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Effects of sequential exposures of sub-lethal doses of amitraz and thiacloprid on learning and memory of honey bee foragers, *Apis mellifera*

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ABSTRACT

Pollinators, honey bees in particular, are continuously exposed to various mixtures of pesticides, which contribute to their population decline. Both amitraz and thiacloprid have been proven less toxic to honey bees and are frequently applied in- and out-hive, respectively. We examined the sub-lethal effects of amitraz, thiacloprid and their sequential exposure on learning, memory and sugar responsiveness in *Apis mellifera* using the Proboscis extension response (PER). Sub-lethal doses of amitraz (0.1, 0.2 and 0.4 μg/bee) and thiacloprid (0.05, 0.1 and 0.2 μg/bee) were tested. Sub-lethal effects were observed only at the highest doses of each pesticide treatment; amitraz (0.4 μg/bee) and thiacloprid (0.2 μg/bee) but not in lower doses. In sequential treatment of amitraz and thiacloprid, reduced acquisition and memory retention were significant across all tested doses. The same profile was also obtained on sugar responsiveness of foragers. Our results suggest that the sequential exposure would pose higher risk to honey bee compared to single pesticide exposure by reducing the bees' appetitive olfactory learning, memory and sugar acuity more than individual pesticide exposures.

Introduction

Insects possess a nervous system with a reduced number of neurons. However, this does not limit their ability to execute sophisticated and complex behaviors (Giurfa, 2012). Despite stereotyped and genetically encoded behaviors, insects exhibit a remarkable behavioral plasticity as many species learn and memorize different sorts of sensory cues as prognosticators of reward or punishment (Giurfa, 2015). The honey bee (*Apis mellifera*) evolved to be equipped with complex sensory systems and well-developed learning and memorizing capacities (Farooqui et al., 2003; Zhang et al., 2006). Most research on insect learning and memory has focused on olfactory and visual associative learning in which animals learn to associate a biologically relevant conditioned stimulus (CS) with an unconditioned stimulus (US) (Pavlov and Anrep, 1927). Thus, ability of foragers to quickly associate a particular floral odor or shape with a nectar reward is a critical part of successful foraging that can be affected by prolonged sub-lethal exposure of pesticides in field (Halm et al., 2006).

Physiology and behavior of honey bees can be affected by sub-lethal doses/concentrations of pesticides. A dose or concentration that does not induce a significant difference in mortality between experimental populations can be considered to be sub-lethal (Desneux et al., 2007; Hesselbach and Scheiner, 2019). It is in the interest of researchers to determine sub-lethal doses/concentrations under experimental conditions. For instance, 10 and 100 nmol/L of imidacloprid and coumaphos (Williamson and Wright, 2013); 8, 4 and 0.4 ng/bee of imidacloprid (Hesselbach and Scheiner, 2019) and 0.83, 0.083 and 0.0083 mol/L of flupyradifurone (Hesselbach and Scheiner, 2018) were identified to represent sub-lethal concentration/doses in relation to behavioral changes in honey bees. Residue based sub-lethal exposures can also affect the behavior of honey bees. Imidacloprid as low as 50 ng/g was shown to affect the bees' foraging behavior and if treated with >800 ng/g it led to an abnormal foraging behavior that caused no honey bees to return to the feeding site (Yang et al., 2008).

Thiacloprid, a widely used cyano-substituted AChE inhibitor neonicotinoid known to be less toxic to honey bee (Matsuda et al., 2001; Ulziibayar and Jung, 2019), has various sub-lethal effects. Immunity (Brandt et al., 2016), disease susceptibility (Vidau et al., 2011) and learning and memory (Tison et al., 2017) of bees were impaired by chronic sub-lethal doses of thiacloprid. Beekeepers can deliberately expose honey bees to miticides and fungicides to control pests and pathogens (Chmiel et al., 2020) and amitraz, a formamidine octopamine

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receptor agonist, for instance, is one of the widely applied in-hive chemicals used by beekeepers to control the most serious pest of honey bees *Varroa destructor*. Frequent applications of chemical acaricides are required to control this pest (Kim and Jung, 2008; Jung, 2012; Jeong et al., 2016), but accumulating residues in the hive lead to sub-lethal effects by interacting with other chemicals. 574.2 and 147.9 ng/g of amitraz or its metabolite N-(2,4-dimethylphenyl)-N-methylformamidine (DMPF), detected in wax and pollen, can cause sub-lethal effects if adult and immature bees become chronically exposed to these substances (Bernal et al., 2010; Mullin et al., 2010).

