IMPACTS OF SUB-LETHAL DOSES OF AMITRAZ AND TAU-FLUVALINATE ON SOME PARAMETERS OF HONEY BEE WORKERS AND DRONES

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ABSTRACT: There are various chemicals available for the control of Varroa mites from honey bee colonies including amitraz and tau-fluvalinate. The optimum dose of these chemicals should give the highest Varroa control efficacy with minimal negative impacts on honey bees. The high doses are expected to have destructive impacts on honey bees. However, the impacts of the sub-lethal doses of these chemicals on honey bees are not well known. Therefore, the potential effects of the sub-lethal doses of these two chemicals on the survival of honey bee workers and drones, and on some body parameters were investigated. The study showed that the exposure of honey bee workers and drones to sub-lethal doses of chemical A (amitraz 125 mg/ml) or chemical B (tau-fluvalinate 240 mg/ml) had no clear negative impacts on their survival rates. Only tau-fluvalinate and not amitraz showed adverse impacts on measured body parameters of workers and drones, suggesting impacts on bee physiology. More insights into the potential impacts of tau-fluvalinate at different doses including the optimum one on bee physiology are advisable.

KEY WORDS: Honey bees, Varroa, amitraz, tau-fluvalinate

Honey bee colonies are impacted by many factors including biotic and abiotic ones. Varroa mites, *Varroa destructor*, are among the biotic factors which strongly impact honey bees in a passive way worldwide. These mites were considered as one factor responsible for colony collapse disorder (CCD) as mentioned by Rangel, Tarpy (2016). Many natural, mechanical or chemical methods to control Varroa have been developed and tested as reviewed by Abou-Shaara (2014). However, the use of chemical materials (i.e. acaricides) is almost the most effective way. One of the problems of using acaricides is the high residue level of them in bee products, for example amitraz in analyzed honey samples by Çobanoğlu, Tüze (2008). The chemicals approved for Varroa control are different among countries but chemicals contain either amitraz or tau-fluvalinate among the common ones. For example, chemicals contain tau-fluvalinate is common in North America (Frost et al. 2013) while those contain amitraz is used in some African and European countries. The resistance to these two acaricides by Varroa mites has been found to be different according to apiary location (Kamler et al. 2016). Applied acaricides within beehives have effects on Varroa mites and on honey bee as well. Unfortunately, evaluation the efficacy of acaricides against Varroa mites (Škerl et al. 2011; Semkiw et al. 2013) or other mites within colonies (Vandenberg & Shimanuki, 1990) gained more studies over evaluation of their effects on honey bees. Therefore, evaluating the potential impacts of acaricides on honey bees is essential.

Amitraz and fluvalinate have relatively high efficacy against Varroa mites if compared with other Varroa control options. The efficacy has been found to be from 90.6% to 94.6% for amitraz (Kraus & Berg, 1994) and about 69.21% for fluvalinate strips (Ahmad et al., 2013). However, previous studies have shown some passive impacts of amitraz and fluvalinate on honey bees. Strachecka et al. (2012) have found negative impacts of amitraz on the cuticle proteolytic system of honey bee workers, where hydrophobic protein concentrations were reduced in treated bees. Škerl et al. (2010) have detected acaricides in treated larvae and adult of worker bees including fluvalinate which suggesting negative impact on treated bees. Shouky et al. (2013) have found low sperm count in drones treated with fluvalinate or amitraz. Most studies have concentrated on using lethal or higher doses of the acaricides. Thus, investigating the potential effects of the sub-lethal doses on honey bees is strongly required especially honey bees can frequently be exposed to sub-lethal doses. Therefore, the study aimed to investigate the potential impacts of the sub-lethal doses of two common acaricides (amitraz and tau-fluvalinate) on the survival and some body parameters of honey bee workers and drones.

MATERIALS AND METHODS

1. The experimental conditions.

Metal cages with glass sides and perforated bottoms were used in this study (Fig. 1). During the experiments all cages were left at room temperature about (18±1°C) at the laboratory of the Institute of Apiculture, VÚŽV, Slovakia. The caged bees were supplied with 1 ml of sugar syrup in perforated Eppendorf tubes (Fig. 1) on daily basis. The bees used in the experiments were collected from Carniolan honey bee colonies at the apiary of the Institute of Apiculture. All the bees were approximately with age less than 21 days.

2. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee workers.

Two commercial chemicals contain amitraz or tau-fluvalinate were used in the experiments; 1) chemical A (amitraz 125 mg/ml) and 2) chemical B (tau-fluvalinate 240 mg/ml). These two chemicals were applied in a similar way to their use under field conditions. The standard methods to use chemical A, for one beehive box with bees and without brood, is by adding two drops of it on a small paper sheet (about 2.5 X 10 cm, WXL) and then burn it and close the beehive up to 30 minutes. In the experiment, only 1 µl, 2 µl or 3 µl of chemical A (hereafter amitraz) were added to small paper sheet (2.5 X 3 cm, WXL) and was burnt inside glass box with dimensions (25X 25X40 cm, L X H XW) as shown in Fig. 2 for 10 minutes. The applied amounts were less than the optimum one to ensure that bees exposed to the sub-lethal doses of this chemical. For chemical B (hereafter tau-fluvalinate), it is used by mixing 50 ml of water with 5 drops of it, and then a piece of paper (15X15 cm approximately) is immersed in the solution and placed inside the beehive. In the experiment, only 1, 2 or 3 drops were mixed with 50 ml of distilled water and then a small paper (2X10 cm, WXL) was immersed inside the mixture and then was placed in the cage. Thus, in this experiment 6 treatments were used (3 for amitraz and 3 for tau-fluvalinate) beside one control group with sugar syrup only and without any chemicals. For each treatment, 3 cages were used and in each cage 10 bees were added with a total of 30 bees per treatment (group). Then, some parameters were measured to treated bees.

3. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee drones.

The same experiment done with worker bees was repeated in the same way with drones. For each treatment, three cages were used and in each cage a group of 10 workers and 5 drones were added. Thus, for each treatment 15 drones were tested. The workers were added only to feed bee drones and to make the necessary thermoregulation within cages.

4. Measured parameters.

For workers and drones, daily survival rate was recorded up to one week after the treatment. The dead bees were removed from the cages daily. Also, head weight was recorded for one worker or drone per cage (three per treatment) using sensitive balance (KERN ABJ 220/4NM, Germany). Moreover, fresh weight (W1) was determined and then bees were placed in an oven at 100°C for 48 hours to record the dry weight (W2) in line with Abou-Shaara (2015) method. Then, the body water content was determined as: (W1-W2/W1) X 100. For workers only, the developmental degree of the hypopharyngeal glands was recorded using Hess (1942) scale, considering undeveloped glands as degree 1 while fully developed glands as degree 4.

5. Statistical analysis.

Three replicates (or cages) were assigned per each treatment in a complete random design (CRD). Means and their standard errors (S.E.) were calculated for measured parameters. The arcsine transformation was utilized to transfer % into degrees before the statistical analysis. The means were compared using Duncan's multiple range test at 5% degree of probability using SAS (Version 9.1.3.,2004, SAS Institute. Cary. NC, USA). Due to some conditions, readings of day 5 and 6 were not recorded.

RESULTS

1. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee workers.

The % of surviving bees declined over one week as shown in Fig. 3. The% of surviving bees declined from 100% at day 1 to 16.66, 53.33, 66.66, 26.66, 26.66, 3.33, and 60% at day 7 for treatments of 3µl of amitraz, 2µl of amitraz, 1 µl of amitraz, 1 drop of tau-fluvalinate, 2 drops of tau-fluvalinate, 3 drops of tau-fluvalinate, and control group, in respect (Fig. 1). Treatments had no significant impact on % of surviving bees (df= 6, F= 0.69, P= 0.6610 > 0.05). At day 7 only, treatments of 3µl of amitraz and 3 drops of tau-fluvalinate had the less surviving % with significant differences (P<0.05) than the other treatments. All treatments did not differ significantly than the control group over the experiment period, except the aforementioned treatments at day 7. It is apparent that increasing the amount of the chemical material caused high decrease in % of surviving bees. It could be said that the sub-lethal doses showed no passive impacts on the survival of worker bees.

Most of the measured parameters showed insignificant difference (P>0.05) than the control group as presented in Table 1. Only tau-fluvalinate treatments differed significantly less than control group in body dry weight. Moreover, bee group treated with 3 drops of tau-fluvalinate were less significant in head weight than the control group. It seems that the impact of tested treatments of amitraz had no adverse impacts on measured body parameters of honey bees while tau-fluvalinate showed some adverse impacts.

2. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee drones.

Drones either in the control group or in the other treatment groups were not able to survive for a long period (only up to 6 days). Fig. 4 compares among survival rates of the drones at different days (2, 3 and 4 days after the treatments). Treatments, in general, impacted survival rates of bee drones significantly (df=20, F=2.35 and P= 0.0100 <0.05). All drones of the control group had died by day 3 while those of the other groups survived during day 3 and 4 with significant differences (P<0.05) than the control group. The figure clearly highlights that the highest survival rates (13.33%) were to groups of 1 µl of amitraz and 1 drop of tau-fluvalinate while the other groups impacted the survival rates in a similar way (6.67% for day 3 and 4). According to these results, treatments of amitraz or tau-fluvalinate have no negative impact on the survival of bee drones.

Measured body parameters showed no significant differences (P> 0.05) among treatments, except treatments of 3 drop of tau-fluvalinate and 2µl of amitraz which were less significantly (P< 0.05) than the control group in fresh body weight. Also, treatments of 1 drop of tau-fluvalinate and 3 drop of tau-fluvalinate were less significantly (P<0.05) than the control group in dry body weight and body water %, respectively (Table 2). The tau-fluvalinate showed some adverse impacts on measured body parameters of honey bee drones, in a way similar to this found for the workers.

DISCUSSION

1. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee workers.

The tested doses of amitraz or tau-fluvalinate showed no significant impact on the survival of honey bee workers than the control group. It could be said the sub-lethal doses have no destructive impact on worker bees. These results are supported by the work of Rangel, Tarpy (2016), they found that colonies provided with combs contaminated with acaricides had significantly more activities (i.e. comb building and food storage) than untreated colonies. Thus, treatments sometimes have no negative impact on honey bees, but on the contrary their impacts could be positive. Increasing the amount of the chemical material either of amitraz or tau-fluvalinate caused higher decrease in % of surviving bees. This trend is expected and could be considered as normal because increasing the amount of chemical material could have some physiological impacts leading to impact the survival ability. In a similar way, Vandenberg and Shimanuki (1990) found higher bee mortality when high doses of amitraz (about 0.01 g) were applied on worker bees in small mailing cages.

Amitraz treatments showed no adverse impacts on measured parameters of bee workers. Treatments of tau-fluvalinate had significantly negative impacts on measured parameters of bee workers than the control group. It is expected that tau-fluvalinate had passive impacts on the physiology of honey bees. Similarly, Škerl et al. (2010) detected high quantities of fluvalinate in worker bee heads and in worker larvae after 8 days (105 ng/g) and 4 days (110 ng/g) from treatment, respectively suggesting negative impacts while very few quantities of amitraz were detected. Moreover, negative effects of fluvalinate were found by Frost et al. (2013) on learning, memory, responsiveness to sucrose, and survival of honey bee. This supports the idea that tau-fluvalinate has negative impacts on the physiology of honey bees.

2. Impacts of sub-lethal doses of amitraz and tau-fluvalinate on honey bee drones.

Treated drones or those of the control group were not able to survive for a long period or at least for one week. This could be explained by the experimental conditions where the study was done at about 18°C. It is known that temperature of honey bee colonies is around 34.5°C (Jones et al. 2005). Therefore, the experimental temperature is lower than the optimum one which could impact the ability of drones to survive for a long time passively regardless of treatments. In accordance with this, Neves et al. (2011) found no drones left the colony at temperatures of 26.5, 28.3, or even 31.3°C, suggesting high impact of low temperature of bee drones.

Treated drones with either fluvalinate or amitraz were able to survive more than control group during day 3 and 4. This result is somewhat resemble to those obtained by Rangel and Tarpy (2016), they found treated colonies had significantly better activities than untreated ones. This supports the idea that treatments could have a positive impact on honey bees especially when sub-lethal doses are used. Here, tau-fluvalinate also showed some negative impacts on measured parameters of honey bee drones. This result is in line with results obtained for worker bees. Moreover, the results of Shouky et al. (2013) are in agreement with the present study, they found that the least body weight of 0.186 gm was to drones treated with fluvalinate. They also found that treated drones with fluvalinate or amitraz had significantly low sperm number than untreated ones. The present study showed the absence of adverse impacts of amitraz or tau-fluvalinate on honey bee workers and drones except tau-fluvalinate which could has some physiological impacts on honey bees. Also, it could be expected that amitraz is better than tau-fluvalinate. Especially, the efficacy of amitraz is somewhat stable over long period of application as found by Semkiw et al. (2013). Similarly, Abou-Shaara (2014) concluded that amitraz is best acaricides against Varroa.

