Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

# The effects of deltamethrin (a synthetic pyrethroid insecticide), an anionic surfactant alone, and a co-formulated mixture of these substances on the honeybee (*Apis mellifera)* temperature preference, $CO_2$ emission, and expression of detoxification-related genes

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**DOI: 10.24425/jppr.2025.153825**

Received: May 06, 2024
Accepted: October 09, 2024
Online publication: June 03, 2025

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Responsible Editor:
Paweł Węgorek

## Abstract

Honeybees in crops are exposed to active ingredients of pesticides (plant protection proucts – PPPs) and adjuvants used in agriculture. The aim of this research was to estimate the effect of a sublethal dose of a pyrethroid insecticide (deltamethrin; Deltam), a plant protection product – adjuvant (alkylbenzene sulphonic acid sodium salt; Superam 10 AL), and a co-formulated mixture of those substances on the honeybee temperature preference, $CO_2$ emission, and expression of detoxification-related genes (CYP9Q1, CYP9Q2, CYP9Q3). When the measurement results were analyzed using outcome-matched statistical tests such as the Wilcoxon test, the conclusion was reached that Deltam, Superam 10 AL and the mixture of the two preparations significantly statistically differentiated the bees' thermal environment preference (a decrease in the preferred temperature by 0, 4, 0.9 and 0.5°C, respectively), altered $CO_2$ levels (an increase by 4.2% and a decrease by 10% and 13.5%, respectively) and statistically significantly increased the levels of CYP9Q1, CYP9Q2 and CYP9Q3 transcripts in the insects' head, but not in their thorax. The results indicate that the condition of honeybees can be affected by the finished formulation of the plant protection product, its adjuvant, and the mixture of both acting together, with a direction of change varying according to the studied parameters.

**Keywords**: alkylbenzene sulphonic acid sodium salt, deltamethrin, respirometry, thermoregulation, CYP9Q

## Introduction

In crops, pyrethroids are usually applied in spring because they have a negative temperature coefficient and lose their effectiveness to a significant extent at temperatures above 20°C (Malinowski 1982). Because they are characterized by a relatively low toxicity to warm-blooded organisms (Singh *et al.* 2022), these substances are also used to control parasites in humans (Hołyńska–Iwan and Szewczyk-Golec 2020), pets, and livestock (Bertero *et al.* 2020), including the Varroa destructor bees' parasite (Vlogiannitis *et al.* 2021). Because pests develop resistance to plant protection products (PPPs), including pyrethroids (Chen *et al.* 2020; Machani *et al.* 2020), new alternatives to formulations that are commonly available in the market are being sought today.

Since development and implementation of new active ingredients (AIs) for plant protection products (PPPs) is complex, challenging, and costly, new PPPs based on ingredients already approved for use are being developed (Kaczmarek *et al.* 2019). Adjuvants are not PPPs, but influence PPPs behavior in the environment and their effectiveness. These substances include surfactants, anti-foaming agents, compounds that improve the active ingredient stability in the environment, and adjuvants responsible for the effective absorption of AIs into the body, as well as prolonging their effect, or modifying the way and rate at which the substance is eliminated from the body (Gao *et al.* 2019). PPPs can be used with adjuvants, often making up to 90% of the composition of finished insecticide formulations (Hewitt 2024).

Honeybees, one of the most important crop pollinators, are exposed to PPPs and PPP-additive combinations (Piechowicz *et al.* 2022a; Hrynko *et al.* 2021). The presence of residues of plant protection products and their adjuvants in insect bodies can result in their varying influence on the rate of metabolic transformations (Maliszewska *et al.* 2010); although, as indicated by Terriere (1983), detoxification, as an energy demanding process, usually accelerates the rate of metabolic processes. One of the factors contributing to this situation is an increase in the expression of genes responsible for xenobiotics elimination from the body. These genes include, among others, CYP9Q1-Q3, as the first specific P450, which contributes to pesticide detoxification in honeybees (Mao *et al.* 2011; Piechowicz *et al.* 2021a, 2021b).

Disruptions in the body metabolism and internal homeostasis or a direct effect of toxic substances on the thermoregulation center in the nervous system can be manifested as changes in the insects' thermal preferences (Tęgowska 2003). Pyrethroids also have a similar effect (Vandame and Belzunces 1998). Bees are among animals whose thermal preferences change as a consequence of an action of those compounds (Piechowicz *et al.* 2022b).

The present experiment aimed at investigating and evaluating the effects of a pyrethroid insecticide (deltamethrin; Deltam), a commercial PPP-adjuvant (alkylbenzene sulphonic acid sodium salt; Superam 10 AL), and their co-formulated mixture on the thermal environment preference, metabolic levels measured by an analysis of the $CO_2$ emission rate, and the expression of detoxification genes (CYP9Q1, CYP9Q2, CYP9Q3) in *Apis mellifera* workers.

## Materials and Methods

Laboratory tests were carried out concerning the preferred ambient temperature, $CO_2$ emissions, and gene expression in 2021 and 2022.

### Animals used in laboratory tests

*Apis. mellifera carnica* workers was collected from an apiary located in the area owned by the University of Rzeszow, in Werynia village (Podkarpackie voivodeship, Poland), where there were no nectar-producing plants treated with PPPs within a radius of 2 km. In these studies, honeybees from four colonies were used (hives in the apiary were set at 3 m apart and marked, to limit wandering of individuals returning to them). For 4 weeks before and throughout the experiment, no protective treatments related to bee health (acaricides, acids, etc.) were conducted. The honeybee colonies which were studied showed no clinical symptoms of parasite or pathogen infection, except for a low level of *Varroa* infection.

Laboratory tests were conducted using honeybees obtained from frames placed in the honey super. A worker was understood as a foraging honeybee and/or a honeybee processing nectar. To assess ambient temperature preferences, $CO_2$ emissions, and expression of detoxification-related genes, 72, 400 and 400 honeybee individuals were used, respectively.

### Bee's plant protection products treatment procedure

The honeybees were transferred to the laboratory within 15 minutes of being collected from a hive, and cooled at a temperature of 4°C for 30 min in constant darkness to sedate them. Then, they were treated with distilled water (the control group) or with freshly prepared solutions of Deltam (Deltamethrin-Based Insecticide – DBI), AI – deltamethrin from pyrethroid group, 15 g · $l^{-1}$; an emulsion diluted with water before

application; producer: SBM Life Science, Poland Batch No.: EV63002396), and of Superam 10 AL [Adjuvant – AD), AI – alkylbenzene sulphonic acid sodium salt, 10%; producer: Danmar, Poland. Batch Nos.: 08.01.19 and 25.04.2022]. The concentration of both working solutions was 20 µl of the preparation in 1 l of water.

The solutions prepared in this way (with water as the control sample) were used at the amount of 5 µl per individual, topically on the ventral part of the prothorax because, under natural circumstances, that part of the honeybee body is most frequently in contact with pesticides when it lands on plants or the ground.

## Determination of honeybees' temperature preference

Thermal preference studies were performed similarly to those presented in the publication by Piechowicz *et al.* (2022b).

The honeybees were placed in a thermal gradient system immediately after treatment, at noon (12:00 ± 5 min). For each experimental group (control, DBI treated, AD treated, and treated with both PPPs), 18 recording cycles were performed.

In each group, the determination of the honeybees' thermal preference took 48 hours.

## Metabolic rate

The honeybee metabolic rate was measured indirectly by estimating the $CO_2$ emission rate from honeybee subsamples, for the control ($n$ = 10), DBI-treated ($n$ = 10), AD-treated ($n$ = 10), and treated with both PPPs ($n$ = 10) groups. Each experimental sample consisted of 10 workers from the same hive. Intoxicated bees (in the control, treated with water) were placed in a respirometric chamber (a volume of 80 ml, a diameter of 3.2 cm, and a length of 9.95 cm) placed in a laboratory incubator S 711 (Chłodnictwo-Madej, Poland) at 33°C, in constant darkness. Each measurement began at noon (± 15 min). We fed honeybees *ad libitum* with a 50% sucrose solution (50/50 vol/vol) throughout the measurement period.

