Evaluation of Hygienic Behaviour in Different Status of Managed Honey Bee Colonies

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ABSTRACT

Hygienic behaviour is a desirable trait in honey bees and involves the detection of diseased, infected broods and their quick removal from the nest by worker honey bees. The pin-killed test and gene expressions of five primers for hygienic behaviour using Real-Time PCR were used to compare colonies from swarming, queen-less colonies, and dividing headed by a queen unifying the beekeeping process, colony strength, and genetic origin of the three types of studying nuclei and returning the hygienic behaviour differentiation to the colonies' population status. Our findings revealed that the removal of dead broods in swarming colonies was significantly higher than that of both dividing colonies headed by queen and queen-less dividing colonies. Swarming colonies exhibiting different rates of hygienic behaviour using pin killed test (HB %) correlated with its different genetic structures using gene expression. We recommend that this trait be considered in queen-rearing programs and that hives be left to swarm, which is a stimulant and promotes the genes of hygienic behaviour inductions associated with swarming.

Keywords: Honey bee colonies, Hygienic behaviour, swarming, dividing, queen-less, laying worker, brood pin killed test, Gene expression.

INTRODUCTION

Hygienic behaviour is a mechanism of quickly uncapping, removing diseased broods and interrupting the infectious cycle (Uzunov *et al.*, 2014 and Abou-shaara *et al.*, 2018). Furthermore, disease resistance if bees can remove brood from the nest before the pathogen becomes infectious, which is a desirable trait in honey bees that confers colony-level resistance against various brood diseases (Balhareth *et al.*, 2012; Chandran *et al.*, 2015 and Nganso *et al.*, 2017).

Naturally, honey bees developed some mechanisms to defend against invaders. The term "hygienic behaviour" was originally first mentioned (Rothenbuhler, 1964), and means the ability of worker bees to identify dead broods and remove them from the cell, thus reducing the infestation (Bigio, 2014 and Chandran *et al.*, 2015). But its genetic basis was first suggested by Rothenbuhler (1964), who proposed a two-loci model to explain hygienic behaviour inheritance (Rothenbuhler, 1964 and Thompson, 1964). Hygienic behaviour in honey bees is controlled at least partly by two recessive genes, one for uncapping cells and the other for removing brood remains (Panasiuk *et al.*, 2009; Balhareth *et al.*, 2012 and Bigio, 2014). The degree of hygienic behaviour varies between colonies, both because of the genetic composition of the worker bees and because of the strength and age distribution of the colony population (Simone *et al.*, 2009 and Büchler *et al.*, 2013). Not worthy, the hygienic behaviour of honey bees has been described as a two-step process bees uncap wax-covered cells containing diseased brood (fifth-instar larvae and pupae) and then remove the brood (Bigio, 2014 and Boutin *et al.*, 2015).

It is known that in the honey bee colony, the queen has a dominant influence on the behaviour and the physiology of the worker bee's community (Simon *et al.*, 2001), the presence of the queen in a group of worker bees inhibits the ovarioles development in the bee workers by its pheromones (Khodairy & Moustafa, 1963 and Pinto *et al.*, 2000). The workers establish a retinue behaviour as a special behaviour towards their queen (Velthuis & Van Es, 1964; Velthuis, 1970 and Slessor *et al.*, 1988) recognizing her by the special queen pheromones leading to the queen right behaviour, which in turn influences the development of the worker ovaries. When the queen is taken away or lost from its queen-less colony, ovarioles development in the orphan workers begins and may result in the so-called laying worker (Khodairy and Moustafa, 1963).

DOI: 10.21608/asejaiqjsae.2024.388838

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Received, September 10, 2024, Accepted, October 10, 2024.

The dividing system (artificial swarm) and nuclei production are some of the most profitable products of the beekeepers, either for sale to others for establishing new colonies in apiaries or in the same apiary used for increasing the number of colonies. Divided the strong colonies is considered one of the controlling methods of swarming (Winston, 1980; Winston, 1991; Winston *et al.,* 1991 and Lewis & Schneider, 2008). The production of package bees and nuclei needs high experience and knowledge from the beekeeper to identify several important points associated with the success of the division process as; the most suitable seasons for the division, the best colonies that will be divided, the type of queens and the ways of introducing queens in the new colonies (Masry *et al.,* 2015; Masry & Abdelaal, 2016; Al Naggar *et al.,* 2018 and Taha *et al.,* 2019).

