Ecological Impacts of Pesticides in Agricultural Ecosystem

Khalil Talebi¹, Vahid Hosseininaveh¹ and Mohammad Ghadamyari²

¹Department of Plant Protection, College of Agriculture and Natural Science, University of Tehran, Iran
²Department of Plant Protection, College of Agriculture, University of Guilan, Iran

1. Introduction

Pesticides are essential tools in integrated pest management (IPM) programs which can have the great influence if they are used properly. However, the adverse impacts of these compounds on the environment and ecosystem should not be ignored. The ecological effects of pesticides can be discussed from different points of view. Some of the significant consequences of use of pesticides are side effects of the pesticides on non-target organisms, sub-lethal effects of the pesticides on target and non-target organisms, emergence of resistant populations and pesticide residue and their entry into the trophic network. Side effect of the pesticides is a controversial issue in pesticides applications. They kill natural enemies present in the field and ecosystem and destroy the natural equilibrium between the hosts and their natural enemies. In the absence of natural enemies, pest populations increase rapidly and makes more controlling efforts, usually pesticides, necessary. In spite of pests, pesticide resistance in natural enemies is not common due to lower exposure to pesticides. Sub-lethal deposits of pesticides can change some biological traits of the organisms exposed to low and highly low concentrations of the toxicants. Sublethal impacts of pesticides are mostly ignored in ecological pesticide assessment because most pesticide assessments are performed as individual-level bioassays and population-level of toxicants has not been considered. Insects (pests and natural enemies) exposed to sub-lethal concentrations of pesticides show some changes in their life history’s traits. Resistant populations emerge due to the misuse of pesticides. The populations with high ecological potential are gradually selected generation by generation and subsequent populations are remarkably or completely insensitive to pesticides. Resistant populations are usually different from natural population in their fertility life table characteristics. Nowadays, the existence of pesticides residue in agricultural crops and their entrance into the trophic network has endangered human health and environment, and it has also necessitated the correct use of the pesticides. In the current chapter, the most significant ecological impacts of pesticides in agricultural ecosystems have been discussed.

2. Impacts of pesticides on natural enemies

The concept of Integrated Pest Management (IPM) was initially defined as the combined use of natural enemies and pesticides to manage pests (Stern et al., 1959). The IPM
concept later includes coordinated use of all possible tactics to suppress pest damage (Smith et al., 1976, as cited in Ruberson et al., 1998). Use of selective pesticides or rates, temporal separation of pesticides and natural enemies, and spatial separation of pesticides and natural enemies are three main areas of integrating natural enemies with pesticides in pest management programs (Ruberson et al., 1998). Conventional use of insecticides can have deleterious effects on natural enemy populations because beneficial arthropods can have greater susceptibility to low concentrations of insecticides than their prey or host (Ruberson et al., 1998; Torres & Ruberson, 2004). Pesticide compatibility with biological control agents is a major concern to practitioners of IPM, and knowledge about the activity of insecticides toward pests, non-target insects and the environment is a necessity (Stark et al., 2004).

Pesticides exert a wide range of lethal (acute and chronic) and sublethal (often chronic) impacts on natural enemies (Rezaei et al., 2007; Ruberson et al., 1998; Stark et al., 2004). Talebi et al. (2008) have published a comprehensive review on the impacts of pesticides on arthropod biological control agents. Sublethal effects are expressed as some changes in the insect's life history attributes (Ruberson et al., 1998). Many studies have been performed on the evaluation of the toxicity of various pesticides to beneficial organisms (Kavousi & Talebi, 2003; Lucas et al., 2004; Medina et al., 2003; Oomen et al., 1991; Paine et al., 2011; Rezaei et al., 2007; Steiner et al., 2011; Urbaneja et al., 2008; Van de Veire et al., 2002; Van den Bosch et al., 1956; Walker et al., 1998). Some important issues including natural enemy species, life stages/sexes, routes of pesticide entry, life history parameters, plot size for field screenings and pesticide formulations and rates must be considered for designing bioassays evaluating the effects of pesticides on natural enemies (Ruberson et al., 1998). One of the commonly used methods in testing the side effects of pesticides on natural enemies, recommended by the International Organization of Biological Control (IOBC), is a tiered approach whereby initial pesticide screening is done in the laboratory, and, depending on the results obtained, semi-field or field tests may be conducted (Dohmen, 1998; Hassan, 1998). This method has been designed to evaluate the acute residual toxicity as well as sublethal effects of the pesticides on the reproductive performance (Vogt et al., 1992). In this method, dead subjects are recorded (often daily) and the total mortality is calculated. The value of mortality (M) for the treated series is determined as the corrected mortality according to Abbott (1925). The average number of progenies (R) is measured as fecundity affected by exposing to a pesticide. The total effect of a pesticide (E) is calculated by the formula \( E = 100\% - (100\% - M) \times R \) proposed by Overmeer & Van Zon (1982). Based on the total effects, a pesticide is classified using IOBC evaluation categories (Sterk et al., 1999). Rezaei el al. (2007) investigated the effects of imidacloprid, propargite and pymetrozine in laboratory experiments using IOBC-system on the common green lacewing, Chrysoperla carnea (Stephens). All three tested pesticides produced significant adverse effects on pre-immaginal survival (\( p<0.01 \)). Imidacloprid had no significant effect on fecundity, but propargite and pymetrozin caused significant reductions (\( p<0.05 \)). According to IOBC classification, imidacloprid was found to be harmless (E=27.44%), propargite (E=49.78%) and pymetrozine (E=66.9%) were determined as slightly harmful.