Changes in the $CO_2$ levels in the air flowing around each of the studied groups of bees were measured by flow-through respirometry. Dry $CO_2$-free air (Air Liquide, Poland) flowed through the chambers to an infrared $CO_2$ analyzer (model: S157; Qubit Systems Inc., Canada). The C950-MCGES (version 3.8.7) software (Qubit Systems Inc., Canada) recorded the results using a single measurement cycle (measurements of a reference and a test sample) of 60 minutes (1100 s for each channel with animals and 300 s for the reference measurement – no chamber connected). The average from the last 50 seconds of each recording for the analysis was used. Each experiment lasted 48 hours.

## Expression of detoxification-related genes

The subsamples of honeybees, control ($n$ = 10), DBI-treated ($n$ = 10), AD-treated ($n$ = 10), and treated with both PPPs ($n$ = 10), were placed on Petri dishes in a laboratory incubator S 711 (Chłodnictwo-Madej, Poland) at 33°C and in constant darkness, with *ad libitum* access to a 50% sucrose solution (50/50 vol/vol). After 24 h of incubation, the honeybees were flash-frozen and immediately stored at –80°C until use. The expression of detoxification-related genes in 120 animals (30 control, 30 DBI treated, 30 AD treated, and 30 treated with both PPPs) was determined.

Total RNA was extracted from 10 heads or abdomens (in each group). Samples were put in a 2 ml RNase DNase-free tube containing zirconium/silica beads and 500 µl of TRIzol Reagent. Homogenization was performed on a FastPrep-24 Instrument (MP Biomedicals), in 2–4 cycles at 6 m/s for 30 to 40 s, with 5-minute incubation on ice between cycles. The RNA was isolated using the TRIzol reagent method (Invitrogen, USA) (Chomczyński and Sacchi 1987), and precipitated with ethanol.

The RNA extracts (1 µg) were reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA; catalog number: 4374966), following the manufacturer's protocol, with a thermal cycler (Biometra, Germany). A real-time PCR was performed with SYBRGreen PCR Master Mix (Applied Biosystems, USA: catalog number: 4367659) using primers CYP9Q1, CYP9Q2, and CYP9Q3. Ten µl reactions were set up with 1 µl of each cDNA sample diluted 10x, 5 µl of SYBRGreen PCR Master Mix, 0.4 µl 10 µm of each forward and reverse primer, and 3.5 µl of distilled water. All reactions were performed in triplicate. Real-time quantitative PCR amplification reactions were carried out using the StepOne Plus real-time system (Applied Biosystems, USA) using a program which started with a single cycle of 10 min at 95°C and 40 cycles of 15 s at 95°C and 60 s at 56°C. Afterwards, the PCR products were heated to 95°C for 15 s, cooled to 60°C for 1 min,

**Table 1.** Starter sequences of the genes CYP9Q1, CYP9Q2, CYP9Q3, and the reference gene eIF3-s8, used in the real-time qPCR technique

| Primers | | Starter sequences (5'-3') |
|---|---|---|
| CYP9Q1 | forward: | TCGAGAAGTTTTTCCACCG |
| | reverse: | CTCTTTCCTCCTCGATTG |
| CYP9Q2 | forward: | GATTATCGCCTATTATTACTG |
| | reverse: | GTTCTCCTTCCCTCTGAT |
| CYP9Q3 | forward: | GTTCCGGGAAAATGACTAC |
| | reverse: | GGTCAAAATGGTGGTGAC |
| eIF3-s8 | forward: | GAGTGTCTGCTATGGATTGCAA |
| | reverse: | TCGCGGCTCGTGGTAAA |

and heated to 95°C for 15 s to measure the dissociation curves to eliminate nonspecific amplifications. The gene expression results were analyzed using the StepOnePlus system (Applied Biosystems, USA) and normalized against the eiF3-S8 gene, used as the reference gene. eiF3-S8 as an endogenous control gene was chosen, because it was previously demonstrated that it is characterized by a limited variation in expression in tissues of honeybees of foraging age (Harrison *et al.* 2019).

## Statistical analysis

### Determination of honeybees' temperature preference/metabolic rate

The results were analyzed using STATISTICA Tibco 13.3 (StatSoft Inc., Tulsa, OK, USA) and R 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria) software. For both independent and dependent sample comparisons, non-parametric equivalents such as the Kruskal-Wallis test, the Friedman test, and Bonferroni-corrected multiple comparison tests or the Wilcoxon test were used in the statistical analyses, after establishing that the results did not meet the assumptions for the application of parametric tests. The ADF test was used in longitudinal analyses of the studied phenomena, to establish stationarity. If non-stationarity was established, estimation methods were used to determine the best-fitting function out of linear, logarithmic, exponential and power functions. If stationarity was established, the Holt-Winters model was used to illustrate changes in the studied phenomena over time. A significance threshold of two-sided p-values below 0.05 was used in all analyses.

### Expression of detoxification-related genes

Data underwent the analysis of variance (ANOVA) and Bonferroni post-test for pairwise comparison of means, where applicable. Statistical parameters were calculated using Prism 6 (GraphPad). P values below 0.05 were considered as statistically significant. Values are means ± SEM

## Results

### Temperature preference of *Apis mellifera*

The Augmented Dickey-Fuller test was used to check if the series describing changes in thermal environment preference over 48 hours in control bees and in bees treated with DBI, AD, and both substances simultaneously could be considered stationary or if a trend describing changes over time could be distinguished. In addition, basic parameters were calculated for the experimental data, and the Kruskal-Wallis test and multiple comparison tests with Bonferroni correction were used to determine if there were significant differences between groups of bees for the studied parameter (Table 2; Fig. 1).

The honeybees in the control group preferred the warmest thermal environment (33.3°C). Intoxicated insects preferred the environment with a lower temperature – of 32.9°C in the DBI-treated group, 32.4°C in the AD-treated group, and 32.8°C in the DBI + AD-treated group (Table 2).

The results show that the trend function can only be directly identified for the DBI + AD group ($p = 0.01$) (Table 2) (in this case, when the best-fit trend function was analyzed, it was found that of linear, power, and exponential functions, the best-fit function was a logarithmic function of the form $y = aln(x) + c$, where $a = -1.1894$, $c = 38.3396$; error $c = 0.2717$; and $a = 0.057$).

For the stationary series, the method of decomposing the series was used to identify a separate trend, random fluctuations and cyclical fluctuations. The Holt-Winthers model was then used to identify the best-fitting time course of the phenomenon, so that not only was a description of the changes in the phenomenon over time obtained but also it was possible to predict the phenomenon in the future (Table 3). Table 3 also contains information about the obtained trend models for the phenomenon after applying exponential equalization of random fluctuations with the best-fit parameter $\alpha$.

Additionally, the Kruskal-Wallis test shows a statistically significant difference between the variables distributions in individual groups. Using multiple comparison tests, it was demonstrated that the

**Table 2.** Preferential temperatures of the control and pesticide-intoxicated bees and types of trends identified

| Name | Kind of trend | *p*-value for adf test | q1 | q3 | Mean ±SD [°C] | Median |
|---|---|---|---|---|---|---|
| Control | stationary | 0.153 | 32.1 | 34.1 | 33.3 ± 1.54 | 33.2 |
| DBI | stationary | 0.505 | 31.3 | 34.3 | 32.9 ± 2.11 | 32.8 |
| AD | stationary | 0.060 | 31.6 | 33.3 | 32.4 ± 1.21 | 32.2 |
| DBI + AD | not stationary | 0.01 | 31.7 | 33.6 | 32.8 ± 1.48 | 32.6 |

