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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

# EFFECTS OF CONCENTRATIONS OF SUGAR, VITAMINS, AND AMINO ACIDS ON SURVIVAL AND TOLERANCE OF HONEY BEES TO LOW-TEMPERATURE STRESS

## Bal Arılarının Düşük Sıcaklık Stresine Karşı Hayatta Kalma ve Toleransı Üzerinde Şeker, Vitamin ve Amino Asit Konsantrasyonlarının Etkileri

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## ABSTRACT

**Honey bees (*Apis mellifera*) may experience brief periods of cold stress, particularly in autumn and winter. Meanwhile, beekeepers often supplement their colonies with artificial nutrition during these periods. This practice can expose honey bees to varying sucrose concentrations, vitamins, and amino acids in their diet. Therefore, this study examines how these nutritional components influence the honey bees' ability to withstand cold stress in laboratory settings. This study comprised three sub-experiments: I) Comparing three concentrations (40%, 60%, and 80%) of sugar syrup + water as control group, II) Comparing three concentrations (0.02%, 0.06%, and 0.12% (equivalent to 10, 30, and 60 µL / 50 mL of 60% sugar syrup) of vitamins + 60% sugar syrup only as control group, and III) Comparing three concentrations (0.02%, 0.06%, and 0.12% (equivalent to 10, 30, and 60 µL / 50 mL of 60% sugar syrup) of amino acids/vitamins mixture + 60% sugar syrup only as control group. The concentration of 60% sugar syrup was selected as the solvent for vitamins and amino acids/vitamins mixture because preliminary sucrose experiments showed that 60% provided the highest cold stress tolerance. Three parameters were used to evaluate the ability of honey bees to withstand cold stress conditions: time until narcosis, time until recovery, and survival after recovery. Bees were exposed to 4°C until narcosis and then transferred to room temperature (25 ± 1 °C) for recovery. The results showed that bees fed on 60% sugar syrup performed better in terms of the measured parameters compared to other concentrations or the control group. Supplementation of sugar syrup with vitamins had an impact on the measured parameters, showing superior results in the vitamin 0.06% (30 µL/50 mL) group. Regarding the amino acids/vitamins mixture, some improvements in the ability of honey bees to tolerate cold stress conditions were recorded. This study provides insights into the effects of nutrition on honey bees' tolerance to low-temperature stress, laying the groundwork for further research.**

**Key Words: Nutrition, Cold, Stress, Honey bees, Tolerance**

## ÖZ

**Arılar (*Apis mellifera*), genellikle sonbahar ve kış aylarında kısa süreli soğuk stresi deneyimleyebilirler. Bu sırada arıcılar genellikle kolonilerini yapay besinlerle desteklerler. Bu uygulama bal arılarını diyetlerinde değişen şeker konsantrasyonları, vitaminler ve amino asitlere maruz bırakabilir. Bu nedenle, bu çalışma bu besin bileşenlerinin bal arılarının laboratuvar ortamında soğuk stresle başa çıkma yeteneklerini nasıl etkileyebileceğini araştırmaktadır. Bu çalışma üç alt deneyi içermektedir: I)**

**Şeker şurubunun üç konsantrasyonunun (40%, 60%,ve 80%) su kontrol grubu ile karşılaştırılması, II) Vitaminler üç konsantrasyonunun (10 µL, 30 µL ve 60 µL / 50 µL %60 şeker şurubu, sırasıyla %0,02, %0,06 ve %0,12) %60 şeker şurubukontrol grubu ile karşılaştırılması, ve III) Amino asitler / vitamin karışımının üç konsantrasyonunun (10 µL, 30 µL ve 60 µL / 50 µL %60 şeker şurubu, sırasıyla %0,02, %0,06 ve %0,12) %60 şeker şurubu seçilmiştir çünkü ön deneylerde farklı sakkaroz konsantrasyonları karşılaştırıldığında %60, soğuk stres toleransında en yüksek performansı göstermiştir. Bal arılarının soğuk stres koşullarına dayanma yeteneğini değerlendirmek için üç parametre olarak; narkoz süresi, iyileşme süresi ve iyileşme sonrası hayatta kalma kulanılmıştır. Arılar 4°C'de narkoz olana kadar tutulmuş ve ardından iyileşme için laboratuvar sıcaklığına (25±1°C) aktarılmıştır. Sonuçlar, %60 şeker şurubu ile beslenen arıların diğer konsantrasyonlara veya kontrol grubuna göre ölçülen parametreler açısından daha iyi performans gösterdiğini ortaya koymuştur. Şeker şurubu vitamin takviyesi, ölçülen parametreler üzerine etkili olmuş ve 30 µL, / 50 µL (%60) vitamin grubu en iyi sonuçları göstermiştir. Amino asitler / vitamin karışımı ile ilgili olarak, bal arılarının soğuk stres koşullarını tolere etme yeteneklerinde bazı iyileşmeler kaydedilmiştir. Bu çalışma , baslenmenin bal arılarının düşük sıcaklık stresine karşı toleransı üzerindeki etkilerine dair öngörüler sunarak ileri araştırmalar için altyapı oluşturmaktadır.**

