Effect of hydroxymethyl furfural (HMF) on the Longevity and food consumption of Honeybee (Apis *mellifera* L.) workers

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DOI: https://doi.org/10.36077/kjas/2025/v17i2.17765

Received date: 26/10/2024 Accepted date: 4/2/2025

Abstract

The study was carried out at the apiary and in the laboratory of the Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt, during the year, 2023. Honey bee workers, the supporting force of the colony, and a measure of the queen's efficiency, as the increase in the size of the colony population and the presence of age sequence, are evidence of the efficiency of the queen and the goodness and efficiency of the pasture. The absence of natural nectar or pollen sources may force beekeepers to use alternatives, some beekeepers may resort to feeding bees with things that are not suitable for human use as a result of poor storage, long-term storage, or heat treatment of honey, which increases the levels of HMF. Accordingly, the level of HMF content in the fresh clover honey sample was estimated using a spectrophotometer, which was estimated at 1.92, it was divided and exposed to heat to reach HMF levels of 40 mg/Kg. 80mg/Kg, 200mg/Kg, 400mg/Kg, 700mg/Kg, 3700mg/Kg. The experiment was conducted on newly hatched bees inside small cages, containing 100 workers. The results obtained show that food consumption is inversely proportional to the levels of HMF in honey which are recorded as 7.02±0.07 for fresh honey and 1.78±0.30 for honey with HMF level of 3700 mg/Kg, the proportion of longevity is also directly proportional to its proportion HMF (33, 27, 24, 24, 18, 15 and 9 days for fresh honey, 40mg/Kg, 80mg/Kg, 200mg/Kg, 400mg/Kg, 700mg/Kg, 3700mg/Kg, respectively). Therefore, using honey with HMF High in feeding bee colonies is not recommended as an alternative in scarce pasture or during the winter season.

Keywords: honeybee, 5-hydroxymethylfurfural (HMF), mortality, longevity, food

consumption.

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Introduction

Honey is produced by bees collecting nectar from flowers. The nectar matures and becomes honey, which is rich in antibiotics. enzymes, phenolic antioxidants, aroma compounds, amino and organic acids, flavonoids, phenolic acids, gluconic acid, minerals, vitamins, 5hydroxymethylfurfural (HMF), and other phytochemicals. Honey also contains a complex mixture of nitrogenous compounds, lactones, proteins, and inhibin (7 and 1). Honey is considered a nutritional and therapeutic product. However, contamination with heavy elements or high levels of its components, such as some alkaloids and 5-hydroxymethylfurfural, might make honey and its derivatives nutritionally toxic.

HMF is a well-known factor influencing honey freshness because it is typically absent from fresh honey or present in trace levels. Its concentration tends to increase during heat processing and long storage periods. Previous studies have shown that honey stored under good conditions or at low temperatures has low levels of HMF (22, 5, and 15). Very little research has indicated that high levels of HMF harm honeybees. Jachimowicz et al. report that within 16 days of the feeding regimen starting, the 150 mg/kg HMF content in commercially produced acid-hydrolyzed inverted sugar syrup resulted in a 50% mortality rate (17). longevity of honey bee workers is an important indicator of the development of honey bee colonies and ensuring the existence of age and functional sequence within the colony, nutrition may be a major factor in this, (12) evaluated the HMF dose-response effect on bee mortality using caged honey bees and

high-fructose corn syrup (HFCS), an alternative saccharose substitute for honey bees in the United States. After 19 days, they found that 50% of the bees died from HFCS with 150 mg/kg HMF. This closely resembles the findings of (7). When they examined bee mortality after 26 days at various HMF doses (57, 100, 150, 200, and 250 mg/kg), they discovered that only HFCS enriched with 250 mg/kg caused a noticeably lower survival rate. One trait of honeybee workers that is significant to the economy is their food consumption tendencies (4). Bee preference plays an important factor in storing the sugar syrup, depending on the sugar percentage as well as the additives that in turn increase the preference properties such as Flavors, amino acids, or even bee venom, which recorded an increase in the food consumption levels (8 and 21). Therefore, this work aims to study the effect of HMF on the longevity and food consumption of honey bee (A. *mellifera*) workers underfeeding with stored honey containing different levels of HMF.

Material and methods

The present investigation was carried out at the apiary and in the laboratory of the Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt, during the year, 2023.

