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PP-094 [Bee Health]

Altering the expression of *hsp* and *amp* genes to potentially enhance stress tolerance by curcumin and quercetin containing nutritional supplement in honey bees (*Apis mellifera*)

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Introduction

Exposure of cells to stressors such as **high/low temperature, pesticides** and **pathogens** can damage cellular proteins. **Heat shock proteins (HSPs)** respond to various **abiotic** and **biotic stressors**, act as molecular chaperons to protect cells against apoptosis therefore they are essential for avoiding stress damage. However, in case of viruses elevated production of HSPs can be detrimental because **viruses** can hijack HSPs and utilize the native HSPs of the host to help them invade cells and nucleus. In addition, the different stressors affect the regulation of genes coding for **antimicrobial peptides (AMPs),** which are part of the **innate immune system**.

Aim of the study

We hypothesize that changes in the expression of *hsp* and *amp* genes can lead to increased stress tolerance depending on the type of stress. The aim of our research was therefore to **change the expression of genes encoding HSP**s and **AMP**s in honey bees, following treatment with **curcumin**, as a potentially applicable cytoprotective agent and with **quercetin**, as a plant flavonoid.

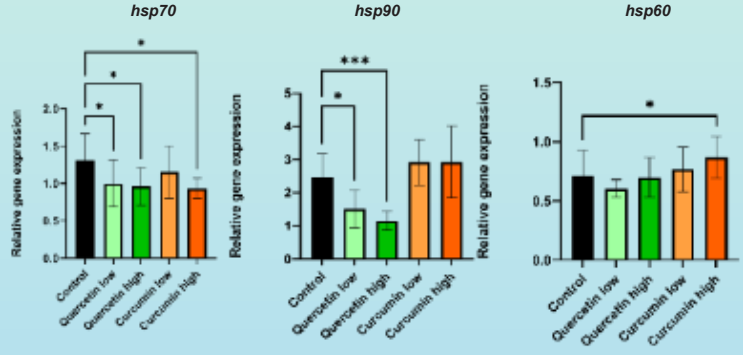
Materials and methods

5 treatment groups, n=15 bees/ group
48-hour treatment with **feeding solutions**:

- Curcumin in low (0.03 mmol) and in high concetration (0,3 mmol)
- Quercetin in low (0,1 mmol) and in high concentration (1mmol)
- Controll with normal feeding solution

RNA isolation from 3 head tissue homogenate *(Zymo Research, Direct-zol™ RNA Microprep)*
cDNA synthesis *(Thermo Scientific™ RevertAid First Strand cDNA Synthesis Kit)*
Determination of **relative gene expression by real-time PCR** *(Thermo Scientific™ DreamTaq PCR Master Mixes)* with the use of gene specific and β-tubulin primers (selected based on literature data).

Results



Relative gene expression of *hsp70*, *hsp90* and *hsp60*. The mean difference is statistically significant at P < 0.05 level, marked by asterisks. Statistical analysis was performed using one-way ANOVA and Dunnet's posthoc test. * p < 0.05, *** p < 0.001

Conclusion

- **Curcumin** and **quercetin** containing feeding solutions have **significant effect** on the **relative expression** of researched genes compared to their controls.
- With **curcumin treatment** we successfully **increased** the expression of *hsp60* and *hsp70* while **quercetin treatment** caused **reduced** mRNA levels of *hsp70* and *hsp90*.
- Interestingly in the case of *amp* genes no significant changes were observed due to the different treatments.
- Based on our research **curcumin** and **quercetin** can serve as nutritional supplements for bees to **help** them **tolerate** the various **stress factors** depending on the type of stressor that should be avoided.

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Background/purpose
Honey bees (Apis mellifera) play a crucial role in agriculture as pollinators of crops and plant species, however the different abiotic and biotic stressors are greatly affecting them. Exposure of cells to stressors such as high/low temperature, pesticides and pathogens can damage cellular proteins. Heat shoch proteins (HSPs) respond to various abiotic and biotic stressors, act as molecular chaperons to protect against apoptosis, thus, they are vital for cellular protein homeostasis and regulate the cell cycle. Although, viruses can hijack HSPs and can utilize the native HSPs of the host to help them invade cells and nucleus. In addition, the different stressors affect the regulation of genes coding for antimicrobial peptides (AMPs), which are part of the innate immune system.

Problem Description
We assume that changes in the expression of hsp and amp genes can lead to increased stress tolerance depending on the type of stress. The aim of our research was therefore to change the expression of genes encoding HSPs and AMPs in honey bees, following treatment with curcumin, as a potentially applicable cytoprotective agent and with quercetin, as a plant flavonoid.

Methodology
For alteration of gene expression, bees were provided feeding solutions containing curcumin (0.3 and 0.03 mmol) and quercetin (0.1 and 1 mmol) for 48 hours. After the treatment, whole head tissue homogenate was used for RNA isolation then cDNA synthesis was performed and the relative expression of hsp (hsp60, hsp70, and hsp90) and amp (defensin, vitellogenin and apidaecin) were determined using real-time PCR.

Results, CONCLUSION: Our results suggest that curcumin and quercetin containing feeding solutions have significant effect on the relative expression of researched genes compared to their controls. With curcumin treatment we successfully increased the expression of hsp60 and hsp70 while quercetin treatment caused reduced mRNA levels of hsp70 and hsp90. In the case of amp genes, however, no significant changes were observed due to the different treatments.

Practical Implications for Beekeepers
Based on our research curcumin and quercetin can serve as nutritional supplements for bees to help them tolerate the various stress factors depending on the type of stressor that should be avoided.