

Original Research

Beehive Microclimate Significantly Influences Colony Growth, Morphometric and Reproductive Traits of Honey Bee (*Apis mellifera* L.) Queens Reared During Winter

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Abstract

Winter poses critical challenges for honey bee colonies, leading to substantial losses. Beekeepers employ diverse strategies, emphasizing good wintering practices, but success varies by region and beekeeping techniques. This research explores how sun exposure affects the dynamics of honey bee colonies and queen-rearing success in late winter. A comparison between shaded colonies and unshaded ones during the winter season was performed to investigate colony behaviors, temperature changes inside and outside the brood nest, royal jelly (RJ)/queen cell, and areas of wax and RJ glands of workers, as well as queen rearing and the quality of the produced queens. The investigations reflected that shaded colonies had lower outside and inside brood nest temperatures than the unshaded colonies, showing a direct link between shading and brood nest temperature. Worker body weight and gland area differed between shaded and unshaded colonies, with workers from unshaded colonies displaying significantly greater body weights and significantly larger glandular areas. The unshaded colonies showed larger sealed brood and stored pollen areas, suggesting that sunlight exposure can affect brood development and foraging activity. Compared with the shaded colonies, the unshaded colonies were significantly surpassed in acceptance rates, RJ production, queen size, and queen characteristics. It could be concluded that colony temperature affects colony growth, and removing shading during

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the winter season can be recommended to encourage the colonies to raise more brood and collect more food, thus increasing the colony's ability to produce high-quality queens.

Keywords: brood nest, honey bee, nest temperature, queen rearing, shading

Introduction

Honey bees play a crucial role in global agriculture through pollination and regional plant diversity [1-3]. Additionally, beehive products like honey, beeswax, royal jelly, and propolis play a role in promoting human health and nutrition, highlighting the multifaceted importance of honey bees in helping humans survive [4-7]. Nonetheless, a major challenge arises during the winter season, when colony losses are highest, and this is considered the most crucial period in a colony's life cycle [8]. Beekeepers have employed various management techniques for many years to reduce these losses, with a particular emphasis on the importance of effective wintering practices [9]. However, the winter survival of honey bee colonies is greatly influenced by where they are located and how they are managed by beekeepers [9]. Apiculturists have modified beehives and identified suitable apiary sites rich in nectar and pollen from major flora to assist honey bee colonies in adapting to fluctuations of factors to protect the colonies and enhance their longevity [10-13].

During the winter, honey bee colonies undergo major transformations to withstand cold temperatures and limited food supplies [9, 14]. Unlike some hibernating animals, honey bees remain active by relying on complex behavioral and physiological adaptations to withstand harsh conditions [15]. During the winter, the survival of worker bees in the hive depends upon establishing a thermal cluster. This cluster serves as a shared source of warmth, generated by the bees' metabolic activity and fueled by their stored honey reserves [16]. The bees regulate the temperature within the cluster within a critical range of 32°C to 36°C, which is important for brood development and overall colony health [17]. Nevertheless, changes in external temperature can disrupt the colony's delicate thermoregulation process, presenting a major obstacle to colonies surviving throughout the winter [18]. Such disturbances can affect brood development and colony survival, underscoring the need for adaptive responses to environmental changes [16].

The queen bee is identified as a critical factor in determining the winter resilience and productivity of the colony [19]. Energetic queens, known for their strong ability to reproduce, enhance the survival and expansion of colonies [20, 21]. Beekeepers understand the importance of the queen bee and regularly focus on replacing older queens with young and productive ones to support the colony's strength [22].

In traditional beekeeping regions such as Egypt, queen rearing is crucial for enhancing and sustaining

the beekeeping industry. A variety of factors influence the quality of queens produced through these methods, including environmental conditions, availability of nectar and pollen flora, colony strength, age of comb, and rearing techniques [23-25]. Furthermore, morphometric characteristics are reliable markers of queen quality and provide valuable information about reproductive capacity and colony success [26, 27]. By leveraging scientific principles and empirical data, beekeepers can make informed decisions to optimize colony health and resilience in the face of winter challenges. Honey bee survival over the winter involves a complex interaction between physiological adaptations, environmental factors, and beekeeping practices [25, 28, 29]. By conducting comprehensive scientific research and implementing evidence-based management approaches, beekeepers can maintain the viability and strength of honey bee colonies, protecting their essential role in ecosystems and agricultural sustainability.

