Nosema Control with 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 honey bee mortality when they are infected with Nosema; and
2). To determine whether essential oils can reduce *nosema* spore load in honey bees.

Interpretive Summary:

We studied the effects of three essential oils (two different formulations) on honey bee survival and their *nosema* loads. Bees infected with *nosema* lived significantly shorter than those not infected with *nosema*; however, treating with essential oils did not improve survival 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* (14), 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 survival compared to control group (19 ± 3) but these differences were not significant. Essential oils, whether delivered in syrup or pollen, did not reduce spore load in inoculated bees. We therefore conclude that the essential oils we tested, at the specific doses and formulations under our laboratory testing conditions, do not suppress *nosema* spore load, nor do they improve honey bee survival.

Materials and 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 isolated in individual glass vials for 30 min to reduce *nosema* transmission among

bees. The bees were then 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, but individually fed 50% sugar syrup. 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, with a total of 2800 bees tested.

Results and Discussion:

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 1**.

Table 1. 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
16	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% sugar 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) (**Figure 1**).

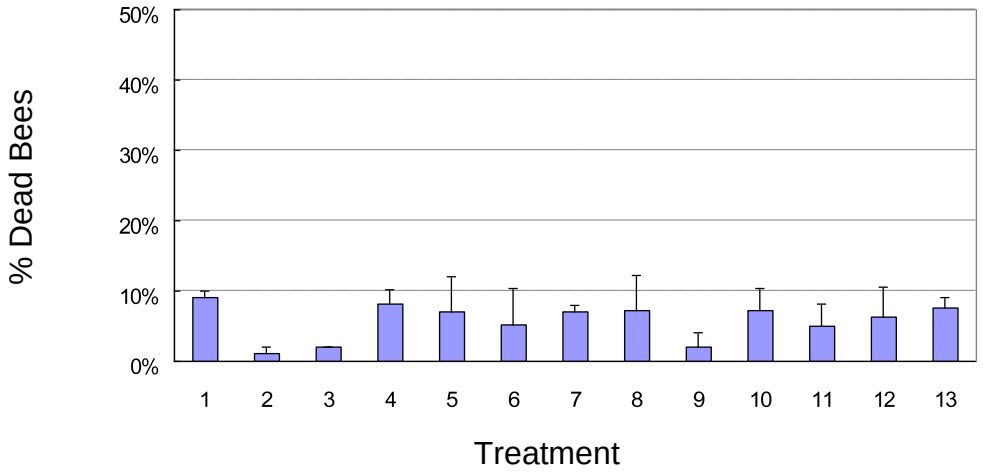

Figure 1. Average (+SE) percentage of dead bees in three controls and 10 treatments. (Treatment legend the same as in **Table 1**).

The daily bee intake of pollen was 4.93 mg on average and no significant differences were detected among all treatments (3 control groups, 10 treatments, 1 non-*nosema* inoculated group) (F = 1.4; df = 12, 12; P = 0.29) (**Figure 2**). The daily bee intake of sugar syrup was 22.90 mg on average and no significant differences were detected among the controls and treatments (F = 1.27; df = 12, 12; P = 0.35) (**Figure 3**). Therefore, the doses of each type/formulation of essential oils used in the experiments are not overly toxic to adult bees (but see survival analysis later). The worker bees also did not consume less pollen and sugar syrup when they contained selected essential oils.