**Anahtar Kelimeler: Beslenme, Soğuk, Stres, Bal arıları, Tolerans**

## GENİŞLETİLMİŞ ÖZET

**Giriş:** Bal arıları, *Apis mellifera*, özellikle sonbahar ve kış aylarında kısa süreli soğuk stresi deneyimleyebilirler. Bu sırada, arıcılar yeterli nektar kaynağı bulunmadığı dönemlerde arı kolonilerine şeker şurubu sağlarlar. Aynı zamanda, arıcılar kolonilerine hayatta kalabilmeleri için özellikle yetersizlik dönemlerinde soğuk stresle karşılaşabilecekleri zamanlarda vitaminler veya amino asitler/vitamin karışımı ekleyebilirler. Şeker konsantrasyonunun, amino asitlerin ve vitaminlerin, soğuk stres altında dolaşıcı arıların hayatta kalma yeteneğindeki rolüne dair çalışmalar hala sınırlıdır ve odaklanmış deneyler gerektiren önemli bir bilgi boşluğunu temsil etmektedir.

**Amaç:** Bu çalışma, bal arılarının soğuk stres koşulları altında hayatta kalma yeteneği üzerinde farklı şeker şurubu konsantrasyonlarının etkilerini araştırmaktadır. Ayrıca, vitaminlerin ve amino asitlerin kaynakları olarak hizmet eden ticari ürünler bu bağlamda test edilmiştir. Bu çalışma, bal arısı beslenmesine ilişkin daha fazla içgörü sunmakta ve arıcılar tarafından sağlanan yapay beslemenin bal arılarının soğuk stres koşullarına dayanma yeteneğini nasıl etkileyebileceğini anlamada yardımcı olabilir.

**Yöntem:** Bu çalışma üç alt deneyi içermiştir: I) Kontrol grubu ile karşılaştırılan üç konsantrasyon (40%, 60% ve 80%) şeker şurubu (su kontrol grubu ile), II) Kontrol grubu ile karşılaştırılan üç konsantrasyon (10 µL, 30 µL ve 60 µL / 50 mL %60 şeker şurubu, sırasıyla %0.02, %0.06 ve %0.12) (%60 şeker şurubu kontrol grubu ile) vitamin, ve III) Kontrol grubu ile karşılaştırılan üç konsantrasyon (10 µL, 30 µL ve 60 µL / 50 mL %60 şeker şurubu, sırasıyla %0.02, %0.06 ve %0.12) ( 60% şeker şurubu kontrol grubu ile) amino asitler/vitamin karışımı olarak kullanılmıştır. Vitaminler ve amino asitler/vitamin karışımı %60 şeker şurubunda çözülmüştür, bu konsantrasyon ise ön deneylerde farklı sakkaroz konsantrasyonları (40%, 60%, 80%) karşılaştırıldığında soğuk stres toleransı açısından en iyi performansı göstermesi nedeniyle seçilmiştir. Bal arılarının soğuk stres koşullarına dayanma yeteneğini değerlendirmek için üç parametre kullanılmıştır: narkoz süresi, iyileşme süresi ve iyileşme sonrası hayatta kalma. Arılar 4°C'de narkoz olana kadar tutulmuş ve ardından iyileşme için laboratuvar sıcaklığına (25 ± 1 °C) aktarılmıştır.

**Bulgular:** Şeker beslenmesi ile ilgili olarak, düşük (40%) ve yüksek (80%) konsantrasyonlar, orta 60% konsantrasyonunun aksine soğuk stres koşullarına maruz kalan bal arılarının hayatta kalmasını desteklemedi. 60% şeker şurubu ile beslenen işçi arılar, diğer test gruplarına göre en uzun narkoz süresine ve iyileşme süresine sahiptir. Şeker şurubuna vitamin eklenmesi durumunda, 30 µL / 50 mL (%0.06) vitamin grubu, düşük 10 µL / 50 mL (%0.02) ve yüksek 60 µL / 50 mL (%0.12)miktarlardan, ayrıca sadece şeker şurubundan daha uzun narkoz ve iyileşme süresine olumlu etkisi olmuştur. Ayrıca, bal arılarının 72 saatlik bir dönemde hayatta kalma oranı, vitamin

gruplarında kontrol grubuna kıyasla daha yüksektir. Vitaminlere amino asitler eklenmesi, 60 µL / 50 mL (%0.12) amino asit/vitamin karışımı içeren şeker şurubu ile beslenenlerde narkoz süresinde bir iyileşme göstermiştir. Ancak, bu karışım, kontrol grubuna kıyasla iyileşme süresini artırmamıştır. 72 saatlik bir süre içinde ölen arı sayısı, amino asit/vitamin karışımı gruplarında kontrol grubuna kıyasla iyileşme sonrası bal arılarının hayatta kalma yeteneğini artırmamıştır.

**Sonuç:** Laboratuvar ortamlarında, %60 şeker şurubu, düşük (40%) veya yüksek (80%) şeker konsantrasyonlarına kıyasla arıları desteklemede daha etkilidir. Vitaminler, arıların soğuk stres koşullarına dayanma yeteneklerini artırabileceğini göstermiştir, ancak bu konuda daha fazla çalışma gerektiren uygun bir sınır bulunmaktadır. Belirli amino asitlerin, vitaminlerle birlikte, bal arılarının soğuk stres koşullarına dayanma yeteneklerini artırmak için gerekli olmayabileceği sonucuna varılmıştır. Bu çalışma, beslenmenin bal arılarının düşük sıcaklık stresine karşı toleransı üzerindeki etkilerine dair içgörüler sunarak ileri çalışmalar için altyapı oluşturmaktadır.

