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Sublethal effects of neonicotinoids in bees: a review

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Abstract. Beginning in 2006, beekeepers on the east coast of the United States began to report severe declines in their honeybee colonies. Because of the severity and unusual circumstances of these declines, scientists have called this phenomenon colony collapse disorder (CCD). In 2019, 500 million dead bees were found in Brazil. Analyzes of dead bees identified agrochemicals in approximately 80% of them. Thus, it is believed that one of the main causes for CCD is the intensive use of agrochemicals. Neonicotinoids are the most widely used class of insecticides in the world, they are used for pest control in a variety of crops. However, they can not only affect insects considered pests, but also non-target organisms, such as pollinators. This class of insecticides is divided into five main active ingredients: imidacloprid, thiamethoxam, clothianidin, thiacloprid and acetamiprid. Several studies demonstrate that sublethal concentrations of these insecticides affect bees in different ways, such as navigation memory and muscle movements. Thus, this review aims to report the studies published between 2014 and 2019 regarding the contamination of bees with sublethal doses of the five active ingredients of the neonicotinoid class. Imidacloprid and thiamethoxam are the most used insecticides of this class and show high toxicity to bees. On the other hand, clothianidin showed the least sublethal effects on bees on the studies reported on this review. Thiacloprid and acetamiprid, although less used in agriculture, also impair several aspects of bee health. Thus, it is possible to infer that neonicotinoids are contributing to the disappearance of bees worldwide.
Keywords: imidacloprid, thiamethoxam, clothianidin, thiacloprid, acetamiprid

Introduction

Bees are divided into seven families, 28 subfamilies, 67 tribes, 529 genres and more than 20,000 species, there are five times more species of bees than species of mammals (Danforth et al., 2019). The most well-known bee species is *Apis mellifera*, because of its massive production of honey and other bee products such as propolis, wax, pollen and royal jelly.

These insects have an important ecological role, being one of the main pollinators, both in natural and agricultural environments. Bees are responsible for the pollination of several important crops, such as watermelon, apple, orange, coffee, blueberry, tomato and sunflower. It was estimated that in the United States the services provided by bees are worth 14 billion US$ per year (Danforth, 2007). Worldwide, the economic value of pollination services in agricultural production and biodiversity is estimated to be 150 billion US$ per year (Delaplane & Mayer, 2000). Crops that depend on pollinators had grown 16.7% in developed countries and 9.4% in undeveloped countries between 1961 and 2006 (Aizen et al., 2008).

At the end of 2006, a massive decline in bees was identified on the west coast of the United States, mainly in *A. mellifera* colonies, this unusual and unpredicted event was called colony collapse disorder (CCD). Most losses occurred because bees did not return to the colony and there are three most likely reasons for this behaviour, (1) parasites, (2) stresses caused by infections, poor nutrition, low availability of pollen and nectar, water contamination and migratory stress, (3) indiscriminate use of agrochemicals. Currently, the last reason is considered one of the most relevant factors (Johnson, 2010).

Pollinating insects are being constantly exposed to a cocktail of agrochemicals. Frazier et al. (2008) analyzed 108 pollen samples and detected

46 different types of agrochemicals. In a single pollen sample from an *A. mellifera* colony, 17 different types of agrochemicals were identified. Thus, further studies regarding the use of insecticides and their effects on pollinating insects are needed to understand the impact on biodiversity and crops (Brittain & Potts, 2011). It is already known that some agrochemicals may not kill bees in low concentrations but can cause sublethal effects, leading to development impairment and behaviour changes (Frazier, 2008).

Neonicotinoids are a class of insecticides widely used for pest control in crops and contain various types of active ingredients such as imidacloprid, acetamiprid, thiamethoxam, clothianidin, thiacloprid and acetamiprid. This class of insecticides acts as antagonists of the nicotinic acetylcholine receptors (Blacquière et al., 2012). The high translocation of neonicotinoids in plants causes this insecticide to reach the flowers, leaving residues in nectar and pollen, which feed the bees (Williams, 2008).

