Journal of Entomology

ISSN 1812-5670

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS

Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2020.84.92

Review Article
Behavioral Changes Due to Sub-lethal Doses of Pesticides in Bees

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Abstract

Pollination services provided by the different species of bee prove that they are indispensable to agriculture as well as natural ecosystems. However, the decline or collapse of the bee population is a global concern. Among different reasons, pesticides, especially insecticides, are responsible for bee decline or collapse. Not only exposure to lethal doses causes multiple negative impacts on the bee colony but the sub-lethal doses are also severely harmful to the bees. This paper presents the review of previously published studies on the effect of sub-lethal doses of different commonly used pesticides in bee's behavior.

Key words: Honeybee, behavior, insecticides, sub-lethal doses, natural ecosystems, pesticides

Citation: Rameshwor Pudasaini, 2020. Behavioral changes due to sub-lethal doses of pesticides in bees. J. Entomol., 17: 84-92.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bees are indispensable components for both natural as well as agricultural ecosystems[1,2]. About 75% of crops are animal pollinated[1,3] and bees significantly contribute to the pollination[4,5]. Bee pollination improves both the quality and quantity of crops production[6-8]. Similarly, insect-pollinated crops are a vital source of nutrition throughout the world[9] and among the insects, bees are important groups of pollinators[4].

However, the decline in the bee population is a huge concern rising all over the world[10-12]. Among the different reasons, the widespread use of pesticides is majorly responsible for the decline of bees[13-16]. Pesticides exposure to bees causes several negative impacts such as altered gut microbiota[17], susceptible to pest and diseases[18,19], brood development and function[20], behavioral changes[21-24] and bee colony health[25,15]. Pesticides also impair on worker performance as foraging efficiency and navigation ability[26-30]. Further, it is reported that pesticide residue in brood comb decreased adult life span, increased brood mortality and they became more susceptible to pathogens which resulted in reduced honeybee colony affecting their health[31]. These effects disturbed normal colony cycle[32] which led either in bee decline or colony collapse.

Honeybees are social insect and all behaviors are essential to run normally in a healthy colony. For example, foraging behavior to collect pollen, nectar, water and resin[33], hygienic behavior for disease and parasite resistance[34,35], communication to inform the location of food source to other foragers[36], reviewed by I'Anson *et al*.[37]. Similarly, in a normal healthy honeybee colony, nurse workers clean the cells, care the broods and support to store the food whereas middle-aged bee maintains the hive, receives and processes nectar. Besides, they guard the nest entrance and after 21 days go for forage[38-41]. Although there may not be immediate mortality after exposure to sub-lethal doses of pesticides, it does have chronic effects on bee health. Multiple review papers discussed the negative impact of pesticides on bees e.g.[38,42]. In addition to this recent review on the effects of sub-lethal dose of pesticides on bees is hardly found. Although, Thompson[43] and Desneux *et al*.[44] reviewed on effects of sub-lethal doses of pesticides after those multiple new findings have been explored. So, this is essential to keep in account that doing the review is the utmost need for the proper direction in further studies. Hence, this paper mainly reviewed previously published results and behavioral impairment due to different types of pesticides on bee species.

FORAGING BEHAVIOR

Foraging behavior is important to collect the nectar, pollen, water and propolis to the bees and to pollinate the crop[6,45,33]. Several cultivated crops and wild plants depend on the bee for effective and proper pollination so efficient bee foraging is essential factor[4,45,46]. However, several previous studies showed that sub-lethal doses of pesticides impair the foraging behavior of bee[24,47,48]. Nectar or pollen detection in bees is conducted by sophisticated nervous activity which was found disrupted by neurotoxic pesticides[49]. The sub-lethal dose of imidacloprid and clothianidin impaired the homing and foraging activities of worker honeybees as they failed or delayed to return in the hives or their feeding sites[23,24,47,48]. For example, imidacloprid treated orally with sugar water at the lowest concentration of 50 μg L^{-1} and higher than 1,200 μg L^{-1} resulted in delayed normal foraging interval and abnormalities in revisiting the feeding sites[24]. Similarly, another experiment reported that Cry1Ab protoxin, deltamethrin and imidacloprid has been affecting the foraging activity[50]. According to them, the mean number of foraging honeybees visited was 43.4, 30.7 and 23.7 before, during and after treatment by protoxin respectively. Likewise, in the case of the deltamethrin test, the mean numbers were 1.7, 0.7 and 1.0 before, during and after the treatment respectively. Finally, in imidacloprid mean visited numbers initially was 23.7, during the treatment 4.8 and 20.4 after the treatment.

Other activities in foraging behavior were also found to be affected by the sub-lethal dose of imidacloprid like less nectar or pollen collection[23], delay in revisiting for foraging[51]. Also, due to the reduction of olfactory capacity worker bees became incapable to locate the food source[49]. In the case of bumblebees, exposure to imidacloprid reduced foraging motivation. They visited the less robotic flowers, slowed down to commence for foraging and did not visit all three provided flower colors compared to the control[52].

