

# Effect of Sub-lethal Doses of Imidacloprid on Learning and Memory Formation of Indigenous Arabian Bee (*Apis mellifera jemenitica* Ruttner) Adult Foragers

J IQBAL<sup>1,2</sup> , AS ALQARNI<sup>1</sup>, HSA RAWEH<sup>1</sup>

<sup>1</sup>Melittology Research Lab, Dept of Plant Protection, College of Food and Agriculture Sciences, King Saud Univ, Riyadh, Saudi Arabia

<sup>2</sup>Dept of Entomology, MNS Univ of Agriculture, Multan, Pakistan

## Keywords

Insecticide, neonicotinoid, associative learning, honey bee, *Apis mellifera jemenitica*

## Correspondence

Melittology Research Lab, Dept of Plant Protection, College of Food and Agriculture Sciences, King Saud Univ, Riyadh, Saudi Arabia; jiqbal@ksu.edu.sa

Edited by Alberto S Corrêa – ESALQ/USP

Received 21 March 2018 and accepted 1 November 2018

© Sociedade Entomológica do Brasil 2018

## Abstract

The indigenous bee race *Apis mellifera jemenitica* Ruttner of Saudi Arabia can learn and retain memories established by the classical conditioning of proboscis extension response (PER). The insecticide imidacloprid has shown a drastic effect on the olfactory behavior of *A. m. jemenitica* in the harsh arid climatic conditions of central Saudi Arabia. The oral feeding of single imidacloprid sub-lethal doses (1.0 ng, 0.5 ng, or 0.1 ng) under laboratory conditions significantly impaired associative learning during the 2nd and 3rd conditioning trials compared to control bees (0 ng). The memory tests also revealed significant impairment in memory formation at 1 h, 2 h, and 24 h after conditioning compared to control bees. Even the lowest dose (0.1 ng/bee) can significantly impair the bees' ability to learn and memorize. This impairment effect was dose dependent and increased with increasing doses. The higher dose (1.0 ng) completely impaired the learning but still showed a little memory and reflected the potential recovery of bees from insecticide-induced impairment with the passage of time. To our knowledge, this is the first study in *A. m. jemenitica* that demonstrated the drastic effect of neonicotinoids on associative learning in indigenous bees. This study further expresses the possible severity of insecticidal exposure to bees in actual field conditions and its effect on the neural functions used in important behavior involved in the foraging of bees.

## Introduction

Insecticides are extensively used in agriculture to control insect pests of different crops. However, they also confer toxic effects to humans, the environment, and other non-target beneficial insects and arthropods (Desneux *et al* 2007, Aktar *et al* 2009, Nicolopoulou-Stamati *et al* 2016). The decline in pollinators is a potential threat for global and food security and may result in the destabilization of ecosystems through food shortage and hunger (van der Sluijs & Vaage 2016).

Honey bees are major pollinators in agriculture. However, their population faces a seasonal loss in some parts of the world due to many factors, including insecticides (Potts *et al*

2010, Krupke *et al* 2012, Kiljanek *et al* 2016, Sanchez-Bayo & Goka 2014). The mite *Varroa destructor* Anderson & Trueman is another economic honey bee pest that is accused for most of the global colony losses (Rosenkranz *et al* 2010, Jacques *et al* 2017, Genersch *et al* 2010). Insecticidal residues were found in the nectar and pollen of several crops consumed by bees (Goulson 2013). During foraging, the bees can be exposed to contaminated nectar and pollen that are later transported to the hive and shared within the colony and among young brood (Rortais *et al* 2005). This situation may lead to the production of contaminated honey that has traces of insecticides (Sanchez-Bayo & Goka 2014). Insecticides can negatively influence the foraging activity of

bees, which corresponds to less of the pollination that is vital for the production of many crops (Desneux *et al* 2007, Ndakidemi *et al* 2016).

In certain field studies, realistic exposure levels to insecticides do not harm the bees at the colony level. But, when investigated at individual level, insecticide-induced effect can be observed (Blacquiere *et al* 2012, Fischer *et al* 2014, Lundin *et al* 2015, Retschnig *et al* 2015, Odemer *et al* 2018, Siede *et al* 2018, Rolke *et al* 2016). Social resilience or buffering obviously plays a crucial role at colony level which is not possible at individual level and these mechanisms are not yet fully understood (Manjon *et al* 2018, Straub *et al* 2015).

Imidacloprid is one of the most widespread neonicotinoid insecticides in the world; it affects the cholinergic pathways, disrupting the insect nervous system and acting as a neurotoxin (Jeschke *et al* 2011, Blacquiere *et al* 2012, Wood & Goulson 2017). It is a systemic insecticide that is readily absorbed in plant tissues, nectar, and pollen (Codling *et al* 2016, Bonmatin *et al* 2015). Honey bees are vulnerable to imidacloprid at its lethal and sub-lethal doses, with broad range of detrimental effects on the performances of honey bees under laboratory conditions (Kiljanek *et al* 2016, Pisa *et al* 2017, Laurino *et al* 2013).

Honeybees have unique cognitive abilities to associate odor, color, and floral pattern with food source during foraging, and this olfactory behavior can be assessed by following Pavlovian conditioning techniques, using the proboscis extension response (PER) (Menzel & Müller 1996, Giurfa & Sandoz 2012, Müller 2013). *Apis mellifera jemenitica* Ruttner is a widespread and successful indigenous bee race in the hot climate of Saudi Arabia, where the summer temperature often exceeds 45°C with low humidity (Alqarni *et al* 2011, Ali *et al* 2017). These bees are well known for high heat and water loss tolerance, and approximately 70–80% of the bee colonies that are used for domestic honey production in Saudi Arabia belong to *A. m. jemenitica* (Alqarni *et al* 2014).