In 2016, concerns about the application of these insecticides led the European Union (EU) to restrict the use of imidacloprid, clothianidin and thiamethoxam (European Food Safety Authority, 2016). In the same year, the United Kingdom Food and Environmental Research Agency conducted its first field study and showed that bee colonies were affected by neonicotinoids (Ellis et al., 2017). Also, several EU countries, such as France, Germany, Italy and Slovenia, have agreed to a ban neonicotinoids. Similar positions have been taken by Canada and the United States (Moreira et al., 2017). Studies revealing the relationship of this class of insecticides with CCD are growing. Between 2014 and 2019 more than 170 scientific papers were written containing the keywords "neonicotinoid/neonicotinoids" and "bee/bees". Thus, this review aims to summarize the articles about sublethal effects of the main neonicotinoid active ingredients in bees.

Imidacloprid

Imidacloprid showed that up to 100 µg/kg of insecticide had no significant effect on foraging soon after contamination. However, after two or three life cycles the colonies that were contaminated with concentrations from 20 to 100 µg/kg of imidacloprid showed higher rates of queen failure and non-reproductive periods, which leads to weaker colonies. Besides that, colony survival after winter was proportional to the increase of insecticide concentration.

In addition to insecticides, viral diseases are among the numerous causes that threaten bee survival, such as chronic bee paralysis (Coulon et al., 2018), where the adults lose the ability to fly and starts to crawl in front of their colonies, leading to death.

Thus, Diao et al. (2018) observed that viral load in bees with chronic paralysis was significantly higher in adult bees after 96 hours of imidacloprid contamination at a concentration of 66.9 ng/bee. This provides clear evidence that imidacloprid exposure on bees infected with the virus has a significant negative effect on bee survival. These results indicate that acute environmental stressors may be one of the major causes for rapid viral replication, which may progress to mass spread of the virus and lead to colony decline.

Infestations caused by the ectoparasite mite *Varroa destructor* are significantly higher after sublethal contamination (Dively et al., 2015, Abbo et al., 2017). Blanken et al. (2015) found that contamination with imidacloprid and *V. destructor* reduces flight performance of foraging bees by 24%, although van Dooremalen et al. (2018) did not found this correlation. However, the interaction between insecticide exposure and *V. destructor* infestation increased the chances of infection with *Nosema spp*, a fungus that affects workers' digestive systems (van Dooremalen et al., 2018).

The expression of several genes, mainly related to body detoxification, such as Glutathione S-transferases (*gst*) and cytochrome P450s (*cyp450*), also changes after exposure to sublethal doses of imidacloprid. Wu et al. (2017) demonstrated that the expression level of two *gst*s and nine *cyp450*s in *A. mellifera* larvae were affected after contamination. Up and downregulation of different *cyp450*s can induce chaotic behaviour among bees.

De Smet et al. (2017) point out that differences between laboratory and field bioassays must be considered before conclusions are generalized. After *A. mellifera* contamination with sublethal doses of imidacloprid, colonies show a population decline after the first year of field rearing and increased expression of *cyp9q* and *cytp450* genes. Laboratory-contaminated bees showed immunosuppression and detoxification mechanism were not significantly triggered, but field bees showed a strong immune response (De Smet et al., 2017).

Detoxification processes require a lot of metabolic energy (Rand et al., 2015). It has been observed that the expression of the vitellogenin is significantly reduced, suggesting that increased energy cost for detoxification during insecticide exposure may result in altered nutritional status, as evidenced by significant vitellogenin reduction (Abbo et al., 2017, De Smet et al., 2017).

Major royal jelly proteins (MRJPs) are a group of multifunctional proteins present in royal jelly that performs significant roles in colony development. Imidacloprid contamination during larval stage suppresses *mrjp*s expression in adult bees, especially *mrjp1-4*. Downregulation of *mrjp*s can lead to nutritional deficiencies in larvae since royal jelly production is highly dependent on *mrjp*s genes (Wu et al., 2017a).

Transcriptome studies have found 197,861 coding regions in *A. mellifera* brain. After 48 hours of contamination with 0.3 ng/bee of imidacloprid 7 regions showed upregulation, and 19 regions showed downregulation. When the study was conducted using 3 ng/bee of the insecticide, 36 regions were upregulated and 77 regions downregulated (Christen et al., 2018) This result shows that different concentrations of insecticide cause different genes to be transcribed.