Swarming is an advantage to the honey bees, which use swarms to increase their numbers, doubling their chances of survival and ensuring the survival of their species. However, it is a distinct disadvantage for beekeepers. Consequently, beekeepers manage bee hives to reduce the incidence of swarming to the extent possible. It usually occurs in spring or early summer and sometimes at other times of the year when local conditions permit and begins in the warmer hours of the day (Winston *et al.,* 1991; Woyciechowski & Kuszewska, 2012; Richards *et al.,* 2015; Tahmasbi *et al.,* 2015 and Zhu *et al.,* 2019). The nest site selection process starts with several hundred scout bees flying from the swarm cluster to search for tree cavities and other potential nest sites. Then they use the waggle dances to steer them to the swarm's new home. Once the scouter bees have completed their deliberations, they stimulate the other members of the swarm to launch into flight and to the chosen site (Avitabile *et al.,* 1975; Winston, 1980; Lensky & Slabezki, 1981; Ferrari *et al.,* 2008; Bencsik *et al.,* 2011; Richards, 2012; Uzunov *et al.,* 2014 and Andonov *et al.,* 2019**).** Swarmed colonies have good hygienic behaviour tested by pin killed test (HB %) which correlated with more biological activities of a higher amount of brood, pollen grains, honey and bee population than dividing colonies (Kandel *et al.,* 2024).

The objective of this study is to compare the hygienic behaviour of HB% using the pin-killed test of three tested groups of honey bee colonies' populations of swarms, divisions headed by queens, and queen-less divisions. Gene expressions of five primers for hygienic behaviour using Real-Time PCR and RNA analysis are the molecular tools used in this study to compare colonies from swarming, orphan colonies, and dividing headed by a queen. Unifying the beekeeping process and genetic origin of the three types of studying nuclei and returning the hygienic behaviour differentiation to the colonies' population status was demonstrated in this investigation.

MATERIAL AND METHODS

A total of twelve mother colonies were established and headed by mated queens that have the same genetic origin obtained from Menzala, the previous region of isolated Carniolan bees in Egypt (Fathy *et al.,* 2019), transferred to the experimental apiary of Al-Sabahia Research Station, Alexandria, Egypt on March 2021.

In May 2021, six natural swarms were caught separately from the mother colonies. Furthermore, two types of divisions as well (six colonies with queens, and six queen-less colonies (orphan colonies or laying worker colonies) were established and housed in a Langstroth hive simultaneously with the swarm mimicking approximately equal in their strength (stored honey, stored pollen, number of frames covered with bees, brood production, and queen status).

Queens were introduced only in six colonies, using a semi-circle cage, and released after 48 hr (Masry & Abdelaal, 2016). The released queens were inspected daily for recording the starting of laying eggs to be confident the queen was accepted.

The emphasis lies in unifying the genetic origin factor and beekeeping process of both types of studying colonies and returning the Hygienic behaviour differentiation to the colony status whether it was a natural swarm diving colony or queen-less colony (laying workers – orphan colony).

3.1. Methods for testing hygienic behaviour (pin killed test):

Pin killed test was carried out one time simultaneously with samples collected during the active seasons (Evans *et al.,* 2013 and Uzunov *et al.,* 2014), considered an indicator of hygienic behaviour was estimated in each colony cleaning cell numbers were counted after 12, 24, 48 hr of the 100 sealed worker cells that were pierced by a tiny needle described (Gramacho *et al.,* 1999; EID, 2013; Abou-Shaara *et al.,* 2018 and Kandel *et al.,* 2024). One sealed brood comb was chosen from each of the three experimental colonies, then 10 x 5 cm were marked using a marker pen and counted as a total number of 100 marked brood cells (X) (Fig. 1). A pin was used to kill the marked cells. Then, those treated combs were returned to their colonies and after 12, 24, and 48 hr, the number of removed dead broods from marked brood cells by worker bees was counted and recorded as (Z) (Fig. 2). This observation was made one time in the early summer for both 18 tested nuclei. The percentage of hygienic behaviour (pin test) calculated by this formula HB %= Z/X*100 (Kandel *et al.,* 2024).

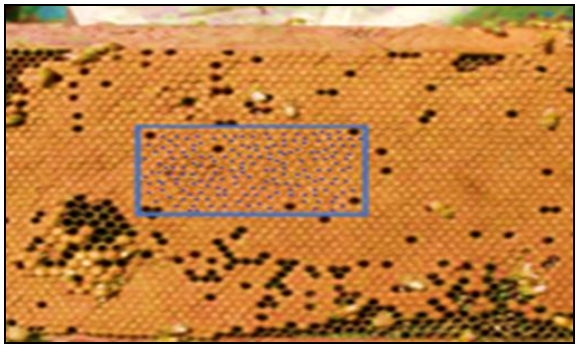

Fig.1. Marked brood cells on honey bee-sealed brood comb for the pin-killed test

Fig. 2. Honey bee workers detect and remove the dead sealed brood

3.2. Gene expression of hygienic behaviour

3.2.1. Sampling

Honey bee workers were collected from the 18th experimental colonies separately two times a week after housing the treatment colonies, and during the early summer of 2021 with a composite pooled sample of approximately 50 worker bees equally derived from the same colony (ca. 25 worker bees per colony in one time). Honey bee workers were chosen from the hive entrance (foragers) and maintained alive in ventilated cages, transported cold to the laboratory, where they were stored at -80°C until processing (Evans *et al.,* 2013 and Scheiner *et al.,* 2013). In total, 36 samples (6 colonies x 3 treatments x 2 times) were collected.

