



Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com



Original article

Nutritional efficacy of different diets supplemented with microalga *Arthrospira platensis* (spirulina) in honey bees (*Apis mellifera*)

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ARTICLE INFO

Article history:

Received 4 November 2021

Revised 19 December 2021

Accepted 30 December 2021

Available online 5 January 2022

Keywords:

Pollen

Nutrition

Consumption

Laboratory-controlled experiments

Mortality

ABSTRACT

In honey bees, food, gut microorganisms, and their nestmates may contribute to the health status of newly emerged worker bees. However, relatively little data are available regarding the extent to which supplemental protein feeding impacts bee colony health and mortality. The present research compared the efficacy of different diets supplements, i.e., bee pollen, ajeena (commercially available pollen substitute), and date paste either alone or admixed with varying percentages of microalga *Arthrospira platensis* (spirulina) for caged honey bees (*Apis mellifera jemenitica*). In addition, to investigate diet preference and mortality percentage in honey bees, the physicochemical analysis of these diets were also performed. Our results indicated that the honey bees consumed bee pollen (11.51 ± 2.22 mg/bee) and ajeena diet (10.68 ± 1.29 mg/bee) significantly higher than other diets.

In contrast, the maximum consumption was 4.68 ± 2.82 mg/bee for date paste admixed with 2.5% spirulina than date paste diet only (2.41 ± 0.91 mg/bee). In addition, the mortality percentage was significantly lower when bees fed pollen ($56.67\% \pm 3.88\%$) and ajeena diet ($39.33\% \pm 2.08\%$) in comparison to combination with spirulina supplement. Overall, the mean maximum diet consumption for ajeena was 15.72 ± 3.65 mg/bee followed by bee pollen diet (11.97 ± 2.41 mg/bee) and date paste (2.08 ± 0.94 mg/bee), respectively, resulting mean percentage of mortality of $13.37\% \pm 3.77\%$. Physicochemical analysis revealed that glucose and fructose were higher in the date paste diet. In contrast, total sugar content, sucrose, and protein content were higher in the ajeena diet. Future research is needed to determine the impact of diet supplementation with spirulina on colony health and bee physiology.

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1. Introduction

Apis mellifera jemenitica Ruttner (Hymenoptera: Apidae) is the most heat-tolerant eusocial insect native to Saudi Arabia and is extensively used throughout the kingdom (Alqarni et al., 2011). It is naturally adapted with various behavioural and ecological adaptations; therefore, 70%–80% of total bee colonies in Saudi Arabia are occupied by *A. m. jemenitica* (Abou-Shaara et al., 2017; Iqbal et al., 2019). Its behavioural and ecological succession have been

reported to endure and thrive in the hot and dry environment of central Saudi Arabia, where the temperature surpasses $45\text{ }^{\circ}\text{C}$ (Alqarni et al., 2011). In addition, *A. m. jemenitica* has a high fecundity capability, and its searching ability to collect pollen is also remarkable (Taha and Al-Kahtani, 2019). During dearth and drought periods, *A. m. jemenitica* can enhance its colony with many worker cells (Woyke, 1993; Alqarni, 1995; Iqbal et al., 2019).

The invention of the Langstroth beehive in 1851 ushered in the modern era of beekeeping (Langstroth, 1853), and before that time, the bees were kept in the hive of earthen pots for rearing (Crane, 1983). In Saudi Arabia, 4,000 beekeepers owned 700,000 hives and produced 3,500 tons of honey per year. This honey production fulfils 26% of the required honey demand of consumers. The need for honey by consumers is expected to reach approximately 29,784 tons in 2025 (Adgaba et al., 2014; Ismaiel et al., 2014; Poole, 2021). Almost 10,000 tons of honey (that fulfil the local demand) are imported yearly from the United States, Australia, Turkey, Iran and European countries (Poole, 2021). However,

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various factors are responsible for less honey production in Saudi Arabia, mainly dietary problems (Ismaiel et al., 2014; Khan and Ghramh, 2021; Paray et al., 2021).

