Propolis and Honey: Examining Antiviral Mechanisms Against *Israeli acute paralysis virus*

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Introduction

Why does honey have antiviral properties? Honey has multiple chemical and physical properties that may contribute to its inhibitory effects. Previous studies have examined a low pH level as a potential cause for its inhibitory properties. Propolis is a waxy substance that honey bees, *Apis mellifera*, produce and place throughout the hive to protect themselves from various infections. Propolis contains numerous plant-derived chemicals from the bee's environment, including water-soluble chemicals that end up leeching into their food, honey[1]. For this experiment, we collected data on the effects of a sucrose-propolis extract diet compared to honey on *Israeli acute paralysis virus* (IAPV) infection in honey bees. The purpose of this research was to better understand the mechanisms behind the antiviral properties of honey through the lens of chemical content in propolis. Understanding how to combat viruses like *Israeli acute paralysis virus* which is linked to Colony Collapse Disorder a major threat to many agricultural crops[2]. If honey's inhibitory effects on IAPV infection are primarily attributed to the chemical content derived from propolis, then propolis extract will show equal or greater inhibitory effects.

Project Description

- Divided into three groups based on food provided: honey (H), sucrose - propolis extract diet (2,500 micrograms/mL; PE) and a 50% (w/v) sucrose diet (C).
- Divided those three groups into three subgroups (nine total treatments) based on injection type: live virus (IAPV), heat killed virus (HKV), and handling control or no virus (HC).
- 5 cage replicates were used per treatment, with 25 bees per cage
- Recorded mortality data from each cage at 24- and 48-hours post-infection.
- Collected 5 live bees per cage at 6-, 12-, and 24-hours post-infection for quantification of IAPV genome equivalents.

Figure 1: *Apis mellifera* collecting and recycling propolis.

Methodology

- Mortality assay
- IAPV titer quantification assay
  - RNA extraction & qPCR
- Statistical analysis
  - Cox proportional-hazards regression analysis

Figure 2: Injection of *Apis mellifera* worker with inoculum

Data

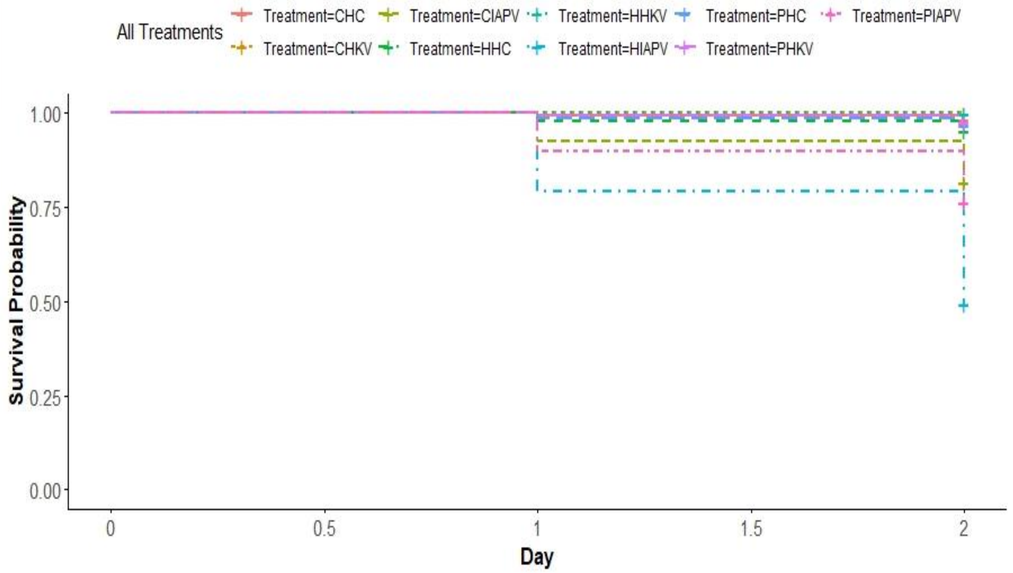

Figure 3: Cox proportional-hazards regression showing mortality data of all nine treatment groups over a span of two days.
HC; handling control, HKV; heat-killed virus, IAPV; IAPV infected, P; propolis extract and sucrose diet, C; sucrose diet, H; honey diet