3. Population-level impacts of pesticides

Sublethal effects of pesticides on the fitness of individuals are usually assessed using laboratory bioassays with insects (Grant, 1998) due to reduced variation among subject
insects and high validity of statistical analyses (Robertson & Preisler, 1992). On the contrary, insecticide bioassays with field-collected insect subjects reduce reliability on the real effects of insecticides because of heterogeneity among tested individuals (Robertson & Worner, 1990). In fact, the main research aim in ecotoxicological studies is predicting of insect field populations faced with sublethal concentrations/doses of pesticides (Ferson et al., 1996). However, most studies deal with the impact of insecticides on individuals or some components of individuals (Stark & Banks, 2003). In laboratory tests, individual responses to chronic toxicity may be evaluated from morphological, biochemical, physiological, molecular and ecological point of view. Some responses such as reduction of growth or fecundity or increase in mortality rates or development times (Grant, 1998) are more easily measured in most insecticide bioassays. Although, mortality is the endpoint of interest for many acute studies and it may be used as an endpoint criterion in chronic exposure bioassays, reproductive inhibition or growth retardation are generally considered more sensitive measurements, particularly for the estimation of sublethal responses (Villarroel, 1999; Stark & Banks 2003). Sublethal doses/concentrations of toxicants may change life span, development rates, fecundity, egg viability, sex ratio, consumption rate and behavior of subjects (Dempster 1968; Ruberson et al., 1998; Stark & Banks, 2003, Stark & Rangus 1994; Stark et al. 1992a, 1992b; Vinson, 1974). Individual (sub-population) level responses and population level consequences can be related using life table response experiments (Caswell, 1989). Data generated within life table response experiments give valuable information to the assessment of population-level consequences of toxicant sublethal effects (Caswell, 1989b; 1996a, 1996b; Ferson et al., 1996; Grant, 1998; Stark & Banks 2003).

3.1 Life table response experiments

Lethal dose/concentration of an insecticide that kills 50% of a population (LD$_{50}$ or LC$_{50}$) is commonly used as a simplistic criterion for determining and comparing the effects of toxicants (Stark et al., 2007). This approach relies on the death of individuals and ignores many consequent impacts of a toxicant on survivors. In addition to death, exposure to a toxicant may result in simultaneous manifestation of multiple sublethal effects (Stark & Banks, 2003; Stark et al., 2004). Under the phenomenon population compensation, if sublethal effects do not occur but the population density is reduced, survivors may have more resources available and actually produce more offspring than untreated populations (Stark et al., 2007). Effective concentration/dose of a toxicant that affect x% of a population (EC$_x$ or ED$_x$) is also used when sublethal effects are scored. (Kammenga & Laskowski, 2000). Demographic toxicological analyses or life table response experiments is another approach, which takes into account total effects that a toxicant might have at the levels of organization higher than the individual (Stark et al., 2004). The advantage of this approach is that a total measure of the effect is determined that incorporates lethal and sublethal effects into a single endpoint, the intrinsic rate of natural increase or $r_n$ (Kammenga & Laskowski, 2000; Stark & Banks, 2003), which can detect subtle, individual-level effects of contaminants that alter the growth of populations at rates below the lethal concentration limits (Bechmann, 1994, as cited in Rezaei et al., 2007). The first life table response experiment was performed by Birch (1953) to study the impacts of temperature, moisture and food sources on flour beetles (as cited in Kammenga & Laskowski, 2000). The approach has been widely used in ecotoxicological studies; however few studies have been published on the use of demography and similar measures of the population growth rate for evaluating the effect of pesticides on insects, especially insect natural enemies (Kammenga & Laskowski, 2000;
Rezaei et al., 2007; Stark & Banks, 2003). Life table response experiments are being increased to measure multiple endpoints of effects and have been recommended as a superior laboratory toxicological endpoint (Stark et al., 1997). In general, the main reason to use life table response experiments in toxicological studies is revealing of total effect (lethal, sublethal and too subtle impacts) of a toxicant on an insect at the population level. In a few investigations, especially in pesticide side effect studies, total effect of a pesticide is measured using the index E which incorporates mortality and fecundity (Overmeer & Van Zon, 1982; Rezaei et al., 2007). However, the index E is not like the demographic parameters (such as $r_m$) which measure the impact of a toxicant at population level.

### 3.2 Construction of a life table

Demography has been used in a small number of toxicological studies to evaluate lethal and sublethal effects of toxicants on insect populations (Stark & Banks, 2003; Stark et al., 2007). The basic principal in insect toxicological demography is construction a fertility table. The construction of a number of life tables is an important component in the understanding of the population dynamics of a species (Carey, 1993).

A life table, for each treatment (toxicant concentration or dose), is constructed by following an insect cohort (egg, larva or adult), till the death of all individual members of a cohort, individually, and recording the age of each female ($x$), the probability that a new individual is alive at age $x$ ($L_x$), and the number of female offspring produced by a female with attributed $x$ ($m_x$) were recorded. Each individual from the initial cohort is treated according to a convenient procedure depends on test subject, toxicant and purpose. The survived individuals from the treated individuals are maintained and monitored individually to collect necessary data for construction life tables.

The precise value of the intrinsic rate of increase ($r_m$) is obtained by solving the Euler equation (Andrewartha & Birch, 1954):

$$\sum_{x=0}^{y} L_x m_x e^{-rx} = 1 
\tag{1}$$

In this equation, $y$ is the oldest age class, $L_x$ is the survival of a newborn female to the midpoint of an age interval, and $x$ is the age of each female at each age interval. In addition to $r_m$, the other main fertility life table parameters including net reproductive rate ($R_0$), generation time ($T$), doubling time ($DT$), and finite rate of increase ($\lambda$) are also computed using the following formulas, respectively:

$$R_0 = \sum L_x m_x \n\tag{2}$$

$$T = \sum xL_x m_x / \sum L_x m_x \n\tag{3}$$

$$DT = \ln(2) / r_m \n\tag{4}$$

$$\lambda = e^{r_m} \n\tag{5}$$

In fact, these parameters are estimations for a given population; therefore, the uncertainty associated with them must be estimated. Uncertainty associated with the parameters can be estimated using two techniques; jackknife and bootstrap. However, jackknife technique is
more popular and nearly all estimations are performed according to this method. The jackknife technique is used for ease of statistical comparisons among life table parameters related to each treatment and for estimating the standard errors (SE) associated with the parameters. First, the precise value of $r_m$ is calculated for all of the raw data ($r_{total}$). Then, one of the insect subjects is omitted and an $r_m$ is computed for the remaining insects ($n-1$). Based on the suggested equation by Meyer et al. (1986) the jackknife pseudo-values were calculated for this subset of the original data according to:

$$\tilde{r}_i = n r_{total} - (n - 1) \hat{r}_i$$  \hspace{1cm} (6)

The value of $n$ is the number of insects needed to construct a fertility life table. This process is repeated until pseudo-values were calculated for all $n$ possible omissions of one insect from the original data set. Finally $n$ number of calculated $\tilde{r}_i$ are provided to calculate the mean ($r_j$) and its SE.

$$r_j = \frac{1}{n} \sum_{i=1}^{n} \tilde{r}_i$$  \hspace{1cm} (7)

$$\hat{SE}(r_j) = \sqrt{\frac{s_j^2}{n}}$$  \hspace{1cm} (8)

In the equation 8, $s_j^2$ is the variance of the $n$ jackknife pseudo-values. This algorithm is used for estimating uncertainties associated with the four other parameters. All jackknife pseudovalues for each treatment are usually subjected to analysis of variance (ANOVA) followed by a convenient mean comparison test. The nonparametric tests are also used for some pseudovalues which are not meet ANOVA perquisites (Rezaei et al., 2007).