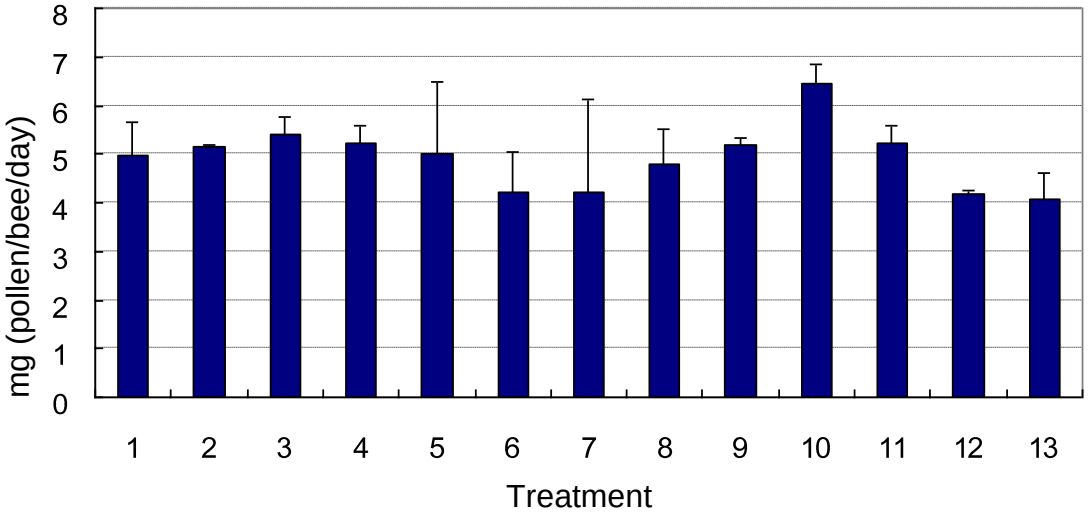

Figure 2. Average (+SE) daily pollen consumption per bee (mg) with or without essential oils. (Treatment legend the same as in **Table 1**).

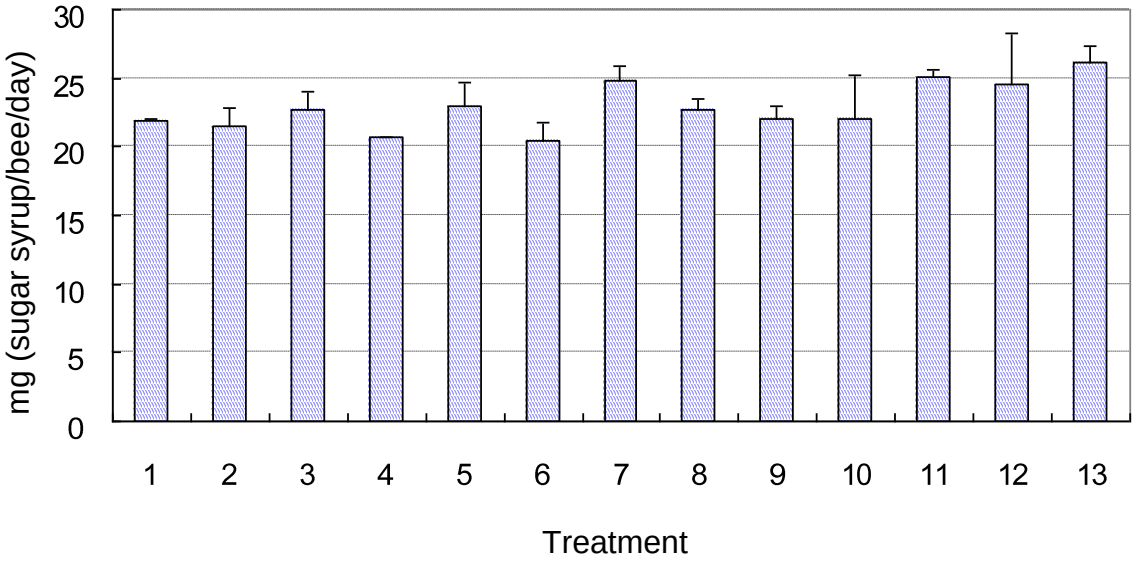

Figure 3. Average (+SE) daily sugar syrup consumption per bee with or without essential oils (Treatment legend the same as in **Table 1**).