Many biotic and abiotic stressors, including insecticides, can potentially affect the behavioral performances of bees during foraging (Kiljanek *et al* 2016, Abou-Shaara *et al* 2017). Studies with a diversified experimental setup and parameters have provided evidence that imidacloprid and other insecticides induce changes in the olfactory behavior of different honey bee species (Frost *et al* 2013, Gonalons & Farina 2015, Tan *et al* 2015). However, to our knowledge, no one has yet checked the effects of a widely used neonicotinoid insecticide (imidacloprid) on the olfactory behavior of the indigenous bee race *A. m. jemenitica*. The Arabian bees are known for their better adaptation in hot-dry climate of Saudi Arabia. The use of traditional insecticides in the field poses a threat to these bees that may lead to their alteration in physiology and behavior. Therefore, the present study was planned on a hypothesis for investigating the effects of oral feeding of field-realistic sub-lethal doses of imidacloprid on the

associative learning and memory of *A. m. jemenitica* exclusively under laboratory conditions in an arid climatic zone of central Saudi Arabia.

## Materials and Methods

### Handling of bees

The colonies of the indigenous bee race *A. m. jemenitica* (AMJ) were raised from queens of native origin (Balhareth *et al* 2012) and maintained at an educational farm (24.73° N, 46.61° E) at King Saud University, Riyadh, Saudi Arabia. A morphometric characterization confirms the existence of bee races in Saudi Arabia (Alattal *et al* 2014, Ruttner 1976). These colonies were free from any diseases and not treated with any pesticides shortly before the start of the experiment.

Adult foragers of AMJ were caught from the entrance of three bee hives, immobilized on ice, and harnessed in small plastic tubes for behavioral analysis (Iqbal & Mueller 2007, Smith & Burden 2014) during April 2017. The harnessed bees were left for 30 min in a darker moist plastic container at controlled room temperature ( $25 \pm 2.0^\circ\text{C}$ ) and relative humidity ( $40 \pm 10\%$ ). The antennae of each bee were tested with 0 M water solution, and any bee that responded to the water was discarded.

### Insecticide and test concentrations

Formulated chloronicotinyl insecticide (Confidor 350SC, Bayer®) containing 350 g imidacloprid (active ingredient/liter) was obtained from a local agricultural chemical store. The bottle of insecticide was well shaken and distilled water was used to formulate the stock solutions. These stock solutions were further diluted in sucrose solution (0.5 M) or distilled water to formulate the applicable doses of insecticide. Imidacloprid was orally administered because the oral feeding is the most likely exposure route for bees during foraging. Each bee in the respective groups was orally fed 2 µl of imidacloprid-mixed sucrose solutions (0.5 M) as a single dose containing one of the following imidacloprid doses: 1 ng/bee (high dose), 0.5 ng/bee (medium dose), and 0.1 ng/bee (low dose). In addition, the control bees in each respective group received only 2 µl of 0.5 M sucrose solution without imidacloprid (0 ng). All doses were administered with a micro pipette in parallel experiments 1 h before the learning trials. The insecticide doses were chosen from the range used in previous studies (Gonalons & Farina 2015, Tan *et al* 2015) and from the residues of imidacloprid found in the nectar and pollen of different crops that any forager could encounter during foraging (Johnson *et al* 2010, Goulson 2013, Tan *et al* 2014).

### Behavioral tests

The olfactory proboscis extension response (PER) conditioning was started 1 h after the oral administration of imidacloprid. The initial motivation of the bees was performed 10 min prior to the actual learning trials with 0.5 M sucrose solution by touching the antenna without feeding. The bees were transferred to the experimental site and allowed to face stimulus-free air for 15 s to ensure that bees were not responding to any mechanosensory stimulation (Frost *et al* 2013). The odor stimulus was prepared by applying 10 µl clove oil on filter paper in a 20 ml plastic syringe (Smith & Burden 2014). The training consisted of three successive conditioning trials with 2-min inter-trial intervals by presenting the odor stimulus paired with a sucrose (1 M) reward that represents strong conditioning to elicit all the properties of long-term memory (Müller 2002, Iqbal & Mueller 2007, Merschbaeche *et al* 2012, Müller 2013, Michely *et al* 2017, Iqbal *et al* 2018). Only the odor stimulus was used to perform the memory retention test at 1 h, 2 h, and 24 h after conditioning. A conditioned PER was recorded if the bee fully extended its proboscis during the first 3 seconds of odor stimulus. All harnessed bees were fed 0.5 M sucrose solution (without imidacloprid) until satiation to maintain their dietary requirement and survival prior to overnight incubation in a moist plastic container for the 24-h memory test the following day. The PER percentage was later used as a monitor to exhibit the level of learning and memory formation in bees.

### Data analysis

The behavioral data on the percentage PER between groups were compared with chi-square tests at  $p < 0.05$  using Statistix 8.1 software. A significant difference in the PER reflects the effect of the insecticide on learning and memory retention at different time periods in the indigenous bee race.

### Results

Indigenous bees (*A. m. jemenitica*) were submitted to olfactory conditioning 1 h after oral feeding of single doses of imidacloprid. The olfactory learning was significantly impaired in the imidacloprid-fed bees (1 ng, 0.5 ng, or 0.1 ng) with reduced proboscis extension response (PER) during the 2nd and 3rd conditioning trials compared to control bees (0 ng) (Fig 1). The higher dose of imidacloprid (1 ng) completely impaired the learning followed by a dose-dependent learning response with 0.5 ng, 0.1 ng, and 0 ng in both conditioning trials (Fig 1a, b). Thus, the increasing insecticide dose corresponds to a decrease in the PER of *A. m. jemenitica*. No bees elicited a learning response in the 1st conditioning

trial (Fig 1a), and this ruled out any pre-sensitivity of the bees towards the conditioning odor used in the experiment. The PER of imidacloprid-fed bees was significantly different from control bees in the 2nd conditioning trial (0.5 ng:  $\chi^2 = 29.0057$ ,  $p < 0.00001$ ; 0.1 ng:  $\chi^2 = 8.4349$ ,  $p = 0.00368$ ) and 3rd conditioning trial (0.5 ng:  $\chi^2 = 37.5281$ ,  $p < 0.00001$ ; 0.1 ng:  $\chi^2 = 12.0792$ ,  $p = 0.00051$ ).

