International Journal of Latest Engineering and Management Research (IJLEMR)
ISSN: 2455-4847
www.ijlemr.com || Volume 05 - Issue 06 || June 2020 || PP. 36-45

Analysis of the effect of different pesticides on *Tetragoniscaangustula* (Jatai Bee) through oral contamination

Naiane Antunes Alves Ribeiro*
Gilson Barbara**
Dr. Dagmar A. de Marco Ferro***

[*]*Undergraduate in Agronomic Engineering, Centro Universitário de Santa Fé do Sul – SP, UNIFUNEC,*
[**]*Undergraduate in Agronomic Engineering, Centro Universitário de Santa Fé do Sul – SP, UNIFUNEC,*
[***]*University Professor, Centro Universitário de Santa Fé do Sul – SP, UNIFUNEC,*

Abstract: The bees perform many different activities besides pollination, bringing positive impact both social and economic. The indiscriminate use of pesticides have caused a negative impact on them bringing excessive reduction on the number of bees and possible extinction. Therefore, the goal of this project was to identify the effects of different groups of pesticides and chemicals on the native bees *Tetragonisca angustula*when exposed to contaminate feeding. The methods used were the ones described by Lourenço (2012), with some modifications. Bees removed from three colonies were placed in plastic jars with ventilation holes and fed by eppendorfs with drillings at the ends. Three groups of pesticides were tested Neonicotinoids, Pyrethroids and Organophosphates.In its highest and lowest concentrations. Each experimental group made of three repetitions and ten bees of each colony per jar were exposed to diets based of sugar diluted in deionized water and different concentration of pesticides. After 18 hours, the contaminated solutions were replaced by other ones without contamination and observed in intervals of 2, 4, 6, 12 and 24 hours. Initially the experimental groups with the highest death rate were the Organophosphates and Neonicotinoids in highest concentration. On the second run, the Organophosphates in high concentration presented 100% death rate and the Pyrethroids showed the lowest death rate. A third observation showed that the whole group ofOrganophosphates of both concentrations showed total death rate. In the fourth analysis both the Neonicotinoids and Organophosphates already presented 100% of mortality in both concentrations. The Pyrethroids presented full mortality in only one repetition of each concentration and excessive self-sanitation as abnormal behavior minimized after the change of the feeders for other ones not contaminated. The conclusion was that the chemical group that presented highest danger was the Organophosphates followed by the Neonicotinoids. The Pyrethroids were shown to be the least lethal. It was also observed excessive self-sanitation as a recurrent sublethal effect.
Keywords: Natives bees. Pollination. Pesticides. Contamination

Introduction

The species*Tetragoniscaangustula*(Jatai bee) known as the “stingless bees” are important for the maintenance of the biodiversity being responsible for pollinating 30% to 90% of the native flora, specially species of the Caatinga, Pantanal and Atlantic Forest. They also present great potential as pollinators in greenhouses because of being easily tamed as well as having the ability to perform “vibration pollinating” where they can release pollen by vibrating their thoracic muscles which is indispensable in the pollination of flowers with porous anthers, like some cases of plants of the *Solanaceae*genre that have great agricultural importance (LOURENÇO, 2012). According to Lima; Rocha (2012), in the last years the concern with the preservation of the bees have increased a lot due to the decline of pollinators and as a consequence the pollination of landscapes highly dominated by economical activities.

