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Original article

Honey bee (*Apis mellifera jemenitica*) colony performance and queen fecundity in response to different nutritional practices

Khalid Ali Khan^{a,b,c,*}, Hamed A. Ghramh^{a,b,c}, Zubair Ahmad^{a,b,d}^a Research Center for Advanced Materials Science (RCAMS), King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia^b Unit of Bee Research and Honey Production, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia^c Biology Department, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia^d Biology Department, College of Arts and Sciences, Zehran Junub, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia

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ABSTRACT

Honey bee colony nutritional dynamics depend on the availability of floral resources throughout a countryside with varying forage circumstances. Few studies quantify the queen fecundity and colony performance about certain management approaches on a broad scale. The present study was conducted to investigate the queen bee fecundity and various colony performance parameters in response to different nutritional practices, i.e., Group-I, supplied with sucrose solution (1:1; w/v), Group-II, provided with locally available commercial pollen substitute, Group-III, supplied with both sucrose solution + locally available commercial pollen substitute, and Group-IV without any sugar solution and pollen substitute. Our results demonstrated that eggs laid by queen bees were significantly higher (1241.83 ± 46.24) in Group-III than in other groups over the time of observations. Similarly, a significant difference was noticed in the mean sealed worker brood area and honey store area between the different groups of management practices. Both, the max mean sealed worker brood area ($2153.53 \pm 29.18 \text{ cm}^2$) and max mean honey store area ($1713.33 \pm 12.06 \text{ cm}^2$) were observed in Group-III while, the mini mean sealed worker brood area ($1066.53 \pm 20.18 \text{ cm}^2$) and mini mean honey store area ($1058.86 \pm 4.07 \text{ cm}^2$) were observed in Group-IV. In contrast, a non-significant difference was observed in pollen stores between Group-II and Group-III ($p > 0.005$). Current findings add to our understanding of the mechanisms that underpin large-scale controlled colony performance when the natural pollens resources are insufficient.

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1. Introduction

The world is majorly populated with pollinator-dependent plants. Around 200,000 distinct animal species serve as pollinators on a global scale (Ahmad et al., 2021a; Devi et al., 2021). Among these, 1000 are vertebrates such as birds, bats, and tiny mammals, while the remainder is invertebrates such as bees, beetles, butterflies, flies, and moths (Abrol, 2012). Pollinators pollinate approximately 180,000 different plant species worldwide (Council, 2007; Abrol, 2012). Notably, honey bees are among the most efficient

pollinator species and pollinate 87.5% of angiosperms. A honey bee can visit around 2000 flowers daily during foraging of nectar and pollen (Ollerton et al., 2011; Abrol, 2012; Abbasi et al., 2021; Devi et al., 2021). In addition, honey bees produce various natural products such as honey, royal jelly, bee bread, and propolis (Ahmad et al., 2020; Jagdale et al., 2021). The *Apis mellifera jemenitica* governed the entire beekeeping industry of Saudi Arabia due to its good foraging ability to produce more honey than *A. m. carnica* and *A. m. lamarki* of Egypt (Al-Ghamdi et al., 2013; Al-Ghamdi et al., 2021). On the other hand, Saudi Arabian beekeepers are not fully exploiting beekeeping's competitive advantages with *A. m. jemenitica* due to the low performances of bee colonies (Adgaba et al., 2014). Moreover, the decline of *A. m. jemenitica* colonies is attributed to three key factors that badly influence the colony health of honey bees; poor nutrition, pesticide exposure, and pathogens (Albarrak and Gray, 2017; Ansari et al., 2017; Iqbal et al., 2019). The honey bee queen is a central part of the colony and solely regulates the population of honey bees and the unity of the colony (Perumal et al., 2021).

* Corresponding author at: Unit of Bee Research and Honey Production, Faculty of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia.

E-mail address: khalidtalpur@hotmail.com (K.A. Khan).

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The queen's fecundity is a critical factor for the long-term survival of the honey bee colony, mainly depending upon younger queens (Winston, 1980; Winston, 1991; Amiri et al., 2017). A high-fecundity queen allows a colony to have a maximum bee population that is very productive. The queen bee relies on worker bees for constant care because sustained queen egg-laying does not occur without worker bees. The egg-laying by the queen bee is the result of the queen's and workers' synchronized efforts (Fine et al., 2018). Colony performance includes colony strength and productivity (Chemurot and de Graaf, 2019). The colony strength is determined by the number of adult worker bees in the hive, brood pattern, and the flight activity around the hive entrance (Pokhrel et al., 2006; Delaplane et al., 2013; Taha and Al-Kahtani, 2019; Grant et al., 2021) while colony productivity is measured in honey production and pollen collection (Hoover and Ovinge, 2018). The physical characters of the queen are associated with colony performance and were investigated using a different behavioral attribute by Akyol et al. (2008) and Hatjina et al. (2014). The honey bee colony performance is crucial in beekeeping (Al-Ghamdi et al., 2021). The sharp decline of *A. m. jemenitica* is documented by Al-Ghamdi et al. (2017). It is associated with a mated queen's low reproductive potential or fecundity (Al-Sarhan et al., 2019).

