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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

EFFECT OF MITICIDES AMITRAZ AND FLUVALINATE ON REPRODUCTION AND PRODUCTIVITY OF HONEY BEE *APIS MELLIFERA*

Akarisit Amitraz ve Fluvalinat'ın Bal Arısı *Apis mellifera*'nın Üreme ve Verimliliğine Etkisi

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ABSTRACT

***Varroa destructor* is a well-known ectoparasite of the honey bee *Apis mellifera*. Amitraz and fluvalinate are highly effective miticides used against *V. destructor* infestation in colonies of honey bee *A. mellifera*. Though honey bees more resistant to miticides, there are side effects of these chemicals on the reproduction, olfaction, and honey production of honey bees. We showed a negative impact of miticides amitraz and fluvalinate on honey production and reproduction of honey bee colonies. Also, we assumed the reduction of olfaction of honey bees by fluvalinate due to changes of expression of olfactory related neuropeptide genes short neuropeptide F sNPF, tachykinin TK, short neuropeptide F receptor sNPFR. The external treatment of honey bee colonies by miticides amitraz and fluvalinate along with a positive effect of pest control harms reproductivity, honey productivity, and, probably, can reduce learning and memory, gustation and olfaction of honey bees. When used for a short time and with care, miticides can be less harmful to honey bees. Breeding varroa-resistant honey bees allow to reduce the use of miticides and produce organic honey. Therefore, the further development of beekeeping should be in the direction of selection for disease and Varroa resistance and adaptation to the environment.**

Keywords: Amitraz, Fluvalinate, Honey Production, Reproduction, *A. mellifera*, *V. destructor,* Short Neuropeptide F sNPF, Tachykinin TK, Short neuropeptide F receptor sNPFR, RT-PCR, Gene expression.

ÖZ

Varroa, bal arısı *Apis mellifera*'nın iyi bilinen bir ektoparazitidir. Amitraz ve fluvalinat, bal arısı *A. mellifera* kolonilerinde *V. destructor* istilasına karşı kullanılan oldukça yüksek etkili akarisitlerdir. Bal arıları, akarisitlere karşı daha dirençli olsalar da, bu kimyasalların bal arılarının üreme, koku alma ve bal üretimi üzerinde yan etkileri vardır. Bu çalışma ile Akarisitler olan amitraz ve fluvalinatın bal üretimi ve bal arısı kolonilerinin üremesi üzerinde olumsuz bir etkisi olduğu belirlenmiştir. Ayrıca, bal arılarının

Fluvalinat'a bağlı olarak koku alma duyusunun azalması durumunu ilgili nöropeptid genlerinin kısa nöropeptid F sNPF, taşikinin TK, kısa nöropeptid F reseptörü sNPFR ifadesindeki değişiklik olduğunu varsaydık. Bal arısı kolonilerinin akarisitler olan amitraz ve fluvalinate ile kontrol edilmesi, haşere kontrolünün olumlu etkisiyle birlikte üremeye, bal verimliliğine zarar verir ve muhtemelen bal arılarının öğrenmesini ve hafızasını, lezzetini ve kokusunu muhtemelen azaltabilir. Kısa bir süre ve özenle kullanıldığında, akarisit kullanımı bal arılarına daha az zarar verebilir. Varroaya dirençli bal arılarının yetiştirilmesi, akarisit kullanımını azaltmaya ve organik bal üretmeye izin verir. Bu nedenle, arıcılığın daha da geliştirilmesi için seçim; hastalıklara, Varroa'ya dirençli ve çevreye uyum yönünde olmalıdır.

Anahtar Kelimeler: Amitraz, fluvalinat, bal üretimi, üreme, A. mellifera, V. destructor, kısa nöropeptid F sNPF, takikinin TK, kısa nöropeptid F reseptörü sNPFR, RT-PCR, gen ifadesi.

GENIŞLETILMIŞ ÖZET

Çalışmanın amacı: Bu makalede, amitraz ve fluvalinat gibi akarisitlerin bal arısı kolonileri üzerindeki etkisi yumurtlama, bal üretimi ve koku alma özelliklerinin gözlemlenerek tahmin edilecektir.

