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Chapter 2

The Sublethal Effects of Insecticides in Insects

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Additional information is available at the end of the chapter

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Abstract

Studies related to the effect of insecticides on insect pests and nontarget organisms, such as natural enemies, are traditionally accessed by the estimative of lethal effects, through mortality data. Due to the limitations of the traditional methods, recent studies in the past three decades are assessing the sublethal effects of insecticides upon several important biological traits of insect pests and natural enemies. Besides mortality, the sublethal dose/concentrations of an insecticide can affect insect biology, physiology, behavior and demographic parameters. In this chapter, many sublethal effects of insecticides were addressed for several chemical groups, such as botanical insecticides, carbamate, diamide, insect growth regulators, neonicotinoid, organochlorides, organophosphates, pyrethroid and others. An accurate assessment of these effects is crucial to acquire knowledge on the overall insecticide efficacy in the management of pest insect populations, as well as on their selectivity toward nontarget organisms.

Keywords: sublethal concentrations, pest insects, natural enemies, biological effects, physiological effects, behavioral effects, demographic studies

1. Introduction

Despite numerous novel control agents available at integrated pest management programs, insecticides remain as the most reliable method for insect control. The effects of insecticides and other toxicants on insect pests and other arthropods have been the subject of innumerous studies in the past several decades [1]. Methods to test the side effects of toxicants have been developed as a function of insect control evaluations. For a long time, the classical laboratory

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method for estimating the side effects of insecticides on insect pests, natural enemies and beneficial arthropods was to determine the median lethal dose (LD_{50}) or lethal concentration (LC_{50}) [2]. The assessment of lethal dose/concentrations is a very useful tool to compare the toxicities of different active ingredients and different formulations of insecticides containing the same active ingredient. The lethal estimates may also be an important information when evaluating the development of resistant pest populations to insecticides.

Although the results of such estimates in laboratory have been extremely valuable, interpretation of the data is severely limited. In field crops, lower insecticide dose/concentrations usually occur after the initial application, as they degrade by several abiotic factors, such as rainfall, temperature and sunlight. In this way, under field conditions, insects can be exposed to sublethal doses/concentrations of insecticides and may experience related to sublethal effects [3].

Sublethal effects are defined as biological, physiological, demographic or behavioral effects on individuals or populations that survive exposure to a toxicant at lethal or sublethal dose/concentration. A sublethal dose/concentration is defined as inducing no apparent mortality in the experimental population [2]. In general, insecticide dose/concentrations under the median lethal (LD_{50}/LC_{50}) are considered to be sublethal. The sublethal effects may be manifested as reductions in life span, development rates, population growth, fertility, fecundity, changes in sex ratio, deformities, changes in behavior, feeding, searching and oviposition [4, 5]. Thus, toxicants can exert subtle as well as overt effects that must be considered when examining their total impact.

Due to the recognition of limitations associated with traditional methods for studying sublethal effects, a growing body of the literature has aimed at assessing insecticide sublethal effects on various important biological traits of pests in the past three decades. An accurate assessment of these effects is crucial to acquire knowledge on the overall insecticide efficacy in the management of pest insect populations, as well as on their selectivity toward nontarget organisms, such as natural enemies [6].

Sublethal effects were reported in several insect orders upon different biological, physiological, behavioral and demographic aspects, such as the effect of the aqueous extract of *Trichilla* sp. upon survival, development and larval and pupal weight of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) [7]. Physiological and behavioral effects were observed upon *Aphis mellifera* L. (Hymenoptera: Apidae) that when exposed to sublethal doses of permethrin exhibited lower learning response levels than untreated bees, but regained normal learning ability with time after the insecticide degradation [8]. Demographic parameters, such as net fecundity rate, intrinsic rate of increase (rm) and the intrinsic birth rate, were affected in *Brevicoryne brassicae* (Hemiptera: Aphididae) when exposed to sublethal concentrations of imidacloprid and pymetrozine [9]. In addition, natural enemies may be affected by sublethal dose/concentrations of insecticides, as *Catolaccus grandis* (Burks) (Hymenoptera: Pteromalidae), an ectoparasitoid of *Anthonomus grandis* Boheman (Coleptera: Curculionidae), developed no pupae from parasitism during a 24-h treatment period with malathion or spinosad [10].