The factors that contribute the most to the reduction of the diversity of bees are the fragmentation of their habitats that begin with the deforestation, use of pesticides in agriculture and the introduction of species able to compete with the native bees, especially for the floral resources. The lost of pollinators of a biotic community may not be easily reversible. Studies about the toxicity of chemical agents to stingless bees are still relatively scarce, being that such species do not exist in countries with temperate climates, usually the ones to conduct this type of study (LIMA; ROCHA, 2012). According to Fernandes (2012), recently the test to evaluate the toxicity of pesticides in bees, done by IBAMA, follow recognized international protocols. The measurement of the toxicity of the pesticides in bees orally and through contact is done through the definition of the DL_{50}(GUEVARA PUEYO, 1995) only for the exotic species *Appis melífera*. The goal of this project is to evaluate the impact of the ingestion of food contaminated by certain groups of pesticides in different concentrations on the native bee *Tetragoniscaangustula.*

Methodology

The Methodology used in this research was based on the one described by Lourenço (2012), with some modifications. In order to evaluate the lethal and sublethal effects on the native bees the defensives of chemical groups Neonicotinoids, Pyrethroids and Organophosphates in their maximum and minimum recommended concentration, given orally during a period of 72 hours. To execute the tests 3 (three) hives of Jatai bees were used, named as hive “X”, “Y” and “Z”, placed in a vegetation area inside the Centro Universitario de Santa Fe do Sul (UNIFUNEC) Campus in the State of Sao Paulo as illustrated on Image 1.

Image **1**: Location of the hives das X, Y, Z e W.

Source: The authors

The experiment began with the preparation of the collecting jars and the experimental jars. 500ml plastic jars were used, pierced so there was proper ventilation. A paper tube was fixated on the side to make it easy to collect the bees in the field and on the experimental jars,an 1,5ml eppendorf was inserted into the lid to be used as a feeder. The contaminated solutions used in the tests were prepared with deionized water with sugar with the sugar proportion being 1:1 with the pesticide concentrations (BOX 1). Next 0,5 ml of the solutions in each eppendorf was pipetted according to the suggested repetitions.

Box 1: Details of the commercial products and their maximum and minimum utilized doses.

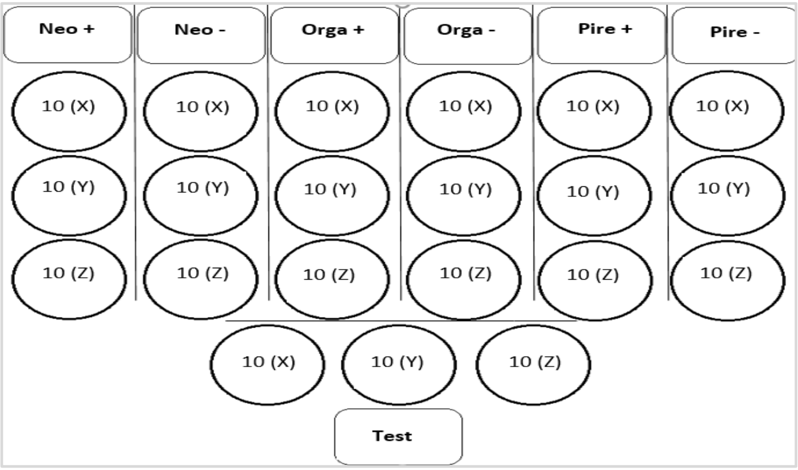
Chemical Group: NEONICOTINOIDS		
COMERCIAL PRODUCT (C.P.): PROVADO ACTIVE INGREDIENT (A.I.): IMIDACLOPRID		
Concentration of A.I.. (g/L)	200	
Culture: Citrus		
DOSAGE	↑ Dose (c.p.) 35 - 50 ml/100L water	↓ Dose (c.p.) 15 ml/100L water
CONTROLED PLAGUES	Cochonilha-Orthezia (*Orthezia praelonga*)	Pulgão-preto-dos-citrus (*Toxoptera citricida*)
Class: Pesticide Action mode: Sistemic Formulation: SC Toxicologic Classification: III – Medium toxic		

