Study of Different Resistance Mechanisms in Stored-Product Insects: A Review

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***Abstract*-** Stored grain pests have developed resistance against almost all kinds of pesticides and fumigants due to frequent and non-proper use of pesticides and fumigants. In postharvest ecosystems, the development of insecticide resistance among stored grain pests has threatened the global food security. Comprehensive detail of common mechanisms behind pesticide resistance in stored grain pests has been described in this review. The different resistance mechanisms stored grain pests usually developed to cope with pesticidal stress, include physiological mechanism, behavioral mechanism and biochemical mechanism. All the aspects of resistance mechanisms should be known in order to design effective strategy for the proper control of target pest.

I. Introduction

Global food production cannot be enhanced by only increasing the yield of food crops but also reducing all the elements which have adverse impact on food productivity. Major cause of post-harvest stored grain condiments losses are due to stored grain pests especially by insects estimated approximately 30% of 1800 million tons of stored grain. High rate of reproduction and short generation period usually make insects most ruinous pest of stored grain commodities as compared with pest. Although it is reported that 20 species of insects (excluding psocids) out of the 100 are evaluated as most destructive pests and are cosmopolitan by distribution (Haubruge *et al*., 1997; Andrew, 2004).

Among the stored grain insect pests, *T. granarium*, *R. dominica*, *S. oryzae*, *Sitotroga cerealella* and *T. castaneum*, are ruinous to great extent bringing about at least 5-10% loss over a year due to non-proper use of pesticides (Baloch *et al*., 1994; Tubiello *et al*., 2007).

Traditional strategies like use of plant extract, ginger, garlic lemon leaves typically are low-cost strategies of post-harvest handling of the crops but they have limited use because of many circumscriptions (Dakshinamurthy, 1988).

Mostly insect infestation of cultivated crops, plants and stored grain is controlled by chemical control methods with insecticides which are deployed mainly for the eradication target insect in specific area. Majority of insecticides are very noxious that is why specific pesticides with acceptable recommended doses for stored grains and possess no health concerns are mainly used. On the basis of chemical group present in insecticides are categorized as (OP) carbamates, organochlorines (OC), Organophosphates and pyrethroids among them organophosphate and pyrethroids are deployed on ample scale now days. Other pesticides deployed against insect pests are chlorpyrifos methyl, primiphos methyl, methyl parathion, lindane, piperonyl butoxide, diazinon dichlorvos, pyrethrins and malathion *etc.* (White *et al*., 1995; Lessard *et al*., 1998).

Fumigation is reasonable strategy by which a target pest can be killed by taking advantage from its respiration and plays very crucial part for the security of stored food products against rodents, insects and mites (UNEP, 2002). Commonly used fumigants for stored grain pests are, carbonyl sulphide (Desmarchelier 1998; Xianchang *et al.* 1999), ozone (Mason *et al.* 1999), hydrogen cyanide and ethyl formate (Haritos *et al.* 1999) and phosphine. However, phosphine is most advantageous for the proper management of stored grain pest due to cheap cost, safe appliance to a lot of stored food condiments, and it is also acknowledged as a buildup free treatment on a global scale (Schlipalius *et al*., 2002).

Although development of insect resistance among stored grain pests has become a universal phenomenon in recent years due to frequent and non-proper use of pesticides and fumigants. Insect resistance is described as the adaptation that target pests have developed in order to makes its survival possible at the recommended dose of an insecticide that could be toxic to rest of pests in a normal population (Subramanyam and Hagstrum, 1995; Gwinner *et al*., 1996).

There are numerous elements which contribute in conferring resistance to insect pest among stored grain pests such as biochemical, physiological and behavioral. Biochemical factors govern resistance by inducing enzyme which may cause activation and detoxification of insecticides thus ultimately makes target enzyme insensitive. Mutations induce overproduction enzymes of detoxification system of insects. Physiological mechanism confers insect resistance by penetration (inhibition of transport of insecticide), and insensitivity of nerves. Development of behavioral resistance in insects depends on the period of contact with insecticidal residue (Georghiou, 1972).

This study focuses about resistance mechanisms and comprehensive information especially on biochemical, molecular and behavioral resistance mechanisms in stored grain insects.

