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**STUDY OF THE BIO-ACCUMULATION OF PESTICIDES IN APISMELLIFERA IN SOUSSMASSA REGION (SOUTHERN MOROCCO)**

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**ABSTRACT**

The aim of this study is to assess the bioaccumulation of pesticides in the bee *Apismellifera* living by farming methods in the Souss Massa Region, known as an excellent agricultural area in South of Morocco. The study was conducted on bees collected from five sites in the region (Agadir and Ida Ougnid if considered as a control sites and Sebt El Guerdane, Houara and Taroudant, which are agricultural sites, suspected to be contaminated by pesticides).

Our preliminary results show the presence of pesticides: Neonicotinoids (Acetamiprid and Imidacloprid) and Organophosphorus (ChlorpyrifosEthyl) in samples of bees collected in the Sebt El Guerdane, Houara and Taroudant sites: These results lead to the supposition that behind the appearance of mortality rates, the behavioral and physiological disorders observed in bees in these sites can be probably due to the intoxication by the use of pesticides

**Keywords:** *Apismellifera*, Beekeeping, Bioaccumulation, Pesticides, Souss Massa Region.

**1. INTRODUCTION**

The Souss Massa is an agricultural region in the south of Morocco in which the use of pesticides is a routine practice. However, this orientation is not without consequences for the environment and human health. Indeed, several useful animal and plant species that are not targets of pesticides are often impacted by these products. In this context, beekeeping is one of the most affected sectors and often experiences mortalities, weakening and collapse of bees which are partly linked to pesticides. Beekeepers incriminate bee pathologies, in particular *Varroa*, bee intoxication by insecticide treatments and to the degradation of the floral cover of the environment. Faced to this situation, it is necessary to assess the risks associated to pesticides in order to contribute to set up a strategy for monitoring, preventing and protecting the state of health of human, environment and beekeeping resources.

The bee plays a key role in the production of citrus fruits. Orange blossoms produce abundant nectar (some flowers contain an average of 20 µl) (Pouvreau, 1984; Crane & Walker, 1984). The flowering is very abundant, there are more than 60,000 flowers on an orange tree. The honey bee

is the most abundant pollinating insect. Bees collect either nectar, pollen, or both. Each visit on a flower lasts 15 to 20 sec for the nectar foragers and 5 to 8 s for the 34 pollen foragers.

Pesticides are a source of mortality in the bee, this essential insect for maintaining biodiversity. The bee participates in almost 80% in pollination, which makes it a key element in the survival of the plant world. For example, in France, the bee mortality rate increased from 5% in the 1990s, to 30% in 2017, when neonicotinoids were introduced to the market.

Pesticides are used preventively and massively; they have a very high toxicity for invertebrates and a high toxicity for vertebrates; they persist in the environment for a very long time. For these reasons, they are a major cause of the loss of pollinators and they compromise the stability of the ecosystem. They are also a threat to food security.

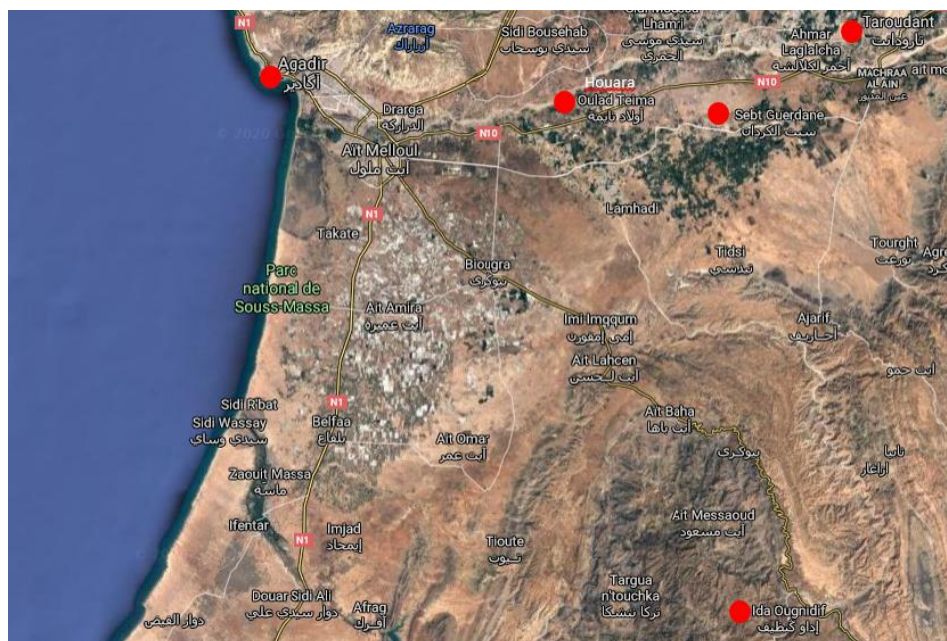
In this context, the objective of our work is to study the effect of using pesticides on the serious disturbances and collapses observed in beekeepers hives and put forward the responsibility for pesticides used in the treatment of orchards around.