3.3 Life table parameters

Intrinsic rate of natural increase, $r_m$, is the main and the best estimator for growth rate of insect populations. When values of $r_m$ are positive, a population is increasing exponentially; when $r_m$ is equal to zero, a population is stable and when $r_m$ is negative, a population is declining exponentially and headed toward extinction (Kammenga & Laskowski, 2000). In toxicological studies, values for $r_m$ are statistically compared among different cohorts (toxicant-treated and control). Rezaei et al. (2007) in life table response experiments of *C. carnea* with some pesticides revealed that imidacloprid and propargite had no significant effects on the intrinsic rate of natural increase, while pymetrozine caused a 34% reduction in $r_m$ value ($p<0.05$). Propargite was non-toxic to *C. carnea* under the tested conditions. The life table assay showed more adverse effects of pymetrozine than a non-life table response experiment method (IOBC method). Lashkari et al. (2007) studied the efficiency of imidacloprid and pymetrozine on population growth parameters of cabbage aphid, *Brevicoryne brassicae* L. (Homoptera: Aphididae). They revealed that $r_m$ were lower in imidacloprid and pymetrozine treatments than in controls. In such investigations, simple statistical comparisons of $r_m$ values among cohorts determine efficiency of toxicants. However, a more precise and complicated method is estimating of a concentration/dose of a toxicant at which $r_m$ value is reduced by 50% (population-level EC$_{50}$ or ED$_{50}$) or specific proportions (population-level EC$_x$ or ED$_x$) under laboratory conditions. (Suter & Glenn, 1993; Tanaka & Nakanishi, 2001).
3.4 Age-stage two-sex life table
In construction of a fertility life table, raw data is commonly collected from survival and reproduction of female individuals. In this method, males are completely ignored and only used for fertilizing females in a cohort. Ignoring the sex of individuals can result in errors (Chi, 1988). Chi & Liu (1985) and Chi (1988) developed a new method, age-stage two-sex life table, for construction a life table with taking into consideration both female and male sexes. In a small number of investigations, two-sex life table theory have been used for construction of the life table and data analysis (Chi & Su, 2006; Kavousi et al., 2009; Refaat et al., 2005; Schneider et al., 2009; Yang et al., 2006; Yu et al., 2005). As far as the authors aware, there is only one investigation, Schneider et al. (2009), on the effect of a toxicant on an insect according to the age-stage two-sex life table theory. Schneider et al. (2009) determined the side-effects of glyphosate (a herbicide) on development, fertility and demographic parameters of C. externa (Neuroptera: Chrysopidae) in the laboratory. They revealed that glyphosate will decrease arthropod population performance and the major detrimental effect observed on C. externa was on fecundity and fertility.

3.5 Drawbacks to the use of life table response experiments
Although life table response experiments may provide the most complete data for the impacts of a pesticide on an animal subject at population-level, there are some disadvantages associated with this method. The most important one is that life table response experiments are expensive and time consuming. Construction of a life table is difficult or impossible for some species (long-lived species) when exposed to a pesticide because of the low rate of reproduction. The other major disadvantage is unrealistic conditions under which a life table is constructed. These conditions are far from the natural conditions in field (Kammenga & Laskowski, 2000; Stark & banks, 2003).

4. Resistance of pests to pesticides
Pesticides are used extensively for control of invertebrate pests, plant pathogens, weeds and rodents and other pests in a wide range of crops and for veterinary purpose. Resistant to pesticides develop in insects, mites, fungi, weeds, bacteria and rodents. Repeated applications and extensive use of the synthetic pesticides has toxicity toward natural enemies and cause resistance development in pest species against major classes of pesticides throughout the world. The repeated and extensive application of pesticides caused majority on susceptible individuals in population and only some resistant individuals survive from pesticide exposure. The offspring genotype of survival individual is homozygous or heterozygous that depends on history of pesticide application and type of pesticides. The offspring inherit the resistant genes and survival ability from the exposure to the pesticides. The surviving individuals multiply in absence of their natural enemies and finally replace the non-resistant population. The development of pesticide resistance is a Darwinian evolutionary process at a rate that rare genes conferring resistance to pesticides are selected by the high selection of pesticides. Resistance to pesticides is defined as “the development of an ability in a population of a pest to tolerate doses of pesticides that would prove lethal to the majority of individuals in a normal population of the same species” (Stenersen, 2004). The first case of resistance occurrence in insect pests was reported in 1908. This document reported the failure in the control of Quadraspidotus perniciosus (Hem.: Diaspididae) by sulphur. After this report, Melander (1914) reported resistance of three scale strains in...
United State to sulphur and sulphur–lime (as inorganic pesticide) (as cited in Stenersen, 2004). The organochlorine and synthetic insecticides were commercialized for chemical control of pests in the 1940’s. The first case of DDT resistance in insect was reported in *Musca domestica* few years after introduction. After that, new insecticides such as cyclodiene, pyrethroids, organophosphates (OP), carbamates, formamidines, *Bacillus thuringiensis*, avermectins, spinosyns, insect growth regulators (IGR) and neonicotinoids were introduced for pest control and the cases of resistance to these compounds appeared a few years after their application. Now, more than 504 key pest species were resistant to pesticides and the resistance to pesticides has become a major contemporary problem in pest management programs (IRM) worldwide. Stuart (2003) reported resistance of 520 insect and acari species, 150 plant pathogen species and 273 weed species to pesticides. Pesticides resistance reduces the ability control of pesticides on pests and leads to higher application rates to achieving satisfactory pest control. Pimentel (2003) estimated the major economic and environmental losses due to the application of pesticides on crops and veterinary purpose in the USA and showed the following costs: “public health, $1.1 billion year-1; pesticide resistance in pests, $1.5 billion; crop losses caused by pesticides, $1.1 billion; bird losses due to pesticides, $2.2 billion; and ground water contamination, $2.0 billion” (Pimentel, 2005).