Mechanism of development of resistance to insecticides

Ishaaya (2001) classified the insecticide resistance mechanism into three major groups that are generally found in

insects included biochemical mechanism, physiological mechanism and behavioral mechanism. Although biochemical mechanism of insecticide resistance is divided into two types:

1. Target site mechanism
2. Detoxification mechanism

1. Target-site resistance

Target-site resistance is major mechanisms of resistance among insects by which they handle with several classes of insecticides. This mechanism involves the substitution/alterations in the sequences of genes encoding for the insecticide target proteins, adversely affect the binding property of the toxic compound. Conserved target-site mutations have been reported in genes encoding for voltage-gated sodium channel for example, ryanodine receptor, acetylcholinesterase, nicotic receptor, and GABA receptor of insect pests which give different degree of insensitivity to the insecticides (Hollingworth and Dong 2008; Yu, 2008).

i. Voltage gated sodium channels

Voltage-gated sodium channels (VGSC) regulate the electrical signaling in nerve cell membranes. It belongs to class of large trans-membrane spanning proteins. Such kinds of protein usually have one pore-forming α-subunit of about 260 kDa and up to four smaller α-subunits of about 30-40 kDa. The α-subunit is the main structural element and composed of only one trans membrane polypeptide chain with four internally repeating homologous domains (I to IV), each with six hydrophobic trans membrane segments (S1 to S6) connected by intracellular or extracellular loops. S5 and S6 helices form the central pore, whilst S1-S4 helices form the voltage sensing domains. Such kind of conformation makes the channel permeable to the sodium ion that is crucial for the normal transmission of nerve impulses (Catterall, 2000). Because it plays crucial role in electrical signaling, sodium channels are served as major target for several natural or synthetic neurotoxins, like pyrethroid insecticides. It is reported that pyrethroids, pyrethrins and DDT act on the VGSC and induce a changes in the gating kinetic, usually by reducing the rate of deactivation which lead to prolonged opening of the individual channel which cause repetitive discharges through the nerve and ultimately lead to the paralysis and the death of target insect pests (Davies *et al*., 2007; Soderlund, 2012).

Several sort of target-site mutations in the sodium channel protein have been reported in numerous insect species. However few of them have been studied functionally by expressing mutant gene in Xenopus oocytes, and their role in reducing sodium channel sensitivity to pyrethroids have been ascertained; however, majority of them were not characterised (Rinkevich *et al*., 2013).

In *M. domestica* non synonymous mutations were first reported and described as "knock-down resistance" as they govern resistance to the paralytic effect in resistant insect in response to (knock-down) pyrethroids and DDT (Busvine, 1951; Williamson *et al.*, 1996).

ii. Acetylcholinesterases

Acetylcholinesterase (AChE) is the enzyme that catalysis the hydrolysis of the excitatory neurotransmitter acetylcholine (ACh) which is responsible for the nerve impulse transmission across the cholinergic synapses. It is reported that organophosphate (OP) and carbamate insecticides usually denature acetylcholinesterase by phosphorylating or carbamylating the critical serine residue in the active site of enzyme thus inhibition of AChE activity lead to the accumulation of ACh in the synapse causing a continuous stimulation and ultimately the death of the insect (Eldefrawi, 1985; Casida and Quistad, 2003). Modification in the primary structure of the AChE due to mutation makes it insensitive to OPs and carbamates and provides to the insect some levels of resistance (Fournier and Mutero, 1994). In resistant insect several point mutations have been reported especially in the region of AChE genes (*ace*) that code for the active site of the enzyme. Such kinds of mutations generally govern resistance of different degree due to this reason variable effects have been observed in response to different insecticide among insects (Fournier, 2005). Although, it is also reported that different insects have variable number of AChE gene but most of the insect species have two genes, *ace-1* (paralogous to *ace*) and *ace-2* (orthologous to *ace*) encoding for two different acetylcholinesterases, AChE1 and AChE2 while higher Diptera have only one gene (*ace*) for AChE (Fournier, 2005).

iii. Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChR) are mainly found in nervous system of insects.

They are proteins by nature and contain at least two binding sites for neurotransmitter ACh. These binding sites should be bound by ACh to initiate the channel opening (Karlin, 2002). Nicotine and the neonicotinoid insecticides activate the receptor because they mimic the neurotransmitter ACh and subsequently induce the influx of sodium ions with the stimulation of action potentials. As it is described above those insecticides denature the AChE and thus prevent the destruction of neurotransmitter at synapses. Subsequently continuous generation of synaptic action potential leads to hyperexcitation, convulsion, paralysis and death of the insect (Jeske and Nauen, 2005). Although, modified nAChR and its correlation with respect to alteration in the sensitivity to the effect of the insecticides has been evaluated in several insect species (Nauen and Denholm, 2005; Crossthwait *et al*., 2014).

iv. GABA receptors

GABA gated-chloride channels receptors (GABAR) are class of membrane-bound proteins belongs to the superfamily of ligand-gated channels known as "Cys-loop" and have of receptors 5 subunits that form a central ion pore; each subunit has a long N-terminal domain that is a part of the GABA binding site. They are mainly found in the central nervous system and also at the peripheral neuromuscolar junctions. GABA gated-chloride channels receptors are deactivated by Ɣ-aminobutyric acid (GABA) and subsequently nerve impulse inhibition took place (Buckingham and Sattelle, 2005). Cyclodiene insecticides, usually bound to the GABA binding site, prevent the binding of GABA neurotransmitter at the same receptors and subsequently inhibit the impulse transmission (Bloomquist, 2001).