Classification of the potential environmental danger: III – Dangerous product to the environment		
Chemical Group: ORGANOPHOSPHATES		
COMERCIAL PRODUCT (C.P.): CHLORPYRIFOS ACTIVE INGREDIENT (A.I.): CHLORPYRIFOS		
Concentration of A.I.. (g/L)	480	
Culture: Beans		
DOSAGE	↑ Dose (c.p.) 1.250 ml/100L water	↓ ↑ Dose (c.p.) 800 ml/100L water
CONTROLED PLAGUES	Broca-das-vagens (*Etiella zinckenella)*/ Largata-da-vagem (*Michaelus jebus*)	Cigarrinha-verde (*Empoasca kraemeri*)
Class: Pesticide Action mode: Contact and ingestion Formulation: EC Toxicologic Classification: I – Extremely Toxic Classification of the potential environmental danger: II – Very Dangerous product to the environment		
Chemical Group: PIRETHROID		
COMERCIAL PRODUCT (C.P.): TALSTAR ACTIVE INGREDIENT (A.I.): BIFENTRINE		
Concentration of A.I.. (g/L)	100	
Culture: Cotton		
DOSAGe	↑ Dose (c.p.) 600 - 800 ml/100L water	↓ Dose (c.p.) 300 ml/100L water
CONTROLED PLAGUES	Lagarta Helicoverpa (*Helicoverpa armigera*)	Curuquerê (*Alabama argillacea*)
Class: Pesticide e acaricide Action mode: Contact and ingestion Formulation: EC Toxicologic Classification: III – Medium toxic Classification of the potential environmental danger: III – Dangerous product to the environment		

Source: Pesticidepackageleaflet

The collection of the bees was done using the collecting jars. 90 field bees were collected from each hive (X, Y and Z) and taken to the microscopic laboratory of the university and then anesthetized in refrigeration with an average temperature around 4°C for approximately 5 minutes. The anesthetized bees were then relocated to their respective experimental jars. The experiment counted with 6 treatments plus the control test, 3 repetitions per treatment, each repetition corresponding to one experimental jar, with 10 bees per jar, as illustrated on Image 1.

Image 1: Explanatory scheme of how the experiment was set.



Source: The authors

The observations were done in intervals of 2, 4, 6, 6, 6, 12, 12 and 24 hours (TABLE 1), completing 72 hours of the experiment. The bees had contact with the contaminated feeding only for the first 18 hours, as shown on Table 1. After that period, the feeders were replaced by other ones without contamination, dispensing only the sugary solution.

Table 1: Observation Intervals.

OBSERVATIONS		
*Conclusion of the proceduresat 12:30		
Date	Interval	Time
28/07/2019	2hr	14:30
28/07/2019	4hr	18:30
28/07/2019	6hr	00:30
29/07/2019	6hr	06:30
29/07/2019	6hr	18:30
30/07/2019	12hr	06:30
30/07/2019	12hr	18:30
31/07/2019	24hr	18:30

Source: The authors

During the observations the value of the mortality rate were noted as well as the sublethal effects observed by repetition, caused by the different exposure times of the bees to the pesticides.

Results and Discussion

During the experiment, it was possible to observe that besides the lethal effects some sublethal effects also took place some of them being paralysis, shaking, motion difficulties and excessive self-sanitation. These results are shown on Tables 2, 3, 4, 5, 6, 7, 8, 9, where the first line identifies the treatments and under them are the three repetitions that came from each hive (X, Y and Z) with the number of subjects affected by each symptom, being "0" represented by"*". Therefore, the first column contains the observations, being them mortality represented by the letter "A", paralysis "B", shaking "C", mobility difficulties "D" and excessive self-sanitation "E".