A honey bee colony collects 10–26 kg of pollens per year from flowers as a primitive source of protein content and amino acids composition for their colony's well-being (Wille et al., 1985; Brodschneider and Crailsheim, 2010). In addition, a sufficient amount of protein and carbohydrate stocks in the colony is thought to help the honey bees fight or tolerate various stressors associated with modern apiculture (Brodschneider and Crailsheim, 2010). In the dearth period, the necessary diet of bees is not available in high quantity—the consequences of forage scarcity periods resultantly bee starvation, consumption of stored honey, and pollen. Therefore, commercially available pollen substitutes are provided to honey bees to fulfil the essential diet to maintain the colony's strength (Saffari et al., 2004; Kumar et al., 2013; Agrawal, 2014; Abbasi et al., 2021; Ahmad et al., 2021). Many artificial diets were formulated as a pollen substitute for the survival and development of honey bee colonies. The different parameters associated with colony health such as fecundity, reproductive performance, pollen load, sealed brood area, and honey yield have been analyzed when feeding various pollen substitutes to honey bees (Paiva et al., 2016; Ahmad et al., 2021; Jagdale et al., 2021; Paray et al., 2021; Qasim et al., 2021). Soybean flour is a core part of the honey bee diet due to the high percentage of protein (49.3%) and carbohydrates (18.6%) present in it (Farzana and Mohajan, 2015). Its products have been extensively employed by many scientists worldwide, with promising outcomes for honey bees (Dastouri et al., 2007; Saffari et al., 2010; Kumar and Agrawal, 2014; Qasim et al., 2018; Shawer et al., 2021). The date palm fruits are enriched with sugars and are widely cultivated in Saudi Arabia. The dried fruit comprises more than 50% carbohydrates by weight and less than 2% protein, fat, or mineral components (Al-Redhaiman, 2014). The aquatics, photosynthetic, and nucleus-bearing organisms called spirulina (*Arthrospira platensis*) are used as nutritional supplements for humans and livestock. These are grown on industrial scales as a nutrition supplement due to more than 93% protein in them (Soni et al., 2017; Böcker et al., 2021). Also, spirulina is a rich source of essential components such as amino acids, lipids, carbohydrates, minerals, and vitamins. There is minimal literature highlighting the importance of cyanobacteria (spirulina) in honey bees. Therefore, the objective of this study was to evaluate the potential of various diet supplements admixed with spirulina as a nutrition supplement (especially protein) for honey bees in the control condition. In addition to determining the preference of honey bees to different diet supplements and their impact on honey bee mortality, the physiochemical properties of diets consumed by honey bees were also studied.

2. Materials and methods

2.1. Honey bees (*Apis mellifera jemenitica*)

Sealed brood combs from bee colonies were incubated at 34.5 °C under laboratory conditions to get newly emerged worker bees (0–24 h). Newly emerging bees from these brood combs were mixed and kept in test cages at random. This research was carried out at the Unit of Bee Research and Honey Production (UBRHP), King Khalid University Abha, Saudi Arabia.

2.2. Type of supplement diets

These experiments evaluated three different types of diets (Ajeena = commercially available pollen substitute, date paste, and bee pollen) separately and mixed with 2.5%, 5%, and 10% spirulina as supplements.

Ajeena and date paste were purchased from the local bee cooperative market of Abha, Saudi Arabia, while bee pollen was collected from beehives using pollen traps. Spirulina was purchased through online order. The entrance-mounted plastic pollen traps were utilized in the apiaries of Bee Research and Honey Production, King Khalid University, Abha Saudi Arabia, to collect pollens from the foraging bees. Pollen traps were set up in the morning with favourable weather and emptied late afternoon. The collected pollens were directly deep-frozen (–18 °C). All diets were preserved at 4 °C till the bioassays were performed.

2.3. Experimental cages

The wooden cages were selected for the experiments had a size of 15 × 15 × 5 cm (Fig. 1). It was equipped with glass on one side and an iron grid that enabled air to travel through on the other. One hole was provided with a 20 mL syringe filled with tap water, and another hole was provided with a 20 mL syringe filled with 50% (w/v) sucrose solution (Evans et al., 2009; Williams et al., 2013).