3.2.2. Total RNA extraction and cDNA synthesis:

According to the manufacturer's protocol, total RNA was isolated from worker bees using an RNA extraction kit (Thermo scientific). RNA concentrations were determined by using a Nanodrop ND-1000 spectrometer (Nanodrop Technologies, Wilmington, DE) (Fig. 3). Then cDNA was synthesized using oligo-dT primers (Thermo Fisher Scientific, Schwerte, Germany) and reverse transcriptase (M-MLV and Revertase, Promega, Mannheim, Germany) following the manufacturer's instructions. For cDNA synthesis, 800 ng of RNA were used, after which the resultant cDNA was diluted 1:10 prior to use in quantitative real-time PCR (qPCR) (Fig. 4) (Dainat & Neumann, 2013; Evans *et al.,* 2013; Kandel & Paxton, 2023 and Mahmoud *et al.,* 2024).

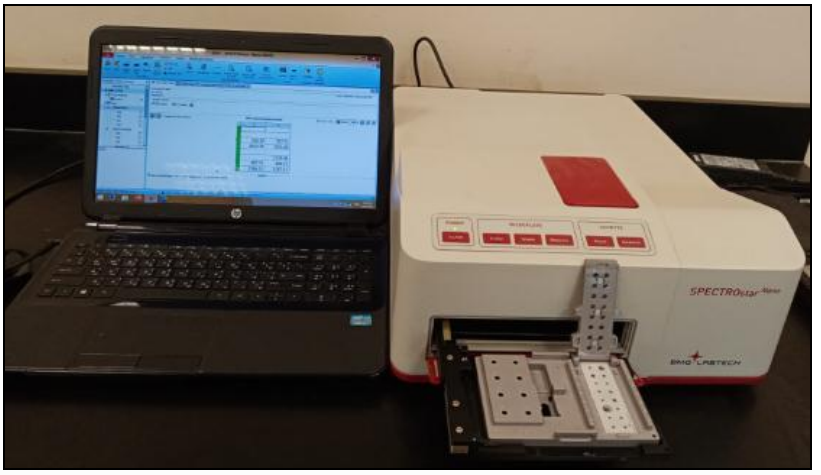

Fig.3. Nanodrop for determination of RNA concentrations

***Fig. 4. PCR* or *Polymerase Chain Reaction (PCR)* is used to create several copies of a certain DNA segment and cDNA synthesis**

3.2.3. Expressions of genes (Amplification of quantitative real-time PCR)

Quantitative real-time PCR qPCR was performed in a 20 µL reaction mixture consisting of 1X Sso Advanced TM SYBR Green supermix (Bio-Rad), 0.2 µL of each primer, and 1 µL (100 ng) of cDNA template (Fig. 5). The oligonucleotide primers for qPCR are shown in Table (1) (Hamiduzzaman *et al.*, 2017). The reaction was carried out in 96-well plates using a Bio-Rad I cycler (Bio-Rad Crop., Hercules, CA.) programmed with the following temperature profile: 95 °C for 30 sec followed by 50 cycles of 95 °C for 5 sec, 60 °C for 30 sec, melt curve from 65 to 95 °C in 0.5 °C/5 sec increments. The melt curve segregation was analyzed to confirm each amplicon. Relative expression levels were calculated by the DCT method. Threshold cycle (CT) numbers for target genes were deducted from the reference gene for each sample. Ribosomal protein subunit 5 was used for normalization and chosen as the reference gene. According to the primer efficiencies via serial dilutions of known templates, a low transcript level (10 copies) was detected at 42 cycles (Kandel *et al.*, 2024 and Mahmoud *et al.*, 2024). Thus, a CT value of 35 cycles was assigned above 35.

Table 1.The primers used to amplify the hygienic and grooming behaviour genes evaluated

Gene name	Sequence `5-`3	Gene ID	Reference
HYM*	F: 5′- CTC TTC TGT GCC GTT GCA TA-3′ R: 5′- GCG TCT CCT GTC ATT CCA TT-3′	GB17538	(Evans *et al.*, 2006)
PUf68*	F: 5′- CAA GAC CTC CAA CTA GCA TG-3′ R: 5′- CAA CAG GTG GTG GTG GTG-3′	GB13651	(Hamiduzzaman *et al.*, 2012)
CYP9Q3*	F: 5′- GTT CCG GGA AAA TGA CTA C-3′ R: 5′- ACT CTC GAC GCA CAT CCT G-3′	XM_006562300	(Mao *et al.*, 2011)
BICh*	F: 5′- GTG CTT GGG TTA GGA TGT GTAC-3′ R: 5′- GTT AAT CTT CTT CCG CTA CTG-3′	GB10249	(Hamiduzzaman *et al.*, 2012)
Vg*	F: 5′- CTG TCG ATG GAG AAG GGA ACT-3′ R: 5′- CTT GCC TAC GAG TCT TGC TGT-3′	NM_001011578	(Hamiduzzaman *et al.*, 2017)
(β-actin) Housekeeping	F: 5′- ATGCCAACACTGTCCTTTCTGG-3′ R: 5′- GACCCACCAATCCATACGGA-3′		(Forsgren *et al.*, 2009)