### 4.1 Detection and monitoring of resistance

Reduced pesticide selection pressure for each resistance mechanism is necessary for avoiding and delaying control failure prior to occurrence of resistance. For achieving this purpose, successful detection techniques are required for avoiding resistance developing and a control failure. "These techniques could be able to detect of resistant individual at low frequency in natural population" (Scott, 1995). Techniques for monitoring resistance to different pesticides in pest population gathering valuable information for insecticide resistance management (IRM) employer. Detection and identification of resistance mechanisms to pesticides require monitoring approach with appropriate bioassay method. Monitoring of resistance is required in order to sustainable management of pesticide resistance and to know the status of resistance. Therefore, developing precision and reliable susceptibility test must be developed. These tests must be also accurate, chip, easy to perform in variety of conditions in laboratory and on farm site. So far, many standardized susceptibility test method were presented by Food Agriculture Organization (FAO) and World Health Organization (WHO) such as exposure to standard residue treatment on glass scintillation vial or filter paper, plastic bags, topical application, spray application of standard solutions, and resistance detection kits and strip. Resistance frequencies can be detected and monitored by bioassays using diagnostic (discriminating) dose (LD$_{99}$) and estimating resistance factor (Rf= LD$_{50}$ of resistant population/LD$_{50}$ of susceptible population). The diagnostic dose (ie. LD$_{99}$) can be calculated from regression line of log dose probit-mortality data using appropriate software such as POLO-PC. This dose discriminate the tested population as susceptible and resistant and the pests that die after exposures with LD$_{99}$ of pesticide are classified as susceptible and those individual that survive from exposure considered as resistant. The discriminating-dose assay is a chip, less time consuming approach for monitoring resistance in natural pest populations. These bioassay procedures provide valuable data for monitoring of resistance but this method is not practical for detection of resistance in low frequencies in field population of pests (Roush and Miller, 1986).
The mechanisms of resistance are behavioral, reduced penetration, metabolism of toxicant to inactive product and target site insensitivity. These mechanisms can be detected using biochemical assay techniques (spectrophotometric and fluorometric methods) and molecular assays (base on DNA diagnostic) in one individual or small number of insect. Identification of resistance mechanisms is critical for determining of the cross resistance spectrum (Brogdon and McAllister, 1998).

"Molecular methods and traditional assays (ie. bioassay) used for distinguish heterozygotes (SR), homozygous susceptible (SS) and homozygous resistant (RR) genotypes" (Scott, 1995). The environmental conditions such as temperatures, humidity, pH and light increase errors in biochemical and bioassay results but these conditions can not affect the results of molecular methods (Scott, 1995). Now, PCR-based techniques have been designed for field detection of modified acetylcholinesterase (AChE) and knock down (Kdr) in individual Myzus persicae (Field et al., 1996). "The amplified E4 or FE4 genes can be identified by restriction enzyme analysis or polymerase chain reaction (PCR)-based methods” (Field et al., 1996).

### 4.2 Mechanisms of resistance to pesticides

Biochemical and molecular basis of resistance mechanisms to pesticides in insects, acari, fungi, bacteria, weeds and vertebrate pests are similar. An exhaustive knowledge on biochemical and molecular resistance mechanisms in pests are useful for designing insecticide resistance management (IRM) strategies. Also, identification of resistance mechanisms is necessary for developing discriminating techniques for detecting and monitoring resistance genes and cross resistance spectrum in the field populations of pests (Hammock and Soderlund, 1986). The factors affecting pesticides effectiveness were distinguished in two classes: The first class decreases the amount of pesticide dose in action site including behavioural resistance, reduced penetration or adsorption, sequestration and detoxification. The second class is decreased target site sensitivity to pesticides that reduce the affinity of target protein toward activated pesticide (van leeuwen, et al., 2009). “In practice, probably more than 90% of all resistance cases in insects and mites are caused by a less sensitive target site and/or an enhanced pesticide detoxification” (Roush and Tabashnik, 1990 as cited in van leeuwen, et al., 2009). The relative importance of these mechanisms depends on pest species and history of chemical application.

#### 4.2.1 Genetic mechanisms

Genetic mechanisms of pesticide resistance involve some point mutations in genes and their over expression. These mechanisms were elucidated as follow:

##### 4.2.1.1 Gene amplification

Devonshire and Moores, 1982 showed that the gene amplification of one of two closely related carboxylesterases (E4 and FE4) in M. persicae were associated with resistance to OP, carbamates and pyrethroids. Carboxylesterases sequester or degrade carbamate and OP insecticides before they reach to AChE in the nervous system. E4 and EF4 overproduction in resistant strains of M. persicae is due to amplification of structural genes encoding these enzymes (Field et al., 1988).

##### 4.2.1.2 Up- and down-regulation

The research showed that cytochrome P450 enzyme were over expressed in some resistance strain of M. domestica through the increase of gene transcription by up-regulated
mechanism. The up-regulation of a cytochrome P450 enzyme led to resistance when an insecticide is used in its toxic form on *M. domestica*. If pro-insecticide, i.e. a chemical must be converted in pest through metabolism to the active form, used against *M. domestica*, down-regulation of cytochrome P450 or other metabolizing enzymes will increase resistance (Scott, 1995).

### 4.2.1.3 Structural change in insecticide-target molecules

AChE, the gamma-aminobutyric acid (GABA) receptor, Voltage-gated sodium channels, nicotinic acetylcholine receptor, octopamine receptor and the juvenile hormone (JH) receptor are known as targets of pesticides and substitution of amino acid residues in these sites led to insensitivity of structural protein toward pesticides (Kono and Tomita, 2006).