2.4. Experimental design

2.4.1. Diet consumption in caged bees

Twenty newly emerged bees were kept in different cages. Artificial diets were placed in small Petri plates given to honey bees in cages. They were kept in the dark in an incubator at 34.5 °C and 50% relative humidity under laboratory conditions for 18 days. Dead bees were counted every day and removed from the cages throughout the experiment. Consumed diet was measured, the difference between the weight of the diet before introduction and the weight after 24 h was measured on an analytical balance (Mettler Toledo XS205 dual range).

2.4.2. Diet preferences

The preferences of all three types of diets without the spirulina supplement were estimated during March 2021. Each diet was used as a dough. Each cage contained 20 newly emerged bees. Mortality was recorded daily, and remove dead honey bees from caged. Daily consumed diet was recorded and replaced with a new diet. Consumed diet measured by the difference between the weight before introduction and after 24 h was measured on an analytical balance (Mettler Toledo XS205 dual range). Time duration in which the bees had contact with the diets was calculated precisely to the minute and was recalculated for the total consumption of food over 24 h. The experiment was done in three replicates.

2.5. Chemical analysis of diets

2.5.1. Sugar analysis of different diets using HPLC-RID

Each diet sample of 1 g and 250 mg were weighed with electric balance (Shimadzu, Kyoto, Japan) and kept in a glass beaker (100 mL). Add the glass HPLC grade water to create a total volume of 25 mL. Diets were dissolved using vortex and filtered by 0.22 mm syringe filters (Iso-lab, Laborgeräte GmbH). Different saccharides (sucrose, maltose, fructose, and glucose) were measured by HPLC system (Agilent 1260 Infinity II, Agilent Technologies, California, USA) fitted with pump (1260 Quat Pump VL, Agilent Technologies, California, USA), vial sampler (1260 vial sampler, Agilent Technologies, California, USA), and a refractive index detector (1260 RID, Agilent Technologies, California, USA) by ZORBAX Carbohydrate analyzing column (4.6 × 150 mm, 5 μm; Agilent Technologies, California, USA), with an isocratic mobile phase of Acetonitrile: water (75:25, v/v), retained at a flow rate 1.0 mL/min. The 10 μL volume of sample was injected and analyzed



Fig. 1. Visual representation of experimental bee cages and different diets applied.

at 35 °C. Standard curves for fructose and glucose were primed in HPLC grade water at concentrations range of 0.0625%–2.00% (w/v) and for sucrose and maltose from 0.03125% to 1.00% (w/v). The instrument was operated OpenLab CDS, ChemStation Edition. Rev. C.01.10 (201) software installed (Agilent Technologies, California, USA). The chromatographic peaks matching each sugar were synchronized with the retention time of the standard. A calibration curve fitted by linear regression analysis was prepared using serial dilutions of standards to define the correlation in peak area and concentration. These sugar contents were presented in percentages.

2.5.2. Chemical analysis of diets using HPLC-DAD

Diet solutions of 5% were prepared as mentioned in the previous section of sugar analysis of different diets using HPLC-RID. Chromatographic analyses were conducted on an HPLC system (equipped with pump, vial sampler, and a Diode Array HPLC Detector by an Infinity lab Poroshell 120 EC-C18 analyzing column, with a mobile gradient phase, i.e., 90% water at 1% of acetic acid and 10% methanol kept at a flow rate 0.300 mL/min. The 5 μ L volume of sample was injected and analyzed at 35 °C. The 284 nm wavelength was adjusted, and the chromatograms were observed at 284 nm. The instrument was operated using the OpenLab CDS, ChemStation Edition . Rev. C.01.10 (201) software (Agilent Technologies, California, USA) installed. The experiment was done in three replicates.

2.6. Statistical analysis

All statistical data were investigated by the statistical application SPSS (version 26), and the results were given as mean \pm standard error (S. Error). The significance of a difference between three or more groups was determined using the one-way ANOVA followed by the Tukey post-hoc test. A significant difference was considered at the 95% ($p < 0.05$) confidence level

3. Results

3.1. Diet consumption in caged bees

Our results indicated that the cumulative consumption was significantly higher for the pollen diet than pollen with the spirulina diet. The maximum mean consumption was 11.51 ± 2.22 mg per honey bee for pollen only, whereas less mean consumption was observed 7.03 ± 1.56 mg per honeybee for pollen with a 10% spirulina diet. The mean consumption was 10.70 ± 1.43 mg per honeybee and 8.69 ± 1.43 mg per honey for the pollen + 2.5% spirulina diet and pollen + 5% spirulina diet, respectively.