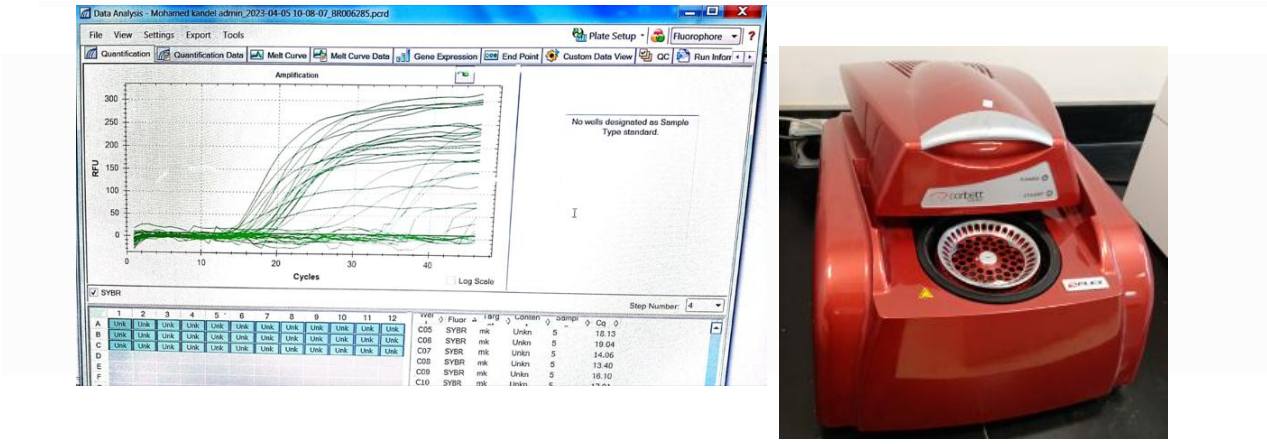

Fig.5. Reverse transcription-polymerase chain reaction (RT-PCR) is a relatively simple and inexpensive technique to determine the expression level of hygienic genes

RESULTS AND DISCUSSION

Eighteen bee hives were evaluated for hygienic behaviour using a brood pin-killed test one time according to previous studies, hygienic behaviour varied between years and seasons (Bigio, 2014; Boutin *et al.*, 2015; Gempe *et al.*, 2016 and Kandel *et al.*, 2024).

Our results show that the mean of HB % in the swarming colonies was 35.58 %, 66.58 %, and 91.08 % after 12, 24, and 48 hr, respectively. However, in dividing colonies with the queen 26.83%, 60.25%, and 80% after 12, 24, and 48 hr, respectively. Furthermore, it was 19.67 %, 50.83 %, and 73.33 % after 12, 24, and 48 hr, respectively in queen-less dividing colonies (laying workers).

The total mean for swarming colonies, dividing queen colonies, and queen-less dividing colonies was 63.7, 55.4, and 48 %. There was a significant difference noticed between swarming, queen-dividing colonies and queen-less dividing colonies in the hygienic behaviour test described in Fig. (6).

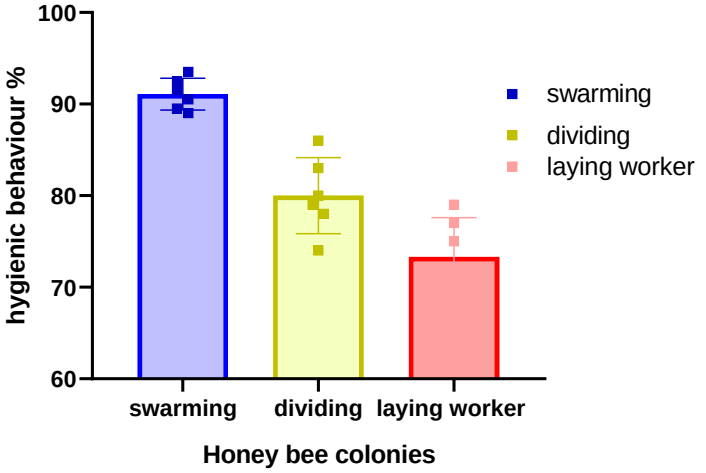

Fig.6. Hygienic behavior test (HB%) of eighteen colonies headed by queens both of natural swarm, dividing, and orphan colonies carried out at the same time.

Our results show that the expression of hygienic behaviour genes was better expressed in swarming colonies than in dividing queen colonies, and queen-less dividing colonies. Hygienic behaviour significantly differs between the three tested colonies population relies on quantitative real-time PCR with CT value above 35 as shown in Fig. (7).