3. Effects of Essential oil on Honey Bee Survival

Life table for each group and median survival time were obtained by using survival analysis function in SPSS statistics 17.0. Overall analysis showed there were significant differences among different treatments (Wilcoxon statistic = 265.133; df = 13; P<0.0001, **Figure 4**). The bees without *nosema* (Treatment # 16) lived significantly longer than the bees infected with *nosema* spores. Pairwise comparisons showed that the survival of control group 1 (S, with *nosema* and syrup only) was significantly longer

than that of treatment 10 (Orig (St)-S, Starch-encapsulated Origanum (25%) in sugar syrup for 25 days, Wilcoxon statistic = 5.176; df = 1; P = 0.023, **Figure 5**). Survival of control group 2 (SE, with *nosema* feeding) was significantly longer than that of treatment 4 (T(0)-S, Thymol crystals in 1% ethanol in sugar syrup for 25 days. Wilcoxon statistic = 5.05; df = 1; P = 0.025) (**Figure 6**). Survival of control group 3 (PE, with *nosema* feeding) was significantly longer than that of treatment 9 (Orig(0)-P, origanum oil in pollen patty for 25 days, Wilcoxon statistic = 17.58; df = 1; P < 0.0001, **Figure 7**).

4. Effects of Essential Oil on *nosema* spore load

We found no difference among the treatment groups for *nosema* spore load at various ages (**Figure 8**). Treatment 16, which was not fed *nosema* spores, showed a significantly lower number of spores, as expected. These data are based on 8 live bees sampled for each treatment on specific days as indicated on **Figure 8**.

Conclusions:

In summary, bees infected with *nosema* lived significantly shorter than those not infected with *nosema* however treating with essential oils did not improve survival 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* (14), 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 survival compared to control group (19 ± 3) but these differences were not significant. Essential oils, whether delivered in syrup or pollen, did not reduce spore load in inoculated bees. We therefore conclude that oils we tested, at the specific doses and formulations under our laboratory testing conditions, do not suppress *nosema* spore load, nor do they improve honey bee survival.

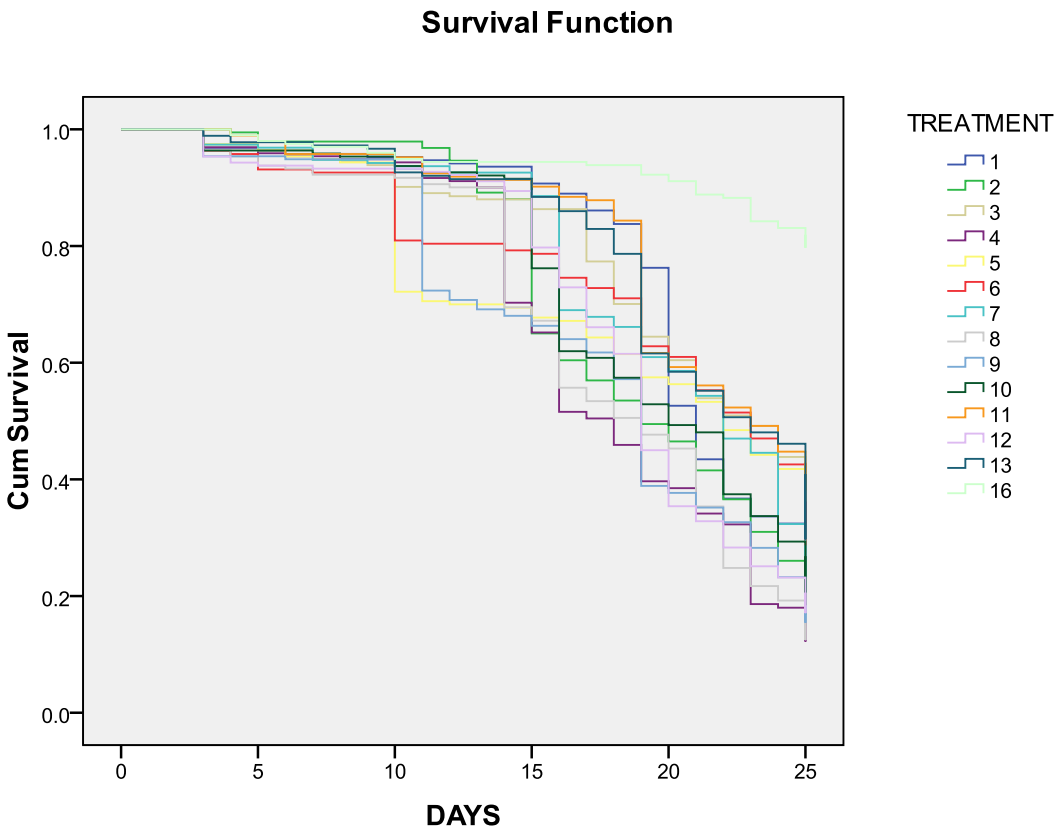

Figure 4. Survival curves of all the treatments. Bees in treatment 1-13 were force-fed with *nosema* spores. Treatment legends are the same as **Table 1**; group 16 was not infected with *nosema* spores, but fed with regular pollen and 50% sugar syrup).

