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Original article (Orijinal araştırma)

Comparative toxicity of two neonicotinoids and a pyrethroid to forager honeybees (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) by different exposure methods[1]

Toplayıcı bal arılarının (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) farklı maruz kalma yöntemleri ile iki neonikotinoid ve bir piretroidin karşılaştırmalı toksisitesi

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Abstract

Honeybees are exposed to insecticides by direct contact with spray droplets or residues on plant, or through ingestion of contaminated pollen or nectar. Direct contact with foliar spray might be the most common exposure route and contact bioassays are preferred as they better simulate field situation. Bioassays were conducted during 2018 at Sultan Qaboos University, Oman. The acute contact and oral toxicity of commercial formulations of deltamethrin 2.5 EC, thiamethoxam 25 WG and acetamiprid 20 SL to *Apis mellifera* subsp. *lamarckii* Cockerell 1906 (Hymenoptera: Apidae) foragers were measured by three exposure methods (contact by a 1-µL droplet on thorax, contact by Potter spray tower and oral ingestion). Potter tower exposure gave significantly higher mortality at lower concentration of deltamethrin than contact exposure by single droplet on thorax. Thiamethoxam showed significantly higher mortality through oral exposure at all concentrations. HQ_{oral} values were also calculated. Acetamiprid did not give more than 50% mortality even with the highest concentration. Potter tower produced fine droplets (0.286±0.071 µm) and a total of 0.829 µL was deposited on a single honeybee. Forager honeybees are more likely be exposed to the very fine droplets in field and toxicological results obtained by Potter tower or similar devices will be more realistic than a single droplet on thorax.

Keywords: Acetamiprid, *Apis mellifera lamarckii,* deltamethrin, exposure methods, thiamethoxam, toxicity

Öz

Bal arıları sprey damlamaları veya bitkilerdeki kalıntılarından doğrudan temas ile, ya da bulaşık polen veya nektar alımı ile insektisitlere maruz kalmaktadır. İlaçlama ile doğrudan teması en yaygın maruz kalma şeklidir ve arazideki durumu daha iyi simüle ettiği için temas biyolojik denemeleri tercih edilmektedir. Biyolojik denemeler, 2018 yılında Umman Sultan Qaboos Üniversitesi'nde yürütülmüştür. Deltamethrin 2.5 EC, thiamethoxam 25 WG ve acetamiprid 20 SL'nin ticari formülasyonlarının *Apis mellifera* subsp. *lamarckii* Cockerell 1906 (Hymenoptera: Apidae) toplayıcılara akut teması ve ağızdan zehirlenmesi üç yöntem (thoraks üzerinde 1-µL damlacık ile temas, Potter sprey kulesi ile temas ve oral alınım) ile ölçülmüştür. Potter kule uygulaması, daha düşük deltametrin konsantrasyonunda, thoraks üzerindeki tek damlacık ile temasta etkilenmeye göre önemli ölçüde daha yüksek ölüm oranı sağlamıştır. Thiamethoxam, tüm konsantrasyonlarda oral yoldan maruz kalma ile önemli ölçüde daha yüksek ölüm oranı göstermiştir. HQ_{oral} değerleri de hesaplanmıştır. Acetamiprid, en yüksek konsantrasyonda bile %50'den fazla ölüm oranı vermemiştir. Potter kulesi, ince damlacıklar (0.286 ± 0.071 µm) üretmiştir ve tek bir bal arısı üzerinde toplam 0.829 µL biriktirilmiştir. Toplayıcı bal arıları, tarladaki çok ince damlacıklara maruz kalmaya daha yatkındır ve Potter kulesi veya benzer cihazları kullanarak elde edilen toksikolojik sonuçlar, thorakstaki tek bir damlacığa göre daha gerçekçi olacaktır.

Anahtar sözcükler: Acetamiprid, *Apis mellifera lamarckii,* deltamethrin, maruz kalma yöntemleri, thiamethoxam, toksisite

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Introduction

Honeybees (*Apis mellifera* L., 1758) (Hymenoptera: Apidae) produce valuable commercial products (honey, pollen, royal jelly, propolis and wax) and crop pollination largely relies on managed colonies of honeybees (Free, 1993; Gallai et al., 2009). Insecticides which are normally designed to control insect pests can also affect non-target organisms, including the honeybees.