Likewise, the memory test also revealed impairment in memory retention in imidacloprid-fed (1 ng, 0.5 ng, or 0.1 ng) bees. The imidacloprid-fed bees showed significantly reduced PER at 1 h, 2 h, and 24 h compared to control bees (0 ng) (Fig 2a–c). The memory response was also dependent on the insecticide dose, and an increase in dose corresponded to decrease in memory retention. Interestingly, the bees fed a high imidacloprid dose (1 ng) showed zero learning response (Fig 1) but still retained some memory at the 1-, 2-, and 24-h memory tests (Fig 2). The PER of imidacloprid-fed bees during memory tests was significantly different from control bees: 1-h memory test (1 ng:  $\chi^2 = 52.6721$ ,  $p < 0.00001$ ; 0.5 ng:  $\chi^2 = 17.8121$ ,  $p < 0.000024$ ; 0.1 ng:  $\chi^2 = 5.3908$ ,  $p = 0.020243$ ), 2-h memory test (1 ng:  $\chi^2 = 52.0965$ ,  $p < 0.00001$ ; 0.5 ng:  $\chi^2 = 34.4623$ ,  $p < 0.00001$ ; 0.1 ng:  $\chi^2 = 15.492$ ,  $p = 0.00083$ ), and 24-h memory test (1 ng:  $\chi^2 = 38.097$ ,  $p < 0.00001$ ; 0.5 ng:  $\chi^2 = 21.054$ ,  $p < 0.00001$ ; 0.1 ng:  $\chi^2 = 5.0225$ ,  $p = 0.02502$ ).

### Discussion

The present laboratory study evaluated the effect of a commonly used neonicotinoid (imidacloprid) on the olfactory behavior of the indigenous bee race *A. m. jemenitica* in the harsh arid climatic conditions of central Saudi Arabia. We used oral feeding because it is the most likely route of poisoning during the foraging of bees on nectar and pollen. Using a classical conditioning assay, this study established that *A. m. jemenitica* also possesses associative olfactory learning and memory formation as reported in other bee species (Iqbal & Mueller 2007, Giurfa & Sandoz 2012, Wang & Tan 2014, Iqbal *et al* 2018). The indigenous bees are well known for foraging and honey production in Saudi Arabia (Ali 2011, Alqarni *et al* 2011, Abou-Shaara 2014, Alqarni *et al* 2014).

The data revealed an impairment in the learning and memory retention of *A. m. jemenitica* after oral feeding of single sub-lethal doses of imidacloprid (1, 0.5, or 0.1 ng/bee). These results are compatible with the findings of Gonalons & Farina (2015), where 0.25 ng and 0.5 ng orally fed imidacloprid produced impairment in the acquisition and retention in *Apis mellifera* L. However, they also compared the relationship of bee age and feeding method of imidacloprid, which was beyond the scope of our study. In *Apis cerana* F., sub-lethal doses of imidacloprid can alter the cognition,