Gereç ve Yöntemler: Bu çalışmada 46 bal arısı *A. mellifera* kolonisi (Rusya'dan 40 koloni ve Kore'den altı koloni) deney grubu olarak kullanılmıştır. Kontrol grubu olarak yirmi altı bal arısı *A. mellifera* kolonisi (Rusya'dan yirmi koloni ve Kore'den altı koloni) kullanıldı. İşçi arılar 2019 yılında kovanların girişinden toplanmıştır.

Rusya'dan 20 bal arısı *A. mellifera* kolonisi ve Kore'den 6 koloniye haricen ortalama 2 µg/arı fluvalinat dozu uygulanmıştır. Rusya'dan gelen diğer 20 bal arısı *A. mellifera* kolonisine harici olarak ortalama 20 µg/arı amitraz dozu uygulanmıştır. Kontrol deneylerinde, Rusya'dan 20 bal arısı *A. mellifera* kolonisi ve Kore'den 6 kolonide her hargi bir uygulama yapılmamıştır. Rusya'daki *A. mellifera* kolonilerinde haricen amitraz ve fluvalinate uygulamalarının ortalama yumurtlama ve bal üretimi üzerindeki etkisi değerlendirilmiştir. Kore bal arısı *A. mellifera* kolonileri, fluvalinat uygulamasının koku alma ile ilgili nöropeptit genleri kısa nöropeptit F sNPF, taşikinin TK, kısa nöropeptit F reseptörü sNPFR ekspresyonu üzerine olan etkisi değerlendirilmiştir. Ortalama yumurtlama ve bal üretiminin değerlendirilmesi, Biyokimya ve Genetik Enstitüsü, Ufa Federal Araştırma Merkezi, Rusya Bilimler Akademisi'nde (Rusya) gerçekleştirilmiştir.

RT-PCR, Incheon Ulusal Üniversitesinde (Kore) gerçekleştirildi. Her bal arısı kolonisinden on beş işçi arıdan izole edilmiş antenler alındı. Qiagen RNeasy Mini Kit üreticinin (Qiagen, Almanya) talimatlarına göre kullanılarak antenlerden toplam RNA'lar izole edilmiştir. cDNA, toplam 500 ng RNA'dan oligo-dT ve Superscript III enzimi (Invitrogen, Yeni Zelanda) ile sentezlenmiş ve RT-PCR, Brilliant III Ultra-hızlı SYBR Green qPCR Master Mix (Agilent Technologies, ABD) kullanılarak AriaMx Real-Time PCR Systemi üzerinde gerçekleştirilmiştir. RT-PCR primerleri, (ribosomal protein 49 gene, TK, sNPF ve SNPFR) Macrogen firması tarafından sentezlenmiştir. RT-PCR: 95°C-1 dakika, 95°C-5 sn'lik 40 döngü, 55-60°C-10 sn, 72°C-10 sn koşullarında gerçekleştirilmiştir. Her bir RT-PCR, üç tekrar halinde gerçekleştirildi. Genlerin ekspresyon seviyesi delta-delta Ct yöntemi kullanılarak değerlendirilmiştir.

Bal arısı kolonisinde, anaarının ortalama yumurtlama oranı, EP = E/D olarak bulunur; burada E- kolonideki ana arı tarafından yumurtlanan toplam yumurta sayısı, EP-kraliçelerin ortalama yumurtlama, D-yumurtlama günlerin sayısı göstermektedir. Kolonilerdeki ortalama üretkenlik HP = H/M olarak tahmin edildi, burada H-kolonide toplam bal üretimi, HP-ortalama bal üretimini, M-balın üretildiği ay sayısını gösterir. Varyans (ANOVA) analizi, standart sapma SS, standart hata SE, güven aralığı CI, Student t-testi ve olasılık P analizi JMP 13 (SAS, ABD) paket programı kullanılarak hesaplanmıştır.

Sonuç: Deneysel bal arısı *A. mellifera* kolonileri, kontrol grubu ile karşılaştırmasında ölümcül olmayan dozlarda akarisit amitraz ve fluvalinat ile muamele edildi. Kontrol grubunda yumurtlama ortalama 1650 adet, bal verimi ortalama 31.1 kg elde edilirken, Fluvalinat uygulaması yapılan bal arıları grubunda yumurtlama kontrole göre %9,7 oranında azalmıştır (t-testi=2,55, p≤0,05). Amitraz ile muamele edilen bal arısı grubunda yumurtlama, kontrole göre %7,9 oranında azalmıştır (t-testi = 2,20, p ≤ 0,05) (Şekil 1, Tablo 1).