Target site mutation in GABAR gene makes the binding site of receptor insensitive for the insecticide in resistant insect. Such kind of mutation has been analyzed in many insect pests for example gene called *Rdl* (Resistance to Dieldrin), have been studied from a field collected population of *Drosophila*

melanogaster (fFrench-Constant *et al*., 1993; Buckingham and Sattelle, 2005).

v. Ryanodine receptors

Ryanodine receptors (RyR), which is also known as ryanodine-sensitive calcium release channels, belong to class of large tetrameric proteins mainly found in sarcoplasmic/endoplasmic reticulum membrane in muscles and nervous tissue. They have the same basic structure of the sodium channels, with 4 homologous domains arranged around a central ion pore. RyR is important for many physiological activities such as muscle contraction by calcium regulation because when these receptors are stimulated by their ligand cause withdrawal of calcium ion from intracellular stores (Hamilton, 2005). Efficacy of both flubendiamide and anthranilic diamides, have been recently determined against lepidopteran pest species. They target the RyR activators and adversely affect the calcium homeostasis release causing which disrupt the muscles contraction of target insects pests, which cause paralysis and death (Nauen, 2006). Although resistance to such insecticides (flubendiamide and anthranilic) with respect to target-site mutation in the membrane-spanning domain of the RyR has recently been evaluated in the diamondback moth *Plutella xylostella*, a global lepidopteran pest of cruciferous crop (Troczka *et al*., 2012).

2. Detoxification mechanism

Majority of the enzymes involve in the detoxification of xenobiotics are part of large multigene families generally known as mixed function oxidases, esterases and glutathione S-transferases (Van, 1962).

Generally xenobiotics such as insecticides and other toxicants in insects are detoxified by oxidation *via* mixed function oxidases (MFO) that is why such enzymes are very crucial. Mixed function oxidases (MFO) govern resistance to insect pests against xenobiotics because it is observed that resistant insects have high level of MFOs than in their susceptible member and such elevated level of MFOs is contributed by gene expression not amplification (Carino *et al*., 1994; Tomita *et al*., 1995). The mechanism of detoxification of xenobiotics has been correlated with MFOs after the discovery of multifunctional enzymes of cytochrome P-450 complex.

Although studies have also been revealed that different sorts of xenobiotics cause induction of several kinds of cytochrome P-450 capable of distinct catalytic properties (Cooney, 1967).

Approximately, 62 families of cytochrome P-450 have been identified in animals and plants and whereas four families (*i.e.* 4, 6, 9 and 18) have been studied among insects. It has also been found that cytochrome P-450 oxidases (occur in cluster of genes) which is a member of family 6 are mainly involve in detoxification of xenobiotics and hence give resistance to target insect pest. However, NADPH and oxygen are required for proper activity of MFOs (Gunsalus, 1972).

Whereas, alternations in activities of different esterases are also correlated with pesticide induced resistance among insects (Needam and Sawicki, 1971; Hughes and Devonshire, 1982). Esterases usually catalyze the hydrolytic reactions which involve the cleaveage of halide esters, peptides, thioesters and amides so it is concluded that insecticides usually have esters of substituted carbamic, phosphoric or cyclopropane carboxylic acids that is why they are hydrolyzed by these esterases (Devonshire, 1991).

There are six families of esterases found in the form of gene cluster on same chromosome (Cygler *et al*., 1993; Russel *et al.*, 1996; Cambell *et al*., 1997). Point mutation in just one member of gene cluster, transformed the esterases into insecticide hydrolase. It is also observed in resistant population of insects that elevated activity of esterase is responsible for the detoxification of insecticides (Oppenoorth and Van, 1960; Casida, 1973). Likewise, Carboxyl esterases, acetylcholine esterases, aryl estearse and choline esterase are also involved in the neutralization of neutralization of xenobiotics and govern resistance to insect (Ellman *et al*., 1961; Fournier and Mutero, 1994).