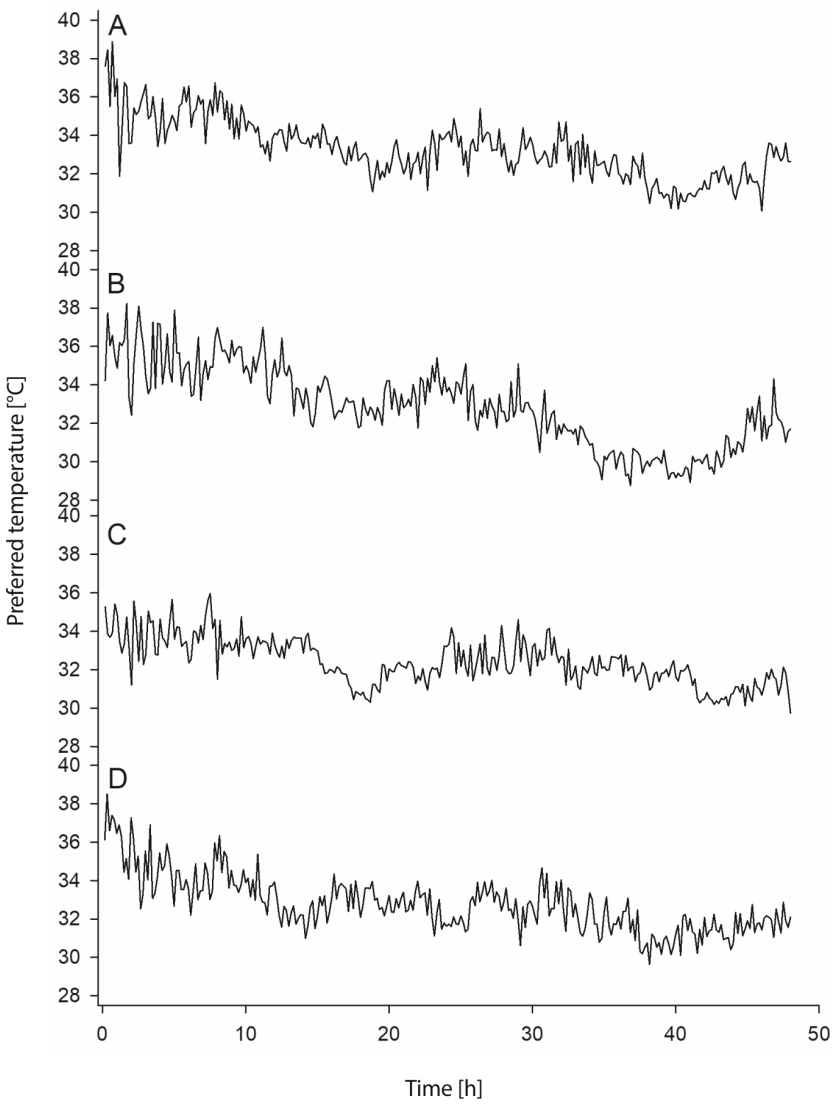


**Fig. 1.** Honeybee temperature preference. A – control group; B – DBI-treated; C – AD-treated; D – DBI + AD-treated bees

**Table 3.** Bees' thermal preferences. Results were obtained using the Holt-Winthers method

| Name | Alpha | Beta | Model | Intercpt | Coefficient *a* | *F* | *p*-value |
|---|---|---|---|---|---|---|---|
| Control | 0.488 | 0.107 | linear | 35.4 | –0.015 | 475.2 | 0.000 |
| DBI | 0.681 | 0.226 | linear | 36.1 | –0.022 | 561.3 | 0.000 |
| AD | 0.48 | 0.207 | logarithmic | 35.3 | –0.624 | 105.9 | 0.000 |

control group was statistically significantly different from other groups [$p = 0.012$(DBI), $p < 0.0001$ (AD), $p = 0.0005$ (DBI_AD), and $p = 0.004$ between the DB and DBI groups].

The results show that there is no cyclic variation in the thermal environment preference either in the control or in the intoxicated bees (the gamma coefficient = 0 in all cases). For these three cases, a statistically significant trend was identified in the phenomenon after adjusting for random fluctuations. This trend is a linear function in the control and DBI, and a logarithmic function for AD.

In addition to the studies described above, which determined the temporal variation in the phenomenon of honeybee workers' thermal preference, dependencies between selected temperature values in individual groups were also analyzed.

Using Pearson's linear correlation coefficient, it was assessed if there was a relationship between the thermal environment preferences of the different study

groups. In each case, there was a statistically significant and high (ranging from 0.507 for AD-DBI + AD, to 0.737 for control-DBI) positive correlation between the results obtained.

Another question was if distributions of these values differed significantly between the two identified groups. In order to compare the distribution of results for thermal environment preference between different groups of bees two different tests were applied. On one hand, it was the test described under Table 2 showing if the distributions of results differed between groups. The results were treated as repeated measurements for individual treatment groups, and the Friedman test was used to determine if at least one result of a measurement after treatment with a specific preparation differed from the others. The Wilcoxon test was used to compare the differences between each pair of results. In this study, the Friedman test showed a statistically significant difference in at least one distribution of results ($\chi 2 = 93.48$, $p < 0.0001$). The Wilcoxon test showed that, with the exception of DBI-DBI + AD ($p = 0.721$), all groups revealed statistically significant differences in the results obtained ($p = 0.000$ in each case).

The same statistical reasoning was applied to $CO_2$ emissions.

## *Apis mellifera* worker $CO_2$ emission

The average $CO_2$ emission was the highest (3039 µl/g b. wt. × $h^{-=-1}$) in animals treated with DBI throughout the measurement cycle, while it was slightly lower (2917 µl/g b. wt. × $h^{-1}$) in the control group. Honeybees treated with AD and with a mixture of the two formulations, emitted $CO_2$ in the amount of 2626 and 2524 µl/g b. wt. × $h^{-1}$, respectively (Table 4; Fig. 2).

A similar analysis of the temporal variation in $CO_2$ emissions showed that a trend could only be distinguished in the bees treated with DBI. Given that the significance in this case was on the borderline of the accepted significance level for the whole study, it was decided that treating this time series as stationary was a more appropriate approach (Table 4).

In addition, contrary to the temperature, no statistically significant differences in $CO_2$ levels were found between the study groups with the Kruskal-Wallis test.

As in the case of the temperature, the method of decomposing the series was used for stationary series to identify a separate trend, random fluctuations and cyclical fluctuations. Then, the Holt-Winthers model was applied to determine the best-fitting time course of the phenomenon. In this way, not only was a description of the changes obtained in the phenomenon over time, but it was also possible to predict the phenomenon in the future (Table 5). Table 5 also provides information on the obtained trend models for the phenomenon after applying exponential equalization of random fluctuations with the best-fit parameter $\alpha$.

In all cases, gamma equaled 0, indicating a lack of cyclicity in the bees' $CO_2$ emission in each studied group. Although cyclical changes in $CO_2$ emission could occur in both control and intoxicated bees, the number of cycles was too low to allow proper statistical analysis of the results obtained.

In this case, neither the use of Holt-Winthers models nor an attempt at modelling using ARiMA methods resulted in finding statistically significant models to describe changes over time in the studied phenomenon. This was probably due to the periodicity of the data, which became apparent after fitting polynomial trends to the raw data. The only trend detected was a polynomial of the sixth degree. On this basis, it can

**Table 4.** $CO_2$ emissions of control and intoxicated bees and designated trend types

| | Kind of trend | *p*-value for adf test | q1 | q3 | Mean ±SD [µl/g b. wt. × $h^{-1}$] | Median [µl/g b. wt.. × $h^{-1}$] |
|---|---|---|---|---|---|---|
| Control | stationary | 0.321 | 2122 | 3763 | 2917 ± 848 | 2999 |
| DBI | not stationary | 0.049 | 2285 | 3777 | 3039 ± 773 | 3173 |
| AD | stationary | 0.548 | 2047 | 3157 | 2626 ± 636 | 2528 |
| DBI+AD | stationary | 0.214 | 1920 | 3080 | 2524 ±667 | 2507 |

**Table 5.** Basic results of $CO_2$ analysis of control and intoxicated bees using the Holt-Winthers method

| | Alpha | Beta | Model | Intercpt | Coefficient *a* | Coefficient *b* | *F* | *p*- value |
|---|---|---|---|---|---|---|---|---|
| Control | 0.711 | 0.499 | square | 3484 | –58.69 | 49.13 | 0.715 | 0.250 |
| DBI | 0.895 | 0.379 | logarithmic | 2742 | 0 | 0 | 0.267 | 0.608 |
| AD | 1.000 | 0.052 | square | 2447 | 21.87 | 32.42 | 0.517 | 0.400 |
| DBI + AD | 1.000 | 0.016 | square | 2316 | 7.16 | 33.15 | 0.238 | 0.960 |