### 4.2.2 Behavioral resistance

“Behavioral mechanisms, defined as evolved behaviors that reduce an insect’s exposure to toxic compounds or that allow an insect to survive in what would otherwise be a toxic and fatal environment” (Sparks et al., 1989). There is little literature on behavioural resistance mechanisms in insects due to difficulties in detection (as cited in Jensen, 2000). It seems the significance of this mechanism for resistance is less than other resistance mechanisms.

### 4.2.3 Reduced penetration

Reduced penetration of insecticide as a resistance mechanism has been studied in a few insect species such as *Leptinotarsa decemlineata*. Reduced insecticide penetration via cuticle led to decreased the amount of dose in action site. The resistance ratio by this mechanism was lower than 3-fold (Scott, 1990), but because several different mechanisms are responsible for resistance to an insecticide and multiple resistance mechanisms may coexist in an insect and act either additively or synergistically. Patil & Guthrie, 1979 compared the composition of the cuticular lipids of two resistant strains of *M. domestica* and their results showed that "total lipids, monoglycerides, diglycerides and sterol esters, sterols, fatty acids and phospholipid phosphorus were higher in resistant strains than in the susceptible strain". Three methods for detecting of this mechanism include: Wash-off, diffusion cell and disk technique. In wash-off radiolabelled insecticide was topically applied to the insects and then, at fixed times after application, un-penetrated insecticide was washed off with an appropriate solvent and quantified (as cited in Jensen, 2000).

### 4.2.4 Metabolism of toxicants

Three enzyme groups involved in metabolic resistance to pesticides: esterases, glutathione S-transferases (GST) and mixed function oxidases (MFO). The following technique can be used for detection of these mechanisms.

#### 4.2.4.1 Esterase

Esterases metabolize a variety of pesticides such as OP, carbamate, pyrethroids with ester linkages. "These enzymes confer resistance to pesticides in over 50 species of insects, ticks and mitesa" (Devorshak and Roe 1998; as cited in van Leeuwen et al., 2009). Detection and investigation of esterases-based mechanism can be achieved from synergistic bioassays and biochemical assays. For synergistic bioassays, some synergists such as DEF (S,S,S-tributylphosphorotrithioate), TPP (O,O,O-triphenylphosphate), and IBP (O,O-bis[1-
methylethyl] S-phenylmethylphosphorothioate) were used to inhibit esterases (Raffa & Priester, 1985 as cited in Jensen, 2000). However, the synergistic bioassays are useful to achieve valuable data, but DEF in higher concentrations inhibits MFO and esterase activity (Scott, 1990).

In biochemical assay, increased esterase activity in resistant insect can be checked with some artificial substrates such as α- and β- naphthyl acetates, α- and β- naphtyl butyrates, α- and β- naphthyl propionates and p- nitrophenyl acetates. These substrates hydrolyzed by general esterases and the involvement of esterase in resistance must be checked by more than one substrate. Also, the specific elevated esterase can be detected with immunological methods and an antiserum for example antiserum of E4 carboxylesterase *M. persicae* (Devonshire et al., 1986). An affinity purified immunoglobulin G (IgG) fraction from this antiserum has been used in a immunoplate assay to distinguish between the different resistant strains of *M. persicae* (Devonshire et al., 1986).

### 4.2.4.2 GST

The GSTs are involved in the detoxification of a wide range of xenobiotics including insecticides, fungicides, acaricides and herbicides (Salinas & Wong, 1999). GSTs catalyze the conjugation of hydrophobic electrophile compounds such as pesticides and their metabolites with the thiol group of reduced glutathione (GSH) (Habig et al., 1974).

Elevated GST activity has been associated with resistance to the major classes of pesticides and the involvement of GSTs in resistance to insecticides is well reviewed in Enayati et al., (2005). Because GSTs can metabolize a wide variety of xenobiotics such as insecticides and plant allelochemicals, increased GST activity may be due to exposure of insect with foreign compound in environment not resistance mechanisms. Diethyl maleate (DEM) is used as synergist for inhibiting of GSTs involved in resistance in bioassays.

GST activity can be measured from direct measurement of the conjugation of reduced glutathione with non-fluorescent monochlorobimane (MCB), l-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB) as substrates using a spectrophotometer at 340 nm.

### 4.2.4.3 Cytochrome P450

Cytochrome P450 plays important role in metabolism of pesticides and confers resistance to many classes of pesticides in mite, insect, weeds and fungi. These enzymes widely distributed in fungi, bacteria, yeast, insect, mite, weeds and invertebrate. Piperonyl butoxide (PBO) and sesamex was used in synergistic bioassays for involvement of MFO in resistance. However, PBO can inhibit both MFO and esterases-based resistance mechanisms in some insect and mite species (Gunning et al., 1999; Young et al., 2005).

There are many P450-monooxygenase isoenzymes with different substrate specificity within an insect. Therefore, in measuring activity of MFO- based resistance mechanisms must use different substrates and methods (Rose et al., 1991). Different biochemical methods have been used to study P450-monooxygenase activity in insects and mites. One of these methods is measuring the total amount of heme containing protein using a heme-peroxidase assay (Brogdon et al., 1997). Another method uses the aldrin as substrate to measure P450- monooxygenase activity. O-demethylase can be detected using p-nitroanisole as substrate using spectrophotometer. O-deethylation activity of the artificial substrates, 7-ethoxycoumarin (7-EC) and ethoxy-4-trifluoromethylcoumarin, by MFO can be measured with fluorometric microplate assay (van Pottelberge et al., 2009).
4.3 Fitness costs associated with pesticide resistance

Genetic changes confer insecticide resistance in insects can affect their developmental time and reproductive potential. Resistance genes can alter some life table and physiology parameters of pests and thus causing a fitness cost (as cited in Gazavi et al., 2001). In some insect and mite species, genetic changes that enhance survival due to pesticides exposure reduce pest fitness in the absence of pesticides (Roff and Derose, 2001; Higginson et al., 2005). Because insecticides caused a huge selection on pest population in a short time, therefore susceptible and resistant populations are useful models for evaluating fitness trade-off (Crow, 1957; McKenzie, 1996; Higginson et al., 2005; Ghadamyari et al., 2008). Mutation associated with insecticide resistance can disturb pest physiology (MacCarroll et al., 2000).

