OP-111 [Bee Health]
The use of thymol as nano-emulsion in honey bee supplemental feed (Apis mellifera)

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Background and problem description: The two species of Nosema (Vairimorpha) microsporidian parasite found in honey bees are widespread, being present in Romania as well. Considering that in beekeeping the contamination of hive products with fumagillin is not allowed, the only effective and accepted antibiotic in combating the vegetative form of Nosema is thymol. There are many concerns to search for alternatives to classic antibiotics that are based on natural and effective substances, especially plant extracts. Thymol (C10H14O) is a phenolic monoterpene found in plants and especially in thyme oil (Thymus spp), known for its powerful antiseptic properties. In beekeeping, it is used both for its adjuvant role in the nosemosis control and in ecological treatments for the varroa mite control. PURPOSE: To increase the effectiveness of thymol, a new formulation approach as nano-emulsion was developed by which the natural compound extracted from the essential oil of thyme (Thymus vulgaris) is dispersed in a support substance as nanometric particles (below 200 nm) by sonication, and subsequently stabilization. METHODOLOGY: To determine the influence of energetic feed with thymol as nano-emulsion on the honeybees' lifespan, preliminary researches were conducted on honeybees kept in cages, fed with carbohydrate feed (syrup and fondant) to which thymol was added in form of nano-emulsion (experimental group 1), with syrup to which a mix of hydro-alcoholic extract of thymol was added (experimental group 2) and syrup without thymol (control group). Results and CONCLUSION: According to the Kaplan-Mayer regression curve, bees fed with syrup with thymol extract lived, on average, five days longer than bees in the control group. According to the same regression model, bees fed with thymol nano-emulsion survived, on average, one day longer than bees fed with thymol extract syrup. The results were similar when bees were fed with fondant to which thymol extract was added as per se and as nano-emulsion. The differences between the groups were statistically significant ($p<0.01$). Practical implications for beekeepers: Given the results obtained, the administration of thymol as nano-emulsion represent an improved administration method of thymol in honeybee artificial feed.

The use of thymol as nano-emulsion in honey bee supplemental feed (Apis mellifera)

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A short address about what thymol is and how it is used in beekeeping?

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What is thymol?

Thymol (C10H14O / 3-hydroxy-p-cymene) is a phenolic monoterpene found in plants and especially in thyme oil (Thymus spp), known for its powerful antiseptic properties.

Some other plants that contain thymol:

- Creeping Thyme (Thymus serpyllum)
- Oregano (Origanum vulgare)

Image source: https://arochemexports.com/wp-content/uploads/2024/01/Thymol-Crystal.jpg

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What are now the uses of thymol in beekeeping?

Preservative for bee syrup

In the 1940s, it was recommended to add to the syrup intended for feeding bees 5 ml disolved in a litre of syrrup of a solution prepared from 30 grams of thymol and 150 ml of alcohol.

Nosema control

Administered to bees in syrup as food, it leads to a decrease in nosema infestation.

Varroa control

Sublimated in the hive, it reduces varroa infestation.

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How is thymol used in bee feeding?

Thymol is administered in bee feed after being dissolved in alcohol to reduce the effects of nosema infestation. Ex. Tymolysed syrrup

Previous studies have shown a weaker efficacy than fumagillin, but have **demonstrated positive effects on the overall health of the bee.**

Some previous studies

Glavinic, Uros (2022) *et al.* showed:

- Positive effects of thymol on health (increasing levels of immune-related genes and values of oxidative stress parameters, and decreasing Nosema spore loads) when applied to Nosema-infected bees.
- Supplementation with thymol did not induce negative effects in Nosema-infected bees.
- Nosema-free bees, thymol itself could cause certain disorders (affecting bee survival, decreasing oxidative capacity, and downregulation of some immune-related gene expressions), showing that one should be careful with preventive, uncontrolled, and excessive use of thymol.
- Thus, further research is needed to reveal the effect of this phytogenic supplement on the immunity of uninfected bees.

