# Survival rate in the honeybee workers (*Apis mellifera* L.) additively fed with polypore mycelial extract

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*Abstract:* The deteriorating health status of honeybee colonies is considered to be the result of the interaction among several stress factors, in particular pathogens, pesticides and malnutrition. Secondary metabolites from mushrooms were shown to have the potential to mitigate the impact of some of these stressors. However, possible side effects mushroom extracts must be evaluated. The aim of this experiment was to test the survival rate in worker bees fed by mycelial extract from *Fomes fomentarius*. 1% (v/v) extract was well ingested by honeybees and did not cause them any harm. There was no difference in the survival rates in the experimental and control groups. These results did not confirm the previously reported effect of mycelial extracts on the substantially increasing bee longevity. Potential variability of the mycelial extracts is discussed.

*Key Words: Fomes fomentarius*, mycelium, extract, *Apis mellifera*, survival

## INTRODUCTION

Mushrooms are rich source of specific pharmaceutical substances (Grienke et al. 2014). The health of honeybee colonies has been getting worse in the last decades due to several stress factors. It means especially pathogens (Nazzi and Pennacchio 2014) with emphasis on the bee viruses (Genersch and Aubert 2010) causing covert infections (Benaets et al. 2017). The reduction of *Varroa destructor* population, as a vector of the bee viruses, is only indirect and uncertain method how to prevent the virus infections (Roth et al. 2020). However, acaricides have side effects and are source of additional stresses (Tihelka 2018).

Stamets et al. (2018) had shown that mycelial extracts from Polyporales probably act as an antiviral substance. They determined that the extracts from *Fomes fomentarius* and *Ganoderma resinaceum* are highly effective against two bee viruses (DWV and LSV). There are also other mushrooms with an antiviral potential: *Fomitopsis officinalis*, *F. pinicola*, *Ganoderma applanatum* and outside of Polyporales group also *Schizophyllum commune* or *Inonotus obliquus* (Stamets 2018). The mushrooms are attractive for bees. It was repeatedly observed in case of many mushrooms, e.g. *Xerocomus* sp., *Sepedonium* sp. and *Hypomyces chrysospermus* (Takahashi et al. 2019). The explanations why bees are lured by mushrooms are rather speculative. In the case of *Zygosaccharomyces* sp., this yeast was found as a source of steroids important for development of bee brood (Paludo et al. 2018).

It is expected that mycelial mushroom extracts containing p-coumaric acid can be involved in activating the expression of cytochrome P450 detoxifying path or induction of antimicrobial bee proteins (Mao et al. 2013, Gong and Diao 2017). That is why the mycelial extracts were tested as a hopeful matter for support of bee immunity (Stamets 2018).

In summary, the antiviral effect of the mycelial extracts was demonstrated (Stamets et al. 2018) and the extracts were tested in a beekeeper practice (Pătruică et al. 2017, Stevanovic et al. 2018). There are only limited results about possible side effects of the mycelial extracts (Stamets 2018), therefore, we tested the mycelial extract from *Fomes fomentarius* in a cage experiment with the aim to evaluate a survival rate in the honeybee workers.

## MATERIAL AND METHODS

### Cultivation and mycelial extraction

The extract was prepared from polypore mushroom (Polyporales) *Fomes fomentarius* (collected as the basidiocarp in a birch forest Kurimska Nova Ves, Czech Republic, N49°20'52", E16°17'6"). Cultivation procedure was carried out according the method by Stamets et al. (2018). The method was not described in all details, therefore, temperature 24 °C and humidity 60–70% were chosen, inspired by Masaryková et al. (2009). Mycelium from basidiocarp of *F. fomentarius* was isolated by cultivation of dissected cube from trama with an edge of about 1 cm. Uninfected part of the mycelium grown from trama was aseptically transferred on 2% malt yeast agar (HiMedia Laboratories GmbH, Germany) for cultivation of the pure mycelial culture. The extraction of the mycelium was carried out also according the methodology by Stamets et al. (2018) with only one difference, i.e. the fresh mycelium was directly extracted without its freezing.

### Cage experiment – founding and supplementation

For the cage experiment, a large box was filled with approximately 2000 workers honey bees pooled from multiple colonies and catched on a brood comb. Each cage (n = 3 per treatment, triplicate) was randomly populated with 60 worker bees. In the experimental cages, the worker bees were maintained in the incubator with 32 °C and 60% RH with *ad libitum* sources of candy (honey and powdered beet sugar in proportions 1:4) given in a plastic tube to prevent sticking of bees. The water was supplied in a drinker (6 ml) with 1% (v/v) of the mycelial extract in the experimental group and only pure water was supplied in the control group. The drinkers were changed and newly filled every 3$^{rd}$ day of the experiment to prevent any decomposition of the content. The solution rests in the drinkers was recorded and so the total intake of water was measured. Dead bees were counted and removed from a cage once per day over the course of the experiment. The experiment was stopped 15$^{th}$ day when a first cage collapsed, i.e. more than 50% bees were dead.