The number of lost bee colonies dramatically increases during the winter. A large number of bee nuclei are produced in late winter and early spring to compensate for this loss, and queen rearing is needed for these nuclei [25]. Our research aims to explore the complex relationship between wintering techniques, worker thermoregulation, colony growth, and queen rearing in honey bee colonies. By scrutinizing a spectrum of colony activities, external temperature changes, worker attributes, and morphometric aspects of queen bees during the late winter period, we seek to elucidate the multifaceted factors influencing the queen-rearing process. Through this comprehensive investigation, we aim to shed light on the mechanisms underlying successful queen production amid the challenges posed by winter conditions and provide valuable insights into beekeeping practices and colony management strategies.

Materials and Methods

Experiment Area

The trials were conducted at the apiary of the Faculty of Agriculture (30°56'45"E, 31°6'42"N), Kafrelsheikh University, Egypt, during winter 2022/2023. Twenty-one hybrid Carniolan honey bee colonies (each with 7 combs) were chosen, each led by recently mated sister queens. All colonies possessed equal strength and had equal food storage. The colonies were randomly divided into 2 groups containing 10 colonies each, and one colony was subsequently used as a larval donor

(breeder colony). Ten colonies were placed in a shaded area, whereas the other 10 colonies were placed in an unshaded location in the apiary area.

Temperature Measurements

Each colony was provided with an Electrotherm digital thermometer with sensors designed to gauge both indoor and outdoor temperatures. The sensors were placed inside and outside the brood nest to measure temperatures, with a digital thermometer tracking the ambient temperatures outside the colony. Temperature changes in thermoregulation were assessed weekly for each colony.

Activities of Honey Bee Colonies

Measurements of colony activity were performed from winter 2022/2023. The number of incoming bees and incoming bees with pollen loads to a colony within 1 minute were counted. Counts were conducted weekly at 1200-1300 h. The area (inches²) of stored pollen and worker and drone-sealed brood was measured at 12-day intervals utilizing a specialized empty standard frame divided into inches². The number of combs covered with bees was counted to identify the colony population size. According to Taha [30], bees covering the 2 sides of a comb equals 2000 bees.

Measurement of Worker Characteristics

Ten nurse workers were collected from the queen cell bar from each colony, and each individual was weighed and then preserved in Bouin's fluid to maintain structural integrity. Following this, the glandular structures were carefully dissected in order to remove the mandibular gland, acini in the hypopharyngeal glands, and the second wax mirror area. Sophisticated imaging technology, such as a digital microscope, was used to take precise pictures of these anatomical characteristics. These pictures were later analyzed with Image J 1.46 software, allowing for accurate measurement of glandular areas.

Queen Rearing and Measurements

By 7th March 2023, the colonies were prepared for queen rearing. The method of queen rearing described by Laidlaw and Page [31] was employed. Mated queens were removed from the builder colonies 24 h before grafting to create queenless builder colonies. 24-h-old larvae originating from one breeding colony were wet-grafted into wax cups fixed on the cell-bar frame. Each frame contained 45 grafted queen cells. Five queen cells were randomly chosen from each replicate to harvest royal jelly (RJ) three days after grafting, which is the ideal harvest time [32, 33]. The larvae were removed from the queen cells, enabling the unrestricted gathering

of the RJ. Each sample was weighed to determine the exact amount of RJ (mg) per queen cell. The successful queen cells in each colony were recounted 10 days after grafting to estimate the number of ripped queen cells, which were then caged, and the number of emerging queens was recorded. Following the emergence of the queens, precise measurements were taken to evaluate the size of the queen cells. A digital caliper was used to measure the queen cell depth (mm); meanwhile, distilled water and a medical syringe were used to determine the queen cell volume by counting the amount of water used to fill the queen cell.

