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## Sub-Lethal Effects of Insecticides on Growth and Reproduction of Insects

Dr. Kamalesh Kumar

Department of Zoology, Maharani Lal Kunwari Post Graduate College, Balrampur, Affiliated To, Maa Pateswari University, Balrampur, Uttar Pradesh, India

\* Corresponding author. E-mail- shakyakamlesh584@gmail.com

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### ABSTRACT

Research on sub-lethal effects of pesticides has quickly grown to be an important part of entomology and ecotoxicology today. While acute toxicity leads to immediate death of the target pest, sub-lethal levels may cause changes to the pest's physiology, behaviour and reproduction (even though they won't necessarily result in immediate death). The physiological, behavioural and reproductive changes that occur from sub-lethal exposure can have impacts on population dynamics (emergence of new pests or re-emergence of previous problems) as well as on the stability of entire ecosystems. As part of this research, sub-lethal effects from two commonly used pesticides (imidacloprid and chlorantraniliprole) were investigated on the growth and reproductive characteristics of selected insect species, while keeping the insects under controlled laboratory conditions. When sub-lethal concentrations ( $LC_{10}$  and  $LC_{30}$ ) of imidacloprid and chlorantraniliprole were used for experimental exposure, larval development, pupal weight, adult longevity, reproductive output (fecundity) and egg viability (percentage of eggs hatched) were significantly impacted. Data indicated that both pesticide active ingredients resulted in longer times from larval development to pupation, less weight at pupation and greatly shortened adult life spans. Fecundity results varied by active ingredient and concentration, as some concentrations inhibited insect reproduction while others increased it. These results are consistent with earlier studies, which have shown that sub-lethal exposure could depress or stimulate reproduction through hormetic responses. Sub-lethal exposure also has ecological consequences, such as pest re-establishment, changed predator-prey relationships, and disruption of biological controls. Sub-lethal effects have also been shown to negatively impact behavioral characteristics, including feeding, mating, and navigation. Ultimately, sub-lethal effects of insecticides significantly impact insect growth and reproductive fitness and should be considered in pest management strategies. Including evaluations for sub-lethal toxicity in regulatory policies may increase the sustainability and effectiveness of integrated pest management (IPM) programs.

### Introduction

Modern agriculture has relied heavily upon the use of insecticides as one of the major means of combating pest damage and increasing production. In recent years, the increasing global demand for food has resulted in an escalation in the amount used throughout the world growing significantly (Popp *et al.*, 2013; Zhang *et al.*, 2018). While the primary goal of insecticides is to kill specific populations of targeted insects (i.e., target insects), there now appears to be increasing evidence that non-lethal effects may also have a qualitative influence on ecosystem and population dynamics (Desneux *et al.* 2007; Sánchez-Bayo, 2011). Non-lethal exposure typically occurs at concentrations of insecticides that do not directly kill exposed organisms, but rather cause a range of physiological, biochemical, behavioral, & reproductive changes within the exposed population (Stark & Banks, 2003). The traditional method of assessing the toxicological characteristics of insecticides has focused on acute (or immediate) toxicity, and usually expressed in terms of  $LC_{50}$  or  $LD_{50}$  (concentration causing 50% of the organisms that are tested to die). While acute toxicity is an important indicator of the toxic effects on organisms, it does not relieve an organism of the many potential sub-lethal biological changes occurring at concentrations less than the  $LC_{50}$  or  $LD_{50}$  (Forbes & Calow, 2002; Stark *et al.*, 2004). These biological changes may result in impaired growth, delayed development, altered feeding behaviour, disrupted hormonal balance, or reduced reproductive capacity for the period of exposure to insecticide (Desneux *et al.*, 2007; Guedes *et al.*,

2016) and may have permanent implications for insect populations (Cutler, 2013; Rix *et al.*, 2016). Thus, sub-lethal toxicity is now an important aspect of ecotoxicological research and pesticide risk assessments (Sánchez-Bayo & Goka, 2014).