Table 2: Observations on 28/07/19 at 14:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organo +**			**Organo -**			**Test**		
Rep	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 Z	1 x	2 y	3 z
A	*	6	*	*	*	*	7	1	6	1	*	*	8	10	4	9	*	10	*	*	*
B	1	*	1	*	2	*	3	1	3	*	6	8	2	*	6	*	2	*	*	*	*
C	*	*	*	*	*	*	*	*	1	3	*	1	*	*	*	*	1	*	*	*	*
D	1	*	1	2	1	3	*	*	*	*	1	*	*	*	*	*	*	*	*	*	*
E	4	4	8	7	3	9	*	8	*	*	4	*	*	*	1	*	1	*	*	*	*

Source: The authors

Table 3: Observationson 28/07/19 at 18:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	1	6	2	3	*	*	10	8	9	10	6	10	10	10	10	10	8	10	*	1	*
B	*	*	*	*	2	*	*	2	1	*	4	*	*	*	*	1	2	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	1	*	*	*	*	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	9	4	8	4	8	10	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Table 4: Observations on 29/07/19 at 00:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	1	6	2	3	5	*	10	10	9	10	10	10	10	10	10	10	10	10	*	1	*
B	*	*	*	*	2	*	*	*	1	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	1	*	1	*	*	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	9	3	7	3	4	9	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Table 5: Observations on 29/07/19 at 06:30

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1-x	2-y	3-z	1-x	2-y	3-z	1-x	2-y	3-z	1-x	2-y	3-z	1-x	2-y	3-z	1-x	2-y	3-z	1-x	2-y	3-z
A	1	6	3	3	10	1	10	10	10	10	10	10	10	10	10	10	10	10	*	1	*
B	5	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Table 6: Observations on 29/07/19 at 18:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof-**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	1	10	3	5	10	1	10	10	10	10	10	10	10	10	10	10	10	10	*	1	*
B	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	2	*	7	2	*	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Table 7: Observations on 30/07/19 at 06:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	3	10	4	6	10	1	10	10	10	10	10	10	10	10	10	10	10	10	*	1	*
B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	3	*	6	3	*	2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Table 8: Observations on 30/07/19 at 18:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	3	10	4	6	10	1	10	10	10	10	10	10	10	10	10	10	10	10	*	1	*

B	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	4	*	2	*	*	3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

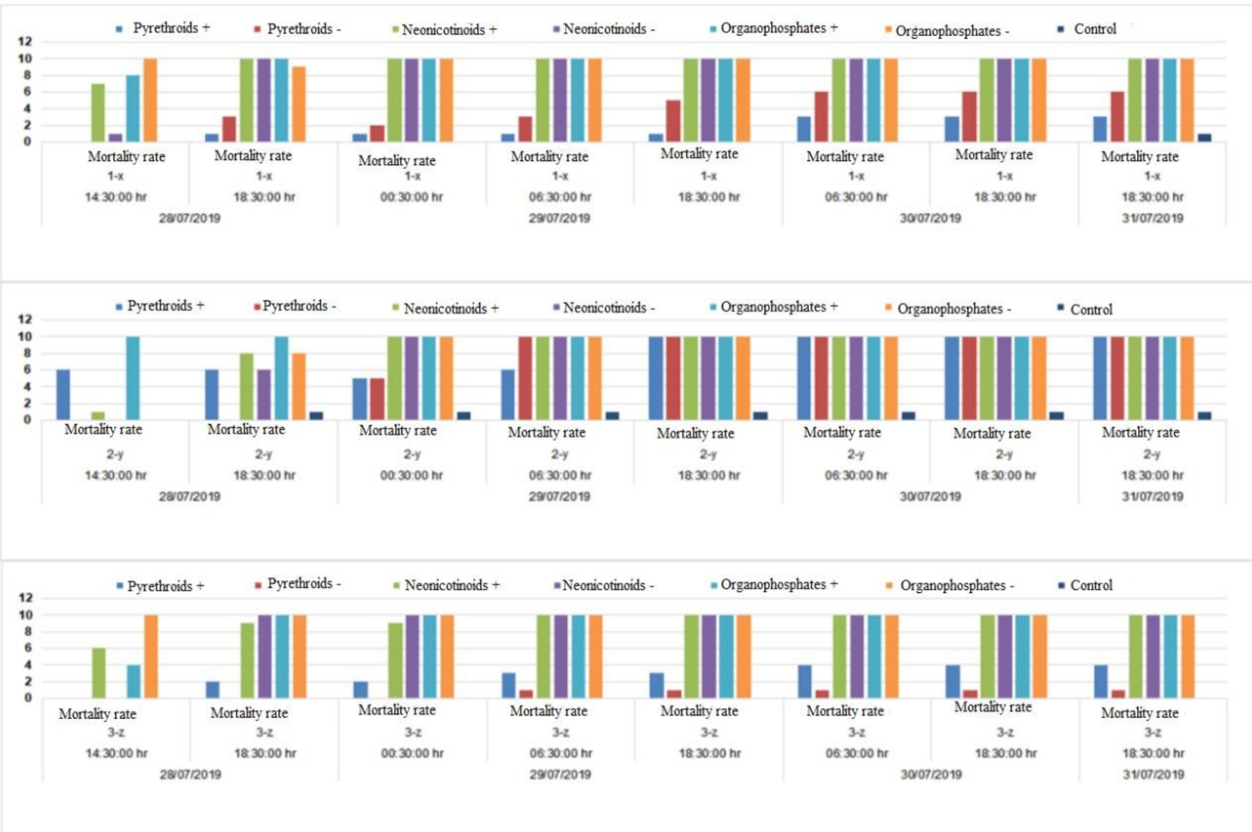
Table 9: Observations on 31/07/19 at 18:30.