Neonicotinoid insecticides are used on several crops including pome fruits, stone fruits, citrus, grape, other horticultural and ornamental plants to control different insect pests, for example, aphids, whiteflies, plant hoppers, scale insects, moths and soil insects (Muccinelli, 2008). Neonicotinoid insecticides permanently bind to the nicotinic acetylcholine receptors which result in blocking the passage of nerve impulses (Tomizawa & Casida, 2005). Neonicotinoids, applied foliar or seed coating, translocate to pollen and nectar and are consumed by foraging honeybees.

Pyrethroids are also widely used in agriculture and primary target is the voltage-dependent sodium channel (Soderlund & Bloomquist, 1989). The neonicotinoids have higher selectivity factor for insects versus mammals while pyrethroids are non-selective (Tomizawa & Casida, 2005). Both classes of insecticides show high toxicity to pollinating insects particularly the honeybees (Meled et al., 1998; Laurino et al., 2011).

Honeybees may be exposed to insecticides in several ways, including direct contact with spray residues on plants or through ingestion of contaminated pollen or nectar, whether from the crop plants or from the weeds around the fields (Sanchez-Bayo & Goka, 2014). Direct contact of insecticides to honeybees occurs when the spray droplets directly deposit on honeybees. This can occur when applications are made while honeybees are actively foraging on blooming crops, cultivated understory, weeds, cover crops, or habitat areas. Direct contact with foliar spray may be the most obvious exposure route for honeybees.

The dose-response laboratory toxicity bioassays and assessing the toxicity of pesticides to adults by establishing oral and contact LD_{50} and calculate hazard quotients (HQ) is suggested as risk assessment process (EFSA, 2013). Contact bioassays with a 1-µL droplet on the thorax of a honeybee is generally used. However, Potter spray tower produces a droplet size which is closer to the recommended size produced by spray equipment. Oral toxicity is measured by feeding honeybees with pesticide-contaminated honey or sucrose solution. The contact bioassays with a droplet on thorax and Potter tower have not been compared. In this study we measured the acute contact and oral toxicity of three commonly used insecticides to forager honeybees and compared the measured toxicity using three exposure methods (contact by a droplet on thorax, contact by Potter spray tower and oral ingestion).

Materials and Methods

Source of forager honeybees

Bioassays to assess both acute contact and oral toxicity to honeybee foragers were conducted during 2018. Forager honeybees (*Apis mellifera* subsp. *lamarckii* Cockerell, 1906) used in these bioassays were collected from one well-fed, healthy and disease-free colony maintained at the Agriculture Experiment Station, Sultan Qaboos University, Muscat, Oman. The forager honeybees were collected from a single colony.

Insecticides

Commercial insecticide formulations available in Oman were used. The insecticides used in the study were: Delta (deltamethrin) 2.5 EC from Arab Pesticides and Veterinary Drugs Mfg. Co, Jordan, Actara (thiamethoxam) 25 WG from Syngenta, India and Clipper (acetamiprid) 20 SL from Hexter chemicals Sdn. Bhd, Malaysia (Table 1). All preparations were made using deionized (DI) water as solvent. The concentrations (ai) used were: deltamethrin 1.11, 3.33, 10, 30 and 90 µg/mL; thiamethoxam 0.04, 0.12, 0.37, 1.11, 3.33, 10, 30 and 90 µg/mL; acetamiprid 0.37, 1.11, 3.33, 10, 30 and 90 µg/mL active ingredient. Five to eight concentrations were used for each insecticide to obtain mortality between 15 to 85%.

Table 1. Characteristics of commercial insecticide formulations available in Oman and used in the bioassays

Active ingredient	Trade name	Formulation (ai)	Label concentration	Insect pest
Deltamethrin	Delta 2.5EC	2.5 w/w emulsifiable concentrate	80 mL/100 L (a.i. 10 mg/L)	Aphids, thrips, beetles and others
Thiamethoxam	Actara 25WG	25 w/w water dispersible granules	8 g/20 L (a.i. 100 mg/L)	Aphids, psyllids, leaf miners and others
Acetamiprid	Clipper 20SL	20 w/w soluble liquid	10 mL/20 L (a.i. 100 mg/L)	Whiteflies, thrips, and others

Contact and oral bioassays

Contact and oral bioassays were carried out using three methods of exposure to insecticides (Figure 1). A set of 10 forager honeybees was placed in a Petri dish and immobilized by placing them on a chilling pad for contact bioassays. Acute contact toxicity was measured by either placing a 1-µL droplet on thorax using micropipette or spray using a Potter tower. In Potter tower (Burkard Scientific, Uxbridge, UK) bioassay, 2 mL of each insecticide concentration was sprayed at 70 kPa. Deionized water treatment served as control for contact bioassays.