Similarly, the mean consumption was significantly higher for ajeena diet only than ajeena with spirulina diet. The highest mean consumption was 10.68 ± 1.29 mg per honey bee for ajeena only, while low mean consumption was 4.35 ± 1.50 mg per bee for ajeena + 2.5% spirulina diet. The mean diet consumption was 5.53 ± 0.29 mg per bee for ajeena + 5% spirulina diet and 6.06 ± 1.73 mg per honey bee for ajeena + 10% spirulina diet (Fig. 2A).

In the case of the date paste diet, the mean consumption was significantly more for date paste + 2.5% spirulina diet than other diets. The maximum mean consumption was 4.68 ± 2.82 mg per bee for date paste + 2.5% spirulina diet, and low mean consumption was 2.41 ± 0.91 mg/bee for date paste only diet (Table 1).

3.2. Diet preference and mortality percentage

Our results showed that the mortality percentage was significantly lower when bees were fed on bee pollen than that of bee pollen admixed with spirulina diet (Fig. 2B). In the case of bee pollen, the maximum mortality ($73.09 \pm 2.27\%$) was recorded when bees were fed on bee pollen + 10% spirulina diet, and the most negligible mortality was observed when bees fed on bee pollen diet only. Similar, the percentage of mortality was significantly lower when bees fed on ajeena diet only than that of ajeena admixed with spirulina diet. In case of ajeena supplement, the maximum

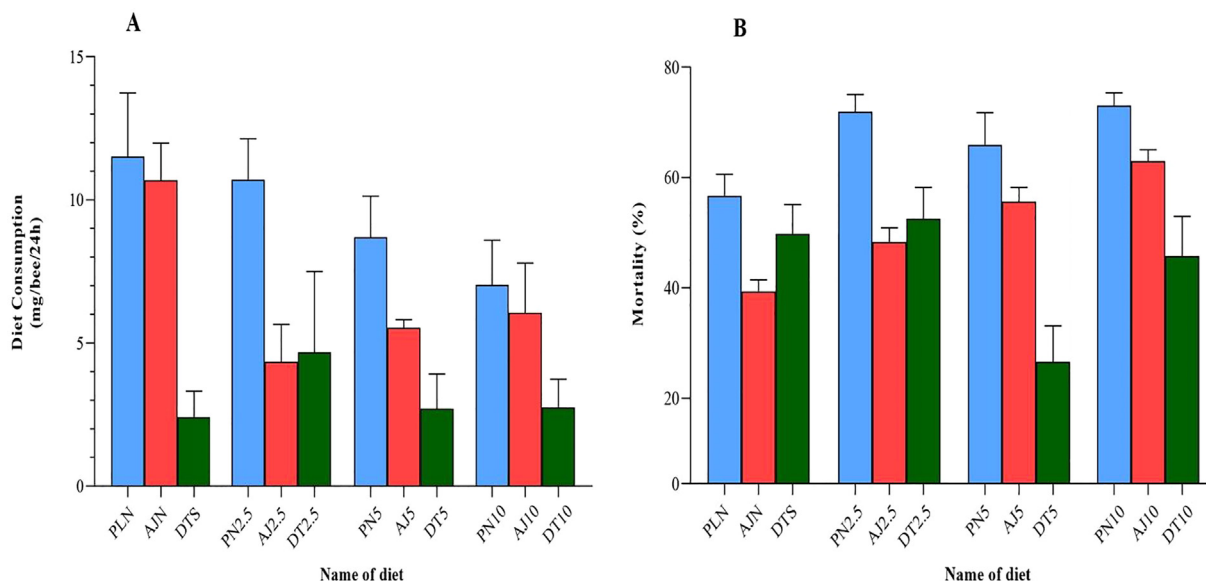


Fig. 2. (A, B). Consumption of different diets by honey bees and their impact on mortality. Whereas PLN = Bee pollen; AJN = Commercially available pollen substitute; DTS = date paste; PN2.5; PN5; and PN10 = Bee pollen admixed with 2.5%, 5%, and 10% spirulina respectively; AJ2.5; AJ5; and AJ10 = Commercially available pollen substitute admixed with 2.5%, 5%, and 10% spirulina respectively; and DT2.5; DT5; and DT10 = date paste admixed with 2.5%, 5%, and 10% spirulina respectively.