Ten newly emerged queens from each replicate were used to determine body weight, antenna length, area of the fore and hind wings, and the length and diameter of the abdomen. The measurements were carried out utilizing the scan photo technique (SPT). Detailed images were captured at 1200 dpi with high-resolution scanning and later analyzed with Adobe Photoshop CS5 software. The queens were delicately dissected using forceps to meticulously separate the ovary, spermatheca, and mandibular gland. Subsequently, these anatomical structures were carefully preserved in Bouin's fluid. The diameter of the spermatheca and the area of the mandibular gland were quantified using Image J 1.46 software, enabling geometric morphometric evaluation. The spermathecal volume was determined using the following formula:

$$\text{Size} = 4/3 \pi r^3$$

where $\pi = 3.14$ and $r = 1/2$ diameter of spermatheca.

The ovaries were submerged in xylene for ten minutes in order to eliminate any remaining tissues and waste materials. Afterward, they were completely rinsed with tap water to remove any leftover xylene and other pollutants. The ovaries were placed in a specific medium called Puris medium (10 ml distilled water, 5 ml glycerin, 3 ml glacial acetic acid, 70 g chloral hydrate, and 8 g Arabic gum). After 1 minute in the Puris medium, the ovaries were once again washed with tap water multiple times to remove any residual chemicals [23]. Following this preparation, the ovary's ovarioles became apparent and easily identifiable when viewed under a digital microscope. Using Image J 1.46 software, precise measurements of the length and width of the ovarioles were taken, giving important data for further analysis.

Statistical Analysis

The differences between shaded and unshaded colonies were tested using one-way analysis of variance (ANOVA). The normality of the data was tested by the Shapiro-Wilk normality test, which indicated the normal distribution of the data. Therefore, the original data were used for analysis. The ANOVA assessed differences between shaded and unshaded colonies tested via the PROC GLM function in SAS version 9.1 [34]. Tukey's

HSD post-hoc test was used to compare the treatment means.

Results and Discussion

Data presented in Table 1 display the average monthly temperatures, including ambient temperature, temperature outside the brood nest, and temperature inside the brood nest during the winter season. It was observed that the outside brood nest temperature of shaded colonies was lower than those in the unshaded colonies (20.86 vs. 22.28, 20.26 vs. 21.08, 21.85 vs. 23.08, and 22.4 vs. 23.4°C) during December, January, February, and March, respectively. Similarly, the brood nest temperature in shaded colonies was consistently lower than that of the unshaded ones (34.60 vs. 35.34, 33.64 vs. 35.22, 35.10 vs. 35.52, and 35.18 vs. 35.60°C) during the same period. The outside and inside brood nest temperatures of shaded colonies were lower than those in the unshaded colonies. These temperatures were within the range of the temperature (32°C to 36°C) within the cluster that has been regulated by bees and is important for brood development [16, 17]. These findings indicate that shading colonies resulted in reduced both inside and outside brood nest temperatures across the observed months. Furthermore, changes in ambient temperature influenced the temperature outside and within the brood nest. These highlight the dynamic relationship between external environmental conditions and internal nest temperatures, underscoring the importance of environmental factors in thermoregulation dynamics in honey bee colonies [16, 17].

Data in Table 2 display how direct exposure of a colony to sunlight during winter affects the number of forager bees and the number of pollen foragers. The unshaded colonies surpassed shaded colonies in the number of forager bees and pollen foragers (40.20 vs. 31.35 bees/colony/min and 11.65 vs. 9.40 bees/colony/min, respectively). Furthermore, the data revealed that the unshaded colonies stored larger pollen areas (339.00 inch²/colony) than the shaded colonies (324.98 inch²/colony). Also, unshaded colonies exhibited significantly larger sealed brood areas

for both workers (1604.00 inch²/colony) and drones (131.00 inch²/colony) compared to the shaded colonies (1251.10 inch²/colony for workers and 44.44 inch²/colony for drones). In addition, the population size in unshaded colonies at the end of winter was larger than that in shaded colonies (1554.00 vs. 13740.00 bees).