The diameter of droplets (on the honeybee body) from the Potter tower spray was measured by a stereomicroscope, and the volume of droplets was calculated by following formula (Cunha et al., 2013):

$$Vg = \pi Dg^3 / 6$$

where Vg is the volume of each droplet (µL) and Dg, the droplet diameter (µm). This calculated droplet volume was used to calculate the amount (µL) received by individual honeybee. The volume median diameter (VMD) was calculated using a spreadsheet. The number of droplets deposited on head, thorax, abdomen, legs and wings of a honeybee were also recorded.

In acute oral bioassay, 2 mL of each insecticide concentration (prepared with 20% honey) was applied to a cotton ball which was kept in a small lid placed inside a container (9 cm upper diam., 6 cm lower diam., 7 cm high) (Figure 1). Honeybees were starved for 1 h and then let to feed for 2 h (www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm). In this setup, honeybees place front legs on the edge of the lid and use proboscis to feed thus avoiding any unnecessary contact with the insecticides. After 2 h, honeybees were transferred to new containers and provided with only 20% honey solution. A 20% (w/v) honey solution was provided to honeybees in control for oral bioassay. Each concentration for each insecticide was replicated four times.

The lids with cotton balls were weighed before and after 2 h to measure the amount of each insecticide at each concentration consumed by honeybees during oral exposure. The ingestion LD_{50} values were obtained from the relative LC_{50} values by multiplying with the amount of food consumed in 2 h (Laurino et al., 2011).

The prepared containers with honeybees were kept at 24±2°C and 60±5% RH inside a box in complete darkness. Data about number of dead and live honeybees were recorded at 24, 48 and 72 h after treatment. A honeybee was considered dead when it remained motionless for ten seconds after touching it gently by a fine brush (Laurino et al., 2011). Two hundred, 320 and 240 forager honeybees were used for deltamethrin, thiamethoxam and acetamiprid, respectively, and 40 forager for each control.

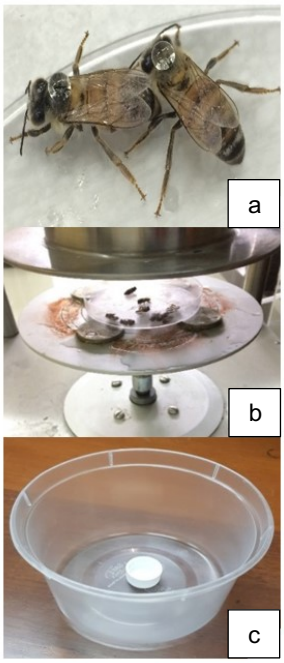


Figure 1. Different methods of honeybee exposure to insecticides: a) a 1-µL droplet on thorax, b) potter spray tower, and c) container used for acute oral bioassay and honeybee feeding.