## INTRODUCTION

The role of honey bees, *Apis mellifera*, as the primary plant pollinator is well established (Hung et al. 2018). Thanks to their vital role, honey bees make significant contributions to the global agricultural economy, underscoring the need for their protection (Halvorson et al. 2021, Marnasidis et al. 2021, Porto et al. 2020). Honey bees are crucial not only for plant pollination and agricultural production but also for their valuable products derived from bee colonies, which have been utilized for numerous nutritional and medicinal purposes (Durazzo et al. 2021, Giampieri et al. 2022, Martinello and Mutinelli 2021). This highlights the importance of beekeeping as a significant agricultural activity (Etxegarai-Legarreta and Sanchez-Famoso 2022, Prodanović et al. 2024). The health of honeybees is a major concern, as it affects their ability to pollinate plants and produce sufficient bee products. There are various environmental stressors that can have adverse effects on honey bees and their crucial roles (Abou-Shaara 2024, Lin et al. 2024, Minaud et al. 2024).

It is well known that nutrition is important for honey bee colonies. Honey bees forage on flowers to collect nectar and pollen, which are the primary food sources for colonies, in addition to collecting water and plant resins (Abou-Shaara 2014). These tasks are carried out outside the colonies, exposing forager bees to various stressors (dos Santos Araújo et al. 2023, Goulson and Nicholls 2022, Ward et al. 2022). Cold stress can affect colonies during late autumn and winter due to reduced hive temperature, while foragers are particularly exposed to low temperatures during foraging in transitional periods (Abou-Shaara et al. 2017). Given the issue of climate change and potential temperature shifts, stress from temperature extremes is considered a significant concern (Ali et al. 2023, de Jongh et al. 2022, Neumann and Straub 2023).

In addition to cold stress, honey bees can be exposed to various other stressors. Given that nectar provides the fuel for forager bees to fly and survive under environmental stressors (Abou-Shaara 2014), the variations in food components are crucial. This includes variations in nectar sugar content (Nicolson 2022, Yessoufou 2023, Venjakob et al. 2022). Additionally, nectar contains several other nutrients such as amino acids and vitamins (Nicolson 2022, Venjakob et al. 2022). Beekeepers provide bee colonies with sugar syrup during periods when not enough nectar sources are available. Additionally, beekeepers may supplement their colonies with vitamins or a mixture of amino acids and vitamins to support their survival, especially during periods of dearth when exposure to cold stress is more likely. Studies on the role of sugar concentration, amino acids, and vitamins in the ability of forager bees to survive under cold stress are still limited, representing a significant knowledge gap that warrants further investigation through focused experiments. Therefore, this study examines the impact of varying sugar syrup concentrations on honey bees' ability to survive under cold stress conditions. Additionally, commercially available products serving as sources of vitamins and amino acids were tested in this context. This study provides further insights into honey bee feeding and can help in understanding how artificial feeding provided by beekeepers affects the ability of honey bees to withstand cold stress conditions.

## MATERIALS AND METHODS

### Honey bees

Hives housing hybrid Carniolan honey bees located

in El-Behera governorate, Egypt, were used in the study. Such hybrids are common in Egypt (Ahmed et al. 2025). Forager honey bees were captured in front of the hive entrance and placed directly in Falcon tubes equipped with half of the Eppendorf tubes at each end of the tube (Figure 1). One end of the tube was fitted with a cotton piece to deliver the treatment (sugar syrup, vitamins, or amino acids/vitamins mixture), typically equal to 1.5 mL of the test treatment. In each tube, 10 bees were added, and a total of 6 tubes were used per test group, as described in the following laboratory experiments. The number of worker bees per tube was selected to facilitate the observation of bee behaviors within the tube. Foragers were deliberately chosen because they are the worker group most exposed to cold stress and other environmental challenges, making them the most relevant for evaluating nutritional supplementation. Although variability in age and internal reserves may exist, this limitation was minimized through simultaneous collection, sufficient replication (six tubes with ten bees each per treatment), and the inclusion of a control group.

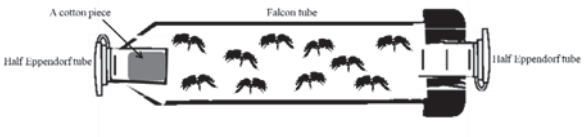


**Figure 1.** The modified Falcon tubes were used in the experiments. Each Falcon tube was equipped with half of an Eppendorf tube at each end. One end of the tube was fitted with a cotton piece to deliver the treatment. In each tube, 10 forager bees were placed.

**Sugar concentrations**

Three sugar syrup concentrations were tested: 40%, 60%, and 80%. To prepare these concentrations, table sugar (sucrose) was dissolved in water and mixed thoroughly using a hand mixer. This process mimics the artificial feeding of honey bees with sugar syrup, a common practice among beekeepers. The control group was supplied with water only, without any sugar.

