Article

Effect of glyphosate on honey bee *(Apis Mellifera)* performance

Mostafa Faghani, **Yaser Rahimian**
Department of Animal Sciences, Faculty of Agriculture, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran
E-mail: yas.rahimiyan.yr@gmail.com

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Abstract
To determine the effect of glyphosate (GLY) on honey bee (*Apis mellifera*) Carnica breed performance, at the Chaharmahal and Bakhtiari province of Iran, some randomized selective beekeepers were asked to participate in honeybee research. They were asked to provide a sample of seven to 12 honeybees and a 5 cm piece of comb from the same hive. The experiment was conducted from August to September 2016. Samples of bees and comb from each experimental hive were analyzed using a liquid-chromatography mass spectrometer. Both experimental groups, which were the glyphosate and Roundup groups, were found to exhibit significantly greater mortality when compared to the none-supplemented group. The mortality rate for the group that was given sugar water with glyphosate was an average of one honeybee per day, whereas the average mortality rate for group that was given sugar water with Roundup was an average of 2 honeybees per day. In conclusion it is impossible to categorically state that glyphosate products cause colony collapse disorder. Revealed data indicates that it is certainly plausible that glyphosate herbicides may contribute to the phenomenon. In conclusion we could demonstrate that hives with glyphosate supplemented diets would exhibit a greater rate of mortality than hives. Hives with Roundup supplemented diets would exhibit a greater rate of mortality than hives with no supplementation.

Keywords glyphosate; honey bee; mortality; performance; roundup.

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1 Introduction

Honeybees (*Apis mellifera*) are the main pollinators in agricultural settings (Aizen et al., 2009; Kamel et al., 2013) and as such are highly exposed to any perturbation occurring in the surroundings of crop fields. *Apis mellifera*, the honeybee, is vital to a healthy, balanced environment and the economy of the World. Honeybees pollinate nearly 130 species of plant life (Kaplan, 2008). The glyphosate (N-phosphonomethyl glycine) is a non-selective systemic herbicide, first commercialized in 1974. Formulations of glyphosate have been extensively tested for a wide range of potential environmental effects and have proven safe for a wide range of

organisms, including honey bees. Both glyphosate and glyphosate formulations were assessed to be practically nontoxic to honeybees during the U.S. Environmental protection agency review of glyphosate for re registration (EPA, 1993). A field study in which honeybee hives and blooming vegetation were over sprayed with the original Roundup herbicide at 3 kg glyphosate per acre supports this conclusion (Giesy et al., 2000). Glyphosate and glyphosate-based formulations have been extensively tested in the laboratory and in the field to evaluate potential toxicity to honeybees. The results from these studies with these herbicides demonstrate no acute and chronic adverse effects to honey bees under good agricultural practices (Giesy et al., 2000). Three decades ago, field studies were conducted on two continents to investigate the potential for acute and chronic effects of glyphosate and a glyphosate-based formulation on honey bee hives (Ferguson, 1987). Glyphosate is a herbicide that is widely used in agriculture for weed control (Zhang, 2018). Although reports about the impact of GLY in snails, crustaceans and amphibians exist, few studies have investigated its sub lethal effects in none target organisms such as the honeybee, the main pollen vector in commercial crops. Here, some researchers tested whether exposure to three sub-lethal concentrations of GLY (2.5, 5 and 10 mg l−1: corresponding to 0.125, 0.250 and 0.500 μg per animal) affects the homeward flight path of honeybees in an open field. Published studies show that the neonicotinoids imidacloprid, clothianidin and thiamethoxam have high acute toxicity to bees, and sub-lethal amounts interfere with foraging and reproduction (Whitehorn et al., 2012; Zhang, 2018). Exposure of bumble bees to neonicotinoid concentrations found in pollen and nectar of treated plants reduces colony growth and the number of bumble bee queens (Gill and Raine, 2014). Beekeepers and scientists have been unable to explain the reason for colony collapse disorder (Kaplan, 2008). Although glyphosate inhibits aromatic amino acid pathways found only in plants, fungi and microorganisms, some studies have shown different negative effects in invertebrate and vertebrate species. The researchers felt that their findings demonstrate that both single exposures and repeated exposures to glyphosate have a detrimental effect on the retrieval and formation of memory. Typical causes of minor hive loss might include starvation, parasites, or freezing; however, these causes are insufficient to explain the massive hive losses that began in 2006 described by Kaplan (2008). The aim of this study was to evaluate the effect of glyphosate on experimental honey bees Performance.

2 Material and Methods

At the Chaharmahal and Bakhtiari province of Iran some selective beekeepers were asked to participate in honeybee research from August to September 2016. They were asked to provide a sample of seven to 12 honeybees and a 5 cm piece of comb from the same hive. They were mailed a package containing instructions for sample collection, sample collection containers, and a questionnaire asking about the location, health, and age of the bee hives from which samples were taken. Most of the beekeepers mailed one or more samples, providing a total of 12 samples.8 cages were constructed and then each cage was 20 cm high by 20 cm wide by 18 cm deep. The two larger faces of the cube were made of screen wire, and the other faces were made of wood. One cm circle was drilled on the top face of each cage. Ajar with a flat and ring lid was placed upside down in each hole. Previously, a drill had been used to puncture each lid with fifteen small holes. A triangular piece of comb foundation was attached to the inside of the rear screen panel using a soldering iron. A water source was created by filling a test tube with water, attaching the test tube to the front screen panel with wire, and placing a strip of cotton fabric into the test tube to act as a wick; one water source was attached to each cage. Equal volumes of sugar and water were combined and heated until the sugar dissolved completely to create a sugar solution. The solution was allowed to cool and was kept in a standard refrigerator for one day. The solution was divided into nine 236-mL portions and poured into the jars to be placed in the hive. The test tubes were filled with approximately 7.5 mL water. Approximately three hundred live Carnica breed