Gauthier et al. (2018) observed a decrease in α-carotene, α-cryptoxanthin and α-tocopherol after 10 days of oral contamination with low doses of imidacloprid in *A. mellifera*. Carotenoids act like antioxidants and precursors of retinoic compounds on vertebrates. Carotenoids and retinoids are extensively studied because retinoic acid is important for several processes during embryonic development and throughout adulthood, such as vision and regeneration.

Regarding proteins related to the motor function of bees, it was observed that after eight days of contamination with sublethal concentrations of imidacloprid *A. mellifera* climbing ability was significantly reduced. Analyzes have shown that genes related to muscle function were downregulated, confirming the mobility impairment (Wu et al., 2017b). Motor alterations have also been reported in laboratory trials with sublethal doses of imidacloprid (Lunardi et al., 2017 and Bovi et al., 2018).

Another parameter related to imidacloprid contamination is the increase of programmed cell death in *A. mellifera* brain cells. Apoptosis is commonly studied in neurological disorders and has been detected in bees contaminated with different types of agrochemicals (Gregorc & Ellis, 2011). Because imidacloprid blocks neurons conduction, its target site is located in the brain. Thus, Wu et al. (2015) demonstrated that after contamination with sublethal doses of imidacloprid, workers had an increase in caspase-1 enzyme levels, suggesting induction of the caspase-dependent apoptotic pathway.

Numerous insecticides act as neurotoxins that alter synaptic function in the insect's central nervous system (Goulson et al., 2015). For instance, neonicotinoids and sulfoximines bind to nicotinic acetylcholine receptors (NAChRs), disrupting cholinergic transmission, which may lead to the non-development or inactivation of neural cells (Peng & Yang, 2016).

The mushroom bodies of bees are a neural region specifically associated with olfactory learning and memory (Devaud et al., 2015). There is strong evidence that the development and function of mushroom bodies may be directly impaired by exposure to NAChR agonists (Peng & Yang, 2016).

Therefore, after exposure to sublethal doses of imidacloprid Peng & Yang (2016) demonstrated that there was a decrease in density of synaptic units in the brain of *A. mellifera* in regions related to smell and vision. Consequently, there was a decrease in olfactory learning and abnormal neural connectivity, providing evidence that imidacloprid impairs the development of the nervous system in regions responsible for smell and vision during the larval stage.

Similarly, Zhang & Nieh (2015) observed that after four days of exposure to 1.5 µg/bee of imidacloprid, short-term learning ability was reduced by 87% and memory retention was reduced by 85%. Even lower concentrations of imidacloprid (0.1 ng/bee) in the Arabic subspecies *A. m. jemenitica* significantly impaired learning ability and memory (Iqbal et al., 2019).

The immune responses in insects depend in part on the activities of the haemocytes that are found in the haemolymph. After infections, haemocytes exhibit alteration in morphology and behaviour, modifying the free-circulating cells to adherent cells, tending to form aggregates. Still, cellular responses include phagocytosis, nodulation, and encapsulation (Lanot et al., 2001, Satyavathi et al, 2014).

Brandt et al. (2016) concluded that there is an increase in haemocytes in *A. mellifera* after 24 hours of oral contamination with 1 µg/L and 10 µg/L of imidacloprid. In contrast, when the stingless species *Melipona quadrifasciata* was orally exposed to 292 ng of active ingredient (i.a.)/mL there was no difference in haemocyte amount (Ravaiano et al., 2018).

Cresswell et al. (2014) analyzed the capacity for clearance of imidacloprid in *A. meffilera* and *Bombus terrestris*. The bees were contaminated orally with 125 µg/L of imidacloprid for eight days. Each *A. mellifera* ingested an average of 2.2 ng/day of imidacloprid and maintained 1.4 ng/g of residue, which was not significantly different from the control group. As a result, it was estimated that *A. mellifera* cleared 100% of agrochemical residues daily. *B. terrestris* cleared 88% of the insecticide daily and showed sublethal effects on locomotory activity and intake impairment. However, after 48 hours without contact with the insecticide, the residues were entirely cleared.