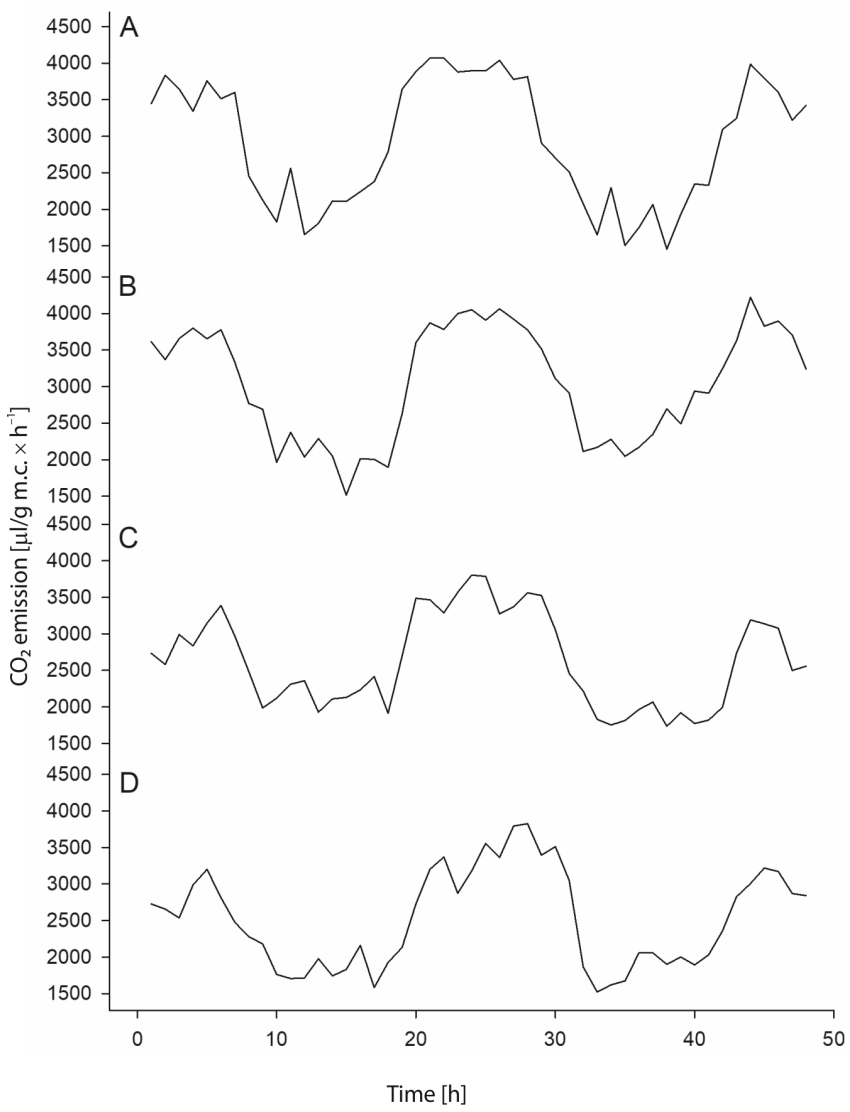


**Fig. 2.** $CO_2$ emissions in control and intoxicated bees. A–control group; B – DBI-treated; C – AD-treated; D – DBI + AD-treated bees

be concluded that none of the treatments affected the periodicity of the bees' $CO_2$ emissions.

Pearson's linear correlation coefficient assessed whether there was a correlation in $CO_2$ emissions between the different study groups. The results show that in each case, there was a statistically significant and pronounced (ranging from 0.799, control-DBI + AD, to 0.874, control-DBI) positive correlation between the results obtained.

As with the temperature, the data was treated as repeated measurements after treatment, and the Friedman test was used to determine if at least one post-treatment measurement differed from the others, and the Wilcoxon test to compare the differences between each pair. In this case, the Friedman test showed a statistically significant difference in at least one distribution of results ($\chi 2 = 42.05$, $p < 0.0001$). Using the Wilcoxon test, statistically significant differences in the results were found in some cases (control-DBI, $p = 0.023$, other differences: $p = 0.000$), excluding the AD treatment group and the DBI + AD treatment group, where no significant differences were observed ($p = 0.117$).

On the basis of the results obtained, the measurement cycle was divided into three periods: 1 to 9, 10 to 36 and 37 to 48 h from the start of the experiment. During the initial period (up to 9 h), the honeybees in each measurement group emitted the highest levels of $CO_2$. The lowest emissions were observed in the middle period of the measurement (10 to 36 h of the recording) for the control, DBI-treated and DBI + AD-treated honeybees, and in the last hours of the recording (37–48 h) for AD-treated honeybees (Table 6).

The Wilcoxon signed-ranks test for nonparametric analyses was used to compare the distribution of thermal environment preference results between different groups of honeybees over the measurement periods. For the intervals of 1 to 9 h and of 37 to 48 h of the

**Table 6.** Basic parameters for the analysis of $CO_2$ emissions from control and intoxicated bees over the three measurement periods

| | Measurement parameters in hours 1–9 | | | |
|---|---|---|---|---|
| | mean | median | q1 | q3 |
| Control | 3302 ± 599 | 3513 | 3344 | 3646 |
| DBI | 3408 ± 417 | 3614 | 3334 | 3659 |
| AD | 2790 ± 411 | 2835 | 2581 | 2989 |
| DBI + AD | 2651 ± 326 | 2658 | 2476 | 2813 |
| | Measurement parameters in hours 10–36 | | | |
| Control | 2808 ± 918 | 2560 | 2076 | 3878 |
| DBI | 2817 ± 868 | 2374 | 2046 | 3782 |
| AD | 2683 ± 710 | 2413 | 2110 | 3466 |
| DBI + AD | 2486 ± 805 | 2132 | 1743 | 3363 |
| | Measurement parameters in hours 37–48 | | | |
| Control | 2874 ± 818 | 3156 | 2197 | 3513 |
| DBI | 3261 ± 600 | 3242 | 2802 | 3768 |
| AD | 2375 ± 560 | 2282 | 1869 | 2906 |
| DBI + AD | 2515 ± 522 | 2592 | 2017 | 2938 |

measurement, the experimental groups differed significantly in $CO_2$ emissions in three (Control-DBI + AD, $p = 0.011$; DBI-AD, $p = 0.006$ and DBI-DBI + AD, $p = 0.001$) and two (DBI-AD, $p = 0.001$; DBI-DBI + AD, $p = 0.004$) cases, respectively. In the interval between 10 to 36 h, $CO_2$ levels were similar in all groups.

## Expression of selected genes of the P450 group

In the conducted experiments, the expression profiles of genes encoding CYP9Q1, CYP9Q2, and CYP9Q3, were analyzed in Apis mellifera either treated or not with Deltam, adjuvant, or a combination of both. The mRNA levels were analyzed separately in the head and the abdomen.