### Statistical evaluation

The survival curves for 14 days of experiment were fitted by the Kaplan-Meier method. Significance of a difference between the survival curves was tested by log-rank test which compares observed number of the events with events what would be expected under null hypotheses (i.e. identical survival curves). The difference of intake of the water/solution was evaluated by t-test. Statistical analysis was performed using the SAS software.

## RESULTS AND DISCUSSION

The content of the drinkers was sucked by bees by the same intensity among all cages during the whole experiment ($p = 0.893$). The survival rate was similar in both groups (Figure 1) and the differences were insignificant (Table 1).

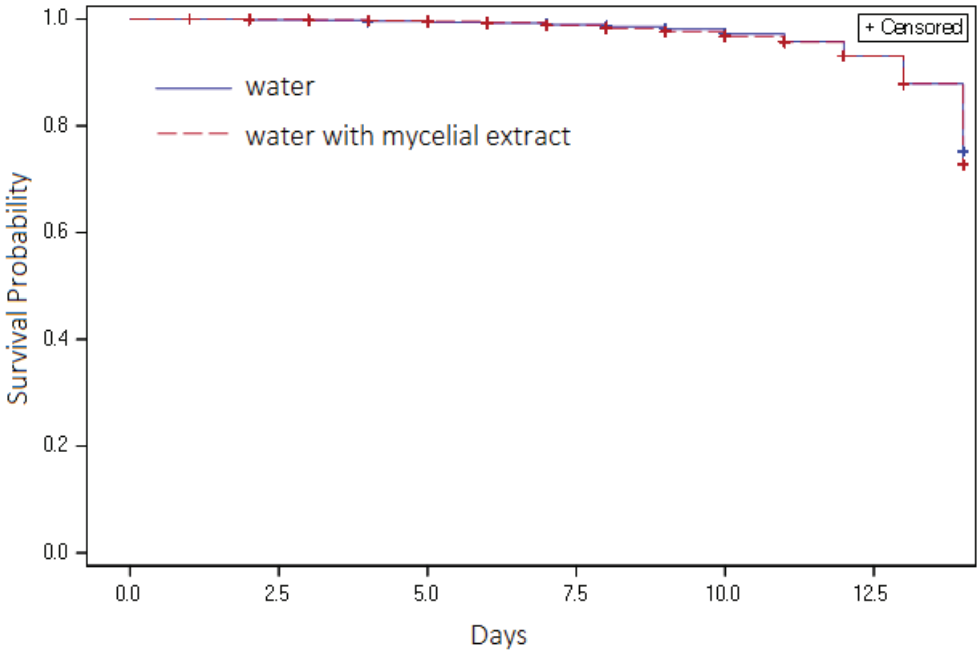
*Table 1 The log-rank test comparing survival curves*

| Treatment | Degrees of freedom | Chi-square statistic | p-value |
|---|---|---|---|
| Control/Experimental | 1 | 0.23 | 0.63 |

The results showed that the 1% (v/v) mycelial extract from *Fomes fomentarius* had no adverse effect on caged worker bees. Stamets (2018) mentioned that the extracts at 100% are far too potent and toxic. Even, the 10% extracts had some toxicity. However, when further diluted to 1% or 0.1% or less, longevity substantially increased. However, the preferred effective dose varies from species to species. Our results did not confirm any increase of honeybee worker longevity or any other apparent potentially beneficial effect (e.g. increased water or feed intake). The neutral effect of the extract could

have several causes. The experimental bees were of an unknown health status, i.e. they were not tested for presence of viruses or pesticides. Therefore, the bee health could somehow interact with the extract's effect (Stamets 2018). Further, our result can be interpreted only in respect to the used strain of *Fomes fomentarius*. It was shown that there are several distinct internal transcribed spacer lineages/sublineages of *Fomes fomentarius* which differ among continents (Gaper et al. 2016).

*Figure 1 Survival rate by Kaplan-Meier survival analyses*




Polyphenols, which are considered to be beneficial to bees in the diet, are often synthesized as protection against extreme environmental conditions (Mao et al. 2013, Gong and Diao 2017). Content of polyphenols differs not only among *F. fomentarius* strains (Gaper et al. 2016) but there are the effects of cultivation conditions, substrates or UV rays (Stamets 2018). The intraspecific variability of bioactive compounds in dependence on cultivation performance parameters has already been demonstrated in *Pleurotus ostreatus* (Koutrotsios et al. 2017). Therefore, the partial finding on the mycelial extract effects must be interpreted with respect of the taxonomy, the cultivation conditions, and the extraction methods. These parameters are important for improvement of biotechnological applications

## CONCLUSION

The tested mycelial extract did not cause any change in water or feed intake of honeybees. The survival rates of worker bees were the same in the experimental group additively watered with the extract and the control group. The extract is safe for further testing of potential biological effects.

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