring metabolic resistance in pests. Resistance of an aphid is primarily associated with multiple duplication of the single-cytochrome P450 (*CYP6CY3*) gene. The resistant aphids possess 18 copies of the gene while susceptible consist of two copies of the gene. A single cytochrome p450 gene increased resistance 22-fold in resistant aphids. The over-expression of cytochrome p450 has revealed mutation in resistant pests. Cytochrome P450 genes are over-expressed in most common pests such as *Myzus persicae*, which cause neonicotinoid resistance [44]. The insertions/deletions occur in the sequences of "cis" as an acting promoter and in trans-acting regulatory loci. Cytochrome P-450 enzymes detoxifying in the resistance were primarily implicated in using synergism termed as Piperonyl butoxide (PBO) [12]. A higher level of differences between catalytically and spectral characteristics of cytochrome P-450s have been detected in the resistant pests as well in the *Musca domestica* housefly [45]. Neonicotinoid resistance is

associated with over expression of (*CYP6A1*, *CYP6D1* and *CYP6D3*) genes in *Musca domestica*. The subunit *CYP6D1* is over-expressed in the male resistant housefly while *CYP6D3* is over-expressed in the female resistant fly [46]. Two resistance loci of p-450 genes are responsible for resistance in *Drosophila melanogaster* subunits, i.e., *CG10737*, *Cyp6w1* and causing resistance to DDT [47].

Over-production of Glutathione-S-transferase

Glutathione-S-transferases (GSTs) are known as ligandins and are extensively familiar in catalyzing conjugation of minor forms of glutathione to xenobiotic substrates that are mainly used for detoxification purposes. The central role of GSTs is the detoxification of various compounds such as endogenous and xenobiotics. They are also involved in intracellular transportation, protection against oxidative stress and biosynthesis of hormones [48]. The GSTs consist of three super families: mitochondrial, cytosolic and microsomal (also known as MAPEG proteins). Cytosolic are involved in detoxification and consist of sub classes (Delta, Epsilon, Omega, Sigma, Theta, Mu and Zeta) [49]. Mitochondrial GSTs are also known as Kappa GSTs. They are found everywhere but are absent in pests [50]. The genes encoding glutathione-S-transferase amplify in many crop pests that evolved resistance to insecticides [51]. The enzyme glutathione-S-transferase is over-produced in insecticide-resistant clones of the fly *Musca domestica*. Furthermore, enhanced expression of GSTs are associated with Neonicotinoid resistance. 40 GSTs genes have been identified in *D. melanogaster*. The over-expression of these genes evolved resistance to DDT [52].

Pest Management Approaches

Insecticides

Insecticides are widely used for management of hazardous pests [53, 54]. Various insecticides are available to counter pest resistance such as Neonicotinoids, organophosphate, Pyrethroid, etc. Currently, Neonicotinoids are essential in declining pests as well as wild bee resistance [55, 56]. Despite the use of insecticides across the world, numerous problems have been raised, i.e., environmental contamination, hazardous effects on human/animal and future outbreak of resistance in pests. The exposure of insecticides and heavy metals (As, Cd, Cu, Ni, Pb, Sn, and Zn) in a combination form immensely affect human health [57-59].

Integrated Pest Resistance (IPM)

The enhancing severe problems of a pest's resistance and contamination of the biosphere are associated with the use of synthetic pesticides. Therefore, it is highly

essential to use effective biodegradable pesticides to combat pest resistance. Previously, various pesticides such as DDT and synthetic pyrethroids have been used, but unfortunately no single management strategies have revealed effective results against pests. Integrated pest management uses various methods against pest populations, which lessens the dependency of conventional pesticides in crop protection [60].

Biopesticides

Biopesticides belong in the significant group of pesticides that proficiently reduces risks of pesticides. They are obtained from plants and animals as well as microorganisms. Plant-extracted chemicals are highly biodegradable, less pollutant to the environment, apparently less toxic to non-target organisms and have lower effects as compared to synthetic pesticides [61]. The use of phytochemical products are supremely efficacious in controlling various hazardous pests. Currently, local plants are used world-wide for crop protection against a pest's infestation [62]. Plant-derived insecticides are used commercially (i.e., ryania, rotenone, pyrethrin, nicotine, azadirachtin and sabadilla) [63].

Biological Control

Biological controls are considered a highly significant approach for management of pests. The importance of this strategy stems from its effectiveness, safety and sustainability as compared to chemical insecticides [64]. Biological control agents are used to control environmental contamination of synthetic insecticides. Biological control agents (predators, parasitoids, and pathogens) target specific pests or introduce these organisms using inoculative techniques that might effectively reduce a pest's population [65]. Baculoviruses (Nucleopolyhedroviruses (NPV) and Granuloviruses (GV) are significant microbial agents used as biopesticides [66]. HaNPV are isolated locally and used on various plants in China, Australia and India [67]. Furthermore, SliNPV are very effective in controlling *S. littoralis* [68]. Entomopathogenic fungi are imperative factors to counteract a pest's population. The outbreak of fungi occurs mostly in favorable conditions. Approximately 700 species of fungi cause pathogenesis of pests [69, 70].

Conclusions

The conclusion of this review indicates that:

A) Resistance in pests is developed by the selective pressure of insecticides, and new individual (pests) are born inherently insecticide-resistant.

B) Two basic mechanisms of resistance exist in pests: target-site and metabolic. During target-site resistance the binding sites of insecticides are mutated

(SNP) and thus the binding affinity of insecticides is lost. Similarly, in most pests over-expression of metabolic enzymes occur, which neutralize insecticides before reaching their target region. Therefore, in future generations pests acquire resistance against insecticides. The pests utilize the target-site resistance and metabolic resistance as their principal strategies against insecticides.

C) Various prevention approaches are used for management of pests, i.e., insecticides, bio-pesticides, biological control agents, etc. These methods are somehow successful but unfortunately different limitations are associated such contamination of the environment, low capacity of management and reduction of binding affinity.

D) During management of pests using insecticides, several genes were discovered in order to combat resistivity. However, further studies would be needed to identify more candidate genes and some other novel strategies that pests may be utilizing for their survival against new classes of insecticides. This information will further explore the mechanism of resistance breakdown in insect/pests species that may lead to prevent future breakage of resistance.

Abbreviations

nAChR: Nicotinic acetylcholine receptors; KDRs: Knockdown resistance; PBO: Piperonyl butoxide; MACE: Modified Acetylcholinesterase; DDT: dichlorodiphenyltrichloroethane; RDL: Resistance to dieldrin; ACHE: Altered Acetylcholinesterase; GSTs: Glutathione S-transferases; GABA: Gamma-Amino Butyric Acid; HaNPV: Helicoverpa armigera Nuclear Polyhedrosis Virus; SliNPV: Spodoptera litura multinucleocapsid nucleopolyhedrovirus

Conflict of Interest

The author confirms that they have no conflict of interest.

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