Glavinic, Uros & Blagojevic, Jovan & Ristanić, Marko & Stevanovic, Jevrosima & Lakic, Nada & Mirilovic, Milorad & Stanimirovic, Zoran. (2022). Use of Thymol in Nosema ceranae Control and Health Improvement of Infected Honey Bees. Insects. 13. 574. 10.3390/insects13070574.

Some previous studies

Vargas-Valero, Azucena (2022) *et al.* showed:

A study from 2021 on Africanized bees compared the effectiveness of thymol with that of fumagillin, another antifungal agent. It was found that fumagillin had a much higher efficacy (95.2%) in reducing the level of infection, while thymol showed an efficacy of 31.1%.

Vargas-Valero, Azucena & Barrientos Medina, Roberto Carlos & Medina-Medina, Luis. (2021). Efficacy of thymol in control of the fungus Nosema ceranae in Africanized Apis mellifera. Revista Mexicana de Ciencias Pecuarias. 12. 633-6

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What is nano thymol?

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What is nano-thymol?

Thymol nano-emulsion or nanoparticle is a product derived from crude thymol, obtained through nanotechnology. The size of the thymol particle is reduced and stabilized through various physicochemical procedures to a size between 10 and 200 nm, and is then used in various industries such as medicine and cosmetics.

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Why nano-thymol?

CONVENTIONAL THYMOL

Composition: A crystalline phenolic extract from oregano or thyme.

Properties: Well-known for its antiseptic, antimicrobial, antifungal and antioxidant properties.

Challenges for use in beekeeping:

- Low water solubility
- High volatility
- Low bioavailability
- Bee repellent

NANO THYMOL

Composition: Conventional thymol transformed into nanoparticles or nanoemulsions. The thymol particles are introduced into water and reduced to a nanometric scale (typically between 10 and 200 nm).

Properties: The same as conventional thymol.

Improvements from nanotechnology:

- Enhanced solubility and stability
- Increased bioavailability
- Superior efficiency
- Controlled release
- Less repellent to bees

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What are the advantages of using thymol nanoemulsion **in agriculture**?

Thymol nanoemulsion has been intensively studied as an alternative to classic antibiotics. In the poultry industry, it has demonstrated:

- antimicrobial effects
- improved digestion
- healthy intestinal tract
- antioxidant and anti-inflammatory properties
- promoted weight gain
- immunomodulatory effects.

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By analogy...

By analogy, we want to experimentally test some effects of thymol as a nano-emulsion on bees.

A shift in perspective?

Should beekeepers use thymol only for the three stated purposes (syrup preservative, nosema control and varroa control) or should also be used for its antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties?

Two experimental studies

Research question

1. Do bees consume nano thymol better compared to conventional thymol?
2. What are the effects of thymol in the form of a nano-emulsion on the lifespan of the bee?
3. Does thymol nanoemulsion increase the lifespan of bees or does it decrease it?
4. Does the form of feed (liquid or solid) influence the effects of nano thymol in any way?

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Experiment 1

The attractiveness of thymol nano-emulsion in bee feed

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Research methodology

Experiment 1:

To see if there are differences in the consumption of energy feed with nano-emulsion thymol and conventional thymol, the same thymol concentrations (160 ppm) were used in the feed and the same type of energy feed (3:1 syrup and solid feed), consisting of a standardized mixture of fructose, glucose (invert sugars), and sucrose (non-inverted sugar). The nano-emulsion thymol was obtained through the sonication in water method (without alcohol or emulsifiers). The conventional thymol was prepared by diluting thymol in isopropyl alcohol (Manley method).

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Research methodology

Making thymol nanoemulsion: The thymol nanoemulsion was obtained by sonicating a mixture of water and thymol. The suspension was subjected to high-intensity ultrasound from a sonicator. No surfactants or stabilizing agents were used. To prevent agglomeration, it was quickly incorporated into solid or liquid feed with a higher viscosity.