A different expression of the hygienic behaviour trait in the honeybee since we recorded different levels of brood removal in both honeybee experimental colonies. Our findings corroborate the results of a previous study of the pin-killed test (Kandel *et al.*, 2024), and others which found a different expression of hygienic behaviour between honey bee races, varroa infestation response (Boutin *et al.*, 2015; Gempe *et al.*, 2016; Hamiduzzaman *et al.*, 2017 and Nganso *et al.*, 2017)

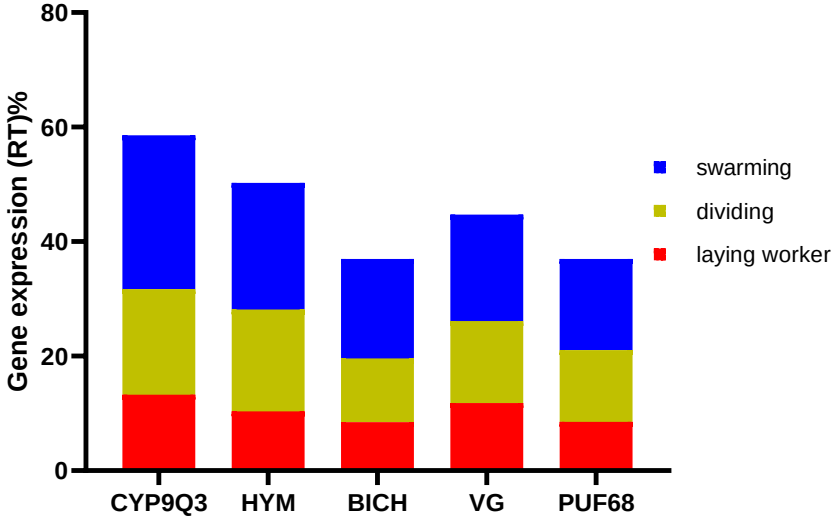

Fig.7. RT- PCR reaction of five genes specific for hygienic behaviour from six colonies in each status of swarm, dividing, orphan honey bee colonies

None of the 18 study colonies had a mean removal level over 93.5 % after 48 h, which is a convenient threshold level above which colonies are considered fully hygienic. However, one colony had a mean of 93.5 % over the experiment. These results agree with previous studies that reported variable hygienic behaviour levels in swarming honey bee colonies and confirm that hygienic behaviour is at a level higher in colonies established from swarming than those that have the same production, population strength, time start-up, and genetic origin (Kandel *et al.,* 2024).

Regarding the time of hygienic behaviour test performance, according to the previous studies hygienic behaviour was strongly influenced by the season in which the HB test was performed, and the highest level of HB was in summer (Bigio, 2014 and Uzunov *et al.,* 2014). Furthermore, there was a significant variability between the genotypes from different subspecies therefore in our study the HB test was carried out only in the summer on the same genetic origin colonies.

It is well known that honey bee colonies' decline was affected by several stressors including pesticides, pathogens and parasites such as Varroa destructor, fungal diseases (*Nosema ceranae* /*Nosema Apis*, *Ascosphaera Apis*), bacterial disease (American foulbrood, European foulbrood), viral diseases (DWV, SBV, BQCV, etc..), and Protozoans (*Crithidia Mellificae, Lotmaria Passim*, *etc*..). Previous study demonstrated that the hygienic honey bees were linked to decreased Varroa mite populations and a lower prevalence of honey bee viruses at the colony level. These bees also exhibited enhanced individual immunity, which may have helped reduce virus levels, furthermore, the lower Varroa numbers due to social immunity likely played a role as well (Bigio, 2014 and Erez *et al.,* 2022).

In summary, these results demonstrated that the hygienic behaviour level might be related to the enhancement and induction of vital gene expression during swarming behaviour that might be useful as a biomarker for behavioural traits in bees. We recommend that hives must be left to swarm or prepared to swarm, which is a stimulant and promotes the genes of hygienic behaviour inductions associated with swarming behaviour. Hygienic behaviour must be considered in queen-breeding programs because a strong immune system ensures resistance to disease pathogens such as fungal diseases, viral diseases, bacterial diseases, and protozoans. Furthermore, this behaviour enables workers to quickly eliminate external pests or pests such as varroa mites.

CONCLUSION

In conclusion, swarming colonies have higher levels of dead broods' removal from the nest than both dividing colonies headed by queen and queen-less dividing colonies, which correlated with its different genetic structures of hygienic behaviour gene expression rate. We recommend that hives be left allowed to swarm which is a stimulant and promotes the genes of hygienic behaviour inductions associated with swarming. This trait should be taken into consideration in queen-breeding programs. The hygienic breeding should be from swarmed colonies with active functional genes specific to hygienic behaviour. This is better than breeding from dividing colonies. We also do not recommend breeding from colonies of previously queen-less colonies and in which queens were introduced.

REFERENCES

Abou-Shaara, F. Hossam, M. Poliak, and T. Čermáková. 2018."Field and laboratory assessment to hygienic behavior of carniolan honey bees with studying impacts of oxalic acid on grooming behavior." *13*(1):256–265.

