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Context-Dependent Effect of Dietary Phytochemicals on Honey Bees Exposed to a Pesticide, Thiamethoxam

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Abstract

Honey bees continue to face challenges relating to the degradation of natural flowering habitats that limit their access to diverse floral resources. While it is known that nectar and pollen provide macronutrients, flowers also contain secondary metabolites (phytochemicals) that impart benefits including increased longevity, improved gut microbiome abundance, and pathogen tolerance. Our study aims to understand the role of phytochemicals in pesticide tolerance when worker bees were fed with sublethal doses (1 ppb and 10 ppb) of thiamethoxam (TMX), a neonicotinoid, in 20% (w/v) sugar solution supplemented with 25 ppm of phytochemicals—caffeine, kaempferol, gallic acid, or *p*-coumaric acid, previously shown to have beneficial impacts on bee health. The effect of phytochemical supplementation during pesticide exposure was context-dependent. With 1 ppb TMX, phytochemical supplementation increased longevity but at 10 ppb TMX, longevity was reduced suggesting a negative synergistic effect. Phytochemicals mixed with 1 ppb TMX increased mortality in bees of the forager-age group but with 10 ppb TMX, mortality of the inhive-age group increased, implying the possibility of accumulation effect in lower sublethal doses. Given that the phytochemical composition of pollen and nectar varies between plant species, we suggest that the negative impacts of agrochemicals on honey bees could vary based on the phytochemicals in pollen and nectar of that crop, and hence the effects may vary across crops. Analyzing the phytochemical composition for individual crops may be a necessary first step prior to determining the appropriate dosage of agrochemicals so that harm to bees *Apis mellifera* L. is minimized while crop pests are effectively controlled.

Key words: honey bee, phytochemical, plant-pollinator interaction, pollinator, synergism, thiamethoxam

The ability of an organism to tackle environmental challenges is strongly dependent on its nutrition. In honey bees and bumblebees, it has been shown that poor or imbalanced nutrition could lead to increased susceptibility to various biotic and abiotic stressors (Schmid-Hempel 2005, Alaux et al. 2010). Of the different abiotic stressors, exposure to harmful agrochemicals is an existential threat that endangers the maintenance of biodiversity and ecosystem stability by adversely affecting populations of beneficial insects including pollinators such as managed honey bees (Potts et al. 2010, Godfray et al. 2014, Potts et al. 2016). Pollen and nectar gathered by foraging honey bees provide essential macronutrients (proteins, carbohydrates, and lipids) that ensure proper brood development (Di Pasquale et al. 2013). In addition to these macronutrients, honey bees also obtain various plant secondary metabolites (phytochemicals) from floral nectar and pollen. Specific phytochemicals in pollen and nectar provide a variety of health benefits to bees, including extended longevity, improved pathogen and environmental stress tolerance (Masai Biller 2015, Palmer-Young et al. 2017a, Giacomini et al. 2018, Bernklau et al. 2019), pesticide detoxification (Mao et al. 2013, Liao et al. 2017), building gut microbiome abundance (Geldert et al. 2021) and enhanced cognition and memory (Wright et al. 2013).

The coevolutionary mutualism between pollinators and plants has likely enabled pollinators including honey bees to derive benefits from these compounds (Parachnowitsch et al. 2018, Negri et al. 2019). It has been demonstrated that plant secondary metabolites being plant defense compounds are toxic to some floral visitors (Adler 2000, Adler et al. 2006, Palmer-Young et al. 2019). While some visitors may be deterred by the presence of such compounds in floral nectar, such deterrence may depend on the ecological context based on the availability of alternative sources of nectar (Gegear et al. 2007). It has also been shown that foraging on toxic nectar leads to the accrual of benefits for pollinating bumblebees where the anti-microbial properties of plant secondary compounds reduce the intensity of pathogen infections (Manson et al. 2010, Richardson et al. 2015, Thorburn et al. 2015, Palmer-Young et al. 2017c). Similar benefits of plant secondary compounds have been demonstrated in laboratory studies where honey bee workers artificially infected with a pathogen while receiving supplemental doses of certain

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phytochemicals, demonstrated a decrease in spore load (Bernklau et al. 2019), or had upregulated immunity genes (Mao et al. 2013).