Including these effects, precocious foraging was reported by multiple studies[53,54]. For example, imidacloprid treatment with a concentration of 5 μg kg^{-1}, ppb induced precocious foraging, fewer orientation flights and reduced lifetime foraging flights by 28%[52]. Due to sensitivity towards the stressors, less effective foraging and precocious foraging remained seriously responsible for the colony collapse in honeybee[25,55,56]. Similarly, the application of Diazinon also found inducing precocious foraging in honeybees[53].

COMMUNICATION

The application of pesticides also affected communication in honeybees. Treatment with imidacloprid was found to affect the waggle dance perform by foragers[57]. Similarly, it is reported that communication in honeybee was impaired which resulted in the decline of social behavior after 30-60 min with single-dose and *ad libitum* administration of 500 and 100 ppb imidacloprid respectively[58]. Likewise, contamination with 20 ppb imidacloprid decreased the frequency of waggle dances of forager bees[59]. Also, treatment with parathion with 0.3 µg/bee caused the incorrect direction (angle of dance) on a vertical surface and incorrect distance on a horizontal surface[60]. Forager bees must remember the location, direction and amount of the food source to inform the colony with dance[36]. But after the application of the sub-lethal dose of pesticide, the memory of the forager bees got to be impaired[61,62] and they often forgot the location or direction of the food source. Also, the application of pesticides affected the development and function of the mushroom body[21,63,64] which resulted to affect communication in the bee colony. Also, nAChRs is responsible for memory and learning[65,66] and social behavior which are present in mushroom bodies[67,68]. However, nAChRs were found impaired by the application of pesticides[69]. Moreover, according to Schmuck [57] and Kirchner [70] probably due to impairment by the insecticide on motor neurons signal transmission communication in honeybees gets affected.

HYGIENIC BEHAVIOR

Hygienic behavior is a behavioral response of worker bees that exhibit removing dead, diseased or pesticides infected individuals from the colony[71]. Disease and parasite resistance of the bee colony depends on the hygienic behavior of workers[34,35]. There are very few studies on the effect of sub-lethal doses of pesticides on hygienic behavior in the bee. However, it is reported that the hygienic behavior of the colony was significantly decreased when exposed to the pesticides[72]. For example, honeybee colonies treated with 50 ppb and 100 ppb imidacloprid significantly reduced the hygienic behavior along with the removal of freeze-killed brood by 63.3 and 73.7%, respectively compared to 97.4% in control[72].

OTHER BEHAVIOR

All usual activities must go normally in a normal colony but it has been found that the pesticides change such activities. Neonicotinoids (imidacloprid, thiamethoxam, clothianidin, dinotefuran) affects in the motor function and behavioral changes of adult workers of honeybees (*Apis mellifera*)[73]. According to Migdał *et al.*[74] aggression, walking, grooming, reversal, mobility, rebuild of comb behavior of honeybee colonies were found affected by the application of different pesticides. Moreover, imidacloprid treated with 100 and 500 ppb a.i., bees were less active, communication capacity got affected and this also results to impair in social behavior[53]. Sub-lethal doses of pesticides caused more time in self-cleaning, trembling dance, abdomen trucking in comparison to the control bees[75]. Pesticides cause direct intoxication as trembling, tumbling, abdomen tucking and cleaning, rubbing the hind legs together[76].

LEARNING AND MEMORY

As presented the summary of sub-lethal doses of pesticides on bees in Table 1, multiple previous studies reported that learning and memory in bees were found impaired by the application of pesticides[10,49,62]. For example, exposure of 25.6 µg L^{-1} (20.8 ppb) imidacloprid concentration for four days significantly impaired short-term learning by 87% and memory retention by 85% as compared with control bees[62]. In another separate study, testing a sub-lethal dose of imidacloprid affected learning performance in honeybee and increased the cytochrome oxidase labeling in the calyces of the mushroom bodies[21]. Similarly, both neonicotinoid and non-neonicotinoid pesticide groups found causing a significant negative impact on memory and learning in Apis species and Bombus under both chronic and acute application[22]. Testing of sub-lethal toxicity of different nine pesticides from 2.2-940 µg L^{-1} pesticides concentrations in sucrose solution in *Apis mellifera* fipronil, deltamethrin, endosulfan and prochloraz, reduced the learning performance whereas bees found to be lacking learning abilities in k-cyhalothrin, cypermethrin, s-fluvalinate, triazamate and dimethoate[77]. It is also reported that exposure to sub-lethal doses of pesticides (imidacloprid, coumaphos or their combination) significantly reduced the olfactory learning in bees[61,73].