However, analogous tests with *A. mellifera* conducted by Sánchez-Bayo et al. (2017) demonstrated that after 10 days of contamination residues were between 2.7 and 5.7 ng/g, the mortality rate was high and modified behaviour (restless, apathetic and shaky). Although these results contrast with the findings reported in the previous study for spring or summer bees, they are consistent with the results reported for other bees.

In a study conducted by Nahar & Ohtani (2015), *A. mellifera* were injected with 1, 5 and 10 ng/bee of imidacloprid, the bees showed altered behaviours, such as tremors, agitation, lack of normal movement and lack of coordination. Some contaminated bees were taken 50 meters from the colony and most of them failed to return to the colony. Colony growth and activity were also affected after imidacloprid contamination, as demonstrated by Meikle et al (2016).

Imidacloprid is used in a wide variety of crops, resulting in several sources of contaminated nectar and pollen that bees can forage. In some crops, such as maize, insecticide concentration can easily exceed the average lethal dose (LD_{50}) for *A. mellifera*. And while neonicotinoid concentrations found in nectar and pollen are in minimal quantities compared to the bee's LD_{50}, evidence suggests that these low doses have negative effects on colonies (Karaha et al., 2015).

Despite pollen and nectar contamination, foragers continue to visit imidacloprid-treated crops, making regular trips from the colony to the flowers and back to the colony, only at a slightly slower rate. Cognitive processes of foragers are not significantly compromised to prevent further foraging in these crops, so the nectar odour brought back to the colony will recruit more bees to visit the flowers, increasing insecticide reserves in honey (Stoner & Eitzer, 2012).

When colonies are to pass through winter and need to use their honey deposits, widespread poisoning of the colony by pesticides accumulated in honey can occur, causing general mortality (Lu et al., 2014). Ironically, if imidacloprid were very toxic to bees and immediately killed foraging bees, the colony as a whole would do better because foragers would not be recruited into contaminated regions. Karaha et al. (2015) concluded that with a scenario of high toxicity, colony losses may be limited to some foragers and the colony would be pesticide-free.

Although *A. mellifera* is the most studied species because of its economic importance, other species have also been studied. Soares et al. (2015) found the LD_{50} and the lethal concentration (LC_{50}) of imidacloprid for the native Brazilian bee *Scaptotrigona postica*. The LD_{50} values for 24 and 48 hours of contamination were 25.2 and 24.5 ng a.i./bee, respectively. The values for LC_{50} after contamination for 24 and 48 hours were 42.5 and 14.3 ng a.i./µL, respectively.

Another native species from Brazil, *Melipona scutellaris*, also had its LD_{50} and LC_{50} calculated. The established topical LD_{50} was 2.41 ng/bee after 24 hours of contamination and 1.29 ng/bee after 48 hours. Oral LC_{50} was 2.01 ng a.i./µL for 24 hours and 0.81 ng a.i./µL for 48 hours (Costa et al., 2015). Thus, we can observe that *S. postica* is more tolerant when compared to *M. scutellaris*.

Solitary bees are the most abundant bee species in the world, most of which nest in the ground. Prolonged contact with neonicotinoid contaminated soils may represent a significant route of exposure for many species. Observations on solitary bee species *Megachile rotundata* and *Osmia lignaria* showed changes in adult longevity, developmental speed and mass in response to increasing imidacloprid concentrations. Suggesting that chronic exposure to nesting substrates contaminated with neonicotinoids represents an important route of exposure, with considerable physiological and ecological consequences for bees and plant-pollinator interactions (Anderson & Harmon-Threatt, 2019).

Considering the studies cited in this review that analyze the results of bee contamination with the imidacloprid, we can conclude that the sublethal effects caused in different bee species are extremely harmful in many ways. Such in the expression of different genes and behavioural changes, thus altering social relations in the colonies, which can lead to huge losses.

Thiametoxam

Thiamethoxam is one of the most widely used neonicotinoids as it is highly toxic to insects (Simon-Delso et al., 2014). Because thiamethoxam is a systemic agrochemical and persistent in the environment, this insecticide is found in many resources that bees collect, such as nectar, pollen and water. Thiamethoxam acts by binding with high affinity to insect nicotinic acetylcholine receptors, thereby inducing a variety of sublethal effects on bees and colonies, even at low doses and concentrations (Godfray et al., 2015).