## **2. MATERIALS AND METHODS**

### ***Sampling sites*** (Figure 1)

The study covered five sites with three beekeepers per site. The choice of sites was based on their nature :

- **Idaougmidif**: which is a site characterized by an area (fixed limit of the foraging area), typical vegetation, limited road traffic and agricultural activity and no industrial activity.
- **Agadir**: which is an urban site characterized by a majority of urbanized area, small flora, significant automobile traffic, industrialization and absent or limited agricultural activity.
- **Houara, SebtEl Guerdane and Taroudant**: these sites are characterized by large areas of large field crops (dominated by orange trees) with beehives close to citrus orchards.



**Figure 1:** Sampling sites: Idaougnidif, Agadir, Houara, Sebte El Guerdane and Taroudant

### *Beekeeping matrix collected*

The sampling plan was established in order to obtain the most faithful image possible of the contaminants brought back to the hive by the bee and to apprehend their possible transfers within the hive itself. Adult bees *Apis mellifera* were collected directly by shaking a frame over a clean and dry aerate box and transported to our laboratory.

### *Sample preparation*

Pesticides were extracted using the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe). It is a technique for extracting pesticides and has the advantage of being quick, easy, inexpensive, effective, robust and reliable. This method was developed for the first time by Anastassiades *et al.* (2003) for the analysis of pesticide residues. It is based on the principle of liquid-liquid extraction with an organic solvent in the presence of QuEChERS salts and buffers followed by purification.

The identification is done by the presence of the two transitions (2 MRM for the MS / MS system) using the liquid chromatography coupled with mass spectrometry (LC MS / MS).

## **3. RESULTS AND DISCUSSION**

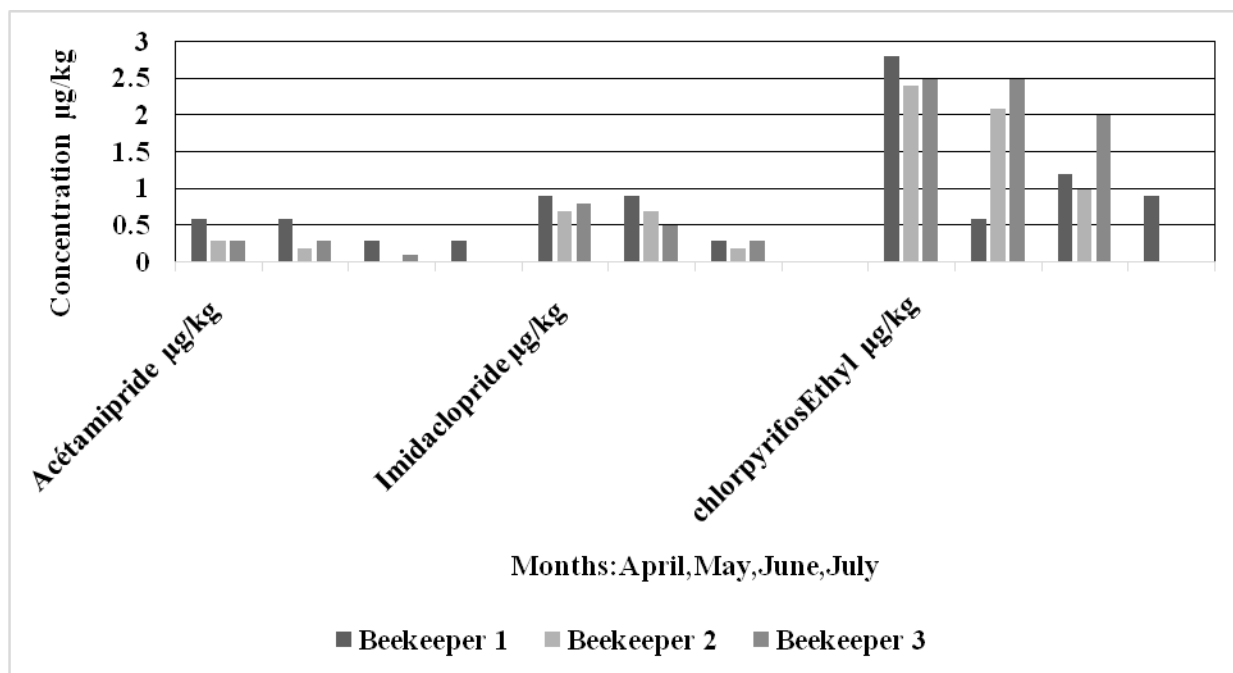
*Apis mellifera* collected in sites of Agadir and Ida Ougnidif, previously considered as a control sites, show no contamination by pesticides but in the sites of Houara, Sebte El Guerdane and Taroudant pesticides were detected: two neonicotinoids (Acetamiprid and Imidacloprid) and an organophosphorus (Chlorpyrifos Ethyl). The analysis results of samples collected during this study using LCMS / MS are given in the table 1:

**Table 1: Results of variance pesticides LCMS / MS analysis of *Apis mellifera* collected in sites of Houara, SebtEl Guerdane and Taroudant (B :Beekeeper ; LOD : Limit of Detection ; LOQ :Limit of Quantification).**

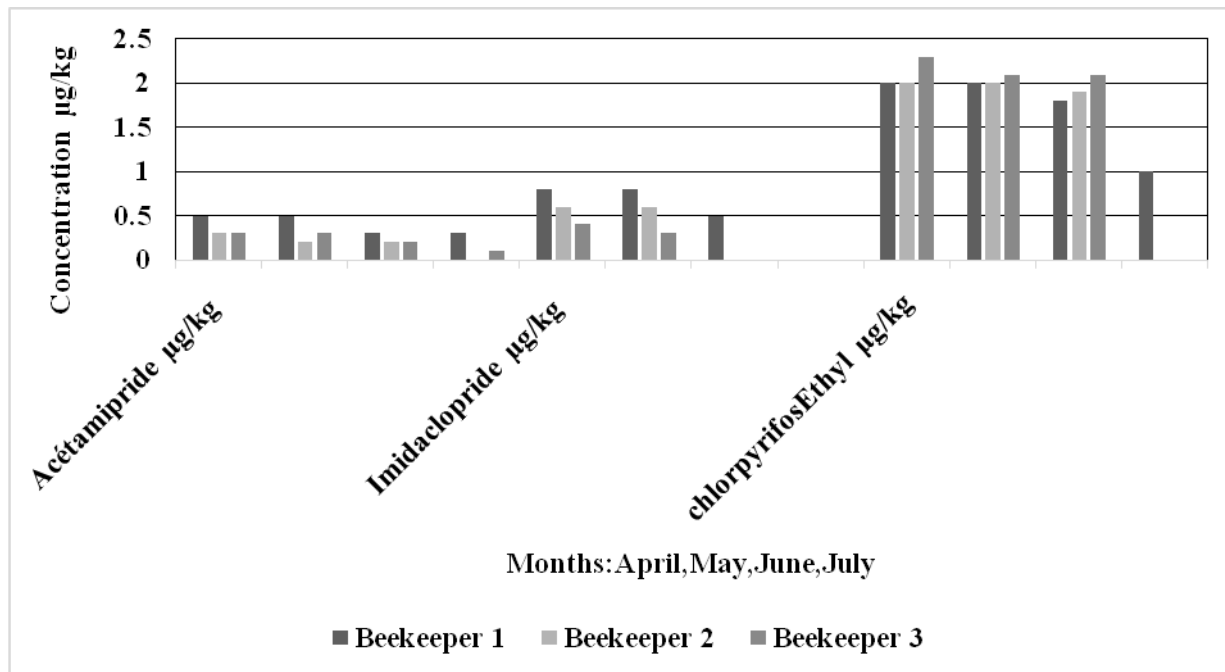
	LO D	LO Q	Mont h2017	Taroudant			SebtEl Guerdane			Houara		
				B1	B2	B3	B1	B2	B3	B1	B2	B3
Acétamipride (µg/Kg)	0,3	1	April	0,5	0,4	0,4	0,6	0,3	0,3	0,5	0,3	0,3
			May	0,4	0,2	0,2	0,6	0,2	0,3	0,5	0,2	0,3
			June	0,3	0,1	0,1	0,3	<LQ	0,1	0,3	0,2	0,2
			July	0,3	<LQ	<LQ	0,3	<LQ	<LQ	0,3	<LQ	0,1
Imidaclopride (µg/Kg)	0,3	1	April	0,8	0,7	0,6	0,9	0,7	0,6	0,8	0,6	0,4
			May	0,7	0,6	0,5	0,9	0,7	0,5	0,8	0,6	0,3
			June	0,5	0,4	0,3	0,3	0,2	0,3	0,5	<LQ	<LQ
			July	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
ChlorpyrifosEthyl (µg/Kg)	0,3	1,2	April	2,5	2,5	2,5	2,8	2,4	2,5	2,0	2,2	2,3
			May	2,7	2,2	2,4	2,6	2,1	2,5	2,0	2,0	2,1
			June	1,0	2,0	2,1	1,2	1	2	1,8	1,9	2,1
			July	1,0	<LQ	<LQ	0,9	<LQ	<LQ	1,0	<LQ	<LQ