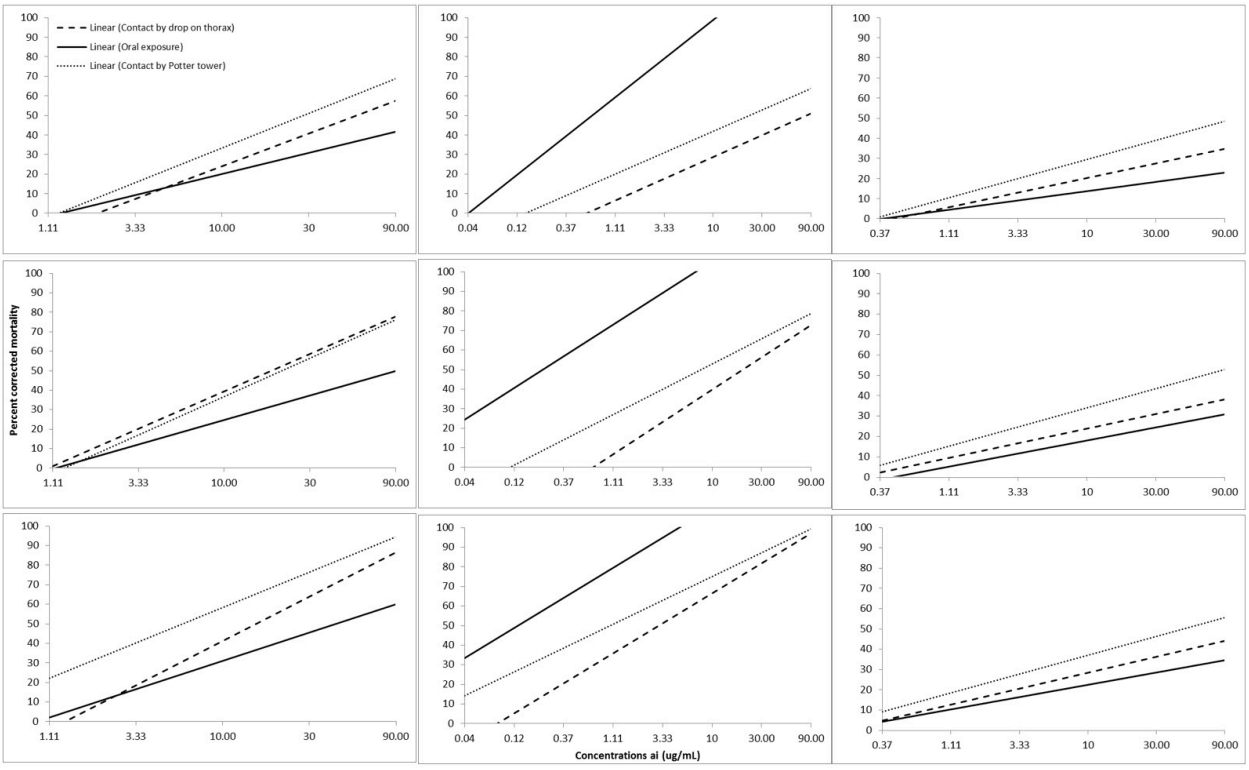
Data analysis

Mortality data was corrected for control using Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} were calculated using PoloPlus software. Probit regressions were plotted by SPSS ver. 18. The number of drops deposited on different body parts of a honeybee after spray by Potter tower was recorded. The number of drops on different body parts was analyzed by ANOVA and Tukey's test was used for means separation in SPSS. A two factor ANOVA was done for the exposure methods for each insecticide at different concentrations; separately for 24, 48 and 72 h. LD_{50} were used to calculate the HQ as field application rate (g/ha) divided by oral LD_{50} (µg/bee) relative to the field application adopted for field concentration determination (Table 1) (OEPP/EPPO, 2010).

Results

Direct observation of the behavior of the honeybees in containers during the trials showed symptoms of poisoning, such as tremors, uncoordinated and uncontrolled movements, and prolonged frenetic movement of the legs at field concentration (30 µL/mL) of deltamethrin and lower concentration (1.1 µL/mL) of thiamethoxam.

After 24 and 48 h of exposure, deltamethrin percent corrected mortality was similar between the exposure methods at lower concentrations. At higher concentrations oral exposure mortality was lower than the contact mortality (Figure 2). At 72 h, Potter tower exposure gave significantly higher mortality ($F = 12.1$, $P = 0.021$) at lower concentrations than contact exposure by single droplet on thorax or oral exposure. This difference was not significant ($F = 1.04$, $P = 0.71$) at the highest concentration from single droplet on thorax exposure (Figure 2).



a b c

Figure 2. Percent corrected mortality caused by a) deltamethrin, b) thiamethoxam and c) acetamiprid by different exposure methods at 24, 48 and 72 h.

Thiamethoxam showed significantly higher mortality through oral exposure at all concentrations than both the contact methods at 24 h ($F = 34.1$, $P = 0.003$), 48 h ($F = 24.3$, $P < 0.001$) and 72 h ($F = 14.2$, $P=0.003$). Potter tower exposure, after 48 and 72 h, gave significantly higher mortality ($F = 16.7$, $P = 0.002$) than droplet on thorax exposure at lower concentrations; the difference was non-significant at higher concentrations. Acetamiprid did not give more than 50% mortality even with the highest concentration at 72h which did not allow calculation of LC_{50} values. Potter tower exposure gave higher mortality which was not significantly different than other exposure methods at 24, 48 and 72 h at lower concentrations. At higher concentration the Potter tower mortality was significantly higher ($F = 7.63$, $P = 0.03$) than oral exposure (Figure 2).