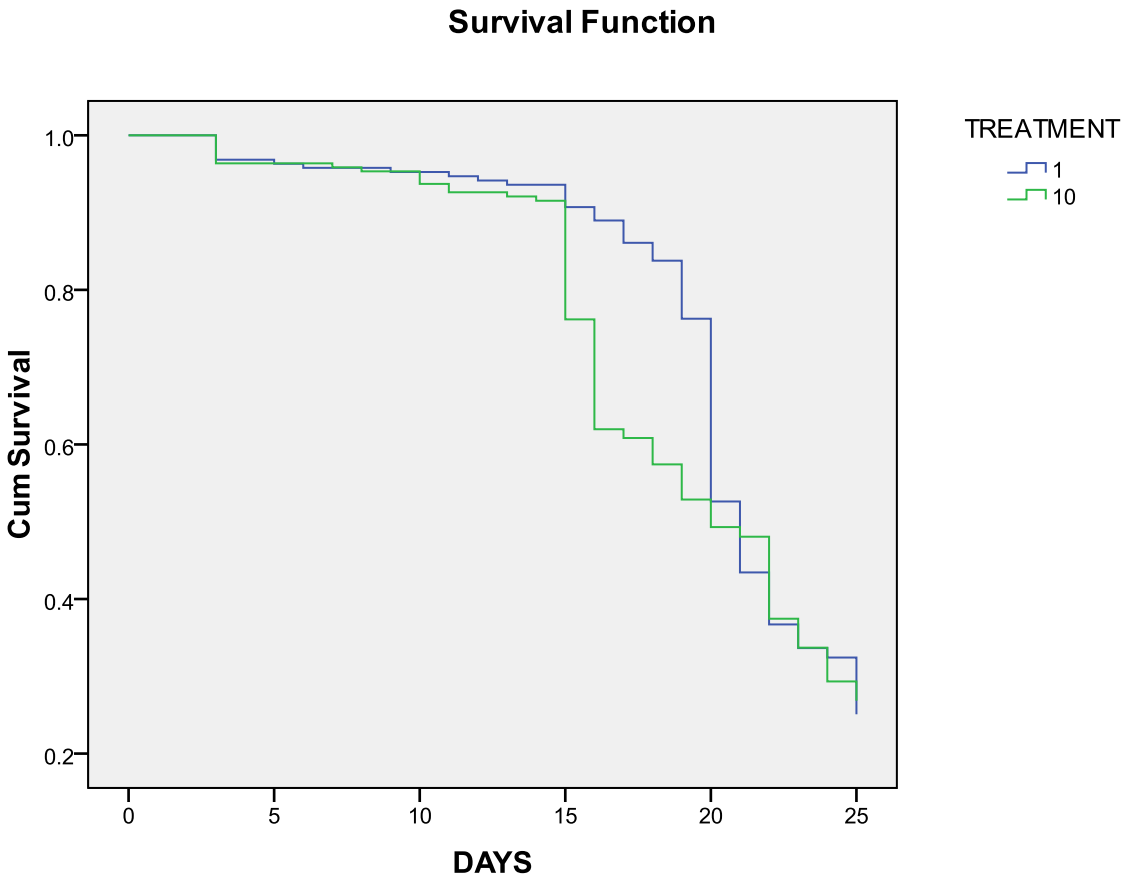

Figure 5. Survival curves of treatments 1 and 10. Survival analysis showed that the survival of control group 1 (S, sugar syrup with *nosema* feeding) is significantly different from that of treated group 10 (Orig (St)-S, Starch-encapsulated Origanum (25%) in sugar syrup for 25 days, Wilcoxon statistic = 5.176; df = 1; P = 0.023). This suggests that starch-encapsulated origanum oil has a negative impact on bees.