Al Naggar, Y., G.Codling, J. P.Giesy and A.Safer. 2018.Beekeeping and the Need for Pollination from an Agricultural Perspective in Egypt. *Bee World*, *95*(4): 107–112. https://doi.org/10.1080/0005772x.2018.1484202

Andonov, S., C. Costa, A. Uzunov, P. Bergomi, D. Lourenco and I. Misztal. 2019. Modeling honey yield, defensive and swarming behaviors of Italian honey bees (*Apis mellifera ligustica*) using linear-threshold approaches. *BMC Genetics*, *20*(1):1–9. https://doi.org/10.1186/s12863-019-0776-2

Avitabile, A., R. A. Morse and R. Boch. 1975.Swarming Honey Bees Guided by Pheromones1. *Annals of the Entomological Society of America*, *68*(6): 1079–1082. https://doi.org/10.1093/aesa/68.6.1079

Balhareth, H. M., A. S. Alqarni and A. A.Owayss. 2012.Comparison of hygienic and grooming behaviors of indigenous and exotic honeybee (*Apis mellifera*) races in central Saudi Arabia. *International Journal of Agriculture and Biology*, *14*(6): 1005–1008.

Bencsik, M., J. Bencsik, M. Baxter, A. Lucian, J. Romieu and M.Millet. 2011. Identification of the honey bee swarming process by analysing the time course of hive vibrations. *Computers and Electronics in Agriculture*, *76*(1): 44–50. https://doi.org/10.1016/j.compag.2011.01.004

Bigio, G. 2014. *Hygienic Behaviour in Honey Bees*. (Doctoral dissertation, University of Sussex). 97.

Boutin, S., M. Alburaki, , P. L.Mercier, P.Giovenazzo and N. Derome. 2015. Differential gene expression between hygienic and non-hygienic honeybee (*Apis mellifera* L.) hives. *BMC Genomics*, *16*(1): 1–13. https://doi.org/10.1186/s12864-015-1714-y

Büchler, R., S. Andonov, K. Bienefeld, C. Costa, F. Hatjina, N. Kezic, P. Kryger, M. Spivak, A.Uzunov and J. Wilde. 2013.Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research*, *52*(1). https://doi.org/10.3896/IBRA.1.52.1.07

Chandran, P. A., P. J. Chandran and S. Kandamuthan. 2015. Journal of international academic research for multidisciplinary. *Journal of International Academic Research for Multidisciplinary*, *3*(7): 319–328.

Dainat, B. and P. Neumann. 2013.Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. *Journal of Invertebrate Pathology*, *112*(3): 278–280. https://doi.org/10.1016/j.jip.2012.12.009

EID, K. S. A. 2013. *KHALID. S. A. EID Plant Protection Department, Faculty of Agriculture, Damanhour University. 12*(1).

Erez, T., E. Bonda, P. Kahanov, O. Rueppell, K.Wagoner, N. Chejanovsky and V. Soroker. 2022. Multiple benefits of breeding honey bees for hygienic behavior. Journal of Invertebrate Pathology, 193. p.107788.

Evans, J. D., K. Aronstein, Y. P. Chen, C. Hetru, J. L. Imler, H. Jiang, M. Kanost, G. J.Thompson, Z. Zou and D. Hultmark. 2006.Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, *15*(5):645–656. https://doi.org/10.1111/j.1365-2583.2006.00682.x

Evans, D. Jay, R. S. Schwarz, Y. P. Chen, G. Budge, R. S.Cornman, P. De La Rua, J. R. De Miranda, S. Foret, L. Foster, L. Gauthier, E. Genersch, S. Gisder, A.Jarosch, R. Kucharski, D.Lopez, C. M.Lun, R. F. A. Moritz, R. Maleszka, I. Muñoz and M. A.Pinto. 2013. Standard methods for molecular research in *Apis mellifera*. *Journal of Apicultural Research*, *52*(4). https://doi.org/10.3896/IBRA.1.52.4.11

Fathy, H.M., A.M.I. Zohairy and M.A.I. Hamada. 2019. Impact of Different Workers Population in Queenless Rearing Colonies on the Quality of Produced Apis mellifera carnica Queen in Manzala Region. Journal of Plant Protection and Pathology, 10(7), pp.355-358.

Ferrari, S., M. Silva, M. Guarino and D.Berckmans. 2008.Monitoring of swarming sounds in bee hives for early detection of the swarming period. *Computers and Electronics in Agriculture*, *64*(1):72–77. https://doi.org/10.1016/j.compag.2008.05.010

Forsgren, E., J. R. De Miranda, M. Isaksson, S. Wei and I.Fries. 2009. Deformed wing virus associated with Tropilaelaps mercedesae infesting European honey bees (*Apis mellifera*). *Experimental and Applied Acarology*, *47*(2): 87–97. https://doi.org/10.1007/s10493-008-9204-4

Gempe, T., S.Stach, K. Bienefeld, M.Otte and M.Beye. 2016. Behavioral and molecular studies of quantitative differences in hygienic behavior in honeybees. *BMC Research Notes*, *9*(1):1–8. https://doi.org/10.1186/s13104-016-2269-y