**Vitamins**

A vitamin mixture commercially available from a local pharmaceutical company (Industries 10th Ramadan, Egypt) was used at concentrations of 0.02%, 0.06%, and 0.12% (equivalent to 10, 30, and 60 µL/50 mL), dissolved in 50 mL of 60% sugar syrup. The choice of 60% sugar syrup as a solvent was based on the results of the first sub-experiment, in which this concentration showed superior performance in survival, time until narcosis, and recovery compared to 40% or 80%. The vitamin mixture contained vitamin A (5,000,000 IU), vitamin D3 (1,000,000 IU), vitamin E (5,000 mg), and vitamin C (5,000 mg), along with propylene glycol (50,000 mg), dissolved in 100 mL of distilled water. This mixture was selected for its availability to beekeepers for the artificial feeding of honey bees. The control group was supplied with 60% sugar syrup without any vitamins.

**Amino acids/vitamins mixture**

An amino acid and vitamin mixture available commercially from a local pharmaceutical company (Industries 10th Ramadan, Egypt) was used at concentrations of 0.02%, 0.06%, and 0.12% (equivalent to 10, 30, and 60 µL / 50 mL) dissolved in 50 mL of 60% sugar syrup, which proved to be the most effective concentration for supporting cold-stress tolerance compared to 40% and 80%**.** The mixture contained vitamin A (1,000,000 IU), vitamin D3 (200,000 IU), vitamin E (9,600 mg), vitamin C (49,500 mg), methionine (9900 mg), lysine (3,803 mg), sorbitol (50,000 mg), along with propylene glycol (50,000 mg) dissolved in 1,000 mL of distilled water. This mixture was chosen for its availability to beekeepers for honey bee artificial feeding. The control group was supplied with 60% sugar syrup, devoid of any amino acid or vitamin mixture.

**Evaluation parameters**

**Time until narcosis and time until recovery:** For each treatment, 6 tubes were used, totaling 60 bees. After collecting the bees inside the tubes, they were left without food for 6 hours. Subsequently, the bees were provided with 1.5 mL of food per tube and allowed to feed on the treatments for 2 hours. Providing an equal volume of syrup to each tube ensured equal feeding opportunities across all treatments, even though exact consumption values were not recorded. Following this, the bees were collectively exposed inside the Falcon tubes to a low temperature of 4°C until narcosis, and the time taken for this state to occur was recorded (the time until narcosis, or TUN). Humidity was not precisely monitored, but conditions were consistent across all tubes. Six tubes, each containing ten bees, were used per treatment to minimize random variation. Then, the bees were placed at room temperature (around 25°C ± 1°C) until they had fully recovered, as evidenced by full-body movement, and the time

required for recovery was recorded (the time until recovery, or TUR). This experiment was conducted according to previously established protocols for the cold tolerance of honey bees (Abou-Shaara 2017,Abou-Shaara et al. 2023).

**Survival after recovery:** Following the previous exposure procedure, the survival of bees after recovery from cold stress at 4˚C was tracked over 24, 48, and 72 hours. The numbers of dead bees were compared among treatments.

### Statistical analysis

The normality of the data was evaluated through the Shapiro-Wilk test. Subsequently, parametric tests were employed to analyze the time until narcosis and time until recovery, utilizing ANOVA followed by Tukey's test to compare means. To evaluate survival after recovery, Kaplan-Meier survival analysis was performed, with mean comparisons made using the Log Rank statistical test. The statistical analysis was conducted using SPSS version 25, with a significance level set at $p \leq 0.05$.

## RESULTS

### Sugar concentrations

**Time until narcosis (TUN):** Workers fed on 60% sugar syrup showed the longest TUN, ranging from 2675 to 2812 seconds (44.58 to 46.86 minutes) with a mean of 2735.83 seconds (45.59 minutes). This was followed by those fed on 80% sugar syrup, with a range from 1753 to 1891 seconds (29.21 to 31.51 minutes) and a mean of 1839 seconds (30.65 minutes). Next were those fed on 40% sugar syrup, with a range from 962 to 1080 seconds (16.03 to 18 minutes) and a mean of 1033 seconds (17.21 minutes). The control group had a range from 427 to 569 seconds (7.11 to 9.48 minutes) with a mean of 498 seconds (8.3 minutes), as shown in Figure 2. Significant differences were detected between the sugar concentration treatments and the control group (df=3, F=1905.92, p<0.05). The group fed on 60% sugar syrup exhibited significantly higher TUN values compared to the other treatments and the control group (Figure 2).

**Time until recovery (TUR)**: Worker bees in the 60% sugar syrup group exhibited the shortest time needed for recovery, ranging from 125 to 172 seconds (2.08 to 2.86 minutes) with a mean of 157.33 seconds (2.62 minutes). This was followed by those fed on 80% sugar syrup, with a range from 246 to 289 seconds (4.1 to 4.81 minutes) and a mean of 266.33 seconds (4.43 minutes). Next were those fed on 40% sugar syrup, with a range from 349 to 400 seconds (5.81 to 6.66 minutes) and a mean of 373.67 seconds (6.22 minutes). The control group had a range from 477 to 578 seconds (7.95 to 9.63 minutes) with a mean of 520.17 seconds (8.66 minutes), as shown in Figures 3. Significant differences were observed among the treatments and the control group (df=3, F=221.80, p<0.05). The 60% group was identified as the group with the lowest TUR, significantly compared to all the other treatments and the control group, as depicted in Figure 3.