Preparation of HMF concentration

Honey Samples were stored under various conditions and evaluated for hydroxymethyl furfural by spectrophotometer according to Winkler (1955). Fresh clover honey (Tripholium alexandrinum) samples were harvested, homogenized, and divided into six sections. One section was kept at 4°C until



the experiment started. The remaining parts were exposed to continuous high temperatures (from 50°C to 100°C) to stimulate increased HMF concentration to reach the following levels (fresh: 1.92, 40, 80, 200, 400, 700, and 3700 mg/Kg).

Feeding honeybee workers

Dissolved 10 grams of honey in 10 ml of distilled water for every concentration for HMF for feeding workers in cages.

The longevity of honeybee workers

Susceptibility of honeybee workers fed on a honey solution containing different concentrations of HMF and sugar syrup (control). Hybrid Carniolan bees collected from combs of healthy kept in the experimental apiary, summer 2023. Tests were conducted using 100 newly hatched honeybee workers placed in special wooden cages (16 cm x 12 cm x 6 cm) with wire-screened sides and glass fronts. Twenty-one cages were divided into seven groups, each comprising three cages/conc. as the replicates and 3 cages used as (control). The boxes treated were incubated at 34°C and 65% RH. Dead workers were counted and removed every three days, and the experiments were recorded as continuing for thirty-three days.

Assessing the food consumption

Under laboratory conditions, the food consumption of honeybee workers (Carniolan hybrids F1) was assessed during the incubation period, which ran from the summer of 2023. The standard procedure was followed to prepare honey syrup with varying concentrations of HMF, which was then given to the cages under investigation in graduated cups at a rate of 10 milliliters per cage. The day after the honey solution was given to the test cages, the amounts of hoarded honey solution in each experimental cage were measured and noted. When necessary, known amounts of honey solution were added to the test cages. (16).

Statistical Analysis

The obtained data was subjected to a twoway ANOVA analysis using SAS's proc ANOVA (19). The same statistical program was used to perform a means separation using p=0.05. The multiplerange test was used to compare all means at the significance level of 0.05 (6).

Results and discussion

Effect of HMF on the longevity of honeybee workers

One of the factors affecting the development and growth of the honey bee colony is the age of the worker bees, which includes many internal factors and external influences that may have a negative or positive effect. Internal nutrition is one of the most important factors affecting workers' longevity. The results showed that workers treated with low concentrations of the HMF lifespan of the workers inside the cages extended to 33 days, and half of the individuals died between the 18th and 21st days. While the HMF percentages, estimated at 40mg/Kg, of the workers died under treatment between 12 and 15, the entire ten collapsed after 27 days. results showed that HMF levels of 80 mg/Kg and 200mg/Kg, all workers died after 21 days and half of the colony was lost between day 12 and day 15. Half the test subjects were lost between days 9 and 12 for both concentrations. 400mg/Kg and 700mg/Kg, while the death of all individuals was recorded on the 21st and 15th day after highest treatment. respectively. The concentrations used under the test.



estimated at 3700 mg/Kg, showed the death of half of the individuals between the third and sixth days and the complete collapse of the treatment on the ninth day, while the results of the control group, which was fed sugar syrup, showed that the individuals did not live longer than 21 days, while half of the individuals died between 12 and 15 days (Table and Fig. 1).

All treatments showed significant differences compared to the control, however, the treatments 80 mg/Kg and 200 mg/Kg were equal in the significant differences. (p = 0.0001, Randomized Complete Block Design (RCBD) analysis).

Table 1. Daily honey bee worker's dead percentage under the influence of feeding honey containing several concentrations of HMF

Conc. HMF	2 dova	s 6 days	9	12	15	18	21	24	27	30	33	Death
(mg/kg)	5 days		days	after/day								
Fresh (1.92)	1.00	4.33	9.66	18.99	33.32	47.32	56.82	70.82	84.82	95.82	100.0	33.0 ^a
40	9.33	21.00	29.00	48.33	63.33	76.66	89.00	93.00	100.0	0.00	0.00	27.0 ^b
80	11.67	15.67	23.0	44.33	60.33	76.66	87.00	100.0	0.00	0.00	0.00	24.0 ^c
200	15.33	18.00	26.33	49.66	66.33	83.00	92.00	100.0	0.00	0.00	0.00	24.0 ^c
400	15.67	23.67	36.34	61.34	85.01	98.34	100.0	0.00	0.00	0.00	0.00	18.0 ^d
700	9.33	21.00	44.67	76.00	100.0	0.00	0.00	0.00	0.00	0.00	0.00	15.0 ^e
3700	23.67	65.34	100.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.0 ^f
Control	16.0	20.00	20.00	23.00	63.00	88.00	95.00	100.0	0.00	0.00	0.00	24.0 ^c