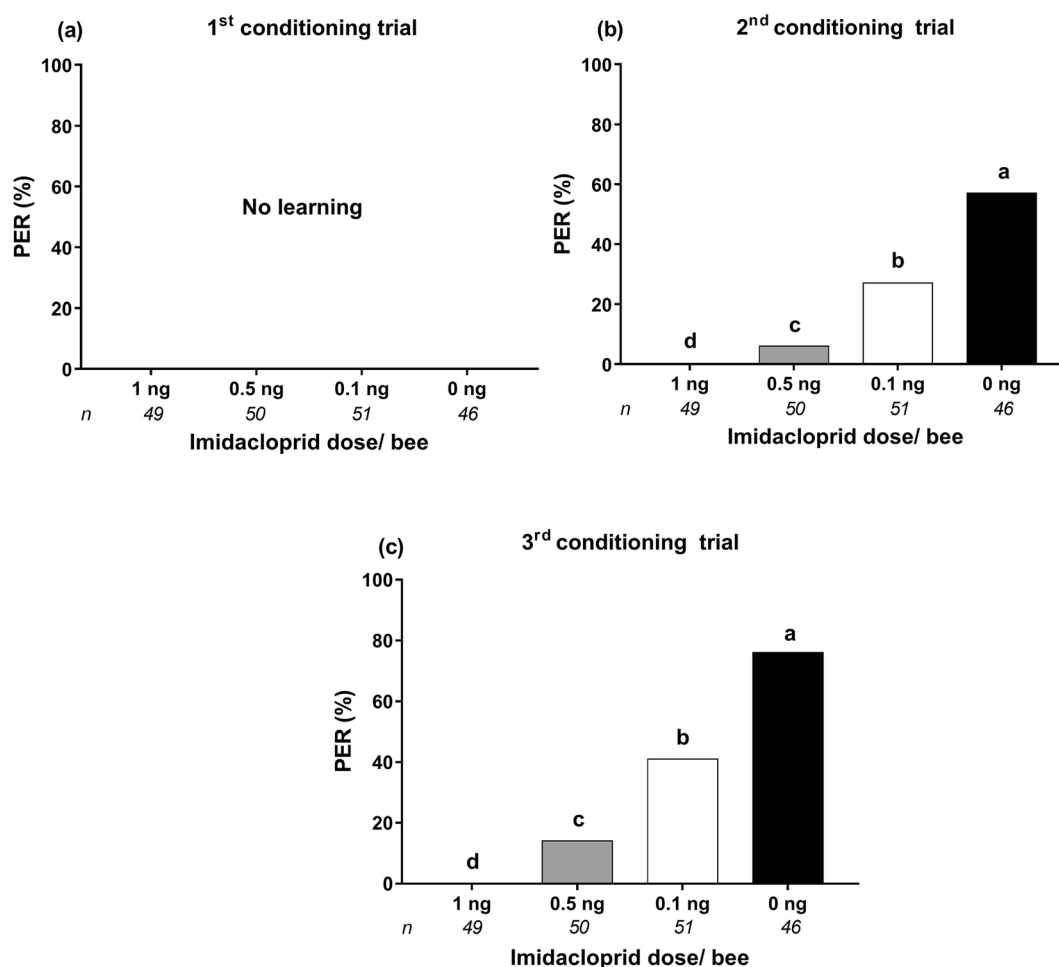


Fig 1 Effect of oral feeding of imidacloprid on learning of *Apis mellifera jemenitica* Ruttner. (a) 1<sup>st</sup> conditioning trial, (b) 2<sup>nd</sup> conditioning trial, and (c) 3<sup>rd</sup> conditioning trial. The percentage of proboscis extension response (PER) showed a significant impairment in the learning of adult foragers of *A. m. jemenitica*. Sample size (*n*) is given below the x-axis. Different letters stand for significant differences between imidacloprid-treated groups of bees ( $\chi^2$  test; \* $p < 0.05$ ).

foraging, and decision-making in danger avoidance (Tan et al 2014, 2015). A similar study was performed by Decourtye et al (2003), but with higher doses of imidacloprid in cage honey bees. It is reported that the adjuvants present in the formulated insecticides could also be a reason for the impairment of learning ability in *A. mellifera* (Ciarlo et al 2012). The future comparative studies may focus in parallel for the potential impact of pure active ingredient and adjuvants on olfactory behavior of *A. m. jemenitica*.

The impairment in the cognitive behavior of *A. m. jemenitica* was directly dependent on the imidacloprid dose: an increase in dose corresponds to a reduction in learning and memory, which is in line with previous findings for imidacloprid dose-related behavioral changes in *A. mellifera* L. and *A. cerana* F. (Gonalons & Farina 2015, Tan et al 2015). A number of studies have identified a severe impact of imidacloprid on the olfactory behavior of other bee races in diverse experimental setups (Decourtye et al 2004, Williamson & Wright 2013, Yang et al 2008). However, we

have shown for the first time that the indigenous bees of Saudi Arabia, *A. m. jemenitica*, also exhibit associative olfactory learning that is vulnerable to imidacloprid.

The oral toxicity of imidacloprid for learning and memory reflects the potential threat to bees from insecticidal residues present in nectar and pollen in a variety of crops consumed by bees (Desneux et al 2007). Thus, the bees exposed to agricultural environments are vulnerable to insecticides, and the plausible route is via pollen and nectar. In addition, the bees can also receive imidacloprid via multiple routes near agricultural fields (Krupke et al 2012) as it is systemic in plants and persistent in the environment (Bonmatin et al 2015). Imidacloprid residues are present in a wide range of locations, such as bee hive pollen (912 ppb) (Johnson et al 2010), and the nectar (1–50 ppb), pollen (Goulson 2013, Tan et al 2014), and nectar load (0.024–0.3 ng per nectar load) (Cresswell 2011) of different crops. LD<sub>50</sub> values of imidacloprid are ranging from 3.7 to 81 ng/bee (Nauen et al 2001, Schmuck et al 2001, Sanchez-Bayo & Goka 2016). Our

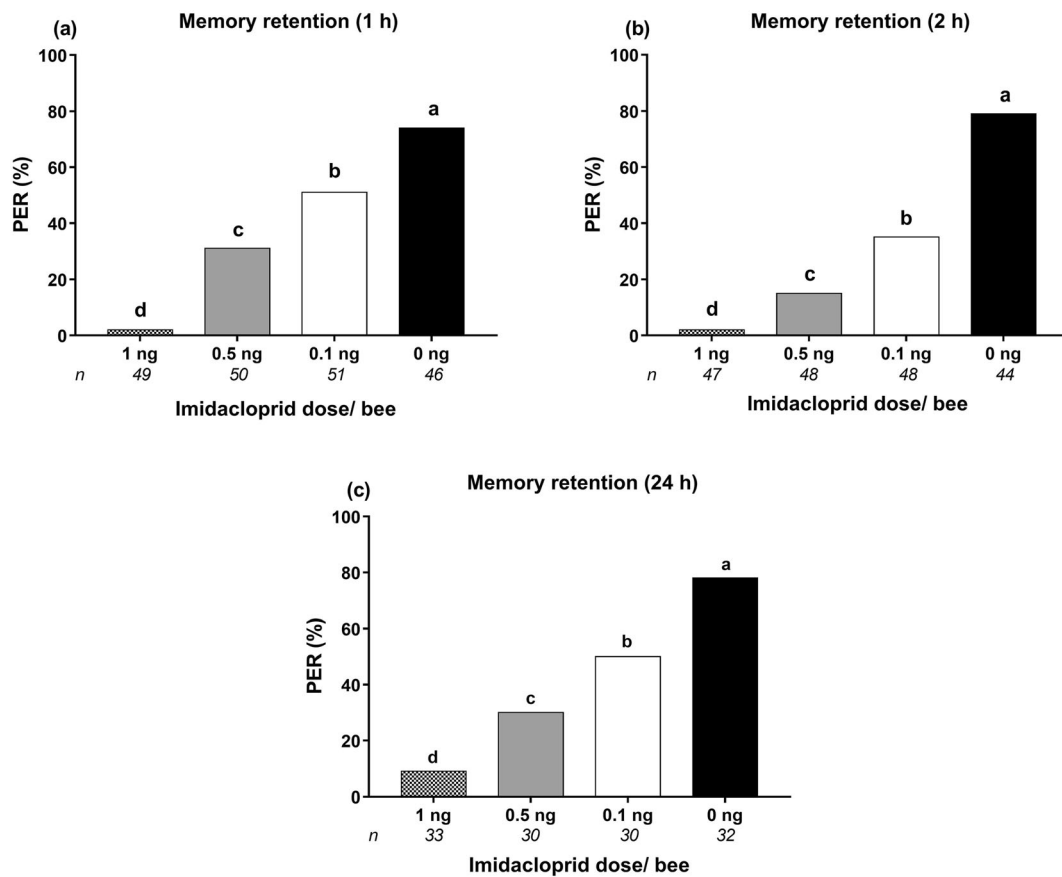


Fig 2 Effect of oral feeding of imidacloprid on memory retention of *Apis mellifera jemenitica* Ruttner (a) at 1 h, (b) at 2 h, and (c) at 24 h after conditioning. The percentage of proboscis extension response (PER) showed a significant impairment in memory retention of adult foragers of *A. m. jemenitica*. Sample size ( $n$ ) is given below the x-axis. Different letters stand for significant differences between imidacloprid-treated groups of bees ( $\chi^2$  test;  $*p < 0.05$ ).

tested doses fall in the sub-lethal range, with zero mortality of foragers during the course of experiments.