Resistant and susceptible strains differ in some properties due to their adaptation to insecticides, such as developmental time, fecundity and fertility, overwintering success and sensitivity to alarm pheromone. Differences in the biological parameters affecting the net reproductive rate of insect populations are important for insecticide resistance management (IRM) (Haubruge and Arnaud 2001). Altered acetylcholinesterase (AChE) and esterase-associated resistance in peach aphids have life history disadvantages compared with susceptible counterpart (Ghadamyari et al., 2008). Also, altered GABA receptor, esterase, MFO and GST associated abamectin resistance in *Tetranychus urticae* showed life history disadvantages.

Field and laboratory studies on different strains of *M. persicae* have been showed that adverse selection by pesticides caused “poorer winter survival, maladaptive behaviour and reduced reproductive fitness” (Foster et al., 2000).

4.3.1 Maladaptive behavior

Some resistance mechanisms can confer more fitness disadvantages to resistant strains than others. Some strains of *M. persicae* with over expressing high levels of carboxylesterase (due to structural gene amplification) show a reduced tendency to move away from senescing leaves compared with susceptible counterpart (Foster et al. 1997, 2003). This behaviour caused higher mortality during worst winter weather conditions and so can be regarded as a deleterious pleiotropic effect of pesticide resistance. Studies have been shown that peach-potato aphids caring both gene amplification and the knock down mutation has reduced response to alarm pheromone (Foster et al., 2003).

4.3.2 Reduced reproductive success

Many experiments measured reproductive fitness in the absence of pesticides in resistant and susceptible strains. The results of these experiments showed that individuals carrying resistant genes have lower reproductive rate than susceptible. When pressure of insecticide is diminished on resistant strain, the number of resistant individual in population quickly reduce due to fitness cost (Crow, 1957; Carrière and Roff, 1995; McKenzie, 1996; as cited in Arnaud et al., 2005). Although the majority of insecticide resistance strains show fitness cost (McKenzie, 1996; Foster et al., 2003; Berticat et al., 2004; as cited in Arnaud et al., 2005; Ghadamyari et al., 2008), a few researches present species with no fitness cost (McKenzie, 1996; Oppert et al., 2000; McCant et al., 2005). For example “some resistant strains of mosquitoes in absence of pesticides showed only one quarter of the reproductive potential of susceptible strains” (Georghiou and Taylor, 1977). *Varroa destructor* has little or no reproductive fitness cost associated with pyrethroid resistance in Texas (Martin et al., 2002).
Tetranychus urticae resistance to abamectin showed reduced reproductive success compared with susceptible populations and the \( r_m \) of susceptible population was higher than \( r_m \) of resistant population (unpublished data).

In contrast, in Tribolium castaneum “resistant to malathion, susceptible male individuals show reduced reproductive success compared with resistant lines” (Arnaud et al., 2005). Foster et al. (2000) showed reduced reproductive success in M. persicae expressing the highest levels of carboxylesterase.

4.3.3 Reduced overwintering ability

Winter field trials by Foster et al., 1996 showed that UK M. persicae clones expressing high levels of esterase-based resistance (i.e. R2 and R3) present higher mortality than their susceptible (S) and -R1 counterparts during worst weather conditions (Foster et al., 1996). In Heliothis virescens, resistance to Cry1Ac is recessive and associated to cadherin gene (Morin et al., 2003), research showed fitness costs associated resistance affecting overwintering success and survival on non-Bt cotton (Carriere and Tabashnik, 2001). The frequency of pink bollworm resistance to Bt cotton has not increased in the field compared with laboratory that show fitness cost (Tabashnik et al., 2003). Monitoring Culex pipiens mosquitoes overwintering in a cave in southern France (in an area where OP insecticides are widely used) showed a “decrease in the frequency of insecticide-resistant mosquitoes compared with susceptible counterpart, indicating a huge fitness cost” (Gazave et al., 2001). In the pink bollworm, Pectinophora gossypiella, has been shown fitness costs at low temperatures associated with resistance to Bt and this can delay resistance of this pest to Bt cotton (Carrie’re et al., 2001; Tabashnik et al., 2005).

4.3.4 Why insecticide resistance caused a fitness cost?

The occurrence of fitness costs in insecticide resistant strain is reported for many pests such as M. persicae (Ghadamyari et al., 2008), T. urticae (unpublished data) and Sitophilus zeamais (Coleoptera: Curculionidae) (Araujo et al., 2008a, 2008b; Guedes et al., 2006). Populations of T. urticae, S. zeamais and M. persicae with different levels of resistance to different pesticides have shown to be good subjects and models for evaluating the physiological base of fitness cost associated pesticide resistance.

Recently, some attempt was done to show the relationship between the energy consumption and the energy reserves available for metabolism of pesticides. The energy consumption could be measured using the electron transport activity (at the mitochondrial level), while reserve energy for metabolism could be achieved by measuring total lipids, protein and sugar contents by spectrophotometric method. The differences between energy consumption and the energy reserves represent the energy available for growth and biomarker of fitness cost in resistant populations. Our research on fitness cost of T. urticae resistant to abamectin showed that no significant differences were presented between the amounts of fuel nutrients macromolecules (carbohydrate, protein and lipid) in the resistant and susceptible populations of T. urticae, the amount of energy consumed was higher for resistant population when compared to its susceptible counterpart. Also the susceptible population exhibits a significantly higher \( r_m \) than the resistant population. These suggested that the resistant population may be less fit than the susceptible compartment.

The following theory was presented by Guedes et al., 2006, Araujo et al., 2008a, 2008b and Lopes et al., 2010 and we attempt to discuss their theory with their justifying fitness cost in S. zeamais.
Populations of *S. zeamais* with different levels of susceptibility to insecticides were used as a model for evaluating the mechanisms of fitness cost associated with resistance. Demographic and competition studies carried out on different strains of *S. zeamais* susceptible and resistant to pyrethroids showed fitness costs associated with insecticide resistance in some strains and no fitness costs in other strain (Fragoso et al., 2005; Oliveira et al., 2007). The susceptible, resistant no-cost and resistant cost strains showed some differences in some biochemical parameters as follow:

I. Some differences in the accumulation and consumption of fuel nutrients macromolecules were observed between *S. zeamais* pyrethroid-resistant and susceptible strains. These differences caused *S. zeamais* could be able to detoxify insecticides without reduction in its reproductive potential. Pyrethroid-resistance cost strain has greater stored total proteins and carbohydrates compared with susceptible and resistant cost strains (Araujo et al., 2008a, 2008b). Finally Araujo et al. (2008a, 2008b) concluded that increased energy reserves may be due to increased digestive enzyme activities.