CONCLUSION

The study showed that the exposure of honey bee workers and drones to sub-lethal doses of chemical A (amitraz 125 mg/ml) or chemical B (tau-fluvalinate 240 mg/ml) had no clear passive impacts on their survival rates. Also, amitraz showed no adverse impacts on measured body parameters of workers or drones while tau-fluvalinate showed the vice versa. It could be expected that tau-fluvalinate has some impacts on bee physiology. More insights into the potential impacts of tau-fluvalinate at different doses including the optimum one on bee physiology are recommended. Also, investigating the impacts of the sub-lethal doses on Varroa mites is also advisable.

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Table 1. Means ± SE. of some parameters measured for bee workers exposed to different amounts of amitraz and tau-fluvalinate.*: Means followed by the same letter within the same column are not significantly varied according to Duncan's multiple range test$_{0.05}$.

Treatment	**Means ± S.E.***				
	Fresh weight (g)	**Dry weight (g)**	**Body water (%)**	**Head weight (g)**	**Glands develop ment (degree)**
Control	0.10±0.02 a	0.03±0.003 A	64±7.09 a	0.01±0.001 a	2.66±0.33 a
1µl of amitraz	0.11±0.005 a	0.026±0.003 Abc	75.33±3.92 a	0.01±0.001 ab	3.00±0.00 a
2µl of amitraz	0.09±0.01 a	0.03±0.00 Ab	65.66±4.33 a	0.01±0.001 a	3.00±0.00 a
3µl of amitraz	0.10±0.0 2 a	0.026±0.003 Abc	73.33±3.28 a	0.01±0.001 a	3.00±0.00 a
1 drop of tau-fluvalinate	0.08±0.003 a	0.02±0.00 C	77.00±1.00 a	0.01±0.001 a	3.00±0.00 a
2 drops of tau-fluvalinate	0.09±0.01 a	0.02±0.00 C	76.33±4.70 a	0.008±0.00 1 ab	3.00±0.00 a
3 drops of tau-fluvalinate	0.09±0.008 a	0.02±0.003 Bc	74.66±4.33 a	0.006±0.00 2b	3.00±0.00 a

Table 2. Means ± SE. of some parameters measured for bee drones exposed to different amounts of amitraz and tau-fluvalinate.*: Means followed by the same letter within the same column are not significantly varied according to Duncan's multiple range test$_{0.05}$.

Treatment	**Means ± S.E.***			
	Fresh weight (g)	**Dry weight (g)**	**Body water (%)**	**Head weight (g)**
Control	0.24±0.006 a	0.06±0.001 ab	73.00±1.00 a	0.016±0.001 a
1µl of amitraz	0.23±0.003 ab	0.06±0.001 ab	72.33±0.33 a	0.01±0.001 a
2µl of amitraz	0.20±0.02 b	0.062±0.001 ab	67.66±3.28 ab	0.01±0.001 a
3µl of amitraz	0.22±0.007 ab	0.06±0.001 ab	70.33±1.20 ab	0.01±0.001 a
1 drop of tau-fluvalinate	0.21±0.01 ab	0.06±0.0003 b	71.00±2.00 ab	0.01±0.001 a
2 drops of tau-fluvalinate	0.21±0.01 ab	0.06±0.0003 ab	69.33±1.66 ab	0.01±0.002 a
3 drops of tau-fluvalinate	0.19±0.01 b	0.065±0.001 a	66.00±1.52 b	0.01±0.002 a

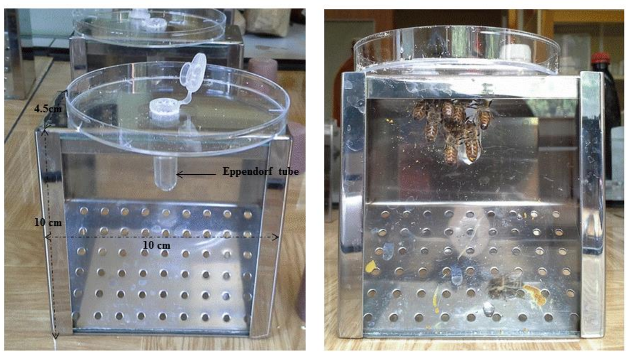

Figure 1. Cages used in the experiments, left: dimensions of the cages, and right: caged bees.