As evidence mounts for the beneficial properties of plant secondary compounds on pollinators (McArt et al. 2014, Gillespie et al. 2015, Masai Biller 2015, Palmer-Young et al. 2017b, Palmer-Young et al. 2017c), it is also becoming evident that the composition of secondary compounds in floral nectar varies across different plant species (Palmer-Young et al. 2019). The impact of these floral chemicals on pollinators and other floral visitors will depend on the chemical compound, its dose, and the chemical context specifically the other chemicals in the nectar and how they interact (Palmer-Young et al. 2019). In addition to synergistic interactions between naturally occurring compounds in floral nectar, it is likely that agrochemicals applied to crops could lead to additional interactions that are not currently well understood. Of the different agrochemicals, neonicotinoids, a class of neurotoxic pesticides, have received considerable attention. Systemic application enables these toxins to pervade floral pollen and nectar and find an easy route to queens, larvae, and newly emerged workers (Schmuck et al. 2001, Johnson et al. 2010, Osterman et al. 2019). Sublethal effects impact foraging success, cognitive capacities, and development of brood, workers, and queens (Decourtye et al. 2003, 2004a, b, Henry et al. 2012, Wu-Smart and Spivak 2016). Furthermore, with the knowledge that phytochemical composition varies across plant species (McArt et al. 2014, Giacomini et al. 2018, Palmer-Young et al. 2019, Fowler et al. 2020), it is likely that the range of impact that agrochemicals have on bees may be crop-specific relating to the nectar composition but this has been seldom investigated.

Our earlier studies demonstrate the benefits of four phytochemicals—caffeine, kaempferol, gallic acid, and *p*-coumaric acid on worker honey bees. Supplementing sucrose solutions with these phytochemicals at 25 ppm concentrations increased longevity and pathogen tolerance in newly emerged bees (Bernklau et al. 2019) and improved gut microbiome abundance and diversity (Geldert et al. 2021). Two of these compounds, gallic acid, and *p*-coumaric acid are phenolic acids, caffeine is an alkaloid while kaempferol is a flavonol. To address whether agrochemicals interact with these floral compounds, the current study explores in a controlled setting, the interaction of two sublethal doses of Thiamethoxam, a neonicotinoid, and the four phytochemicals previously shown to benefit honey bees. If phytochemical supplementation during pesticide exposure is beneficial, we predict improved survival in bees receiving the supplementation. Leading from studies that indicate minor tolerance to pyrethroid pesticides from *p*-coumaric acid (Liao et al. 2017) and the potential for "precision nutrition" to strengthen managed pollinators (Negri et al. 2019), our study aims to test the impacts of low doses of four phytochemicals—caffeine, kaempferol, gallic acid, and *p*-coumaric acid, on the longevity of worker honey bees fed with two sublethal doses of the Thiamethoxam.

Methods

Age-Cohort Bees

Three full-sized colonies of *Apis mellifera* were used for this study. Single age-cohort bees were obtained using the standard queen restraining procedure wherein queens in experimental colonies were provided with empty, uniquely marked frames to lay their eggs. The queen was caged in this empty frame for 24 h to ensure that all the eggs on the frame were laid during that duration. The date when the queen was caged and hence the date of egg-laying was marked on the frame. The queen was released the following day. The marked frames containing late-stage pupae were removed from source colonies after 18 d and placed in an incubator at 32℃ and 50% humidity until the day of emergence (Arathi et al. 2000, Arathi and Spivak 2001). Worker bees from all three colonies were used for the study, taking care to ensure that bees from the same source colony were placed together in feeding cages. Data from different colonies were pooled for each feeding treatment following no detectable colony effects on worker bee survival.

