

Changes in the *lysozyme-1* gene and lysozyme activity in development of honeybee (*Apis mellifera*) in response to infection with *Varroa destructor*

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Phenomenon of mass disappearance of honey bees (CCD-Colony Collapse Disorder) is the result of co-interaction of many factors, but especially large negative part in its essence has mite *Varroa destructor*. This is a parasitic mite feeding on hemolymph of capped brood and mature individuals of *Apis mellifera.* Effectors for the immune in honey bees include a compliment of antimicrobial peptides, the melanizing agent phenoloxidase and three lysozymes. Lysozymes are qualified to perform a dual role in insects, a digestive function in the gut and an immune defense.

The aim of the research was the analysis expression of *lysozyme-1*(GB10231) gene and lysozyme activity in development of *A. mellifera* infected with *V. destructor*.

The research include 5 stages of *A. mellifera* include: larvae (L5), pre-pupae (PP), pupae (P3, P4) and brood out imago naturally infestationed and free from parasite. Isolation RNA from tissues with kit total RNA (A&A Biotechnology, Poland). RT-PCR, cDNA synthesis with TranScriba Kit (A&A Biotechnology). Quantitative real-time PCR was performed using a SYBRGreen PCR-MIX Taq™ (A&ABiotechnology) according to the manufacturer's instructions. The mean value±SD was used for analysis of relative transcript levels for each time point using the ΔΔCt method. The data were analyzed and normalized relative to *rp49* (stages free from mite) transcript levels by an AB analysis software (7500v2.0). All samples were tested in triplicate on light cycler (Applied Biosystem, FAST7500). Lysozyme activity was determined according to the methods proposed by de Azambuja et al. (1991).

Up-regulated expression of *lysozyme-1* genes was observed in all studied stages except imago. The highest expression of this gene was detected in P5 stage (30.0-times). Lysozyme activity was lower in infected bees than in the control bees in all developmental stages. The only exception was stage P5 where lysozyme activity was 2.0-fold in infected than control.

The results provide information on the immune response in larvae, pupaes and imago of *A. mellifera*, confirming decrease of the activity of the immune system to *Varroa* infestation.