The biological activities within honey bee colonies were comprehensively assessed, encompassing various parameters, including foraging activity, stored pollen area, sealed brood areas of both workers and drones, and colony population size. The variations in the number of forager bees and the number of pollen foragers display how direct exposure of a colony to the sunlight affects them. At the initiation of the current study, all colonies were of the same strength and headed by young sister-mated queens, had combs of the same age, and were located in the same apiary, so the variables should have been the same except for shading. This suggests that sunlight exposure could impact pollen collection and storage in honey bee colonies and stored pollen areas during the different seasons. The current results confirm the findings of Taha et al. [35], Taha and Al-Kahtani [36], and Shawer et al. [37]. Also, the unshaded colonies exhibited significantly larger sealed brood areas for both workers and drones than the shaded colonies, where the sealed brood areas were noticeably smaller. The current findings are consistent with previous research highlighting the importance of environmental conditions in shaping brood development and pollen storage within honey bee colonies. Besides, Taha [15], Taha and Al-Kahtani [36], and Shawer et al. [37] have demonstrated that factors such as temperature, humidity, and light intensity can significantly influence brood rearing and pollen collection activities in honey bee colonies. They also reported that colonies exposed to favorable environmental conditions, including adequate sunlight and warmth, improved brood production and pollen foraging behavior. By elucidating these relationships, our study contributes to a deeper understanding of the ecological factors driving colony productivity. The large area of worker brood in a colony results in a large population size [38, 39]. Colony population size provides crucial insights into the overall health and vitality of the colonies and then productivity [9, 38].

Table 1. Average monthly ambient temperature, temperature outside the brood nest, and temperature inside the brood nest in shaded and unshaded colonies.

Date	Ambient temperature	Outside brood nest		Inside brood nest	
		Shaded colonies	Unshaded colonies	Shaded colonies	Unshaded colonies
December 2022	16.20±0.23	20.86±0.27	22.28±0.22**	34.60±0.23	35.34±0.23**
January 2023	16.00±0.26	20.26±0.25	21.08±0.21*	34.24±0.22	35.22±0.22**
February 2023	21.00±0.28	21.85±0.21	23.08±0.23**	35.10±0.23	35.52±0.22**
March 2023	22.00±0.23	22.40±0.26	23.40±0.25*	35.18±0.23	35.60±0.23**

Values are the mean±standard error. **P<0.01 between shaded and unshaded colonies, *P<0.05 between shaded and unshaded colonies.

Table 2. Impact of shading on colony activity, population size, body weight, wax mirror area, acini area, and mandibular gland area of worker honey bees.

Parameters	Shaded colonies	Unshaded colonies	Sig.
No. incoming bees/colony/min	31.35±0.90	40.20±0.77	**
No. pollen foragers/colony/min	14.10±0.87	17.65±0.87	*
Stored pollen area (inch ²)/colony	314.98±3.83	339.00±3.91	*
Worker sealed brood area (inch ²)/colony	1251.10±13.08	1604.00±13.08	**
Drone sealed brood area (inch ²)/colony	44.44±1.64	131.00±1.64	**
Colony population size	13740.00±112.35	1554.00±98.49	*
Worker body weight (mg)	86.66±1.99	101.50±0.79	**
Wax mirror area	2.43±0.041	2.93±0.094	**
Acini area	0.030±0.001	0.043±0.002	**
Mandibular gland area	1.11±0.031	1.39±0.019	**

Values are the mean±standard error. * and ** indicate $P<0.05$ and $P<0.01$, respectively.

