Reduction in *Nosema* spore counts with use of Advance Science's MacroAlgae/Thymol/Lemongrass blend

Results from long-term field trials in Greece 2012-2013

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Introduction:

Nosema is a small parasite that resides in the gut of honeybees and is a worldwide problem for bees and beekeepers. There are two species of *Nosema*, *Nosema apis* and *Nosema ceranae*, both of which are highly associated with incidence of Colony Collapse Disorder (vanEngelsdorp *et al.*, 2009). Current treatments are limited with some demonstrating little or no activity and/or only having no long term benefits (Huang *et al.*, 2013).

Recent results from cages trials carried out by Advance Science in Italy showed an impressive reduction in spore counts in the guts of honeybees fed a with a MacroAlgae/Thymol/Lemongrass blend compared to control. To further assess the benefits of this blend, field trials were carried out by the Hellenic Institute of Apiculture, Greece.

Method:

Each group comprised of ten colonies. All colonies had queens of similar age with each colony receiving a new queen on the last week of March 2012. Spore counts were taken for all colonies in November 2012 (immediately before treatment began) with each colony having an approximate population of six frames.

There were five treatment groups:

-MacroAlgae/Thymol/Lemongrass in syrup
-Drenching MacroAlgae/Thymol/Lemongrass and feeding MacroAlgae/Thymol/Lemongrass in Candy
-Drenching MacroAlgae/Thymol/Lemongrass and feeding control candy
-Fumidil-B
-Control candy

All groups that incorporated MacroAlgae/Thymol/Lemongrass were at a concentration of 2.5 ml of blend per litre syrup.

Feeding of colonies began end of November 2012 and all were fed within 2 weeks.

All colonies fed syrup at this time were fed 4 litres. Colonies fed candy at this time were fed 6kg of candy from this time until March (approx. 1.5kg per month).

Colonies receiving drenching of MacroAlgae/Thymol/Lemongrass did so at this time also, as well as feeding (see table 1).

Spring feeding began in April 2013. Drenching groups received a second drenching treatment in the spring. Some groups received no further treatments. All feeding was stopped towards the end of April and a week later, all colonies were moved for a spring honey flow (May 2013).

Table.1. Treatments groups

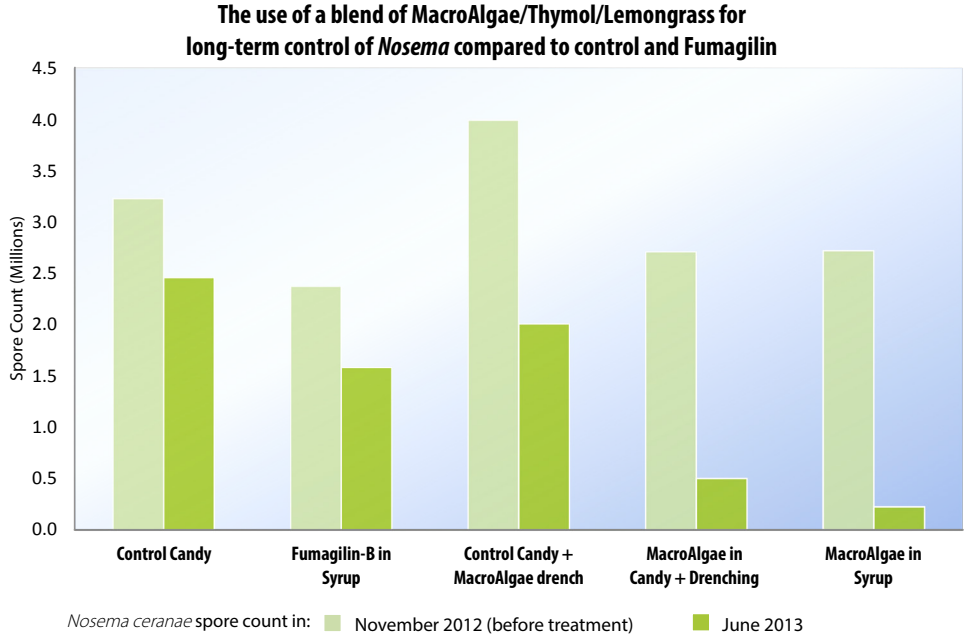
November treatment	Overwinter	April treatment
MacroAlgae/Thymol/Lemongrass in syrup: 5ml in 2L per week for 2 weeks Total: 10 ml in 4 liters	Control candy	Nothing
Fumidil 2.5g/ 2L, twice. Total: 5g/4L	Control candy	Nothing
Drenching with MacroAlgae/Thymol/Lemongrass: 0.5ml/50ml syrup/ colony/ 2 times per week for 2 weeks. Total: 4 treatments	MacroAlgae/Thymol/Lemongrass in Candy: 5ml/kg Total: 6kg	Drenching with MacroAlgae/Thymol/Lemongrass 1ml/100ml syrup/colony/2 times per week, 2 weeks. Total: 4 treatments
Drenching with MacroAlgae/Thymol/Lemongrass: 0.5ml/50ml syrup/colony/2 times per week, 2 weeks. Total: 4 treatments	Control candy	Nothing
Nothing	Control candy	Fed syrup

Nosema ceranae spore counts were taken in November and June. Spore counts were taken following the standard methods now published in Fries *et al.*, 2013.