Imidacloprid is an agonist of insect nicotinic acetylcholine receptors (nAChRs) and downregulate the nAChRs in bee brain (Gauthier 2010, Blacquiere *et al* 2012, Wood & Goulson 2017, Zhou *et al* 2013). The possible explanation for behavioral changes in *A. m. jemenitica* could be due to the effect of sub-lethal doses of imidacloprid in regions of the bee brain responsible for olfactory and visual function, which leads to a reduction in the olfactory learning ability (Peng & Yang 2016). Other insecticides such as deltamethrin, malathion, and abamectin can disrupt the midgut epithelium and longevity of *A. m. jemenitica* foragers (Aljedani & Almeahmadi 2016, Aljedani 2017). Imidacloprid might have a similar effect and need more comprehensive testing for potential physiological changes in indigenous bees. It can reduce the genetic diversity among worker bees, mating frequency of queens (Forfert *et al* 2017), and its chronic sub-lethal exposure at the higher range of field doses (20 to 100  $\mu\text{g}/\text{kg}$ ) can negatively impact colony health and overwintering success (Dively *et al* 2015).

In the present study, the imidacloprid dose of 1 ng resulted in zero learning but interestingly still some memory recall. This result means that bees can recover from the impairment effects of sub-lethal doses with the passage of time. This result also indicates that sub-lethal doses did not completely impair the brain function, and learning information was transferred to the brain that was later recalled during memory tests. Manjon *et al* (2018) identified an enzyme (a single cytochrome P450s) which is the key determinants responsible for detoxifying the neonicotinoids insecticides in the bees. This revealed a self-biochemical defense system that define the sensitivity of bees towards insecticides and can be helpful for their survival against environmental stressors. It is worth to mention that within the last decade, a substantial difference in bee responses towards pesticide exposure is noticed when tested in field and laboratory experiments. The possible reason would be that bee colonies are able to buffer many environmental effects such as pesticide exposure within their social entity whereas individual bees may suffer damage (Straub *et al* 2015, Sponsler & Johnson 2017).



Our study also reveals that a low single dose of imidacloprid (0.1 ng) is enough to interrupt the olfactory behavior of *A. m. jemenitica*. Although the bees can continue their foraging, their foraging time, flight distance, longevity, and number of floral visitations may be affected with increases in the imidacloprid dose. The toxicity of imidacloprid towards the cognitive behavior of native bees will help to limit the exposure of insecticides in Saudi Arabia for the better management of apiculture to avoid a decline in bee populations and honey production. In conclusion, the learning and memory formation of indigenous Arabian honeybees, *A. m. jemenitica* is vulnerable to neonicotinoid insecticide. The sub-lethal doses of imidacloprid can drastically impair associative learning and memory formation. These laboratory-based findings pointed towards the potential threat of insecticides towards Arabian honey bee's foraging behavior in the actual field conditions that need to be explored in a larger scale.

**Acknowledgement** This work was supported by the Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia, project number RGP-189.

**Author Contributions** JI designed, executed, analyzed the data and wrote the initial manuscript, ASA reviewed the results, figures, and the final manuscript, and HSAR helped in the execution and data collection of the experiments.