Queen-size is almost regulated by the larval rearing situation, mostly the royal jelly consumed by the larvae during their growth (Hartfelder et al., 2015). The queen's fertility is determined by examining her ovaries and spermatheca. The size of these two organs is linked with more bodyweight when fed with sugar-based supplementary diets during beekeeping (Tarpay et al., 2011; De Souza et al., 2013; McAfee et al., 2020). Honey bee colonies are fed on commercially available pollen substitute diets when the natural pollen resources are scarce. Artificial pollen substitutes may affect bee physiology in addition to their primary nutritional role (Al-Ghamdi et al., 2011; Ahmad et al., 2021b). For instance, sugar syrup is the most often used artificial diet for honey bees. It is made by combining sugar and water in a 2:1 ratio (Genç and Aksoy, 1993). The different types of carbohydrates also significantly enhance the gut bacteria of the queen (Taylor et al., 2019). The sugar-based supplemental diet increased the honey bee's reproductive development and fertility potential when mixed with juvenile hormones (Daiana et al., 2019; Frizzera et al., 2020).

The present study was conducted to demonstrate the effect of various nutritional management practices on the fecundity of honey bee queens. In addition to investigating the potential impacts of different nutritional practices on colony performance, including sealed brood area, pollen, and honey stored area. This study helps determine the optimal sucrose solution for maintaining the optimal population of the colony and its performance in the scarcity of natural pollen.

2. Materials and methods

2.1. Experimental honeybee colonies and treatments

The experiment was conducted at the Unit of Bee Research and Honey Production (UBRHP), King Khalid University, Abha, Saudi Arabia. This study used native honey bee (*Apis mellifera jemenitica*) colonies. All bee colonies were kept in standard Langstroth hives containing eight frames and aligned concerning equal colony strength, sealed brood area, and food stocks in June 2021. Every investigational group comprised five bee colonies and the mated queen age with one year. Group-I was supplied with sucrose solution (1:1; w/v) by using an internal sugar feeder, and Group-II was provided with a locally available commercial pollen substitute. At the same time, Group-III was given both sucrose solution (1:1)

by using an internal sugar feeder and locally available commercial pollen substitute. Group-IV was controlled without any sugar solution and pollen substitute. All other management applications applied to each bee colony were the same throughout the experiments. In addition, allow the bees to forage around crops and urban residential neighborhoods freely.

2.2. Effect of various management practices on fecundity

Above mentioned four groups were selected for this experiment. An old frame was placed in the queen cage barrier with a healthy queen to count the egg numbers. The queen laid eggs on an old comb within one day in the queen cage barrier. The number of eggs laid by the queen per frame per day was recorded (Fig. 1). Eggs laid by the queen was tracked in each colony for 15 days.

2.3. Worker sealed brood area (cm²)

The worker sealed brood area was assessed with a modified grid system. Squares of the grid had a surface area of 25 cm². The sealed brood frame area was marked and measured with a grid.

2.4. Food stores (pollen and honey)

Pollen stored in each colony was measured by placing a frame grid consisting of 25 cm² over each side of every frame inside each bee colony and calculated the summing the number of grid cells occupied with pollen.

Similarly, honey stored in each colony was measured by placing a frame grid consisting of 25 cm² and summing the number of grid cells with occupied honey.

2.5. Statistical analysis

Fecundity, sealed brood area, food stored were compared between the treatments. All statistical results were analyzed and calculated by SPSS software according to the analysis of variance (ANOVA). The figures were prepared by GraphPad Prism software (version 8). The Tukey posthoc test was conducted for multiple comparisons between treatment groups at a significance level ($p = 0.05$).

3. Results

3.1. Effect of different nutrition practices on fecundity

Overall, a statistically significant difference was detected between the effect of different nutritional practices and eggs laid by the queen bee over the time of observations ($F(3, 296) = 37.971, p = 0.001$). The queen laid max eggs (1241.83 ± 46.24) when fed on pollen substitute plus sucrose solution, and the lower number of eggs were observed in the control group (822.82 ± 31.62). The eggs laid by the queen were (1127.33 ± 10.56) and (908.01 ± 26.17) when either fed separately on commercially available pollen substitute and sucrose solution, respectively (Fig. 2).