Sublethal doses of thiamethoxam significantly alter several characteristics of bees. For *A. mellifera*, the LD_{50} for oral administration of thiamethoxam over 48 hours is 5 ng/bee, and the LD_{50} for 24-hour contact is 29 ng/bee (Decourtye et al., 2005).

After 1 hour of contamination with 1.34 ng/bee of thiamethoxam *A. mellifera* showed excitation, increase of flight duration (78%) and 72% increase in distance. After chronic contamination for two days, bees presented a decrease in flight duration (54%), a decrease in the distance (56%) and a decrease in flight speed (7%). These results demonstrate that acute and chronic contamination with thiamethoxam alters significantly flight capacity in honeybees (Tosi et al., 2017).

The movement of bees stimulated by light has an important role in their life and in the division of work inside the colony. Yang bees are photophobic negative and are usually found inside the colony, on the other hand, foragers are photophobic positive and typically stay at the entrance or outside the colon, showing a preference to areas with more light (Tosi & Nieh, 2017).

Contamination with insecticides can impair several behaviours of bees, including photo sensibility. After 30 minutes of contamination with 1.34 ng/bee of thiamethoxam honeybees showed hyperactivity and after one hour it was observed abnormal behaviour and lack of motor skills. After two days of contamination, bees lost their capacity of ascension. Thiamethoxam increased the movement of the bees to the light, thus this insecticide can reduce the health of the colonies by impairing workers' mobility and potentially alter the division of labour (Tosi & Nieh, 2017).

Gajger et al. (2017) argue that the knowledge about the effects of agrochemicals in bees it is crucial to understand the high mortality of individuals and colonies that are happening all around the world in the last years, especially regarding the development of queens. This study showed that after contamination of queen larvae with 4.28 ng/queen of thiamethoxam there was a decrease in body weight of emerging queens, a decrease of ovary weight and less sperm after mating with drones.

Agrochemicals in the environment can harm not only foraging bees, but also those that perform activities within the colonies and larvae that feed on contaminated pollen and nectar. *A. mellifera* larvae contaminated with 0.001 ng/µL thiamethoxam showed no significant differences in pupal survival, but digestive cells and Malpighi tubule cells of contaminated bees showed alterations, indicating possible tissue degeneration. These data demonstrate the cytotoxicity of thiamethoxam in target and non-target cells of newly emerged bees, suggesting impairment of cellular organelles and their functions (Friol et al., 2017).

Tavares et al. (2015) found an LC_{50} for *A. mellifera* larvae equal to 14.34 ng/µL of thiamethoxam in the bee diet. After contamination with the sublethal concentration of 1.43 ng/µL, morphological and immunocytochemical analyzes of the bee brain showed condensed cells and early cell death in the optic lobes. Additional effects on larval development were also observed, indicating that thiamethoxam may modulate bee development, affecting colony maintenance and survival.

Some biomarkers are used to evaluate the physiological effects of environmental stressors, such as acetylcholinesterase (AChE), an enzyme that controls the neuronal activity of cholinergic synapses, carboxylesterases (CaE) and glutathione S-transferase (GST). After contamination with 0.00001 ng/µL, 0.001 ng/µL and 1.44 ng/µL thiamethoxam there was AChE activity in all developmental stages and increase of CaE and GST activity in the pupal stage, indicating that contamination triggered the stress responses and reducing larval and pupal survival (Tavares et al., 2019),

Synapsins are phosphoproteins acts almost all synapses in the nervous system. Thus, studying the distribution of these proteins may help to assess the impacts of insecticides on the brain structure of bees (Fahrbach & Van Nest, 2016). After contamination of *A. mellifera* larvae with sublethal doses of thiamethoxam, Tavares et al. (2019) observed that there was a decrease in synapsin in newly emerged pupae and bees, which may lead to disturbances in nervous system functions, such as olfactory learning and neurotransmitter release control, impairing the behaviour and survival of bees.

After contamination with insecticides there is an increase in catalase and GST activity, showing that thiamethoxam promotes oxidative stress in bees. Oxidative stress occurs when the antioxidant defences of an organism are unable to counterbalance free radicals and can alter DNA and protein constituents, leading to disorders of organ and tissues (Gauthier et al., 2018).