Table 1
Mean, minimum, and maximum diet consumption by honey bees.

Treatment	Name of Diet	Diet Consumption (mg/bee/24 h)		
		Mean	Min.	Max.
T1	Bee pollen only	11.51 ± 2.22 ^a	9.26	13.71
T2	Bee pollen + 2.5% Spirulina	10.70 ± 1.43 ^a	9.27	12.14
T3	Bee pollen + 5% Spirulina	8.69 ± 1.43 ^{ab}	7.47	10.27
T4	Bee pollen + 10% Spirulina	7.03 ± 1.56 ^{abc}	5.32	8.36
T5	Ajeena only	10.68 ± 1.29 ^a	9.54	12.09
T6	Ajeena + 2.5% Spirulina	4.34 ± 1.30 ^{bcd}	3.06	5.06
T7	Ajeena + 5% Spirulina	5.53 ± 0.29 ^{bcd}	5.21	5.76
T8	Ajeena + 10% Spirulina	6.06 ± 1.73 ^{bcd}	4.40	7.85
T9	Date Paste only	2.41 ± 0.91 ^d	1.50	3.31
T10	Date Paste + 2.5% Spirulina	4.68 ± 2.82 ^{bcd}	2.15	7.72
T11	Date Paste + 5% Spirulina	2.71 ± 1.20 ^{cd}	1.92	4.10
T12	Date Paste + 10% Spirulina	2.75 ± 0.99 ^{cd}	1.84	3.80

Results are means ± standard deviation of triplicate determinations. Means in a column with different letters are significantly different (P < 0.05). Ajeena = commercially available pollen substitute.

mortality rate (63.0% ± 2.00%) was noted when bees were fed on ajeena + 10% spirulina diet, and the lowest percentage of mortality was 39.33% ± 2.08% when bees were fed ajeena diet only. In the case of the date paste diet, the mortality percentage was significantly lower when bees fed on date paste + 5% spirulina diet compared to other diets. The highest mortality rate was recorded when bees were provided date paste + 2.5% spirulina diet. When honey bees were fed on date paste + 5% spirulina diet, the lowest mortality percentage (26.57% ± 6.53%) was observed (Table 2).

3.3. Comparison between different diet consumption and their impact on honey bee mortality

The mean maximum diet consumption without spirulina supplement was 15.72 ± 3.65 mg/bee for the ajeena diet, and the less consumption was 2.08 ± 0.94 mg per bee for the date paste diet. At the same time, the mean diet consumption without spirulina supplement was 11.97 ± 2.41 mg per bee for bee pollen diet. The mean percentage of mortality was 13.37 ± 3.77 when bees were provided all three diets, i.e., ajeena, date paste, and bee pollen without

mixing spirulina. While, the mean maximum diet consumption with spirulina supplement was 8.80 ± 2.13 mg/bee for bee pollen diet, and the lowest was 3.38 ± 1.91 mg per bee for date paste diet. At the same time, the mean diet consumption supplement with spirulina was 5.31 ± 1.46 mg per bee for the ajeena diet. The mean percentage of mortality was 55.86 ± 7.24 when bees were fed on ajeena, date paste, and bee pollen diets admixed with spirulina (Table 3).

3.4. Physicochemical analysis

Our results related to the physicochemical analysis of three different kinds of diets, i.e., ajeena, date paste, and bee pollen, are shown in Table 4. The percentage of fructose (34.40 ± 0.27) was significantly higher in the date paste diet in comparison to other ajeena diets (13.76 ± 0.07) and pollen diet (19.68 ± 0.31). The highest percentage of glucose (34.51 ± 0.29) was noticed in the date paste diet, whereas the lowest percentage (13.81 ± 0.14) was observed in the ajeena diet. The percentage of sucrose was significantly more (69.68 ± 1.87) in the ajeena diet than that of the date

Table 2
Mean, minimum, and maximum mortality (%) in honey bees due to consumption of different types of diet.