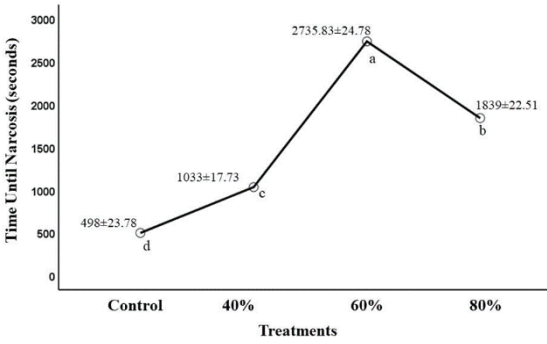


**Figure 2**. Means ± SE for the time until narcosis (seconds) of honey bee workers exposed to low-temperature stress after feeding on different sugar syrup concentrations (40%, 60%, and 80%) and a control group (water). Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N = 6).

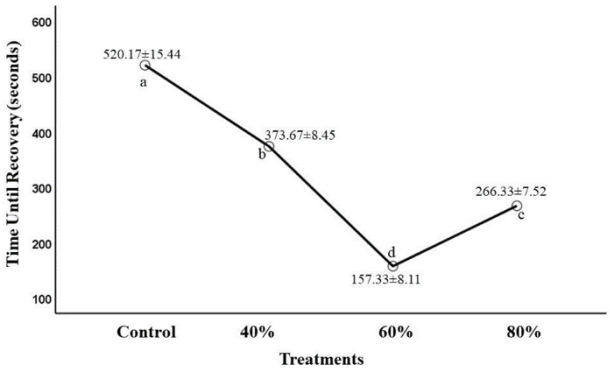


**Figure 3.** Means ± SE for time until recovery (seconds) of honey bee workers exposed to low-temperature stress after feeding on different sugar syrup concentrations (40%, 60%, and 80%), and a control group (water). Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N=6).

**Survival after recovery:** At 24 hours, significant differences were detected among all treatments,

with the highest number of deaths recorded in the control group, followed by the 40% sugar syrup group, then the 80% sugar syrup group, and finally the 60% sugar syrup group (Means± SE were 9.67±0.21, 4.83±0.30, 0.17±0.16, and 02.50±0.22 bees for control, 40%, 60%, and 80% sugar syrup, respectively). The same pattern of significance and differences in death numbers was observed at 48 hours, with an increase in the number of deceased bees across all groups (Means± SE were 10.00±0.00, 6.67±0.33, 2.00±0.36, and 4.67±0.71 bees for control, 40%, 60%, and 80% sugar syrup, respectively). By 72 hours, the number of dead bees had risen in all groups, with the control group showing significantly higher death rates than all other groups (Means± SE were 10.00±0.00, 7.33±0.21, 4.50±0.22, and 6.00±0.36 bees for control, 40%, 60%, and 80% sugar syrup, respectively) (Figure 4). Throughout the three time points, the 60% sugar syrup group consistently had the lowest number of deceased bees. The groups can be ranked in terms of descending death numbers as follows: control group, 40% sugar syrup, 80% sugar syrup, and finally 60% sugar syrup.

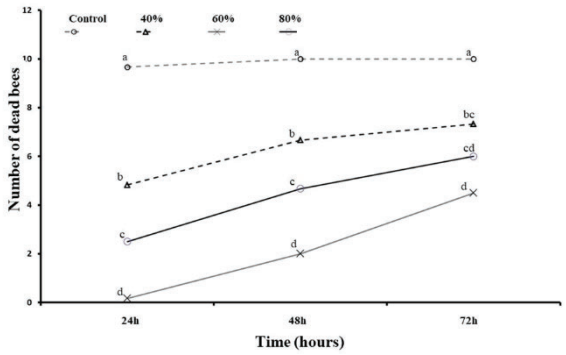


**Figure 4.** Variations in the mean number of deceased bees among honey bee workers exposed to low-temperature stress after consuming different sugar syrup concentrations (40%, 60%, and 80%), as well as a control group (water). At each time point, means followed by different letters indicate significant differences according to the Log Rank statistical test (95% significance level, N=6).

### Vitamins

**Time until narcosis (TUN):** Workers fed with 30 µL of vitamin exhibited the longest TUN, ranging from 4935 to 5071 seconds (82.25 to 84.51 minutes) with a mean of 5009.17 seconds (83.48 minutes). This was followed by those fed with 60 µL of vitamin, with a range from 4226 to 4327 seconds (70.43 to 72.11 minutes) and a mean of 4256.83 seconds (70.94 minutes). Next were those fed with 10 µL of vitamin, showing a range from 3570 to 3696 seconds (59.5 to 61.6 minutes) and a mean of 3624.83 seconds (60.41 minutes). The control group exhibited a range from 2668 to 2806 seconds (44.46 to 46.76 minutes) with a mean of 2718.83 seconds (45.31 minutes), as depicted in Figure 5. Significant differences were observed between the vitamin treatments and the control group (df=3, F=2671.64, p<0.05). Treatment with 30 µL showed significant variations compared to the other treatments (Figure 5).