With high potential of foraging bees simultaneously exposed to chemicals in the natural environment, it is important to consider interactions between acaricides and insecticides (Gill et al., 2012; Johnson et al., 2013). Thus, amitraz with its higher residue amount in hive material (Mullin et al., 2010) metabolizing rapidly (Hillier et al., 2013) could interact with thiacloprid, the less toxic neonicotinoid used outside the hive (Matsuda et al., 2001). We demonstrated negative effects on leaning, memory and sugar responsiveness in honey bees by sequential exposures of the latter to sub-lethal doses of amitraz and thiacloprid, which can reach a level of high toxicity when interacting (Williamson and Wright, 2013), To best of our knowledge, the effects of sequential sub-lethal exposures of bees to amitraz and thiacloprid on learning, memory and sugar responsiveness have not been studied before.

Materials and methods

Honey bees

Returning adult *A. mellifera* pollen foragers, recognizable by pollen loads on their corbiculae, were individually collected into glass vials (22.5 mm × 75.5 mm × 12.5 mm) at the entrance from three healthy honey bee colonies in the experimental apiary of Andong National University. The glass vials were then transferred to the laboratory and placed in a refrigerator (3–5 min) at a temperature of 4 °C to render the foragers motionless. When the honey bee foragers stopped moving, they were inserted into properly cut 1 mL plastic pipette tips in which only the antennae and mouthparts were free to move. Harnessed *A. mellifera* foragers were fed to satiation with 50% sugar solution and left in darkness at room temperature, 60% RH, until the analysis commenced (Piiroinen and Goulson, 2016).

Pesticides

Amitraz emulsion insecticide, containing 20% a.i, Arysta Life Science; Thiacloprid formulation Calypso® containing 10% a.i, Bayer, Acetone (purity 99.5%; CAS No. 67–64-1; Daejung, South Korea) and 1-Nonanol (purity > 98%; CAS No. 143–08-8, Sigma Aldrich, Korea) were purchased.

Determining sub-lethal dosage

Preliminary experiments were conducted to set a series of sub-lethal doses. To ascertain the sub-lethal doses under our testing condition, pollen foragers were caught and fixed as the conditioning test above indicated. The sub-lethal doses were identified per Williamson and Wright, (2013) method. Harnessed pollen foragers were fed 5 μl of (20 μg/ml = 0.1 μg/bee, 40 μg/ml = 0.2 μg/bee, 80 μg/ml = 0.4 μg/bee and 160 μg/ml = 0.8 μg/bee) of amitraz; (10 μg/ml = 0.05 μg/bee, 20 μg/ml = 0.1 μg/bee, 40 μg/ml = 0.2 μg/bee and 80 μg/ml = 0.4 μg/bee) of thiacloprid, and 50% sugar solution as a control. Individually harnessed bees were kept on the rack and fed 5 μl of 50% sucrose syrup three times a day using a micropipette (Gilson's pipetman classic™ Korean analytical instruments, Korea) (Gashout et al., 2020). The number of bees surviving for 72 hrs was recorded (Fig. 3). Fifty individuals were used for each treatment group.

Motivation test

Thirty minutes before the first conditioning trial, the antennae of the foragers were stimulated with a toothpick soaked in 50% sugar solution (Piiroinen and Goulson, 2016). Only bees that responded by extending their proboscis were included in further learning and memory experiments (Rix and Christopher Cutler, 2017).

Learning and memory test

The proboscis extension response (PER) is a useful tool to evaluate significant elements of an individual bee's foraging abilities in the laboratory. Learning and memory tests were carried out according to the method described by (Matsumoto et al., 2012). 30 min before starting the acquisition and memory test, a group of ten harnessed bees was placed at the experimental site in the laboratory to familiarize them with the surroundings under open air conditions. During conditioning in all trials each bee was placed at the conditioning site for 25 s. 2 M of conditioned stimulus (CS) was presented from a 5 μl aliquot of 1-nonanol applied to a piece of filter paper (10 mm × 30 mm) placed within 20 mL syringe (Korean vaccine Co.,LTD) for 4 s to each bee during each trial. The tip of a syringe was placed at the same height 1.5 cm from the bee's antennae. The US, 50% sugar water was presented for 3 s after the odor onset with a 1-second overlap with the CS, i.e., the antennae of the harnessed bee were stimulated with US, and when it extended its proboscis the bee could lick the sucrose solution for 3 s. The bee was left at the acquisition site 25 more seconds after the unconditioned stimulus (US) presentation, then removed and replaced by the next bee. Each bee received 6 paired CS-US conditioning trials with a 10-minute inter-trial interval (ITI) of PER conditioning.

