Biosafety of Deltamethrin Sublethal Doses for Honeybee *A. Mellifera* L. in Iraq

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Abstract

This study has been conducted in the faculty of Agriculture-University of Kufa from March-November 2021. The main objectives of the current study are to determine the deltamethrin residues in honey samples collected from 5 regions of Najaf, middle of Iraq, and to investigate the effect of sublethal doses of deltamethrin on honeybee survival. The results of the current study showed that the residues were 10.6, 8.6 and 7.7 ppm in sample 1,3 and 4 respectively. However, deltamethrin residues from another area (sample 2 and 5) were not detected. In forager bees, the high rate of food consumption of 1M sucrose containing deltamethrin at different concentrations was 86.7 µl/bee/day at 0.25ppm compared to 69 µl/bee/day in control. Whereas, the maximum food consumption for nurse bees was 58.2 µl/bee/day compared to 39-43 µl/bee/day at 1-100 ppm. Bee survival ranged between 93-96% in nurse bees and 70-83% in foragers at 0.25-100ppm deltamethrin. It can be concluded that the sublethal doses of deltamethrin on honeybee survival was low and can be used against insect pests after testing them in the laboratory and field.

Key word: honeybee, survival, pesticides, residues, honey

Introduction

Supplemental feeding is needed for honeybees to overcome the shortage of nutritional requirements (Al-Esawy, 2020; Alshukri and Al-Esawy, 2021; Stabler et al., 2021). As honey bees forage for nectar and pollen, they are incidentally exposed to pesticides which accumulate in the hive (Chmiel et al., 2020) . Exposing to sub-lethal levels of pesticides has adverse health effects including disruption of foraging activities, decreasing brood rearing, impairing the learning and memory, and colony collapse disorder (Lu et al., 2020; Williamson and Wright, 2013). This view is supported by Harwood and A.G.Dolezal. (2020) who noted that One of the environmental pressures on honeybees which make them more susceptible to the biological infection and reduce their survival is exposure to low levels of pesticides.

Deltamethrin is one of the pyrithroids, a natural insecticide derived from some plant species *Pyrethrum* spp. (Rehman et al., 2014). Deltamethrin is highly toxic to honeybees under laboratory conditions (Johnson et al., 2010), and this noted by Aljedani (2017) who found

that deltamethrin had an adverse effect on honeybees *Apis mellifera jemenatica*, where the lethal time (LT_{50}) was 72.01h.

Very little work has been done in Iraq related to the biosafety of pesticides using honeybees under laboratory conditions. Therefore, the aim of the current study is evaluating the adverse effect of consuming deltamethrin on bee survival at sublethal doses.

Materials and methods

Collection area

Bee honey samples were collected in May,2021 from 5 locations in Najaf Ashraf,161 km southwest of Baghdad. These locations are: sample 1, Al-Mishikhab (30 km south of Najaf); sample 2, city center; sample 3, Alqadisiyah (50 km southeast of Najaf); sample 4, Abbasiyah (16 km northeast of Najaf); and sample 5, Alhaidariya (37 km northwest of Najaf) (Figure 1).

