# EFFECTS OF THIAMETHOXAM ON THE BEHAVIOR OF FORAGING HONEY BEES WITH ARTIFICIAL FLOWER CHOICES

## Thiamethoxam'ın Yapay Çiçeklerde Seçenekli Olarak Verildiğinde Yayılmacı Arıların Davaranışı Üzerindeki Etkileri

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Geliş Tarihi: 20.01.2018 Kabul Tarihi: 15.02.2018

**ABSTRACT**

**The effects of thiamethoxam were studied on the foraging behavior of free-flying bees (*Apis mellifera anatoliaca*) visiting artificial flower patches of blue and white flowers. Thiamethoxam doses from 2 % to 40 % of the reported $LD_{50}$ value were given to bees. The study consisted of three experimental parts performed sequentially without interruption. In part 1, we offered bees 6 µL of a 1M sucrose reward in both flower colors. In part 2 we offered bees 6 µL of 1.5 M sucrose solution in blue flowers and 6 µL of 0.5 M sucrose solution in white flowers. In part 3 we reversed the sucrose solution rewards values with respect to flower color. Each experiment began 30 min after administration of the insecticide. The number of bees foraged was recorded, as was flower patch visitation rate, number of flowers visited and flower choices of the bees that did return. The forager return rate declined linearly with increasing thiamethoxam dose and number of foraging trips of returning bees was also affected adversely. Out of 96 bees, the majority of unreturned (50) bees belonged to higher dosages of thiomethoxam groups. However, flower fidelity was not affected by thiamethoxam dose. Foragers visited both blue and white flowers extensively in experimental part 1 and showed greater fidelity for the flower color offering the higher molarity reward in parts 2 but there were less visits to flowers offering the higher molarity reward in part 3 indicating that the bees failed to learn what were the flowers with higher reward. Our study showed that thiamethoxam affected: the number of returning bees, the number of foraging trips and reward re-learning.**

**Keywords:** *Apis mellifera, Honey Bee, Foraging Behavior, Thiamethoxam, Neonicotinoids*

**ÖZ**

**Mavi ve beyaz yapay çiçekleri ziyaret eden yayılmacı arıların davranışı üzerinde thiamethoxam etkileri çalışılmıştır. Thiamethoxam dozları %2 -% 40 'e kadar rapor edilen LD50 değerleri olarak verilmiştir. Çalışma kesintisiz olarak uygulanan test fazı 3 kısımdan oluşmaktadır. Ilk kısımda arılar 6 ul ve 1 M her iki renk çiçeklerde sukroz verdik.Ikinci kısımda arılara 6 ul ve 1.5 M şeker solüsyonu mavi çiçeklerde 6 ul ve 0.5 m şeker solüsyonu beyaz çiçeklere verdik.Üçünü kısımda ise arılara çiçek rengine göre ikinci kısımın tam tersi olarak şeker solüsyonu verdik.Her çalışma arılara ve çiçeklere yayılışları insektisitler verdikte 30 dk sonra başlamıştır.Yayılmacı arıların sayıları, her çiçeğe ziyaret sayısı, çiçek tercihleri ve çiçeklere geri dönmeyen arılar kayıt edilmiştir.Yayılmacı arıların çiçeklere**


U. Arı D. – U. Bee J. 2018, 18 (1): 2-13
http://dx.doi.org/10.31467/uluaricilik.427583

**geri dönüş seviyeleri artan thiamethoxam dozu ile azalmıştır.Çalışmada kullanılan toplam 96 arıdan 50 arı yüksek doz thiaamethoxam verilen guruba aittir.Çiçeklere geri dönen yayılmacı arıların ziyaret sayıları olumsuz etkillenmiştir.Fakat arıların çiçek bağımlılığı thiamethoxam dozundan etkilenmemiştir. Beyaz ve mavi çiçekleri çalışmanın ilk kısmında çok sayıda ziyaret eden yayılmacı arılar ikinci kısımda daha koyu şekeri olan ödül olan çiçeğe ziyaret etmişler ve üçüncü kısımda ise daha koyu şeker solüsyonu olan çiçekelere ziyaret etmeyi öğrenememişlerdir. Sonuç olarak bu çalışma göstermiştirki thimaethoxam çiçeklere geri dönen arıların sayısı, çiçeklere ziyaret sayısı ve ödülün yayılmacı arılar tarafından yeniden öğrenilmesini etkilemiştir.**

**Anahtar Kelimeler:** *Apis mellifera, Bal Arısı, Yayılma Davranışı, Thiamethoxam, Neonicotinoids*

## GENİŞLETİLMİŞ ÖZET

**Amaç:** Thiamethoxam dünyada bir çok ülkede satışı yapılan ve en çok satılan ikinci yeni nesil neonikotinoid tarım ilaçlarından biridir ve patates, pirinç, ayçiçeği gib birçok alanda kullanılmaktadır. Ayrıca bu ilacın metaboliti olan daha zehirli clothianidinoldukça etkili bir tarım ilacıdır. Bu yenil nesil tarım ilaçları veya böcek öldürücler topraktan bitkinin kökleri ile alınır gövde, dal ve yapraklar ve sonra çiçeklere kadar ulaşır. Bir çok kültür bitkisinde oldukça yaygın olarak kullanılmaktadır.