There are both chronic and sub-lethal doses of pesticides that harm the brain as well as mushroom body[64,78,79] which lowers down the learning ability and memory[80-82]. In addition, nicotinic acetylcholine receptors (nAChRs) respond to cholinergic neural signaling play a vital role in honeybee learning and memory[65] was found affected by the application of pesticides[70]. As a result, it harms the olfactory learning and memory in honeybees[21,47] because of the change in the way that neurons in the honeybee's mushroom bodies function[83]. Moreover, the application of the neonicotinoid, imidacloprid down-regulates nAChRs in their brains[70], causes brain cell death[84], motor function reduced[85,73], decreased the hive

Table 1: Summary of the effect of a sublethal dose of pesticide on bee behavior

Pesticides	Bee species	Doses	Effects	References
Imidacloprid	*Apis mellifera*	100 ppb	-Delay workers to returning to the hive or to the feeding site	Decourtye *et al.*[47]
		500 ppb	-The minimum dose that causes to fail to return to the hive	
Imidacloprid	*A. mellifera*	-	-Impaired learning performance, increased the cytochrome oxidase labeling in the calyces of the mushroom bodies	Decourtye *et al.*[21]
Nine different insecticides	*A. mellifera*	2.2-940 μg L^{-1}	-Some pesticides reduced on learning performance, whereas some had no effect	Decourtye *et al.*[77]
Imidacloprid	*A. mellifera*	Lowest-50, Highest-1200 μg L^{-1}	-Delayed in foraging and abnormalities in revisiting the feeding site	Yang *et al.*[24]
Imidacloprid	*A. cerana*	40 mg L^{-1}	-Returned to the feeder was lower by 23%	
		20 mg L^{-1}	-63% less nectar	
			-46% less nectar respectively	Tan *et al.*[23]
Imidacloprid	*A. mellifera*	500 ppb and 1000 ppb	-Foraging behavior affected	
			-None bees returned to the hive	Romero *et al.*[50]
Imidacloprid	*A. mellifera*	5 μg kg^{-1} ppb	-Performs fewer orientation flights, reduced lifetime foraging flights by 28% and foraging performance	Lämsä *et al.*[52]
Neonicotinoid and non-neonicotinoid	*A. mellifera* and Bumblebees	Acute and Chronic	-Hamper learning and memory	Siviter *et al.*[22]
Imidacloprid	Bumblebees	-	-Foraging behavior affected	Bortolotti *et al.*[51]
Cry1Abprotoxin, Deltamethrin Imidacloprid	*A. mellifera*	1000, 500 and 48 μg kg^{-1}, respectively	-Learning capacity, foraging activity	Haynes[49]
Imidacloprid	Honeybees	-	-Learning ability, orientation, foraging	Thompson and Maus[99]
Imidacloprid	*A. mellifera*	0.15–6 μg/bee	-Foraging activity and flights	Schneider *et al.*[48]
Clothianidin	*A. mellifera*	0.05–2 μg/bee		
Thiamethoxam	*A. mellifera*	Sublethal	-Biological processes affected	Shi *et al.*[90]
Imidacloprid	*A. mellifera*	Sublethal	-Affected learning by 87% and memory retention by 85%	Zhang and Nieh[62]
Imidacloprid and coumaphos	*A. mellifera*	100 nmoll^{-1}	-Olfactory learning reduced	Williamson and Wright[61]
Imidacloprid	*A. mellifera*	20 ppb and 100 ppb	-Communication, waggle dance affected	
			-Foraging behavior impaired	Ndakidemi *et al.*, Kirchner and Friebe[57,70]
Imidacloprid	*A. mellifera*	500 and 100 ppb	-Social behavior impaired	Bortolotti *et al.*[58]
Imidacloprid	*A. mellifera*	0.04 μg	-Mushroom body calyx affected	Peng and Yang[64]
Imidacloprid	Stingless Bee	56 μg a.i./bee	-Mushroom body volume reduced by 36%	Tomé *et al.*[96]
Imidacloprid	Bumblebee	5 ppb	-Mushroom body calyces affected	
			-Learning behavior impaired	Smith *et al.*[97]
Imidacloprid	*A. mellifera*	8100 ppb	-Mushroom bodies cell death	Rossi *et al.*[98]
Pyrethroids	*A. mellifera*	6 % LD_{50}	-Homing behavior affected	
			-More time for self-cleaning, trembling dance, abdomen trucking, rotating	Cox and Wilson[75]
Deltamethrin	*A. mellifera*	4 % LD_{50}	-Homing flight ability affected	
			-Flight muscles and their coordination impaired	Van Dame *et al.*[100]
Pyrethroid	*A. mellifera*	10-30 g ai/ha	-Foraging behavior affected	Shires *et al.*[101]
Serine	*A. mellifera*	0.01-0.1 mg mL^{-1}	-Slower learning of odour	Delegue *et al.*[102]
Parathion	*A. mellifera*	0.3 μg/bee	-Incorrect direction (angle of dance) on vertical surface, incorrect distance on horizontal surface	Williamson and Wright[61]
Different fungicides, herbicides, insecticide	*A. mellifera*	-	-Affected behavior like aggression, walking, grooming, reversal, still, rebuild of comb	Migdal *et al.*[74]
Imidacloprid	*A. mellifera*	100 ppb and 500 ppb a.i.	-Less active, communication capacity and social behavior affected	Bortolotti *et al.*[58]
Imidacloprid	*A. mellifera*	5 μg kg^{-1}, ppb	-Precocious foraging	Lämsä *et al.*[52]
Diazinon	*A. mellifera*	25% LD_{50}	-Precocious foraging	Colin *et al.*[53]
Imidacloprid	*A. mellifera*	50 ppb and 100 ppb	-Reduced hygienic behavior (freeze-killed brood remove) by 63.3 and 73.7%, respectively compared to 97.4% control	Smart and Spivak[72]
Imidacloprid, Thiamethoxam, Clothianidin, Dinotefuran	*A. mellifera*	10 nM	-Motor function and behavioral changes like grooming	Williamson *et al.*[73]