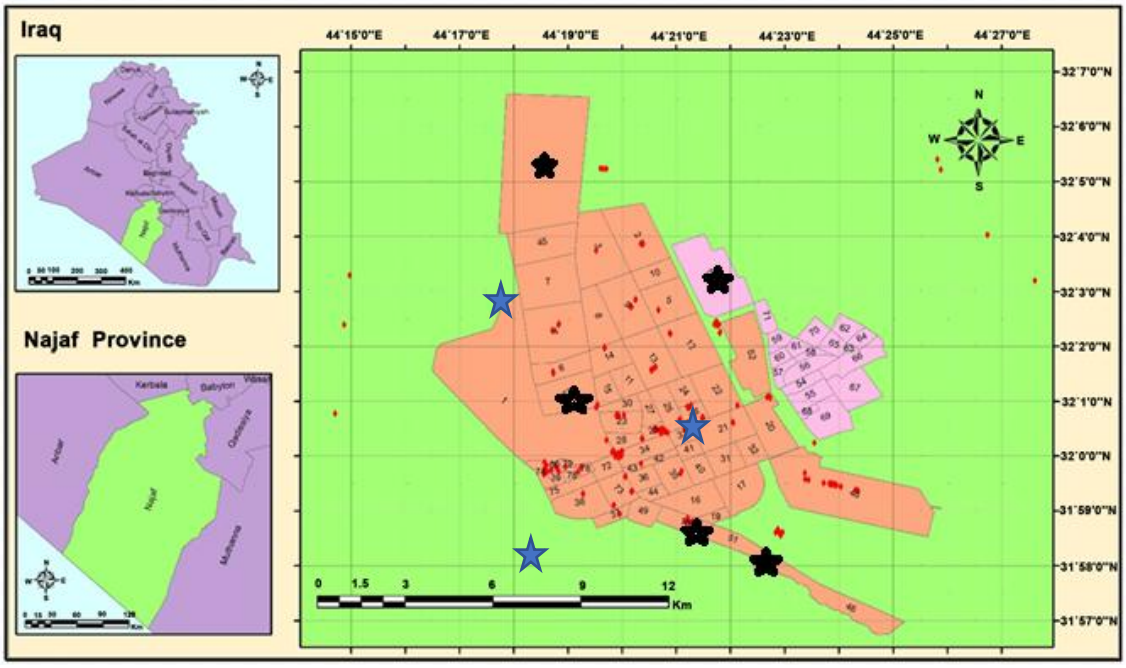

Figure 1: Collection areas of Bee honey samples in Najaf province/ Iraq. Stars indicating the collection area. The map from (Al-Maliki et al., 2018).

Bees collection

Stocks of hybrid Italian race, *A. mellifera ligustica* were obtained from our apiary at faculty of Agriculture/University of Kufa, Najaf/ Iraq. Brood frames containing capped cells (or foraging bees collected from the hive entry) were brought into the honeybee laboratory. Twenty newly emerged bees were gently collected and put in a cage (figure 2) and incubated at 34 °C and 66% RH in a darkness incubator.

Figure 2: Hoarding cage of honey bee *A. mellifera* (photographed by the author).

Food preference and survival

Six different treatments (100,10,1.0,0.5, 0.25 and 0.0) were used of deltamethrin (CAS NO. 52918-63-5, Rencheng Technology Co., Ltd. China) was used by mixing with 1M sucrose. Bees were assigned randomly to wooden cages (15 x 6 x 12 cm) with mesh on two sides, 20 bees per cage. Thirty-five cages in total were set up: five replicates x 6 treatments and one evaporation. Each cage was provided with six Eppendorf tubes, two containing water and two (1M sucrose+ deltamethrin), which were replenished every day during the experiment. For each cage, the tubes were weighed at the beginning of the experiment and at 24-h intervals for 3 days to record the food consumption. Dead bees were removed every day and the numbers of surviving bees in each cage were recorded.

LC_{50} Determination

determination of the oral LC_{50} was based on the OECD (1998) developed for *A. mellifera* after 72h.

Statistical Analysis

Daily and total consumption data were analyzed using fit general linear model ANOVA using Minitab ®19 (Minitab, LCC, USA) with diets as the main effect. Comparisons were made using the Tuckey method and 95% confidence with a significance at $p \leq 0.05$. The impact of diets on survival was analyzed using a Cox regression (Coxreg) analysis to calculate the hazard ratio (HR) analysis. Mortality data obtained from the survivorship were subjected to statistical analysis using the Probit method(Finney, 1952) using SPSS (IBM SPSS Statistics v.23). LC_{50} value was determined, as well as their respective 95% confidence intervals values.

Results

Deltamethrin residues in honey

The residues amount of deltamethrin in honey samples were calculated by standard chromatogram (Figure 3, F). The results of the current study showed that the residues were

10.6, 8.6 and 7.7 ppm in sample 1,3 and 4 respectively (Figure 3, A-C). However, deltamethrin residues from another areas (sample 2 and 5) were not detected (Figure 3, D and E).

Nutritional behaviour and diet consumption

Food consumption rate of deltamethrin was not significantly different among treatments even with the control in foragers ($F_{5,23}$ = 1.26, p = 0.313), where the high rate was 86.7 µl/bee/day at 0.25ppm compared to 69 µl/bee/day at control (figure 4, a). However, there was a significant difference in food consumption for nurse bees ($F_{5,24}$ = 4.85, p = 0.003), where the maximum consumption rate was 58.2 µl/bee/day compared to 39-43 µl/bee/day at 1-100 ppm (figure 4, b).

