Assessment of the *in-vitro* inhibitory activity of HiveAlive™ against *Ascosphaera apis*, the causative agent of Chalkbrood infection in honeybees

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Abstract

Chalkbrood is one of the most common and widely recognised fungal diseases that affects honeybee brood, the causative agent of which is *Ascosphaera apis*. Currently, there are no approved treatments for the management of Chalkbrood infection in beekeeping operations. Anecdotal evidence from beekeepers suggests that use of the feed supplement HiveAlive may cause a reduction in Chalkbrood infection. Here, a number of *in-vitro* tests were carried out to assess this hypothesis including agar dilution assays and 96 well plate microdilution assays. A concentration of 0.25% inhibited the growth and sporulation of *A. apis* on agar plates. Results from the 96 well microdilution assays suggest that even lower concentrations may be effective at inhibiting growth. Further research is required to validate the findings presented here and to examine effects in a hive setting.

Introduction

Chalkbrood usually occurs in damp weather and is most common in the spring. Inadequate nutrition and exposure to other diseases or conditions such as chilled brood or colony stress may also promote Chalkbrood. Currently, there are no approved treatments for this fungal infection. Reports from beekeepers suggest that supplementing hives with HiveAlive, a feed for bees, decreases levels of Chalkbrood observed.

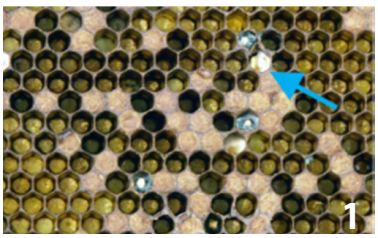

Image 1: Blue arrow indicates chalkbrood infection in brood on a frame.
Image 2: Chalkbrood mummies taken from hive infected with chalkbrood.
Images taken from Aronstein & Holloway, 2013

Materials & Methods

Plugs of agar from cultures of positive and negative mating strains (ARSEF7405 and ARSEF7406) of *Ascosphaera apis* were placed approximately 4cm apart from each other on Sabouraud Dextrose agar supplemented with varying concentrations of HiveAlive. Plates were subsequently incubated at 30°C and growth was monitored every 24 hours.

Spore solution was obtained from 7 day old plates containing both mating strains with visible spores present on the plate by flooding the plate with 5ml of sterile H_2O and swirling. The liquid was removed and placed in a tube to allow spore cysts (ascoma) to be ruptured to release spores balls (asci) by mechanical crushing. Spores balls (asci) contain ascospores which are the primary source of infection. This solution was added to a 96 well plate in SD media supplemented with varying concentrations of HiveAlive. Growth was monitored spectrophotometrically.

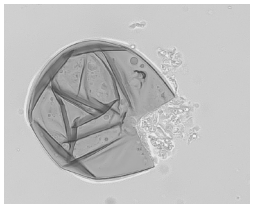

Image 3: Spore cyst which has been mechanically burst to release spore balls. 1000x magnification.

Results & Discussion

Results from agar dilution plates suggest that a concentration of 0.25% HiveAlive prevents growth of *A. apis* (see image 4). At a lower concentration of 0.05%, growth is slightly inhibited but is not inhibited at all when 0.005% HiveAlive is present. This would suggest that HiveAlive is suited to prevent sporulation of *A. apis*. A range of concentrations were also tested in the microdilution assay and it was found that even lower than 0.25% inhibited growth. Further experiments are required to determine minimum inhibitory concentration *in vitro* and determine effects in a hive setting.

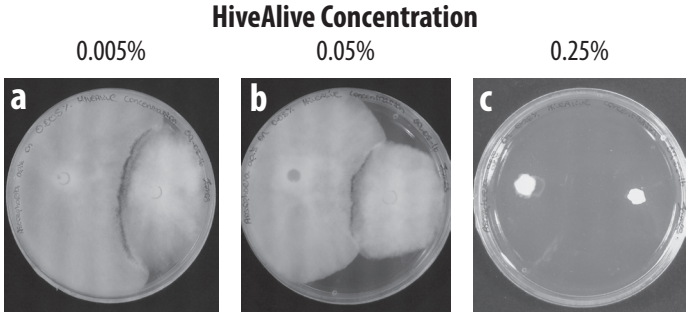

Image 4: Effect of addition of HiveAlive to agar medium on *A. apis* after 7 days incubation. Lower concentrations as seen in image a and b did not inhibit the growth of *A. apis*. Fungal growth can be observed around each of the agar plugs, with spore production visible where the two mating strains meet. A concentration of 0.25% HiveAlive appeared to inhibit growth as seen in c.

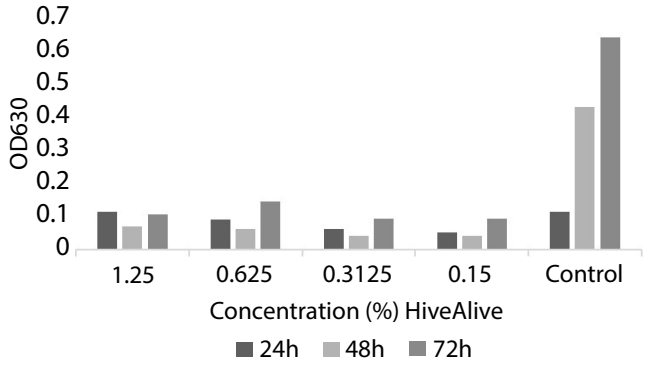

Image 5: Growth of *A. apis* in media supplemented with HiveAlive over 72 hours represented by absorbance at 630nm. All concentrations of HiveAlive used here appear to inhibit growth.

References: Aronstein, K.A., Holloway, B.A. 2013. Honey bee fungal pathogen, *Ascosphaera apis*; current understanding of host-pathogen interactions and host mechanisms of resistance. Microbial Pathogenesis. ISBN (13)1:402-410.