The results of the quantitative real-time PCR showed different expression patterns for a transcription level of analyzed genes, depending on the tissue. In the control group, the transcription level of

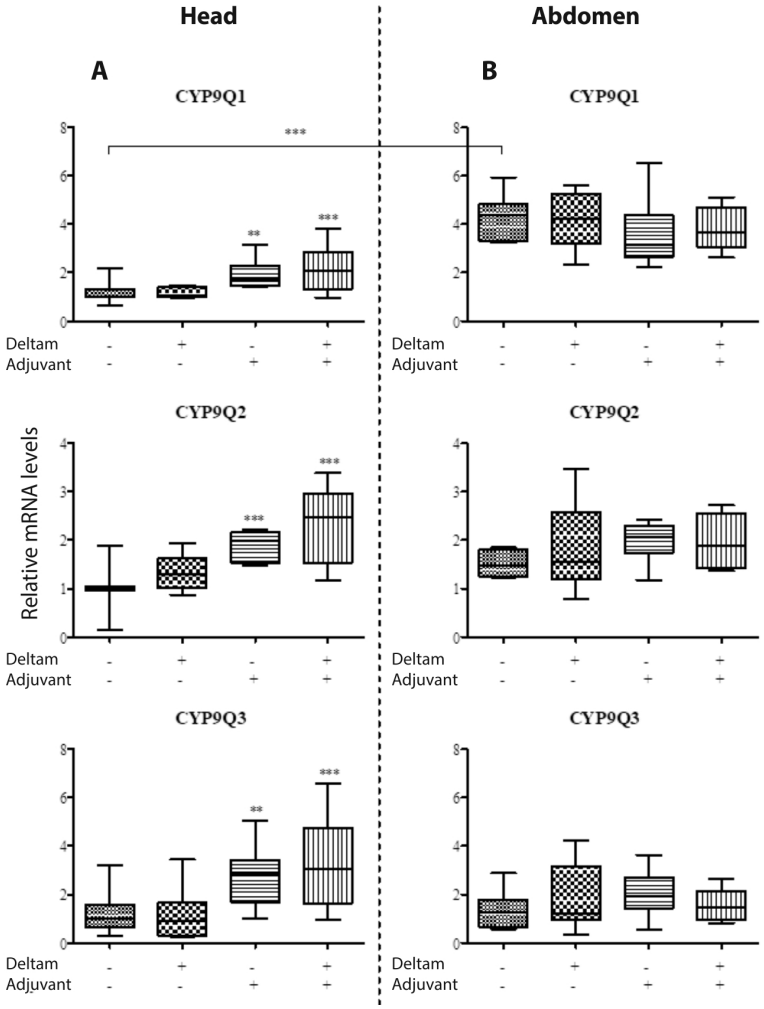


**Fig. 3.** qPCR results of the CYP9Q1-3 genes in the head (A) and abdomen (B) of honeybees after Deltam and/or adjuvant treatment. Statistical analysis was performed using GraphPad Prism 6.0 with a one-way analysis of variance, followed by Bonferroni's post hoc test. The results are presented as mean ± standard error of the mean (SEM). Significant differences were marked with **($p \leq 0.01$) and ***($p \leq 0.001$)

CYP9Q1 was significantly higher in the bees' abdomens ($p < 0.001$). The effect of Deltam and adjuvant on genes encoding CYP9Q1, CYP9Q2, and CYP9Q3 was different in the head and in the abdomen (Fig. 3).

When heads were compared, groups treated with adjuvant or a combination of Deltam and adjuvant showed higher transcription levels of CYPQ1, CYPQ2 and CYPQ3, than the control (Fig. 3A).

No changes in CYPQ1, CYPQ2 and CYPQ3 mRNA levels were observed in the abdomens of bees in each analyzed treatment group, in comparison to the control (Fig. 3B).

## Discussion

### Determination of the honeybee temperature preference

The ability to maintain their body temperature at a suitable, constant level is a tremendous evolutionary achievement for honeybees, as it allows the insect to feed efficiently under changing environmental conditions, as well as to maintain a continuous, optimal temperature in the nest for brood rearing. It is important, because honeybees from a brood that has developed under inappropriate thermal conditions have a poorer memory (Jones *et al.* 2005), are less resistant to environmental pollutants (Medrzycki *et al.* 2010) and finally, provide less food for the hive, which can affect the condition of the entire colony.

The results of this study (Table 2; Fig. 1) show that honeybees, regardless of the treatment group, chose an environment with a relatively constant temperature, which in the control group (33.3 ± 1.54°C) was similar to that of an earlier study (33.1 ± 1.2°C) (Piechowicz *et al.* 2022b) and slightly higher than that observed in the study by Grodzicki and Caputa (2014) (31.5°C). In this study, however, significant changes in honeybees' thermal preference in relation to the diurnal rhythm were not observed (Table 3), but had been visible in previous studies (Piechowicz *et al.* 2022b). Observed differences in thermal preference may result from the fact that in the past only older guarding and foraging workers were used with a marked diurnal cyclicity of physiological processes. In the current study, nest honeybees were investigated that included individuals performing all functions in the colony. As indicated by Moore *et al.* (1998), in honeybees the daily rhythm develops with age and depends both on the tasks performed in the hive and genotypic variation in the rate of behavioral development. The presence of juveniles among the honeybees, with a diurnal cycle of activity and rest (nest cleaning and larval feeding take place throughout the day), could reduce the amplitude of the diurnal variation in thermal preference. In the same way the sudden exposure of nest honeybees to constant light could also weaken the rhythm. For this reason, the study period was not divided into intervals that differed significantly in the ambient temperature chosen by the honeybees.

The application of PPPs influenced the thermal preferences of honeybees (Table 2; Fig. 1) by decreasing the temperature selected in the thermal gradient. Pyrethroids can influence the fluidity of cellular membranes (Moya-Quiles *et al.* 1996). Similarly, surfactants bind to bioactive macromolecules such as peptides, enzymes, and DNA. They can alter the polypeptide chain folding and the surface charge of the molecule, modifying the biological functions of these structures (Cserháti *et al.* 2002). This, and other factors, are the effect of oxidative stress (Shen *et al.* 2015), because both pyrethroids (Ding *et al.* 2017), and surfactants (Susmi *et al.* 2010) are generators of this effect. Tęgowska (2003) indicates that sufficient cell membrane fluidity in excitable cells is restored by selecting an appropriate thermal ambient. Also, the ratio of sodium to calcium ions in the thermoregulatory center significantly impacts an animal's thermoregulation, as the primary mechanism of pyrethroids' action is related to their modification of the function of sodium, as well as of calcium ion channels (Soderlund 2012). Shafer *et al.* (2005) claim that one of the mechanisms underlying the effect of pyrethroids is the induction of hypothermia. Research shows that chemicals such as pyrethroids (Soderlund 2012) or surfactants (Bardach *et al.* 1965) modify the functioning of an animal's receptors, interfering with the animal's perception of the environment. The observed changes in the thermal preference can result from impaired perception of external stimuli in honeybees.

Bees (i.e., single workers and the bee colonies forming superorganisms) can generate fever. That is an energetically costly response of the body to intoxication or effects of pathogens (Johnson 1899). As Kovac *et al.* (2007) believe, honeybees maintain their optimal body temperature at rest mainly by choosing an environment with an appropriate temperature, activating intensive thermogenesis only when their body temperature decreases significantly. During the research, while the studied insects were in a temperature gradient, they were provided with an environment having a wide temperature range, which allowed them to limit energy-costly endogenous processes to achieve the optimal temperature and making it possible to observe changes in their thermal environment preferences.

Interestingly, this study showed that the combination of AD + DBI had a weaker effect on honeybee thermoregulation than these compounds acting alone (these differences were not statistically significant). This is in contrast to studies of Vandame and Belzunces (1998), who reported no thermoregulatory

changes in deltamethrin-treated honeybees. However, they were present when the insects were co-treated with difenoconazole or prochlorazoles, suggesting an interaction between these products that increases their toxicity. It is hard to pinpoint the reasons underlying the obtained results for thermal preferences in honeybees (Table 2; Fig. 1), since the $CO_2$ emissions (Tables 4 and 6; Fig. 2) and P450 expression studies (Fig. 3) showed an increase in the effect of both substances tested when they acted together.

## Respirometry

Every living organism needs to fulfil its vital needs and, in social organisms, this involves tasks related to community survival. In honeybees, they include all daytime activities related to caring for broods, cleaning, wax extrusion, honey evaporation, or maintaining the correct temperature and humidity of the nest, and during the day, the processes of foraging, receiving and processing food brought by foragers, or protecting the nest from attacks of other insects. These activities of the honeybees probably had a much more pronounced effect on the observed diurnal variation in $CO_2$ cyclicity (Fig. 2; Table 6) than on the diurnal variation in the thermal preference (Table 4). The presence of pronounced cyclicity in $CO_2$ emissions may result from the fact that thermal preference studies were carried out in single individuals, whereas those concerning $CO_2$ emissions were conducted in groups of honeybees, which, moreover, possibly included older honeybees with clearly developed diurnal cyclicity. The absence of a brood, which uses chemical communication to force young worker honeybees to perform certain activities throughout the 24 hours, with the simultaneous presence of highly cyclical sentinels and collectors, could be the reason for the change in the daily rhythm in the honeybee nest. However, further studies are needed to confirm this hypothesis.