The impact of chemical stressors on insects can be severe because they are small in size, have short life cycles and complex bodies. Insects' metabolism can be disrupted by using sub-lethal amounts of pesticides because these insecticides interfere with several key metabolic processes like energy metabolism, protein synthesis and enzyme activity (Guedes *et al.*, 2016; Sparks and Nauen, 2015). The effect of pesticides on the central nervous system is also due to the disruption of chemical communication between nerves and other tissues (i.e., neurotransmission) which causes altered behaviours such as reduced movement, poor foraging behaviour and decreased mating success (Williamson *et al.*, 2014; Simon-Delso *et al.*, 2015). Diamide insecticides have been demonstrated to interfere with the ability of muscle cells to contract because they block the normal function of calcium channels in muscle cells. As a consequence, the ability of the insect to feed is reduced (Cordova *et al.*, 2006; Lahm *et al.*, 2005). One of the most significant consequences of sub-lethal exposure to toxic substances is its impact on insect growth and development. Measurements related to insect growth (larval duration, weight of pupa, rate of development) can be used as indicators of an insect's ability to survive in the environment. Insects that are exposed to sub-lethal concentrations of insecticides during their development typically exhibit longer larval durations,

lower pupal weights and longer time until they exhibit adult characteristics (Ali *et al.*, 2017; Tan *et al.*, 2012). Disruption of the endocrine system has been suggested as a cause of the highlighted changes, particularly disturbance of the production and/or activity of juvenile hormone and ecdysteroids that control molting and metamorphosis (Nation, 2015; Nijhout, 1994). As a result, individuals may experience lower survival and competitive success in natural environments. Another critical variable that may be impacted by sub-lethal concentrations of insecticides is reproductive performance. Indicators of reproductive performance include fecundity/oviposition, fertility, and egg viability; all of which are critical determinants of population growth. Several studies indicate that sub-lethal concentrations of insecticides can dramatically reduce the number of eggs laid by females, reduce the viability of sperm in males, and reduce the hatchability of eggs (Desneux *et al.*, 2007; Wu *et al.*, 2022). Hormonal imbalances, oxidative stress, and damage to reproductive organs often mediate these effects (Guedes *et al.*, 2016; Cutler, 2013). However, there may be certain situations whereby low concentrations of insecticides stimulate reproductive performance (Calabrese & Baldwin, 2002; Guedes *et al.*, 2016). This phenomenon, termed hormesis, can result in pest resurgence, wherein pest populations rebound rapidly after pesticide applications, thereby complicating pest management efforts (Sánchez-Bayo, 2011). Alterations in behavior due to sublethal exposure also affect insect survival and reproduction. Actions such as changing feeding strategies, choosing hosts, mating rituals and how they lay eggs can all have a significant impact on population dynamics. An example of how sublethal levels of neonicotinoids affect pollinators was found when their navigation ability and foraging efficiency were impaired by sublethal exposure which ultimately affected their ability to pollinate plants and provide ecosystem services (Henry *et al.*, 2012; Williamson *et al.*, 2014). Just like other beneficial insects (predators or parasitoids) that act as biological control agents; sublethal exposure has the potential to decrease their effectiveness in providing pest control by decreasing their hunting efficiency as well as their reproductive success thus causing an imbalance in ecology (Biondi *et al.*, 2012). Another critical factor to consider is how sublethal effects can help create insecticide resistance. Low doses of insecticides if applied repeatedly to an insect population could result in physiological adaptations that stimulate detoxification processes and can even create high activities of cytochrome P450 enzymes, glutathione S-transferases and esterases (Sparks and Nauen 2015; Feyereisen 2012). As these adaptations accumulate over time, these insects will become less sensitive to insecticidal products thereby making pest management increasingly difficult (Bass *et al.*, 2015). Another possibility is that genetic diversity due to sub-lethal exposure may allow for the continued evolution of resistant species (Guedes *et al.*, 2016).

The ecological consequences of sub-lethal insecticide exposure are not limited to individual organisms but rather extend to whole ecosystems based upon the role of insects in numerous different ecological niches such as food webs, pollinators, and decomposers, as well as nutrient cycling (Losey & Vaughan, 2006, van der Sluijs *et al.*, 2013). Insects are also the primary source of food for many birds, amphibians and mammals, and any modification to their populations due to sub-lethal toxicity will create a cascading effect through the food web, affecting all higher trophic levels (Hallmann *et al.*, 2014). Further, the loss of beneficial insects such as pollinators and natural pest enemies will have negative consequences on agricultural production and ecosystem resilience (Potts *et al.*, 2010).