Transcriptome analyses of thiamethoxam-contaminated honeybees identified 609 differentially expressed genes, including downregulation of *Vg* (lifetime regulation), *CSP3* (pheromone transport in the offspring), *defensin-1* (fighting pathogens), *mrjp1* (nutrition and differentiation) and upregulation of *Cyp6as5* (thiacloprid resistance) (Shi et al., 2017b). Besides, seven microRNAs were differentially expressed after contamination and some putative target genes were related to behaviour, immunity and neural function (Shi et al., 2017a).

When *A. mellifera* infected with the chronic bee paralysis virus were contaminated with 5 ng/bee/day of thiamethoxam, mortality increased after 8 to 10 days, but there was no increase in viral loads. When contaminated with half of the dose (2.5 ng/bee/day), there was no increase in mortality, but viral loads were significantly higher in naturally dead bees when compared to bees that were sacrificed. Thus, it can be observed that the interaction between pathogens and bee contamination with agrochemicals is quite complex (Coulon et al., 2018).

Analyzes of the sublethal effects of thiamethoxam on stingless bees are scarce. Moreira et al. (2018) studied these effects in *Scaptotrigona bipunctata* and concluded that although there was no high mortality in the tested concentrations, there was a significant change in the expression of esterase and intestinal morphology.

Thiamethoxam is the second neonicotinoid with the largest amount of scientific studies relating its sublethal effects on bees. Based on the scientific articles published between 2014 and 2019, it is possible to observe that contamination with thiamethoxam affects several characteristics of bees, directly influencing colony survival.

Clotianidine

Clotianidine is a highly effective insecticide used for a broad spectrum of sucking and biting insects. Even relatively small amounts of the substance are sufficient to control pests in major crops (Elbert et al., 2008). Due to its systemic properties, it is suitable for seed treatment. Clotianidine is easily transported within the xylem to the untreated parts of the plant (Bonmatin et al. 2015).

Studies analyzed the contamination of *A. mellifera* and *B. terrestris* with canola treated with clotianidine in Germany and Canada and found no sublethal effects on colony health, bee development and winter survival (Cutler et al., 2014, Sterk et al., 2016, Dietzsch et al., 2019). However, the learning ability and semen quality of *A. mellifera* drones were impaired by sublethal doses of clotianidine (Abderkader et al., 2018, Tison et al., 2019).

Odemer et al. (2018) studied the synergistic effect of sublethal doses of clotianidine in *A. mellifera* infected with *Nosema spp*. and observed that there was no synergistic effect. Also, contamination with this neonicotinoid at sublethal doses did not change bee mortality or flight capacity.

It was also observed by Siede et al. (2018) and Morfin et al. (2019) that there is no synergism between clotianidine contamination and *V. destructor* infection. However, sublethal contamination with clotianidine, similar to that expected under field conditions, may impair the self-cleaning behaviour of bees infected with *V. destructor* (Morfin et al., 2019).

Clotianidine also showed no sublethal effects on development, winter survival and adult metabolism in the solitary bee *Osmis bicornis* (Nicholls et al., 2017). However, the species *Osmia cornuta* showed impairment in visual orientation and use of navigational memory when contaminated with sublethal doses (0.76 ng/bee) of clotianidine (Jin et al., 2015).

Sublethal doses of clotianidine increase CYP450 activity, but do not alter esterase and GST activity, indicating that CYP450s are involved in clotianidine detoxification in bees. Further analysis revealed that *CYP9q1* gene expression (important in detoxification) was significantly induced by insecticide (Yao et al., 2018). Thus, the latest studies on bees contaminated with sublethal doses of clotianidine showed that this insecticide has lower bee toxicity when compared to imidacloprid and thiamethoxam.

Thiacloprid and Acetamiprid

Thiacloprid is a neonicotinoid-class agrochemical used on leaves and seeds of crops to control insect populations (Siede et al., 2017). As well as imidacloprid, thiacloprid acts against nicotinic acetylcholine receptor. It is the first neonicotinoid that acts on sucking insects such as aphids, whiteflies and also weevils, leafminers and various species of beetles (Omirou et al., 2009).