Supplementary Diet

A concentration of 25 ppm of each of the four phytochemicals (caffeine (C), kaempferol (K), gallic acid (G), or *p*-coumaric acid (P)) in 20% (w/v) sucrose, previously shown to improve longevity, pathogen tolerance (Bernklau et al. 2019) and gut microbiome abundance (Geldert et al. 2021) in similarly aged worker bees, were used as dietary supplements in this study. Bees were exposed to two sublethal doses (1 ppb and 10 ppb) of the common neonicotinoid pesticide, thiamethoxam (TMX) (Laycock et al. 2014, Woodcock et al. 2017). Stock solutions (50 mg/ml) of individual phytochemicals (C,G,K,P) and TMX (0.1 μg/ml) were prepared separately in acetone and used for making feeding solutions in 20% (w/v) sucrose following established protocols (Bernklau et al. 2019). A total of 11 different treatment feeding solutions as shown in Table 1 were prepared and provided to bees *via* syringe feeders inserted into individual cup-cages (Evans et al. 2009, Bernklau et al. 2019). Bees exposed to these dietary treatments were held in cup-cages such that each cage had 5 to 10 bees (Evans et al. 2009; Bernklau et al. 2019). The cup-cages were maintained at 30–32°C where bees had ad libitum access to the respective treatment diet. The different treatments, number of cup-capes per treatment, and the number of bees in each treatment are detailed in Table 1.

Feeding Assays

Newly emerged bees were acclimatized to cages by providing *ad libitum* access to 20% sucrose solution for a 2-day period after which 10 bees were allocated into each of the cup cages that contained 25 ppm of a single phytochemical feeding solution in 20% (w/v) sucrose or sucrose-only control feeding solution such that, there were a total of six cages—one for each of the four phytochemicals and two cages for sucrose-only. This was done to provide all bees with the phytochemical diet prior to exposing them to the pesticide. Bees were maintained on the individual phytochemical in sucrose solution diet for seven days, after which, the individual phytochemical feeding solutions were replaced with the treatment solutions—phytochemical + TMX (1 ppb or 10 ppb) diet, sucrose-only controls,

Table 1. Different treatment solutions, numbers of bees in the different treatment diets and the total number of cup-cages (numbers in parentheses) set up for the 11 different treatment solutions

Feeding treatments in 20%(w/v) sucrose solution	1 ppb TMX (cages)	10 ppb TMX (cages)
Caffeine (25 ppm) + TMX	88 (10)	57 (7)
Gallic acid (25 ppm) + TMX	83 (10)	66 (7)
Kaempferol (25 ppm) + TMX	91 (10)	63 (7)
p-coumaric acid (25 ppm) + TMX	93 (10)	61 (7)
TMX + sucrose	92 (10)	65 (7)
Sucrose-only control*	95 (10)	65 (7)

*Two sucrose-only control cages were setup, paired with each TMX sublethal dose.

and pesticide controls (Table 1). Thus, 9-d old bees were exposed to the phytochemical + TMX diet and continued to receive that diet until they died. Accordingly, bees that died later in the experiment had consumed more of the treatment diet. Dead bees were removed, the date of death was recorded each day, and the solutions were refreshed weekly. Therefore, bees that died later in the study fed on the pesticide + phytochemical diet for a longer period than bees that died earlier in the study. Although consumption of dietary solutions was not recorded, bees feeding on 1 ppb TMX are expected to have consumed lower total amounts of the active ingredient of TMX as compared to bees feeding on 10 ppb TMX.