## References

- Abou-Shaara HF (2014) The foraging behaviour of honey bees, *Apis mellifera*: a review. Vet Med-Czech 59(1):1–10. <https://doi.org/10.17221/7240-VETMED>
- Abou-Shaara HF, Owayss AA, Ibrahim YY, Basuny NK (2017) A review of impacts of temperature and relative humidity on various activities of honey bees. Insect Soc 64(4):455–463. <https://doi.org/10.1007/s00040-017-0573-8>
- Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdiscip Toxicol 2(1):1–12. <https://doi.org/10.2478/v10102-009-0001-7>
- Alattal Y, Al Ghamdi A, Al Sharhi M, Fuchs S (2014) Morphometric characterisation of the native honeybee, *Apis mellifera* Linnaeus, 1758, of Saudi Arabia. Zool Middle East 60(3):226–235. <https://doi.org/10.1080/09397140.2014.944431>
- Ali MAM (2011) Comparative study for evaluating two honey bee races, *Apis mellifera jemenitica* (indigenous race) and *Apis mellifera carnica* (carniolan race) in brood production, population development and foraging activity under the environmental conditions of the central region of the Kingdom of Saudi Arabia. Ann Agric Sci 56(2):127–134. <https://doi.org/10.1016/j.aoas.2011.07.006>
- Ali H, Alqarni AS, Owayss AA, Hassan AM, Smith BH (2017) Osmotic concentration in three races of honey bee, *Apis mellifera* L. under environmental conditions of arid zone. Saudi J Biol Sci 24(5):1081–1085. <https://doi.org/10.1016/j.sjbs.2016.12.006>
- Aljedani DM (2017) Effects of abamectin and deltamethrin to the foragers honeybee workers of *Apis mellifera jemenitica* (Hymenoptera: Apidae) under laboratory conditions. Saudi J Biol Sci 24(5):1007–1015. <https://doi.org/10.1016/j.sjbs.2016.12.007>
- Aljedani DM, Almeahmadi RM (2016) Effects of some insecticides on longevity of the foragers honey bee worker of local honey bee race *Apis mellifera jemenitica*. Electronic Physician 8(1):1843–1849. <https://doi.org/10.19082/1843b>
- Alqarni AS, Hannan MA, Owayss AA, Engel MS (2011) The indigenous honey bees of Saudi Arabia (Hymenoptera, Apidae, *Apis mellifera jemenitica* Ruttner): their natural history and role in beekeeping. Zookeys 134(134):83–98. <https://doi.org/10.3897/zookeys.134.1677>
- Alqarni AS, Balhareth HM, Owayss AA (2014) Performance evaluation of indigenous and exotic honey bee (*Apis mellifera* L.) races in Assir region, southwestern Saudi Arabia. Saudi J Biol Sci 21(3):256–264. <https://doi.org/10.1016/j.sjbs.2013.10.007>
- Blacquiere T, Smagghe G, van Gestel CA, Mommaerts V (2012) Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21(4):973–992. <https://doi.org/10.1007/s10646-012-0863-x>
- Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EA, Noome DA, Simon-Delso N, Tapparo A (2015) Environmental fate and exposure; neonicotinoids and fipronil. Environ Sci Pollut Res Int 22(1):35–67. <https://doi.org/10.1007/s11356-014-3332-7>
- Ciarlo TJ, Mullin CA, Frazier JL, Schmehl DR (2012) Learning impairment in honey bees caused by agricultural spray adjuvants. PLoS One 7(7):e40848. <https://doi.org/10.1371/journal.pone.0040848>
- Codling G, Al Naggar Y, Giesy JP, Robertson AJ (2016) Concentrations of neonicotinoid insecticides in honey, pollen and honey bees (*Apis mellifera* L.) in central Saskatchewan, Canada. Chemosphere 144:2321–2328. <https://doi.org/10.1016/j.chemosphere.2015.10.135>
- Cresswell JE (2011) A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology 20(1):149–157. <https://doi.org/10.1007/s10646-010-0566-0>
- Decourtye A, Lacassie E, Pham-Delegue MH (2003) Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. Pest Manag Sci 59(3):269–278. <https://doi.org/10.1002/ps.631>
- Decourtye A, Devillers J, Cluzeau S, Charreton M, Pham-Delegue MH (2004) Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol Environ Saf 57(3):410–419. <https://doi.org/10.1016/j.ecoenv.2003.08.001>
- Desneux N, Decourtye A, Delpuech JM (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106. <https://doi.org/10.1146/annurev.ento.52.110405.091440>
- Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS (2015) Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PLoS One 10(3):e0118748. <https://doi.org/10.1371/journal.pone.0118748>
- Fischer J, Müller T, Spatz A-K, Greggers U, Grünewald B, Menzel R (2014) Neonicotinoids interfere with specific components of navigation in honeybees. PLoS One 9(3):e91364. <https://doi.org/10.1371/journal.pone.0091364>
- Forfert N, Troxler A, Retschnig G, Gauthier L, Straub L, Moritz RFA, Neumann P, Williams GR (2017) Neonicotinoid pesticides can reduce honeybee colony genetic diversity. PLoS One 12(10):e0186109. <https://doi.org/10.1371/journal.pone.0186109>
- Frost EH, Shutler D, Hillier NK (2013) Effects of fluralinate on honey bee learning, memory, responsiveness to sucrose, and survival. J Exp Biol 216(15):2931–2938. <https://doi.org/10.1242/jeb.086538>
- Gauthier M (2010) State of the art on insect nicotinic acetylcholine receptor function in learning and memory. In: Thany SH (ed) Insect nicotinic acetylcholine receptors. Springer, New York, pp 97–115. [https://doi.org/10.1007/978-1-4419-6445-8\\_9](https://doi.org/10.1007/978-1-4419-6445-8_9)
- Genersch E, von der Ohe W, Kaatz H, Schroeder A, Otten C, Böhler R, Berg S, Ritter W, Mühlen W, Gisder S, Meixner M, Liebig G,