**Gereç ve Yöntem**: Bu çalışmada mavi ve beyaz yapay çiçekleri ziyaret eden yayılmacı arıların davranışı üzerinde thiamethoxam etkileri çalışılmıştır. Thiamethoxam dozları %2 - %40 ‘e kadar rapor edilen LD50 değerleri olarak verilmiştir. Bu çalışma 4 aşamada ara verilmeden yapılmıştır. 1. Bağımlılık fazı 2. İnsektisit fazı 3.Yayılmacı geri dönüş fazı 4. Test fazı (Şekil 1.). Birinci fazda çiçek bağımlılığı oluşması için arıların çiçekleri öğrenip geri dönmeleri için sağlanmaktadır.Bunun için arılara yapay çiçekler üzerinde 6 ul ve 1 M kokusuz şeker (sukroz) solüsyonu verilmiştir.Çiçekler üzerinde arıların şeker solüsyonu ödülü bittikçe tekrar doldurulmaktadır.Ardından çiçekler üzerine arı konduğunda ödül olan solüsyonu bitirmeden insektisit fazı ile devam etmiştir ve 30 dk süre ile beklenmektedir.Daha sonra yayılmacı arıların geri dönüp dönmediği tespit edilmektedir. Son olarak test fazı uygulanmaktadır. Çalışmada kesintisiz olarak uygulanan test fazı 3 kısımdan oluşmaktadır. İlk kısımda arılar 6 ul ve 1 M olarak her iki renk çiçeklerde sukroz verilmektedir.Ikinci kısımda arılara 6 ul ve 1.5 M şeker solüsyonu mavi çiçeklerde 6 ul ve 0. 5 m şeker solüsyonu beyaz çiçeklere verilmekte,.üçüncü kısımda ise arılara çiçek rengine göre ikinci kısmın tam tersi olarak şeker solüsyonu verdilmiştir. Her çalışma arıların yapay çieçeklere ziyareti sırasında verilen insektisitlerden 30 dk sonra başlamıştır.Yayılmacı arıların sayıları, her çiçeğe ziyaret sayısı, çiçek tercihleri, ve çiçeklere geri dönmeyen arılar kayıt edilmiştir.

**Bulgular**: Yayılmacı arıların çiçeklere geri dönüş seviyeleri artan thiamethoxam dozu ile azalmıştır. Çiçeklere geri dönen yayılmacı arıların ziyaret sayıları olumsuz etkillenmiştir.Fakat arıların çiçek bağımlılığı thiamethoxam dozundan etkilenmemiştir. Beyaz ve mavi çiçekleri çalışmanın ilk kısmında çok sayıda ziyaret eden yayılmacı arılar ikinci kısımda daha koyu şekeri olan ödül olan çiçeğe ziyaret etmişler ve üçüncü kısımda ise daha koyu şeker solüsyonu olan çiçekleri ziyaret etmeyi öğrenememişlerdir. İlaç alınmadığında yayılmacı arılar koyu şeker solüsyonu olan çiçekleri öğrenebilmektedirler.

**Sonuçlar:** Sonuç olarak bu çalışma göstermiştirki thiamethoxam çiçeklere geri dönen arıların sayısı, çiçeklere ziyaret sayısı ve ödülün yayılmacı arılar tarafından yeniden öğrenilmesini etkilemiştir. Thiamethoxam öldürücü seviyelerin altında olduğu zaman bile bal arısı yayılma davranışını bu ilacın miktarı ve süresine bağlı olarak etkilemektedir. Bu yüzden bu ilaç kovanda depolanan bal ve polende uzun zaman kaldıklarında genç arılar ve larvalar üzerinde öneimli etkileşim söz konusudur. Bu yüzden bu yeni nesil ilaçların öldürücü seviyelerin altında oldukları zaman bile bal arılarının farklı yaşam devrelerinde uzun süre kalmaları durumunda hem davranış ve hem de fizyolojik etkilerinin araştırılmasına ihtiyaç duyulmaktadır.