Among the insecticides used in sublethal effect studies, the botanical and biological insecticides, organochlorides, organophosphates, carbamates, diamides, hydrazines, growth regulators, neonicotinoids and pyrethroids demonstrate several adverse effects presented throughout this chapter (**Table 1**). Therefore, we aim to discuss the importance of sublethal effects of insecticides for integrated pest management programs, through the effects upon pest insect biology, physiology, behavior, demographic parameters and natural enemies.

Chemical group	Active ingredient	Mechanism of action
Antibiotic insecticide (Spinosyn)	Spinosad	Nicotinic acetylcholine receptors and y-aminobutyric acid receptors
Botanical insecticides	Azadirachtin	Ecdysis inhibitor
	Essential oils and major compounds in general	Ecdysis inhibitor, acetylcholinesterase inhibitor, octopamine mimic
Carbamate	Methomyl	Acetylcholinesterase inhibitors
Hydrazines		
Bisacylhydrazine	Bisacylhydrazine (RH 5849)	Ecdysteroids agonists
Diacylhydrazines	Methoxyfenozide	
	Tebufenozide	
Diamide	Cyantraniliprole	Ryanodine receptors (affecting calcium channels in the sarcoplasmic reticulum)
	Chlorantraniliprole	
Insect growth regulators		
Juvenile hormone mimics	Pyriproxyfen	Inhibition of the development of insect adult characteristics
Urea derivatives	Hexaflumuron	Chitin synthesis inhibitor
	Lufenuron	
	Novaluron	
	Triflumuron	
Thiadiazines	Buprofezin	
Neonicotinoid	Acetamiprid	Acetylcholine mimic
	Clothianidin	
	Imidacloprid	
	Thiacloprid	
Organochlorides	Endosulfan	Interfere with the transmission of nervous impulses (flux of Na and K)
Organophosphates	Chlorpyrifos	Acetylcholinesterase inhibitors
Pyrethroid	Deltamethrin	Channel sodium modulators

Table 1. Chemical group, active ingredient and mechanism of action of the insecticides used in sublethal effects studies presented in this chapter.

2. Sublethal effects upon insect biology

The effects of insecticides sublethal doses/concentrations upon insect biology may present itself through reduced oviposition, increased development period of immature stages or decreased life span. Nevertheless, the effect of sublethal doses/concentrations of some neuro-

toxic insecticides upon insect fecundity and fertility may be related to behavioral changes, particularly during their reproductive stage [11]. Several biological effects are reported in the literature due to the use of sublethal dose/concentrations of insecticides, for example, the sublethal effects of the insecticides lufenuron, methoxyfenozide, spinosad, endosulfan, novaluron and tebufenozide upon *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), reducing the pupal weight, adult longevity and fertility [12]. The insecticide hexaflumuron decreased the total number of eggs, oviposition period, pupation and adult emergence of *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) [13]. The sublethal effects of cyantraniliprole on *Helicoverpa assulta* Guenée (Lepidoptera: Noctuidae) decreased the pupal weight and adult fecundity of the parental generation at LC_{30}. However, cyantraniliprole did not significantly affect the pupal period, the percentage of females and longevity of adults in other generations [14].