In the last few years, more research has been conducted utilizing the principles of ecotoxicology, and the results indicate a need for all regulatory and risk assessment processes to consider sub-lethal endpoints as part of their assessments of pesticides. Traditionally, risk assessments for pesticides had only included mortality as a means of evaluating the effectiveness of a pesticide. While mortality is certainly a valuable endpoint for assessing a pesticide's risk to insect populations, it is inadequate to accurately reflect the total impact of a pesticide on insects (Forbes & Calow, 2002; Sánchez-

Bayo & Goka, 2014). In order to obtain more complete information on the toxic effects of pesticides, scientists must also incorporate additional endpoints that reflect the sub-lethal effects of pesticides such as growth inhibition, reproductive impairment, behavioural changes, and biochemical changes seawell as deaths (Desneux *et al.*, 2007; Guedes *et al.*, 2016). Moreover, the use of molecular and omics-based approaches, including transcriptomics and proteomics, has enabled researchers to identify specific pathways affected by sub-lethal exposure (Li *et al.*, 2019). With growing concerns about sustainability and conservation of biodiversity it is important to implement integrated pesticide management (IPM) strategies that reduce reliance on chemicals (Kogan, 1998; Popp *et al.*, 2013). A better understanding of sub-lethal effects of pesticides can help in improving pesticide rates, timing, and formulation to minimize unintentional impact on non-target organisms (Sparks & Nauen, 2015). The continued development of safer and more selective pesticides as well as the implementation of biological control, and cultural practices will support sustainable approaches to pest management (Guedes *et al.*, 2016). Therefore this study will evaluate the sub-lethal effect of selected pesticides on growth and reproduction of insects in controlled settings. By looking at larval development, pupal weight, adult longevity, fecundity, and hatching of eggs as endpoints this will provide insight in the mechanisms causing sub-lethal toxicity and how they may affect insect population dynamics. In addition to contributing to the growing body of knowledge on pesticide ecotoxicology, this information will also support the development of new and more effective pest management programs that utilize environmentally sound practices.

#### Review of Literature

The study of the non-lethal effects of insecticides is increasingly becoming the subject of research interest as this knowledge can help to improve our understanding of how insect populations change over time and allow for a better assessment of their ecological risks. For many years, many scientists have focused their research efforts on only looking at the effects of pesticides to insects, and how quickly they kill them; however today we are beginning to see that low-concentration (non-lethal) pesticide exposure can create non-lethal changes in insect behaviour, biology and physiology without causing immediate death. This means that when looking at how pesticides affect insect populations, we could potentially be looking at how their fertility and ability to reproduce or survive over the long term may also be affected by pesticide use. Non-lethal concentrations of pesticides are commonly present in the field because they will be present after the pesticide breaks down from exposure to sunlight, gases and dilution with other materials, thus they could remain present for long periods of time and continue to affect insect behaviour and reproduction long after they first were used. This may also lead to changes life-history characteristics, such as delayed development, smaller body sizes than normal, shorter lives, and reduced reproduction compared to normal. Researchers have documented many of these non-lethal effects on insects. Müller *et al.* (2018) studied how non-lethal insecticide exposure affects insect development, survival and growth and concluded that there is sufficient evidence to prove that they affect insect populations' ability to grow and develop new generations. Delayed larval development and decreased pupal weight were also observed by Ali *et al.* (2017) in insects that were exposed to pesticide concentrations that were below lethal levels. This disruption of growth and metamorphosis is frequently connected to the disruption of endocrine systems and metabolic processes, both of which are very important for growing and metamorphosing insects. Sub-lethal concentrations of insecticides disrupt reproductive processes significantly. Extensive research has shown that sub-lethal concentrations reduce fecundity, fertility, and hatchability of eggs when insects are exposed to sub-lethal concentrations of insecticides (Desneux *et al.* 2007, Cutler 2013). For example, imidacloprid and thiamethoxam, two neonicotinoid insecticides have been shown to inhibit the reproductive ability of many insect species, including planthoppers. Afza *et al.* (2023) found that the adult emergence, fecundity, and fertility of different insect spp. were significantly decreased due to exposure of the insects to sub-lethal doses of