Glutathione S-transferases are usually crucial for the detoxification of cyclodienes organochlorines and organophosphates by resistant insect (Yu, 1996; Yu, 2002; Boyer *et al.*, 2007; Fragoso *et al.*, 2007). Many organisms possess several kinds GST; it is taken into account that that elevated catalytic activity of GSTs is correlated with resistance to insecticide. It is also proposed that GSTs may act as binding proteins or may elevate the activity of other detoxification enzymes like esterases (Grant and Matsumura, 1989; Kostaropoulos *et al.*, 2001). Gene of GST are also found as cluster of genes that shuffled through recombination of genome, and so a member of multiple genes responsible (Grant *et al.*, 1991; Ranson *et al*., 1997; Prapanthadara *et al*., 2000).

The role of GSTs has been demonstrated in insecticide resistance in the mite *Varroa jacobsoni* (Hillesheim *et al.*, 1996), *Helicoverpa armigera* (Hubner) in German cockroach species (Wu *et al.*, 1998) and in *Plutella xylostella* L. (Yu and Nguyen, 1996; Ali and Turner,2001).

3. Behavioral resistance

Behavioral resistance developed in insects when they learn to repudiate pesticide. This phenomenon is required stimulus and resistant insects become able to identify the danger and simply avoid feeding or leave the treated area, walking or flying away. Usually such kinds of insects possess well developed receptors by which they can detect even lower concentrations of insecticide as compared with susceptible insects (Yu, 2008).

4. Physiological resistance

Physiological resistance involves the interactions of various metabolic factors. Mainly specific genes conferred physiological resistance to insecticide that is accomplished either by increased metabolism or reduced sensitivity of target sites that are usually acetyl cholinesterase, gamma-amino butyric acid and para-sodium ion channels (Miller, 1988).

5. Other resistance mechanisms

Main mechanisms of resistance among insects are target site and metabolic resistance. Although there are many other mechanisms of insect resistance that are given less important because they are considered to confer minor resistance to insecticides. However, they may be only modest role in insecticide resistance can be manipulated by conjoined with major mechanisms in the same insect.

1. Pgp pumps

P-glycoprotein (Pgp) transporters are integral membrane proteins and member of ATP binding cassette (ABC) superfamily. They translocate several kinds of metabolites and xenobiotics across cellular membranes with the expenditure of the energy derived from hydrolysis of ATP (Hollenstein *et al.*, 2007). The role of Pgp pumps in the removal of variety of toxic compounds from cells have been demonstrated in term of mechanism of antibiotic resistance in bacteria and of fungicide resistance in fungi. Although its physiological role have not yet been properly understood in insects (Lage, 2003). However, role of ABC transporters have recently been studied in insects as a putative mechanism which govern resistance in insects by facilitating efflux transport of insecticides and their metabolites derived from phase I and II reactions (O'Donnell, 2008). The role of Pgp pumps in insecticide resistance has been evaluated in term of increased expression of genes encoding ABC transporters in many insect species (Porretta *et al*., 2008; Aurade *et al*., 2010; Bariami *et al.*, 2012). Dermauw and Van Leeuwen (2014) has recently been reviewed many cases of survey which suggest the involvement of ABC transporters in insecticide resistance. ABC transporters govern resistance to insecticides by several kind of mechanisms based on the quantification of transcript or protein levels and by synergism studies using ABC inhibitors (Buss and Callaghan, 2008; Dermauw and Leeuwen, 2014). However, in different lepidopteran species a mutant allele has recently been recognized with respect to its role in conferring resistance against the pore-forming Cry1Ac toxin from *Bacillus thuringiensis* (*Bt*) by a mechanism that causes the the loss of Cry1Ac binding to membrane vesicles rather than toxin extrusion (Gahan *et al*., 2010; Heckel, 2012).

2. Penetration resistance

Penetration resistance is a sort of adaptation which prevents the insecticide to reach its target by penetrating through the cuticle of resistant insect. Penetration resistance develops due to physico-chemical alterations in the structure of cuticle that subsequently decrease the absorption of the chemicals thus the modest amount of the insecticide can pass through such physical barriers. Although, this mechanism govern small degree of resistance to insect despite of the fact that it protects insects from many kinds of xenobiotics. Rather, it exerts its impact through its combination with other resistance mechanism, intensify their effects. It is suggested that detained and low rate of penetration may provide more time for the detoxification of the xenobiotics (Oppenoorth and Welling, 1976; Scott, 1990).

