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EFFECT OF HERBICIDE ON THE SURVIVAL OF STINGLESS BEES IN THE STATE OF RONDÔNIA

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ABSTRACT

As the main pollinating agents, bees are responsible for pollinating most of the native plants in Brazil. The objective of this study was to verify the mortality rate of Melipona seminigra seminigra bees through contact and ingestion with different concentrations of the herbicides Reglone® and Trop®. The bees were collected in the inlet tube and placed in glass jars with a volumetric capacity of 1L, capped with voile fabric. The experimental model used was a Completely Randomized Design, the control in three replications, containing 15 bees per replication. In the experiment, Candy food was provided (a mixture of 40 mL of honey and 70 g of powdered sugar). Inside each jar, food, filter paper, and a container containing cotton soaked in water were placed. For contact contamination, filter paper soaked in 1000 µl of Aqueous Reglone® solution was used, as well as for Trop®, placed at the bottom of the glass flasks and tested at different concentrations (100% and 50%). Contamination by ingestion was used 1000 µl of aqueous solution of Reglone® and also for Trop®, mixed with the food and tested at different concentrations (100% and 50%) of the concentration of active ingredient diluted according to the package insert for use in soybean crops. The flasks were kept at a temperature of 28ºC and 70% humidity, the observations were carried out in 24h, 48h, and 72h. All the bees survived the control experiment. The results of the contaminations for the two herbicides evaluated did not show differences. The contact experiment showed lower lethality potential when compared to ingestion. The highest lethality occurred at 72 hours of the experiment when compared to the rates observed in 24 hours and 48 hours. The highest concentration (100%) was the one with the highest lethality, but the number of dead individuals was low and was only recorded after 48 hours of the experiment. Considering the contamination by contact, the bees of the species Melipona seminigra seminigra did not have their mortality affected by exposure to the herbicide, but more studies are needed.

Keywords: Bees. Pesticide. Amazon. Reglone®. Trop®.

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INTRODUCTION

Bees are insects known in the world for the various benefits they provide, such as the ability of some species to produce honey and their natural role of pollination (SANTOS, 2010). In cultivated crops, bees are the main pollinators. Most fruits, small seeds, and many vegetable crops require pollination for production and increased economic yields (ABROL, 2012).

Bees are divided into two groups, the stinging bees known as *Apis mellifera* and the stingless bees which are known as indigenous bees, the breeding of these bees is called beekeeping and meliponiculture, respectively. Stingless bees are among the most common pollinators in tropical environments and in certain regions they are dominant, visiting various crops used by humans (MACÍAS-MACÍAS *et al.,* 2009). Comprising a diverse group of insects that includes more than 400 species that have high variability in their physiology, morphology, and size (MOURE *et al*., 2007).

The use of Meliponines in the service of pollination is increasingly increasing, as there is a great diversity of species in this group, which makes it possible to select species that are more efficient in pollinating certain crops (SILVA *et al*., 2014). Bees play a fundamental role in maintaining environmental balance, being responsible for 73% of cross-pollination in most ecosystems, and increasing the vigor of the species (COUTO and COUTO, 2002). However, the constant deforestation, added to the extraction of honey trees, has been increasing the pressure on this important natural resource, making it necessary to use advanced strategies that help in its conservation (SANTOS, 2010). A decline in these species or inadequate pollination in some crops can cause yield losses of 50% or more (Klein *et al*., 2007).

In the State of Rondônia, concern about deforestation and its influence on bee populations led the authors BROWN; ALBRECHT (2001) to develop a study (in the southeast of the Amazon basin) to find out if deforestation affects the incidence of lipomas in the Amazon forest. Studying seven species of melipones, these authors revealed that the richness and abundance of meliponines are directly related to the area of vegetation cover and inversely proportional to the size of the deforested area.

The diversity of stingless bees in the State of Rondônia is great, but the impact of agriculture and deforestation on stingless bees in the State affects the quantity, diversity, and composition (BROWN; OLIVEIRA, 2014). Some species can survive in disturbed environments, such as *Tetragonisca angustula* (FIERRO, 2012).

The decline of bee populations has been reported, and the intensive use of pesticides is pointed out as one of the main factors responsible for this impact (GOMES *et al*., 2017). The toxicological stress caused by agrochemicals and their consequences on bees has been an intense focus of debate. However, the emphasis on honey bees and pesticides has neglected the relevance of stingless bees, the main pollinators of natural tropical ecosystems (LIMA *et al*., 2016).

Small concentrations and doses of pesticides may not cause the immediate death of bees, however, they can interfere with their behavior, in addition to reducing the foraging activity or increasing the disorientation of these insects, harming the entire colony. Therefore, relevant changes can be caused by this long-term exposure, even at low concentrations and doses, known as sublethal (NOCELLI *et al*., 2012).

