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External immune stimulation and its impact on the honey bee *Apis mellifera jementica* in Saudi Arabia

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The primary aim of this study was to investigate the antimicrobial immune response of the adult honeybee worker *Apis mellifera jementica* stimulated with *Micrococcus luteus* (ATCC 10240) bacteria or the immune elicitor, Lipopolysaccharide (LPS) and comparing their antimicrobial activity after feeding bees on standardized dose of thymoquinone, the active ingredient of black seed oil *Nigella sativa*, for 72 hours, then bees were injected with LPS or *M. luteus*. Different antimicrobial peptides elicited in worker hemolymph treated groups were evaluated *in vitro* by an agar well diffusion assay against *Escherichia coli* strain (ATCC 25922) as Gram negative bacteria, *Micrococcus luteus* as Gram positive bacteria. The adult crude hemolymph of worker bees *Apis mellifera jementica* were obtained from naturally mated queen colonies in the apiary of the Research Institute in King Saud University. They were collected from hives in May 2012. The age of bees was determined by colour labeling of 11-19 h old workers which were isolated of combs maintained in an incubator at 34°C. Workers of particular ages gathered into plastic tubes and divided into three groups, the first was left untreated (control), the second immunized by 0.5 µl of 20 ng LPS/10 µl of APS (*Apis* physiological saline, pH: 4.5) or 0.5 µl of 1.15×10⁶ cells/ml *M. luteus*. The third group were first allowed to feed on 10% glucose solution (w/v) containing 0.3% of thymoquinone (W/v) for 72 hours, and then were injected with LPS or *M. luteus*. Results of solid agar inhibition zone assays revealed significant effects of antimicrobial peptides elicited in worker *Apis mellifera jementica* immunized with LPS against *E. coli* (ATCC 25922), the diameter of growth inhibition zone reached (18.0±0.00 mm) compared to control non stimulated group (13.33±0.667 mm). However, in LPS-injected, group post-fed on Thymoquinone, non significant increase was observed. Comparable results are detected towards *M. luteus* with inhibitory zone diameter of (20.667±0.66 mm), that continued the same increase in the Thymoquinone fed group with a diameter of (24.7±0.42 mm) compared to the intact non injected group. Comparably worker bees challenged with the infectious dosage 1.1x10⁶ cells/ml of *M. luteus* (ATCC10240), showed that significant inhibition zone diameters were detected towards *E. coli* (ATCC 25922) of (17±1.33) and also the inhibitory zone continued until the highest range of it appeared on feeding with Thymoquinone as the diameter of (22.667±0.667 mm) was obtained. The current study also included the use of Flow Cytometry technique to determine the rate of *Apis mellifera jementica* worker hemocytes that are subjected to programmed cell death post injection with infectious dosage of 1.1x10⁶ cells/ml *M. luteus* (ATCC 10240). Results showed a moderate increase in the rate of programmed cell death of hemocytes in comparison to control group (non- induced bees). Such findings are considered the groundwork in an initial step for future study of the programmed cell death gene. Further experiments are in progress to evaluate the cytotoxicity of a standardized thymoquinone dose stimulation on the worker bees to avoid the adverse effects of this enhancer on bees trade off and vitality.

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