Statistical Analyses

All statistical analyses were performed using IBM Statistics SPSS 26. Kaplan-Meier survival probability estimates followed by post-hoc comparisons evaluated the differences in worker honey bee survival as affected by treatment diets. Breslow Generalized Wilcoxon tests were used for post-hoc comparisons of survival curves between treatments. Additionally, the median longevity values between treatments were compared by Independent samples medians test after instituting Bonferroni correction for multiple comparisons (Sokal and Rohlf 2012). The survival data for each treatment were categorized into two groups at 20 d on the survival curve—(i) number of bees that died prior to the 20-d point (younger or in-hive bees: 9–20 d) and (ii) number of bees that died after the 20-d point (older or forager-aged bees >21 d). The 20-d point was used to categorize the mortality data for analyzing survival difference because, at the age of about 20 d, bees begin to work outside the hive as foragers and are physiologically and behaviorally different from those younger than 20 d that mainly perform inhive tasks (Seeley 1982, 1995). This categorization allowed us to determine whether the proportion of dead bees in the two groups was the same as that expected from random mortality or if treatments affected mortality non-randomly. If proportion mortality was random, then the expectation is that half the treatment bees would die under the age of 20 d and the other half would die over the age of 20. Since bees that died later in the experiment consumed more of the treatment solution than bees that died earlier in the study, this categorization allowed for testing whether there was a potential accumulation effect of TMX seen as non-random mortality frequency of forager-aged bees. G test of heterogeneity compared the proportion of dead bees in the two groups for each of the phytochemical + TMX treatment (Sokal and Rohlf 1999, 2012).

Results

Our results indicate that the effects of dietary phytochemical + TMX on worker honey bees depend on the concentration of TMX and the phytochemical. When worker bees received the lower dose of TMX, the phytochemical + 1 ppb TMX diet increased longevity (Fig. 1; Table 2). Significantly higher median longevity values were recorded for all phytochemicals + 1 ppb TMX treatments as compared to bees in the sucrose solution + TMX control and sucrose-only control. There was no significant difference in median longevity between sucrose-only control bees and sucrose solution + 1 ppb TMX control. Bees receiving caffeine + 1 ppb TMX showed the highest median longevity of 34 d. A similar increase in longevity was however not seen in bees that received phytochemical + 10 ppb TMX (Fig. 1; Table 2). Median longevity values for bees receiving phytochemical + 10 ppb TMX were significantly lower than both sets of control bees. Bees receiving sucrose-only diet showed the highest median

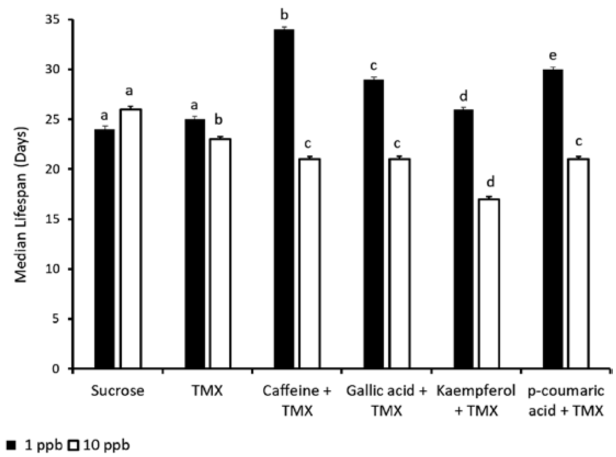

Fig. 1. Median (+SE) survival of worker honey bees exposed to thiamethoxam (TMX) in sugar solution supplemented with phytochemicals at 25 ppm. Statistical comparisons were across all treatments within each TMX dose (Table 2). Bars with the different alphabets are significantly different by independent samples medians test at $P < 0.05$.

longevity of 26 d which was significantly higher than the median longevity of bees that received 10 ppb TMX in sucrose solution control. Median longevity was 21 d in the three treatments: caffeine + 10 ppb TMX, gallic acid + 10 ppb TMX, and *p*-coumaric acid + 10 ppb TMX and was not significantly different between each other. Bees receiving kaempferol + 10 ppb TMX exhibited the lowest median longevity of 17 d showing that phytochemicals can synergize with the higher sublethal 10ppb dose TMX and reduce longevity.