II. The pyrethroid -resistant strains showed increased serine- and cysteine-proteolytic and cellulolytic activity. Also, kinetic parameters of these enzymes were different in susceptible, resistant no-cost and resistant cost strains. These differences suggested that cysteine-proteinase and cellulase activities were more important for justifying the cost of insecticide resistance in *S. zeamais* strains (Araujo et al., 2008b). The activity of carbohydrases specially amylase was higher in the resistant no-cost strains suggesting that a more efficient energy storage may justify the fitness costs due to the over expression of detoxify enzymes (Lopes et al., 2010).

III. The pyrethroid-resistant no-cost strain of *S. zeamais* show higher grain loss, higher respiration rate, higher body mass, and larger energy reserve cells than the pyrethroid-resistant cost strain and susceptible strains (Guedes et al., 2006). These advantages cause resistant no-cost strain has additional reserved energy for detoxifying insecticides without any adverse effect on their life table parameters (Guedes et al., 2006).

Some resistant strains of green peach potato aphid in the UK showed various fitness costs, such as reduced overwintering ability, lower rate of movement away from senescing plant leaves at low temperatures, reduced responses to alarm pheromones and reduced reproduction (Foster et al., 2000, 2002, 2005; Ghadamyari et al., 2008). Foster et al., 2000 concluded that differences in behavior reducing *M. persicae* survival due to pleiotropic effects of the *kdr* mechanism and over expression of E4 and FE4 gene (Foster et al., 2000) Also behavioural characteristics is associated with knock down resistance in the *M. domestica* (Foster et al., 2003).

5. Pesticides residue in the environment

Chemical pesticides are used to control target pests. Extensive use of pesticides after World War II has substantially increased the agricultural production. However non target organisms including human and wildlife are affected. Pesticides are bioactive molecules that interfere with vital biochemical and physiological processes in organisms. Some are lethal to exposed organisms and many can cause disorder at sub lethal level. Extensive research is necessary to clarify the side effects of pesticides on organisms. About 3 billion kg of pesticides is applied each year with a purchase price rose to $47 billion in 2008, worldwide (Pimentel, 2009; Frabotta, 2009).
The environmental persistence is different from pesticide to pesticide. Some are persistent and remain in the environment either as a parent compound or transferred products. The fate of pesticides in soil depends on the value of Koc, carbon sorption coefficient. High values of Koc indicate a pesticide that strongly adsorbs to the soil particles and less likely to move with water. Moreover, soil composition, pH, moisture content and microbial activity affect pesticide persistence.

5.1 Insecticides

The most toxic and environmentally persistent compounds are found among insecticides; therefore, the emphasis is on groups of insecticides that have been studied in detail.

5.1.1 Chlorinated insecticides

The first synthetic insecticide was DDT with a wide spectrum of insecticidal action that was used in agriculture and against insect vectors of deadly diseases. DDT solubility in water is very low, about 0.006 mg/l, which makes it one of the most hydrophobic insecticides. DDT residues either as parent compound or its metabolites DDD and DDE are stable and have high persistence in the environment. It has a great tendency to be stored in fatty tissue of different organisms. After the introduction of DDT, HCH was marketed. HCH has eight isomeric forms of which γ-isomer is called Lindane. Lindane is a volatile insecticide and was used against agricultural and households pests. Lindane is less persistent than the other organochlorine insecticides especially under moist conditions. The cyclodiens are stable organochlorine soil applied insecticides. These included aldrin, dieldrin, endrin, chlordane, heptachlor and endosulfan. Cyclodiens are environmentally persistent compounds that have raised concern about adverse effects on human health and wildlife. Residues of DDT and its metabolites DDD and DDE, dieldrin and heptachlor epoxide were detected in high percentage of soil and water samples from agricultural areas decades after their use were banned. Extensive studies on organochlorine pesticides has shown the environmental persistence of these Compounds (LeaMond et al. 1992; Reiser & O'Brien, 1999).

5.1.2 Organophosphorus Insecticides

Organophosphorus insecticides replaced persistent organochlorine compounds. Utilization of these insecticides increased rapidly and for several decades comprised high proportion of total insecticide use. Organophosphates are unstable compounds, however some of these insecticides are more acutely toxic to invertebrate than chlorinated insecticides. Parathion was the first marketed product that was effective against a wide variety of pests. Some organophosphates caused severe toxicity associated with many deaths especially in developing country, whereas a few compounds such as malathion are relatively safe to mammals and degrade fairly rapidly in the environment. Most organophosphates are harmful to beneficial arthropods, though few compounds such as phosalone and dimethoate are considered as harmless compounds.

The occurrence and movement of some organophosphate pesticides are reported in rivers and streams. Several studies conducted to find out the presence of organophosphate residues in California rivers during 1993-1994. Diazinon, methidathion, dimethoate and chlorpyrifos residues were detected in water samples. The detection occurred mostly during rainy season, showing how run off influences the presence of pesticide residues in rivers and streams (Ganapathy et al. 1997).
5.1.3 Carbamate Insecticides
Carbamate insecticides are a group of synthetic compounds derived from carbamic acid. The first carbamate carbaryl was an N-methyl carbamate with high insecticidal activity against many insect pests and ectoparasites of animals. Carbamates especially N-methyl carbamates are extremely toxic to hymenoptera and are lethal to exposed foraging bees. Carbamates biodegradation in environment is relatively rapid.

Oxime carbamates are a group of carbamate with systemic action. Aldicarb, an oxime carbamate is the most potent toxic substance (LD₅₀=0.9 mg/kg) ever used in crop protection. Because of high toxicity it is used as granular formulation. Aldicarb sulfoxide is its oxidative metabolite that may undergo further oxidation to the sulfone. Oxidative residues and its parent compound (Tatal aldicarb) are toxic and highly mobile in the environment. Total aldicarb is detected especially in shallow ground water since 1979. Ground water quality monitoring has shown that many samples contain aldicarb residues and some of them exceeded maximum acceptable concentration (Priddle et al. 1989; Marade & Weaver 1994).