## INTRODUCTION

Honey bee colony losses have been increasing in the United States of America (USA), Europe and other countries. A number of factors have been suggested as the cause of massive colony losses referred to as CCD (Colony Collapse disorder). In addition, a team of researchers who investigate recent honey bee colony losses has been established known as COLOSS (member over 100 countries) and this is supported by both the European Union (EU) and the private sector. One of the main factors responsible for colony losses that been suggested includes agricultural pesticides particularly a new line of insecticides called neonicotinoids. Neonicotinoid use has been suspected as one of them main culprits for the colony declines resulting in a two-year moratorium on their use in the EU until more research can be completed to make the final decision on a permanent ban. A final decision to ban or nor to ban to use of neonicotinoids will be made by EU that will be primarily driven by new research findings, which will be based on their effects on pollinator health (VanEngelsdorp et al. 2009; Neumann and Carreck 2010; Cresswell and Thompson 2012).

Recently, honey bee colony losses have been widely publicized in scientific articles and also by media in the USA and Europe. However, the underlying causes of massive colony losses have not been explained sufficiently. Some of these dramatic large-scale colony losses of honey bees have been designated as Colony Collapse Disorder (CCD). CCD is characterized by clear symptoms (absence of adult bees, capped brood, no evidence of dead bees, having a queen present with insufficient workforce to maintain brood) but causation has been difficult to determine from among an array of pathogens, parasites, and chemicals (VanEngelsdorp et al. 2007; Cox-Foster et al. 2008; Cox-Foster et al. 2008; Neumann and Carreck 2010).

Toxicological studies of honey bees are most often conducted on the common subspecies in North America and Europe (i.e., *Apis mellifera mellifera*, *Apis mellifera ligustica)*. With the global use of neonicotinoid insecticides increasing, the 25 subspecies of honey bee, each adapted to native climates and conditions (e.g, Ruttner 1988, 1992, Kandemir et al. 2000, 2006), may have variation in sensitivity to insecticides and their effects remain unknown because relatively few different kinds of subspecies have been tested.

The neonicotinoids pesticides now comprise about a quarter of the global insecticide market with thiamethoxam (being the second most commonly used one (Jeschke et al. 2011). They are projected to become the top selling insecticide in the world (Neonicotinoid Insecticide Report 2010). The water solubility of the neonicotinoids leads to their uptake and systemic action in plants, where substantial amounts are being found even in nectar and pollen (Schmuck et al 2001; Bonmatin et al 2005; Cutler & Scott-Dupree 2007). Even though the neonicotinoid concentrations found in nectar and pollen are minute compared to the honey bee $LD_{50}$ values for these pesticides, growing evidence suggests they have dramatic effects on honey bee colonies, including being one of the causes of colony collapse (Gill et al. 2012; Osborne 2012; Cressey 2012, 2013). The effects of thiomethoxam was also observed in freshwater insect near agricultural areas (Saraiva et al. 2017).

Resistance development to the pesticide treatment is a serious issue for neonicotinoids due to its pervasive use. Thiamethoxam resistance has already been reported for the Sweetpotato Whitefly, *Bemisia tabaci*, and the Cotton Aphid *Aphis gossypii* (Horowitz et al 2004; Wei et al. 2017). Molecular studies also suggests that thiamethoxam affects a number of genes, metabolic pathways and biological functions in honey bees (Shi et al. 2017).

Systemic neonicotinoid insecticides such as imidacloprid and thiomethoxam can be found in nectar and pollen of cultivated crops when spraying and drip irrigation was used in squash


U. Arı D. – U. Bee J. 2018, 18 (1): 2-13
http://dx.doi.org/10.31467/uluaricilik.427583

Cucurbita pepo cultivars. When pollen and nectar samples were analyzed, high imidacloprid and thiomethoxam levels were found comparison to what was used for the original seed treatments (Stoner and Eitzer 2012).