Several studies also report the sublethal effects of essential oils and their compounds upon insect biology. The insecticidal activity of essential oils is based on the high concentrations of major compounds that belong to the classes of terpenes, phenolics and alkaloids [15]. The essential oils of long pepper and clove demonstrated the activity of these substances on several biological parameters of *Spodopetra frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), such as reduced survival periods, alterations in larval and pupal weight and period, and decreased longevity, fecundity and fertility [16]. The essential oils of *Eucalyptus staigeriana* F. Muell. (Myrtaceae), *Ocimum gratissimum* L. (Lamiaceae) and *Foeniculum vulgare* Mill (Apiaceae) demonstrated several sublethal effects upon the biology of *S. frugiperda* reducing the larval and pupal weight in the sublethal doses of LD_{10}, LD_{20} and LD_{40} [17]. The neem oil (10 g L^{-1} azadirachtin A) presented different sublethal effects upon *Bonagota salubricola* (Meyrick) (Lepidoptera: Tortricidae), such as prolonged larval period, reduced pupal viability and fecundity [18]. In *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), an increase in the pupal period was reported, when the moths were exposed to the neem oil by ingestion [19].

The assessment of the sublethal effects of insecticides upon insect biology is of great importance for the integrated pest management programs, as sublethal doses/concentrations do not cause the insect death, but through the interference in biological traits may reduce the insect populations of next generations in the crops.

3. Insect behavior as a measurement of insecticide sublethal effects

The exposure to insecticide sublethal dose/concentrations may cause changes in several behavioral parameters of insects, such as food foraging, choice of oviposition sites, pheromonal communications and others. The production and emission of pheromone by females, males and its detection depend on complex physiological mechanisms involving hormones and neurohormones. Some insecticides that act on the endocrine system may also influence reproductive behavior.

Sublethal dose/concentrations of insecticides may change the chemical communication system and, therefore, decrease chances of reproduction in insects that largely rely on olfactory

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communication. For example, the effects of deltamethrin on the calling behavior and production of sex pheromone in *Ostrinia furnacalis* Guenée (Lepidoptera: Crambidae) showed that *O. furnacalis* developed a compensation system in which males who survived the insecticide exposure present a low response to pheromone, while surviving females produces and releases more pheromone [20].

Besides adverse effects, the insecticides at sublethal doses/concentrations may cause positive responses at reproduction, known as hormesis and hormoligosis. However, there is still little information regarding the effects of sublethal dose/concentrations on insect behavior [21]. The sublethal doses of clothianidin on males of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) presented a "biphasic effect" with increased or decreased male pherome response depending on the insecticide dosage [22].