Due to the high chemical potential of crop protection products, there are indications that some substances used in pest control in agriculture may be involved in cases of bee poisoning. The effects of these products may not be noticed, but they can cause serious physiological and behavioral effects, compromising individuals and the viability of the colony in general (TOMÉ *et al*., 2020; PIRES *et al*., 2016)

In general, herbicides and fungicides have specific mechanisms of action, thus, due to this specificity, few studies are still carried out studying the effects of herbicides on bees. The fact that herbicides do not appear to pose a risk to insects does not make them the target of more in-depth experiments, only the possible sub-lethal effects are treated (FREITAS; PINHEIRO, 2010).

Most studies on the effects of herbicides and fungicides on bees have been conducted in North America and Europe, especially from the mid-2000s onwards; a trend similar to that observed in the literature on neonicotinoids and bees LUNDIN *et al.,* (2015) apud CULLEN *et al.,* (2019). In addition, most insect-pollinated plants are grown in countries outside the EU and North America according to GALLAI *et al*., (2009 and CULLEN *et al.,* (2019) All these factors suggest that it is important to investigate the impacts of fungicides and herbicides on pollinators in the conditions in which they are used globally, to build a complete picture of any potential impacts. The objective of this study was to evaluate the toxicity of insecticides in stingless bees and to compare the effects of contamination by ingestion and contact with the herbicides Reglone® and Trop®.

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METHODOLOGY

MATERIAL AND METHODS

Adult worker bees of *Melipona seminigra seminigra* were collected from colonies located in the meliponary in an urban area of the municipality of Porto Velho, Rondônia. The captured bees were exposed to different concentrations of agrochemicals, the herbicides Reglone® and Trop®. The forms of exposure to the material were via contact and ingestion.

The experiments were carried out with two different herbicides, Reglone® and Trop®, commercially, which have concentrations of 480 g.i.a/L of glyphosate and 200 g.i.a/L of diquat dibromide, respectively. The commercial products were diluted in distilled water until the concentrations were obtained, corresponding to 50% and 100% of the concentration of the active ingredient recommended in the package insert of the products for use in soybean crops.

Both experiments, evaluating ingestion and contact, were conducted in a randomized block design, as well as each experiment was carried out containing three replications. The experiments were conducted under a 2 x 2 factorial scheme with an additional treatment, in which the factors tested were the products of the doses of 50% and 100% of the concentration of active ingredient used for the soybean crops, in addition to control, without contamination with herbicides.

The bioassays were mounted in glass jars, the individuals were placed in glass flasks with a volumetric capacity of an average of 1L, capped with *voile* fabric (PEREIRA, 2008). Inside each bottle, there was a filter paper soaked with the herbicide under test, when exposed via contact, a container with wet cotton, and a container with candy food, without contamination. As for the bioassay via ingestion, the food was contaminated and not the filter paper, nor the wet cotton.

For each concentration and form of exposure, three replicates were performed, containing fifteen individuals per sampling unit. In the control treatment, there was no contamination. In the evaluations, the mortality rate was counted; For this, bees that did not show movements at the time of the observations were taken into account, even when touched with the aid of a fine-tipped brush.

The data obtained were submitted to survival analysis, carried out by counting dead worker bees in the determined intervals of 24, 48, and 72 hours.

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RESULTS

MORTALITY BY INGESTION

Initially, through exploratory analysis, it was possible to verify how the variation in the percentage of mortality of *Melipona seminigra seminigra* occurred, for the product-dose factorial. Bee contamination by herbicides affected bee mortality in the control condition. However, no difference was observed between the herbicides, especially at the 50% dose.

All the bees survived the control experiment. The result of contamination by ingestion showed a higher lethality potential after 72 hours of experiment than when compared to the rates observed in 24 hours and 48 hours. The highest concentration (100%) was the one with the highest lethality, but the number of dead individuals was low and they were only recorded after 48 hours of experiment.

The lack of significance for the simple effect of the Product demonstrates that the herbicides had a similar effect on the mortality of *Melipona seminigra seminigra.* In the average dose of 100% of both products, the mortality was 40%, while when half dose was used, the average mortality of the products was 20%. This result demonstrates that the higher dose has a higher impact on the deaths of these bees when ingested.

CONTACT MORTALITY

The result of contamination by contact showed a higher lethality potential within 72 hours of the experiment when compared to the rates observed in 24 hours and 48 hours. The highest concentration (100%) was the one with the highest lethality, however, the number of dead individuals was low and they were recorded after 24 hours of experiment.

Considering the contact mortality of *Melipona seminigra seminigra*, there was no significant effect for the product-dose interaction, as well as for the simple block, product, and dose effects. Likewise, no significant effect was found for the effect of the factorial about the control.

Comparing the herbicides, there was no difference between the products for the 50% and 100% doses. In addition, the difference in mortality between the 50% and 100% doses for the products was the same in both doses (30%). For the bees used in the control experiment, without contamination, no mortality was recorded.