Chronic exposure to sublethal doses of thiacloprid in A. mellifera compromised foraging behaviour, return to the colony, navigation and social communication. Thiacloprid residue levels increased in both foragers and nestmates over time (Tison et al., 2016).

A. mellifera fed with thiacloprid significantly reduced the centrality of the interaction network in the colony, however, exchanged more food with other members of the group, resulting in dilution of contaminated food. Thus, although thiacloprid may act as a general disruptor of social network structure, it can still play a role in the dynamics of disease transmission in the colony if pathogens are transmitted via food exchange (Forfert & Moritz, 2017).

Siede et al. (2017) studied colony performance during three years of contamination of *A. mellifera* colonies with sublethal concentrations of thiacloprid and concluded that under field conditions the colonies were not harmed. Tison et al. (2017) demonstrated that acute contamination by both the active ingredient thiacloprid and its commercial composition pose a substantial risk to bees, disrupting learning and memory functions.

Metabolic analyses of *A. mellifera* after sublethal contamination with thiacloprid for three days showed that 115 metabolites were significantly affected in treated bees when compared to control. These metabolites are associated with oxidative stress and detoxification, suggesting that bees activated their detoxification system when contaminated with thiacloprid. Within these insecticide-altered metabolites, the reduction in serotonin suggests that thiacloprid may hinder brain activity related to learning and behavioural development (Shi et al., 2018).

In *B. terrestris* thiacloprid was absorbed in the bees when the crops were sprayed during flowering but were not detected when the crops were sprayed in the budding phase. Bees in late-sprayed crops also developed weaker colonies than in non-sprayed crops. Dead bees with a high internal concentration of thiacloprid were found in an overnight spray culture with 35% flowering. This shows that thiacloprid is not safe for bees if sprayed after anthesis and that spraying should be performed in the budding phase to reduce nectar and pollen contamination (Havstada et al., 2019). Despite the small number of articles published regarding thiacloprid toxicity in bees, it can be observed that this insecticide alters several characteristics of bees.

Acetamiprid is a new neonicotinoid insecticide, used to control agricultural pests, especially in vegetables, fruits and tea. This agrochemical acts target insects from the Hemiptera order, mainly aphids, Thysanoptera and Lepidoptera. Because this insecticide has relatively low acute and chronic mammalian toxicity, acetamiprid is more competitive than some conventional insecticides and has been considered an important substitute for organophosphate insecticides that causes severe environmental pollution and resistance, and it is prohibited in many countries (Yao et al., 2006).

Only two articles published between 2014 and 2019 that studied the sublethal effects of bees contaminated with acetamiprid were found. Thany et al. (2015) studied the retraction of *A. mellifera* proboscis and concluded that acetamiprid impaired this movement at the tested doses (10 and 100 ng/bee). Telangre et al. (2018) observed that in sunflower crops sprayed with half of the recommended dose of acetamiprid there was higher foraging by *A. mellifera* when compared to foraging on flowers that were treated with the full dose of acetamiprid. Thus, we can observe that in these two studies acetamiprid impaired the behaviour of bees.

Conclusion

Imidacloprid and thiamethoxam are the neonicotinoids most used in crops, that is why they are the most studied for sublethal effects on bees. In the studies cited in this review, it is observed the detrimental effect of these two agrochemicals on the behaviour and development of bees.

Clotianidine less toxic insecticide to bees when contaminated with sublethal doses. On the other hand, thiacloprid and acetamiprid were as harmful to bees as imidacloprid and thiamethoxam.

Although there are numerous studies on the effects of insecticides on bees, little is known about the long-term consequences of insecticide contamination. It is also important to point out that in agriculture several different agrochemicals are used in a single crop, so bees are exposed to a cocktail of these substances and the combination of their effects can lead to other unknown consequences.

Moreover, most studies illustrate the effects of agrochemicals on commercially important bees, especially *A. mellifera* and few studies are focused on the consequences on other species of bees, which are also very important for pollination in the environment.

In conclusion, sublethal effects on bees contaminated with neonicotinoids are highly relevant in the disappearance of bees all over the world.

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