Gramacho, K. P., L. S. Gonçalves, P. Rosenkranz and D. De Jong.1999. Influence of body fluid from pin-killed honey bee pupae on hygienic behavior. *Apidologie*, *30*(5–6): 367–374. https://doi.org/10.1051/apido:19990502

Hamiduzzaman, M. M., B. Emsen, G. J. Hunt, S.Subramanyam, C. E.Williams, J. M.Tsuruda and E.Guzman-Novoa. 2017. Differential Gene Expression Associated with Honey Bee Grooming Behavior in Response to Varroa Mites. *Behavior Genetics*, *47*(3):335–344. https://doi.org/10.1007/s10519-017-9834-6

Hamiduzzaman, M. M., A.Sinia, E. Guzman-Novoa and P. H.Goodwin. 2012. Entomopathogenic fungi as potential biocontrol agents of the ecto-parasitic mite, *Varroa destructor*, and their effect on the immune response of honey bees (Apis mellifera L.). *Journal of Invertebrate Pathology*, *111*(3): 237–243. https://doi.org/10.1016/j.jip.2012.09.001

Kandel, M., S. Masry, E.-S. Hafez, M. El- kady and N. Hassona. 2024. Genetic Diversity and Biological Activities of Nuclei from Swarming and Dividing of Honey Bee *Apis mellifera* L. Colonies. *Journal of Plant Protection and Pathology*, *0*(0): 33–43. https://doi.org/10.21608/jppp.2024.259561.1206

Kandel, Mohamed and R. J.Paxton. 2023.Nationwide Screening for Bee Viruses in *Apis mellifera* Colonies in Egypt:1–12.

Khodairy, M. M. and A. M. Moustafa. 1963. Influence of Certain Types of Bee-Stored Pollen on Ovarian Development of Honey Bee Workers under Queenless Conditions. 37(4).

Lensky, Y. and Y. Slabezki. 1981. The inhibiting effect of the queen bee (*Apis mellifera* L.) foot-print pheromone on the construction of swarming queen cups. *Journal of Insect Physiology*, *27*(5):313–323. https://doi.org/10.1016/0022-1910(81)90077-9

Lewis, L. A. and S. S.Schneider. 2008. “Migration dances” in swarming colonies of the honey bee, *Apis mellifera*. *Apidologie*, *39*(3):354–361. https://doi.org/10.1051/apido:2008018

Mahmoud, S.H., M. Kandel, H. El-Seedi and Y. Al Naggar. 2024. Original article Honey bee venom promotes the immune system and reduces Vairimorpha (Nosema) ceranae infection in honey bees (Apis mellifera L .). https://doi.org/10.1007/s13592-023-01048-2

Mao, W., M. A.,Schuler and M. R.Berenbaum. 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). *Proceedings of the National Academy of Sciences of the United States of America*, 108(31): 12657–12662. https://doi.org/10.1073/pnas.1109535108

Masry, S. H.D. and A. A. A. Abdelaal. 2016. Impact of arid land conditions on biological activities of honeybee colonies. *Journal of Entomology*, *13*(4): 148–154. https://doi.org/10.3923/je.2016.148.154

Masry, Saad H. D., T. E.Abd El-Wahab and N. M. Hassona. 2015. Origin, Weight at Emergence of Virgin Honey Bee Queens and its Effect on Acceptance During Introduction. *Academic Journal of Entomology*, *8*(4): 174–182. https://doi.org/10.5829/idosi.aje.2015.8.4.96198

Nganso, B. T., A. T. Fombong, A. A. Yusuf, C. W. W. Pirk, C. Stuhl and B. Torto. 2017. Hygienic and grooming behaviors in African and European honeybees - New damage categories in Varroa destructor. *PLoS ONE*, *12*(6).

https://doi.org/10.1371/journal.pone.0179329

Panasiuk, B., W. Skowronek and D. Gerula. 2009. Effect of period of the season and environmental conditions on rate of cleaning cells with dead brood. *Journal of Apicultural Science*, *53*(1):95–103.

Pinto, L. Z., M. M. G. Bitondi and Z. L. P. Simões. 2000. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *Journal of Insect Physiology*, *46*(2):153–160. https://doi.org/10.1016/S0022-1910(99)00111-0

Richards, Jessica, M.Carr-Markell, A. Hefetz, C. M.Grozinger and H. R. Mattila. 2015. Queen-produced volatiles change dynamically during reproductive swarming and are associated with changes in honey bee (*Apis mellifera*) worker behaviour. *Apidologie*, *46*(6): 679–690. https://doi.org/10.1007/s13592-015-0358-x

Richards, JY. 2012. Chemical communication and genomics of swarming behaviour in honey bees *(Apis mellifera L.)*. *May*. https://etda.libraries.psu.edu/catalog/16241

Rothenbuhler, W. C. 1964. Behavior genetics of nest gleaning in Honey bees. IV. responses of F1 and backcross generations to disease-killed brood. *Integrative and Comparative Biology*, *4*(2): 111–123. https://doi.org/10.1093/icb/4.2.111