Results:

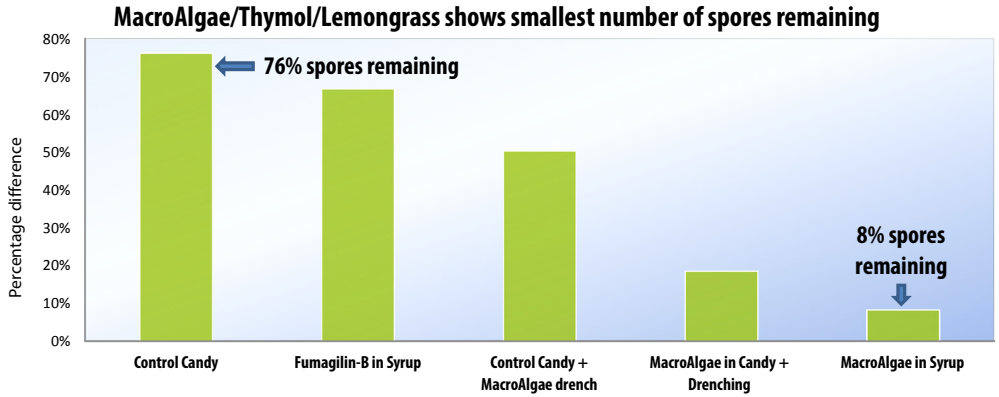
The graph below shows spore counts for November and June.
MacroAlgae/Thymol/Lemongrass in syrup demonstrates the greatest impact on spore reduction.

Graph.1. Difference in spore counts between November and June



	Control Candy	Fumagilin-B in Syrup	Control Candy + MacroAlgae drench	MacroAlgae in Candy + Drenching	MacroAlgae in Syrup
Nov-2012 (Before treatment)	3,226,400	2,372,900	3,990,700	2,708,500	2,719,700
Jun-2013	2,457,350	1,582,600	2,006,143	499,800	223,111

Graph.2. Percentage difference in spore reduction



	Control Candy	Fumagilin-B in Syrup	Control Candy + MacroAlgae drench	MacroAlgae in Candy + Drenching	MacroAlgae in Syrup
% of spores present in June when compared to November (pre-treatment)	76.16%	66.69%	50.27%	18.45%	8.20%

Conclusion:

Results generated in these field trials confirm previous results obtained in cage trials. The data clearly shows the benefits of using MacroAlgae/Thymol/Lemongrass for all three methods of application with administration of syrup activated with MacroAlgae/Thymol/Lemongrass being the most effective method.

Spore counts were taken prior to treatment just before the winter and then again after winter. It was expected that spore counts would be highest over winter and then decrease in spring. This is confirmed by the slight decrease in spore counts present in the control group. As shown in Graph.1, addition of the MacroAlgae/Thymol/Lemongrass blend to the bees diet results in a clear decrease of spores over the winter. While the addition of this blend to colonies through candy or by drenching was effective, best results were seen when it was fed in sugar syrup.

In order to make the difference in spore reduction clearer, the percentage difference in spore counts before and after treatment was obtained and plotted in Graph.2. Here, it is shown that only 8% of spores are remaining after treatment with the MacroAlgae/Thymol/Lemongrass blend in syrup. This is a 92% reduction in spore counts after the treatment.

As only one treatment was administered before Winter and results were seen after Winter, this shows that the blend demonstrates long-term efficacy against *Nosema ceranae*. Further long-term tests will be carried out to see if improvements are noted with continued use.

In conclusion, the MacroAlgae/Thymol/Lemongrass blend represents a natural, effective alternative for *Nosema* control in colonies with long-lasting benefits.

References:

Fries *et al.* (2013) Standard methods for *Nosema* research. Journal of Apicultural Research 52(1)

Huang *et al.* (2013) *Nosema* ceranae escapes Fumagillin control in honeybees. PloS Pathog 9(3)

VanEngelsdorp *et al.* (2009) "Colony Collapse Disorder: A Descriptive Study", PLoS ONE,4(8): e6481. doi: 10.1371/journal.pone.0006481