3.2. Effect of various nutritional management practices on brood area

A significant effect of various nutritional management was observed on sealed brood area of bee colonies ($F(3, 176) = 257.93, p = 0.001$). There was a fluctuating trend in the sealed brood area over the time of observations (Fig. 3). The mean highest sealed brood area ($2153.53 \pm 29.18 \text{ cm}^2$) was recorded in

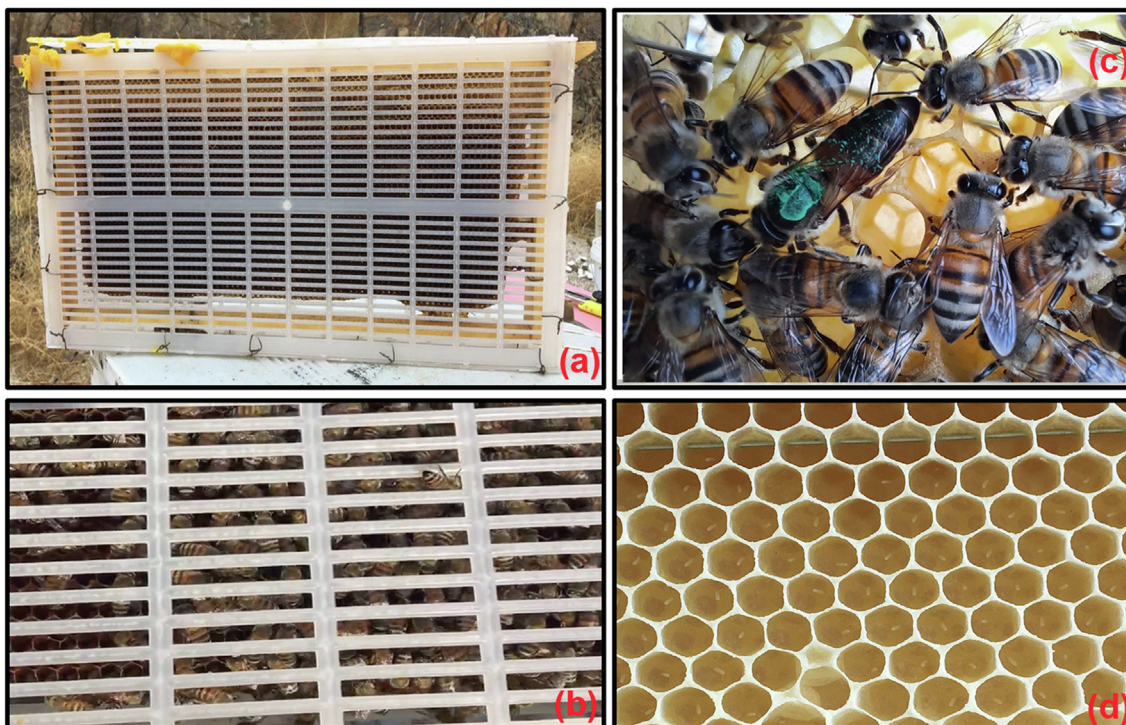


Fig. 1. A frame inside the queen cage barrier (a); bees and queen working on the frames inside the queen cage barrier (b); a queen bee with some attendants prior to be caged (c); eggs laid by the queen bee (d).

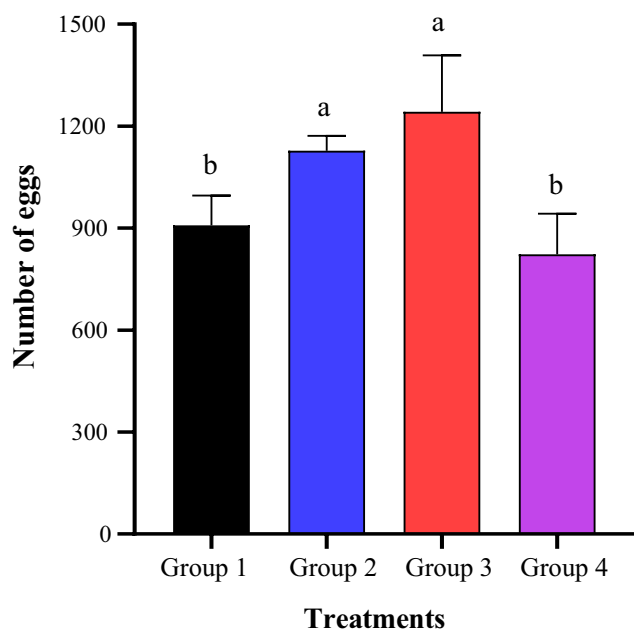


Fig. 2. Honey bee (*Apis mellifera jemenitica*) queen fecundity in response to various nutrition practices, i.e., Group-I = bee colonies provided with sucrose solution (1:1; w/v), Group-II = bee colonies supplied with locally available commercial pollen substitute, Group-III = bee colonies given with both sucrose solution + locally available commercial pollen substitute, and Group-IV = bee colonies without any sugar solution and pollen substitute.