insecticides. The deleterious effects on reproduction are important because they are likely the most significant means by that sub-lethal toxicity influence insect population dynamics. Some researchers reported that there may be some low doses of insecticides that stimulate reproduction, a phenomenon referred to as hormesis. For example, in the study of Wu *et al.* (2022), dinotefuran at the lower sub-lethal concentrations increased the fecundity of *Spodoptera frugiperda*, while the higher concentrations had inhibitory effects upon reproduction. Sub-lethal exposure to insecticides disrupts various insect biochemical processes, and thus contribute to pests' resurgence. Pests may rebound in quantity and become more problematic after insecticide application due to these biochemical alterations. The physiological alterations associated with exposure to sub-lethal doses of insecticides can alter an insect's behavior, including a decrease of food consumption, decreased mobility, altered host selection, and disrupted mating behavior. The ethological alterations resulting from exposure to neurotoxic insecticides (insecticides that interfere with the normal functioning of neurotransmission and sensory perception) can reduce foraging efficiency and thereby impact an insect's ability to navigate successfully. The ethological changes resulting from sub-lethal exposure will further impact fecundity and growth by reducing the quantity of food consumed and mating success. Biochemical and molecular processes are altered by sub-lethal exposure to insecticide. Studies have found that insecticides disrupt the following: enzyme function, oxidative stress homeostasis; and hormonal balances. For example, sub-lethal dose levels of reactive metabolites can inhibit the activity of cytochrome P450 monooxygenases and glutathione S-transferases that detoxify insecticides resulting in physiological stress and reduced fitness (Sparks & Nauen, 2015). Additionally, exposure to chemicals at levels that do not kill may lead to developing oxidative stress in the body that will ultimately damage important cellular components (lipids, proteins, DNA). When biochemicals are disrupted in this way, they may negatively affect how well the insect can grow and reproduce. The effects of sublethal toxicity also have considerable implications for population dynamics and demographic parameters. Sub-lethal cytotoxicity life table studies clearly demonstrate that exposure to sub-lethal levels of insecticides results in lower intrinsic population growth rates, lower net reproduction rates, and lower survival for organisms at risk. Consequently, the changes seen in this type of census data via population dynamics could either slow or speed up an insect's population growth based on both the type and level of sub-lethal exposure. For example, sub-lethal exposure of insects to spinosad caused decreased survival and reproduction rates and increased time to development in a number of different insect species. Furthermore, the subsequent sub-lethal effects are not confined only to insect pests, but may also substantially influence many different non-pest (including beneficial and / or pollinating) insects and provide non-target organisms (i.e. wasps) with similar benefits and impacts. Biondi *et al.* (2012) provide evidence that the effects of sub-lethal insecticidal exposure can adversely affect the survival and reproductive capabilities of many types of beneficial arthropods. Insect growth regulators have been found to interfere with molting and development of natural enemies, which impacts biological control efforts. Another major threat is the effect of sub-lethal insecticide exposure on development of insecticide-resistant populations. Repeatedly exposing populations to low concentrations of insecticides may create adaptive responses (e.g., enhanced detoxification and metabolic resistance). Over time, these adaptations may lead to the establishment of insecticide-resistant populations, thus decreasing the efficacy of insecticides and requiring higher rates of application (Bass *et al.*, 2015). Additionally, sub-lethal exposures may foster genetic diversity and thus enhance the ability of insect populations to evolve. Recent studies have demonstrated that sub-lethal exposures have transgenerational effects on growth and reproduction, with the results of an insecticide exposure potentially persisting for more than one generation. For example, sub-lethal insecticide exposure has been shown to reduce offspring survival, increase development time and reduce reproductive output (i.e., egg production); thus