Multiple Comparasion Test	Hazard Ratio	P-Value
CHC vs PHC	1.9974	0.424
CHC vs HHC	2.9881	0.180
CHC vs CHKV	3.14×10^{-11}	1.000
PHC vs PHKV	0.7514	0.708
HHC vs PHC	0.6678	0.532
HHC vs HHKV	1.14×10^{-11}	1.000
CHKV vs PHKV	1.19×10^{10}	1.000
CHKV vs HHKV	0.0000	NaN
CHKV vs CIAPV	1.22×10^{10}	0.999
PHKV vs PIAPV	11.2840	5.88E-05
HHKV vs HIAPV	1.04×10^{11}	0.999
HHKV vs PHKV	1.19×10^{10}	1.000
CIAPV vs PIAPV	1.3247	0.289
CIAPV vs HIAPV	3.5785	3.17E-08
HIAPV vs PIAPV	0.3782	2.91E-06

Table 1: Cox proportional-hazard analysis table displaying the hazard ratio (HR) and P-value. P-values less than 0.0042 are statistically significant.

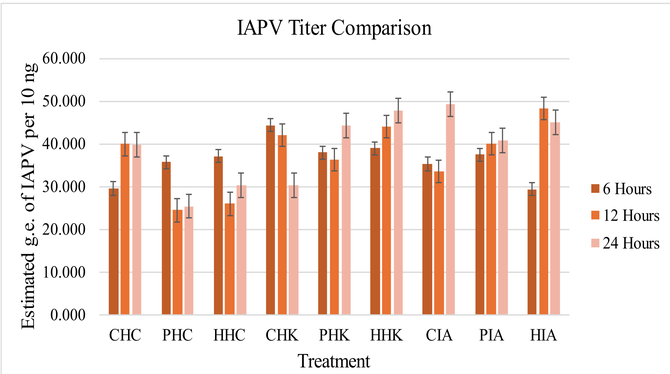

Figure 4: qPCR titer comparison of IAPV between nine treatment groups over a twenty-four-hour period. There are three groups: six hours, twelve hours, and twenty-four-hours.
HC; handling control, HKV; heat-killed virus, IAPV; IAPV infected, P; propolis extract and sucrose diet, C; sucrose diet, H; honey diet

Discussion and Conclusions

The analysis of the cox proportional-hazard regression shows no significant difference between the control IAPV group and propolis IAPV group. There is a statistical difference between the honey and control IAPV groups indicating that the honey IAPV group was at an increased risk compared to both the propolis and control groups. Of all the multiple camparison tests, only three were statistically significant, with the CIAPV and PIAPV against HIAPV demonstrating that HIAPV experienced a much higher even potentially anomalous mortality. A possible reasons behind the anomaly is an issue with dosing the control and propolis groups. For qPCR analysis all the treatment groups resulted in nondetectable levels of IAPV which is likely caused by a group of strong bees or a dosing issue.

Future Work

Future work will include running more trials of this experiment with a greater sample size to get better accuracy . Additional trials can be performed with a higher dose of IAPV to get quantification data. Further studies could also examine the exact mechanisms behind the inhibitory effects of honey, such as osmotic pressure.

References

Berenbaum MR et al. 2021. DOI: 10.1146/annurev-ento-040320-074933.[1]; Chen YP et al. 2014. DOI: 10.1371/journal.ppat.1004261.[2]; Hsieh EM et al. 2020. DOI: 10.3791/61725.[3]; Bankova V et al. 2016. doi: 10.1080/00218839.2016.1222661.[4]

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Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 2349220/2349221, as part of the MICRO-CCS: Microbial Interactions Create Research Opportunities for Community College Students program through the University of Illinois at Urbana-Champaign and Parkland College: https://publish.illinois.edu/micro-ccs/

Thank you to Dr. Adam Dolezal, Sam Mowery, Dr. Sarah Hind and Dr. Chelsea Lloyd for the opportunity to learn and grow as a scientist. Thanks to Kenneth Chaung and Zainub Ahmed for all the help you gave me with injections, RNA extractions and so much more.