Group-III, while less brood area ($1066.53 \pm 20.18 \text{ cm}^2$) was noticed in the control group. The mean sealed worker brood area in Group-I was $1477.23 \pm 15.58 \text{ cm}^2$ and in Group-II was $1682.53 \pm 40.81 \text{ cm}^2$.

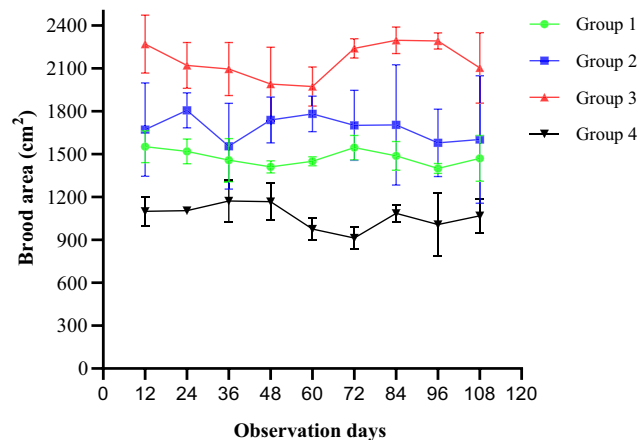


Fig. 3. Effect of different management practices on worker sealed brood area ($\text{cm}^2/\text{colony}$). Group-I = bee colonies provided with sucrose solution (1:1; w/v), Group-II = bee colonies supplied with locally available commercial pollen substitute, Group-III = bee colonies given with both sucrose solution + locally available commercial pollen substitute, and Group-IV = bee colonies without any sugar solution and pollen substitute.

3.3. Effect of various nutritional practices on food store (pollen and honey)

A significant difference was recorded in pollen store between the treatment's groups over the time of observations ($F(3, 176) = 44.83, p = 0.001$). No significant difference was found in the pollen stored between Group-II and Group-III ($p > 0.005$) (Table 1).

The maximum mean pollen store was noticed in Group-III ($780.62 \pm 12.80 \text{ cm}^2$) followed by Group-II ($756.22 \pm 17.91 \text{ cm}^2$) and

Table 1
Effect of different management practices on queen fecundity and honey bee colony performance.

Treatment	Fecundity Mean \pm S. Error	Worker sealed brood area (cm ²) Mean \pm S. Error	Pollen store area (cm ²) Mean \pm S. Error	Honey store area (cm ²) Mean \pm S. Error
Group-I	908.01 \pm 26.17b	1477.23 \pm 15.58 a	646.76 \pm 16.98b	1425.53 \pm 19.31 a
Group-II	1127.33 \pm 10.56 a	1682.53 \pm 40.81b	756.22 \pm 17.91 a	1527.03 \pm 29.15b
Group-III	1241.83 \pm 46.24 a	2153.53 \pm 29.18c	780.62 \pm 12.80 a	1713.33 \pm 12.06c
Group-IV	822.82 \pm 31.62b	1066.53 \pm 20.18 d	571.29 \pm 8.71c	1058.86 \pm 4.07 d

Small letters within each column represent statistically significant differences ($p < 0.05$). Group-I = bee colonies provided with sucrose solution (1:1; w/v), Group-II = bee colonies supplied with locally available commercial pollen substitute, Group-III = bee colonies given with both sucrose solution + locally available commercial pollen substitute, and Group-IV = bee colonies without any sugar solution and pollen substitute.

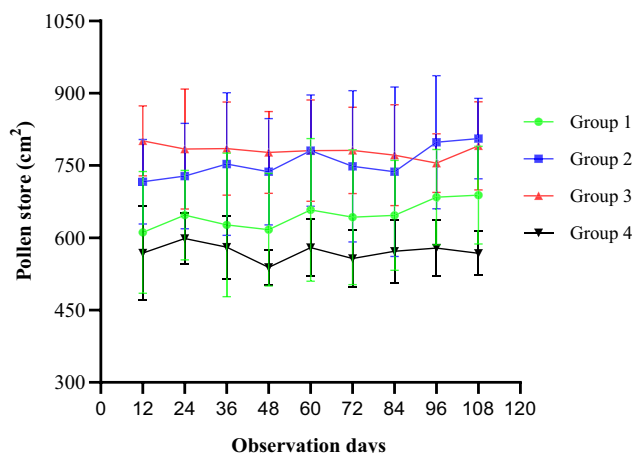


Fig. 4. Effect of nutritional management practices on pollen store area (cm²/colony). Group-I = bee colonies provided with sucrose solution (1:1; w/v), Group-II = bee colonies supplied with locally available commercial pollen substitute, Group-III = bee colonies given with both sucrose solution + locally available commercial pollen substitute, and Group-IV = bee colonies without any sugar solution and pollen substitute.