Using statistical methods, it was shown that none of the applied preparations or their mixtures disturbed the diurnal cyclicity of the honeybees (Tables 4 and 5; Fig. 2). However, Natarajan (1985) and Nicolau (1982, 1983) provided evidence that such an effect can possibly be generated by PPPs.

It is a commonly accepted assumption that metabolic detoxification mechanisms in insects are energy-expensive (Guedes *et al.* 2006). Usually the gene expression and related biosynthesis of amino acids and peptides require an energy supply (Wagner 2005). Protein breakdown is also highly energy-demanding (Kelly and McBridge 1990), and this process probably includes those damaged by the preparations used. Hence, if it is assumed that only detoxification processes (also including those related to the observed expression of CYPQ1, CYPQ2 and CYPQ3 in heads of bees intoxicated with an adjuvant and with the mixture of compounds, Fig. 3) are responsible for the rate of metabolic processes, in each case after intoxication, an increase in the metabolic rate of the tested insects should be observed. The present experiments showed both an increase in $CO_2$ emission in the honeybee group treated with DBI and a decrease in $CO_2$ emission in bees intoxicated with the PPPs synergists and with the mixture of both preparations (Table 4). Those observations are in line with studies of Castañeda *et al.* (2009) and Kliot and Ghanim (2012), who also did not show any increase in the metabolic rate in insects exposed to plant protection products. This also means that some other factors related to mechanisms of action of preparations and bee bodies themselves are responsible for changes in the $CO_2$ emission rate.

Neurons transmit signals by the $Na^+$ and $K^+$ ion flow. That flow generates an electrical impulse called an action potential (AP) (Hodgkin and Huxley 1952). After AP, the $Na^+/K^+$ pump resets the levels of $Na^+$ and $K^+$ ions back to their initial values, to make the neuron ready to transmit another AP, when needed (Glitsch 2001). Kelly and McBride (1990) believe that the $Na^+/K^+$ pump function is one of the highest energy-demanding processes in the organism. The most important mechanism underlying the pyrethroid effect extends the time for which sodium channels in excitable cells are open (Soderlund 2012). In these circumstances, sodium influx to the cell induces a more intense function of the Na+/K+ pump to prevent the cell interior from being overloaded with sodium, and to restore an initial resting membrane potential. For this reason, the increase in the pump operation may be the primary factor influencing the increase in the rate of $CO_2$ emission in the group of animals treated with Deltam (Table 4; Fig. 2). An increase in the metabolic rate of insects treated with pyrethroids was also demonstrated for *Anauplotrupes stercorosus* (Piechowicz *et al.* 2015) and *Tenebrio molitor* (Maliszewska *et al.* 2010). Kakko (2004) showed that pyrethroids reduce the Na-K-ATPase and the total ATPase levels in nerve cells. This means that pyrethroids can restrict the amount of energy available to ion pumps, and thus contribute to the increased rate of $CO_2$ emissions in the first 9 hours after the intoxication, and again just after 37 hours, when the body has probably eliminated most of the deltamethrin. Previous studies on the degradation of another pyrethroid, λ-cyhalothrin, showed that honeybees rapidly detoxify compounds from this group. During the first 6 hours of measurements, degradation rates ranged from 11.43 to 14.29% $\cdot h^{-1}$ before slowing to less than 1% $\cdot h^{-1}$ (Piechowicz *et al.* 2021b).

The mechanism underlying surfactant toxicity is a nonpolar narcosis, in which the presence of the surfactant in the cell membrane interferes with membrane-dependent life processes, such as energy metabolism and transport of nutrients and oxygen across the membrane (Cowan-Ellsberry *et al.* 2014). Thus, the reduced metabolic rate measured by respirometry may result from the interaction between the surfactants and the biological membrane. The disruption of cellular nutritional processes, combined with a slowdown in metabolism and, therefore, probably also processes of biological membranes repair, may be responsible for the overall reduction in $CO_2$ emissions in AD-treated honeybees. However, this requires further and more thorough molecular and biophysical studies. Faster energy depletion as a result of more intense operation of the sodium-potassium pump and activation of detoxification processes after deltamethrine poisoning, combined with the slowing of transmembrane energy flux caused by the surfactant, could contribute to the observed slowing of $CO_2$ emissions in honeybees treated with both preparations in periods of 1–9 h and 10–36 h after treatment. The increased rate of carbon dioxide emissions in the last measurement period can be explained by results of earlier studies (Piechowicz *et al.* 2021a, 2021b), according to which it can be assumed that honeybees eliminated most of deltamethrin. To date, any references specifying the rate of surfactant elimination from the bee organism have not been found.

### P450 expression

Cytochrome P450 monooxygenases are a large and complex group of heme-thiolate proteins found in almost all living organisms (Yu *et al.* 2015). P450 monooxygenase participates in deltamethrin detoxifying, together with esterase and glutathione-S-transferase (Yang *et al.* 2017).

A statistically significant effect of deltamethrin in the applied dose on the transcriptional activity of P450s was not observed (Fig. 3), which is inconsistent with previous studies showing the impact of the pyrethroid λ-cyhalothrin (applied as Karate Zeon 050 CS; Syngenta Limited, UK) on the transcriptional activity of CYP9Q1, CYP9Q2 and CYP9Q3 in whole honeybees, with changes depending on the time of day (Piechowicz *et al.* 2021b) and the temperature used (Piechowicz *et al.* 2021a). Mao *et al.* (2011) observed similar changes, suggesting the involvement of CYP9Q1, CYP9Q2, and CYP9Q3 in the detoxification of pyrethroids. However, other authors suggest that other P450s are also involved in the detoxification of pyrethroids in insects (such as CYP4M5, CYP4M9, CYP6B29 and CYP9A22, CYP305B1, CYP6AB4, CYP6AE and CYP9A19 (Zhao *et al.* 2011); CYP4CB1 and CYP4CC1 (Jiang *et al.* 2012); CYP408B1, CYP9AQ2 and CYP6F (Guo *et al.* 2016); or CYP9A40 (Wang *et al.* 2015)). Unfortunately, it was not possible to verify this assumption for technical reasons.

Surfactants may be potential inhibitors of P450 enzymes. They inhibit, among others, the CYP3A4-mediated metabolism of testosterone, and the CYP2C9-mediated metabolism of diclofenac in human liver cells (Christiansen *et al.* 2011). The inhibitory effect of these compounds was also observed for P450 3A (*in vitro* and *in vivo* studies) (Ren *et al.* 2009). The present research showed a significant increase in CYP9Q1-3 expression in samples taken from the head of honeybees treated with AD solution alone (Fig. 3). A similar relationship was found in studies in mice, where an increase in CYP4A10 and CYPA13 expression was shown after treatment with ToximulTM (a mixture of structurally heterogeneous nonionic and anionic hydrocarbons used as an adjuvant in crop protection products) (Upham *et al.* 2007). The expression level of CYP450s was significantly higher in the abdomen than in the head. At the same time, significant changes in CYP450 expression between the control and the AD-treated group for samples from bee abdomens were not observed.

When applied together, AD and DBI caused the most pronounced increase in CYP450 expression in the honeybee head, although no significant statistical differences were observed in the abdomen (Fig. 3). That confirms that adjuvants modify the efficacy of PPP (Straw *et al.* 2022; Wernecke *et al.* 2022), and in this response, the effect of crop protection ingredients is more highly pronounced in the region of the greatest accumulation of nerve cells, representing the main target for deltamethrin, which primarily disrupts sodium ion conductance across the cell membrane.

## Conclusions

The present study showed that PPPs combined with synergists can affect honeybees differently. On one hand, a slight, nonsignificant weakening of the effect of the applied insecticide when it acted together with the adjuvant (thermoregulation) was observed; on the other hand, in the case of respirometric tests and the expression of selected P450s, an increase in the potency of the preparations when they were applied together was observed. A clear explanation for these results has not been determined, and they require further extensive studies. However, the fact that this preparation, which was used at very low doses, affects bees is very worrying, because under field conditions, the honeybees are exposed to residues of a much larger number of formulations than applied there.