	Piret +			**Piret -**			**Neonic +**			**Neonic -**			**Organof+**			**Organof -**			**Test**		
Rep	1 x	2 Y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 y	3 z	1 x	2 Y	3 z	1 x	2 y	3 z
A	3	10	4	6	10	1	10	10	10	10	10	10	10	10	10	10	10	10	1	1	*
B	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
D	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
E	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Source: The authors

Chart 1 shows the mortality rate caused by the pesticides in each repetition.

Chart 1: Mortality rate in the populations.



Source: The authors

On the first observation, after 2 hours from the beginning of the experiment there was a big mortality in the Organophosphates group, as shown on Chart 1 by the light blue and orange columns. In two of the repetitions that correspond to the hives "X" and "Z" the bees were already showing 100% mortality in the group with the highest concentration of the pesticide and the low concentration had a total of 8 individuals alive. The second group that presented the highest mortality rate in the first hours of observation were the Neonicotinoids, in green and purple, reaching the first day of observation with only 3 bees still alive on the highest concentration treatment and 4 surviving the low concentration treatment.

The lowest mortality rate was observed on the Pyrethroids, dark blue and red, presenting deaths only on the high concentration group, a total of 6 bees dead, being that on the last observation of that day the number went up to 9 and on the low concentration group 3 bees were observed to be dead.