entrance activity[47], predator avoidance ability[23], harms foraging[48], impaired visual learning[86], impaired navigation to the nest[29,87] and deficient rewarded olfactory learning[61,88,89]. Finally, different biological processes like ribosomes, the oxidative phosphorylation pathway, tyrosine metabolism pathway, pentose and glucuronate interconversions and drug metabolism were also found to be affected by the sub-lethal dose of pesticides[90], which also might impair the learning and memory of bees.

Therefore, this clearly shows that even a small amount of pesticides causes severe negative effects on bees learning and memory. As a result, different behavioral changes occurred as they were unable to learn or memorize the activities.

MUSHROOM BODY

The behavior described above primarily depends on the mushroom body in social insects[91,92]. It plays a vital role in olfactory learning, memory and other behavior in honeybees e.g.[93-95]. But the application of pesticides found impaired growth, development and function of the mushroom body[21,63,64] which resulted in different behavioral changes in bees.

The calyces are important sensory input regions in the mushroom bodies, while sub-lethal doses of imidacloprid affected the neural development of the honey bee brain by immune-labeling synaptic units in the calyces of mushroom bodies. The density of the synaptic units in the region of the calyces, responsible for olfactory and visual functions, found to be decreased after being exposed to a sub-lethal dose of imidacloprid[64]. Similarly, it was found that sub-lethal doses of neonicotinoid insecticides decreased the synaptic density of the mushroom body calyx of honey bees and the bees exposed to 0.04 µg imidacloprid per bee larvae in the larval stage exhibit an impaired olfactory associative behavior in the adult stage[89]. Also, the growth and development of the mushroom body of Stingless Bee (*Melipona quadrifasciata anthidioides*) were found negatively affected by the application of imidacloprid[96]. They found that untreated bees mushroom body volume was normally increased, whereas, treated with 56 µg a.i./bee with imidacloprid was reduced by 36% by volume at eight days of emergence. Similarly, the mushroom body calyces, relatively volumes were significantly small in worker bees exposed to pesticides as compared to control[97]. Nicotinic acetylcholine receptors (nAChRs) are responsible for cholinergic synaptic transmission and play vital roles in cognitive and behavioral processes[65] which are located in the mushroom bodies[67,68]. They were also found impaired by the application of pesticides[70]. Also, it is reported that treatment with 8100 ppb imidacloprid induced mushroom bodies cell death after 1 day of treatment[98].

CONCLUSION

It is concluded that pesticides, especially insecticides, impaired the different behavioral activities in honeybees. Not only pesticides contaminated forager bees but the collected nectar or pollen by them contained pesticides even in trace amounts which caused problems in the colony. Bee behavior depends on the higher functionality of the sensory and integrative nervous system which is affected by exposure to even a small amount of pesticides both at larval and adult stages.

SIGNIFICANCE STATEMENT

This review paper will be the basis to the researchers to explore further the effect of sub-lethal doses of pesticides in the bee. This review explored that even trace amounts of pesticides impaired bees' behaviors. Hence, not only exposure to lethal doses causes multiple negative impacts on the bee but the sub-lethal doses are also harmful to the bees.

REFERENCES

1. Klein, A.M., B.E. Vaissiere, J.H. Cane, I. Steffan-Dewenter and S.A. Cunningham *et al*., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. London B Biol. Sci., 274: 303-313.
2. Kremen, C., N.M. Williams and R.W. Throp, 2002. Crop pollination from native bees at risk from agricultural intensification. PNAS, 99: 16812-16816.
3. Ollerton, J., R. Winfree and S. Tarrant, 2011. How many flowering plants are pollinated by animals? Oikos, 120: 321-326.
4. Morse, R.A. and N.W. Calderone, 2000. The value of honey bees as pollinators of US crops in 2000. Bee Cult. Magaz., 28: 1-15.
5. Thapa, R.B., 2006. Honeybees and other insect pollinators of cultivated plants: A review. J. Inst. Agric. Anim. Sci., 27: 1-23.
6. Pudasaini, R. and R.B. Thapa, 2014. Effect of pollination on rapeseed (*Brassica campestris* L. var. Toria) production in Chitwan, Nepal. J. Agric. Environ., 15: 41-45.
7. Pudasaini, R., R.B. Thapa and P.R. Poudel, 2014. Effect of pollination on qualitative characteristics of rapeseed (*Brassica campestris* L. var. toria) seed in Chitwan, Nepal. Int. J. Biol. For. Vet. Agric., 8: 1278-1281.