Fluvalinat ile muamele edilen bal arısı grubunda bal üretimi kontrole göre %21,9 oranında azalmıştır (t-testi = 2,89, p ≤ 0,05). Amitraz ile muamele edilen

bal arısı grubunda, bal verimi kontrole göre %12.1 oranında azalmıştır (t-testi = 2.80, p ≤ 0.05) (Şekil 1, Tablo 2).

Amitraz ve fluvalinat uygulaması yapılan bal arısı kolonilerindeki varyans ANOVA analizi, p değerlerine ve 0.05 anlamlılık seviyesine göre, bal arısı kolonilerinde fluvalinat ve amitrazın yumurtlama ve bal üretimi üzerindeki etkileşim etkisinin istatistiksel olarak anlamlı olduğunu göstermiştir (Tablo 3). Ayrıca, bal arısı kolonilerinde fluvalinat ve amitrazın yumurtlama ve bal üretimi üzerindeki etkileri arasındaki farklar istatistiksel olarak önemli değildir, bu da her iki akarisitin bal arısı kolonileri üzerinde neredeyse benzer olumsuz etkilere sahip olduğu anlamına gelmektedir.

Fluvalinata maruz kalan bal arılarında sNPF ekspresyonu önemli ölçüde artmıştır (t-testi = 4.41, p = 0.01). Fluvalinata maruz kalan bal arılarında TK ekspresyonu önemli ölçüde değişmedi (t-testi = 0.80, p = 0.46). Fluvalinata maruz kalan bal arılarında sNPFR ekspresyonu önemli ölçüde azalmıştır (t-test = 3.49, p = 0.01). Muhtemelen, sNPF'nin spesifik membran reseptörü sNPFR ile etkileşim yoluyla hedef hücrelere etki etmesinden dolayı artan sNPF ekspresyonu. Bu nedenle, fluvalinat muamelesinden sonra sNPFR ekspresyonundaki değişiklikler önemlidir (Şekil 2).

Tüm olasılıklar değerlendirildiğinde, mitisitlerin dezavantajlardan daha fazla avantaja sahip olduğu varsayılabilir. Ektoparazitik akarların bal arıları üzerindeki olumsuz etkisi, sayılarının artması nedeniyle her geçen gün artarken, mitisitlerin bal arıları üzerindeki olumsuz etkisi detoksifikasyon süreçleri nedeniyle azalma eğilimindedir. Bu nedenle arıcılıkta mitisitlerin kullanılması ekonomik olarak faydalıdır. Bal arılarına kıyasla, ektoparazitik akarlar, daha küçük boyutları ve daha az etkili detoksifikasyon sistemleri nedeniyle bu mitisitlere karşı daha hassastır. Bu nedenle bal arıları, ektoparazitik akarlara göre mitisitlerden daha az muzdariptir. Bununla birlikte, mitisitler arılar için güvenli değildir. Amitraz ve fluvalinat gibi akar öldürücülerin bal arılarına, *A. mellifera* benzer, olumsuz etkileri önceki çalışmalarda da gözlenmiştir.

Amitraz ve fluvalinat gibi akarisitlerin bal arıları üzerindeki olumsuz etkisi deneysel olarak bu çalışmada gösterilmiştir. Bunun yanı sıra, Varroa akarlarının amitraz ve fluvalinat akarisitlerine karşı artan direnç söz konusudur. Bununla birlikte, sürekli seçimle Varroa akarlarına dirençli bal arısı popülasyonları elde etmenin mümkün olduğu gösterilmiştir. Varroaya dirençli bal arılarının yetiştirilmesi, akarisit kullanımını azaltmaya ve organik bal üretilmesine izin verir. Bu nedenle, arıcılığın daha da geliştirilmesi, hastalık ve Varroa direnci seçimi ve çevreye uyum yönünde olmalıdır.

INTRODUCTION

The honey bee, *Apis mellifera*, is an essential pollinator that provides ecological services and economic values in agriculture (Klein et al. 2007, Southwick and Southwick 1992). Varroosis caused by *Varroa destructor* mite leads to losses of honey bee colonies and reduces their adaptation (Zhang 2000, Anderson and Trueman 2000).