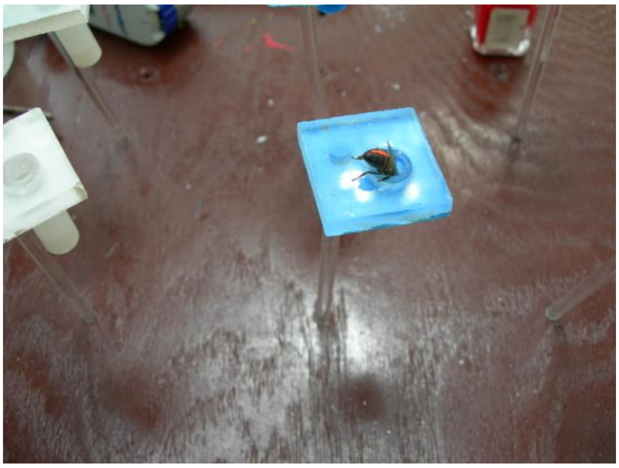
Exposing honey bees to acute (single) and sublethal neonicotinoid doses have been reported to disrupt colony foraging activity (Yang et al. 2008; Colin et al. 2004; Schneider2012; Arena &Sgolastra 2014), by slowing learning, impairing memory as indicated by proboscis extention experiments (PER) (Decourtye et al.2003, Williamson et al. 2014) and decreasing orientation abilities in the field (Henry 2012). For example; a sublethal dosage of imidacloprid has been shown to reduce the mushroom bodies in honey bee brain (Peng and Yang 2016). However, severity of these neurological effects differs substantially depending on the kind of neonicotinoid involved, and thiamethoxam was shown to have relatively mild effects on the mushroom body in the honey bee brain in terms of learning and memory. A single or acute exposures of sublethal doses of thiamethoxam did not impact learning, memory, motor coordination or antennal response of honey bees (Hassani et al, 2008). Nevertheless, several or chronic exposures of sublethal doses of thiamethoxam affected antennal response to sucrose (Aloiouane et al. 2009), and minute (small) sublethal doses have been reported to affect the homing ability of honey bee foragers returning from flower patches (Henry et al. 2012). Here we report on the effect of sublethal thiamethoxam doses on free flying honey bee foraging decisions when they are given floral choices on artificial flower patches.

The aim of our study was to investigate the effects of sublethal doses of thiamethoxam on foraging behavior of the Anatolian Honey Bee (*Apis mellifera anatoliaca*).

**MATERIALS and METHODS**

*Apis melliferaanatoliaca*, an important subspecies of honey bee adapted to different climates and commonly occurring in agricultural settings in in the Marmara Regions of the Republic of Turkey. Experiments were used used free-flying honeybee colonies, as foraging outdoors on artificial flower patches. Each flower patch consisted of 36 flowers spaced 75mm apart in rows and columns of a 6 x 6 Cartesian coordinate system on a brown pegboard. All flower patches consisted of 18 blue and 18 white flowers randomly arranged with respect to color within the array. Each flower consisted of a 28mm x 28mm Plexiglas square that was 6mm thick, placed on a 90mm long dowel with a 5 mm diameter. A 5mm diameter, 5mm deep well was in the center of each flower, and this held the nectar reward.Flowers of different colors were created by painting the lower surface of the flowers with blue or white enamel paint (Testors™ paint Nos. 1208 blue, 1245 white). The reflectance spectra for the paints, and a color hexagon depicting how these colors are perceived by the honeybee, can be found in Hill *et al*. (1997). Flowers were washed with simple, odorless liquid soup in between each experiment and treatment of an experiment

(Picture 1). Artificial flower table with foraging bees



(Picture 2). Marked forager having the reward on blue artificial flower

Foragers from a nine-frame hive were trained to fly 50 m to the experiment location where there was a clear petri dish containing clove-scented 1M sucrose solution (5 µl/L clove oil).The petri dish was removed and replaced with an artificial flower patch where each blue and white flower contained 6 µL of unscented 1M sucrose as a reward. Four bees were used in each trial of an experiment, which translated into having one or two bees on the flower patch at any one time, and thus mimicked a natural foraging environment where hive-mates could be present at the same flower patch. The bees used in an experiment were uniquely marked on the thorax with enamel paint. Additional unmarked bees were removed from the system (Picture 1 and 2).

An experiment had 4 phases that were performed sequentially without breaks in between, these included: 1) the crop attachment phase, 2) a pesticide phase, 3) foraging return phase 4) a test phase (timeline: **Table 1.**). The crop attachment phase lasted 30 min and offered bees 6 µL of unscented 1M sucrose in each flower. Flowers were refilled with the same reward consumed after visitation by a bee. The pesticide phase followed and the start of it was initiated by the capture of each bee as it landed on its first flower, before it could consume the reward. Plastic vials were used to capture and detain bees. Each captured bee was immediately fed 10 µl of unscented 1M sucrose solution containing a specified thiamethoxam dosage. Bees were held in captivity for 15 min, and then released. The flower patch remained in place, but flowers did not have nectar rewards for an addition 15 min. The 30 min pesticide phase allowed the pesticide to be absorbed by bees. The return phase offered bees again 6 µL of unscented 1M sucrose solution in each flower for 30 min. Flowers were refilled with the same reward consumed after visitation by a bee. Bees returned to foraging at different times during this phase.The test phase contained a total of 2 treatments. Treatment 1 offered bees 6 µL of unscented 1.5M sucrose in each blue flower and 6 µL of unscented 0.5M sucrose in each white flower. In treatment 2 the rewards associated with flower color were reversed so that white flowers now offered the 1.5 M sucrose reward and the blue flowers offered the 0.5 M reward. Half of the bees received treatment 2 before treatment 1 and then this was switched. Flowers were immediately refilled with the same reward consumed after visitation by a bee (**Table 1.**).