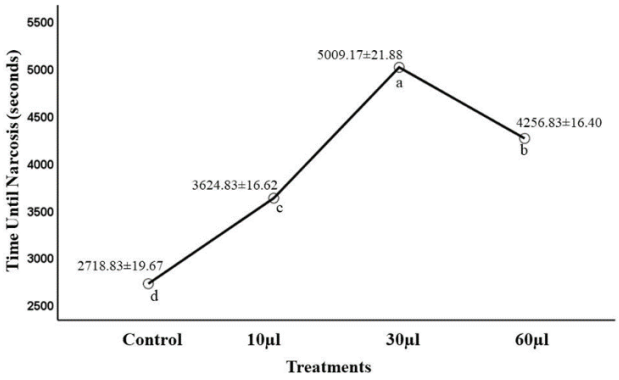


**Figure 5.** Means ± SE for the time until narcosis (seconds) of honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different vitamin concentrations (10 µL, 30 µL, and 60 µL), along with a control group without vitamin. Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N = 6).

**Time until recovery (TUR):** Worker bees in the vitamin 30 µL group exhibited the shortest time needed for recovery, ranging from 37 to 39 seconds (0.61 to 0.65 minutes) with a mean of 38.17 seconds (0.63 minutes). They were followed by those fed with vitamin 60 µL, with a range of 73 to 76 seconds (1.21 to 1.26 minutes) and a mean of 74.67 seconds (1.24 minutes), then those fed with vitamin 10 µL, showing a range of 102 to 104 seconds (1.7 to 1.73 minutes) with a mean of 103.17 seconds (1.71 minutes). Finally, the control group had a range of 167 to 169 seconds (2.78 to 2.81 minutes), with a mean of 167.83 seconds (2.79 minutes), as illustrated in Figure 6. Significant differences were observed among the vitamin treatments and between them and the control group (df = 3, F = 18173.82, p < 0.05), as depicted in Figure 6.

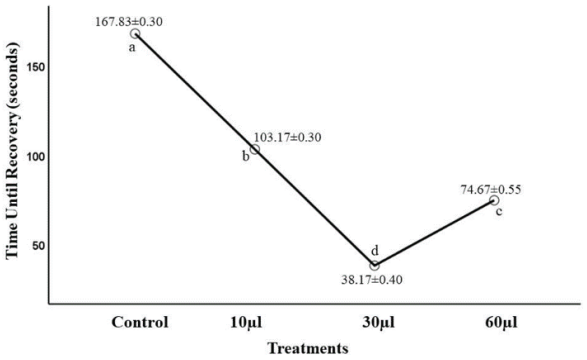


**Figure 6.** Means ± SE for the time until recovery (seconds) of honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different vitamin concentrations (10 µl, 30 µl, and 60 µl), and a control group without vitamin. Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N = 6).

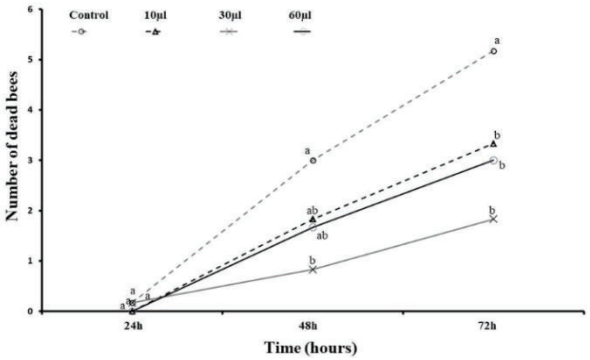


**Figure 7**. Variations in the mean number of dead bees among honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different vitamin concentrations (10µl, 30µl, and 60µl), as well as a control group without vitamins. At each time point, means followed by different letters indicate significant differences according to the Log Rank statistical test (95% significance level, N=6).

**Survival after recovery:** At 24 hours, no significant differences were detected among all treatments (Means± SE were 0.17±0.16, 0.00±0.00, 0.17±0.16, and 0.00±0.00 bees for control, 10µl, 30µl, and 60µl vitamin, respectively). By 48 hours, the vitamin treatment groups exhibited a lower number of dead bees compared to the control group, with no significant differences among the treatment groups except for the vitamin 30 µl group, which was significantly different from the control group (Means± SE were 3.00±0.51, 1.83±0.40, 0.83±0.40, and 1.67±0.33 bees for control, 10µl, 30µl, and 60µl vitamin, respectively). By 72 hours, the number of dead bees had increased in all groups, with the control group having the highest number of deaths, significantly more than all the other treatment groups (Means± SE were 5.17±0.30, 3.33±0.33, 1.83±0.16, and 3.00±0.25 bees for control, 10µl, 30µl, and 60µl vitamin, respectively) (Figure 7). The groups with the highest bee mortality can be ranked in descending order as follows: control group, vitamin 10 µl, vitamin 60 µl, and finally vitamin 30 µl.