8. Stein, K., D. Coulibaly, K. Stenchly, D. Goetze and S. Porembski *et al*., 2017. Bee pollination increases yield quantity and quality of cash crops in Burkina Faso, West Africa. Sci. Rep., Vol. 7, 10.1038/s41598-017-17970-2.
9. Eilers, E.J., C. Kremen, S.S. Greenleaf, A.K. Garber and A.M. Klein, 2011. Contribution of pollinator-mediated crops to nutrients in the human food supply. PLoS ONE, Vol. 6, No. 6, 10.1371/journal.pone.0021363.
10. Biesmeijer, J.C., S.P.M. Roberts, M. Reemer, R. Ohlemuller and M. Edwards *et al*., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313: 351-354.
11. Fitzpatrick, Ú., T.E. Murray, R.J. Paxton, J. Breen, D. Cotton, V. Santorum and M.J. Brown, 2007. Rarity and decline in bumblebees-A test of causes and correlates in the Irish fauna. Biol. Conserv., 136: 185-194.
12. Lebuhn, G., S. Droege, E.F. Connor, B. Gemmill-Herren and S.G. Potts *et al*., 2013. Detecting insect pollinator declines on regional and global scales. Conserv. Biol., 27: 113-120.
13. Budge, G.E., D. Garthwaite, A. Crowe, N.D. Boatman and K.S. Delaplane *et al*., 2015. Evidence for pollinator cost and farming benefits of neonicotinoid seed coatings on oilseed rape. Sci Rep., Vol. 5. 10.1038/srep12574.
14. Pudasaini, R., R.B. Thapa and S. Tiwari, 2016. Farmers perception on effect of pesticide on insect pollinators at Padampur and Jutpani Vdcs, Chitwan, Nepal. Int. J. Appl. Sci. Biotechnol., 4: 64-66.
15. Woodcock, B.A., N.J. Isaac, J.M. Bullock, D.B. Roy, D.G. Garthwaite, A. Crowe and R.F. Pywell, 2016. Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nat. Commun., Vol. 7. 10.1038/ncomms12459.
16. Pudasaini, R., 2014. Abundance of flower visiting insects in rapeseed (*Brassica campestris* L. var. *toria*) field affected by the distance of natural habitat. Sky J. Agri. Res., 3: 223-227.
17. Rouzé, R., A. Moné, F. Delbac, L. Belzunces and N. Blot, 2019. The honeybee gut microbiota is altered after chronic exposure to different families of insecticides and infection by *Nosema ceranae*. Microb. Environ., 34: 226-233.
18. Sánchez-Bayo, F., D. Goulson, F. Pennacchio, F. Nazzi, K. Goka and N. Desneux, 2016. Are bee diseases linked to pesticides? -A brief review. Environ. Int., 89-90: 7-11.
19. T O'Neal, S., T.D. Anderson and J.Y. Wu-Smart, 2018. Interactions between pesticides and pathogen susceptibility in honey bees. Curr. Opin. Insect Sci., 26: 57-62.
20. Thompson, H.M., S.L. Levine, J. Doering, S. Norman, P. Manson, P. Sutton and G. von Mérey, 2014. Evaluating exposure and potential effects on honeybee brood (*Apis mellifera*) development using glyphosate as an example. Integr. Environ. Assess. Manage., 10: 463-470.
21. Decourtye, A., C. Armengaud, M. Renou, J. Devillers, S. Cluzeau, M. Gauthier and M. Pham-Delague, 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pesticide Biochem. Physiol., 78: 83-92.
22. Siviter, H., J. Koricheva, M.J.F. Brown and E. Leadbeater, 2018. Quantifying the impact of pesticides on learning and memory in bees. J. Appl. Ecol., 55: 2812-2821.
23. Tan, K., W. Chen, S. Dong, X. Liu, Y. Wang and J.C. Nieh, 2014. Imidacloprid alters foraging and decreases bee avoidance of predators. PLoS ONE, Vol. 9, No. 7. 10.1371/journal.pone.0102725.
24. Yang, E.C., Y.C. Chuang, Y.L. Chen and L.H. Chang, 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). J. Econ. Entomol., 101: 1743-1748.
25. Tsvetkov, N., O. Samson-Robert, K. Sood, H.S. Patel and D.A. Malena *et al*., 2017. Chronic exposure to neonicotinoids reduces honey bee health near corn crops. Science, 356: 1395-1397.
26. Feltham, H., K. Park and D. Goulson, 2014. Field realistic doses of pesticide imidacloprid reduce bumblebee pollen foraging efficiency. Ecotoxicology, 23: 317-323.
27. Gill, R.J. and N.E. Raine, 2014. Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. Funct. Ecol., 28: 1459-1471.
28. Gill, R.J., O. Ramos-Rodriguez and N.E. Raine, 2012. Combined pesticide exposure severely affects individual- and colony-level traits in bees. Nature, 491: 105-108.
29. Henry, M., M. Beguin, F. Requier, O. Rollin and J.F. Odoux *et al*., 2012. A common pesticide decreases foraging success and survival in honey bees. Science, 336: 348-350.
30. Stanley, D.A., A.L. Russell, S.J. Morrison, C. Rogers and N.E. Raine, 2016. Investigating the impacts of field-realistic exposure to a neonicotinoid pesticide on bumblebee foraging, homing ability and colony growth. J. Appl. Ecol., 53: 1440-1449.
31. Wu, J.Y., C.M. Anelli and W.S. Sheppard, 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. PLoS ONE, Vol. 6, No. 2. 10.1371/journal.pone.0014720.
32. Bryden, J., R.J. Gill, R.A. Mitton, N.E. Raine and V.A. Jansen, 2013. Chronic sublethal stress causes bee colony failure. Ecol. Lett., 16: 1463-1469.
33. Young, H.J., D.W. Dunning and K.W. Hasseln, 2007. Foraging behavior affects pollen removal and deposition in *Impatiens capensis* (Balsaminaceae). Am. J. Bot., 94: 1267-1271.
34. Rothenbuhler, W.C., 1964. Behaviour genetics of nest cleaning in honey bees. I. Responses of four inbred lines to disease-killed brood. Anim. Behav., 12: 578-583.