To prevent damages from ectoparasitic mites, beekeepers commonly use the miticides amitraz and fluvalinate. Fluvalinate is a synthetic pyrethroid that act as a neurotoxin inducing sustained membrane depolarization. Fluvalinate is highly effective against mites and ticks (Wallace 2002, Gupta and Crissman 2013, Gosselin-Badaroudine and Chahine 2017). Amitraz is a synthetic amidine, a derivative of an oxoacid belonging to the group of triazopentadiene. It acts as a neurotoxin with a target of octopamine receptor. Fluvalinate and amitraz are highly effective against mites and are commonly used to control ectoparasitic mites in honey bee colonies (Gregorc et al. 2012, Gracia et al. 2017). In comparison with ectoparasitic mites, honey bees are more resistant to these miticides due to their bigger size and more effective system of detoxification, but despite this, there are various side effects of amitraz and fluvalinate on mortality, productivity, reproduction, and olfaction (Berry et al. 2013, Frost et al. 2013, Ilyasov et al. 2014, Rangel and Tarpy 2015, Dai et al. 2017, Lim et al. 2020). Olfaction is a basic regulation mechanism for honey bees, which important in different aspects of their social life organization (Nässel, 2002; Taghert and Veenstra, 2003; Hauser et al., 2006; Johnson, 2006; Marciniak et al., 2011) and nectar foraging behavior (Menzel 1999, Hewes and Taghert 2001, Johnson 2006, Hummon et al. 2006, Giurfa 2007, Altstein and Nässel 2010, Xu et al. 2016, Schoofs et al. 2017).

The neuropeptides short neuropeptide F sNPF, tachykinin TK, and short neuropeptide F receptor sNPFR are peptidergic regulators in the olfactory systems of insects (Jung et al. 2013, Jiang et al. 2017). Thus, the reduction of olfaction in honey bees caused by miticide fluvalinate must be accompanied

by changes in gene expression of olfactory-related neuropeptide genes and their receptors.

In this paper, the effect of miticides amitraz and fluvalinate on honey bee colonies will be estimated by observation the ovipositioning, honey production, and olfaction.

MATERIALS AND METHODS

Honey bee sampling and experimental groups

Forty-six colonies of honey bee *A. mellifera* (forty colonies from the Republic of Bashkortostan, Russia Federation (54,46N 56,01E), and six colonies from Incheon, the Republic of Korea (37,22N 126,38E)) were used as an experimental group. Twenty-six colonies of honey bee *A. mellifera* (twenty colonies from the Republic of Bashkortostan, Russia Federation, and six colonies from Incheon, the Republic of Korea) were used as a control group. Worker bees were collected from the entrance of hives in 2019.

Twenty colonies of honey bee *A. mellifera* from Russia and six colonies from Korea were externally treated with an average dose of 2 µg/bee fluvalinate. Another twenty colonies of honey bee *A. mellifera* from Russia were externally treated with an average dose of 20 µg/bee amitraz. In control, twenty colonies of honey bee *A. mellifera* from Russia and six colonies from Korea remain untreated. Russian colonies of *A. mellifera* were evaluated for the effect of amitraz and fluvalinate external treatment on average oviposition and honey production. Korean colonies of *A. mellifera* were evaluated for the effect of fluvalinate external treatment on expression of olfactory related neuropeptide genes short neuropeptide F sNPF, tachykinin TK, short neuropeptide F receptor sNPFR. Evaluation of average oviposition and honey production was performed in Institute of Biochemistry and Genetics, Ufa Federal Research Centre, Russian Academy of Sciences (Russia). The experiment with honey bees was carried out for three months (June, July, August) in 2019.

Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR was performed at Incheon National University (Korea). From each honey bee colony were isolated antennae from fifteen worker bees. Total RNAs were extracted from the antenna using a Qiagen RNeasy Mini Kit according to the instructions of the manufacturer (Qiagen, Germany). The cDNA was synthesized with oligo-dT and Superscript III enzyme (Invitrogen, New Zealand) from 500 ng of total RNA. The RT-PCR was performed on the AriaMx Real-Time PCR System using Brilliant III Ultra-fast SYBR Green qPCR Master Mix (Agilent Technologies, USA).