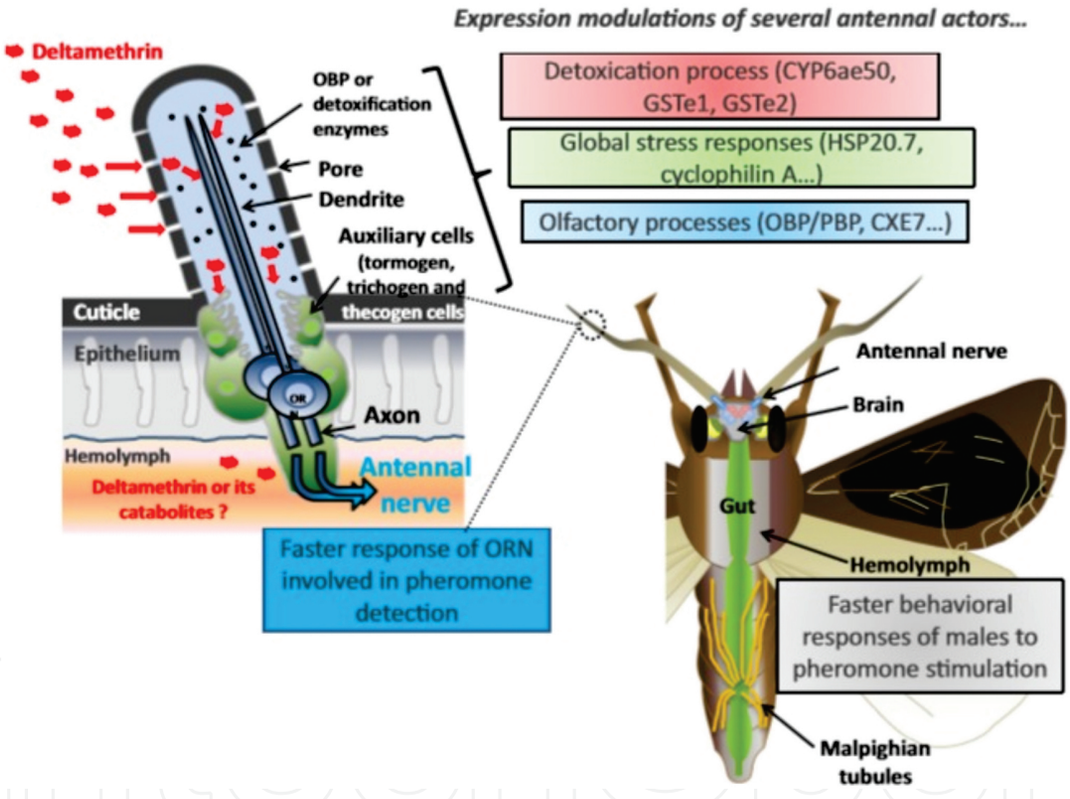


Figure 1. Proposed effect model of sublethal doses of deltamethrin upon *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) olfactory system [23].

Sublethal doses of deltamethrin on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) antennae cause an increased male response to the sex pheromone (hormesis effect), but it did not affect the male response to the host plant. Sublethal doses of deltamethrin can change the expression of several antennal processes involved in insecticide detoxification. These changes may be observed on the transcription levels of several antennal genes involved in the detoxification system and in the odors recognition and transport, such as genes from odorant-binding proteins (OBPs). In *S. littoralis* males treated with deltamethrin, a significant increase in the

transcription levels of genes involved in insecticide detoxification was reported, such as P450 chromosome, glutathione S-transferases (GSTs) and esterase. The regular olfactory process was also affected, since the repolarization on the antenna was reduced, while a rapid response of the olfactory receptor neurons (ORN) was induced. Thus, a faster behavioral response of males to pheromone stimulation was observed (**Figure 1**) [23].

The sublethal dose LD_{01} of chlorpyrifos on *Trichogramma brassicae* Bezdenko (Hymenoptera, Trichogrammatidae) males showed that the their response to the female sex pheromone was significantly decreased. On the other hand, when females were submitted to the insecticide, the response of males to the sex pheromone was slightly but significantly increased [24]. In addition, *T. brassicae* females which survived the exposure to sublethal doses of deltamethrin presented a lower parasitism rate of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs [25].

Altering plants-specific odor bouquet by nonspecific odors may cause oviposition sites rejection. In this way, insecticide sublethal doses/concentrations may present deterrent effect for insect oviposition and feeding. Sublethal concentrations of several essential oils caused the reduction in feeding and oviposition of *A. gemmatalis* by the presence of essential oil volatile components that modified the insect behavior [26]. Sublethal doses of methomyl promoted behavioral disruption of *S. littoralis* for food odors [27]. *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) exposed to *Cinnamomum zeylanicum* L. (Lauraceae) had their locomotor behavior affected, such as time, speed and distance of walk [28].

The use of behavioral control together with chemical control in the integrated pest management is recognized as a promising and efficient tool. For that, the evaluation of sublethal effects of insecticides in insect behavior is essential for the development of new strategies.

4. Physiological responses to insecticides sublethal doses/concentrations

Exposure to sublethal doses/concentrations of insecticides that attack the nervous system or disrupt the hormonal balance can affect insect physiology and reduce survival and reproduction [29]. Potentially, all classes of insecticides can affect insect reproduction through sublethal adverse effects on physiological parameters, such as egg fertilization, oogenesis, ovulation, spermatogenesis and sperm motility [11].