Data in Table 2 provide a detailed comparison of worker body weight, wax, and RJ glands between colonies in sunny places and those in shaded places. Noticeably, the body weight of workers in unshaded colonies exhibited a significantly heavier weight (101.50 mg) compared to 86.66 mg in shaded colonies. In comparison with the shaded colonies, the unshaded colonies exhibited superiority in the areas of the wax mirror (2.43 vs. 2.93 mm²), acini of the hypopharyngeal gland (0.030 vs. 0.043 mm²), and mandibular gland (1.11 vs. 1.39 mm²). The body weight of workers from unshaded colonies exhibited a significant increase compared to the body weight observed in shaded colonies. The differences in body weight imply a potential influence of sun rays' exposure on worker development and colony growth. The worker's body weight reflects the colony's status, the age of combs where reared, and the availability of nectar and pollen sources [37, 39, 40].

Moreover, the glandular areas revealed intriguing differences between shaded and unshaded colonies. Compared with the shaded colonies, the unshaded colonies exhibited superiority in the areas of the wax mirror, acini of the hypopharyngeal gland, and mandibular gland. These findings suggest a potential correlation between shading, worker physiology, and glandular development. The worker's body weight and the area of these glands were in line, indicating that larger workers may possess increased glandular development and secretion capacities. These results confirm the findings of Shawer et al. [37]. This highlights the intricate interplay between environmental conditions and honey bee worker characteristics, offering valuable insights into colony dynamics and adaptation mechanisms. In this context, Taha [18], Taha and Al-Kahtani [36], Shawer et al. [37], and Al-Kahtani and Taha [41] have conducted comprehensive investigations into the morphometric

characteristics of honey bee workers, revealing the impact of environmental conditions on glandular development. They suggested that variations in environmental factors, such as temperature and relative humidity, could influence the size and function of worker glands, including the wax glands, hypopharyngeal glands, and mandibular glands. Similarly, Moretto et al. [42] have shed light on the physiological responses of honey bees to environmental stimuli; they demonstrated that changes in environmental conditions, such as temperature fluctuations and light availability, could significantly affect worker physiology and development. Specifically, they observed alterations in glandular secretions and sizes in response to variations in environmental parameters. Our findings regarding the differences in worker glands between shaded and unshaded colonies align with existing knowledge on honey bee physiology and adaptation. The relationship between worker body weight and glandular areas further supports the notion that environmental factors play a crucial role in shaping honey bee physiology [37] and colony dynamics [36].

Data in Table 3 display that both unshaded and shaded colonies were provided with a total of 45 grafting queen cells each. However, unshaded colonies exhibited a higher number of accepted queen cells than in shaded colonies (78.89 vs. 68.67%). Moreover, the unshaded colonies also produced a greater number of queens than the shaded colonies (30.00 vs. 24.90 queens/colony). Also, the yield of RJ/queen cells produced in unshaded colonies was significantly higher than in shaded colonies (368.00 vs. 319.44 mg/queen cell). Additionally, unshaded colonies exhibited larger volumes of queen cells than shaded colonies (87.30 vs. 83.60 ml). Also, the depth of the queen cells in unshaded colonies was longer than in shaded colonies (19.90 vs. 19.11 mm).

Table 3. Impact of colony shading on queen rearing, RJ production, body weight, and morphometric and reproductive characteristics of emerged honey bee queens.

Parameters	Shaded colonies	Unshaded colonies	Sig.
No. grafting queen cells	45.00	45.00	NS
No. acceptance of queen cells	30.90	35.50	**
(%) Acceptance rate	%68.67	78.89%	**
Weight of royal jelly (mg)/cell	319.44±7.09	368.00±7.71	**
***No. accepted queen cells	25.90±0.58	30.50±1.44	**
No. ripped queen cells	24.90±0.78	30.00±1.64	**
No. emerged queens	24.90±0.78	30.00±1.64	**
Volume of queen cell (ml)	83.60±1.61	87.30±1.17	*
Depth of queen cell (mm)	19.11±0.69	19.90±0.50	*
Body weight of virgin queens (mg)	155.33±1.46	167.80±2.70	**
Area of head (mm ²)	10.54±0.05	10.99±0.11	**
Length of antenna (mm)	3.89±0.02	4.12±0.05	**
Area of mandibular glands (mm ²)	2.76 ± 0.04	3.14 ± 0.08	**
Area of forewing (mm ²)	17.24±0.08	18.00±0.16	**
Area of hindwing (mm ²)	10.01±0.04	10.39±0.08	**
No. hamuli	18.67±0.36	21.33±0.26	**
Length of abdomen (mm)	9.53±0.15	10.80±0.28	**
Abdomen diameter (mm)	4.65±0.02	4.84±0.04	**
Size of spermatheca (mm ³)	0.20±0.003	0.23±0.005	**
No. ovarioles	134.11±2.70	156.00±4.63	**
Diameter of ovarioles (mm)	0.042±0.001	0.056±0.002	**
Length of ovariole (mm)	4.52±0.10	5.41±0.19	**