35. Spivak, M., 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. Apidologie, 27: 245-260.
36. Von Frisch, K., 1967. The Dance Language and Orientation of Bees. Harvard University Press, Cambridge, MA .
37. Price, R.I.A. and C. Grüter, 2015. Why, when and where did honey bee dance communication evolve? Front. Ecol. Evol., Vol. 3, No. 125. 10.3389/fevo.2015.00125.
38. Johnson, B.R., 2010. Division of labor in honeybees: Form, function and proximate mechanisms. Behav. Ecol. Sociobiol., 64: 305-316.
39. Michener, C.D., 1969. Comparative social behavior of bees. Annu. Rev. Entomol., 14: 299-342.
40. Seeley, T.D., 1982. Adaptive significance of the age polyethism schedule in honeybee colonies. Behav. Ecol. Sociobiol., 11: 287-293.
41. Trumbo, S.T., Z.Y. Huang and G.E. Robinson, 1997. Division of labor between undertaker specialists and other middle-aged workers in honey bee colonies. Behav. Ecol. Sociobiol., 41: 151-163.
42. Lundin, O., M. Rundlöf, H.G. Smith, I.Fries and R. Bommarco, 2015. Neonicotinoid insecticides and their impacts on bees: A systematic review of research approaches and identification of knowledge gaps. PLoS ONE, Vol. 10. 10.1371/journal.pone.0136928.
43. Thompson, H.M., 2003. Behavioural effects of pesticides in bees-Their potential for use in risk assessment. Ecotoxicology, 12: 317-330.
44. Desneux, N., A. Decourtye and J.M. Delpuech, 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol., 52: 81-106.
45. Pudasaini, R. and R.B. Thapa, 2014. Foraging behavior of different bee species under cage and open condition in Chitwan, Nepal. New York Sci. J., 7: 94-97.
46. Singh, J., O.P. Agrawal and R.C. Mishra, 2006. Foraging rates of different *Apis* species visiting parental lines of *Brassica napus* L. Zoos Print J., 21: 2226-2227.
47. Decourtye, A., J. Devillers, S. Cluzeau, M. Charreton and M.H. P. Delègue, 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicol. Environ. Saf., 57: 410-419.
48. Schneider, C.W., J. Tautz, B. Grünewald and S. Fuchs, 2012. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging behavior of *Apis mellifera*. PLoS ONE, Vol. 7, No. 1. 10.1371/journal.pone.0030023.
49. Haynes, K.F., 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol., 33: 149-168.
50. Romero, R.R., J. Chaufaux and M.H.P. Delègue, 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. Apidologie, 36: 601-611.
51. Bortolotti, L., R. Montanari, J. Marcelino, P. Medrzycki, S. Maini and C. Porrini, 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bull. Insectol., 56: 63-67.
52. Lämsä, J., E. Kuusela, J. Tuomi, S. Juntunen and P.C. Watts, 2018. Low dose of neonicotinoid insecticide reduces foraging motivation of bumblebees. Proc. R. Soc. B, Vol. 285. 10.1098/rspb.2018.0506.
53. Colin, T., W.G. Meikle, X. Wu and A.B. Barron, 2019. Traces of a neonicotinoid induce precocious foraging and reduce foraging performance in honey bees. Environ. Sci. Technol., 53: 8252-8261.
54. Mackenzie, K.E. and M.L. Winston, 1989. Effects of sublethal exposure to diazinon on longevity and temporal division of labor in the honey bee (Hymenoptera: Apidae). J. Econ. Entomol., 82: 75-82.
55. Henry, M., N. Cerrutti, P. Aupinel, A. Decourtye and M. Gayrard *et al*., 2015. Reconciling laboratory and field assessments of neonicotinoid toxicity to honeybees. Proc. R. Soc. B, Vol. 282, No. 1819. 10.1098/rspb.2015.2110.
56. Perry, C.J., E. Søvik, M.R. Myerscough and A.B. Barron, 2015. Rapid behavioral maturation accelerates failure of stressed honey bee colonies. Proc. Natl. Acad. Sci. USA, 112: 3427-3432.
57. Ndakidemi, B., K. Mtei and P.A. Ndakidemi, 2016. Impacts of synthetic and botanical pesticides on beneficial insects. Agric. Sci., 7: 364-372.
58. Bortolotti, L., R. Montanari, J. Marcelino, P. Medrzycki, S. Maini, C. Porrini and I.N. Di, 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bull. Insectol., 56: 63-67.
59. Dechaume-Moncharmont, F.X., 2003. Butinage collectif chez l'abeille *Apis mellifera* L.: Etude théorique et expérimentale. Ph. D Univ. Pierre-et-Marie-Curie, Paris.
60. Schricker, B. and W.P. Stephen, 1970. The effect of sublethal doses of parathion on honeybee behaviour. I. Oral administration and the communication dance. J. Apic. Res., 9: 141-153.
61. Williamson, S.M. and G.A. Wright, 2013. Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honeybees. J. Exp. Biol., 216: 1799-1807.
62. Zhang, E. and J.C. Nieh, 2015. The neonicotinoid imidacloprid impairs honey bee aversive learning of simulated predation. J. Exp. Biol., 218: 3199-3205.
63. Palmer, M.J., C. Moffat, N. Saranzewa, J. Harvey, G.A. Wright and C.N. Connolly, 2013. Cholinergic pesticides cause mushroom body neuronal inactivation in honeybees. Nat. Commun., 10.1038/ncomms2648.
64. Peng, Y.C. and E.C. Yang, 2016. Sublethal dosage of imidacloprid reduces the microglomerular density of honey bee mushroom bodies. Sci. Rep., Vol. 6. 10.1038/srep19298.