Insect growth regulators (IGRs) are ecdysone agonists and specific for Lepidoptera larvae, being effective against many important crop pests [30]. The HR 5849 bisacylhydrazine and the tebufenozide (RH-5992) IGRs insecticides adversely affect the development of male reproductive system and testicular volume of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) when the larvae were exposed to sublethal doses [31].

Studies with neonicotinoids, which act as agonist of acetylcholine receptor and disturb the neuronal cholinergic signal transduction, demonstrate that thiacloprid, imidacloprid and clothianidin can also interfere with the immune system of honeybees, affecting the total number of hemocytes, the encapsulation response and microbial activity in the hemolymph

[32]. Besides effects on the immune system, neonicotinoids such as imidacloprid have been found to reduce sperm viability by 50% in bees [33]. These factors may also affect disease resistance capacity [34].

Insecticides from the anthranilamide class, such as ciantraniliprole, target the rianodiana receptors in the muscles and the calcium channels [35, 36]. Ciantraniliprole demonstrated sublethal effects upon *A. ipsilon*, reducing nutritional parameters, including lipids, carbohydrates and proteins, affecting larvae development [37]. This same insecticide was also found to reduce the activity of esterase enzymes, glutathione S-transferase and oxidases of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) [38].

The amylase activity in the midgut of *Tribolium castaneum* (Herbst., 1797) (Coleoptera: Tenebrionidae) was reduced by sublethal concentrations of pyrethroids as dimilin and ambush [39]. However, sublethal doses of chlorpyrifos and methomyl did not induce changes in acetylcholinesterase enzyme activities of *S. littoralis* larvae [40].

Natural insecticides also demonstrate sublethal effects in physiological parameters of insects. *Artemisia annua* L. extracts decreased the amylase level of *Xanthogaleruca luteola* Mull (Coleoptera: Chrysomelidae) 24 h after treatment, but significantly increased it after 48 h [41]. The essential oil of *A. annua* significantly reduced protein, carbohydrates and lipids levels of *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae) [42]. Changes in the embryonic development of *S. frugiperda* were verified by scanning electron microscope after exposure to sublethal concentrations of azadirachtin, lufenuron and deltamethrin. The changes consisted of undeveloped embryo, widely dispersed yolk granules, corium disintegration, unorganized blastoderme with the presence of vacuoles and amorphous cells in yolk region [43].

Other physiological parameters such as spermatogenesis and ovarioles histochemistry of *S. frugiperda* were affected by the essential oil of *Piper hispidinervum* C. DC. (Piperaceae) [44] and *Szygium aromaticum* (L.) (Myrtaceae) in sublethal concentrations [45]. The biochemical profile of *S. frugiperda* larvae was also affected when exposed to sublethal dose of *Cymbopogon winterianus* Jowitt (Poaceae), disturbing the insect reproductive histophysiology [46].

Understanding the physiological processes that affect insect life traits is an important step for the evaluation of the overall insecticide effects upon insect pest and natural enemies in integrated pest management programs.

5. Demographic studies for the assessment of insecticide sublethal effects

The use of ecotoxicology approaches is improving the evaluation of insecticides and other toxicants in integrated pest population control programs. The traditional lethal dose/concentration estimates are designed to measure one effect at a time [1]. Demography studies derive better estimates of insecticides impacts on insect pests and natural enemies, since it accounts for all effects a toxicant might have on a population including interactions that are not perceptible in short-term toxicity [47, 48].

The analysis of demographic parameters can evaluate sublethal effects well below the traditional dose/concentration-response curve, resulting in the assessment of population decline and extinction at doses/concentrations previously assumed to have few effects on individuals [49]. On the other hand, sublethal doses/concentrations of insecticides may also result in pest populations outbreaks mediated by reproduction stimulation [50, 51].