Treatment	Name of Diet	Mortality (%)		
		Mean	Min.	Max.
T1	Bee pollen only	56.67 ± 3.88 ^{bcd}	52.50	60.18
T2	Bee pollen + 2.5% Spirulina	71.97 ± 3.10 ^a	68.44	74.26
T3	Bee pollen + 5% Spirulina	65.92 ± 5.80 ^{ab}	60.57	72.10
T4	Bee pollen + 10% Spirulina	73.09 ± 2.27 ^a	70.71	75.24
T5	Ajeena Only	39.33 ± 2.08 ^{ef}	37.00	41.00
T6	Ajeena + 2.5% Spirulina	48.33 ± 2.51 ^{de}	46.00	51.00
T7	Ajeena + 5% Spirulina	55.67 ± 2.52 ^{bcd}	53.00	58.00
T8	Ajeena + 10% Spirulina	63.0 ± 2.00 ^{bc}	61.00	65.00
T9	Date Paste Only	49.81 ± 5.30 ^{de}	46.20	55.90
T10	Date Paste + 2.5% Spirulina	52.54 ± 5.63 ^{cd}	47.20	58.44
T11	Date Paste + 5% Spirulina	26.57 ± 6.53 ^f	20.89	33.71
T12	Date Paste + 10% Spirulina	45.76 ± 7.15 ^{de}	38.40	52.70

Results are means ± standard deviation of triplicate determinations. Means in a column with different letters are significantly different (P < 0.05)—Ajeena = commercially available pollen substitute.

Table 3
Comparison of different kinds of diets (alone and supplemented with spirulina) consumption and their impact on mortality of honey bees.

Treatment	Name of Diet	Diet Consumption (mg/bee/24 h)			Mortality (%)		
		Mean	Min.	Max.	Mean	Min.	Max.
T1	Ajeena only	15.72 ± 3.65 ^a	11.64	18.66	13.37 ± 3.77	10.30	17.58
T2	Date pastes only	2.08 ± 0.94 ^e	1.04	2.88			
T3	Bee pollen only	11.97 ± 2.41 ^b	9.21	13.71			
T4	Ajeena with spirulina	5.31 ± 1.46 ^d	4.34	6.06	55.86 ± 0.00	26.56	73.09
T5	Date paste with spirulina	3.38 ± 1.91 ^e	2.71	4.68			
T6	Bee pollen with spirulina	8.80 ± 2.13 ^c	7.03	10.70			

Results are means ± standard deviation of triplicate determinations. Means in a column with different letters are significantly different (P < 0.05). Ajeena = commercially available pollen substitute.

Table 4
Physicochemical analysis of different kinds of diets consumption by honey bees.

Treatment	Name of Diet	Physicochemical parameters						
		Fructose (%)	Glucose (%)	Sucrose (%)	Maltose (%)	Total Sugar (%)	HMF(mgKg-1)	Protein (%)
T1	Ajeena	13.76 ± 0.07c	13.81 ± 0.14c	69.68 ± 1.87a	0.08 ± 0.06b	97.32 ± 1.73a	0.84 ± 0.18a	43.24 ± 0.65a
T2	Date palm Paste	34.40 ± 0.27a	34.51 ± 0.29a	0.04 ± 0.00b	0.18 ± .03a	69.13 ± 0.51b	0.15 ± 0.05b	1.37 ± 0.29c
T3	Pollen	19.68 ± 0.31b	14.62 ± 0.13b	0.02 ± 0.01b	0.00 ± 0.00b	34.33 ± 0.29b	0.17 ± 0.01b	40.64 ± 0.47b

Results are means ± standard deviation of triplicate determinations. Means in a column with different letters are significantly different (P < 0.05). Ajeena = commercially available pollen substitute.

paste and bee pollen diet. The percentage of maltose was 0.18 ± 0.03 in the date palm diet and was 0.08 ± 0.06 in the ajeena diet, whereas no content of maltose was observed in pollen. The total sugar percentage was higher in the ajeena diet (97.32 ± 1.73) and lower in the bee pollen diet (34.33 ± 0.29). Further, the protein content percentage was higher in the ajeena diet (43.24 ± 0.65), while the lowest rate was observed in the date paste diet (1.37 ± 0.29).