## References


Bardach J.E., Fujiya M., Holl A. 1965. Detergents: effects on the chemical senses of the fish *Ictalurus natalis* (le Sueur). Science 148 (3677): 1605–1607. DOI: 10.1126/science.148.3677.1605

Bertero A., Rivolta M., Davanzo F., Caloni F. 2020. Suspected environmental poisoning by drugs, household products and pesticides in domestic animals. Environmental Toxicology and Pharmacology 80: 103471. DOI: 10.1016/j.etap.2020.103471

Chen M., Du Y., Nomura Y., Zhorov B.S., Dong K. 2020. Chronology of sodium channel mutations associated with pyrethroid resistance in *Aedes aegypti*. Archives of Insect Biochemistry and Physiology 104 (2): e21686. DOI: 10.1002/arch.21686

Castañeda L.E. Figueroa C.C., Fuentes-Contreras E., Niemeyer H.M., Nespolo R.F. 2009. Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*. Journal of Experimental Biology 212 (8): 1185–1190. DOI: 10.1242/jeb.020990

Chomczyński P., Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162 (1): 156–159. DOI: 10.1016/0003-2697(87)90021-2

Christiansen A., Backensfeld T., Denner K., Weitschies W. 2011. Effects of non-ionic surfactants on cytochrome P450-mediated metabolism *in vitro*. European Journal of Pharmaceutics and Biopharmaceutics 78 (1): 166–172. DOI: 10.1016/j.ejpb.2010.12.033

Cowan-Ellsberry C., Belanger S., Dorn P., Dyer S., McAvoy D., Sanderson H., Versteeg D., Ferrer D., Stanton K. 2014. Environmental safety of the use of major surfactant classes in North America. Critical Reviews in Environmental Science and Technology 44 (17): 1893–1993. DOI: 10.1080/10739149.2013.8037

Cserháti T., Forgács E., Oros G. 2002. Biological activity and environmental impact of anionic surfactants. Environment International 28 (5): 337–348. DOI: 10.1016/s0160-4120(02)00032-6

Ding R., Cao Z., Wang Y., Gao X., Luo H., Zhang C., Ma S., Ma X., Jin H., Lu C. 2017. The implication of p66shc in oxidative stress induced by deltamethrin. Chemico-Biological Interactions 278: 162–169. DOI: 10.1016/j.cbi.2017.10.005

Gao Y., Li X., He L., Li B., Mu W., Liu F. 2019. Role of adjuvants in the management of anthracnose – change in the crystal morphology and wetting properties of fungicides. Journal of Agricultural and Food Chemistry 67 (33): 9232–9240. DOI: 10.1021/acs.jafc.9b02147

Glitsch H.G. 2001. Electrophysiology of the sodium-potassium-ATPase in cardiac cells. Physiological Reviews 81 (4): 1791–1826. DOI: 10.1152/physrev.2001.81.4.1791

Grodzicki P., Caputa M. 2014. Diurnal and seasonal changes in thermal preference of single, isolated bees and small groups of bees (*Apis mellifera* L.). Journal of Insect Behavior 27 (6): 701–711. DOI: 10.1007/s10905-014-9460-6

Guedes R.N.C., Oliveira E.E., Guedes N.M.P., Ribeiro B., Serrao J.E. 2006. Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. Physiological Entomology 31 (1): 30–38. DOI: 10.1111/j.1365-3032.2005.00479.x

Guo Y., Wu H., Zhang X., Ma E., Guo Y., Zhu K. Y., Zhang J. 2016. RNA interference of cytochrome P450 CYP6F subfamily genes affects susceptibility to different insecticides in *Locusta migratoria*. Pest Management Science 72 (11): 2154–2165. DOI: 10.1002/ps.4248

Harrison J.W., Palmer J.H., Rittschof C.C. 2019. Altering social cue perception impacts honey bee aggression with minimal impacts on aggression-related brain gene expression. Scientific Reports 9 (1): 14642. DOI: 10.1038/s41598-019-51223-8

Hewitt A.J. 2024. Adjuvant use for the management of pesticide drift, leaching and runoff. Pest Management Science 80 (10): 4819–4827. DOI: 10.1002/ps.8255

Hodgkin A.L., Huxley A.F. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. The Journal of Physiology 117 (4): 500–554. DOI: 10.1113/jphysiol.1952.sp004764

Hołyńska-Iwan I, Szewczyk-Golec K. 2020. Pyrethroids: How they affect human and animal health? Medicina 56 (11): 582. DOI: 10.3390/medicina56110582

Hrynko I., Kaczyński P., Łozowicka B. 2021. A global study of pesticides in bees: QuEChERS as a sample preparation methodology for their analysis - Critical review and perspective. Science of the Total Environment 792: 148385. DOI: 10.1016/j.scitotenv.2021.148385

Jiang H.B., Dou W., Tang P.A., Wang J.J. 2012. Transcription and induction profiles of three novel p450 genes in *Liposcelis bostrychophila* (Psocoptera: *Liposcelididae*). Journal of Economic Entomology 105 (2): 560–572. DOI: 10.1603/ec11324

Johnson J.C. 1899. The pathology of fever. Atlanta Journal-Record of Medicine 1 (6): 433–444.

Jones J.C., Helliwell P., Beekman M., Maleszka R., Oldroyd B.P. 2005. The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. Journal of Comparative Physiology A 191 (12): 1121–1129. DOI: 10.1007/s00359-005-0035-z

Kaczmarek D.K., Rzemieniecki T., Marcinkowska K., Pernak J. 2019. Synthesis, properties and adjuvant activity of docusate-based ionic liquids in pesticide formulations. Journal of Industrial and Engineering Chemistry 78: 440–447. DOI: 10.1016/j.jiec.2019.05.023

Kakko I. 2004. Toxic mechanisms of pyrethroids studied *in vitro*. Acta Universitatis Tamperensis, 1018.

Kelly M., McBride B.W. 1990. The sodium pump and other mechanisms in large mammals. Proceedings of the Nutrition Society 49: 185–202.

Kliot A., Ghanim M. 2012. Fitness costs associated with insecticide resistance. Pest Management Science 68 (11): 1431–1437. DOI: 10.1002/ps.3395

Kovac H., Stabentheiner A., Hetz S.K., Petz M., Crailsheim K. 2007. Respiration of resting honeybees. Journal of Insect Physiology 53 (12): 1250–1261. DOI: 10.1016/j.jinsphys.2007.06.019

Machani M.G., Ochomo E., Zhong D., Zhou G., Wang X., Githeko A.K., Yan G., Afrane Y.A. 2020. Phenotypic, genotypic and biochemical changes during pyrethroid resistance selection in *Anopheles gambiae* mosquitoes. Scientific Reports 10 (1): 19063. DOI: 10.1038/s41598-020-75865-1

Malinowski H. 1982. Wpływ temperatury na aktywność owadobójczą fotostabilnych pyretroidów. Rocznik Nauk Rolniczych 18 (1–2). (in Polish)

Maliszewska J., Tęgowska E., Grajpel B., Adamkiewcz B. 2010. Effect of application of capsaicin and pyrethroid on metabolic rate in mealworm *Tenebrio molitor*. Ecological Chemistry and Engineering 17 (10): 1355–1359.

Mao W., Schuler M.A., Berenbaum M.R. 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). Proceedings of the National Academy of Sciences 108 (31): 12657–12662. DOI: 10.1073/pnas.1109535108

Medrzycki P., Sgolastra F., Bortolotti L., Bogo G., Tosi S., Padovani E., Porrini C., Sabatini A.G. 2010. Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. Journal of Apicultural Research 49 (1): 52–59. DOI: 10.3896/IBRA.1.49.1.07

Moore D., Angel J.E., Cheeseman I.M., Fahrbach S.E., Robinson G.E. 1998. Timekeeping in the honey bee colony: Integration of circadian rhythms and division of labor. Behavioral Ecology and Sociobiology 43 (3): 147–160. DOI: 10.1007/s002650050476

Moya-Quiles M.R., Muñoz-Delgado E., Vidal C.J. 1996. Effects of the pyrethroid insecticide permethrin on membrane fluidity. Chemistry and Physics of Lipids 79 (1): 21–28. DOI: 10.1016/0009-3084(95)02503-0

Natarajan G.M. 1985. Disruption of circadian rhythm of tissue respiration in *Channa striatus* by Metasystox®. Experientia 41 (5): 612–614. DOI: 10.1007/BF02007683

Nicolau G.Y. 1982. Circadian rhythms of RNA, DNA and protein content in the rat thyroid, adrenal and testis in chronic pesticide exposure. I. Effects of a fungicide (Mancozeb). Endocrinologie 20 (4): 249–257.

