

Control of Chalk Brood Disease with Extracts from *Thujaopsis dolabrata* (Aomori Hiba)

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Steam-distilled oil and distilled water from *Thujaopsis dolabrata* var. *hondae* Makino (Aomori hiba) contain hinokitiol, β -dolablin, and other substances which show strong antibacterial and antifungal activities. Hiba oil and hinokitiol were found to inhibit the growth of *Ascosphaera apis* (chalk brood disease fungus) at concentrations 1,600 $\mu\text{g/ml}$ and 25 $\mu\text{g/ml}$, respectively. Heavy infection by the disease was cured by spraying distilled water from aomori hiba on infected frames.

1. INTRODUCTION

Honey chalk disease, found in the larva of honeybees, is caused by the parasite chalk brood disease fungus (*Ascosphaera apis*) resulting in white mummification. This chalk disease was thought to be endemic to Europe, however, has been confirmed in America, Canada, and New Zealand. It first arrived in Japan in 1979, and was transported from Kagoshima to Gifu prefectures in three out of one hundred hives, however, it has since spread and become a significant problem throughout domestic beekeeping enterprises. Following this introduction into Japan, various prevention methods have been tried, however, incineration disposal was found to be most effective.

In the present paper, with the objective of finding a suitable and effective means of preventing chalk disease, hiba oil, distilled water from hiba and hinokitiol, with its superior sterilization properties against the growth of parasitic fungi was tested with regard to chalk disease prevention effectiveness.

2. EXPERIMENTAL METHOD

2.1. Antibacterial test

The antibacterial effectivenesses of hiba oil and hinokitiol against *A. apis* were conducted in the following manner. The bacterial agent *A. apis* was obtained from beekeeper Kougenji. Hiba oil was extracted from the heartwood of aomori hiba by a steam distillation. Hinokitiol was prepared by separated from hiba oil and purified.

The antibacterial tests were conducted under the Japan Science of Chemotherapy, the minimum inhibitory concentrations (MICs) were determined by the agar dilution method using Sabouraud dextrose agar supplied by Eiken Chemical Co., Ltd.. The method of the antifungal tests of volatile matter can be seen in Fig. 1.

2.2. Hinokitiol volatility result test

The hinokitiol volatility test was conducted by gasification of the hinokitiol and testing as an

antifungal material. Using three hives where the white chalk had invaded the entrance of the nests, making collection of honey impossible, three grams of hinokitiol was hung for three months, as shown in Fig. 2. The results were measured after a period of three months.

2.3. Hiba oil volatility result test

To test the disinfectant effect of hiba oil as a volatile matter, in hives with multiple layers of chalk, hiba oil was painted on the dungarees in three hives, as seen in Fig. 3, and on the inner surface of the cover in three hives, as in Fig. 4. The painting was repeated once after a five day period and the results were tested after a period of one month.

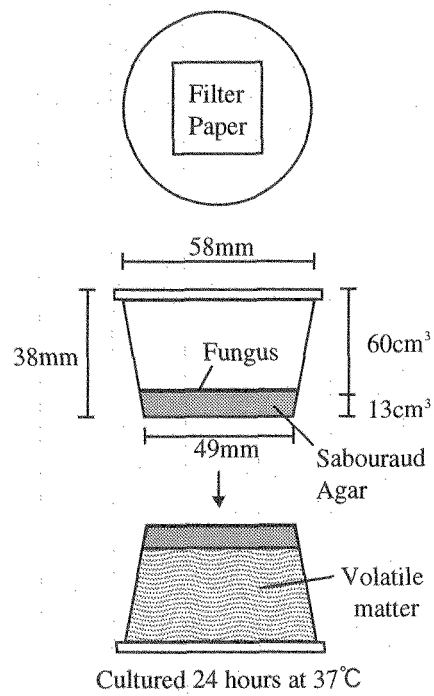


Fig.1 Antifungal test of volatile matter.



Fig.2 The photo of antifungal test suspending hinokitiol with a string in a beehive.



Fig.3 The photo of antifungal test painting hiba oil at the dungarees.

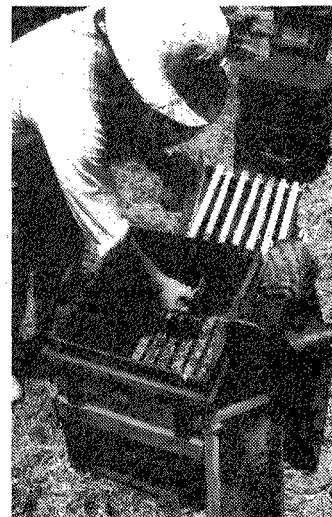


Fig.4 The photo of antifungal test painting hiba oil at a beehive.