Images showing the results of the thymol nanoemulsion process via sonication:

Before sonication

After sonication

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Note:

The whiter the solution obtained through sonication (like milk), the finer the thymol nanoparticles. Obtaining the finest thymol particles depends on the quantities used and the duration of the sonication. The longer the thymol particles are sonicated, the finer they become.

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Research methodology

- Five beehives were fed with thymol nanoparticles added to solid feed, while another five beehives were fed with solid feed containing conventional thymol.
- Additionally, five beehives were fed with thymol nanoparticles added to syrup, and another five were fed with syrup with conventional thymol.

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Results

- Data analysis showed better consumption of products containing thymol nano-emulsion, as can be seen in the attached video clip taken 24 hours after feeding.

- The feed with conventional thymol was consumed with more difficulty.

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Results

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Experiment 2

Impact of Nano-Emulsion Thymol vs. Conventional Thymol on Bee Lifespan

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Research metodology

The experimental study used bees isolated in cages and involved the following stages and procedures:

1. Sterilization of the instruments used for the experiment.
2. Testing for nosema and randomized distribution of bees of a similar age into cages.
3. Daily counting of dead bees.
4. Data processing and analysis.
5. Writing the research report.

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Experimental groups and control groups

Experimental groups

1. Cages with bees fed with syrup containing added thymol nanoemulsion.
2. Cages with bees fed with solid feed (fondant) containing added thymol nanoemulsion.

Control groups

1. Cages with bees fed with plain syrup, without added thymol nanoemulsion.
2. Cages with bees fed with plain solid feed (fondant), without added thymol nanoemulsion.

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Note:

No significant *Nosema* infestation was found in the bees at the beginning of the experiment.

The bees in the experimental groups received fresh sterile food and sterilized water daily, and were provided with similar temperature conditions.

Lots fed with tymolised syrup or solid feed (fondant) contain 160 ppm thymol.

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Nano-Tymolised syrup feed

Experimental results

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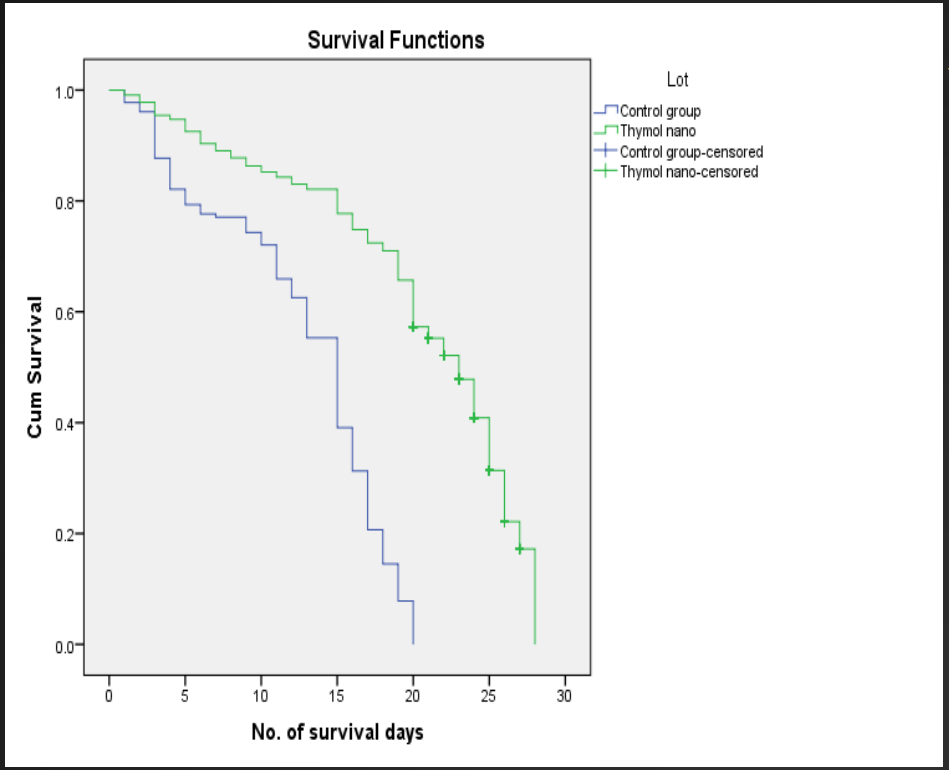
Results

Survival time is significantly higher in the **Thymol nano group** compared to the control group (syrup with no thymol added), for both mean and median. The increase is about **+7.6 days** for the mean and **+8 days** for the median.