### Amino acids/vitamins mixture

**Time until narcosis (TUN):** Workers fed on amino acids/vitamins mixture at 60 µl showed the longest TUN ranging from 4708 to 5740 seconds (78.46 to 95.66 minutes) with a mean of 5357.83 seconds (89.29 minutes), followed by those fed with amino acids/vitamins mixture at 30 µl with a range from 4688 to 4755 seconds (78.13 to 79.25 minutes) and a mean of 4726.33 seconds (78.77 minutes), then those fed with amino acids/vitamins mixture at 10 µl with a range from 3869 to 3920 seconds (64.48 to 65.33 minutes) and a mean of 3895.17 seconds (64.91 minutes), and finally, the control group with a range from 2614 to 2727 seconds (43.56 to 45.45 minutes) and a mean of 2688.33 seconds (44.80 minutes), as shown in Figure 8. Significant differences were detected between the amino acid treatments and the control group ($df=3$, $F=125.37$, $p<0.05$), and the treatment at 60 µl showed significant variations compared to the rest of the treatments (Figure 8).

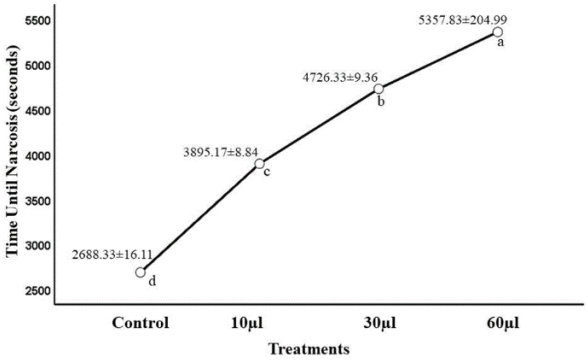


**Figure 8**. Means ± SE for the time until narcosis (seconds) of honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different amino acid/vitamin concentrations (10µl, 30µl, and 60µl), and a control group without amino acids. Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N=6).

**Time until recovery (TUR):** In contrast to the results for TUN, worker bees in the control group showed the shortest time needed for recovery, ranging from 107 to 167 seconds (1.78 to 2.78 minutes) with a mean of 149.50 seconds (2.49 minutes), followed by those fed with amino acids/vitamins mixture at 10 µl with a range from 306 to 354 seconds (5.1 to 5.9 minutes) and a mean of 327.50 seconds (5.45 minutes), then those fed with amino acids/vitamins mixture at 30 µl with a range from 431 to 472 seconds (7.18 to 7.86 minutes) and a mean of 457.33 seconds (7.62 minutes), and finally, those fed with amino acids/vitamins mixture at 60 µl with a range from 608 to 649 seconds (10.13 to 10.81 minutes) and a mean of 623.50 seconds (10.39 minutes) as shown in Figure 9. Significant differences were detected among the amino acid/vitamin treatments as well as between them and the control group (df=3, F=709.98, p<0.05), as shown in Figure 9.

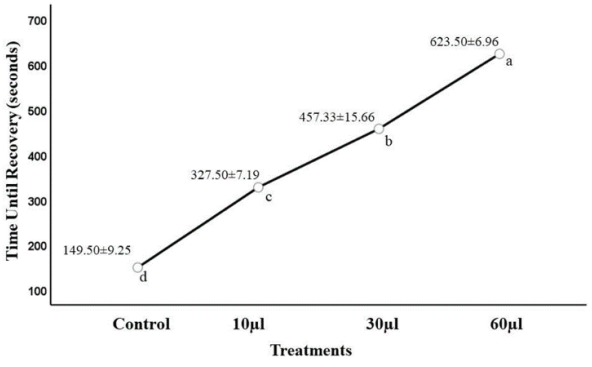


**Figure 9**. Means ± SE for the time until recovery (seconds) of honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different amino acid/vitamin concentrations (10µl, 30µl, and 60µl), and a control group without amino acids. Means followed by different letters indicate significant differences according to the Tukey test (95% significance level, N=6).

**Survival after recovery:** At 24 hours, no significant differences were detected among all treatments (Means± SE were 0.33±0.33, 0.00±0.00, 0.00±0.00, and 0.50±0.34 bees for control, 10µl, 30µl, and 60µl amino acids/vitamins mixture, respectively). At 48 hours, the amino acid/vitamin treatment groups showed a higher number of dead bees compared to the control group, with no significant differences among the amino acid/vitamin groups except for amino acid 60 µl, which was significantly different from the control group (Means± SE were 2.33±0.42, 2.83±0.30, 3.67±0.49, and 4.17±0.40 bees for control, 10µl, 30µl, and 60µl amino acids/vitamins mixture, respectively). By 72 hours, the number of dead bees had increased in all groups, with amino acids/vitamins mixture 30 µl and amino acids/vitamins mixture 60 µl having the highest numbers of deaths significantly higher than all other groups (Means± SE were 5.00±0.25, 5.50±0.56, 8.00±0.68, and 8.33±0.55 bees for control, 10µl, 30µl, and 60µl amino acids/vitamins mixture, respectively) (Figure 10). The groups with the highest bee mortality can be arranged in descending order as amino acids/vitamins mixture 60 µl, amino acids/vitamins mixture 30 µl, amino acids/vitamins mixture 10 µl, and finally the control group.