Group-I (646.76 \pm 16.98 cm²). The least mean pollen stored was observed by the control group colonies (571.29 \pm 8.71 cm²) (Fig. 4).

Similarly, a significant result was noticed on the honey store area between different treatment colony groups over the time of observations ($F(3, 176) = 219.17, p = 0.001$). A significant max mean honey store area was recorded in Group-III 1713.33 \pm 12.06 cm², and the least mean honey store area was observed in Group-IV (control), which was 1058.86 \pm 4.07 cm². The honey store area in Group-I and Group-II was (1425.53 \pm 19.31 cm²) and (1527.03 \pm 29.15 cm²), respectively (Fig. 5).

4. Discussion

Nutrition, particularly pollen, has a well-known impact on honey bee health and colony development (Stevanovic et al., 2010; Omar et al., 2017). In the present study, we compared the effect of various nutritional management practices on queen fecundity and colony performance of honey bees. Our results demonstrated different nutritional management practices significantly impacted queen fecundity and colony performance. The queen fecundity was significantly higher in Group-III (pollen substitute plus sucrose solution) than in other nutritional management practices. The commercially available pollen substitute has a lot of protein content and minerals and vitamins; thus, the availability of protein and sucrose solution to the bee colony resulted in the high fecundity of queen bees. Similarly, a statistically significant difference was found in the sealed worker brood area and honey store area (cm²) between the different groups of honey bees'

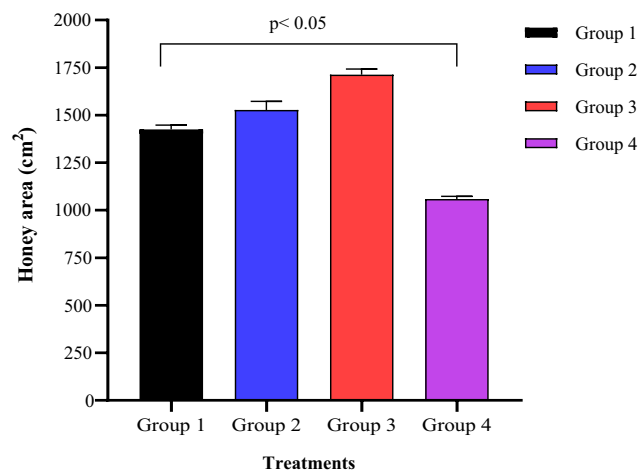


Fig. 5. Effect of various nutritional management practices on honey store area (cm²/colony). Group-I = bee colonies provided with sucrose solution (1:1; w/v), Group-II = bee colonies supplied with locally available commercial pollen substitute, Group-III = bee colonies given with both sucrose solution + locally available commercial pollen substitute, and Group-IV = bee colonies without any sugar solution and pollen substitute.

colonies over the time of observations. However, in the case of the pollen store area, a non-significant difference was recorded between Group-II and Group-III of honey bee colonies.

Remarkably, the honey bee queens are of great importance for bee colonies and beekeeping activities (Dolasevic et al., 2020). Previously, the impact of different diets on the quality of *Apis mellifera* queens has been studied (Dolasevic et al., 2020). In addition, the effect of food on the different traits of the queens of *A. m. anatoliaca* and *A. m. meda* subspecies was investigated (Gençer et al., 2000; Mahbobi et al., 2012). Our study investigated the impact of various nutritional practices on queen fecundity and colony performance. Our findings demonstrated that sucrose syrup solution with artificial pollen substitute enhanced the fecundity, sealed brood area and food storage area than sucrose syrup alone and pollen substitute alone. Ricigliano et al. (2018) suggested that the performance and health of honey bee colonies are heavily influenced by queen quality and availability of nutrition. Recently, Paray et al., (2021) reported that the number of eggs laid by the queen and worker sealed and unsealed brood reared is reduced, but the amount of stored food available determines the extent of the reduction. Many scientists have devised numerous commercially available artificial food recipes for honey bees. These diets contain a balanced nutritious composition for bees and are acceptable, digestible, and materially affordable. However, more research is required to determine which components of commercially available pollen substitute positively affect queen fecundity. In our findings, variation of egg-laying between groups suggested that external factors may impact fecundity.

Similar findings have been reported in the sealed brood area in previous literature (Sihag and Gupta, 2011; Lamontagne-Drolet et al., 2019; Ahmad et al., 2021b); various pollen substitutes increased the worker brood area. However, our results are inconsistent with the findings of Ali (2011), who reported that the worker brood area was 2813.13 cm²/colony, 1730.94, and 1867 cm²/colony in different groups. Our results revealed that less pollen store area was found between all treatments. Our findings are in line with (Ricigliano et al., 2018). A limited pollen store was recorded over the time of observations, suggesting that most bee colonies had at least intermittent access to natural forage, which could have reduced the impact of commercially available pollen substitutes with natural pollen.