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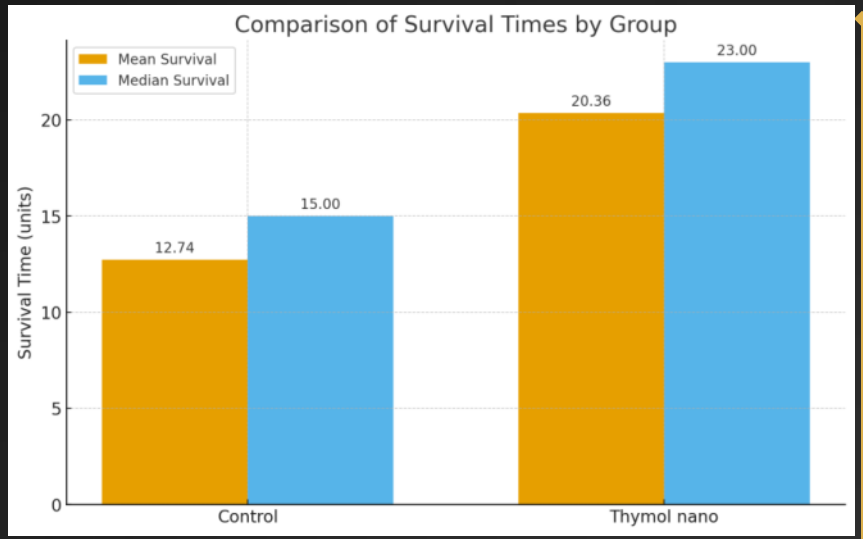
Results

- **Control group:**
  - *Mean*: **12.74** survival days (95% CI: 11.90 – 13.58)
  - *Median*: **15.00** survival days (95% CI: 14.12 – 15.88)

- **Thymol nano group:**
  - *Mean*: **20.36** survival days (95% CI: 19.71 – 21.01)
  - *Median*: **23.00** survival days (95% CI: 22.07 – 23.93)



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Results

Group Comparison Test
Log Rank (Mantel-Cox):

Chi-Square = **269.35**
df = 1
Sig. (p-value) = **0.000**

The result is **highly statistically significant** ($p < 0.001$), confirming that the survival distributions of the two groups are **significantly different**. Thymol nano treatment has a clear positive effect on survival compared to the control group (simple syrup feed).

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Nano-Tymolised solyd feed

Experimental results

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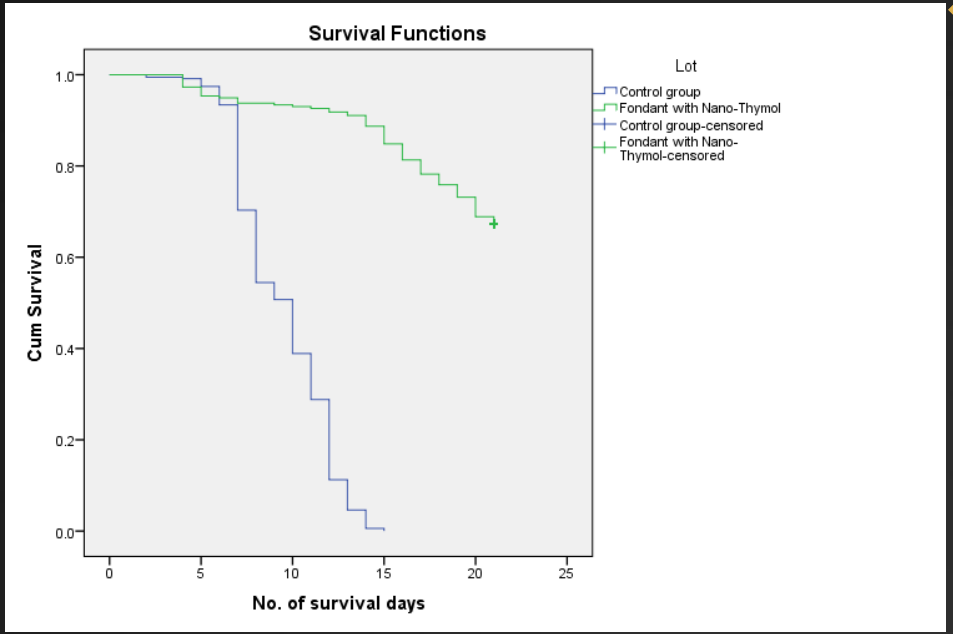
Results