Kaplan-Meier survival analyses also revealed a similar trend with a significant effect of phytochemical + TMX on survival and the Breslow Generalized Wilcoxon pairwise post-hoc comparison of survival curves across treatments followed the same trend as median longevity (Fig. 2; Table 3). Survival curves for each of the four phytochemicals + 1 ppb TMX were significantly different from each other and from the two controls. There was no difference between survival curve comparisons for the two control sets of bees (Table 3). For bees that received phytochemical + 10 ppb TMX, the Breslow Generalized Wilcoxon pairwise post-hoc comparison across treatments revealed significant differences between caffeine + 10 ppb TMX and kaempferol + 10 ppb TMX, sucrose-only control, and 10 ppb TMX + sucrose control. The survival curves were similar for caffeine, gallic acid, and *p*-coumaric acid, all with10 ppb TMX.

Figure 2 indicates a difference in survival decrease for 1 ppb TMX exposure and 10 ppb TMX exposure manifesting after 20 d. To further understand this difference, we categorized bees into two groups, those that died prior to the 20-d point (inhive-aged bees) and those that died after the 20-d point (forager-aged bees). Comparison of the frequency of mortality of bees in these two groups for each of the treatments indicates that higher than randomly expected proportions of forager-aged bees died in phytochemical + 1 ppb TMX diet. Higher than randomly expected proportions of inhive-aged bees died in the phytochemical + 10 ppb TMX diets (Fig.3; Table 4). Mortality frequencies in the sucrose-only control fitted the proportions expected by random with no significant difference in the proportions of bees that died in the two groups. While, in the TMX + sucrose solution control, the mortality frequency in the 10 ppb TMX dose fitted proportions expected by random with no significant difference in the proportions of bees that died in the two groups, a significantly higher than expected proportion of bees in the forager-aged bees

Table 2. Independent sample medians test (Sokal and Rohlf 2012) comparing median age at mortality across treatments after Bonferroni correction for multiple comparisons (Fig. 1)

Treatments	Caffeine + TMX		Gallic Acid + TMX		Kaempferol + TMX		*p*-coumaric acid + TMX		TMX	
1ppb TMX										
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Gallic acid + TMX	231.13	< 0.001								
Kaempferol + TMX	525.07	< 0.001	95.37	< 0.001						
p-coumaric acid + TMX	154.27	< 0.001	6.97	0.008	187.42	< 0.001				
TMX	753.88	< 0.001	204.16	< 0.001	29.83	< 0.001	340.08	< 0.001		
Sucrose only	801.55	< 0.001	228.09	< 0.001	38.21	< 0.001	370.24	< 0.001	0.52	0.47
10ppb TMX										
Treatments	Caffeine + TMX		Gallic Acid + TMX		Kaempferol + TMX		*p*-coumaric acid + TMX		TMX	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Gallic acid + TMX	0.04	0.8								
Kaempferol + TMX	135.62	< 0.001	167.70	< 0.001						
p-coumaric acid + TMX	1.99	0.159	3.24	0.072	194.57	< 0.001				
TMX	49.82	< 0.001	58.83	< 0.001	386.94	< 0.001	33.11	< 0.001		
Sucrose only	251.5	< 0.001	264.85	< 0.001	716.58	< 0.001	226.59	< 0.001	90.44	< 0.001

Bold font indicates statistical significance.