Memory retention was tested 1 and 24 h after the last conditioning trial. For the memory test, each bee was placed at the acquisition site for 25 s; 2 M of CS was presented for 4 s to each forager's antenna with an inter-trial interval of 10 min in which sugar rewards were not given.

Sucrose responsiveness

To examine the effects of sequential exposures to sub-lethal doses of mixture of amitraz and thiacloprid and individual chemicals on sugar responsiveness, honey bees were exposed to insecticides as indicated above. Two hours after the bees had consumed the thiacloprid and 24 hrs after amitraz exposure, they were assayed for a sucrose response (Hladun et al., 2012). The antennae of each honey bee were first stimulated with a droplet of water (0% sugar solution) that used to control any possible effects of repeated sugar stimulation that could lead to increased habituation of the PER. This was followed by ascending sucrose solutions: 0.1%, 0.3%, 1%, 3%, 10%, 30% and 50% (w/w). The inter-stimulus interval was about 4 min to exclude a sugar sensitization effect (Scheiner et al., 2005; Goñalons and Farina, 2018; Jiang et al., 2018). Each treatment was replicated with thirty individuals.

Statistical analysis

Mortality data were analyzed using a Kruskal–Wallis test. Mann-Whitney Tests was applied for pair wise comparisons. The responses in relation to learning, memory, and sucrose responsiveness were scored as a binary variable (no response: 0, proboscis extension response: 1). Binary logistic regression (generalized linear model, GLM) in SPSS version 16 (Chicago, SPSS Inc., 2007) was used to analyze the PER response in motivation test, learning, memory and sugar responsiveness. Treatments and number of trials in learning; treatments and time of testing in memory test and treatment and sugar concentration of sugar were predictors in analyzing PER responses. P-values showing the influence of a fixed effect were obtained by analysis of deviance table (Wald Chi-square tests) between treatments.

Results

Determination of sub-lethal dose

The effects of amitraz, thiacloprid and their sequential mixture exposure on learning, memory and sugar responsiveness of honey bees were carried out at the sub-lethal doses. To this end, four doses of individual pesticide (amitraz: 0.1, 0.2, 0.4 and 0.8 μg/bee and thiacloprid: 0.05, 0.1, 0.2 and 0.4 μg/bee) were initially selected based on preliminary experiments. Overall, there appeared to be significant differences in honey bee mortality between the pesticide groups and the control (Amitraz: $\chi^2 = 15.34$, df = 4, $P = 0.004$; Thiacloprid, $\chi^2 = 15.93$, df = 4, $P = 0.003$). However, a subsequent analysis by the Mann-Whitney pairwise comparison indicated that there were no significant honey bee mortality differences noticeable when honey bees were exposed to amitraz (0.1 μg/bee: U = 10, $P = 0.513$; 0.2 μg/bee: U = 7.5, $P = 0.221$; 0.4 μg/bee: U = 5, $P = 0.072$) and thiacloprid (0.05 μg/bee: U = 10.5, $P = 0.606$; 0.1 μg/bee: U = 8.5, $P = 0.339$; 0.2 μg/bee: U = 4.5, $P = 0.065$) at lower doses. In contrast, both pesticides caused significant differences in bee mortality between the treatment groups and the control at the highest tested doses (amitraz: 0.8 μg/bee: U = 0.0008, $P = 0.004$ and thiacloprid 0.4 μg/bee: U = 0.000, $P = 0.008$). Therefore, our results demonstrated that amitraz concentrations of 0.1, 0.2 and 0.4 μg/bee and those of 0.05, 0.1 and 0.2 μg/bee for thiacloprid were sub-lethal doses (Fig. 3).