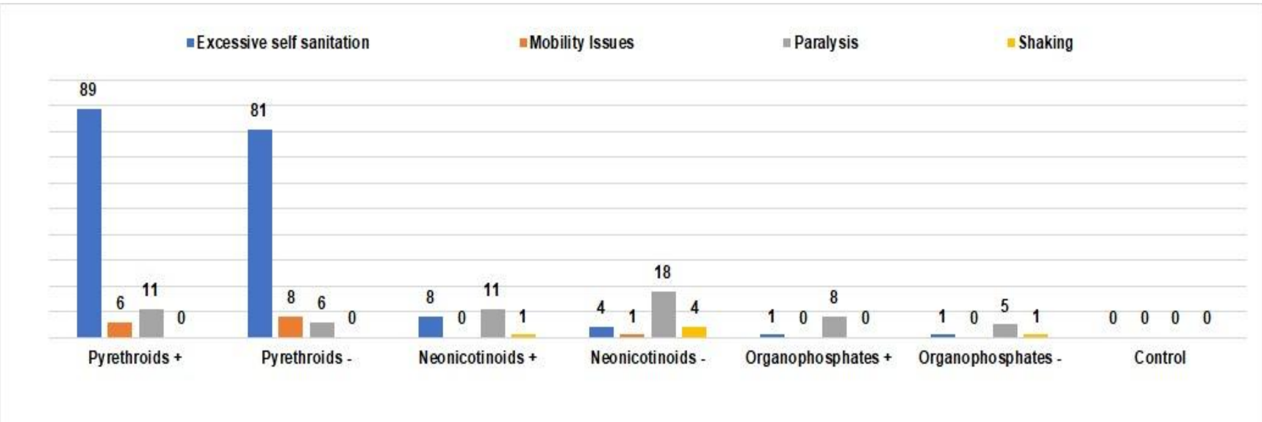
On the second day of the experiment, on the first analysis, the group of theOrganophosphates presented 100% mortality rate in both high and low concentration, being unable to complete the 18 hours to have the contaminated solution be replaced by the non contaminated. The observations then went on with only the Pyrethroids and Neonicotinoids, being that the latest had only one bee alive on the "Z" hive repetition. On the second observation of the second day the feeders were replaced. In this phase, the group of the Neonicotinoids was already showing 100% mortality rate. From those results, the observations continued only with the Pyrethroids group. This group, at that point, had presented a total of 14 dead bees in the low concentration group and on the high concentration 10 dead bees. It was not possible to determine exactly what cause the death rate to be higher in the low concentration group. In the first analysis of the third day, the mortality rate stabilized in the left Pyrethroids group, with 17 bees dead on both concentrations, maintaining that number until the end of the experiment.

At the end of the analysis, a total of 13 of bees remained alive in both concentrations, totaling 26 survivors in a total of 180 bees utilized for the experiment. In the control group, there were only 2 deaths during the whole experiment. Therefore, out of 210 bees used in the whole process only 56 survived and were released back into their hives.

Besides the deaths, other effects we observed on the bees. Those behaviors were denominated sublethal some of them being excessive self-sanitation, mobility difficulties, paralysis and shaking. The effect that stood out the most was excessive self-sanitation, that symptom being observed more frequently in the group of the Pyrethroids, with an average of 85 repetitions in the observations.The group of theOrganophosphates was the one showing the least amount of repetitions of that effect

The second most recurrent sublethal effect was paralysis being the Neonicotinoids the chemical group with the biggest amount of cases of that symptom followed by the Pyrethroids and last the Organophosphates. The symptoms of mobility issues were also observed more in the Pyrethroid group. In addition, the least observed symptom was shaking, observed only in the Organophosphate and Neonicotinoid groups, the latest more frequently with a total of 4 repetitions during the experiment (CHART 2).

Chart 2: Frequency of the sublethal effects observed in the treatments



Source: The authors

Freitas & Pinheiro (2014) also mention a large variation in the tolerance of the stingless bees to the pesticides according to the species of to determine the DL50 *Apismellifera*,besides Malaspina (2019) whose research shows that native bees larvae are less resistant to pesticides than the European *Apismellifera*. The Organophosphates group, shown to be more aggressive to stingless bees according to Costa & Caixeiro (2007) are largely used as pesticides inhibiting the acetylcholinesterase enzyme in the nervous system of vertebrates and invertebrates. The main site of action of that group is the nervous system in the neuromuscular junction interacting with acetylcholinesterase, which has the function of catalyzing the hydrolysis of acetylcholine (ACh) into acetic acid and choline stoping the transmission of nervous impulses in the synapses of the neurons of the central and peripheral nervous system.