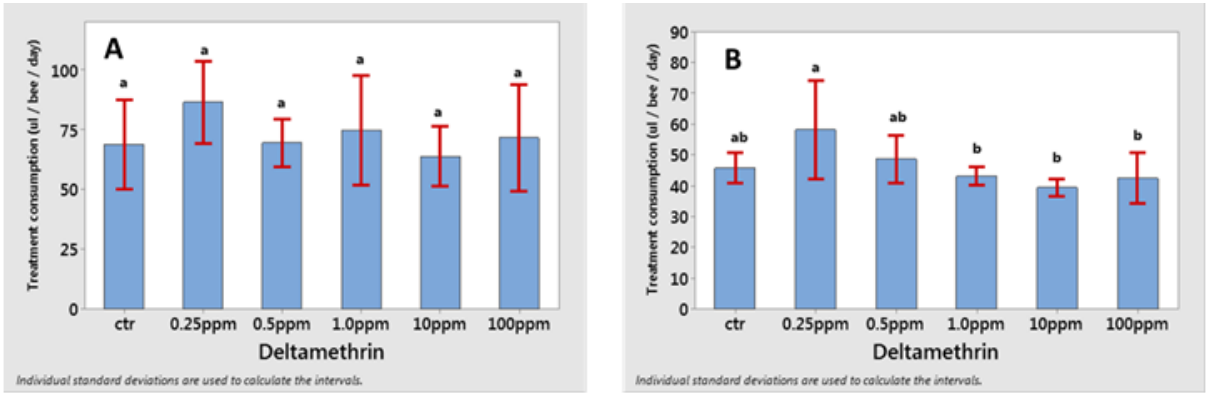

Figure 3: HPLC chromatograms of Deltamethrin in honey. (A-C letters) deltamethrin-detected regions (Samples 1,3,4). (D, E letters) deltamethrin-undetected regions (Sample 2 and 5). (F letter) is the standard curve for used for calculation deltamethrin residues.

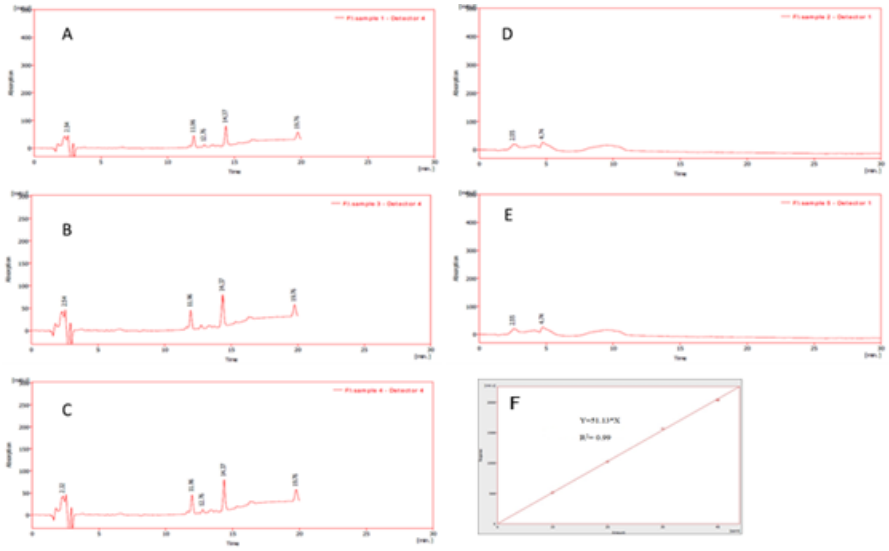

Figure 4:Daily consumption of treatments and sucrose diets for A.mellifera foragers (A) and nurse bees (B). Treatments are represented by their deltamethrin diets , including only 1M sucrose control (ctr). Different letters indicate a significant difference between groups (p=0.05). N= 5 cohorts per treatment with 20 bees each cohort, data presented as means ± SEM.

Bee survival

The present study was designed to determine the effect of deltamethrin on the honeybee survival. Although, all bees dead in the preliminary test at the lethal dose (1000ppm), bees were not harmed largely by ingesting sub-lethal doses. Surprisingly, they preferred to consume them compared to control (Figure 4). Interestingly, bee survival ranged between 93-96% in nurse bees and 70-83% in foragers at 0.25-100ppm deltamethrin (nurse bees, log-rank test, $\chi 2 = 4.5$, $p=0.47$; foragers, log-rank test, $\chi 2 = 14.904$, $p=0.11$, Figure 5).