Motivation test

A motivation test was performed on honey bee foragers by touching their antennae with a toothpick soaked in 50% sugar solution 30 min before learning and memory test executions. *A. mellifera* foragers (668 in total) were initially harnessed, with 77.4% of them responding positively to 50% sucrose solutions and passing the motivation tests. Overall, we did not find any significant differences in the PER proportions of the foragers when they were subjected to sub-lethal doses of amitraz, thiacloprid, sequential or the control groups ($\chi^2 = 12.98$, df = 9, $P = 0.178$) in 50% sugar solution motivation tests. Followed by the comparison of treatments in Wald Chi-square tests table, there were no significant differences in the PER rate between the treatments and the control groups across all sub-lethal doses ($P > 0.05$) except for amitraz at 0.4 μg/bee ($P = 0.012$). In the treatment groups, amitraz (73–92%) thiacloprid (83–88%), and amitraz/thiacloprid sequential exposure (84–86%) led to foragers responding positively to 50% sucrose solutions and passed the motivation test (Table 1).

Learning performance

Upon the first conditioning trial, no honey bees elicited a PER to the CS. However, as the number of trials progressed, the honey bees' PER responses increased to CS in all treatments and control groups during conditioning ($\chi^2 = 1200.94$, df = 5, $P < 0.001$). The significance difference in response to CS in the sixth and first conditioning trials ($P < 0.001$) could be the indication of acquiring experience (learning) the CS in all treatment groups.

It was also evident that learning performance was significantly reduced by the type of treatment ($\chi^2 = 119.77$, df = 3, $P < 0.001$) and dose ($\chi^2 = 187.8$, df = 9, $P < 0.001$) used in our experiments. For instance, in the amitraz-treated group, the maximum level of acquisition (89% PER; n = 54) of foragers was attained when they were exposed to amitraz at its lowest sub-lethal dose of (0.1 μg/bee) and at the sixth conditioning trial. Interestingly, the PER response ($P < 0.001$) of foragers in the sequential amitraz/thiacloprid treatment group was significantly lower than their corresponding individual pesticide treatment groups. We also noted that learning performance of foragers was significantly reduced when they were exposed to higher sub-lethal concentration of amitraz (0.4 μg/bee: $\chi^2 = 49.31$, P < 0.001) and thiacloprid (0.2 μg/bee: $\chi^2 = 34.5$, P < 0.001). However, acquisition PER scores were not significantly affected by each individual pesticide at their lower sub-lethal doses (amitraz: 0.1 μg/bee, $\chi^2 = 0.774$, $P = 0.379$; 0.2 μg/bee, $\chi^2 = 3.51$, $P = 0.058$; thiacloprid: 0.05 μg/bee, $\chi^2 = 0.044$, $P = 0.834$; 0.1 μg/bee, $\chi^2 = 2.63$, $P = 0.105$) (Fig. 1 A-C: Acquisition)

Memory retention

One and twenty-four hours after the end of the conditioning trial, we performed a retention test on the effects of sub-lethal exposures of amitraz, thiacloprid and their sequential mixtures on the ability of honey bee foragers to remember (Fig. 1, Right). Overall, both pesticides and their mixtures significantly reduced the foragers' PER scores to CS ($\chi^2 = 187.8$, df = 9, $P < 0.001$). Indeed, it was only noted that the significant reduction in memory performance of the foragers had occurred when the latter were exposed to higher sub-lethal doses of both individual pesticides (amitraz: 0.4 μg/bee, $\chi^2 = 7.687$, $P = 0.006$ and thiacloprid: 0.2 μg/bee, $\chi^2 = 8.30$, $P = 0.004$). In contrast, the mixture of amitraz and thiacloprid significantly reduced the PER scores of foragers across all tested sub-lethal sequential exposures ($P < 0.001$).

The memory retention of foragers was not significantly affected at lower doses of each individual pesticide (amitraz: 0.1 μg/bee, $\chi^2 = 2.611$, $P = 0.106$; 0.2 μg/bee, $\chi^2 = 1.047$, $P = 0.306$ and thiacloprid: 0.05 μg/bee, $\chi^{2=} 0.809$, $P = 0.369$; 0.1 μg/bee, $\chi^2 = 493$, $P = 0.483$). Our result indicated that there was a significantly lower average rate of PER scores of memory performance at 1hr and 24 hr than on the 6th conditioning trial (thiacloprid: $\chi^2 = 45.283$, df = 2, $P < 0.001$). However, the PER scores did not change between 1 and 24 hr ($P = 0.946$) (Fig. 1 A-C (retention).