When inhibited, the acetylcholine causes paralysis and death of insects, reaffirming the results obtained in this research. On theNeonicotinoids group, studies have been showing the collateral damage of those pesticides in beneficial insects such as bees, who are essential for 90% of the pollination of the angiosperms (ARAÚJO, 2015). It is worth mentioning the importance of the negative effects of the pesticides in non-targeted species. Lastly, the Pyrethroids group act fast on the insects causing immediate paralysis and death (MONTANHA; PIMPÃO, 2012). In general, the Pyrethroids are quickly and extensively absorbed by the gastrointestinal tract after orally administered and through the respiratory tract when inhaled as powder or spray, however not very absorbed through the intact skin. Besides the neurological effects, cardiovascular manifestations can frequently be detected after exposing the individuals to this compound(SANTOS; AREAS; REYES, 2007).

Conclusion

The pesticides, besides being unquestionably useful in agriculture are not exempt of toxicity both environmental and to other living beings. Based on the results obtained, we can conclude that the most aggressive chemical group to the native bees is the Organophosphate, having 100% death rate, being the fastest and not allowing a clear observation of the sublethal effects because of the speed in which it kills the population.

The second chemical group more offensive against the bees is the Neonicotinoids that just as Organophosphate had a fast mortality rate of 100%of the population, allowing us to observe that paralysis was the most frequent sublethal effect. The Pyrethroids group was the only one where some of the individuals remained alive until the end of the experiment, making it the least toxic group for those bees. As for the sublethal effects, it was observed the excessive self-sanitation. However even though it was the group with the least amount of deaths it still had over 50% mortality rate. The stingless bees are more sensitive to the pesticides than *Apismellifera*species utilized to determine the DL50 where the toxicity for bees is evaluated by the law to register pesticides in Brazil, since all the groups had a mortality rate higher than 50%. The determination of the DL50 aims to determine the average lethal rate of the population and the results show that the safety of this non-targeted organism is not being ensured by the current legislation for the registration of pesticides, making it possible for us to observe low rates of those insects in the Brazilian biodiversity.

Resumo

As abelhas desempenham várias atividades além da polinização, provocando impactos positivos, tanto sociais quanto econômicos. O uso indiscriminado de agrotóxicos tem atuado de forma negativa sobre elas, levando à sua diminuição excessiva e possível extinção. Portanto, o objetivo foi identificar os efeitos provocados por diferentes grupos de agroquímicos em abelhas nativas *Tetragonisca angustula* quando expostas a alimentação contaminada. A metodologia utilizada foi a descrita por Lourenço (2012), com modificações. Abelhas, retiradas de três colônias foram colocadas em potes plásticos perfuradosparaventilação,alimentadas por eppendorfs perfurados na extremidade. Foram testados três grupos de agrotóxicos, Neonicotinóides, Piretroides e Organofosforados em concentração máxima e mínimarecomendada. Cadagrupoexperimental, formadoportrêsrepetições, dezabelhas de cada colônia por pote foram expostas a dietas a basedeaçúcardiluídoemáguadeionizada e diferentes concentrações dos agroquímicos. Após 18h as soluções contaminadas foram trocadas por outras sem contaminação e observadas em intervalos de 2, 4,6, 12 e 24 horas. Inicialmente os grupos experimentais com maior taxa de mortalidade foram os Organofosforados e Neonicotinóides em maior concentração. Em uma segunda observação, o grupo Organofosforado de maior concentração apresentou 100% de mortalidade e grupo dos Piretroides, menor taxa de mortalidade. Uma terceira observação mostrou que todo o grupo dos Organofosforados de ambas concentrações apresentaram mortalidade total. Na quartaanálise, tanto os Neonicotinóides como Organofosforadosjá apresentavam 100% de mortalidade nas duas concentrações. O grupo dos Piretroides apresentou mortalidade total em somente uma repetição de cada concentração e auto higienização excessiva como comportamento anormal,minimizadoapós a troca dos alimentadores por outros não contaminados. Chegou-se a conclusão que o grupo químico de maior periculosidade foi o Organofosforado,seguido pelo Neonicotinóide. O Piretroide mostrou-se menosletal.Foiobservado também uma auto higienização excessiva como um efeito subletal recorrente.