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DISCUSSION

MORTALITY BY INGESTION

In the literature, there are several studies using *A. mellifera* as an insect bioindicator of pollutants in the environment. HARDSTONE; and SCOTT (2010) carried out a scientific survey, obtaining toxicity data, to verify the susceptibility of *A. mellifera* to 62 insecticides, from 6 different classes. The results showed that *A. mellifera* is susceptible to the vast majority of insecticides analyzed, but there is no specific susceptibility to a particular insecticide or class. Thus, there is great interest in using *A. mellifera* as a highly sensitive environmental bioindicator insect, as it is a foraging insect and, consequently, is in contact with pollutants applied to the environment.

HASHIMOTO *et al*., (2003) performed several bioassays to detect changes in the relative activity of *Africanized A. mellifera* esterases after contamination by contact and ingestion of the neonicotinoid insecticide thiamethoxam. In this study, it was verified that four esterases (EST-1, EST-2, EST-4, and EST-5) of these honey bees have the potential to be used to detect the presence of thiamethoxam in the environment.

For the jataí bee, few studies have used it as a bioindicator organism, FERMINO (2011) used the stingless bee, *T. angustula, and T. fibrin*, as an environmental bioindicator in the analysis of the influence of herbicides on the expression of isoenzymes, being the esterases sensitive to the presence of nicosulfuron and paraquat, presenting inhibition in their relative activity after contamination. STUCHI (2009) evaluated the stingless bee *T. fibrin* as a bioindicator of the presence of pesticides in the environment, through changes in the expression of esterases, total protein, and chromatin of brain cells of adult individuals, in the face of contamination by fipronil, malathion, neem and thiamethoxam. Fipronil presented the highest value of LC_{50}. The pesticides malathion and fipronil inhibited the EST-1 and EST-4 esterases of *T. fiebrigi.*

Insecticides used correctly can control target organisms without compromising populations of natural enemies or pollinators, as they are more susceptible to chemical compounds, which can affect the ability to collect food and pollinate, as well as honey production. Thus, a conscious way of using insecticides is through the use of selective insecticides (DEGRANDE *et al*., 2002; GODOY *et al*., 2004; MAGALHÃES *et al*., 2002).

CONTACT MORTALITY

In addition to insecticides, fungicides represent another class of pesticides that has been standing out in terms of the effects caused on bees, since some studies have found

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this substance in the matrices collected by bees. As is the case of the study carried out by CARNEIRO (2020), which demonstrated that bees of the species *A. mellifera* can be harmed by the fungicide iprodione since they showed morphological changes in the cells of the intestine. In addition, according to ZALUSKI *et al*., (2017), the exposure of nursing bees to the fungicide pyraclostrobin, belonging to the class of strobilurins, was capable of causing harmful changes in their mandibular and hypopharyngeal glands, which could impair the maintenance of the colony by these bees.

SOARES *et al*., (2015) conducted a study with the bee species *S. postica*, where they observed loss of brush border in the intestine and Malpighian tubules of bees that were exposed to the insecticide imidacloprid, orally, for 48 hours. In addition, GRELLA (2017) also found toothbrush border loss in the intestine and Malpighian tubules of *Melipona scutellaris* (LATREILLE, 1811) exposed to nano doses of the insecticide thiamethoxam for 24 hours, with a concentration equal to 0.0453 ng a.i./µL diet (LC50), which is similar to that used in the present study (0.04392 ng/µL for dimethoate and 0.060 ng/µL for azoxystrobin). The result obtained by these authors reinforces those found in this study since it also verified loss of brush border in the intestine and Malpighian tubules of bees, of the species *S. postica*, exposed to the insecticide dimethoate and the fungicide azoxystrobin.

According to CULLEN *et al*., (2019), the vast majority of studies that investigated the effects of herbicides and fungicides were with the Apis species, most of which with *Apis mellifera*, and two studies with *Apis cerana*. A smaller number of studies were conducted with other bee species, including *Bombus* spp. (13 studies), *Osmia* spp. (7 studies), *Megachile rotundata* (8 studies), and other bee species (4 studies). Within bees, *Bombus* was the most extensively studied (8 studies), followed by *Bombus impatiens* (4 trials). Most studies investigated the effects on just one species, and only nine examined the effects on multiple species in the same paper. Nine bee species studied were social, while four species were solitary.

CONCLUSION

The mortality of bees of the species *Melipona seminigra seminigra is* affected by the ingestion of the herbicides evaluated. We can consider that herbicides do not differ from each other in the mortality of individuals. Comparing the concentrations from 50% to 100% in the two products, we observed an increase in the number of dead bees for herbicides.

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The evaluation of contact contamination in this bee species did not influence mortality when exposed to herbicides at the concentrations tested.

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