Sugar responsiveness

Sucrose responsiveness of individual foragers was determined by applying different sucrose concentrations to the antennae of restrained bees treated with 50% sugar (control), three different sub-lethal doses of amitraz, thiacloprid and their mixtures. We noted that the PER response (the sucrose response) of foragers was significantly lower in the groups treated with at higher sub-lethal doses of amitraz (0.4 μg/bee),

Table 1
Number of harnessed honey bee foragers, dead before motivation test, bees in motivation test, bees with positive and negative response to the antennal stimulation of 50% sucrose prior to the conditioning test.

	Treatments (μg/bee)									
	Control	Amitraz			Thiacloprid			Mixture		
	0	0.1	0.2	0.4	0.05	0.1	0.2	0.1 + 0.05	0.2 + 0.1	0.4 + 0.2
Harnessed bees (n)	70	66	75	72	70	63	60	70	60	62
Dead bees (n)	4	5	4	8	9	6	7	6	8	5
Bees in motivation test (n)	66	61	71	64	61	57	53	64	52	57
Positive response	60	54	65	47	51	50	44	55	43	48
Negative response	6	7	6	17	10	7	9	9	9	9
Motivated bee (%)	91	89	92	73	84	88	83	86	83	84

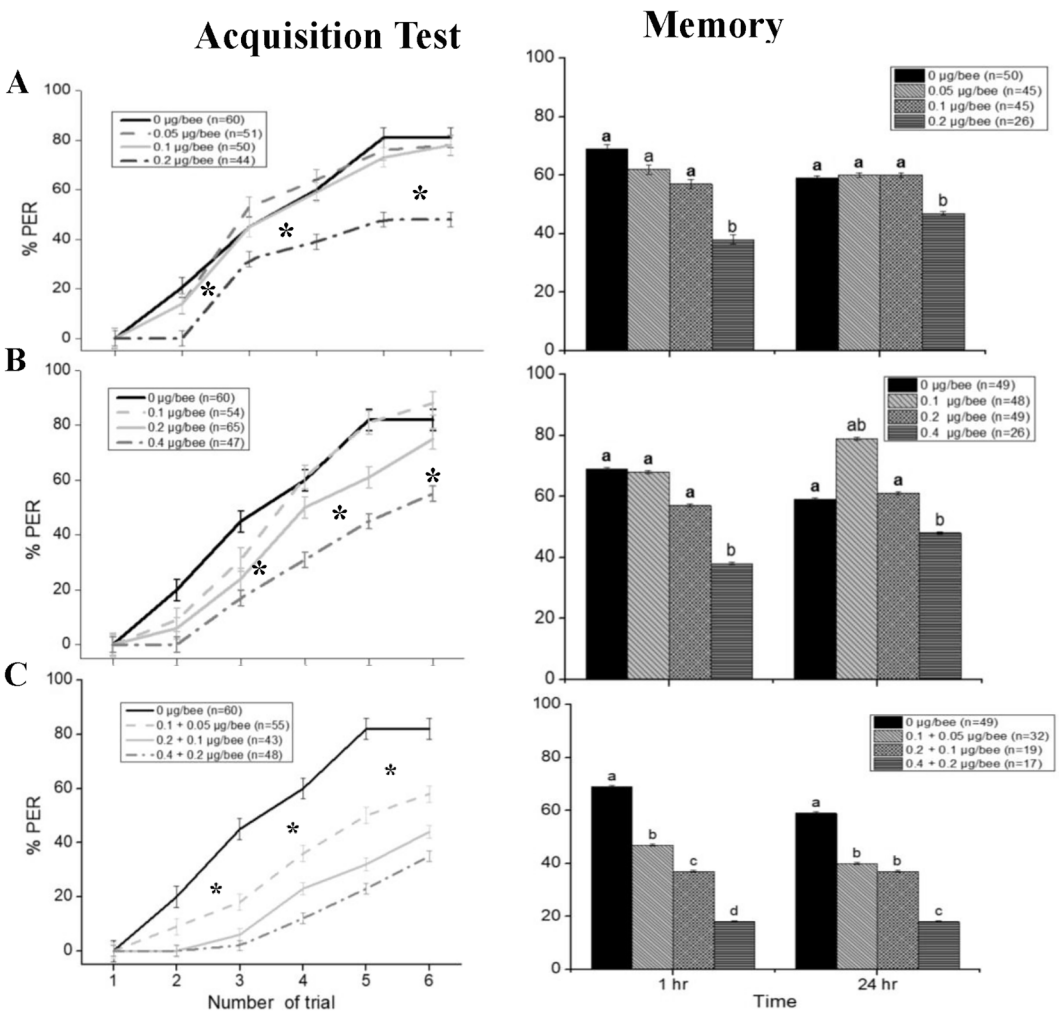

Fig. 1. Effect of sub-lethal exposure of Thiacloprid with doses 0.05, 0.1 and 0.2 µg/bee (A), amitraz with doses 0.1, 0.2, and 0.4 µg/bee (B) and their sequential mixture exposure (C) on associative olfactory learning and memory of pollen forager honey bees. Learning performance was measured as proboscis extension response (% PER ± SE, left) and percentage memory retentions (±SE) were measured at 1 and 24 hr (right) after treatment. Statistical differences are marked as * or different letter ($P < 0.05$).

thiacloprid (0.2 µg/bee) and all sequential mixture of amitraz and thiacloprid than in the control group when the tests were carried out at higher concentration of sugar solution (10, 30 and 50%). However, we did not find any difference in the PER response of foragers when they were subjected to lower doses of amitraz (0.1, 0.2 µg/bee) and thiacloprid (0.05, 0.1 µg/bee) ($P > 0.05$, Fig. 2) as compared with the control group. Sucrose responses of foragers increased with increasing concentrations of sucrose for each treatment group in a dose-dependent manner.

Discussion