Development of resistance in insect pests against insecticides

1. Mechanism of resistance to organochlorines

The organochlorines are primarily detoxified by dehydrochlorination and microsomal detoxification (Agosin *et al.*, 1961). Dehydrochlorination is mainly catalyzed by enzyme dehydrochlorinase and it was first discovered in 1950 in housefly (Sternburg *et al.*, 1950). After that it was also reported in other species. Such enzyme is considered under the control of Semi dominant gene (Deh) in houseflie.

Whereas, microsomal enzymes are also reported in various strains of European housefly and other taxa of insects and such enzymes are under the control of DDTmd gene which is a semi-dominant gene as well. This gene governs resistance to DDT and diazinon by microsomal detoxification. Although such findings showed coexistence of DDT and diazinon resistance in few strains of the housefly, while this gene is not common in this insect taxa. Substitution and alternation in kdr and super kdr gene is also correlated with resistance to organochlorines (Oppenoorth, 1965; Oppenoorth and Houx, 1968).

2. Development of resistance to organophosphates and carbamates

Acetylcholinesterase (AChE) is the target site for organophosphate and carbamate insecticides and mutation in this enzyme usually govern resistance against them (Fournier and Mutero, 1994). However, resistance to organophosphorus insecticides is also correlated associated with the depleted activity of carboxylesterase (CarE) in *Musca domestica* and *Drosophila melanogaster* species (Campbell *et al.*, 1997).

3. Development of resistance to pyrethroids

It is observed that DDT and pyrethroids attack on the central nervous system of the insects, induce convulsions, paralysis and subsequent death, this phenomenon is called knockdown. Although, unlike DDT pyrethroids have not harmful concerns regarding environment, animal and human health that is why they have widespread use. Just like DDT single amino acid substitution in protein encode for the voltage gated sodium channel in the central nervous system, is considered as the main pyrethroid resistance mechanism (the knockdown resistance phenotype, kdr) (Martins, A.J. and Valle, D., 2012).

4. Fumigants

Fumigants kill insects by neurological, metabolically or oxidative stress. It has been observed fumigants toxicity is temperature dependent. Generally high temperature increases the respiration of insects thus more fumigant is inhaled whereas low temperature has inverse impact (Ahmdani, 2009).

a. Mechanism of phosphine toxicity to stored product pests

Mechanism of phosphine toxicity has not yet been clearly understood although physiological and biochemical alternations which occur as a consequence of phosphine exposure can be classified as neural, metabolic and redox related response (Nath *et al*., 2011).

Phosphine inflicts neural response *via* increasing acetylcholine neurotransmission by the denaturation of an enzyme acetylcholine esterase (Al-Azzawi *et al*., 1990; Potter *et al*., 1993). It also is proposed that activation of acetylcholine signaling by phosphine leads to increase in metabolic output and also raise the metabolic demand which subsequently result in hypersensitivity to phosphine (Valmas *et al*., 2008).

Phosphine toxicity is correlated with disturbance in energy production mechanism by mitochondria as well (Chefurka *et al.*, 1976; Jian *et al*., 2000; Dua and Gill, 2004; Singh *et al*., 2006). Phosphine hinders respiration by mitochondria as a result insufficient amount of energy is generated which causes mortality of insects. Though, there is a correlation between energy metabolism and phosphine toxicity. (Chefurka *et al*., 1976; Price and Dance, 1983; Schlipalius *et al*., 2006; Dua *et al*., 2010). It is considered that phosphine exerts its toxic effect either *via* increased rate of metabolism or energy depletion inflicts

mortality of insect after phosphine exposure (Valmas *et al*., 2008).

It has been reported that phosphine brought oxidative damage to biological macromolecules (Chaudhry and Price, 1992) by cytochrome c inhibition. As a consequence reactive oxygen species (ROS) is generated which decreases energy metabolism, so this is evident to ascertain that insect may become resistant to phosphine by metabolic suppression.

The possible mechanism can be linked with uptake of phosphine is either availability of oxygen (Because when oxygen is absent phosphine is not absorbed and loses its insecticidal potential) or rate of respiration and metabolism *i.e* low rate of respiration and metabolism may be proposed as mechanism of resistance to phosphine in *S. granarius* (Bond and Monro, 1967; Bond *et al.*, 1969; Monro *et al.*, 1972; Kashi, 1981; Pimentel *et al*., 2007).

II. Conclusions

The main objective was to give inclusive detail on the resistance mechanisms of stored-product insects. It is suggested that understanding of all elements of resistance mechanisms among stored grain pests are very important for the planning of effective strategy in order to combat resistant insect pest.

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