Further investigation is required to determine optimal sucrose solution and commercially available pollen substitute ratios and demonstrate the optimal timing of artificial diets to colonies to enhance fecundity and colony performance.

5. Conclusions

The main purpose of the present study was to investigate whether commercially available pollen substitutes and sucrose to bee colonies affect queen fecundity and colony performance. Our findings revealed that the different nutritional management practices significantly impacted queen fecundity and colony performance. A significant difference was observed in fecundity, worker sealed brood area, and food storage area in sucrose solution plus commercially available pollen substitute than the other groups. However, further studies are needed to investigate the effect of nutritional management practices on honey bee physiology and colony-level molecular biomarkers.

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.

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References

Abbasi, K.H., Jamal, M., Ahmad, S., Ghranh, H.A., Khanum, S., Khan, K.A., Ullah, M.A., Aljedani, D.M., Zulfikar, B., 2021. Standardization of managed honey bee (*Apis mellifera*) hives for pollination of Sunflower (*Helianthus annuus*) crop. *J. King Saud Univ.-Sci.* 33 (8), 101608. <https://doi.org/10.1016/j.jksus.2021.101608>.

Abrol, D.P., 2012. Non bee pollinators-plant interaction. In: *Pollination Biology*. Springer, pp. 265-310.

Adgaba, N., Al-Ghamdi, A., Shenkute, A., Ismaiel, S., Al-Kahtani, S., Tadess, Y., et al., 2014. Socio-economic analysis of beekeeping and determinants of box hive technology adoption in the Kingdom of Saudi Arabia. *J. Anim. Plant Sci.* 24 (6), 1876–1884.

Ahmad, S., Campos, M.G., Fratini, F., Altaye, S.Z., Li, J., 2020. New insights into the biological and pharmaceutical properties of royal jelly. *Int. J. Mol. Sci.* 21 (2), 382.

Ahmad, S., Khalofah, A., Khan, S.A., Khan, K.A., Jilani, M.J., Hussain, T., Skalicky, M., Ghranh, H.A., Ahmad, Z., 2021a. Effects of native pollinator communities on the physiological and chemical parameters of loquat tree (*Eriobotrya japonica*) under open field condition. *Saudi J. Biol. Sci.* 28 (6), 3235–3241.

Ahmad, S., Khan, K.A., Khan, S.A., Ghranh, H.A., Gul, A., Shah, A.N., 2021b. Comparative assessment of various supplementary diets on commercial honey bee (*Apis mellifera*) health and colony performance. *PLoS One* 16 (10), e0258430. <https://doi.org/10.1371/journal.pone.0258430>.

Akyol, E., Yeninar, H., Korkmaz, A., Çakmak, İ., 2008. An observation study on the effects of queen age on some characteristics of honey bee colonies. *Ital. J. Anim. Sci.* 7 (1), 19–25.

Al-Ghamdi, A.A., Adgaba, N., Tadesse, Y., Getachew, A., Al-Maktary, A.A., 2017. Comparative study on the dynamics and performances of *Apis mellifera jemenitica* and imported hybrid honeybee colonies in southwestern Saudi Arabia. *Saudi J. Biol. Sci.* 24 (5), 1086–1093.

Al-Ghamdi, A.A., Al-Ghamdi, M.S., Ahmed, A.M., Mohamed, A.S.A., Shaker, G.H., Ansari, M.J., Dorrah, M.A., Khan, K.A., Ayaad, T.H., 2021. Immune investigation of the honeybee *Apis mellifera jemenitica* broods: a step toward production of a bee-derived antibiotic against the American foulbrood. *Saudi J. Biol. Sci.* 28 (3), 1528–1538.

Al-Ghamdi, A.A., Al-Khaibari, A.M., Omar, M.O., 2011. Consumption rate of some proteinic diets affecting hypopharyngeal glands development in honeybee workers. *Saudi J. Biol. Sci.* 18 (1), 73–77.

Al-Ghamdi, A.A., Nuru, A., Khanbush, M.S., Smith, D.R., 2013. Geographical distribution and population variation of *Apis mellifera jemenitica* Ruttner. *J. Apic. Res.* 52 (3), 124–133.

Al-Sarhan, R., Adgaba, N., Tadesse, Y., Alattal, Y., Al-Abbadi, A., Single, A., Al-Ghamdi, A., 2019. Reproductive biology and morphology of *Apis mellifera jemenitica* (Apidae) queens and drones. *Saudi J. Biol. Sci.* 26 (7), 1581–1586.

Albarrak, A., Gray, A., 2017. Beekeeping and colony losses in Saudi Arabia". In: *Royal Statistical Society Conference*.