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http://dx.doi.org/10.31467/uluaricilik.427583

**Table 1.**Theexperimental design utilized.Each experiment consisted of 3 phases, which were given sequentially, without a break. The crop attachment phase conditioned bees to visit the flower patch. Then in the beginning of the insecticide phase a dose of thiamethoxam was administered and was allowed be absorbed by the bee for 30 minutes. The test phase examined forager flower-color fidelity under different reward scenarios.

**Experimental Design**

| **Crop Attachment Phase** | **Insecticide Phase** | | **Return Phase** | **Test Phase** *Treatment* 1 | *Treatment* 2 |
|---|---|---|---|---|---|
| 1M sucrose Blue<br>1M sucrose White | Hold | No Reward | 1M sucrose Blue<br>1M sucrose White | 1.5 M sucrose Blue<br>0.5M sucrose White | 0.5M sucrose Blue<br>1.5M sucrose White |
| **30 min** ↑ | **15 min** | **15 min** | **30 min** | **45 min** | **45 min** |

**Insecticide Dose**

Experimental groups were defined by the ceratin dosage of thiamethoxam given to the test bees. Negative control bees (no pesticide) were included in each experiment. Three experimental groups were included: 12.0 ng thiamethoxam (40% of the LD50), 3.0 ng thiamethoxam (10% of the LD50) and 0.6 ng thiamethoxam (2% of the LD50). Several (four) trials of each experiment were performed, each with a new set of bees and total of 96 bees were used in the experiments. In each trial at least 1 bee was given sugar water without pesticide (negative control: 0 ng thiamethoxam). Bees receiving 12 ng thiamethoxam represent the positive control because we would expect at this concentration that it would have sort of an effect at this high dose based on harnessed bee 4 hr post ingestion data (Hranitz *et al*. 2014 unpublished). The flower color sequence that each bee visited was recorded. In addition the number of foraging trips a bee made from the hive was recorded.

A chi square goodness of fit test was used to compare the differences in the number of bees that returned to forage from the 12 ng thiamethoxam, 3 ng thiamethoxam, 0.6 ng thiamethoxam treatments and the negative control bee population. Non-returning bees which are bees that did not come back to the flower patch at all after being released were also accounted for.

A repeated measures MANOVA (two way ANOVA) was used compare the number of blue flowers visited that occurred between the return phase and treatment 1, and between treatment 1 and treatment 2 of the test phase across the negative control and 2% $LD_{50}$ tretaed bees. To normalize the data, an Arcsine square root transformation was used on the relative frequency of blue flowers visited for each of the three phases. Dose (control or 2%), time (return, treatment 1, treatment 2) and interaction effects were tested. Too few of the 10% and 40% $LD_{50}$ treated bees were able to return successfully so these treatment groups were excluded from the analysis.

Finally, we used one-way ANOVAs to compare the number of flowers visited and the number of foraging trips made in the test phase (treatments 1 and 2) across the following treatments, control, 2% and 10%.

## RESULTS

Dosage had a significant effect on number of bees that returned to foraging after being fed an insecticide ($X^2$ =47.290, df=3, P<0.0001). A dose of 40% $LD_{50}$ resulted in only 4% of the bees ever returning to the flower patch after being release. More surprising was the fact that a dose as small as 10% LD50 was effective in shutting down foraging: only 22% of the bees ever returned (Fig. 1).

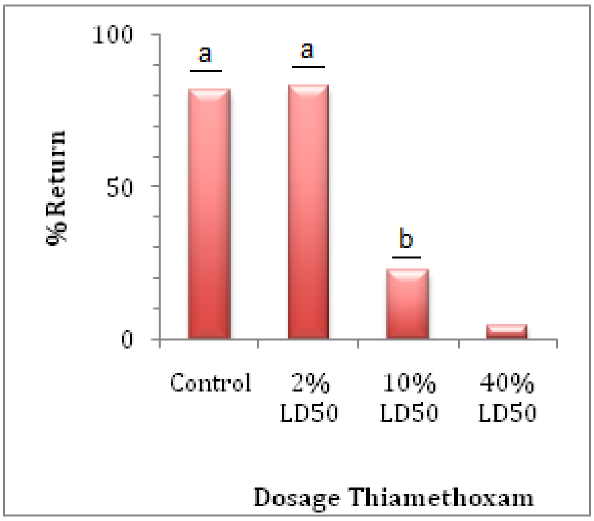


**Fig. 1.**Percentage of foragers returning in the test phase for each dose administered (12,9 ng thiamethoxam = 40 % $LD_{50}$; 3,9 ng =10 % $LD_{50}$; 0.6 ng = 2 % $LD_{50}$; 0 ng = 0 % $LD_{50}$).Dosage has significant effect on returning bees.