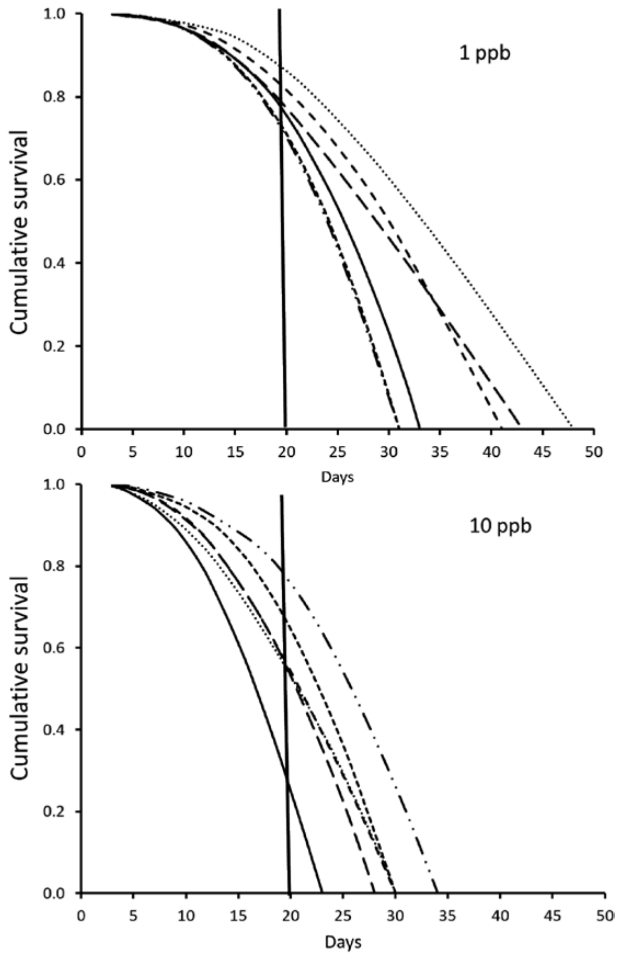

Fig. 2. Survival curves of worker honey bees fed thiamethoxam (TMX) in sugar solution supplemented with phytochemicals at 25 ppm. Kaplan-Meier survival analyses and post-hoc Breslow (Generalized Wilcoxon's) tests compared survival across treatments for each dose of TMX. Table 3 provides the significance values for the different comparisons. Sugar-only control overlapped with TMX + sugar control in 1 ppb. Vertical line at 20 d is the point for categorizing data on frequency of dead worker honey bees prior to and after 20 d (Fig. 3). Sucrose TMX control Caffeine + TMX Gallic Acid + TMX Kaempferol + TMX *p*-coumaric acid + TMX

group died in the 1 ppb TMX + sucrose solution control. (Table 4). G-heterogeneity was significant for both experiments (TMX 1 ppb: $G_H = 13.12$; df = 5, $P = 0.05$; TMX 10 ppb: $G_H = 36.59$; df = 5, $P = 0.01$). Figure 3 shows proportions of dead bees in the in-hive-aged group for each of the treatments and controls.

Discussion

Here, we present evidence showing that the impacts of sublethal doses of a neonicotinoid, thiamethoxam differs depending on the phytochemical supplement in the worker honey bee diet. We suggest that although phytochemicals in pollen and nectar may help improve the ability of honey bees to tolerate the pesticide, the benefits depend on the phytochemical and the dose of agrochemical. Similar results have been reported in previous studies (Berenbaum 2015, Liao et al. 2017, Negri et al. 2019), where access to phytochemicals in the diet allowed bees to live longer even when workers are exposed to sublethal doses of neonicotinoids. Low dose of caffeine has been suggested

Table 3. Kaplan-Meier survival analyses followed by Breslow Generalized Wilcoxon pairwise post-hoc comparison of survival curves (Fig. 2) across treatments

Treatments	Caffeine + TMX		Gallic Acid + TMX		Kaempferol + TMX		*p*-coumaric acid + TMX		TMX	
	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
1ppb TMX										
Gallic acid + TMX	231.13	< 0.001								
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Bold font indicates statistical significance.