The RT-PCR primers were synthesized in company Macrogen Inc. (Seoul, Korea): the ribosomal protein 49 gene (AF441189) RP49 primers RP49-F: 5'-GGGACAATATTTGATGCCCAAT-3' and RP49-R 5'-CTTGACATTATGTACCAAAACTTTTCT-3', product size is 100 bp (housekeeping gene); the neuropeptide gene tachykinin (XM_026441578) TK primers TK-F 5'-GGCGGGGATTTACGGATCAA-3' and TK-R 5'-CCCTCGAAATTCCCATCGTG-3', product size is 166 bp; the neuropeptide gene short neuropeptide F (XM_003250107) sNPF primers sNPF-F 5'-ATAGATTACTCAGATGAAATACCAG-3' and sNPF-R 5'-GCACTCATTGGTTTTGATAGAATAG-3', product size is 218 bp; the short neuropeptide F receptor gene (XM_006561685) sNPFR primers sNPFR-F 5'-GCATTTTGTTACATCTGCGTC-3' and sNPFR-R 5'-TCGTTCGCTTCTTCCTCTC-3', product size is 112 bp (Mao et al. 2011, Lim et al. 2020). The RT-PCR was performed in the following conditions: 95°C-1 min, 40 cycles of 95°C-5 s, 55-60°C-10 s, 72°C-10 s. Each RT-PCR was performed in three replicates. The expression level of genes was evaluated using the delta-delta Ct method (Livak and Schmittgen, 2001).

Statistical analysis

The average oviposition of queen in each honey bee colony was estimated as EP = E/D, where H- total number of laid eggs by queens in the colony, OP-average oviposition of queens, D-number of oviposition days.

The average productivity in each honey bee colony was estimated as HP = H / M, where H-total produced honey in the colony, HP-average honey production of honey bee colonies, M-number of months when honey was produced.

The analysis of variance ANOVA, standard deviation SD, standard error SE, confidence interval CI, Student's t-test, and probability P ($P \leq 0.05$ means statistical significance at 95% reliability) was estimated using JMP 13 (SAS, USA).

RESULTS

The experimental honey bee *A. mellifera* colonies were treated by sublethal doses of miticides amitraz and fluvalinate in comparing control. In the control group, oviposition was an average of 1650 pcs, honey productivity was average 31.1 kg. In the group of honey bees, treated with fluvalinate, oviposition was decreased relative to the control by 9.7% (t-test = 2.55, $p \leq 0.05$). In the group of honey bees, treated with amitraz, oviposition was decreased relative to the control by 7.9% (t-test = 2.20, $p \leq 0.05$) (Figure 1, Table 1).

Table 1. Average oviposition OP of queen bees in honey bee colonies treated with amitraz and fluvalinate

Group	OP ± SE, pcs.	CI, pcs.	SD	t-test
Fluvalinate	1490 ± 11.5	1410 - 1510	51.3	2.55
Amitraz	1520 ± 24.1	1380 - 1590	53.7	2.20
Control	1650 ± 20.6	1440 - 1700	65.3	

OP-average oviposition of queen bees, SE-standard error, CI-confidence interval, CD-standard deviation, t-test – Student's t-test. Each group N = 20.

In the group of honey bees, treated with fluvalinate, honey production was decreased relative to the control on 21.9% (t-test = 2.89, $p \leq 0.05$). In the group of honey bees, treated with amitraz, honey productivity was decreased relative to the control on 12.1% (t-test = 2.80, $p \leq 0.05$) (Figure 1, Table 2).

Table 2. Average honey production HP in honey bee colonies treated with amitraz and fluvalinate

Group	HP ± SE, kg	CI, kg	SD	t-test
Fluvalinate	24.6 ± 2.1	20.3 - 26.4	3.1	2.89
Amitraz	27.7 ± 1.2	25.2 - 31.6	4.1	2.80
Control	31.1 ± 2,1	26.6 - 34.7	1.7	

HP-average honey production of honey bee colonies, SE-standard error, CI-confidence interval, CD-standard deviation, t-test – Student's t-test. Each group N = 20.