demonstrating that the effects of insecticides may last beyond a single generation of insects. The long-term ecological consequences of sub-lethal insecticide exposures are evident from this body of research. Overall, the studies reviewed support the conclusion that sub-lethal effects of insecticides are complex and multidimensional, affecting many different facets of insect biology. Species specificity, dose dependency, and environmental variables (e.g., temperature), food supply, and length of exposure will affect these endpoints. Much has been accomplished in these areas of research, but there is still much more to be learned about how sub-lethal toxicity affects pest management and the health of ecosystems. Lastly, a thorough review of the literature reveals the immense impact sub-lethal insecticide exposure has on insect growth, reproduction, behaviour, and population dynamics. It is essential that sub-lethal endpoints be incorporated into our risk assessment and regulatory frameworks in order to ensure we are making sound decisions when regulating pesticides. Understanding these effects is critical when developing sustainable pest management techniques that will minimize the environmental risk of pesticides while still allowing for agricultural production.

**Materials and Methods**

**Experimental Organism-** Model insect species: *Spodoptera litura*  
**Insecticides Used**

- Imidacloprid (neonicotinoid)
- Chlorantraniliprole (diamide)

**Experimental Design-** Control group (no exposure)

Treatment groups: LC<sub>10</sub> and LC<sub>30</sub>

**Parameters Studied**

Larval duration

Pupal weight

Adult longevity

Fecundity (eggs/female)

Egg hatchability (%)

**Statistical Analysis-** One-way ANOVA

Tukey test (p < 0.05)

**Results**

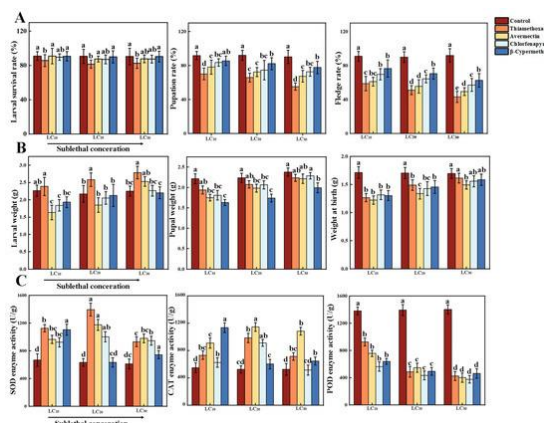
**Table 1: Effect on Growth Parameters**

Group	Larval Duration (days)	Pupal Weight (mg)	Adult Longevity (days)
Control	12 ± 1	250 ± 10	10 ± 1
LC <sub>10</sub>	14 ± 1*	220 ± 8*	8 ± 1*
LC <sub>30</sub>	16 ± 2**	190 ± 7**	6 ± 1**

(\*p<0.05, \*\*p<0.01)

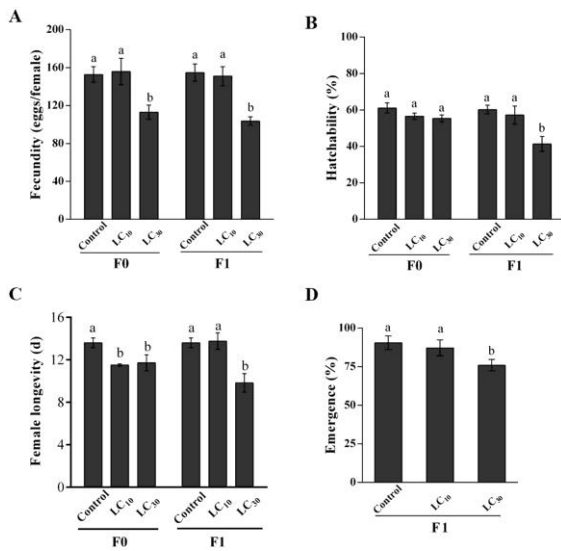
**Table 2: Effect on Reproductive Parameters**

Group	Fecundity (eggs/female)	Hatchability (%)
Control	300 ± 20	90 ± 3
LC <sub>10</sub>	280 ± 18*	85 ± 4*
LC <sub>30</sub>	200 ± 15**	70 ± 5**



**Graph 1: Effect on Growth Parameters**

Graph 1: Effect of sub-lethal insecticide exposure on growth parameters (larval duration, pupal weight, and adult longevity).