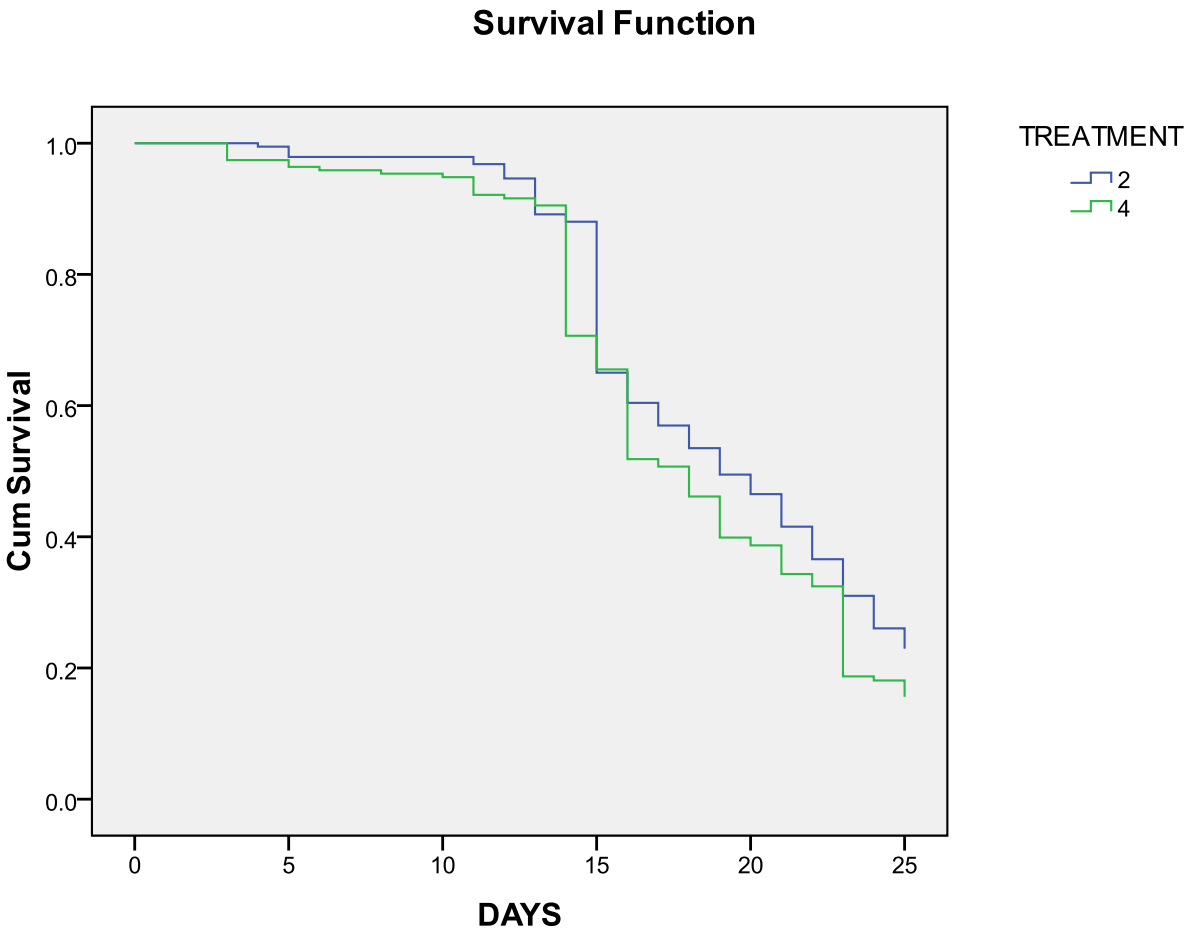

Figure 6. Survival curves of treatments 2 and 4. Survival analysis showed that the survival of control 2 (SE, 1% ethanol in 50% sugar syrup) was significantly different from that of treatment 4 (T(0)SE; Thymol crystals in 1% ethanol in sugar syrup for 25 days, Wilcoxon statistic = 5.05; df = 1; P = 0.025). This suggests that thymol crystals when supplied in sugar syrup have a negative impact on bee longevity.