The study showed an analytical HPLC method for determining and quantifying main sugar contents in ajeena, date paste, and bee pollen diet. In the ajeena diet, fructose, glucose, sucrose, and maltose peaks were observed at retention times 6.080, 7.015, 10.004, and 12.214 min (Fig. 3A). In the case of date paste diet, the peaks of fructose, glucose, sucrose, and maltose were noticed at retention times 6.077, 7.024, 10.098, and 12.085 min, respectively (Fig. 3B). The retention times were 6.081, 7.043, 9.997 min for fructose, glucose, and sucrose, respectively, for the bee pollen diet (Fig. 3C). The detailed description of chromatography using HPLC-DAD for the diets is shown in Supplementary Fig. S1, Fig. S2, and Fig. S3).

4. Discussion

The majority of beekeepers frequently supplement the diet of honey bees' colonies using pollen and nectar substitutes commercially to boost colony growth and prevent honey losses (Mortensen et al., 2019). Several studies focused on diet, gut microbiota, and their nestmates may contribute to newly emerged individual bees (Alaux et al., 2010; Brodschneider and Crailsheim, 2010). The present study was conducted to determine the rate of various artificial diets (ajeena, date paste, and bee pollen) with or without spirulina diet consumption and their impact on mortality in newly emerged caged bees. In addition, physicochemical analysis of different kinds of diets consumption by honey bees was recorded. Our results indicated that honey bees' consumption rate was significantly higher for pollen and ajeena diet only compared to spirulina's addition. These results are consistent with a reduction in spirulina consumption relative to bee pollen and commercially available pollen substitute diets noticed before Ricigliano and Simone-Finstrom (2020).

In contrast, the maximum diet consumption by honey bees for date paste with spirulina diet is more than the date paste diet in