However, the number of flowers visited by those bees that returned and foraged in treatments 1 and 2 did not differ significantly between dosage populations (F=2.7682; P = 0.0769). Neither did the number of trips made from the hive differ between dosage populations for bees that returned and foraged in treatments 1 and 2 (F=2.9418; P=0.0663) Bees appear to continue to forage at the same rate regardless of dosage thiamethoxam given until catastrophically overcome by the pesticide and stop foraging all together(Fig. 2 & 3). Negative control bees (0 ng thiamethoxam) made significantly more trips than bees receiving the pesticide. Differences among the bees receiving thiamethoxam were not significant (Fig. 3).

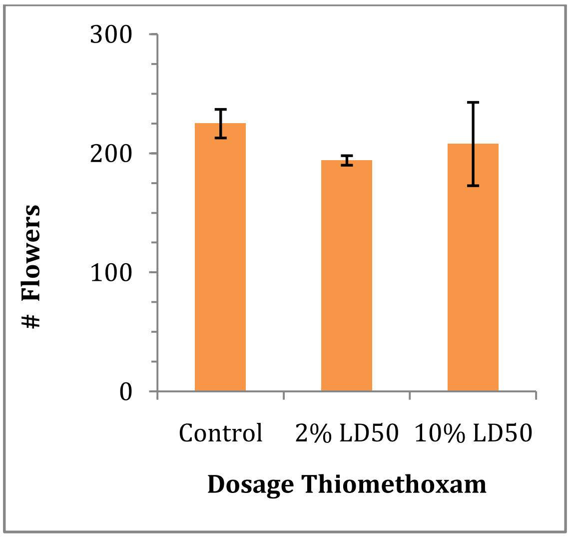


**Fig. 2.**Total number of flowers visited in experimental phases 2 and 3 per bee for each dosage group (mean with SE bars). Only bees that returned to forage after the insecticide phase were included. Differences observed were not significant.

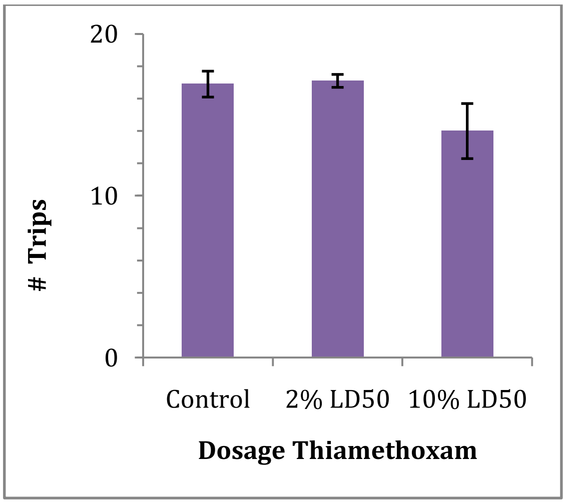


**Fig. 3.**Total number of trips made to the flower patch in experimental Parts 2 and 3 per bee for each dosage group (mean with SE bars). Only bees that returned to forage after the pesticide phase were included. Negative control bees (0 ng



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http://dx.doi.org/10.31467/uluaricilik.427583

Thiamethoxam) made significantly more trips than bees receiving the pesticide. Differences among the bees receiving Thiamethoxam were not significant.

When examining the effect of a very low dose of thiamethoxam on foraging decisions we found that there were significant time ($F=31.8907$; $P=0.0001$) and dose, time, interaction ($F=3.9708$; $P=0.0313$) but not a dose main effect ($F=1.8442$; $P=0.1857$).

