Nosema Control by Thymol and Other Essential Oils

Project No.: **08-POLL2-Huang**

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Objectives:

1). To study the effect of essential oils on *Nosema ceranae*, to determine if certain oil would reduce spore load in bees; and
2). To determine whether essential oils can improve survival in the host honey bees.

Interpretive Summary:

Methods

Newly emerged bees (younger than 24 hr) were fed with 2 ul 50% sugar syrup containing 18,000 freshly extracted Nosema spores (18,000 spores per bee). All bees were starved for 2 hr before Nosema feeding. Immediately after inoculation, the bees were placed in the cages, 50 bees each, and kept at 34 °C, 50% RH, in the dark for 25 days. Group 1 to group 13 were fed with nosema spores and group 16 was a blank control without feeding nosema spores. Dead bees in each cage were counted and removed daily. The syrup and pollen were weighed on the days 2, 8, 14, and 25 to monitor their consumption. In order to monitor the progress of nosema infection, we collected 2 live bees from each cage on day 8, 13, 19, and 25 and checked their spore loads. The experiment was replicated in 4 colonies, therefore each treatment had a total of 200 bees.

Results

1. Essential oils used in the experiment
   Three selected essential oils, thymol, origanum oil, and clove oil, were tested on adult honey bees. Two formulations of thymol and origanum oil and one formulation of clove oil were mixed in pollen patties or 50% sugar syrup to feed worker bees from the day of emergence. The concentrations of the essential oils in the pollen patties or sugar syrup were chosen on the basis of preliminary tests and previous research (Maistrello et al. 2008) and are shown in Table I.

Table I. Controls and essential oils used in the experiment

No.	Treatment	Dose	Formulation
1	S		Control for 6,7,10,11
2	SE		Control for 4,8,12
3	PE		Control for 5,9,13
4	T(0)-SE	0.12 mg/g SE	Thymol crystals in SE
5	T(0)-PE	0.4 mg/g PE	Thymol crystals in PE
6	T(St)-S	0.12 mg/g S	Starch-encapsulated Thymol (25%) in S
7	T(St)-P	0.4 mg/g P	Starch-encapsulated Thymol (25%) in P
8	Orig(0)-SE	0.12 mg/g SE	Origanum oil in SE
9	Orig(0)-PE	0.4 mg/g PE	Origanum oil in PE
10	Orig(St)-S	0.12 mg/g S	Starch-encapsulated Origanum (25%) in S
11	Orig(St)-P	0.4 mg/g P	Starch-encapsulated Origanum (25%) in P
12	Clov(0)-SE	0.35 mg/g SE	Clove oil in SE
13	Clov(0)-PE	1 mg/g PE	Clove oil in PE
14	Nos-		No nosema fed to the bees

S-: 50% (w/w) sugar syrup
SE: 1% (99.5%) ethanol in 50% sugar syrup
P-: pollen patties made by 50% sugar syrup (65% (w/w) sterilized dry pollen in 50% sygar syrup)
PE: pollen patties made by SE (65% (w/w) sterilized dry pollen 1% ethanol in 50% sugar syrup)
T(0) = thymol crystals, T(St) = 25% starch-encapsulated thymol
Orig (0) = neat origanum oil, Orig (St) = 25% starch-encapsulated origanum oil
Clo(0) = neat clove oil

2. Bee susceptibility and diet consumption

In order to test the susceptibility of worker bees to the selected essential oils with different formulations, we fed 50 non-infected bees per cage with different treatments (3 controls and 10 essential oil treatments in table I). The bees were kept at 34 °C, 50% RH, in the dark. Dead bees in each cage were counted and removed everyday, and the food were weighed on the day 2, 5, 8, and 14. Average mortality of the cage bees fed with the experimental diet after 14 days was 5.8% and no significant differences were detected among the groups ($F = 0.87$; $df = 12, 12$; $P = 0.59$) (Fig. 1).