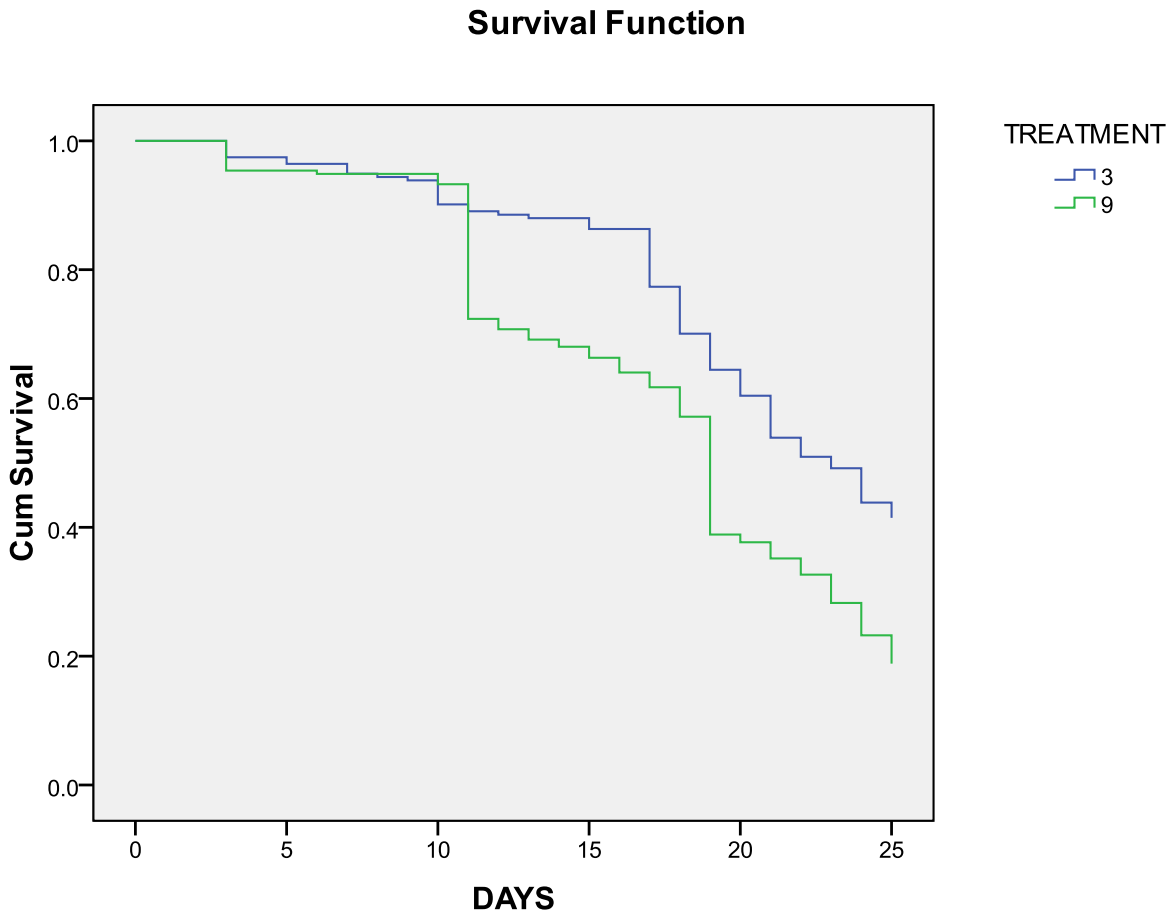

Figure 7. Survival curves of treatments 3 and 9. Survival analysis showed that the survival of control 3 (SE, 1% ethanol in 50% sugar syrup) was significantly different from that of treatment 9 (Orig(0)-PE, 0.4 mg origanum oil in 1 g of pollen patty made with 1% ethanol in 50% sugar syrup, Wilcoxon statistic = 17.58; df = 1; $P < 0.0001$). This suggests that origanum oil, when supplied in pollen, have a negative impact on bee longevity.