The assay results revealed the detection of pesticides only in samples from Houara, SebtEl Guerdane and Taroudant with recording of different and decreasing values from April to July 2017 (Figures 2, 3 and 4).

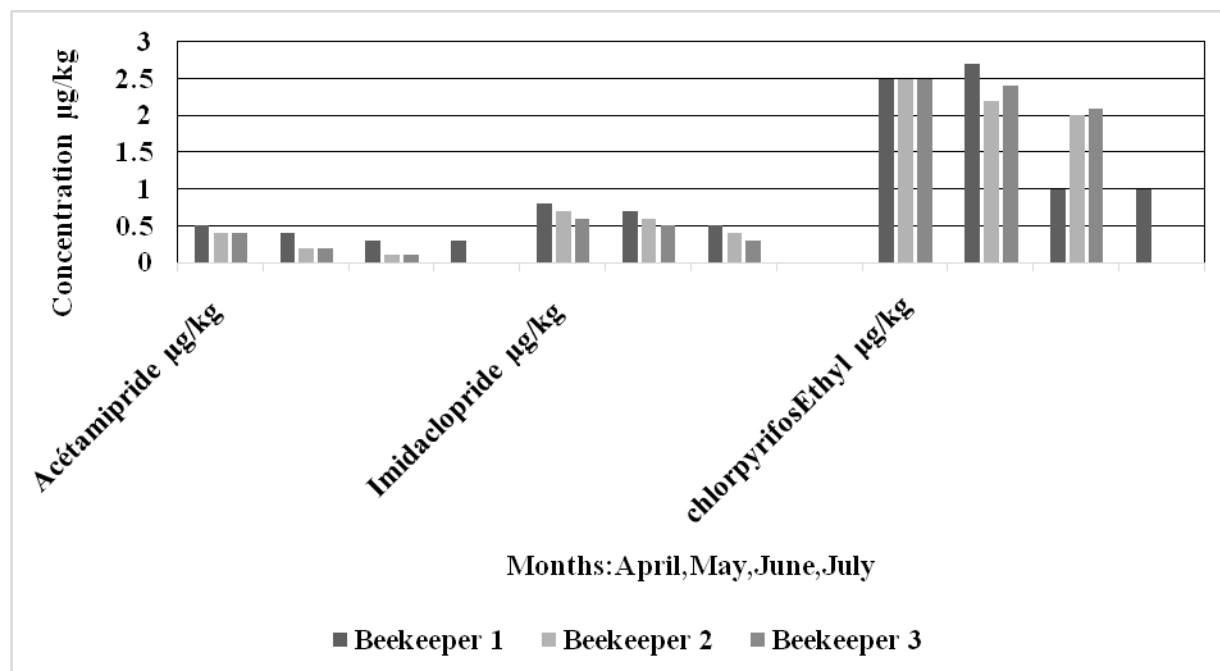
The simultaneous absence of pesticide residues in bees from Agadir and Ida Ougnidif and their strong presence in other beekeepers appeared to be significantly correlated with the high use of pesticides in citrus orchards in these sites.