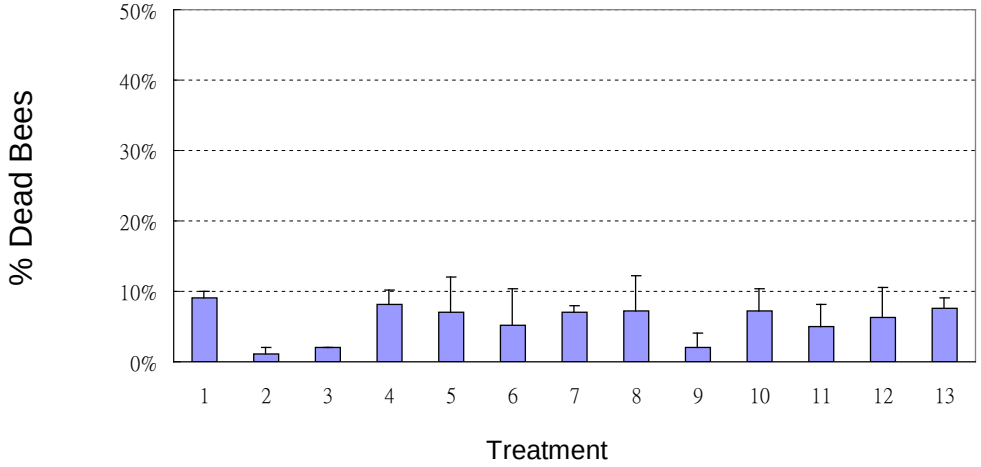

Fig. 1. Average (+SE) percentage of dead bees in three controls and 10 treated groups. (Treatment legend the same as in Table 1).

The daily bee intake of pollen was 4.93 mg on average and no significant differences were detected among the controls and treated groups (F = 1.4; df = 12, 12; P = 0.29) (Fig. 2). The daily bee intake of sugar syrup was 22.90 mg on average and no significant differences were detected among the controls and treated groups (F = 1.27; df = 12, 12; P = 0.35) (Fig. 3). Therefore, the doses of each type/formulation of essential oils used in the experiments are not toxic to adult bees. The worker bees also did not consume less pollen and sugar syrup when they contained selected essential oils.

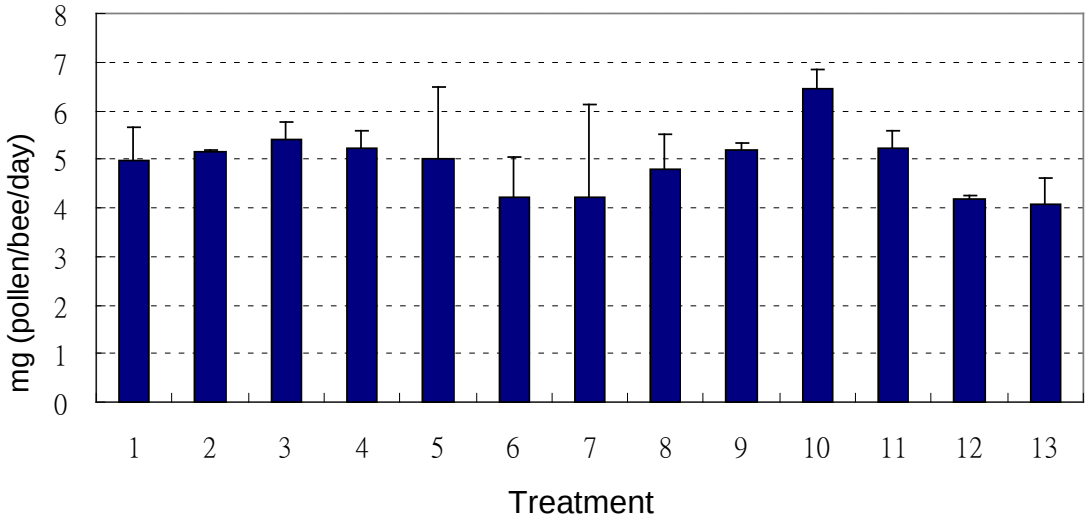

Fig. 2. Average (+SE) daily pollen with or without essential oils intake (mg) per bee. (Treatment legend the same as in Table I).