The **Fondant with Nano-Thymol** group shows a survival time that is approximately **double** that of the control group (no thymol added) (mean survival: 18.88 vs. 9.48). The median survival for the treatment group was not estimated in the output, but the shift in mean survival strongly indicates a major improvement.



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Results

- **Control group:**
  - *Mean*: **9.48** survival days(95% CI: 9.22 – 9.75)
  - *Median*: **10.00** survival days (95% CI: 9.51 – 10.49)

- **Fondant with Nano-Thymol group:**
  - *Mean*: **18.88** survival days (95% CI: 18.35 – 19.41)
  - *Median*: survival days (due to censored data and estimation limits)

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Results

Group Comparison Test
Log Rank (Mantel-Cox):

Chi-Square = **497.91**
df = 1
Sig. (p-value) = **0.000**

The result is **highly statistically significant** ($p < 0.001$). This confirms that the survival distributions between the two groups (Control vs. Fondant with Nano-Thymol) are **significantly different**, with the treated group having superior survival.

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Nano-Thymol syrup feed vs. Nano-thymol solid feed

Comparison

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Comparison table

Group	Mean Survival (95% CI)	Median Survival (95% CI)
Control (syrup study)	12.74 (11.90–13.58)	15.00 (14.12–15.88)
Nano-Thymol (syrup)	20.36 (19.71–21.01)	23.00 (22.07–23.93)
Control (solid feed/fondant)	9.48 (9.22–9.75)	10.00 (9.51–10.49)
Nano-Thymol (solid feed/fondant)	18.88 (18.35–19.41)	Not reported
Overall (syrup)	18.48 (17.90–19.06)	20.00 (19.48–20.52)
Overall (solid feed)	13.48 (13.02–13.94)	12.00 (11.46–12.55)

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Comparison conclusions

- **Efficacy:**
  - Nano-Thymol significantly prolongs survival, whether delivered in syrup or solid/fondant form.

- **Comparison of forms:**
  - The syrup group achieved the highest median survival (23 units).
  - The solid group also showed strong results (mean 18.9), though median was not reported.
  - The difference in control group survival suggests background conditions may have varied between studies.

- **Key takeaway:**
  - Nano-Thymol increase survival compared to controls in both formulations.
  - The syrup formulation may offer a slightly higher survival benefit, but the solid form shows equally robust statistical evidence of efficacy.

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Nano-Thymol syrup
vs.
conventional thymol syrup