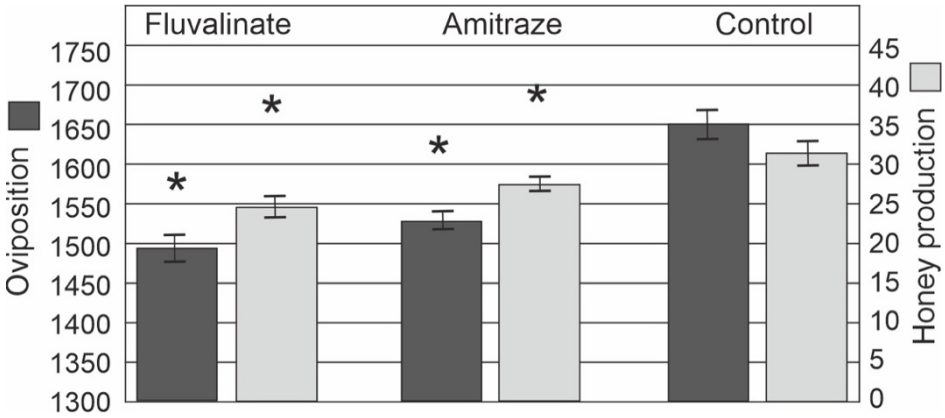

Figure 1. Average oviposition and honey production in honey bee colonies treated with amitraz and fluvalinate. * Statistically significant differences, $p \leq 0.05$. Each group N = 20.

The analysis of variance ANOVA in honey bee colonies treated with amitraz and fluvalinate based

on the p-values and a significance level of 0.05 showed that the interaction effect of fluvalinate and amitraz on oviposition and honey production in honey bee colonies are statistically significant (Table 3). Moreover, the differences between the effects of fluvalinate and amitraz on oviposition and honey production in honey bee colonies are not statistically significant, which means that both miticides have almost similar negative effects on honey bee colonies.

Table 3. Analysis of variance ANOVA the effect of the miticides amitraz and fluvalinate on useful traits of honey bee colonies

Useful traits	Comparison	DF	SS	SE	F-value	P-value
Honey productivity	Fluvalinate / Control	2	11.208	0.221	3.655	0.049*
	Amitraz / Control	2	43.745	0.199	14.265	0.001*
	Fluvalinate / Amitraz	2	6.476	0.232	2.112	0.164
Oviposition	Fluvalinate / Control	2	119544.450	0.233	30.735	0.001*
	Amitraz / Control	2	2446.021	0.228	0.629	0.044*
	Fluvalinate / Amitraz	2	6043.601	0.005	1.554	0.230

DF - degrees of freedom, SS - a sum of squares, SE-standard error, F-value - Fisher's exact test value, P-value – a value of probability, * - statistically significant differences.

Effects of fluvalinate on the expression of olfactory-related neuropeptide genes sNPF, TK, sNPFR.

The expression of sNPF was significantly increased in fluvalinate exposed honey bees (t-test = 4.41, p = 0.01). The expression of TK was not significantly changed in fluvalinate exposed honey bees (t-test = 0.80, p = 0.46). The expression of sNPFR was significantly decreased in fluvalinate exposed honey bees (t-test = 3.49, p = 0.01). Probably, the increased expression of sNPF due to that sNPF acts on target cells, through interaction with specific membrane receptor sNPFR. Therefore, the changes of sNPFR expression after fluvalinate treatment are important (Figure 2).

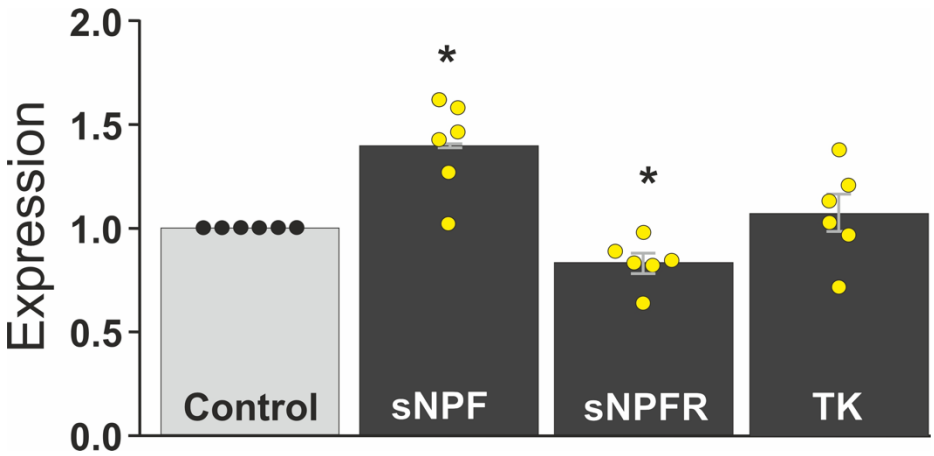

Figure 2. The patterns of sNPF, TK, and SNPFR neuropeptide genes expression. Relative expression levels of sNPF, TK, and sNPFR in antenna from control (light grey) and fluvalinate treated (dark grey) honey bees. Data points represent values from biological replicates. * Statistically significant differences, $p \leq 0.05$. Each group N = 6.