- Rosenkranz P (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* 41(3):332–352. <https://doi.org/10.1051/apido/2010014>
- Giurfa M, Sandoz JC (2012) Invertebrate learning and memory: fifty years of olfactory conditioning of the proboscis extension response in honeybees. *Learn Memory* 19(2):54–66. <https://doi.org/10.1101/lm.024711.111>
- Gonalons C, Farina WM (2015) Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. *PLoS One* 10(10):e0140814. <https://doi.org/10.1371/journal.pone.0140814>
- Goulson D (2013) Review: an overview of the environmental risks posed by neonicotinoid insecticides. *J Appl Ecol* 50(4):977–987. <https://doi.org/10.1111/1365-2664.12111>
- Iqbal J, Mueller U (2007) Virus infection causes specific learning deficits in honeybee foragers. *Proc R Soc Lond B Biol Sci* 274(1617):1517–1521. <https://doi.org/10.1098/rspb.2007.0022>
- Iqbal J, Ali H, Owayss AA, Raweh HSA, Engel MS, Alqarni AS, Smith BH (2018) Olfactory associative behavioral differences in three honey bee *Apis mellifera* L. races under the arid zone ecosystem of central Saudi Arabia. *Saudi J Biol Sci*. <https://doi.org/10.1016/j.sjbs.2018.08.002>
- Jacques A, Laurent M, Ribiere-Chabert M, Saussac M, Bougeard S, Budge GE, Hendrikx P, Chauzat MP, De Graaf D, Meroc E, Nguyen BK, Roelandt S, Roels S, Van der Stede Y, Tonnersen T, Kryger P, Jaarma K, Kuus M, Raie A, Heinikainen S, Pelkonen S, Vahanikilla N, Andrieux C, Ballis A, Barrieu G, Bendali F, Brugoux C, Franco S, Fuentes AM, Joel A, Layec Y, Lopez J, Lozach A, Malherbe DL, Mariau V, Meziani F, Monod D, Mutel S, Oesterle E, Orlowski M, Petit M, Pillu P, Poret F, Viry A, Berg S, Buchler R, de Craigher D, Genersch E, Kaatz HH, Meixner MD, von der Ohe W, Otten C, Rosenkranz P, Schaefer MO, Schroeder A, Agianiotaki E, Arfara S, Boutsini S, Giannouloupoulou M, Hondrou V, Karipidou S, Katsaros D, Katzagianakis A, Kiriakopoulos A, Oureilidis K, Panteli A, Pantoleon F, Papagianni Z, Papalexou E, Perdikaris S, Prapas A, Siana P, Skandalakis I, Stogiou D, Tomazinakis I, Tsali E, Tseliou E, Tsiplakidis A, Tsompanellis E, Vamvakas G, Varvarouta V, Vourvidis D, Dan A, Daroczi G, Lang M, Papp M, Paulus PD, Pupp E, Szalo M, Toth A, Zseli S, Bressan G, Cerrone A, Formato G, Granato A, Lavazza A, Macellari P, Marcello P, Ghittino C, Maroni PA, Possidente R, Mutinelli F, Nassuato C, Pintore A, Ricchiuti L, Ruocco L, Salvaggio A, Troiano P, Voltini B, Avsejenko J, Ciekure E, Deksnė G, Eglite I, Granta R, Olsevski E, Rodze I, Stinka M, Sirutkaitis R, Siriukaitis S, Bobber A, Jazdzewski K, Pohorecka K, Skubida M, Zdanska D, Amador MRR, Freitas S, Quintans S, Santos PT, Brezinova N, Brtkova A, Cuvalova Z, Filipova M, Jurovcikova J, Kantikova M, Kubicova Z, Papiernikova E, Sulejova L, Toporcak J, Ares CCM, Ariza J, Berna SN, Cabeza NA, Casasempere CJ, Cid GC, Corzan RJM, De Abajo DMA, Diaz RR, Esteban RA, Fernandez SP, Garcia PA, Gonzalez BC, Minguez GO, Onate ML, Oteiza OP, Perez CI, Plaza PM, Puy P, Riol GR, Romero GLJ, Soldevilla YJF, Barrasus MSI, Soriano GM, Vigo LV, Villarta RJL, Fabricius-Kristiansen L, Forsgren E, Brown M, Budge G, Grant R, Marris G, Powell M, Wattam A, Whiting I, Cauquil L, Riviere MP, Garin E, Consortium E (2017) A pan-European epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. *PLoS One* 12(3):17. <https://doi.org/10.1371/journal.pone.0172591>
- Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* 59(7):2897–2908. <https://doi.org/10.1021/jf101303g>
- Johnson RM, Ellis MD, Mullin CA, Frazier M (2010) Pesticides and honey bee toxicity - USA. *Apidologie* 41(3):312–331. <https://doi.org/10.1051/apido/2010018>
- Kiljanek T, Niewiadowska A, Posylniak A (2016) Pesticide poisoning of honeybees: a review of symptoms, incident classification, and causes of poisoning. *J Apic Sci* 60(2):5–24. <https://doi.org/10.1515/jas-2016-0024>
- Krupke CH, Hunt GJ, Eitzer BD, Andino G, Given K (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One* 7(1):e29268. <https://doi.org/10.1371/journal.pone.0029268>
- Laurino D, Manino A, Patetta A, Porporato M (2013) Toxicity of neonicotinoid insecticides on different honey bee genotypes. *B Insectol* 66(1):119–126
- Lundin O, Rundlof M, Smith HG, Fries I, Bommarco R (2015) Neonicotinoid insecticides and their impacts on bees: a systematic review of research approaches and identification of knowledge gaps. *PLoS One* 10(8):e0136928. <https://doi.org/10.1371/journal.pone.0136928>
- Manjon C, Troczka BJ, Zaworra M, Beadle K, Randall E, Hertlein G, Singh KS, Zimmer CT, Homem RA, Lueke B, Reid R, Kor L, Kohler M, Benting J, Williamson MS, Davies TGE, Field LM, Bass C, Nauen R (2018) Unravelling the molecular determinants of bee sensitivity to neonicotinoid insecticides. *Curr Biol* 28(7):1137–1143.e1135. <https://doi.org/10.1016/j.cub.2018.02.045>
- Menzel R, Müller U (1996) Learning and memory in honeybees: from behavior to neural substrates. *Annu Rev Neurosci* 19:379–404. <https://doi.org/10.1146/annurev.ne.19.030196.002115>
- Merschbaecher K, Haettig J, Mueller U (2012) Acetylation-mediated suppression of transcription-independent memory: bidirectional modulation of memory by acetylation. *PLoS One* 7(9):e45131. <https://doi.org/10.1371/journal.pone.0045131>
- Michely J, Kraft S, Muller U (2017) miR-12 and miR-124 contribute to defined early phases of long-lasting and transient memory. *Sci Rep* 7:7910. <https://doi.org/10.1038/s41598-017-08486-w>
- Müller U (2002) Learning in honeybees: from molecules to behaviour. *Zoology (Jena, Germany)* 105(4):313–320. <https://doi.org/10.1078/0944-2006-00075>
- Müller U (2013) Memory phases and signaling cascades in honeybees. In: Menzel R, Benjamin PR (eds) *Handbook of behavioral neuroscience*, vol 22. Elsevier press, Philadelphia, pp 433–441. <https://doi.org/10.1016/B978-0-12-415823-8.00031-9>
- Nauen R, Ebbinghaus-Kintscher U, Schmuck R (2001) Toxicity and nicotinic acetylcholine receptor interaction of imidacloprid and its metabolites in *Apis mellifera* (Hymenoptera: Apidae). *Pest Manag Sci* 57(7):577–586. <https://doi.org/10.1002/ps.331>
- Ndakidemi B, Mtei K, Ndakidemi PA (2016) Impacts of synthetic and botanical pesticides on beneficial insects. *Agric Sci* 7(6):364–372
- Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L (2016) Chemical pesticides and human health: the urgent need for a new concept in agriculture. *Front Public Health* 4:148. <https://doi.org/10.3389/fpubh.2016.00148>
- Odemer R, Nilles L, Linder N, Rosenkranz P (2018) Sublethal effects of clothianidin and Nosema spp. on the longevity and foraging activity of free flying honey bees. *Ecotoxicology* 27(5):527–538. <https://doi.org/10.1007/s10646-018-1925-5>
- Peng YC, Yang EC (2016) Sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies. *Sci Rep* 6:192–198. <https://doi.org/10.1038/srep19298>
- Pisa L, Goulson D, Yang E-C, Gibbons D, Sánchez-Bayo F, Mitchell E, Aebi A, van der Sluijs J, MacQuarrie CJK, Giorio C, Long EY, McField M, Bijleveld van Lexmond M, Bonmatin J-M (2017) An update of the worldwide integrated assessment (WIA) on systemic insecticides. Part 2: impacts on organisms and ecosystems. *Environ Sci Pollut Res*:1–49. <https://doi.org/10.1007/s11356-017-0341-3>
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25(6):345–353. <https://doi.org/10.1016/j.tree.2010.01.007>
- Retschnig G, Williams GR, Odemer R, Boltin J, Di Poto C, Mehmman MM, Retschnig P, Winiger P, Rosenkranz P, Neumann P (2015) Effects, but no interactions, of ubiquitous pesticide and parasite stressors on honey bee (*Apis mellifera*) lifespan and behaviour in a colony environment. *Environ Microbiol* 17(11):4322–4331. <https://doi.org/10.1111/1462-2920.12825>