Acknowledgments

The authors are grateful the Paula C. Ferro for translatingthispaper.

References

[1]. Araújo, W. L. de. **Toxicidade de neonicotinóides sobre abelhas (*Apismellifera*)** / Whalamys Lourenço de Araújo. – Pombal, 2015. 49 f. Dissertação (Mestrado em Horticultura Tropical) – Universidade Federal de Campina Grande, Centro de Ciências e Tecnologia Agroalimentar, 2015.

[2]. COSTA, J .B. N. da, CAIXEIRO, J. M. R. **Compostos organofosforados pentavalentes: histórico, métodos sintéticos de preparação e aplicações como inseticidas e agentes antitumorais** Quim. Nova, Vol. 30, No. 1, 159-170, 2007.

[3]. FERNANDES, R. O. **Avaliação ecotoxicológica de agrotóxicos, seus componentes e afins: Teste para o parâmetro abelhas.** Universidade Federal de Viçosa, MG, 2012. Disponívelem:< https://www.locus.ufv.br/bitstream/handle/123456789/3962/texto%20completo%20.pdf?sequence=1&isAllowed=y>. Acesso em:07/10/19.

[4]. FREITAS, B. M.; PINHEIRO, J. N. **Polinizadores e pesticidas: princípios de manejo para os agroecossistemas brasileiros**. Brasília, DF: Ministério do Meio Ambiente, 2012. Disponível em:<https://biblioteca.incaper.es.gov.br/busca?b=ad&id=3168&biblioteca=vazio&busca=autoria:%22FREITAS,%20B.%20M.%22&qFacets=autoria:%22FREITAS,%20B.%20M.%22&sort=&paginacao=t&paginaAtual=1>. Acesso em: 30/08/19.

[5]. Guevara, J.L. de &Pueyo, V.M., 1995. **Toxicología médica**: clínica y laboral, Madrid: Interamericana, McGraw-Hill.

[6]. LIMA, M. C; ROCHA, S. A. **Efeitos dos agrotóxicos sobre as abelhas silvestres no Brasil - Proposta metodológica de acompanhamento.** IBAMA, Brasília, 2012. Disponível em:< http://www.semabelhasemalimento.com.br/wp-content/uploads/2015/02/efeitos_agrotoxicos_abelhas_silvestres_brasil.pdf>. Acesso em: 12/09/2019.

[7]. LOURENÇO, C. T. **Determinação da toxicidade tópica e oral do inseticida friponil e efeitos de suas doses subletais no comportamento de abelhas sem ferrão Melipona scutellaris (Latreille, 1811)**, UFSCar, 2012. Disponível em:< https://repositorio.ufscar.br/handle/ufscar/10?show=full>. Acesso em: 12/09/2019.

[8]. MALASPINA. O. **Padronização de método para testes de toxicidade em larvas de abelhas sem ferrão em condições de laboratório, e potenciais efeitos adversos provenientes do alimento larval contaminado com o neonicotinoide tiametoxam**. Instituto de Biociências (IB). Universidade Estadual Paulista (UNESP). Campus de Rio Claro. Rio Claro, SP, Brasil, 2019. Disponível em:<https://bv.fapesp.br/pt/bolsas/166702/padronizacao-de-metodo-para-testes-de-toxicidade-em-larvas-de-abelhas-sem-ferrao-em-condicoes-de-lab/>. Acesso em: 30/08/19.

[9]. PIMPÃO, F P; MONTANHA, C. T. **Efeitos Toxicológicos de Piretróides (Cipermetrina e Deltametrina) em Peixes**. Revista Científica Eletrônica de Medicina Veterinária. Ano IX – Número 18 – Janeiro de 2012.

[10]. SANTOS, M. A. T. dos; AREAS, M. A.; REYES, F. G. **Piretróides – uma visão geral**. Alim. Nutr. Araraquara v.18, n.3, p. 339-349, jul./set. 2007.