Various dose concentrations were tested to determine sub-lethal levels and it could be shown that amitraz (0.1, 0.2 and 0.4 µg/bee) and thiacloprid (0.05, 0.1 and 0.2 µg/bee) caused no significant differences in honey bee forage mortality as compared to the control group, signifying that these doses represented sub-lethal doses. Our findings were consistent with those previous reported for amitraz (Abou-Shaara et al., 2017; Dai et al., 2018) and thiacloprid (Fischer et al., 2014). The maximum sub-lethal concentration of amitraz identified in the present study, 80 µg/ml, was only less than two times the maximum residue amount of amitraz in the form of its metabolite 2,4-dimethylphenyl-N-methylformamidine (DMPF) in wax (Mullin et al., 2010). This showed that the amount of amitraz before metabolization could be approximately the same as or even more than our highest dose. The sub-lethal doses determined in the presented study were much less than half the acute LD_{50} since those doses were selected by Li et al., (2017) for chlorpyrifos to assess the behavioral changes in the honey bee

The sub-lethal doses employed in our study significantly impaired not only the acquisition and, hence, memory retention, but also reduced the sugar responsiveness in honey bee foragers. Foragers exposed to amitraz (0.4 µg/bee) and thiacloprid (0.2 µg/bee), representing the highest tested sub-lethal doses during our experiments, displayed lower learning and memory abilities. Furthermore, we found that foragers had a higher tolerance to amitraz than thiacloprid. This was supported by a study documented that honey bees can tolerate amitraz up to certain levels by rapidly metabolizing the chemical, when administrated topically (Rix and Christopher Cutler, 2017). For instance, Hillier et al. (2013) confirmed that only 32% of amitraz residue was obtained from honey bees just 1hr after topical treatment. The impaired learning and memory of honey bees by amitraz may be an over-dose effect (0.4 µg/bee) in our study blocking the metabolism of octopamine, a neurotransmitter that may disturb the cholinergic pathway in bees (Rix and Christopher Cutler, 2017). Furthermore, amitraz's lower learning and memory performance could be due to its ability to suppress the octopaminergic pathway in the honey bee brain by binding to octopamine receptors (Farooqui et al., 2003; Gashout et al., 2020). Unlike our findings, Rix and Christopher Cutler, (2017) reported that amitraz had