Demographic toxicological studies through fertility life table bioassays provide a measure of the insecticide effect upon the population growth rate. The sublethal effects on population growth rate after exposure to insecticides are highly influenced by the starting population structure. Because different insect stages/ages may present different susceptibilities to toxicants, it is essential to consider this factor to estimate the population susceptibility [52].

Life table response experiments are conducted by exposing individuals or groups to increasing doses or concentrations of a toxicant over their life span. Daily mortality and reproduction are recorded and used to generate life table parameters [1]. In the fertility life table study, the intrinsic rate of increase (r_m), the finite rate of increase (λ), the net reproductive rate (R_0), the mean generation time (T) and the doubling time (TD) are important parameters [53]. The major disadvantage to the use of demographic toxicology is that the development of life table data is expensive and time-consuming. One way to reduce cost is to use partial life tables [54] or another population growth rate method, such as the instantaneous rate of increase (r_i).

The instantaneous rate of increase is calculated by the following equation: $r_i = \ln (Nf/No)/\Delta T$, where Nf is the final number of insects, No is the initial number of insects and ΔT is the change in time (number of days the bioassay was run). Positive values of r_i indicate a growing population, $r_i = 0$ indicates a stable population and negative r_i values indicate a population in decline, toward extinction [1, 55]. Although this is not demography in the true sense, this approach does yield a measure of population growth.

These demographic approaches have been used in a toxicological context by several authors to assess the sublethal effects of synthetic. The use of fertility life table bioassays demonstrated that sublethal concentrations of cyantraniliprole decreased growth speed and reduced population reproduction of *A. ipsilon* [37]. Fertility life tables for the evaluation of sublethal concentrations of chlorantraniliprole also demonstrated prolonged larval duration and the pupal stages of lepidopteran pests such as *O. furnacalis* and *P. xylostella* [56, 57]. Demographic changes in multigeneration were observed in *P. xylostella* after exposure to sublethal concentrations of spinosad [58]. The sublethal effects of spinosad can affect *S. exigua* population dynamics by decreasing its survival, reproduction and delaying its development [59].

Aphis gossypii Glover (Hemiptera: Aphididae) exposed to botanical insecticides based on azadirachtin, aqueous extract of neem seeds, and castor oil presented negatives values for the instantaneous rate of increase (r_i) [60]. Negatives r_i for *A. gossypii* population were also observed with the use of the botanical insecticides Compostonat®, Rotenat® and Neempro® [61]. Negatives r_i and a decline in the population of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) were reported when using the botanical insecticide based on neem oil, NeemAzal T/S® [62].

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In this way, the study of sublethal effects of insecticides on insect pests and natural enemies through the use of demographic parameters is crucial for guiding the use of new toxicants, delaying the development of resistance and reducing the risk of pest resurgence.

6. Sublethal effects of insecticides on biological control

The studies of insecticide effects on beneficial insects, particularly natural enemies, have grown in recent years. These impacts are not limited to mortality, as they also present sublethal effects on insects that survive the insecticide exposure [2]. These effects may result, for example, in changes of biological parameters, reproduction (fertility, fecundity and sex ratio), development time, longevity and insect behavior [11, 63].

The sublethal effects upon natural enemies can be divided in two groups: physiological and behavioral. Among the physiological effects are changes in neurophysiology, development, adult longevity, fecundity and sex ratio [2]. Among the behavioral effects upon natural enemies are the changes in mobility of insects, although it is still little studied, changes in the ability to search for prey or host and changes in feeding behavior and insect oviposition.