65. Dacher, M. and M. Gauthier, 2008. Involvement of NO-synthase and nicotinic receptors in learning in the honey bee. Physiol. Behav., 95: 200-207.
66. Guez, D., S. Suchail, M. Gauthier, R. Maleszka and L.P. Belzunces, 2001. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). Neurobiol. Learn. Memory, 76: 183-191.
67. Bicker, G., 1999. Histochemistry of classical neurotransmitters in antennal lobes and mushroom bodies of the honeybee. Neurotransmitter Histochem. Insect Brain, Part II, 45: 174-183.
68. Kreissl, S. and G. Bicker, 1989. Histochemistry of acetylcholinesterase and immunocytochemistry of an acetylcholine receptor-like antigen in the brain of the honeybee. J. Comp. Neurol., 286: 71-84.
69. Zhou, T., H. Song, Q. Wang, Y. Wu and J. Sun, 2013. Effects of imidacloprid on the distribution of nicotine acetylcholine receptors in the brain of adult honeybee (*Apis mellifera ligustica*). Acta Entomol. Sinica, 56: 1258-1266.
70. Kirchner, W.H. and R. Friebe, 1999. Nestmate discrimination in the African stingless bee *Hypotrigona gribodoi* Magretti (Hymenoptera: Apidae). Apidologie, 30: 293-298.
71. Wilson-Rich, N., M. Spivak, N.H. Fefferman and P.T. Starks, 2009. Genetic, individual and group facilitation of disease resistance in insect societies. Annu. Rev. Entomol., 54: 405-423.
72. Smart, J.W. and M. Spivak, 2016. Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. Sci. Rep., Vol. 6. 10.1038/srep32108.
73. Williamson, S.M., S.J. Willis and G.A. Wright, 2014. Exposure to neonicotinoids influences the motor function of adult worker honeybees. Ecotoxicology, 23: 1409-1418.
74. Migdał, P., A. Roman, E.P. Pleban, M.K. Góralska and S. Opaliński, 2018. The impact of selected pesticides on honey bees. Pol. J. Environ. Stud., 27: 787-792.
75. Cox, R.L. and W.T. Wilson, 1984. Effects of permethrin on the behavior of individually tagged honey bees, *Apis mellifera* L. (Hymenoptera: Apidae). Environ. Entomol., 13: 375-378.
76. Suchail, S., D. Guez and L.P. Belzunces, 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. Environ. Toxicol. Chem., 20: 2482-2486.
77. Decourtye, A., J. Devillers, E. Genecque, K. Le Menach, H. Budzinski, S. Cluzeau and M.H.P. Delegue, 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. Arch. Environ. Contam. Toxicol., 48: 242-250.
78. Catae, A.F., T.C. Roat, R.A. de Oliveira, R.C.F. Nocelli and O. Malaspina, 2014. Cytotoxic effects of thiamethoxam in the midgut and malpighian tubules of Africanized *Apis mellifera* (Hymenoptera: Apidae). Microscopy Res. Techn., 77: 274-281.
79. Oliveira, R.A., T.C. Roat, S.M. Carvalho and O. Malaspina, 2014. Side-effects of thiamethoxam on the brain andmidgut of the africanized honey bee *Apis mellifera* (Hymenopptera: Apidae). Environ. Toxicol., 29: 1122-1133.
80. Aliouane, Y., A.K. El Hassani, V. Gary, C. Armengaud, M. Lambin and M. Gauthier, 2009. Subchronic exposure of honeybees to sublethal doses of pesticides: Effects on behavior. Environ. Toxicol. Chem., 28: 113-122.
81. Alkassab, A.T. and W.H. Kirchner, 2016. Impacts of chronic sublethal exposure to clothianidin on winter honeybees. Ecotoxicology, 25: 1000-1010.
82. Goñalons, C.M. and W.M. Farina, 2015. Effects of sublethal doses of imidacloprid on young adult honeybee behaviour. PLoS ONE, Vol. 10, No. 10. 10.1371/journal.pone.0140814.
83. Gauthier, M., 2010. State of the art on insect nicotinic acetylcholine receptor function in learning and memory. Insect Nicotinic Acetylcholine Recept., 683: 97-115.
84. Wu, Y.Y., Z. Ting, A.J. Wubie, W. Qiang, D.P. Li and J.H. Ru, 2014. Apoptosis in the nerve cells of adult honeybee (*Apis mellifera ligustica*) brain induced by imidacloprid. Acta Entomologica Sinica., 57: 194-203.
85. Lambin, M., C. Armengaud, S. Raymond and M. Gauthier, 2001. Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. Arch. Insect Biochem. Physiol., 48: 129-134.
86. Han, P., C.Y. Niu, C.L. Lei, J.J. Cui and N. Desneux, 2010. Use of an innovative T-tube maze assay and the proboscis extension response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honey bee *Apis mellifera* L. Ecotoxicol., 19: 1612-1619.
87. Fischer, J., T. Müller, A.K. Spatz, U. Greggers, B. Grünewald and R. Menzel, 2014. Neonicotinoids interfere with specific components of navigation in honeybees. PLoS ONE, Vol. 9, No. 3. 10.1371/journal.pone.0091364.
88. Cresswell, J.E., 2011. A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. Ecotoxicology, 20: 149-157.
89. Yang, E.C., H.C. Chang, W.Y. Wu and Y.W. Chen, 2012. Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. PLoS ONE, Vol. 7, No. 11. 10.1371/journal.pone. 0049472.
90. Shi, T.F., Y.F. Wang, F. Liu, L. Qi and L.S. Yu, 2017. Sublethal effects of the neonicotinoid insecticide thiamethoxam on the transcriptome of the honey bees (Hymenoptera: Apidae). J. Econ. Entomol., 110: 2283-2289.
91. Heisenberg, M., 1998. What do the mushroom bodies do for the insect brain? An introduction. Learn Mem., 5: 1-10.
92. Strausfeld, N.J., L. Hansen, Y. Li, R.S. Gomez and K. Ito, 1998. Evolution, discovery and interpretations of arthropod mushroom bodies. Learn Mem., 5: 11-37.