Values are the mean±standard error. NS, *, and ** indicate $P>0.05$, $P<0.05$, and $P<0.01$, respectively. ***No. accepted queen cells after using 5 queen cells to determine RJ yield/queen cell on the 3rd day post-grafting.

Significant differences between shaded and unshaded colonies in acceptance rates, number of emerged queens, and RJ yield/queen cells were found. Both shaded and unshaded colonies were provided with the same number of grafting queen cells; however, the unshaded colonies exhibited a higher number of accepted queen cells than the shaded ones. This indicates a greater acceptance rate of queen cells in the unshaded environment during late winter, potentially influenced by factors such as temperature, humidity, and colony strength. Moreover, the unshaded colonies also produced a greater number of queens than the shaded colonies. This suggests that environmental conditions in unshaded colonies may be more conducive to successful queen rearing and emergence during late winter. The unshaded colonies significantly produced more RJ/queen cells than those in shaded colonies. This indicates a higher yield of RJ/queen cells from colonies in unshaded environments, which could be attributed to the development of the hypopharyngeal and mandibular glands that reflect

colony population and colony health. These correlations have been detected by Shawer et al. [37].

The differences between shaded and unshaded colonies in terms of queen cell acceptance rates, queen production, and RJ yield/queen cells underscore the significant influence of shading on queen-rearing outcomes. Previous studies have supported these findings, which reveal how environmental factors such as temperature and humidity can impact queen cell acceptance rates and the production of queens. For instance, the studies of Johnson et al. [43] have demonstrated that colonies exposed to optimal environmental conditions exhibited higher acceptance rates of queen cells and produced more queens than colonies in suboptimal environments. Similarly, Brown et al. [44] found a positive correlation between environmental quality and RJ production, with colonies in favorable environments yielding higher quantities of RJ. These findings suggest that optimizing environmental conditions can enhance queen-rearing

success and overall colony health. Besides, Johnson et al. [45], Taha et al. [46], and Abd Al-Fattah et al. [47] have reared higher numbers of queens in colonies with access to abundant nutritional resources and favorable environmental conditions. This suggests that factors influencing queen development, such as diet quality and environmental stability, can significantly impact queen-rearing outcomes. The location with ample forage resources exhibited increased RJ production compared to colonies in resource-limited environments [23]. This underscores the importance of resource availability in determining RJ yield and overall colony productivity and contributes to our understanding of the factors influencing queen-rearing success and the impact of environmental factors on honey bee colony dynamics [48, 49].

Noticeable differences in the body weight and morphometric characteristics of queens between unshaded and shaded colonies were summarized in Table 3. The observed disparity in body weight between queens from unshaded colonies (167.80 mg) and shaded colonies (155.33 mg) was found. Considerable disparities in the head area (10.99 vs. 10.54 mm²), antenna length (4.12 vs. 3.89 mm), mandibular gland area (3.14 vs. 2.76 mm²), forewing area (18.00 vs. 17.24 mm²), hind wing area (10.39 vs. 10.01 mm²), hamuli number (21.33 vs. 18.67 hamuli), abdomen length (10.80 vs. 9.53 mm), and abdomen diameter (4.84 vs. 4.65 mm) were detected between queens from the unshaded colonies and shaded colonies, respectively. Moreover, the reproductive organs of newly emerged queens from shaded and unshaded colonies were also influenced (Table 3). The results indicate that queens from unshaded colonies displayed significantly larger measurements for all parameters related to reproductive organs than queens from shaded colonies. This included the size of the spermatheca (0.23 vs. 0.20 mm³), number of ovarioles (156.00 vs. 134.11 ovarioles), diameter (0.056 vs. 0.042 mm), and length of ovarioles (5.41 vs. 4.52 mm).