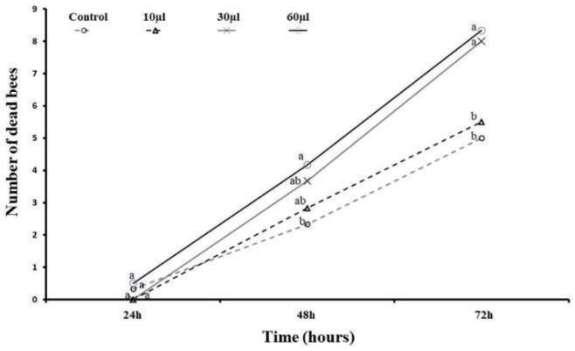


Figure 10. Variations in the mean number of dead honey bee workers exposed to low-temperature stress after feeding on sugar syrup mixed with different amino acid/vitamin concentrations (10 µl, 30 µl, and 60 µl), and a control group without amino acids. At each time point, means followed by different letters denote significant differences according to the Log-Rank statistical test (95% significance level, N=6).

## DISCUSSION

Regarding sugar feeding, low (40%) and high (80%) concentrations did not support the survival of honey bees under cold-stress conditions, unlike the medium concentration of 60%. Workers fed on 60% sugar syrup also showed the longest time until narcosis and the shortest recovery time compared to the other test groups. This can be explained by the ability of caged bees to utilize this medium concentration more easily than low and high concentrations. While a low concentration is not a sufficient source of energy, a concentration of 60% is better and can fulfill the energy requirements of a bee's body. It can be hypothesized that although an 80% sugar syrup may provide more energy than a 60% concentration, its higher viscosity and reduced ingestion capacity could limit its effective utilization by honey bee workers compared to the 60% concentration. Previous studies on sugar feeding of honey bees have shown that sugar syrup yields better results than honey or other alternatives in terms of supporting tolerance to cold-stress conditions (Abou-Shaara 2017). However, it appears that studies on sugar concentration are still insufficient to fully understand the effects on cold-stress tolerance in honey bees. This finding can help in understanding the effects of sugar concentrations on the foraging performance of animals. Consistent with this, the decreased viscosity of artificial nectar has been shown to boost bees' intake rates (Shi et al. 2020). Moreover, augmenting the quantity of nectar or syrup stored in their crops for flight fuel can have a detrimental effect on the pollen weight carried by pollen foragers, with a 60% sugar solution proving more effective than a 30% sugar solution for this purpose (Harano 2020). It seems that 60% sugar syrup can be considered a good fuel for forager bees. In line with this, the highly concentrated syrup or sugar candy presented to bee colonies is diluted by the worker bees before they can utilize it.

The addition of amino acids to vitamins has shown an improvement in the time until narcosis for those fed a sugar syrup containing a 60 µl amino acid/vitamin mixture. However, this mixture did not enhance recovery time at any concentration compared to the control group. This suggests that the use of liquid amino acids may be less important in improving cold-stress tolerance in honey bees, as vitamins alone appear to be sufficient. The number of deceased bees increased in the amino acids/vitamins mixture groups over 72 hours without enhancing the survival ability of honey bees post-recovery compared to the control group. This reinforces the notion that the tested amino acids have not been effective in enhancing the cold tolerance of honey bees. It is possible that the type and form of amino acids used have influenced the bees' ability to utilize these components to bolster their resilience to cold conditions. Studies have shown that foragers are more attracted to flowers with higher amino acid concentrations compared to those with lower amounts (Kavitha and Reddy 2019, Seo et al. 2019). Bees have the ability to discern variations in amino and fatty acid concentrations (Ruedenauer et al. 2021). However, protein concentrations do not seem to be the primary attractant for pollen foragers (Pernal and Curie 2001, Roulston et al. 2000). A deficiency in essential dietary amino acids can lead to an increase in pollen collection (Bonoan et al. 2020). The understanding of the role of amino acids in bolstering cold tolerance in honey bees remains incomplete, and more detailed studies are necessary to shed light on this topic.

## Conclusion

The current research investigates the effects of different sugar concentrations, vitamins, and amino acids on the tolerance ability of honey bees to low-temperature stress. Sugar concentrations play a crucial role in providing honey bees with the necessary energy to withstand cold stress. A 60% sugar syrup is more efficient in supporting bees under laboratory settings compared to low (40%) or high (80%) sugar concentrations. Vitamins have been shown to enhance bees' ability to withstand cold-stress conditions, but a suitable limit requires further study under field conditions. The presence of specific amino acids, in combination with vitamins, may not be essential for boosting the ability of honey bees to withstand cold-stress conditions. This aspect of the study emphasizes that the proper addition of sugar and vitamins in feeding is necessary for adult worker bees during late autumn and the winter period, rather than supplementing protein sources to withstand cold-stress periods. Further studies on the effects of other forms of amino acid sources, such as pollen or its alternatives, as well as liquid amino acids, are necessary for a detailed analysis.

**Author contribution**: Heba I. ELGEBALY (performed the study, data analysis, writing, revision), Atef M.K. NASSAR (designed the study, writing, revision, and supervision), Adnan A.E. DARWISH (designed the study, writing, revision,

and supervision), and Hossam F. ABOU-SHAARA (designed the study, data analysis, writing, revision, and supervision). All authors read and approved the final version of the manuscript.

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**Data availability**: All data that support the study are included in the manuscript

**Ethical issue:** Not applicable.

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