2.4. Hiba oil water suspension test

Hinokitiol with approximately 100 µg/ml included in a water suspension was sprayed on both the hive frame and the interior surface of four hives infected with chalk and three unaffected hives as shown in Fig. 5. Testing was done on three of the infected hives and two of the unaffected hives at a period of one month, and on one of each at a period of two months.

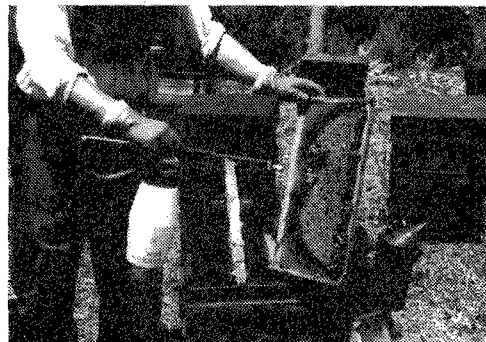


Fig.5 The photo of antifungal test spraying steam-distilled water.

3. RESULTS AND DISCUSSION

3.1. Antibacterial tests

The results of antibacterial tests for hiba oil and hinokitiol, as well as several standard agricultural chemicals against *A. apis* are shown in Table 1. To achieve the same inhibitory results, contrasting amounts are needed, as seen in the amount for standard chemical treatments (3200 µg/ml), as opposed to hiba oil (1600 µg/ml), and hinokitiol (25 µg/ml). Further, as the hinokitiol used in the hiba oil suspension came to be approximately 100 µg/ml, it is clear that even a Hiba water suspension is effective as a growth inhibitor against *A. apis*.

Hiba oil volatility test results as a growth inhibitor against *A. apis* are shown in Table 2. As can be seen, at 60 cm³, effectively 20 mg, which is to say 1 m³ for approximately 337 g usage, *A. apis* inhibition can be considered as potential.

3.2. Hinokitiol volatility test results

In the case of hinokitiol used in the the base of infected hives, growth of chalk disease was suppressed, however, a large number of dead bees was also observed. As the growth of the sickness was controlled,

Table 1 Minimum inhibitory concentrations of various antifungal agents, hiba oil and hinokitiol against *Ascosphaera apis*.

Agents	MIC(µg/ml)
Benlate wettable powder	3,200
Polyoxin AL wettable powder	> 6,400
Lobral wettable powder	> 6,400
Hiba Oil	1,600
Hinokitiol	25

Table 2 Growth inhibitions by volatile method.

Hiba oil concentrations (mg/filter paper)	Growth inhibitions
4 0	—
2 0	—
1 0	+
5	+
2 . 5	++
1 . 0	++
0 . 5	++
0	+++

-:Growth inhibition, +:Growth (+ < ++ < +++).

it is clear that hinokitiol gas is effective in infected hives. However, whether the origin of the bee deaths arose due to a suffocation environment brought about by the hinokitiol gas, or whether the hinokitiol itself disrupted an internal enzyme process is unknown.

3.3. Hiba oil volatility test results

In the case of hiba oil, no positive result could be seen in either the case of the oil being painted on the infected hive frame or in the case of painting of the hive's inner surface. Hiba oil, being a natural wood product, if used with a wood preservative should exhibit good results. However, in this experiment, as opposed to the case of the Hinokitiol anti-bacterial results, the antiseptic properties were not clear. Which is to say, hiba oil may provide a supplementary effect when applied to in the inner surfaces of uninfected hives, as a measure to hinder the rampant growth of the disease.

3.4. Test of hiba in suspension dispersion

Hiba distilled water contains approximately 100 µg/ml of hinokitiol. However, even with full hive coverage, no bee death or evasion was observed. In terms of dispersion to the infected hives, as direct contact works most effectively in an anti-bacterial process, this would be the best method.

One month after treatment of the three infected hives, one hive was completely free of infection, while two showed minor growth. Among the two uninfected hives, neither showed growth, and further, both were filled with bee larvae. After two months, the remaining infected hive was fully reinfected, however, the

remaining uninfected hive, being completely free of infection, was moved close to the infected hive. With this, the previously infected hive made a complete recovery to uninfected status. Thus, it can be predicted that hiba applied in suspension can have a high effectiveness against chalk disease as well as a effect against reinfection by regrowth.

4. CONCLUSION

In the testing of oil extracted from aomori hiba, hiba oil in suspension, as well as hinokitiol as a superior antibacterial agent against honeybee chalk disease, the results are as follows:

- (1) Hinokitiol was effective when used in the interior of hives infected with the disease, however, also resulted in a high number of dead bees.
- (2) Hiba oil, used in painting treatments above and inside the hives, showed effective results.
- (3) Hiba in suspension shows exceptional effect in preventing chalk disease, and shows effectiveness in preventing propagation of the disease as well.

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