Deltamethrin LC_{50} measured by acute contact exposure (both a droplet on thorax and Potter tower) was significantly lower (95% CI did not overlap) than acute oral exposure, however, LC_{90} values were not different between the exposure methods (Table 2). Thiamethoxam gave low acute oral LC_{50} when fed in treated honey. Thiamethoxam oral LD_{50} and LD_{90} values were significantly lower than both of the contact exposure methods (Table 2).

Table 2. Comparison of LC_{50} and LC_{90} of deltamethrin and thiamethoxam to *Apis mellifera lamarckii* forager after 48 h of exposure by different methods. Six concentrations of deltamethrin and eight concentrations of thiamethoxam were used

Insecticides	Method of exposure	Slope ± SEM	Het[a]	LC_{50}[b]	95% CL[c]	LC_{90}	95% CL
Delta 2.5EC (deltamethrin)	Acute contact (1µL drop on thorax)	1.28±0.22	0.069	19.44	11.76-30.92	195.5	99-669
	Acute contact (Potter tower spray)	1.38±0.26	0.071	22.35	12.19-34.38	189.5	99-663
	Acute oral	0.95±0.23	0.044	70.99	37.77-217.91	1564.0	401-53100
Actara 25WG (thiamethoxam)	Acute contact (1µL drop on thorax)	1.73±0.43	0.996	23.36	12.10-34.54	132.0	76-554
	Acute contact (Potter tower spray)	0.77±0.13	0.699	8.05	3.85-15.61	367.0	127-2690
	Acute oral	1.24±0.18	0.816	0.22	0.11-0.36	2.4	1.42-5.33

[a] Het, heterogeneity adjustment factor;
[b] LC, lethal concentration expressed as µg/mL;
[c] 95% confidence limits.

The slopes of regression lines for deltamethrin acute contact (drop on thorax) and acute oral exposure methods were equal and the hypothesis of parallelism was accepted (P = 0.344). For thiamethoxam the slopes were not equal and the hypothesis of parallelism was rejected (P = 0.002). The lethal dose ratio (LDR) 95% confidence limits were <1 for deltamethrin and only acute contact (drop on thorax) was significantly different than acute oral exposure and there was no significantly different between the two contact exposure methods (Table 3). The calculated LDR for thiamethoxam was not meaningful since the hypothesis of parallelism was rejected. However, regression analysis by PoloPlus showed that acute oral regression line was widely separated from the contact lines indicating that oral LC_{50} values for thiamethoxam were significantly lower than both contact LC_{50} (Table 3).

Table 3. Parallelism hypothesis and lethal dose ratio of deltamethrin and thiamethoxam at LC_{50} after 48 h acute contact (drop on thorax). Two hundred and forty, and 320 honeybees were used for acute contact (deltamethrin) and acute oral (thiamethoxam), respectively

Method of exposure	df	Parallelism (Chi-square)	Lethal Dose Ratio (95% CI)	
			Acute contact (Potter tower)	Acute oral (Feeding honey)
Deltamethrin	2	2.13 (P = 0.344)[a]	0.870 (0.450-1.68)	0.274 (0.111-0.677)[b]
Thiamethoxam	2	12.2 (P = 0.002)	2.90 (1.30-6.50)	105 (51.6-215)

[a] Parallelism hypothesis is not rejected at P > 0.05;
[b] If 95% confidence interval does not include 1, then LD_{50} is significantly different between exposure methods and between insecticides.

In total, 1070±106 droplets were deposited on a single worker honeybee after spray by the Potter tower (Table 4, Figure 3). The average number of droplets deposited on head and thorax of a honeybee were similar but significantly greater than abdomen, legs and wings (F = 23.3, P < 0.001) (Table 5). To the best of our knowledge this information was not available before. The average droplet diameter was 0.286±0.071 µm. The total calculated volume of an insecticide deposited on a honeybee was 0.829 µL (0.669-0.990 µL) and was used in contact LD_{50} calculation. About 66% of the total spray volume was deposited on head and thorax (Table 4).