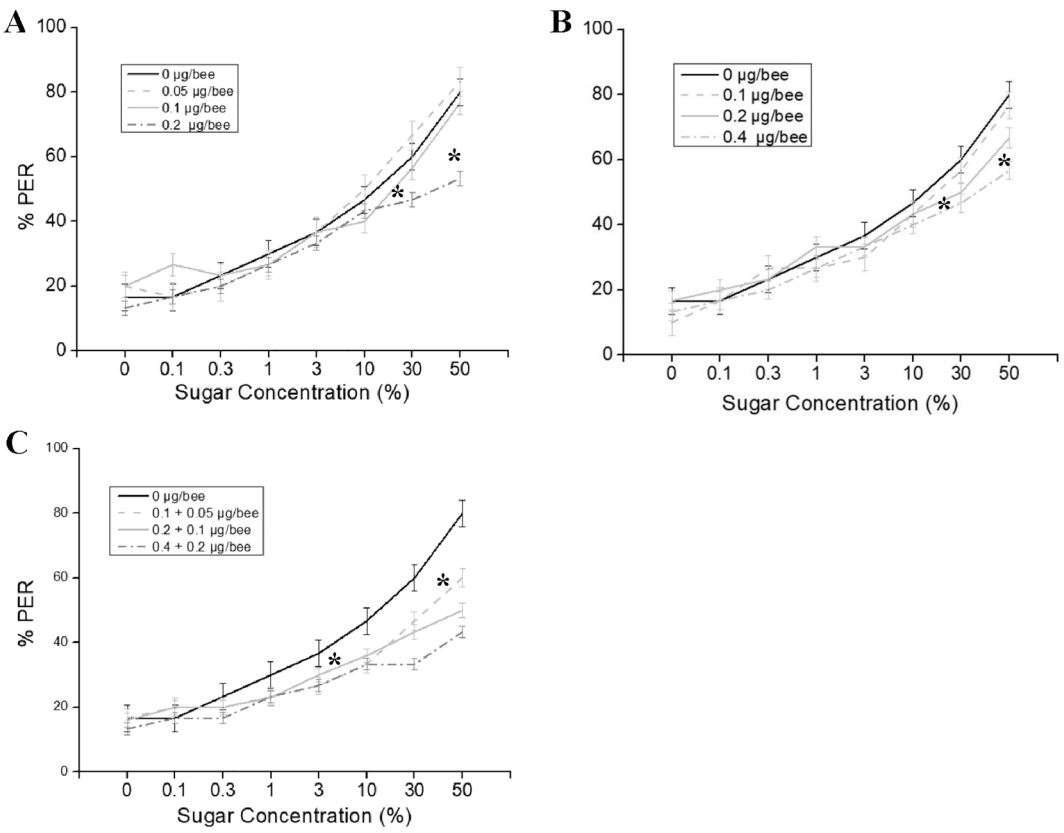

Fig. 2. Effect of sub-lethal exposure of thiacloprid with 0.05,0.1 and 0.2 µg/bee dose (A) amitraz with 0.1, 0.2 and 0.4 µg/bee doses (B) and their sequential mixture exposure (C) on sugar responsiveness of pollen forager honey bees. Sugar responsiveness was measured as proboscis extension response (% PER ± SE) measured at different concentration of sugar. Statistical differences are marked as * ($P < 0.05$).

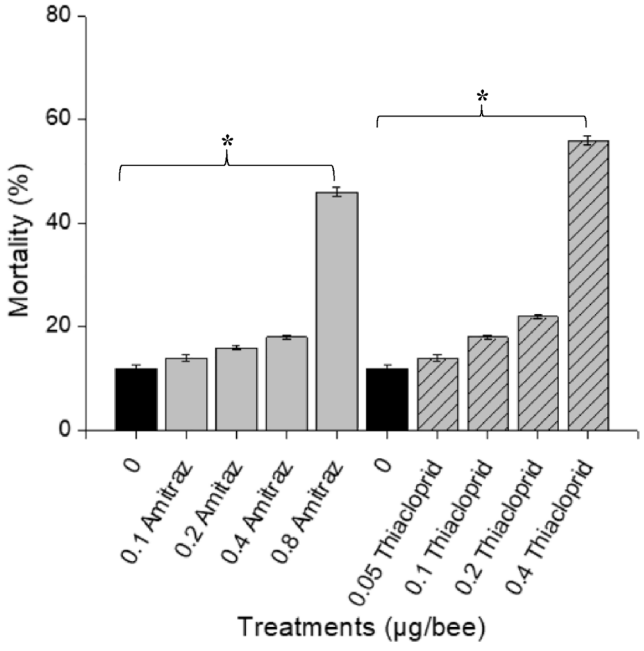

Fig. 3. Survival data for bees fed different concentrations of amitraz and thiacloprid treatments for 72 h prior to conditioning. The mortality of bees measured at 72 hrs of different doses of the treatments was indicated (% mortality ± SD). Statistical differences are marked as * ($p < 0.05$).

no effect on learning and memory of honey bees. Abou-Shaara et al., (2017) also revealed no adverse impacts of sub-lethal doses of amitraz on survival and physiological. However, many authors also documented sub-lethal effect of thiacloprid or its formulation Calypso® on honey bees. For instance, olfactory associative learning and memory (Tison et al., 2017), flight speed and homing success (Fischer et al., 2014), navigation performance, as well as social communication (Tison et al., 2016) were all impaired by sub-lethal doses. The negative pressure of amitraz and thiacloprid on learning and memory of bees in the present study may be contributed by the formulation of the pesticide which is toxic to bees (Stanley and Preetha, 2016; Tison et al., 2017), bearing in mind that formulations of pesticides have been reported to be more acutely toxic than individual active ingredients (Mullin et al., 2015).