Amiri, E., Strand, M.K., Rueppell, O., Tarpay, D.R., 2017. Queen quality and the impact of honey bee diseases on queen health: potential for interactions between two major threats to colony health. *Insects* 8 (2), 48.

Ansari, M.J., Al-Ghamdi, A., Nuru, A., Ahmed, A.M., Ayaad, T.H., Khan, K.A., Al-Waili, N., 2017. Diagnosis and molecular detection of *Paenibacillus* larvae, the causative agent of American foulbrood in honey bees in Saudi Arabia. *Int. J. Trop. Insect Sci.* 37 (03), 137–148.

Chemurot, M., de Graaf, D.C., 2019. Honeybee colony performance in two agro-ecological zones of Uganda is influenced by land-use type around apiary. *Livestock Res. Rural Develop.* 31 (7).

Council, N.R., 2007. Status of pollinators in North America. *National Academies Press*.

De SOUZA, D.A., Huang, M.H., Tarpay, D.R., 2019. Experimental improvement of honey bee (*Apis mellifera*) queen quality through nutritional and hormonal supplementation. *Apidologie* 50 (1), 14–27.

De Souza, D., Bezerra-Laure, M., Franco, T., Gonçalves, L., 2013. Experimental evaluation of the reproductive quality of Africanized queen bees (*Apis mellifera*) on the basis of body weight at emergence. *Genet. Mol. Res.* 12 (4), 5382–5391.

Delaplane, K.S., Van Der Steen, J., Guzman-Novoa, E., 2013. Standard methods for estimating strength parameters of *Apis mellifera* colonies. *J. Apic. Res.* 52 (1), 1–12.

Devi, S., Devi, D., Singh, S.J., Kaushal, B., 2021. Strategies to combat the decline in pollinator's population.

Dolasevic, S., Stevanovic, J., Aleksic, N., Glavinic, U., Deletic, N., Mladenovic, M., Stanimirovic, Z., 2020. The effect of diet types on some quality characteristics of artificially reared *Apis mellifera* queens. *J. Apic. Res.* 59 (1), 115–123.

Fine, J.D., Shpigler, H.Y., Ray, A.M., Beach, N.J., Sankey, A.L., Cash-Ahmed, A., Huang, Z.Y., Austraikaite, I., Chao, R., Zhao, H., Robinson, G.E., Rueppell, O., 2018. Quantifying the effects of pollen nutrition on honey bee queen egg laying with a new laboratory system. *PLoS One* 13 (9), e0203444. <https://doi.org/10.1371/journal.pone.0203444>.

Frizzera, D., Del Fabbro, S., Ortis, G., Zanni, V., Bortolomeazzi, R., Nazzi, F., Annoscia, D., 2020. Possible side effects of sugar supplementary nutrition on honey bee health. *Apidologie* 51 (4), 594–608.

Genç, F., Aksoy, A., 1993. Some of the correlations between the colony development and honey production on the honeybee (*Apis mellifera* L.) colonies. *Apiacta* 28 (2), 33–41.

Gençer, H.V., Shah, S.Q., Firatli, Ç., 2000. Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. *Pakistan J. Biol. Sci. (Pakistan)*.

Grant, K.J., DeVetter, L., Melathopoulos, A., 2021. Honey bee (*Apis mellifera*) colony strength and its effects on pollination and yield in highbush blueberries (*Vaccinium corymbosum*). *PeerJ* 9, e11634. <https://doi.org/10.7717/peerj.11634>.

Hartfelder, K., Guidugli-Lazzarini, K.R., Cervoni, M.S., Santos, D.E., Humann, F.C., 2015. Old Threads Make New Tapestry—Rewiring of Signalling Pathways Underlies Caste Phenotypic Plasticity in the Honey Bee, *Apis mellifera* L. *Adv. Insect Physiol.* 48, 1–36.

Hatjina, F., Bieńkowska, M., Charistos, L., Chlebo, R., Costa, C., Dražić, M.M., Filipi, J., Gregorc, A., Ivanova, E.N., Kezić, N., Kopernicky, J., Kryger, P., Lodesani, M., Lokar, V., Mladenovic, M., Panasiuk, B., Petrov, P.P., Rašić, S., Skerl, M.I.S., Vajsnaš, F., Wilde, J., 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. *J. Apic. Res.* 53 (3), 337–363.

Hoover, S.E., Ovinge, L.P., 2018. Pollen collection, honey production, and pollination services: managing honey bees in an agricultural setting. *J. Econ. Entomol.* 111 (4), 1509–1516.

Iqbal, J., Alqarni, A.S., Raweh, H.S.A., 2019. Effect of sub-lethal doses of imidacloprid on learning and memory formation of indigenous Arabian bee (*Apis mellifera jemenitica* Ruttner) adult foragers. *Neotropical Entomol.* 48 (3), 373–380.