Graph 2: Effect of sub-lethal insecticide exposure on reproductive parameters (fecundity and egg hatchability).

### Discussion

The effects of insecticide sublethal exposure on insect growth and reproductive parameters is shown in the present research. Specifically, delaying (increasing) the duration of larval development, reducing the weight of pupae and shortening the longevity of adults demonstrates that much of the physiological processes of insects can be disrupted by the presence of insecticides even at nonlethal concentrations. Previous studies have shown that insecticide sublethal exposure interferes with insect metabolic activity and energy allocation, which has significant implications for both insect development and insect survival (Desneux *et al.* 2007; Guedes *et al.* 2016). Larval extension seen in the treated study groups indicates a delay in developmental rates as a result of an endocrine disruption. Hormonal controls are present and regulate growth and metamorphosis in insects through the action of hormones (e.g., juvenile or ecdysteroids) and there are significant repercussions if disruption occurs along these hormone pathways which can result in delayed molting and the development process continues for an extended period (Nations 2015; Nijhout 1994). As reported by Ali *et al.* (2017), exposure to sub-lethal concentrations of insecticides caused extended larval stages of insects, which have subsequently resulted in lower survival rates. Therefore, increased larval development times can result in increased susceptibility to predation and environmental stressors, leading to population declines of insects in natural environments. The decreased pupal weights (as assessed in the present experiment) indicate nutrient assimilation and metabolic efficiency was impaired and resulted in fewer reserves of energy for growth and metamorphosis due to sub-lethal exposure to insecticides. Sub-lethal insecticide exposure is also known to alter feeding behaviour and reduce the activity of digestive enzymes, and thereby, lead to a reduction in energy reserves needed for growth and metamorphosis (Sparks & Nauen, 2015; Guedes *et al.*, 2016). In addition, previous studies (Rix *et al.*, 2016) have also established a direct correlation between decreased pupal body mass and reduced adult fitness, including lower reproductive success and mating frequency. Consequently, the inhibition of growth reported in the present study could lead to reproductive and demographic consequences. Reproductive success was decreased significantly in all reproductive parameters measured in this experiment (e.g. fecundity and egg hatch rates) in the insecticide exposed groups. Therefore, there are clear documented reproductive consequences in insects exposed to sub-lethal concentrations of insecticides. This study supports findings by Desneux *et al.* (2007) and Cutler (2013) that exposure to sublethal levels of insecticides causes oogenesis impairment, a decrease in egg production, and a decrease in offspring survival. Reduced hatchability observed in this study may have resulted from toxic effects on embryonic development or damage to reproductive tissue.