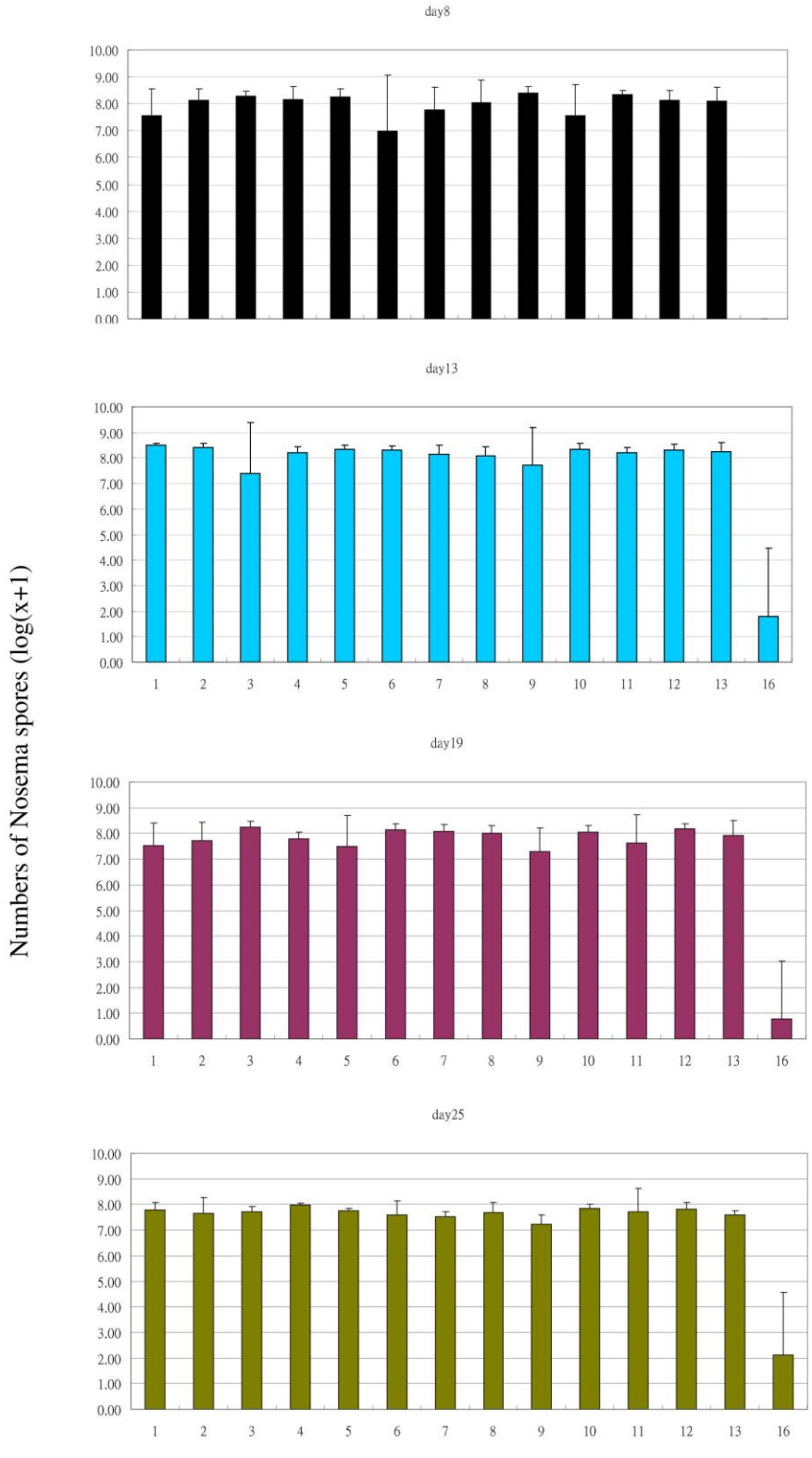

Fig. 8. Effect of various essential oil treatments on the number of nosema spores (log transformed). Except the non-nosema-inoculated control (16) that shows much lower spore levels, there was no significant difference among treatments 1-13 (see Table 1 for treatment annotations).