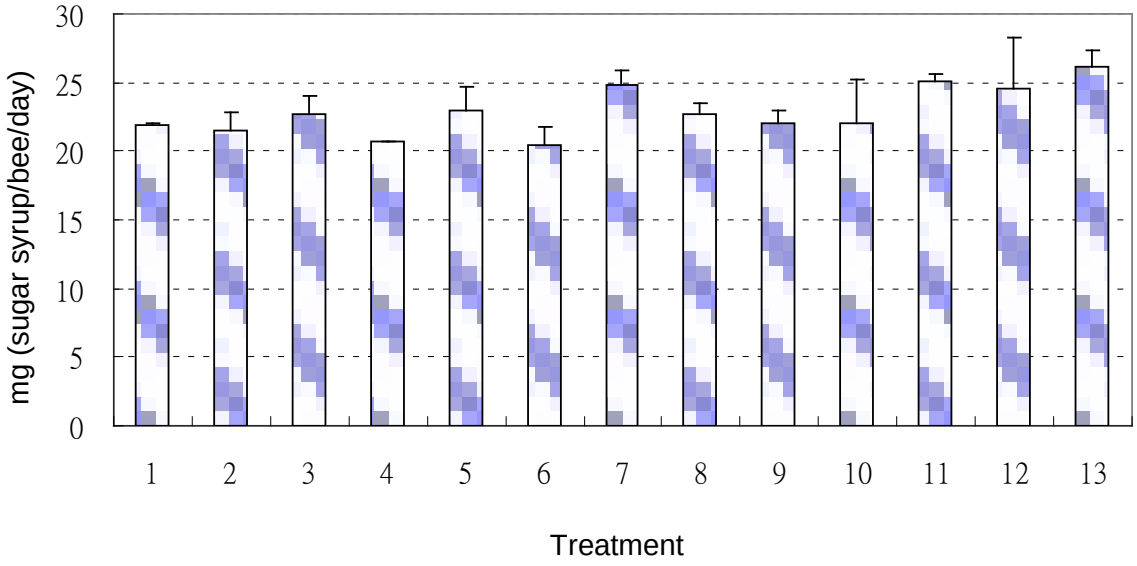

Fig. 3. Average (+SE) daily sugar syrup consumption per bee with or without essential oils (Treatment legend the same as in Table I).

3. Effects of Essential oil on Nosema infection

Life table for each group and median survival time were obtained by using survival analysis function in SPSS statistics 17.0. Overall comparison showed there were significant differences among different treatments (Wilcoxon statistic = 265.133; df = 13; P<0.0001) (fig. 4). The bees without Nosema (Treatment # 14) lived significantly longer than the bees infected with nosema spores. Pairwise comparisons showed that the survial of control group 1 (P with nosema feeding) was significantly longer to that of treated group 10 (Orig(St)-S, Starch-encapsulated Origanum (25%) in sugar syrup for 25 days) (Wilcoxon statistic = 5.176; df = 1; P = 0.023) (Fig. 5). Survival of control group 2 (SE, with nosema feeding) was significantly longer than that of treated group 4 (T(0)-S, Thymol crystals in 1% ethanol in sugar syrup for 25 days) (Wilcoxon statistic = 5.05; df = 1; P = 0.025) (Fig. 6). Survival of control group 3 (PE, with nosema feeding) was significantly longer than that of treated group 9 (Orig(0)-P, origanum oil in pollen patty for 25 days) (Wilcoxon statistic = 17.58; df = 1; P < 0.0001) (Fig. 7).

In summary, bees infected with lived significantly shorter than those not infected with nosema, however, treating with essential oils did not improve survial in the host honey bees. The dosages of selected essential oil used in this experiment did not affect the mortality of bees not fed with nosema, or their food consumption. Some of essential oils such thymol, starch-encapsulated origanum, and origanum oil showed a negative impact on the nosema infected bees. Infected bees treated with starch-encapsulated thymol (25%) in sugar syrup (26% ± 4) and starch-encapsulated origanum (25%) in pollen patty (38% ± 4) showed higher cumulative survial compared to control group (19± 3) but these differences were not significant. We are still determining the spore leves of the sampled live bees at various ages, however based on the negative impact of the oils on bee survival, we do not expect that the essential oils will inhibit the spore levels in these bees.