Fig. 1. Effect of HMF concentration (mg/kg) on the mortality percentage of honeybee workers

The effectiveness of HMF concentration on workers' food consumption

The food consumption of honey bee colonies depends on one of the basic pillars of the degree of preference or acceptance of that food. Therefore, feeding newly hatched workers placed in small cages with honey solutions of different concentrations level of HMF were assessed as the amount of consumption ml/cage, Table (2) shows

Ο

that food consumption in fresh honey is almost with significant equal no differences compared to the control but with a high significant difference with the other treatments, as the food consumption behavior in both showed 7.02 ± 0.07 ml/day and 7.12±0.88 ml/day. The workers that were fed on the HMF conc. 40 mg/kg, 80mg/kg, and 200mg/kg showed a mean consumption of 6.06±0.32 ml/day,5.25±0.31ml/day, and

4.37±0.39ml/day, respectively. The highly

significant difference. The concentration of 700 mg/kg of HMF in test cages means of consumption recorded of 2.38±0.34 ml/day with a significant difference. Also, workers in the treatment group that received 400 mg/kg of HMF 3.38±0.49 ml/day hoarded with a significant difference. 3700 mg/kg of HMF in a honey solution consumed 1.78±0.30 ml./day compared with control sugar syrup showed the highest that mean of consumption 7.12±0.88ml./day.

Table 2. Influence of HMF concentration of honey on the efficiency (means) of honeybee workers' food consumption inside lab cages.

Amount consumption ml/cage						
HMF Concentration (mg/Kg)	Mean± Sd					
(Fresh) 1.92	7.02±0.07 a					
40	6.06±0.32 b					
80	5.25±0.31 c					
200	4.37±0.39 d					
400	3.38±0.49 e					
700	2.38±0.34 f					
3700	1.78±0.30 g					
Control (sugar syrup)	7.12±0.88 a					

Data in Table 4 showed that the highest mean of bee food consumption during the experiment was in control cages and positive correlation with significant differences between the amount of food consumed (ml) from HMF at low concentrations and the mortality rate of worker bees, and a negative correlation tending to no correlation between the amount of food consumed from high workers fed on fresh honey ml/100 worker/cage) compared with cages fed on high concentrations of HMF.

concentrations of HMF and the mortality rate of honey bee workers inside laboratory cages during the experiment period, i.e. with high concentrations of HMF, a large percentage of worker bees die with the withdrawal of a small amount of honey solution.



Concentration of HMF (mg/kg)		Longevity								
		Fresh 1.92 (mg/kg)	40 mg/kg	80 mg/kg	200 mg/kg	400 mg/kg	700 mg/kg	3700 mg/kg	Control (sugar syrup)	
Consumption	1.92 mg/kg	0.973*	0.842*	0.285	-0.948	-0.947	0.999*	-0.999	0.999*	
	40 mg/kg	0.267	-0.063	-0.700	-0.737	-0.179	0.444	-0.999	0.446	
	80 mg/kg	-0.116	-0.435	-0.918	-0.427	0.206	0.074	-0.997	0.076	
	200 mg/kg	-0.978	-0.856	-0.309	0.94*	0.955*	-0.999	0.019	-0.999	
	400 mg/kg	-0.798	-0.951	-0.921	0.358 ns	0.849*	-0.669	-0.743	-0.668	
	700 mg/kg	-0.845	-0.975	-0.882	0.442 ns	0.894*	-0.735	-0.679	-0.733	
	3700	-0.579	-0.814	-0.996	0.06 ns	0.651	-0.415	-0.909	-0.413	
Control (sugar syrup)		-0.982	-0.866	-0.326	0.934	0.96	-0.999	0.001	-0.999	
F c 14		0.207								

< 0.0001

0.607

Table The correlation coefficients 4. simple **(r)** between HMF

Means in the same column followed by the same letter are not significantly different (p>0.05) using LSD in RCBD