Insect growth regulators (IGRs) may promote changes in the development of natural enemies by the interruption of the molting process and cuticle formation, besides acting upon the endocrine system of insects [2]. Fecundity and fertility reduction were observed as sublethal effects of insect growth regulators on the predator larvae of *Ceraeochrysa cubana* (Hagen) (Neuroptera: Chrysopidae) exposed to pyriproxyfen, tebufenozide, methoxyfenozide and buprofezin [64]. Pyriproxyfen is an insect growth regulator that mimics the juvenile hormone in some species. This insecticide significantly reduced the fertility of agricultural and urban pests and may also affect natural enemies [65]. In selectivity studies of insect growth regulators and neonicotinoids to *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), novaluron insecticide was slightly harmful to the emergence of the F1 generation and acetamiprid, imidacloprid, lufenuron and triflumuron were harmless [66]. The insecticide acetamiprid upon immatures and adults of the ectoparasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) did not affect the development of the immature stages of the natural enemy until pupation [67]. The toxicity and sublethal effects upon fecundity and fertility of six insecticides upon on the natural enemies *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Adalia bipunctata* (Linnaeus) (Coleoptera: Chrysopidae) demonstrated that deltamethrin affected the reproduction parameters (fecundity and fertility) of *C. carnea* adults, while caused the total mortality of *A. bipunctata* larvae and adults [68]. The lethal and sublethal effects of lufenuron insecticide on *Diatraea flavipennella* (Box) (Lepidoptera: Crambidae) and its parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) were reported as a delay in the development period of *C. flavipes* when parasitizing *D. flavipennella* larvae that survived to sublethal exposure of the insecticide [69].

Not only synthetic insecticides are likely to affect the natural enemies but also botanical insecticides and essential oils. The effect of the neem-based botanical insecticide Azamax®, the aqueous extract of neem and the emulsifiable oil of *Ricinus communis* (Euphorbiaceae)

demonstrated an adversely affect upon the development of first and fourth instars larvae of the predator *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Chrysopidae) [60]. Lethal and sublethal effects on *Eriopis connexa* (Germar, 1824) (Coleoptera: Chrysopidae) were also observed in laboratory when using neem seeds extract [62]. The evaluation of the effects of four botanical extracts upon the parasitoid *T. galloi* (Zucchi, 1988) (Hymenoptera: Trichogrammatidae) demonstrated that the bark extract of *Aspidosperma pyrifolium* (Apocynaceae) reduced the parasitism rate in *D. sacharallis* eggs (Fabr, 1794) (Lepidoptera: Crambidae) [70]. Several essential oils affected the reproduction of *Euborellia annulipes* (Lucas, 1847) (Dermaptera: Forficulidae), and the essential oils of *F. vulgare* Mill. and *Nicotiana tabacum* L. presented an inhibitory action upon the predator oviposition [71].

Sublethal doses/concentrations of insecticides can also affect beneficial insects such as bees, causing changes in development, behavior, morphophysiology and immune system, affecting the colony functions and decreasing the longevity of individuals [72]. The assessment of selective insecticides to natural enemies is of utmost importance for biological control on integrated management programs.

7. Conclusion

Studies on sublethal effects have been quite elucidated over the last decade, for synthetic and botanical insecticides effects upon pest insects and natural enemies (parasitoids and predators). However, this is still the beginning of the path of knowledge for this particularly area, since each individual and species may present a different response to each insecticide.

Overall, sublethal effects of insecticides may cause biological effects, disturbing the number of eggs, oviposition period, larval and pupal weight, development period, adult emergency, longevity and fertility; behavioral effects on feeding, oviposition, locomotor system and reducing or increasing the production and response to pheromones; and physiological effects upon reproductive and immune systems as well as upon the nutritional status of insects.

The use of demographic parameters in the assessment of sublethal effects came to extend the concept of the total effect of insecticides not only upon individuals, but also on insect populations. In addition, the assessment of sublethal effects upon natural enemies enables the development of integrated pest management programs with safer and effective combined use of chemical and biological control.

For future works, it is also important to target a broader look and observe the effect of sublethal doses/concentrations upon insects life history and expand this impact to a more widely perspective, such as communities and the ecosystem. The study of sublethal effects of insecticides upon insects is of great importance and need to be considered when accessing the total effect of a toxicant.

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