Honey bees are regularly exposed to complex mixtures of pesticides in the natural environments (David et al., 2016). Amitraz, the frequently applied acaricide against *Varroa destructor* (Pohorecka et al., 2018), could become mixed with the less toxic thiacloprid in the field. Our result clearly indicated that all tested sequential sub-lethal doses led to lower PER scores. The research results suggested that the stronger pressure from the mixture of pesticides on learning and memory of bees could be linked to the same mechanisms that are involved in detoxification (Williamson and Wright, 2013). The pretreated pesticides may also enhance the impairments of learning and memory of honey bee in sequential exposures due to a disturbance of the detoxification enzyme. Johnson et al. (2013) reported a 5-fold increase in toxicity when bees were exposed to tau-fluvalinate after having been pre-treated with amitraz. Similarly, Iwasa et al. (2004) reported that Piperonyl butoxide, triflumizole and propiconazole greatly enhanced the toxicity to honey bees of thiacloprid 154-, 1141 and 559-fold, respectively. Generally, toxic effects of pesticides increase when animals are exposed to more than one pesticide interacting with others (Johnson et al., 2013).

Foraging behavior is influenced by sucrose responsiveness, i.e. the assessment of whether a nectar source is sweet enough to feed on (Eiri and Nieh, 2012). The foragers' responses to lower sucrose concentrations are higher than those of newly emerged bees due to the older bees' foraging experience (Pankiw and Page, 1999; Pankiw et al., 2001). The responses of honey bees to higher concentrations of sucrose solutions by high dose of amitraz and thiacloprid and all doses of sequential treatments were lower than those of the control bees. This reduction of PER score may be due to a rapid desensitization of the nAChR leading to temporary sucrose taste responsiveness impairment (Démares et al., 2018). It was also reported that honey bees fed foods containing individual or mixture of toxin showed a reduced responsiveness to sucrose (Pankiw and Page Jr, 2000; El Hassani et al., 2005; Mustard et al., 2008; Aliouane et al., 2009; Jiang et al, 2018). Moreover, Tison et al, (2017) found Calypso® had a repellent effect for sucrose at low concentration. A reduced sugar response can be directly related to a deficit in learning and memory performance caused by sub-lethal exposure on chemosensory information transmission (Goñalons and Farina, 2015). As a result, foraging honey bees would spend more time searching for sources with higher concentrations of sugar in nectar, making longer foraging trips and failing to get back to the hive (Démares et al., 2016). Additionally, restraining of the tested honey bees in a tube could have led to reduced gustatory sensitivity (Mujagic and Erber, 2009). Season (Scheiner et al., 2003), age (Pankiw and Page, 1999), nutrition and experience (Pankiw et al., 2001) also affect sugar responsiveness. Even without noticeable mortality, multiple exposures to chronic sub-lethal doses of similar or different groups of insecticides could impair learning and memory of honey bees, thereby leading foragers into difficulties and consequently causing a decline in colony growth.

Conclusion

In conclusion, we believe that sub-lethal interactions between different pesticides, such as those demonstrated in this report, could be a major contributor worldwide to the loss or impairment of a honey bee's learning and memory abilities and thereby not only increasing honey bee mortality but also affecting whole colonies. To reduce pesticide exposure and to provide clean and healthy nutrition and habitats, one approach focuses on improving the quality of floral resources through supplemental plantings and landscape enrichment with flowering plants (Decourtye et al., 2010; Lee and Jung, 2019). This involves the development of diversified floral provisions to optimize the nutritional needs of honey bees and other pollinators, based on their nutritional profiles of a range of floral resources of native and agricultural plant species. Of course, avoiding any pesticide overuse or misuse of insecticides and proper communication with beekeepers are also required to protect the honey bees as our most important pollinators in the ecosystem generally and the agricultural sector in particular.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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