Table 4. Amount (ng/bee±SEM) of contaminated food (20% honey solution) consumed by individual forager bee. Two hundred and forty honeybees were used for each insecticide

Concentration (µg/mL)	Thiamethoxam	Acetamiprid	Deltamethrin
90	--*	722±81.8	2480±1870
30	--	188±32.1	6750±1190
10	90.0±12.4	40.0±12.8	1750±328
3.33	63.3±2.8	14.5±2.5	500±47
1.11	3.36±0.8	7.4±1.7	167±38
0.37	2.13±0.7		
0.12	2.82±0.2		
0.04	0.72±0.04		

* All honeybees were dead in 30 and 90 µg/mL concentration in thiamethoxam treatment.

Table 5. Number of droplets and droplet volume deposited on different body parts of forager honeybee when sprayed under Potter tower at 70 kPa. In brackets lower and upper limits at 95%. Tukey's test was used for means separation

Body part	Average number of droplets	Total volume (µL)	Percentage
Head	305a* (222-388)	0.236a (0.172-0.300)	28.5a (25.8-30.6)
Thorax	403a (311-494)	0.312a (0.241-0.383)	37.6a (35.8-39.1)
Abdomen	113b (98-127)	0.087b (0.076-0.099)	10.5b (8.3-13.4)
Legs	108b (98-117)	0.083b (0.076-0.091)	10.1b (8.9-11.5)
Wings	143b (106-179)	0.130b (0.082-0.139)	13.3b (10.9-15.7)
Total	1070 (863-1277)	0.829 (0.669-0.990)	

* Values followed by the same letters within column are not significantly different at $\alpha_{0.05}$.

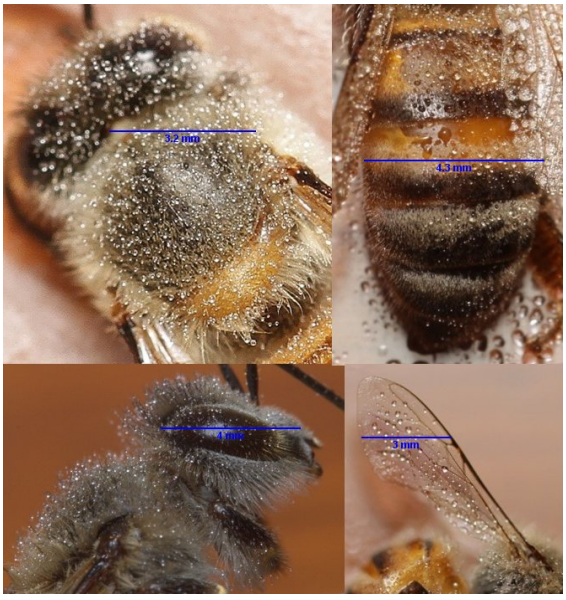


Figure 3. Deposition of droplets on different body parts of a forager honeybee through Potter spray tower at 70 kPa. [Actual sizes: head (4.0±0.5 x 1.0±0.1 mm); thorax (4.7±0.3 x 4.3±0.3 mm); abdomen (6.0±0.5 x 4.3±0.3 mm) and wings (8.3±0.3 x 3.0±0.5 mm)].

The amount of honey solution consumed by a single worker honeybee in 2 h varied according to the concentration and type of insecticide (Table 3). The amount (ai) of deltamethrin consumed decreased from 2480±1870 to 167±38 ng/bee with decrease in concentration. Acetamiprid consumed reduced from 722±81.8 to 7.44±1.7 ng/bee between the highest and the lowest concentration. The consumption of thiamethoxam was very low (90.0±12.4 and 0.72±0.04 ng/bee) compared to the other two insecticides. There was no consumption data for the 30 and 90 mg/mL concentrations of thiamethoxam recorded because of very low non-measurable quantities consumed. The uncontaminated control group consumed 27000±2350 ng of 20% honey/bee (Table 4).