93. Devaud, J.M., T. Papouin, J. Carcaud, J.C. Sandoz, B. Grünewald and M. Giurfa, 2015. Neural substrate for higher-order learning in an insect: Mushroom bodies are necessary for configural discriminations. Proc. Natl. Acad. Sci. USA, 112: E5854-E5862.
94. Fahrbach, S.E., 2005. Structure of the mushroom bodies of the insect brain. Annu. Rev. Entomol., 51: 209-232.
95. Giurfa, M. and J.C. Sandoz, 2012. Invertebrate learning and memory: Fifty years of olfactory conditioning of the proboscis extension response in honeybees. Learning Memory, 19: 54-66.
96. Tomé, H.V.V., G.F. Martins, M.A.P. Lima, L.A.O. Campos and R.N.C. Guedes, 2012. Imidacloprid-induced impairment of mushroom bodies and behavior of the native stingless bee melipona quadrifasciata anthidioides. PLoS ONE, Vol. 7, No. 6. 10.1371/journal.pone.0038406.
97. Smith, D.B., A.N. Arce, A.R. Rodrigues, P.H. Bischoff, D. Burris, F. Ahmed and R.J. Gill, 2019. Developmental exposure to pesticide contaminated food impedes bumblebee brain growth predisposing adults to become poorer learners, BioRxiv, 10.1101/690602.
98. Rossi, C.D.A., T.C. Roat, D.A. Tavares, P.C. Socolowski and O. Malaspina, 2013. Brain morphophysiology of africanized bee *Apis Mellifera* exposed to sublethal doses of imidacloprid. Arch. Environ. Contam. Toxicol., 65: 234-243.
99. Thompson, H.M. and C. Maus, 2007. The relevance of sublethal effects in honey bee testing for pesticide risk assessment. Pest. Manag. Sci., 63: 1058-1061.
100. Van Dame, R., M. Meled, M.E. Colin and L.P. Belzunces, 1995. Alteration of the homing-flight in the honey bee *Apis mellifera* L. Exposed to sublethal dose of deltamethrin. Environ. Toxicol. Chem., 14: 855-860.
101. Shires, S.W., J. Le Blanc, P. Debray, S. Forbes and J. Louveaux, 1984. Field experiments on the effects of a new pyrethroid insecticide WL-85871 on bees foraging artificial aphid honeydew on winter wheat. Pestic. Sci., 15: 543-552.
102. Delegue, M.H.P., C. Girard, M. Metayer, A.L. Picard-Nizou, C. Hennequet, O. Pons and L. Jouanin, 2000. Long-term effects of soybean protease inhibitors on digestive enzymes, survival and learning abilities of honeybees. Entomologia Experimentalis et Applicata, 95: 21-29.