Jagdale, Y.D., Mahale, S.V., Zohra, B., Nayik, G.A., Dar, A.H., Khan, K.A., Abdi, G., Karabagias, I.K., 2021. Nutritional Profile and Potential Health Benefits of Super

- Foods: A Review. Sustainability 13 (16), 9240. <https://doi.org/10.3390/su13169240>.
- Lamontagne-Drolet, M., Samson-Robert, O., Giovenazzo, P., Fournier, V., 2019. The impacts of two protein supplements on commercial honey bee (*Apis mellifera* L.) colonies. J. Apic. Res. 58 (5), 800–813.
- Mahbobi, A., Farshineh-Adl, M., Woyke, J., Abbasi, S., 2012. Effects of the age of grafted larvae and the effects of supplemental feeding on some morphological characteristics of Iranian queen honey bees (*Apis mellifera* meda Skorikov, 1929). J. Apicultural Sci. 56 (1), 93.
- McAfee, A., Chapman, A., Higo, H., Underwood, R., Milone, J., Foster, L.J., Guarna, M. M., Tarpy, D.R., Pettis, J.S., 2020. Vulnerability of honey bee queens to heat-induced loss of fertility. Nat. Sustainability 3 (5), 367–376.
- Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120 (3), 321–326.
- Omar, E., Abd-Ella, A.A., Khodairy, M.M., Moosbeckhofer, R., Crailsheim, K., Brodschneider, R., 2017. Influence of different pollen diets on the development of hypopharyngeal glands and size of acid gland sacs in caged honey bees (*Apis mellifera*). Apidologie 48 (4), 425–436.
- Paray, B.A., Kumari, I., Hajam, Y.A., Sharma, B., Kumar, R., Albeshr, M.F., Farah, M.A., Khan, J.M., 2021. Honeybee nutrition and pollen substitutes: a review. Saudi J. Biol. Sci. 28 (1), 1167–1176.
- Perumal, B., Muneeswaran, V., Pothirasan, N., Reddy, K.R.M., Pranith, K.S.S., Chaitanya, K., et al., 2021. Bee eloper: A novel perspective for emancipating honey bees from its comb using a contrivable technique. In: AIP Conference Proceedings: AIP Publishing LLC, 020003.
- Pokhrel, S., Thapa, R.B., Neupane, F., Shrestha, S., 2006. Abscending behavior and management of *Apis cerana* F. honeybee in Chitwan, Nepal. J. Inst. Agric. Animal Sci. 27, 77–86.
- Ricigliano, V.A., Mott, B.M., Floyd, A.S., Copeland, D.C., Carroll, M.J., Anderson, K.E., 2018. Honey bees overwintering in a southern climate: longitudinal effects of nutrition and queen age on colony-level molecular physiology and performance. Sci. Rep. 8 (1), 1–11.
- Sihag, R.C., Gupta, M., 2011. Development of an artificial pollen substitute/supplement diet to help tide the colonies of honeybee (*Apis mellifera* L.) over the dearth season. J. Apicultural Sci. 55 (2).
- Stevanovic, J., Stanimirovic, Z., Radakovic, M., Kovacevic, S., 2010. Biogeographic study of the honey bee (*Apis mellifera* L.) from Serbia, Bosnia and Herzegovina and Republic of Macedonia based on mitochondrial DNA analyses. Russian J. Genetics 46 (5), 603–609.
- Taha, EL.-K., AL-Kahtani, S.N., 2019. Comparison of the activity and productivity of Carniolan (*Apis mellifera carnica* Pollmann) and Yemeni (*Apis mellifera jemenitica* Ruttner) subspecies under environmental conditions of the Al-Ahsa oasis of eastern Saudi Arabia. Saudi J. Biol. Sci. 26 (4), 681–687.
- Tarpy, D.R., Keller, J.J., Caren, J.R., Delaney, D.A., 2011. Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens. Insectes Sociaux 58 (4), 569–574.
- Taylor, M.A., Robertson, A.W., Biggs, P.J., Richards, K.K., Jones, D.F., Parkar, S.G., Blenau, W., 2019. The effect of carbohydrate sources: Sucrose, invert sugar and components of mānuka honey, on core bacteria in the digestive tract of adult honey bees (*Apis mellifera*). PLoS One 14 (12), e0225845. <https://doi.org/10.1371/journal.pone.0225845>.
- Winston, M.L., 1980. Swarming, afterswarming, and reproductive rate of unmanaged honeybee colonies (*Apis mellifera*) L'essaimage, les essaims secondaires et la taux de reproduction de colonies d'abeilles non domestiquées (*Apis mellifera*). Insectes Sociaux 27 (4), 391–398.
- Winston, M.L., 1991. The biology of the honey bee. Harvard University Press.