The hazard quotients ($HQ_{contact}$ and HQ_{oral}) for deltamethrin and thiamethoxam (Table 6) were calculated based on the average amount of received by a single honeybee or food consumed by a single forager honeybee contaminated with each insecticide. The average (all concentrations) deltamethrin and thiamethoxam contaminated consumed food was 9.87±1.3 and 12.0±1.7 µg/bee, respectively. The $HQ_{contact}$ values were low and the tested insecticides are less harmful to foraging honeybees. HQ_{oral} values indicate that thiamethoxam is extremely toxic to honeybees when ingested (Table 6). Hazard quotients provide an estimate of the risk in comparing the application rate of an insecticide and its intrinsic toxicity and they aim at deciding whether high tier testing is needed (pp1.eppo.int/standards/PP1-170-4).

Table 6. Lethal concentrations (LC_{50}, LD_{50}) and hazard quotients (HQ) for deltamethrin and thiamethoxam 48 h after exposure. In brackets lower and upper limits at 95%

Insecticide	Method of exposure	LC_{50} (µg/mL)	LD_{50} (ng/bee)	HQ
Deltamethrin	Acute contact (drop on thorax)	19.4	19.4 (11.8-30.9)	0.62 (0.38-1.02)
	Acute contact (Potter tower)	22.4	19.0[a] (10.1-28.5)	0.65 (0.51-1.43)
	Acute oral (feeding honey)	71.0	710[b] (378-2180)	16.9 (5.51-31.8)
Thiamethoxam	Acute contact (drop on thorax)	23.4	23.4 (12.1-34.5)	0.51 (0.35-0.99)
	Acute contact (Potter tower)	8.05	6.68 (3.19-13.0)	1.79 (0.92-3.76)
	Acute oral (feeding honey)	0.222	2.66[c] (1.32-4.32)	3750 (2310-7580)

[a] Potter tower deposited 0.83 µL/bee;
[b] Deltamethrin average oral consumption of 9.87 µg/bee;
[c] Thiamethoxam average oral consumption of 12.0 µg/bee.

Discussion

Contact bioassays are preferred as they better simulate the situation in the field. The standard contact bioassay requires a 1-µL droplet placed on the thorax of an insect (e.g., honeybee). The diameter of a 1-µL droplet recorded on honeybee thorax was 1200±150 µm. A medium spray (VMD of 350 µm) droplet size is recommended for insecticides in field which can be achieved by using cone and fan nozzles (Hewitt et al., 1997; Hanna et al., 2009). The Potter tower spray produced a VMD of 16±3.7 µm. The optimum size for insecticide spray droplets for the highest efficacy was about 20 µm in diameter, whereas droplets 50-100 µm in diameter were marginal in efficiency (Himel, 1969). The average droplet size produced by the Potter spray tower at 70 kPa was similar to the most effective droplet size (20 µm). The size of the droplet on thorax was three times larger whereas the droplets produced by the Potter tower were closer to the field spray droplet sizes. The medium spray droplet size reduced to very fine (VMD of 50 µm) soon after application due to environmental conditions. Forager honeybees are more likely experience the very fine droplets. The fine droplets produced by the Potter tower gave good coverage of the body and all body parts, including eyes were covered which would contribute to quicker/greater

absorption and higher mortality with all insecticides. Whenever possible, honeybee toxicology studies should use Potter tower or similar devices instead of a single droplet on thorax.

Mortality of forager honeybee when exposed to different concentrations of deltamethrin, thiamethoxam and acetamiprid varied between the different exposure methods. Contact exposure mortality was higher than oral exposure mortality in deltamethrin and acetamiprid treated honeybees while oral exposure was higher in thiamethoxam treated honeybees because deltamethrin is a contact and thiamethoxam is a systemic insecticide. Acetamiprid is a systemic insecticide with translaminar activity and cause mortality both by contact and ingestion (PPDB, 2019). The small spray droplets by Potter tower could have been easily and rapidly absorbed. Potter tower exposure gave higher mortality than droplet on thorax exposure for all the three insecticides. The nitro-substituted neonicotinoids like thiamethoxam are found to be the most toxic to the honeybee in laboratory studies, however, the cyano-substituted neonicotinoids like acetamiprid exhibited a much lower toxicity (Iwasa et al., 2004). Although acetamiprid is a neonicotinoid insecticide, it is much safer to forager honeybees than thiamethoxam (Laurino et al., 2011).