**Figure 2:** Variation in concentrations of detected pesticides during the study months in *Apismellifera* collected SebtEl Guerdane



**Figure 3:** Variation in concentrations of detected pesticides during the study months in *Apismellifera* collected in Houara



**Figure 4:** Variation in concentrations of detected pesticides during the study months in in *Apismellifera* collected Taroudant

The trend towards a decrease in the concentrations of the three active substances found in the three sites pushed research towards the treatments used in citrus fruits just beside. Indeed, it was found that it the contamination is linked to the use of these treatments along the agricultural season which generally starts from the month of March and ends at the end of June. Such observations could explain the decontamination of the hives at the end of the sampling period and the decrease in the fall hives among beekeepers observed in the region.

Another relevant observation is the high concentration of chlorpyrifosEthyl observed compared to the other pesticides detected and measured during all the months of the study as shown in Figures 2, 3 and 4. This difference can be explained by the increase in frequency of the use of chlorpyrifosEthyl per day, and consequently the contamination of bees by significant quantities of this pesticide by volatilization or during their presence in agricultural crops.

Given this situation and according to the results of our study which revealed the presence of pesticides in sites close to these crops, we can explain the bee disorders observed by the contamination by pesticides detected in the samples, namely the neonicotinoids represented here by Acetamidrid, Imidacloprid, and the organophosphates represented by ChlorpyrifosEthyl.

The result is, within minutes, or even seconds, vision and other sense disturbances, loss of motor control (general paralysis), possibly followed by paralysis of the heart muscle and breathing muscles, leading upon death (Pelletier, 2010).

Studies interested in the effects of imidacloprid have shown that this pesticide and these metabolites are highly toxic to bees.

Imidacloprid is considered to be one of the pesticides that cause toxicity at low doses. In antennal lobe neurons, the characterization of type I currents of nAChR, which has slow desensitization, and type II currents, which exhibit rapid desensitization, strongly suggest the presence of at least two different types of nAChRs. The presence of two types of receptors displaying different affinities for imidacloprid and its metabolites has been proposed on the basis of the complex toxicity profile after chronic exposures in bees; The presence of two types of imidacloprid targets, which could explain the differential toxicity of imidacloprid at low and very low doses observed in bees (Simon-Delso *et al.* 2014).

Imidacloprid also affects the metabolism, reproduction and development of the colony, and the mobility of the bee. It has been shown that doses of 2  $\mu\text{g} / \text{L}$  have an impact on the energy metabolism of larvae and workers by affecting detoxification, the intermediate and energy metabolic pathways, and by this action imidacloprid alters brain metabolism in bees. which leads to an increase in cytochrome oxidase in the peduncle bodies (Pisa *et al.* 2014).

These negative effects of imidacloprid on motor activity depend on the dose of insecticides, the lowest dose 125 ng per bee resulted in increased motor activity, while the highest dose 25 to 20 ng per bees induce a decrease in this activity (Desneux *et al.* 2007). Colin *et al.* (2019) revealed that imidacloprid influences worker foraging during the larval stage strongly.

The second neonicotinoid highlighted by our study is acetamiprid. The harmful effects of this pesticide are tested. The results obtained with acetamiprid have shown that the action of neonicotinoids depends on the level of exposure and cannot be generalized to neighboring destructured compounds. Unlike contact exposure and oral exposure to acetamiprid, which has affected the long-term preservation of olfactory learning (Pisa *et al.* 2014).

Badawy *et al.* (2014) demonstrated that acetamiprid at  $\text{LD}_{50} = 1.60\mu\text{g} / \text{bee}$  causes bee mortality after exposure for 24 hours.

Field and laboratory studies that try to test lethal doses under field conditions have demonstrated that chronic oral or contact exposure for 10 to 11 days to 1  $\mu\text{g} / \text{L}$  of acetamiprid can lead to worker mortality (Pisa *et al.* 2014) other results reveal that a dose of 0.25 g / L of acetamiprid causes 20% mortality in *A. mellifera* bees 48 hours after treatment (Stanley *et al.* 2014).