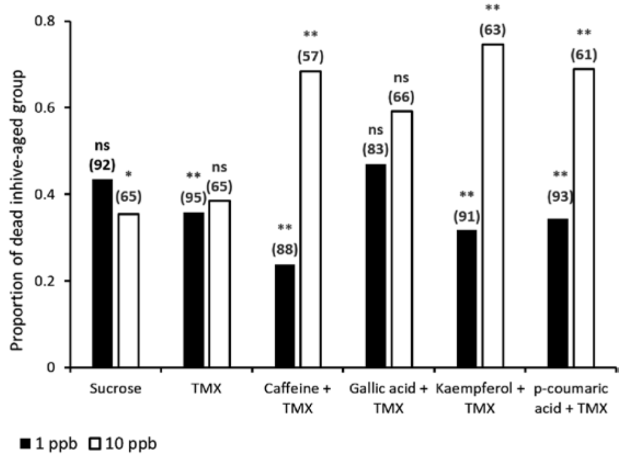

Fig. 3. Proportion mortality of the inhive-aged group of worker honey bees defined by the 20-day point on the survival curve in Fig. 2 for the two doses of Thiamethoxam. G-test of proportions compared frequencies of dead worker honey bees in the two groups (inhive-aged and forager-aged) for each pesticide dose. Statistical significance between the two groups is indicated as * $P < 0.05$, ** $P < 0.001$ and ns non-significant differences. Numbers in parentheses refer to the total number of worker honey bees in the respective treatment (Table 4).

to pharmacologically manipulate bee behavior by improving learning and memory, thereby promoting consumption of caffeine (Wright et al. 2013). Improved longevity in bees receiving the low sublethal dose of thiamethoxam supplemented with caffeine adds to these known benefits. However, the benefit we report being limited to the lower dose of thiamethoxam also implies a negative synergistic effect between phytochemicals and a higher dose of thiamethoxam. We did not record the consumption of treatment solutions and therefore it was not possible to determine the total amount of thiamethoxam consumed by the bees in the two sublethal doses. However, our earlier study demonstrated that bees do not show a differential preference for the phytochemicals (Bernklau et al. 2019). Assuming that worker bees consumed similar amounts of treatment diets, those in the higher sublethal dose of TMX are likely to have ingested higher amounts of the active ingredient than those receiving the lower sublethal dose of TMX impacting the longevity of treatment bees. While some neonicotinoids such as imidacloprid have been shown to suppress consumption of nectar (Cresswell et al. 2012, Laycock et al. 2012), reports on the exposure of thiamethoxam in foraging bumblebees showed similarly reduced longevity even though thiamethoxam may not elicit a strong feeding repression (Laycock et al. 2014). In addition, feeding on neonicotinoids impairs foraging affecting the amounts of active ingredients of the chemical actually consumed by bees while foraging on pesticide-treated crops (Muth and Leonard 2019).

Phytochemicals available to foraging bees depends on the plant species in the foraging range. Each plant species offers a different mix of phytochemicals in its pollen and nectar (Palmer-Young et al. 2019) and the mix depends on the physiological status of the plant (e.g., drought-induced responses Arathi et al. 2018). Therefore, the effect of insecticide exposure on pollinating bees may not be dependent solely on the dose of insecticide but also on the plant species that are receiving the insecticide application and are in the foraging range of bees. Specifically, looking into floral sources for the tested phytochemicals, although there are not many studies documenting the phytochemical composition of nectar and pollen

Table 4. Frequencies of dead bees in the inhive-aged and forager-aged groups

Treatments (1ppb TMX)	Inhive-aged	Forager-aged	G (df)	*P*
Caffeine + TMX	21	**67**	25.28 (1)	**< 0.001**
Gallic acid + TMX	39	44	0.30 (1)	ns
Kaempferol + TMX	29	**62**	12.24 (1)	**< 0.01**
p-coumaric acid + TMX	32	**61**	9.20 (1)	**< 0.01**
TMX + sucrose	34	**61**	7.78 (1)	**< 0.01**
Sucrose only	40	52	1.57 (1)	ns
Treatments (10ppb TMX)				
Caffeine + TMX	**39**	18	7.92 (1)	**< 0.001**
Gallic acid + TMX	39	27	2.19 (1)	ns
Kaempferol + TMX	**47**	16	15.94 (1)	**< 0.01**
p-coumaric acid + TMX	**42**	19	8.89 (1)	**< 0.01**
TMX + sucrose	25	40	3.49 (1)	ns
Sucrose only	23	42	5.64 (1)	ns

Comparisons are within each treatment (Fig. 3) between the observed frequencies against an expected random frequency for each group (Sokal and Rohlf 2012). Bold font indicates statistical significance.

of different plant species, caffeine is found in the nectar of coffee and citrus flowers (Singaravelan et al. 2005). Citrus orchards benefit from pollination services provided by bees and managed colonies are regularly brought into citrus orchards for pollination. Kaempferol is found in canola (Arathi et al. 2018) and sunflowers (Sharma 2019) and both these crops provide significant economic returns from pollination services fulfilled by honey bees (Bond et al. 2014). Our results imply that kaempferol and caffeine may synergize with the higher sublethal doses of thiamethoxam reducing median longevity of worker honey bees, suggesting increased caution in approving dosages of neonicotinoid agrochemicals on these crops.