The 48-h oral LD_{50} of deltamethrin was significantly higher (710 ng/bee) compared to either contact exposure by a droplet on thorax (28.3 ng/bee) or Potter tower (18.6 ng/bee). Deltamethrin can be more toxic to honeybees if directly exposed during spray operations, which is a more likely scenario for forager bees. There is a range of acute contact LD_{50} values found in literature. The topical (1-µL droplet on thorax) LD_{50} of 24 ng/bee, 50.7 ng/bee and 677 ng/bee have been reported (Mayer, 1999; Carvalho et al., 2013; Sanchez-Bayo & Goka, 2014). Our calculated acute contact LD_{50} values for deltamethrin, although on the lower side, are within the reported range. An acute oral LD_{50} of 270 ng/bee and 850 ng/bee of deltamethrin have been reported (Carvalho et al., 2013; Sarto et al., 2014).

Thiamethoxam gave significantly lower oral LC_{50} (0.222 µg/mL) compared to both contact exposure methods. The calculated oral LD_{50} was 2.66 ng/bee based on the average consumption of 12 µL/bee. An oral LC_{50} of 0.150 µg/mL and an LD_{50} of 4.41 ng/bee was calculated based on the average consumption of 35 µL/bee (Laurino et al., 2011). An oral LD_{50} of 5.0 ng/bee has also been reported (Tomlin, 2003; Decourtye & Devillers, 2010). In our control group the average consumption was 29±2.9 µL/bee but consumption of treated honey was different. Since neonicotinoid insecticides are systemic, they have less contact activity. Thiamethoxam acute contact LD_{50} (by a droplet on thorax) of 23.36 ng/bee, 24 ng/bee and 30 ng/bee are also available in literature (Senn et al., 1998; Iwasa et al., 2004; Decourtye & Devillers, 2010).

The thiamethoxam slopes for exposure methods were not parallel and the hypothesis of parallelism was rejected, therefore, the calculated LDR was not meaningful. However, the acute oral regression line was widely separated from the contact lines, which explains the significant difference between oral and contact exposure methods.

The droplets deposited with the Potter spray tower gave good coverage of the body of a bee. The presence of larger number of droplets on head and thorax were because of the presence of hair. The total calculated volume deposited on body was <1 µL which is a standard droplet size on thorax but the recorded mortality was usually higher. The higher mortality can be attributed to even spread and quicker absorption of smaller droplets. The authors could not find any reference for comparison.

At the same highest and lowest concentrations, the consumption of acetamiprid decreased 34 and 22 times, respectively, compared to deltamethrin. There was 34 times and 22 times decrease in the consumption of acetamiprid compared to deltamethrin when offered the same highest and lowest concentrations, respectively. A fiftyfold decrease was recorded in the thiamethoxam consumption at the concentration of 1.11 µg/mL compared to deltamethrin. Acetamiprid can act as a repellent and this repellency effect may increase at lower concentrations (Laurino et al., 2011). Food regurgitation and vomiting by poisoned honeybees at higher concentrations of thiamethoxam can occur (Laurino et al., 2011). The lowest concentration of thiamethoxam 0.12 µg/mL caused 44% mortality. Ninety-one and 6.7 ng/mL

of deltamethrin, and 127 and 17 ng/mL of thiamethoxam have been found in the pollen and nectar, respectively (Scott-Dupree et al., 2001; Chauzat et al., 2011; Stoner & Eitzer, 2013). These low concentrations of thiamethoxam can be toxic to forager honeybees through oral ingestion. The very high HQ_{oral} value of 3750 (2310-7580) indicates that thiamethoxam is extremely toxic to honeybees when ingested. HQ value of 22700 for thiamethoxam with an LD_{50} of 4.41 ng/bee has been reported (Laurino et al., 2011). The higher HQ is due to the higher value LD_{50} which is dependent on the amount consumed.

Bees are generally active from sunrise until a couple of hours before sunset, and pesticide risk exposure to honeybees can be reduced by spraying the crops in the evening when honeybees are not foraging. Some countries, for example Canada and USA, have strict drift prevention protocols and use specific devices, and spray droplets in air may not be of concern. There must be proper communication between the applicators, farmers and beekeepers, and beekeepers should be informed of any spraying operations so they can protect their beehives.

Forager honeybees are more likely experience the very fine droplets in field and acute contact toxicological results obtained by Potter tower or similar devices will be more realistic than a single droplet on thorax. Insecticide-contaminated food consumed by forager honeybees was significantly lower than the control, and the actual amount of food consumed at each concentration could be used in LD_{50} calculations.

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