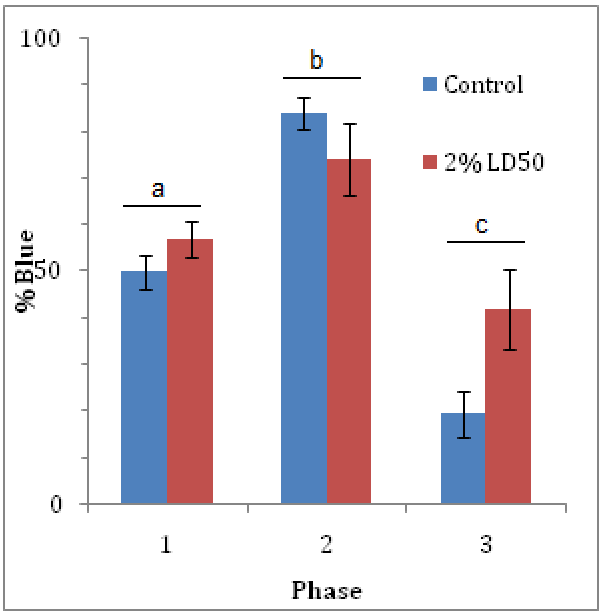


**Fig. 4.**Flower color fidelity of foragers under different reward scenarios. Bars from left to right in each experimental phase represent the dosage populations 0 ng (0 % $LD_{50}$), 0.6 ng (2 % $LD_{50}$) thiamethoxam. Depicted are the mean (with SE bars) percentage of blue flowers visited. Only bees that returned to forage after the pesticide phase were included. Phase 1 offered bees 1 M sucrose solution in both flowers. Phase 2 offered bees 1.5 M sucrose solution in blue and 0.5 M sucrose solution in white flowers. Phase 3 offered bees 0.5 M sucrose solution in blue and 1.5 M sucrose solution in white flowers. Foragers altered fidelity in response to experimental Part, but significant differences among Thiamethoxam dose groups was not observed. The response of forager bees to three phases weresignificant.

Control bees did not favor either flower color when both colors offered 1M sucrose rewards. However, bee showed high fidelity to blue flowers when blue offered the 1.5M sucrose reward and high fidelity to white flowers when white offered the 1.5M sucrose reward This general pattern was observed by the 2% $LD_{50}$ population of foragers, but fidelity to the higher rewarding flower color was not as great, particularly when white flowers offered the 1.5M reward. Treated foragers altered their fidelity in response to experimental phase significantly differently across the three phases, but significant differences among thiamethoxam dose groups was not observed.(Fig. 4).

## DISCUSSION

The doses of pesticide were within the range of what is typically used in agricultural fields (Bonmatin et al. 2005; Blacquiere et al. 2012; Colin *et al*. 2004; Fischer *et al*. 2014; Rortaise*t al*. 2005; Stoner & Eitzer 2012). Our study showed that thiamethoxam affected three factors of foraging behavior and these are as follows; the number of returning bees to foraging after treatment and re-learning the association between flower color and the high food reward. It is important to underline that even as little as 10% of the $LD_{50}$ value resulted in a reduction in honey bee foraging activity. The majority of the bees do not return when to exposed to a 40% of the $LD_{50}$ pesticide dose, only 4% of foraging bees returned to the experimental artificial flower patch after being exposed to this pesticide dose.

Bees appear to continue to forage at the same rate regardless of dosage thiamethoxam given, unless they are catastrophically overcome by a high pesticide dose and stop foraging all together. The number of flowers visited by returning bees was not affected significantly by pesticide thiamethoxam with the 2% and 10% of the $LD_{50}$ doses in comparison to control bees. The flower choice and also trips to each flower by foraging bees were not affected by thiamethoxam doses of 2% and 10%. In addition, fidelity to white flowers when offering a higher food reward, in the third phase of the experiment showed the largest difference in foraging preferences between pesticide treated bees and control bees. Foraging bees treated with 2% pesticide had difficulty in learning the association between flower color and which flower offered the higher food reward. The effect appears to be more pronounced as time goes as there was a significant interaction between the dose of pesticide and time. Therefore, if the experiment were to last longer, foraging performance might have declined even more than what we have observed here. Honey bee foragers that returned to the artificial flower patch with lower doses of pesticide exposure continued with normal foraging activity, but their cognitive ability for associative learning appears to have declined resulting in less efficient foraging trips.

A similar study, where imidocloprid was exposed to foraging bees on artificial flower patches, revealed similar results. Imidocloprid with 10 and 40% of the $LD_{50}$ reduced the number of returning bees to the artificial flower patch and the number of foraging trips was reduced as well. No bees foraged with exposure imidocloprid at a dose of 40% of the $LD_{50}$. Even though the average number of flowers visited by each bee was not affected significantly, imidocloprid ingested bees visited more flowers per trip than the control bees. A sub-lethal dose of imidocloprid did not change the preference of flower color and foragers were able to associate the flower color with the higher food reward. This study suggests that imidocloprid does not affect the bee's learning ability in a free-flying foraging context (Karahan et al. 2015). In addition, it was found that low doses of pesticide exposure (0,35 to 1,80 ng) did not negatively affect the learning ability of foraging bees in the field (Cresell 2011; Charpentier et al. 2014). However, acute or chronic exposure to thiamethoxam did result in negative effects on foraging and homing behavior of honey bee foragers (Tosi et al. 2016).