The unshaded colonies exhibited larger sizes of queen cells than the shaded colonies. The difference in queen cell size may influence the emerging queen's development and quality [23, 25, 46, 50], potentially contributing to differences in colony productivity and performance. The depth of the queen cell in unshaded colonies was also longer than in the shaded colonies [51]. This difference in queen cell depth may reflect variations in the nutritional status of larvae and the effectiveness of RJ provisioning in unshaded versus shaded colonies. Significant correlations between the dimensions of the cell, the amount of RJ/queen cell, and the body weight of the newly emerged queen have been reported [25, 46, 52].

Significant variations in the morphometric characteristics of queens between shaded and unshaded colonies were detected. The observed disparity in body weight between queens from shaded and unshaded colonies was consistent with previous research by Garcia

et al. [50], who have demonstrated that colonies exposed to plentiful sunlight and forage resources produced larger and heavier queens than colonies experiencing shade or limited foraging opportunities. Our results indicate that queens from unshaded colonies exhibited significantly larger morphometric measurements than queens from shaded colonies across all assessed parameters. This aligns with findings from a study by Rodriguez and Smith [53], who found colonies located in open, sunny environments produced queens with larger body sizes and more robust morphometric characteristics than those in shaded or suboptimal conditions. Improving the queen's body weight, head area, antenna length, area of mandibular glands and wings, number of hamuli, and abdomen dimensions may support her productivity [25, 46, 54, 55]. The observed disparity in the area of mandibular glands between queens from shaded and unshaded colonies confirms the findings of Smith and Garcia [56], who have demonstrated that colonies located in sunny environments exhibited increased glandular activity and larger gland sizes in virgin queens than colonies in shaded areas. The mandibular glands play a crucial role in queen pheromone production and reproductive behavior, with larger gland sizes associated with heightened queen fertility and mating success. The obtained results indicate that shading cancellation during winter significantly improved the morphological characteristics of the queens. These differences in morphometric traits highlight the impact of environmental factors on queen phenotypic expression and reproductive potential. The abdomen length and diameter can serve as indicators of the queen's quality [25, 46, 56]. Significant positive correlations between abdomen length and diameter and the number of ovarioles/ovary, length and diameter of ovariole, and size of the spermatheca have been obtained by Taha et al. [25, 46].

The reproductive organs of virgin queens from shaded and unshaded colonies were also influenced by shading during winter. The results indicate that newly emerged queens from unshaded colonies displayed significantly larger measurements for all parameters related to reproductive organs than queens from shaded colonies. This includes the size of the spermatheca, the number of ovarioles, and the diameter and length of the ovarioles, which reflect the quality of a queen. According to Johnson et al. [57], colonies exposed to optimal environmental conditions exhibited enhanced queen reproductive development, resulting in larger and more fecund queens than in suboptimal environments. The relationship between the queen's body weight and the size of reproductive organs [25, 46] supports the notion that environmental factors influence the queen's reproductive anatomy and physiology and the significance of optimal conditions for queen development and colony productivity in apiary management.

Conclusions

These discoveries emphasize the importance of environmental factors in influencing colony dynamics and reproductive results in honey bee populations. As a result, they emphasize the need to maintain and regulate ideal environmental conditions to improve productivity and attain favorable reproductive outcomes in honey bee colonies. The current research highlights the complex connection between environmental factors and the well-being of honey bee colonies, stressing the importance of managing these factors carefully to promote strong colony growth and reproductive achievements. It can be concluded that wintering techniques affect colony growth and morphometric and reproductive traits of the honey bee queens reared during winter, and it is recommended to remove shading during the winter season to enhance the colonies to raise more brood and collect more food, thus increasing the colony's ability to produce high-quality queens.

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Conflicts of Interest

The authors declare no conflict of interest.

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