Experimental results

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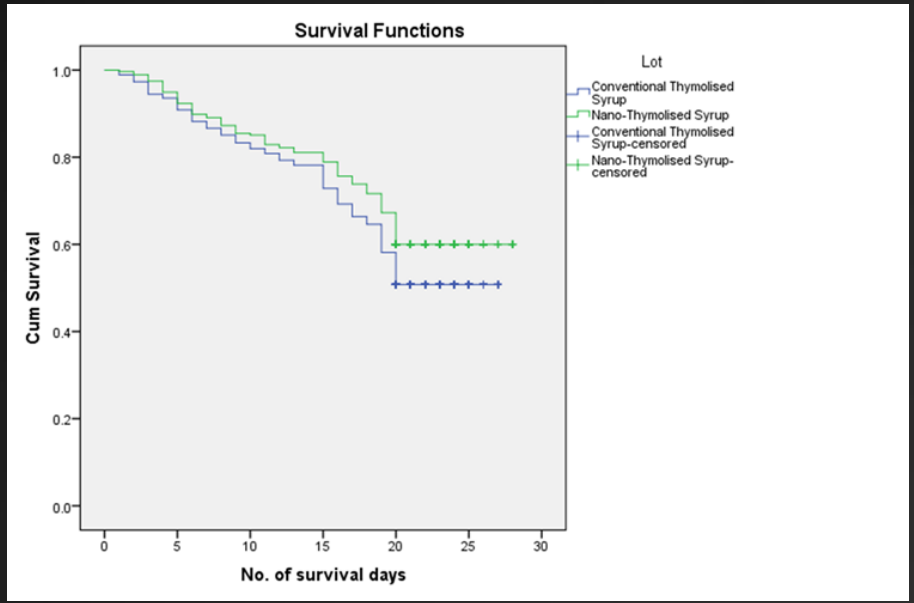
Results

The analysis demonstrates that the **Nano-Thymolised Syrup significantly improves survival time** compared to the conventional formulation. The survival advantage is nearly **two days** on average, and the result is statistically significant ($p<0.05$).

Note: The results are derived from two batches other than those used in the previous experiment.



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Results

Mean Survival Times

- **Conventional Thymolised Syrup:**
  - **Mean survival time = 20.03 units (95% CI: 19.27 – 20.80).**
- **Nano-Thymolised Syrup:**
  - **Mean survival time = 21.95 units (95% CI: 20.96 – 22.94).**

This shows that bees treated with **Nano-Thymolised Syrup survived longer on average** compared to those receiving the conventional Manley formulation. The difference is about **1.9 days**.

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Results

Group Comparison Test

Log Rank (Mantel-Cox) test:

Chi-Square = **5.759**
df = **1**
p = **0.016**

Since the p-value is less than 0.05, there is a **statistically significant difference in survival distributions** between the two groups. This supports the conclusion that the **Nano-Thymolised Syrup provides superior survival outcomes**.

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Study conclusions

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Final conclusions

Based strictly on the results obtained under the conditions of this study:

- Nano-Thymol (160 ppm) is better accepted in bee feed than conventional thymol.
- It significantly increases bee survival in both syrup and solid feed.
- Compared to conventional thymol, Nano-Thymolised Syrup adds ~2 extra survival days.
- Nano-Thymol is a more effective and attractive alternative for bee nutrition.
- Nano-thymol should be regarded as a product that enhances bee longevity, rather than solely serving for the control of *Nosema* and *Varroa*. No significant *Nosema* infestation was found in the bees at the beginning of the experiment or after bee death count.

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Future research directions

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Future research directions

- Potential experimental comparisons could include comparisons between the stock formula (another nanothymol variant), the nanothymol formula obtained through sonication, and conventional thymol for bee feed. Additionally, clear studies must be conducted regarding the safe administered quantities that do not harm the bees.

- Further studies are needed to investigate the effects of nano-thymol on brood, hive bee populations, honey production, and related parameters.

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Industry Collaboration and Product Development

The study was conducted by the Research Department of Dulcofruct Bee Nutrition Science. The results of these experiments, along with other similar studies on bee survival, have been translated into the commercial product Bee Immunity Booster, an apicultural tonic with antimicrobial properties currently undergoing optimization in collaboration with Dunărea de Jos University of Galaţi

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References:

Glavinic, Uros & Blagojevic, Jovan & Ristanić, Marko & Stevanovic, Jevrosima & Lakic, Nada & Mirilovic, Milorad & Stanimirovic, Zoran. (2022). Use of Thymol in Nosema ceranae Control and Health Improvement of Infected Honey Bees. Insects. 13. 574. 10.3390/insects13070574.

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Thanks!