Acetamiprid and its metabolites can also affect the memory process and the metabolism of acetamiprid apparently results in different metabolites in bees, among which 6-chloronicotinic acid is toxic by chronic exposure, but not by acute exposure and remains stable for at least 72 h, especially in the head and thorax. Given the presence of multiple active metabolites over time, it is very difficult to verify which stages of the memory process (acquisition, consolidation or recovery) are affected by acetamiprid (Pisa *et al.* 2014). In addition to the use of synthetic insecticides can also cause changes in the social behavior of bees such as increased agitation, aggression and pollen contamination (Stanley *et al.* 2014).

The toxicity of acetamiprid can also alter the activity of certain key enzymes in the bee's functional processes. Studies have reported that exposure to a dose of 0.6 mg / L of acetamiprid leads to a decrease in the specific activity of AChE by 0.50 and 0.30 nmol hydrolysis of AChE / mg protein / min in the head and thorax respectively. It can be concluded that this pesticide has an inhibitory action on AChE in other enzymes, almost the same results have been found, carboxylesterases are the main enzymes for detoxification of pesticides.

Our previous comparative study of AChE activity of *Apis mellifera* as biomarker of pesticides contamination conducted in Houara region (considered as a contaminated site) and Tassila (considered as a control site) showed a high AChE activity in animals from contaminated sites (Houara), compared to those recolted in the control site (Tassila) (Aboudlou *et al.* 2018).

The third pesticide detected by the analysis in our study is Chlorpyrifos Ethyl, which belongs to the family of organophosphates, it controls the attack of Coleoptera, Diptera, Homoptera and Lepidoptera. It is sprayed on fruit trees (apple, citrus, hazelnut ...), strawberry, banana, potato, tobacco, rice, cotton, alfalfa, cereals, corn, ornamental plants and in forests, chlorpyrifos ethyl is classified as toxic to bees (Rafalimanana, 2003).

Many results show that using doses of neonicotinoids may cause honeybee colony failure, not only because the exposed workers start foraging when younger, which unbalances the age-based division of labor (Jacob *et al.* 2019). Also because of faster biochemical senescence. Its also an important fact that may help explain controversial mechanisms of colony depopulation when it is exposed to sublethal neonicotinoid.

Chlorpyrifos (CPF) has been shown to be metabolically activated by oxidative desulfurization to give its metabolite chlorpyrifos oxon (CPF oxon) which can inhibit acetylcholinesterase (AChE) by phosphorylation of its serine sites (Rehman *et al.* 2012). Inhibition of AChE can induce a general disturbance in all systems because it is an important component in all synaptic transmissions (Nicolas Desneux *et al.* 2007).

Numerous studies have also suggested that CPF could induce the production of ROS in bee nerve tissue, resulting in neurotoxic effects by a mechanism that involves the high toxicity of OH radical instead of inhibition of acetylcholinesterase. The neurotoxicity of CPF increases the formation of malondialdehyde in the bee, malondialdehyde is a reactive substance thiobarbituric acid (TBARS) and a lipid peroxidation marker for cell membrane damage, it is also the end product of oxidative stress in biological systems. After pathophysiology or xenobiotic toxicity, significant increases in malondialdehyde levels in the bee's nervous system by different levels of CPF exposures showed dose dependent slope, meaning that apparent oxidative stress was taking place by action of the pesticide. Other investigations have reported that the toxicity caused by CPF may be due to the induction of oxidative stress in the central nervous system of the bee (Rehman *et al.* 2012).

Another study shows that exposure to a 2g / L dose of chlorpyrifos causes 100% bee mortality 48 hours after treatment, and this can be explained by the high toxicity of chlorpyrifos to bees (Stanley *et al.* 2014).



#### 4. CONCLUSION

Pesticides as a whole are known by their harmful effects on the organisms they affect, our study of the evaluation of the impact of these substances on the bee of the Souss Massa region confirmed this observation through results obtained which showed the presence of the pesticides implicated in the appearance of the disorders observed in bees during field observations.

The information available in the literature shows that these pesticides are classified among the most heavily used and the most toxic for bees. These pesticides can cause many different disorders depending on the dose of these substances in the bee, which can go up to when the organism dies, the toxic effects of these three pesticides can lead to mortality, physiological disorders (development, neurotoxic, fertility, immunity, etc.), and behavioral disorders (navigation and orientation, mobility, etc.).

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