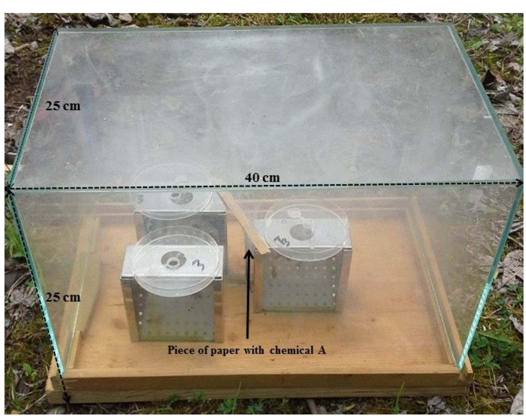

Figure 2. The glass box used during treating caged bees with Amitraz.

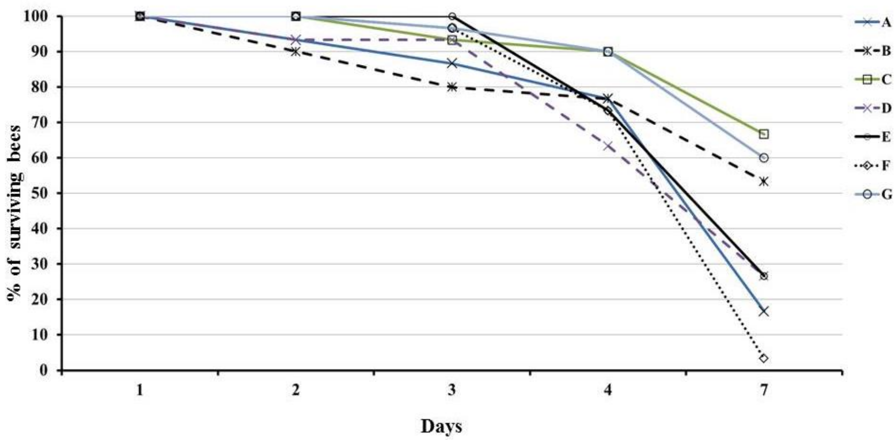

Figure 3. Mean % of surviving bees over one week after the treatments. A: 3µl of amitraz, B: 2µl of amitraz, C: 1 µl of amitraz, D: 1 drop of tau-fluvalinate, E: 2 drops of tau-fluvalinate, F: 3 drops of tau-fluvalinate, and G: control group.

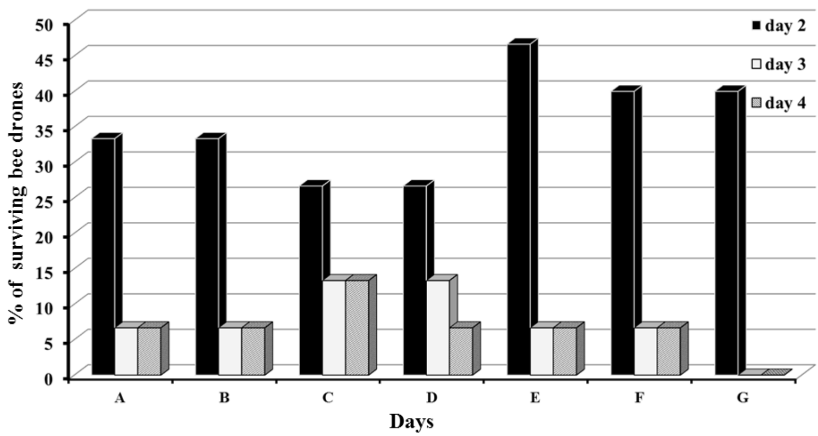

Figure 4. Mean % of surviving bee drones at day 2, 3 and 4 after the treatments. A: 3µl of amitraz, B: 2µl of amitraz, C: 1 µl of amitraz, D: 1 drop of tau-fluvalinate, E: 2 drops of tau-fluvalinate, F: 3 drops of tau-fluvalinate, and G: control group.