Gallic acid is found in flowers of plants from the Malvaceae family which are known to be preferred hosts of certain native bees including *Diadasia* and *Melitoma* (Wilson and Carril 2016) and *p*-coumaric acid is a component of sweet clover, *Mellilotus* which is a weedy plant found growing on field margins and a preferred foraging resource for honey bees. As crop fields and orchards regularly experience pest attacks and receive insecticide application, worker bees feeding on citrus, sunflower, or canola nectar and pollen from fields sprayed with thiamethoxam maybe more likely to experience higher negative impacts than worker bees exposed to the same pesticide in other fields where these phytochemicals may not be available in floral nectar and pollen. While honey bee responses to pesticides are evaluated under exposure to different doses of the pesticide in question, it may be important to evaluate the responses of bees with reference to the specific crop that the agrochemical is being recommended for. Obtaining a clear understanding of the nectar and pollen phytochemicals of crops will allow for the formulation of pesticide doses that are "safer" for bees and provide effective pest control.

Benefits of phytochemical supplementation affect inhive-aged and forager-aged bees differently and the response elicited depends on the phytochemicals. While supplementation with caffeine, kaempferol, and *p*-coumaric acid, may have provided benefits of reduced mortality to inhive-aged bees exposed to lower sublethal doses of thiamethoxam, the increased mortality in forager-aged bees receiving the same dose, suggests a possible accumulation effect of the toxin, that was not improved by phytochemical supplementation. Younger bees becoming exposed to low doses of neonicotinoids could lead to compromised immune responses that manifest as stronger detrimental effects in forager-aged bees (Gill and Raine 2014, Feng et al. 2019). With a better understanding of phytochemical composition of crop pollen and nectar, the damage to bees could be minimized by choosing appropriate agrochemicals and doses. As bees forage on crops, even sublethal doses of toxins they bring back to the hive, exposes newly emerged bees, developing brood and intranidal bees (Wu-Smart and Spivak 2016) resulting in toxin accumulation. As bees age from intranidal to extranidal workers, the risk of direct exposure to pesticides while foraging increases. Accumulation of toxins during their ontogeny makes such bees more likely to exhibit severely compromised responses to the same toxins encountered while foraging on floral nectar. Whether foragers exposed to pesticides are naïve to these chemicals, or whether they have been exposed to it during their ontogeny will determine their response to different doses of pesticides, a critical piece of information while determining pesticide safety for pollinators.

Healthy pollinator populations are vital for the sustenance of natural and agricultural ecosystems (Garibaldi et al. 2013). However, these populations are threatened by several biotic and abiotic factors (Goulson et al. 2015, Ravoet et al. 2015, Calatayud-Vernich et al. 2016) that compromise pollinator nutrition and related responses. Many sources of micronutrients and phytochemicals are non-crop plant species that grow as "weeds" around crop fields (Alburaki et al. 2017, Arathi et al. 2018, 2019, Lundin et al. 2019, Boyle et al. 2020). Further studies are necessary to describe the phytochemical profiles of nectar and pollen in crops, wildflowers, and weeds. Several efforts are underway to improve access to habitat diversity and hence the dietary needs of pollinators. The positive impacts of these efforts can be strengthened through a more thorough understanding of the synergistic effects of phytochemicals and pesticides on bees.

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Author Contributions

AS and EB designed the study, analyzed the data and wrote the manuscript. EB conducted the experiments and collected data.

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