honeybees were measured using a one-cup dry measuring cup and placed into each hive. All honey bees were from the same hive that belonged to the researcher and her family. After the bees were added, the hives were transported for about three miles where they were moved indoors, into a dark, temperature-controlled, noise-free room with an ionizing humidifier. Each hive was randomly assigned to a group: control, glyphosate, or Roundup. All honeybees were allowed to acclimate to their new environment for four days, during which time all groups were fed plain sugar water. The original sugar water was removed from each cage in the Roundup and glyphosate groups. Each cage in the Roundup group received 118-mL of the Roundup, sugar water, and each cage in the glyphosate group received 118-mL of the glyphosate sugar water. Because the honeybees consumed more sugar water than the expected amount, more sugar water was mixed in the same way it had been mixed on the first day. The control group received sugar water with no supplementation, the glyphosate group received sugar water with glyphosate supplementation, and the Roundup group received sugar water with Roundup supplementation. Observations were recorded about mortality, general behavior, and water consumption; each time the sugar water was replaced, the amount of leftover sugar water and amount of added sugar water was measured and recorded. Water was added to the water source every day using a syringe. The quantity of water was recorded each time water was added. The bees were left undisturbed, with the exception of data collection, watering, and feeding maintenance, to function as a normal hive for four days before the chemicals were introduced to the experimental groups. Ten days after the chemicals were introduced to the experimental groups the final data were collected. At least four bees from each cage were collected to be used for testing, and the comb from each cage was collected for testing. Because the honeybees built fewer combs than expected, the comb was not used for testing.

The samples from beekeepers in the Chaharmahal and Bakhtiari Province, as well as the samples from the lab component of the experiment were analyzed with a liquid-chromatography mass spectrometer. It was more useful when detecting a broad variety of chemicals rather than one specific chemical. An LS/MS is more appropriate for 11 detection of glyphosate because an it can detect amino-methylphosphonic acid, the metabolite of glyphosate, and was more sensitive than a liquid-chromatography mass spectrometer and it could detect as little as 17 ppb. Also when all samples were collected, they were delivered to the LS/MS operated and then analyzed. Additionally, mortality data were also analyzed. All data were subjected to a two factor analysis of variance that used an alpha-level of ($p \leq 0.05$) to determine significance .Additionally data on mortality were collected and subjected to a two-way analysis of variance (ANOVA).

3 Results and Discussion

Mortality rate in the lab component of this research, it was determined that the average mortality rate was significantly greater in both groups with supplemented diets when compared to the group with no supplementation. Both experimental groups, which were the glyphosate and Roundup groups, were found to exhibit significantly greater mortality when compared to the none-supplementary group. The mortality rate for the group that was given sugar water with glyphosate was an average of one honeybee per day, whereas the average mortality rate for group that was given sugar water with Roundup was an average of 2 honeybees per day.

As results of this study showed that, it is impossible to categorically state that glyphosate products cause colony collapse disorder. Revealed data indicates that it is certainly plausible that glyphosate herbicides may contribute to the phenomenon. Girolami et al. (2012) noted that neonics have delayed mortality effects on overwintering honey bee colonies. Summer bees are poisoned, and the over wintering colonies die. The delayed mortality effects are similar to those observed with colony collapse disorder. Kessler et al. (2015) have found that both honey bees, Apis Mellifera, and bumble bees, Bombus terrestris, are not repelled by sugar

solution slaced with the neonicotinoids imidacloprid, clothianidin, and thiamethoxam. The results from (Giesy et al., 2000) studies with glyphosate and Roundup herbicides demonstrate no acute and chronic adverse effects to honey bees under good agricultural practices. Bennett (2008) showed that food intake, mortality, locomotive and orientation activity did not vary and all bees, regardless of glyphosate exposure, showed similar behavioral responses and mortality rates. Results of (Balbuena et al., 2015) suggested that, in honeybees, exposure to GLY doses commonly found in agricultural settings impairs the cognitive capacities needed to retrieve and integrate spatial information for a successful return to the hive. Therefore, honeybee navigation is affected by ingesting traces of the most widely used herbicide worldwide, with potential long-term negative consequences for colony foraging success. Herbert et al. (2014) speculate that successful forager bees could become a source of constant inflow of nectar with GLY traces that could then be distributed among nest mates, stored in the hive and have long-term negative consequences on colony performance.

4 Conclusion

We could conclude that hives with glyphosate supplemented diets would exhibit a greater rate of mortality than hives. The hives with Roundup supplemented diets would exhibit a greater rate of mortality than hives with no supplementation. Also we showed that the possibility of detecting glyphosate in honey and bees collected from hives with glyphosate supplemented diets and there would be significantly greater levels of glyphosate in samples from such hives when compared to samples from hives with no supplementation is as of yet unable to be determined. Further studies are needed for more explanations.

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