As we see here the sub-lethal effects of thiamethoxam and Imidocloprid are not the same in a free-flying foraging context. Studies showing the negative effect of imidocloprid when the exposure is 20-40% of the $LD_{50}$ varies based on the different subspecies tested (Colin et al. 2004; Porporato et al. 2013,Scholer and Krischik 2014). Although in general, it has been demonstrated that imidocloprid tends to have some sort of negative effect on navigation, and homing abilities of foragers when exposed to sub-lethal doses (Feltham et al. 2014; Fischer et al. 2014).

Most sophisticated studies with radar tracking of foragers provide more data about foraging flights and disruption due to intoxication of sublethal effects of neonicotinoids (Feltham *et al*. 2014; Fischer *et al*. 2014). Field and lab experiments with honey bees suggest that neonicotinoids negatively affect learning and memory association of scent with reward (Decourye *et al*. 2003; Ramirez-Romeo *et al*. 2005; Blacquiere et al. 2012; Matsumoto 2013). Here, we show that perhaps multimodal senses may be affected as we found a decrease in association with color as opposed to flower odor.

The possibility of synergistic effects from multiple sub-lethal exposure events is certainly possible. The analysis of hive products revealed important research results that honey comb and foundation wax samples were highly contaminated with miticides and agrochemicals, including neonicotinoids. About 98 pesticides and metabolites have been detected in pollen with concentrations as high as 214 ppm. This concentration level is highly dangerous to honey adults and brood. The accumulation of these miticides and pesticides may also cause a great deal of stress on honey bees making them more susceptible to other diseases (Mullin et al. 2010). Some studies report that even low residues of imidocloprid in nectar and pollen harm the


http://dx.doi.org/10.31467/uluaricilik.427583

bees (Oldroyd 2007). In addition, sub-lethal doses of Imidocloprid impair learning and memory of honey bees (Ramirez-Romero et al. 2005; Creswell 2011; Decourtye et al. 2013, Aren a& Sgolastra 2014), but no sub-lethal or synergistic effects of thiomethoxam, a metabolite of clothianidin, and the gut parasite *Nosema* were observed (Odemer et al. 2017).

The sub-lethal effects of neonicotinoids are also linked to colony losses. The most of the recent research suggests that stress of sub-lethal doses of pesticides cause colony mortality in honey bees and winter losses increase significantly, but the exact causes of colony collapse still remains elusive (Maus et al. 2003; Giroloma et al. 2009; Creswell et al. 2012a,b; Bryden et al. 2013; Pilling et al. 2013; Lu et al. 2014). Previous research suggests that sub-lethal exposure to neonicotinoids suppresses the immune system of honey bees which leads to more colony losses. It is thought that the pesticide exposure with the suppression of the immune system allows for viral infections to enter the honey bee and proliferate (Prisco et al. 2013). These viruses are very virulent and there is no treatment available, so consequently this results in more colony deaths. In addition, honey bee fecundity can be impacted by pesticide exposure, exposed queens are known to have lower body weights and lower sperm counts in their spermatheca after thiomethoxam treatments (Gajger et al. 2017).

As it appears that neonicitinoids may not be the best solution for controlling the pests for all crops. There is a need to develop alternative insecticides and employing an integrated pest management strategy where pesticide are only applied if they seem to have some efficacy would be the best overall strategy because it was found that no significant crop yield increase was achieved after treating rice seeds with thiomethoxam (Lanka et al. 2017). Newer pesticide alternatives that take into account pollinator health would be beneficial in general (Chen et al. 2017).

In conclusion, the sub-lethal effects of thiamethoxam affect foraging behavior of honey bees depending on amount and duration of pesticide exposure. This is an important consideration as the pesticide exposure may affect young bees and larva that have spent longer time periods in the hive. Therefore, more research is needed in particular on the sub-lethal exposure to pesticides to clarify their behavioral and physiological effects on different developmental stages of honey bees.

**Ackowledgement**

We would like to thank to NSF National ScienceFoundation (DBI #126332) for financial supportand Assos. Prof. Dr. Himmet Tezcan for adviseand Ali Çakır and Mustafa Ertürk for their help during the study. We also thank to reviewers to improve the manuscript.

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