Nicolau G.Y. 1983. Circadian rhythms of RNA, DNA and protein in the rat thyroid, adrenal and testis in chronic pesticide exposure. III. Effects of the insecticides (dichlorvos and trichlorphon). Physiologie 20 (2): 93–101.

Piechowicz B., Gola J., Grodzicki P. 2015. Selected insecticides as modifiers of the metabolic rate in *Anoplotrupes stercorosus* under various thermal conditions: the effect of indoxacarb and beta-cyfluthrine. Acta Scientiarum Polonorum Silvarum Colendarum Ratio et Industria Lignaria 14 (1): 57–67. DOI: 10.17306/J.AFW.2015.1.6

Piechowicz B., Kobielska M., Koziorowska A., Podbielska M., Szpyrka E., Pieniążek M., Potocki L. 2021a. Dynamics of λ-cyhalothrin disappearance and expression of selected P450 genes in bees depending on the ambient temperature. Open Chemistry 19 (1): 1242–1249. DOI: 10.1515/chem-2021-0104

Piechowicz B., Kuliga A., Kobylarz D., Koziorowska A., Zaręba L., Podbielska M., Piechowicz I., Sadło S. 2022a. A case study on the occurrence of pyrimethanil, cyprodinil and cyflufenamid residues in soil and on apple leaves, blossoms and pollen, and their transfer by worker bees to the hive. Journal of Plant Protection Research 62 (2): 176–188. DOI: 10.24425/jppr.2022.141355

Piechowicz B., Początek E., Woś I., Zaręba L., Koziorowska A., Podbielska M., Grodzicki P., Szpyrka E., Sadło S. 2022b. Insecticide and fungicide effect on thermal and olfactory behavior of bees and their disappearance in bees' tissues. Environmental Toxicology and Pharmacology 95: 103975. DOI: 10.1016/j.etap.2022.103975

Piechowicz B., Sudoł M., Grodzicki P., Podbielska M., Szpyrka E., Zwolak A., Potocki L. 2021b. The dynamics of pyrethroid residues and Cyp P450 gene expression in insects depend on the circadian clock. Environmental Research 194: 110701. DOI: 10.1016/j.envres.2020.110701

Ren X., Mao X., Cao L., Xue K., Si L., Qiu J., Schimmer A.D., Li G. 2009. Nonionic surfactants are strong inhibitors of cytochrome P450 3A biotransformation activity *in vitro* and *in vivo.* European Journal of Pharmaceutical Sciences 36 (4-5): 401–411. DOI: 10.1016/j.ejps.2008.11.002

Shafer T.J., Meyer D.A., Crofton K.M. 2005. Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. Environmental Health Perspectives 113 (2): 123–136. DOI: 10.1289/ehp.7254

Singh S., Mukherjee A., Jaiswal D.K., de Araujo Pereira D.A., Prasad R., Sharma M., Kuhad R.C., Shukla A.C., Verma J.P. 2022. Advances and future prospects of pyrethroids: Toxicity and microbial degradation. Science of The Total Environment 829: 154561. DOI: 10.1016/j.scitotenv.2022.154561

Straw E.A., Thompson L.J., Leadbeater E., Brown M.J.F. 2022. 'Inert' ingredients are understudied, potentially dangerous to bees and deserve more research attention. Proceedings. Biological Sciences 289 (1970): 20212353. DOI: 10.1098/rspb.2021.2353

Soderlund D.M. 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Archives of Toxicology 86: 165–181. DOI: 10.1007/s00204-011-0726-x

Shen C.C., Shen D.S., Shentu J.L., Wang M.Z., Wan M.Y. 2015. Could humic acid relieve the biochemical toxicities and DNA damage caused by nickel and deltamethrin in earthworms (*Eisenia foetida*)? Environmental Science: Processes and Impacts 17 (12): 2074–2081. DOI: 10.1039/c5em00288e

Susmi T.S., RebelloS., Jisha M.S., Sherief P.M. 2010. Toxic effects of sodium dodecyl sulfate on rass carp *Ctenopharyngodon idella*. Fishery Technology 47 (2): 157–162.

Terriere L.C. 1983. Enzyme induction, gene amplification and insect resistance to insecticides. 265–298. In: "Pesticide resistance in arthropods" (R.T. Roush, B.E. Tabashnik, eds.). Springer, Boston, USA. DOI: 10.1007/978-1-4684-4466-7_11

Tęgowska E. 2003. Insecticides and thermoregulation in insects. Pesticides 1: 47–75.

Upham J., Acott P.D., O'Regan P., Sinal C.J., Crocker J.F.S., Geldenhuys L., Murphy M.G. 2007. The pesticide adjuvant, Toximul™, alters hepatic metabolism through effects on downstream targets of PPARα. Biochimica et Biophysica Acta – Molecular Basis of Disease 1772 (9): 1057–1064. DOI: 10.1016/j.bbadis.2007.06.003

Vandame R., Belzunces L.P. 1998. Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation. Neuroscience Letters 251 (1): 57–60. DOI: 10.1016/s0304-3940(98)00494-7

Vlogiannitis S., Jonckheere W., Laget D., de Graaf D., Vontas J., Van Leeuwen T. 2021. Pyrethroid target-site resistance mutations in populations of the honey bee parasite *Varroa destructor* (Acari: *Varroidae*) from Flanders, Belgium. Experimental and Applied Acarology (85) 2021: 205–221. DOI: 10.1007/s10493-021-00665-9

Wagner A. 2005. Energy constraints on the evolution of gene expression. Molecular Biology and Evolution 22 (6): 1365–1374. DOI: 10.1093/molbev/msi126

Wang R.L., Staehelin C., Xia Q.Q., Su Y.J., Zeng R.S. 2015. Identification and characterization of CYP9A40 from the tobacco cutworm moth (*Spodoptera litura*), a cytochrome P450 gene induced by plant allelochemicals and insecticides. International Journal of Molecular Sciences 16 (9): 22606–22620. DOI: 10.3390/ijms160922606

Wernecke A., Eckert J.H., Forster R., Kurlemann N., Odemer R. 2022. Inert agricultural spray adjuvants may increase the adverse effects of selected insecticides on honey bees (*Apis mellifera* L.) under laboratory conditions. Journal of Plant Diseases and Protection 129: 93–105. DOI: 10.1007/s41348-021-00541-z

Yang Z., Zhang Y., Jiang Y., Zhu F., Zeng L., Wang Y., Lei X., Yao Y., Hou Y., Xu L., Xiong C., Yang X., Hu K. 2017. Transcriptional responses in the hepatopancreas of *Eriocheir sinensis* exposed to deltamethrin. PLoS ONE 12 (9): e0184581. DOI: 10.1371/journal.pone.0184581

Yu L., Tang W., He W., Ma X., Vasseur L., Baxter S.W., Yang G., Huang S., Song F., You M. 2015. Characterization and expression of the cytochrome P450 gene family in diamondback moth, *Plutella xylostella* (L.). Scientific Reports 5 (1): 8952. DOI: 10.1038/srep08952

Zhao G., Zhao S., Gao R., Wang R., Zhang T., Ding H., Li B., Lu C.D., Shen W.D., Wei Z. 2011. Transcription profiling of eight cytochrome P450s potentially involved in xenobiotic metabolism in the silkworm, *Bombyx mori*. Pesticide Biochemistry and Physiology 100 (3): 251–255. DOI: 10.1016/j.pestbp.2011.04.0