DISCUSSION

Evaluating all the options, it can be assumed that miticides have more advantages than disadvantages. The negative impact of ectoparasitic

mites on honey bees is increasing every day due to the increase in their numbers, while the negative impact of the miticides on honey bees tends to decrease due to detoxification processes. Therefore, the use of miticides in beekeeping is economically beneficial. In comparison with honey bees, ectoparasitic mites are more sensitive to these miticides due to their smaller size and less effective system of detoxification. Therefore, honey bees suffer less from miticides than ectoparasitic mites. However, miticides are not safe for bees. The analogous, negative effects of miticides amitraz and fluvalinate on honey bees A. mellifera were observed in previous studies (Ilyasov et al. 2014, Lim et al. 2020).

After external treatment, the honey bee colonies with miticides amitraz and fluvalinate their economically useful treats such as honey production, olfaction, and oviposition can be reduced. The expression of TK was not statistically significantly changed by fluvalinate treatment of honey bees, whereas the expression of sNPF was significantly increased and the expression of sNPFR was significantly decreased by fluvalinate treatment of honey bees. Fluvalinate did not affect the TK signaling pathway, but can significantly affect the sNPF signaling pathway, which can decrease learning and memory, gustation and olfaction of honey bees. Olfaction is a basic regulation tool for honey bees' social life organization (Nässel 2002, Taghert and Veenstra 2003, Hauser et al. 2006, Johnson 2006, Marciniak et al. 2011), foraging behavior, and honey production (Menzel 1999, Hewes and Taghert 2001, Johnson 2006, Hummon et al. 2006, Giurfa 2007, Altstein and Nässel 2010, Xu et al. 2016, Schoofs et al. 2017).

When used for a short time and with care, miticides can be less harmful to honey bees. We assumed, the short time treatment of honey bee colonies against *V. destructor* with miticides amitraz and fluvalinate can help honey bees to control pests in the colony and will have more benefits if the treatment will provide by schedule before honey harvesting in spring and after honey harvesting in autumn.

There is the global problem of the growing resistance of *V. destructor* mites to miticides amitraz and fluvalinate, which leads to increasing their dosages, which leads to increased toxicity to honey bees and contamination of beekeeping products (Rinkevich 2020). Fortunately, nine resistant to mite *V. destructor* populations of honey bee *A. mellifera* is obtained by constant selection: 1. Ireland North County Dublin honey bee population, 2. The population of *A. m. scutellata* in Brazil and South Africa, 3. Toulouse honey bee population, 4. Island of Fernando de Noronha honey bee population, 5. Primorsky, Russia honey bee population, 6. Gotland, Sweden honey bee population, 7. Avignon, France honey bee population, 8. Honey bee population of Arnot Forest, Ithaca, NY, USA, 9. Marmara island honey bee population in Turkey (Mondragón et al. 2005; Allsopp 2006; Locke and Fries 2011; Çakmak, Fuchs, 2013; Locke, 2016; Conlon et al., 2018; McMullan, 2018; van Alphen and Fernhout 2020). Thus, the constant selection of honey bee colonies for hygienic behavior and resistance to mite *V. destructor* is more preferable to using increasing amounts of miticides. Moreover, it is assumed, environmental factors may play a big role in reducing the population of Varroa mites, not only the genetics of honey bees (Çakmak, Fuchs, 2013).

CONCLUSION

The negative effect of miticides amitraz and fluvalinate on honey bees have been shown here experimentally. Besides, there is increasing resistance of Varroa mites to the miticides amitraz and fluvalinate. However, it has been shown that it is possible to obtain populations of honey bees resistant to Varroa mites by constant selection. Breeding varroa-resistant honey bees allow to reduce the use of miticides and produce organic honey. Thus, the further development of beekeeping should be in the direction of selection for disease and Varroa resistance and adaptation to the environment.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Consent to participate

The authors agree to participate in this research study.

Consent for publication

The authors agree to publish and a copyright transfer.

Availability of data and material/ Data availability

The qRT-PCR data used in this paper available in the database of GenBank. All other data is available upon request from the corresponding authors.

Code availability

The paper uses data obtained from open access materials in issues on journal websites. All used applications are available online through the internet.

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