The results obtained indicate that feeding bees in laboratory cages with fresh honey (1.92mg/Kg for HMF) led to prolonging the longevity of the worker, unlike high concentrations of HMF, the bees die with the consumption of a very small amount and after a short period of nine days, these findings corroborate those of Gregorc et al., (8), who claimed that fresh honey is rich in proteins, fats, and certain enzymes and vitamins that enhance bee health in general and worker longevity. А

P(0.05) LSD

few years later, Ceksteryte & Racys (4) proposed that wintering honey bees could safely consume sugar syrup made from maize containing 48 mg/kg of HMF, during experiments on the quality of syrups used for bee feeding. Remarkably, they discovered that the amount of HMF in the first syrups dropped in the syrups that the bees left in the comb, indicating that the bee organism can partially metabolize the HMF Krainer et al., (11). These studies

appeared to suggest that HMF contributes to honey bee mortality, but to determine an LD50 HMF for bees. experiment standardization is required. The adult worker at the 3700 mg\kg conc. HMF test groups showed a mortality of 100 % compared to bees fed on fresh honey (0 %, these results mg/kg HMF) 5.7 according to research by (9) commercially available acid-hydrolyzed inverted sugar syrup with a 150 mg/kg HMF content resulted in a 50% mortality rate 16 days after the feeding began. Since honey bees appear to be unaffected by HMF concentrations of up to 30 mg/kg, many experts advised that the maximum amount of HMF in inverted syrup, as in most honey, should not exceed 20 mg/kg. Zirbes et al., (22). The food consumption of honey bee workers is an economically important characteristic Badr et al., (3). Cages whose workers hoard more sugar syrup in cages produce more honey than



those food consume less. Regarding food consumption and storage, the results showed significant differences between feeding bees on conc. Of HMF and workers fed on fresh honey (0 HMF), these results are in agreement with (4). As previously mentioned, there has been an instance where honeybees were exposed to HMF through syrup that was provided to them for winter sustenance. It appears that HMF is toxic to honey bees, as evidenced by the possibility of fatal intestinal tract ulceration. Subsequently, Le Blanc et al., (2009) evaluated the HMF dose-response effect on bee mortality using caged honey bees and high fructose corn syrup (HFCS), an alternative saccharose substitute for honey bees in the United States. After 19 days, they saw a 50% bee mortality rate for HFCS with 150 mg/kg HMF. This closely resembles the findings of Jachimowicz et al. When they compared bee mortality for various HMF doses (57, 100, 150, 200, and 250 mg/kg) after 26 days, they discovered that only HFCS-enriched honey at a dose of 250 mg/kg caused a noticeably lower survival rate. Syrup crystallization enhanced the toxicity of HMF to honey bees. Some of the syrup solidifies during the crystallization process, concentrating the HMF in the liquor—the only phase that honey bees can access. Since information about the toxicity of HMF derivatives became available according to Shapla et al., (18) their interest in these compounds has grown. Numerous structural alerts in HMF have been shown in animal experiments to present potential genotoxic and carcinogenic risks. Nevertheless, very few studies found that HMF was harmful to honey bees. Inverted sugar syrups should have their HMF content under control before being fed to bees, according to previous advice. (10).

Conclusion

In conclusion, hydroxy methyl furfural (HMF) poses a toxicological risk to honey bees, potentially leading to bee mortality either directly or indirectly. Feeding extremely low concentrations of HMF to honey bees lowers their mortality rate and prolongs their life. On the other hand, as HMF concentration rises, so does its toxicity to bees. In high concentrations, bees die at the age of nine days, with a 100% fatality rate. Regarding food consumption, the amounts of food consumed rise when there is a low HMF content in the food, while the amounts consumed fall when there is a high HMF content in the food.

Acknowledgments

The staff of the Bee Research Department, Plant Protection Research Institute, and Agriculture Research Center are acknowledged by the authors.

Conflict of interest

The authors declared that they have no competing interests.

Authors' contribution

The authors contributed equally to the following points (concept and preparation of the manuscript, materials, and methods used, follow-up of results, collection of data resulting from the study, discussion of the research, statistical analysis and collection of references, writing the comprehensive study and preparing the final preparation required for publication) designing and conducting the experiment equally. All authors read and approved the final manuscript.



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