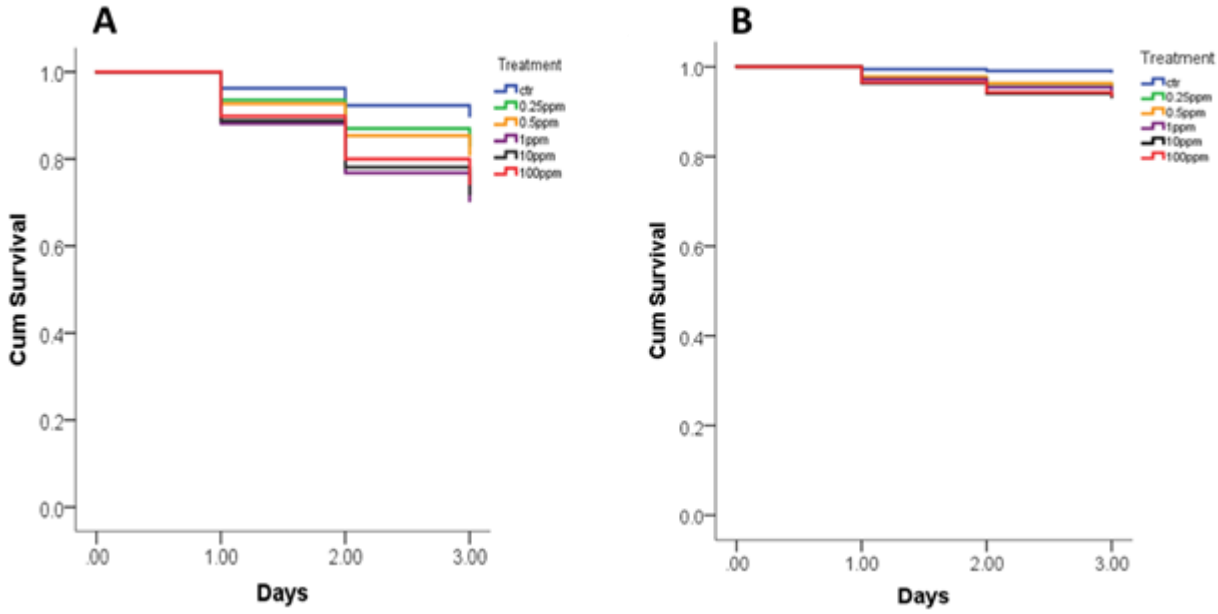

Figure 5: Survivorship of A.mellifera foragers (A) and nurse bees (B). Treatments are represented by their deltamethrin diets , including only 1M sucrose control (ctr). N= 5 cohorts per treatment with 20 bees each cohort.

Discussion

The present study indicated that deltamethrin residues in some regions (sample 1,3 and 4) of Najaf reached more than 200 times (10.6 ppm) the allowed amount in Europe (0.05ppm). However, no deltamethrin residues detected in other areas (sample 2 and 5) at least at the time of the sample collection (Figure 3). It seems possible that these results may be attributed to two factors; firstly, the deltamethrin –detected areas have more agricultural lands than non-detected areas (Central Statistical Organization, 2019) which eventually led to use more pesticides. Secondly, farmer preferences to use deltamethrin than other pesticides. This may be supported by the finding of some studies which proved that Pyrithroids (deltamethrin is one of them) are more commonly used than other pesticides due to the high effectiveness against many target insects (Varloud et al., 2015; Villemin and M.A.Didi., 2015). In accordance with the present results, previous studies have confirmed the contamination of honey samples with pyrithroids in different countries such as France (Chauzat et al, 2009), Turkey (Yavuz et al, 2010), Egypt (Malhat et al, 2015), Estonia (Karise et al , 2017 (Darko et al, 2017), Spain (Lozano et al, 2019), Poland (Gaweł et al, 2019), and India (Kumar et al, 2018). On the other hand, absence of deltamethrin residues from some samples in this study

may belong to the biodegradation occurred by some bacteria such as *Bacillus* spp**.** which are regularly found in the honey (Snowdon and D.O.Cliver., 1996). In addition, the preserving time of honey prior to HPLC test may be vary and had an effect on deltamethrin detection limit. Deltamethrin degradation percentage reached in some studies 51.4% after 3 hours, 59.8% after 6 hours and 63.4% after 9 hours (National Center for Biotechnology information, 2012).

It can be concluded from data of figure 5, that deltamethrin had caused a low risk on honeybee survival especially nurse bees. Unexpectedly, we observed that honeybees showed a preference for solutions containing low concentration of deltamethrin (which is originated from Pyrithroids plant chemicals). Although the role of secondary plant compounds as insect repellants is well documented (Karban and I.T.Baldwin., 2007), their role as pollinating attractants has also been widely studied (Adler, 2000) affecting the fitness of both plants and pollinators. Similarly, in another study, bees preferred low concentrations of a pesticide ,amygdalin, (London-Shafir et al., 2003).

The biosafety of deltamethrin for honey bees was rarely studied (Guo et al., 2020) especially under laboratory conditions, where honey bees are restricted and lack food alternatives compared to foragers in the field where they are able to move freely avoiding toxic substances,. Finally, it can be concluded that the sublethal doses of deltamethrin on honeybee survival was low and can be used against insect pests after testing them in the laboratory and field.

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