5.1.4 Pyrethroids
Pyrethroids are synthesized based on the model of naturally occurring pyrethrins with more stability to light and air. Pyrethroids are used in agriculture, homes, restaurants and hospitals. These compounds are readily metabolized by man but they are effective against insects. Most pyrethroids are esters however non-ester pyrethroids are discovered with good insecticidal activity and low mammalian toxicity. These readily penetrate insects and paralyze their nervous system (Reigart et al., 1999). Since pyrethroids are highly toxic to insects, both the beneficial and pest insects are affected.

Sunlight, microbial activity, heat, and moisture accelerate pyrethroids break down, hence in areas with limited sunlight, pyrethroids persist for a long time. After treatment in the home, cypermethrin persist for about three months (Wright et al, 1993). Pyrethroids are lipophilic compound that strongly absorb to colloids of soil. Dissipation of cypermethrin, fenvalerate, and deltamethrin, were investigated in yellow red soils. The half-life of these compounds were 17, 19, 18 days in unsterilized, compared to 76, 92 and 80 days in sterilized soil (Gu et al, 2008). This experiment shows the effects of biodegradation in pyrethroids life span in soil.

5.1.5 Neonicotinoids
Neonicotinoids are similar to nicotine with the same mode of action. These insecticides have been used worldwide. Most neonicotinoids are absorbed and translocated to the tips of the plants. Imidacloprid is the first widely used insecticide of this group with relatively low mammalian toxicity. However, it is harmful to beneficial arthropods including bees (LD₅₀=0.008 µg /bee). Imidacloprid and clothianidin are more toxic to bees as spray than as seed dressing (Tennekes, 2010). Most neonicotinoids are moderately soluble and so they are mobile in the environment. In ground water 18 feet below sandy loam soil concentrations of imidacloprid ranged from < 0.1 ppb to 1 Ppb (Bacey, J. 2000). This observation shows the potential of imidacloprid to leach downward into shallow groundwater. Imidacloprid has a moderate binding affinity to soil colloids. Half-life in soil varies under different conditions. The half-life of imidacloprid in soil was 48-90 days, depending on the ground cover (Scholz & Spiteller, 1992). Laboratory experiments showed that persistence of another neonicotinoid, thiamethoxam is highly depending on moisture and the half-life varied from
45 to 300 days (Gupta et al. 2008). The half-life of neonicotinoids increases with increasing soil colloids. Overall, neonicotinoids have a low potential to persist in soil and accumulate in the environment.

5.2 Herbicides
Herbicides are the major class of pesticides to control weeds. Little attention is paid to herbicides as a source of pollutants; mainly because with a few exception; most herbicides have not appreciable mammalian toxicity. Among toxic herbicides are paraquat (LD$_{50}$=125 mg/kg) and dinoseb (LD$_{50}$=58 mg/kg); however widely used herbicides including 2,4-D and glyphosate are not highly toxic to mammals. On the other hand groups of herbicides that have potential to persist in soil and enter surface water include triazines, sulfonylureas, phenylureas and uracils. Laboratory experiments have shown that among four triazines; prometryn and terbutylazine half-lives were 263 and 366 days in ground water respectively. The half lives of simazine and atrazine were shorter than prometryn and terbutylazine (Navarro et al. 2004).

Sulfonylureas are high potent herbicides group effective at very low dose (10-15 g/ha), for that reason persistent herbicides from previously sprayed farms may damage the next crop. These herbicides are able to penetrate into deeper layers of the soil profile, where they have a relatively high persistence. A number of sulfonylureas were detected in wetland sediments. Etamsulforun methyl, sulfosulforun and metsulforun-methyl were determined in wetland sediments with mean concentration ranging from 1.2 to 10.0 µg kg$^{-1}$ (Degenhardt et al. 2010). According to Cessna et al (2006) a half-life of 84 days was observed for metsulforun-methyl in farm dugouts. Residues of 10 herbicides were detected in prairie farm dugouts. 2,4-D was the most frequent with median concentration 0.05 µg L$^{-1}$ (Cessna & Elliot, 2004).

Based on these studies, herbicides have different tendency for binding to soil colloids and so have different movement ability.

5.3 Fungicides
Fungicides are substances that destroy or inhibit the growth of fungi. Fungicides are used in agriculture and industry. Early fungicides were organic derivatives of metals such as mercury. Organomercury fungicides were widely used as seed dressing to control diseases of cereals. Although mercury content of these fungicides formulation were less than 5%, the main concern is the side effects of residues remaining in the environment long enough to enter soil and water. Both inorganic and organic compounds of mercury are toxic, however organic compounds are more lipophilic than inorganic and are liable to adsorption by soil colloids and storage in fat depot. Bioconcentration factor up to 100000 times is reported for the methyl mercury content in fish (USEPA, 1980). Dithiocarbamates (e.g. mancozeb, thiram, zineb and mane) are the first synthetic organic fungicides. Some fungicides are toxic to aquatic organisms. Maneb is highly toxic to fish and triadimefon is highly toxic to crustaceans. Dithiocarbamate fungicides have low persistence. Among high persistent group of fungicides are triazoles (penconazole, myclobutanil and flusilazole), carboximides (boscalid) and pirimidines (fenarimol) (Wightwick et al., 2010).

6. Conclusion
The use of pesticides is essential for protecting agricultural products from pest damages; however their adverse effects are inevitable almost on all habitats. From the preceding
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Information it is clear that side effects of pesticides on natural enemies, emergence of resistant populations and entrance of pesticides into the environment are the main issues that have been considering for a long time. More precise methods should be considered to evaluate these adverse impacts at the population-level and ecosystem as well as laboratory-based and individual-level assessment. Life table response experiments reveal total effects of any pesticides on an individual (target or non-target) at the population level. However, most publications in the field of insect toxicology are based on individual-level bioassays. Meanwhile, population genetics and resistance inheritance have mostly been ignored in insect toxicology which can provide great information on the ecological impacts of pesticides in agricultural ecosystems. It is believed that modern sciences such as insect biotechnology and nanotechnology facilitate designing novel and effective pesticides with less adverse effects in the environment.

7. References