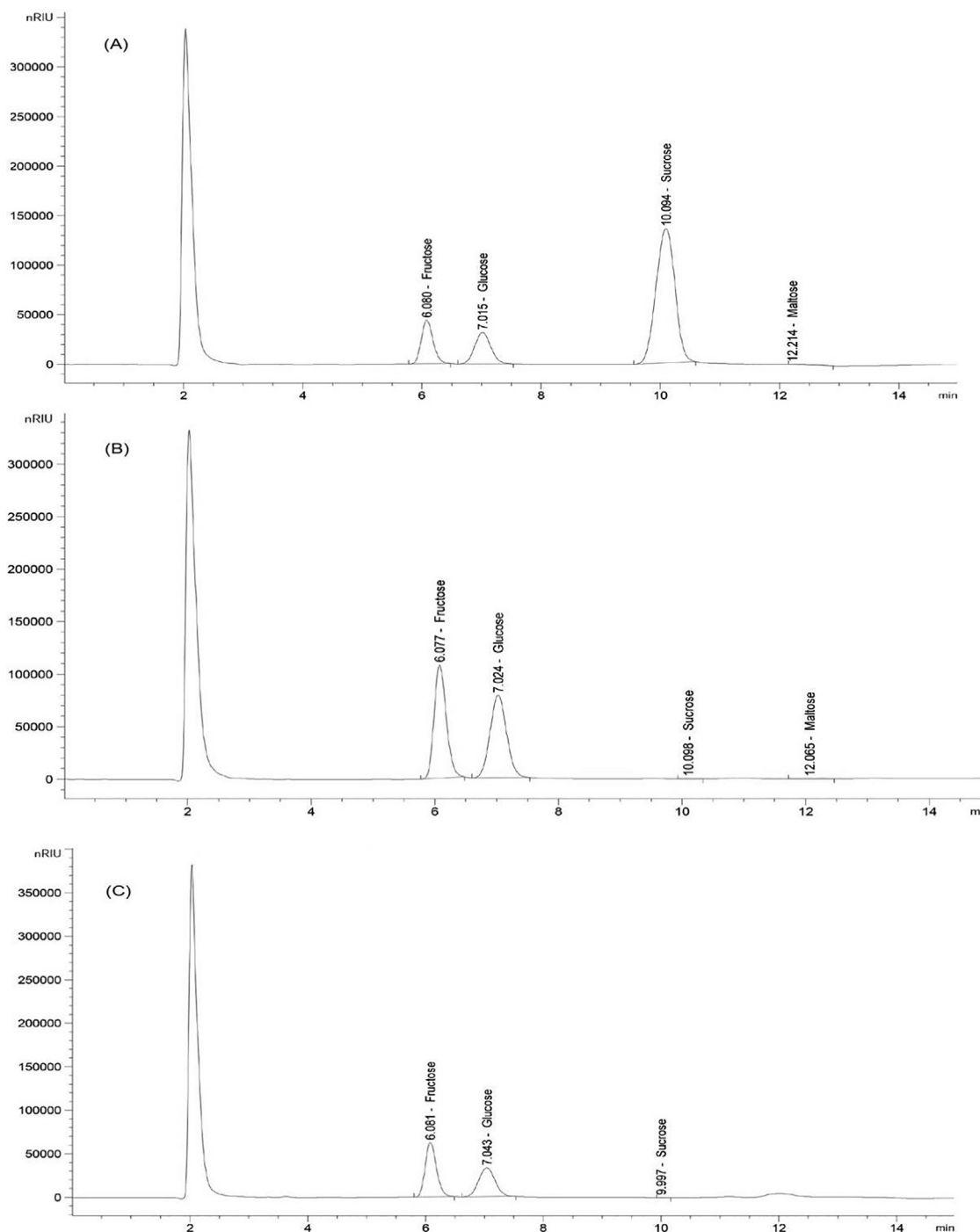


Fig. 3. (A, B, C). High-performance liquid chromatography (HPLC) chromatograms four sugars (fructose, glucose, sucrose, and maltose) found in different diets applied: (A) Commercial diet (Ajeena); (B) Date palm paste; (C) locally collected pollen, using an isocratic mobile phase containing 75% of Acetonitrile and 25% of water maintained at 1 mL/min.

the date paste diet. Ricigliano et al. (2021) suggested that honey bees and their colonies significantly influenced the nutritional response. Spirulina consumption was less than the commercially available pollen supplement diet. Still, it led to more head weights, the equivalent weight of thorax, and level of vitellogenin (Vg) expression and decreased fat body lipids (Ricigliano et al., 2021). Unexpectedly, our findings did not demonstrate the effect of different kinds of diet consumption on bee physiology.

Similarly, the mortality percentage was significantly lower when bees fed on bee pollen, and ajeena diet only compared to combination with spirulina supplement. In the date paste diet,

the mortality percentage was significantly lower when bees provided date paste + 5% spirulina diet than other diets. The longevity of honey bees is likely to be shortened by poor food quality (Gregorc et al., 2019).

In addition, our result indicated the mean maximum diet consumption was 15.72 ± 3.65 mg/bee, 11.97 ± 2.41 mg/bee, 2.08 ± 0.94 mg/bee for ajeena, pollen, and date paste, respectively, resulting mean percentage of mortality was 13.37 ± 3.77 .

Physicochemical analysis of different diets consumption by bees demonstrated that the percentage of glucose and fructose was higher in the date paste diet. In contrast, total sugar content,

sucrose, and protein content were higher in the ajeena diet. In our results, the percentage of mortality was lower in the ajeena diet, which may be due to the presence of higher content of sucrose in the ajeena diet. Our results are in line with Brodschneider and Crailsheim (2010), sucrose allows caged bees to live the longest compared to honey and different diets on high-fructose corn syrup. Further, protein is the primary source for brood rearing, colony development, and longevity of worker bees (Saffari et al., 2004; Khan and Ghramh, 2021; Paray et al., 2021).

This research is based on laboratory reared-bees and elucidates some insight into the impact on bee workers. Further studies are needed to investigate the effect of the various commercial diet supplements with spirulina on the nutritional response at the colony level.

5. Conclusions

Our results indicated diet consumption by bees was higher for pollen and ajeena diet with or without spirulina as compared to date paste diet with or without spirulina. As a result, the percentage of mortality was low when bees were fed on pollen and ajeena diet only. Physiochemical results revealed that glucose and fructose were high in the date paste diet. At the same time, total sugar content, sucrose, and protein content were higher in the ajeena diet. However, future studies are needed to investigate the effect of supplemental diets with spirulina on bee physiology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number IFP-KKU-2020/5.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2021.101819>.

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