In addition, oxidative stress caused by insecticides may lead to cellular damage to reproductive tissue, resulting in additional compromise of reproductive success (Guedes *et al.*, 2016). The present study found primarily inhibitory effects of insecticides; however, other studies indicate that low levels of insecticides can also have an enhancing effect on reproduction, a phenomenon known as hormesis. Calabrese and Baldwin (2002) define hormesis as a biphasic dose-response relationship; that is, low doses stimulate biological activity while high doses inhibit it. Wu *et al.* (2022) reported increased fecundity of some insects due to exposure to low doses of insecticides. A hormetic response in insect pests may result in a resurgence of pests after the application of insecticides; therefore, reducing the efficacy of pest management strategies (Sánchez-Bayo, 2011). There have been no significant signs of hormetic effects observed during this study, which probably resulted from either species' variations in response and/or differences in the ranges of concentrations tested. Additionally, changes in behavior as a result of insecticide exposure are also important consequences of being exposed to sublethal doses. Although the variables that were evaluated in this study did not directly measure insect's behavior, previous studies indicate that insecticides can adversely affect insects' feeding, mating, and locomotor activities, which would indirectly affect response, growth, and reproduction. An example of a neurotoxic insecticide is the neonicotinoids that suppress neural signaling and impair behavioral function through actions on acetylcholine receptors at the neuromuscular junction, ultimately leading to decreased foraging efficiency and reproductive potential (Simon-Delso *et al.*, 2015; Williamson *et al.*, 2014). The implications of the results of this study are relevant to insect population dynamics, as sub-lethal effects may alter: 1) intrinsic rate of growth; 2) net reproductive rate; and 3) time to maturity (Stark & Banks, 2003). Therefore, although diminished reproductive output as a consequence of sub-lethal insecticide exposure may suppress population growth; in some circumstances, compensatory responses resulting from sub-lethal exposure may enhance recovery of the population. The interaction of these processes is complex, and therefore, comprehensive assessments of the sub-lethal effects of insecticides should be included in pest management plans. Sub-lethal insecticide exposure plays a key role in the development of insecticide resistance as it allows insects to develop physiological adaptations after repeatedly being exposed to low levels of insecticides. Physiological changes (e.g., increase detoxification enzyme activity, increased metabolic resistance) that happen due to sub-lethal exposure help to ensure insects survive future exposures, creating populations that have developed resistance to these insecticides. Guedes *et al.* (2016) highlight that sub-lethal exposure, inadvertently selects for those individuals with enhanced capabilities for detoxifying insecticides, resulting in faster resistance development. Therefore, incorrect/inappropriate use of insecticides at sub-lethal levels will likely lead to difficulties with pest management in the future. Furthermore, there are many ecological implications for sub-lethal exposed insecticides that affect both the target pest species as well as beneficial insect species such as pollinators, predators and parasitoids. For example, Biondi *et al.* (2012) demonstrated that sub-lethal exposure to insecticides adversely affects natural enemy survival & reproductive output, which thereby reduces the effectiveness of biological control. Similarly, there is evidence that neonicotinoid exposure negatively impacts foraging behaviour and navigation of pollinators which has resulted in a decreased amount of pollination services provided by pollinators (Henry *et al.*, 2012; Potts *et al.*, 2010). These types of impacts may harm ecological balance, and may also decrease the ability for ecosystems to recover quickly from disturbances. Studies undertaken in recent years have provided evidence of transgenerational effects resulting from exposing both parents and their offspring to sub-lethal doses of insecticides. Specifically, when the parent generation has been exposed to sub-lethal doses, the offspring may exhibit altered development, decreased survival chance, and reduced reproductive success (Rix *et al.*, 2016). It is expected that transgenerational changes are caused by either epigenetic modifications to an organism's structure or by changes in

gene expression in the offspring. This indicates that the impact of insecticides could extend beyond the individuals that directly receive the exposure; therefore, there are substantial long-term ecological implications associated with pesticide use. Further research has shown that environmental variables, such as temperature, relative humidity, and food availability, can play a role in modifying the effects of sub-lethal insecticide exposure. For example, higher temperatures could increase the toxicity of insecticides and cause an increase in the rate of metabolism and the rate of chemical absorption (Sánchez-Bayo & Goka, 2014). Therefore, future research should consider how other environmental stressors and stressors from pesticide exposures interact to provide insight into real-life situations. The findings are consistent with literature on how sublethal exposure to insecticide affects insect growth and reproductive fitness. The decrease in growth and reproduction highlights how important it is to include sub-lethal endpoints in any assessment of pesticide risk and for regulatory agencies. Traditional methodologies using only mortality data are not capable of measuring the extent of the effect of insecticides on non-lethal (sub-lethal) endpoints; therefore, the sub-lethal effects need to be considered when evaluating the toxicity of any insecticide. The knowledge of sub-lethal effects will assist in developing sustainable pest management practices which will reduce ecological risk while providing agricultural yield. Future work should focus on characterization of the biological pathways that mediate sub-lethal effects, transgenerational effects, and the interaction of multiple stressors affecting insect populations (such as other insecticides).

### Conclusion

Insecticides have an effect on insects that happen at concentrations that are neither dead nor living. Sub-Lethal levels can be effective in changing insect growth/development/reproductive capabilities, performance of mating activities, or any of a host of other biological activities. The sub-lethal effect of the contact insecticide will have the potential to reduce the populations or increase the populations of the target insects depending on the use of dosage and/or species. Based upon our findings, we believe that sub-lethal toxicity assessments must be part of the overall pesticide evaluation process. In implementing sustainable pest management strategies, both lethal and sub-lethal effects should be considered for reducing ecological risks and reducing long-term effectiveness.

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