- Rolke D, Fuchs S, Grunewald B, Gao Z, Blenau W (2016) Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in northern Germany: effects on honey bees (*Apis mellifera*). *Ecotoxicology* 25(9):1648–1665. <https://doi.org/10.1007/s10646-016-1725-8>
- Rortais A, Arnold G, Halm M-P, Touffet-Briens F (2005) Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie* 36(1):71–83
- Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. *J Invertebr Pathol* 103:S96–S119. <https://doi.org/10.1016/j.jip.2009.07.016>
- Ruttner F (1976) Les races d'abeilles de l'Afrique. Paper presented at the XXV Congrès International d'Apiculture, Grenoble, France
- Sanchez-Bayo F, Goka K (2014) Pesticide residues and bees - a risk assessment. *PLoS One* 9(4):e94482. <https://doi.org/10.1371/journal.pone.0094482>
- Sanchez-Bayo F, Goka K (2016) Impacts of pesticides on honey bees. In: Chambo DE (eds) *Beekeeping and bee conservation - advances in research*. InTech Publishers, pp 77–97. <https://doi.org/10.5772/62487>
- Schmuck R, Schoning R, Stork A, Schramel O (2001) Risk posed to honeybees (*Apis mellifera* L. Hymenoptera) by an imidacloprid seed dressing of sunflowers. *Pest Manag Sci* 57(3):225–238. <https://doi.org/10.1002/ps.270>
- Siede R, Meixner MD, Almanza MT, Schoning R, Maus C, Buchler R (2018) A long-term field study on the effects of dietary exposure of clothianidin to varroosis-weakened honey bee colonies. *Ecotoxicology* 27(7):772–783. <https://doi.org/10.1007/s10646-018-1937-1>
- Smith BH, Burden CM (2014) A proboscis extension response protocol for investigating behavioral plasticity in insects: application to basic, biomedical, and agricultural research. *Jove-J Vis Exp* 91:e51057. <https://doi.org/10.3791/51057>
- Sponsler DB, Johnson RM (2017) Mechanistic modeling of pesticide exposure: the missing keystone of honey bee toxicology. *Environ Toxicol Chem* 36(4):871–881. <https://doi.org/10.1002/etc.3661>
- Straub L, Williams GR, Pettis J, Fries I, Neumann P (2015) Superorganism resilience: eusociality and susceptibility of ecosystem service providing insects to stressors. *Curr Opin Insect Sci* 12:109–112. <https://doi.org/10.1016/j.cois.2015.10.010>
- Tan K, Chen WW, Dong SH, Liu XW, Wang YC, Nieh JC (2014) Imidacloprid alters foraging and decreases bee avoidance of predators. *PLoS One* 9(7):e102725. <https://doi.org/10.1371/journal.pone.0102725>
- Tan K, Chen WW, Dong SH, Liu XW, Wang YC, Nieh JC (2015) A neonicotinoid impairs olfactory learning in Asian honey bees (*Apis cerana*) exposed as larvae or as adults. *Sci Rep* 5:10989. <https://doi.org/10.1038/srep10989>
- van der Sluijs JP, Vaage NS (2016) Pollinators and global food security: the need for holistic global stewardship. *Food Ethics* 1(1):75–91. <https://doi.org/10.1007/s41055-016-0003-z>
- Wang ZW, Tan K (2014) Comparative analysis of olfactory learning of *Apis cerana* and *Apis mellifera*. *Apidologie* 45(1):45–52. <https://doi.org/10.1007/s13592-013-0228-3>
- Williamson SM, Wright GA (2013) Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. *J Exp Biol* 216(10):1799–1807
- Wood TJ, Goulson D (2017) The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. *Environ Sci Pollut Res Int* 24(21):17285–17325. <https://doi.org/10.1007/s11356-017-9240-x>
- Yang EC, Chuang YC, Chen YL, Chang LH (2008) Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). *J Econ Entomol* 101(6):1743–1748. <https://doi.org/10.1603/0022-0493-101.6.1743>
- Zhou T, Song H-L, Wang Q, Dai P-L, Wu Y-Y, Sun J-H (2013) Effects of imidacloprid on the distribution of nicotine acetylcholine receptors in the brain of adult honeybee (*Apis mellifera ligustica*). *Acta Entomol Sin* 56(11):1258–1266