Scheiner, R., C. I. Abramson, R. Brodschneider, K.Crailsheim, W. M. Farina, S. Fuchs, B. Grünewald, S. Hahshold, M. Karrer, G. Koeniger, N.Koeniger, R. Menzel, S. Mujagic, G. Radspieler, T. Schmickl, C. Schneider, A. J. Siegel, M. Szopek and R.Thenius. 2013.Standard methods for behavioural studies of *Apis mellifera*. *Journal of Apicultural Research*, *52*(4): 1–58. https://doi.org/10.3896/IBRA.1.52.4.04

Simon, U. E., R. F. A. Moritz and R. M.Crewe. 2001. The ontogenetic pattern of mandibular gland components in queenless worker bees (*Apis mellifera capensis* Esch.). *Journal of Insect Physiology*, *47*(7): 735–738. https://doi.org/10.1016/S0022-1910(00)00167-0

Simone, M., J. D.Evans and M. Spivak. 2009. Resin collection and social immunity in honey bees. *Evolution*, *63*(11):3016–3022. https://doi.org/10.1111/j.1558-5646.2009.00772.x

Slessor, K. N., L. A. Kaminski, G. G. S. King, J. H. Borden and M. L.Winston. 1988.Semiochemical basis of the retinue response to queen honey bees. *Nature*, *332*(6162): 354–356. https://doi.org/10.1038/332354a0

Taha, E. K. A., R. A. Taha and S. N.AL-Kahtani. 2019. Nectar and pollen sources for honeybees in Kafrelsheikh province of northern Egypt. *Saudi Journal of Biological Sciences*, *26*(5): 890–896. https://doi.org/10.1016/j.sjbs.2017.12.010

Tahmasbi, G., M. A. Kamali, R. Ebadi, A.Nejati Javaremi, M. Babaei, A. A. Gharadaghi and R.Bahraini. 2015. Genetic trends and parameters of honey production, swarming and defense behaviour in Iranian honeybee (Apis mellifera meda) colonies. *Journal of Agricultural Science and Technology*, *17*: 1735–1742.

Thompson, V.C., 1964. Behaviour genetics of nest cleaning in honeybees. III. Effect of age of bees of a resistant line on their response to disease-killed brood. Journal of Apicultural Research, 3(1), pp.25-30.

Uzunov, A., C. Costa, B. Panasiuk, M.Meixner, P. Kryger , F. Hatjina, M. Bouga, S. Andonov, M.Bienkowska, Y. Le Conte, J. Wilde, D. Gerula, H. Kiprijanovska, J.Filipi, P. Petrov, L.Ruottinen, H. Pechhacker, S. Berg, W. Dyrba and R. Büchler. 2014. Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. *Journal of Apicultural Research*, *53*(2):248–260. https://doi.org/10.3896/IBRA.1.53.2.06

Velthuis, H. H. W. 1970. Queen substances from the abdomen of the honey bee queen. *Zeitschrift Für Vergleichende Physiologie*, *70*(2): 210–221. https://doi.org/10.1007/BF00297717

Velthuis, H. H. W. and J. Van Es. 1964. Some Functional Aspects of the Mandibular Glands of the Queen Honeybee. *Journal of Apicultural Research*, *3*(1):11–16. https://doi.org/10.1080/00218839.1964.11100076

Winston, M. L. 1991. The biology of the honey bee. (Book) Harvard University Press.

Winston, M. L. 1980. Swarming, afterswarming, and reproductive rate of unmanaged honeybee colonies (*Apis mellifera*). *Insectes Sociaux*, *27*(4):391–398. https://doi.org/10.1007/BF02223731

Winston, M.L., H. A. Higo, S. J. Colley, T. Pankiw and K. N. Slessor. 1991. The role of queen mandibular pheromone and colony congestion in honey bee (*Apis mellifera L.*) reproductive swarming (Hymenoptera: Apidae). *Journal of Insect Behavior*, *4*(5): 649–660. https://doi.org/10.1007/BF01048076

Woyciechowski, M. and K. Kuszewska. 2012. Swarming generates rebel workers in honeybees. *Current Biology*, *22*(8):707–711. https://doi.org/10.1016/j.cub.2012.02.063

Zhu, X., X.Wen, S. Zhou, X. Xu, L. Zhou and B. Zhou. 2019.The temperature increase at one position in the colony can predict honey bee swarming (*Apis Cerana*). *Journal of Apicultural Research*, *58*(4): 489–491. https://doi.org/10.1080/00218839.2019.1632149

الملخص العربي

تقييم السلوك الصحي في حالات مختلفة من طوائف نحل العسل

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السلوك الصحي هو صفة مرغوبة في نحل العسل والتي يتضمن اكتشاف الحضنة المريضة والمصابة وإزالتها بسرعة من العش بواسطة شغالات نحل العسل. في هذه الدراسة تم استخدام اختبار القتل بالدبوس، وكذا التعبير الجيني لخمسة بادئات (Primers) للسلوك الصحي باستخدام تفاعل البوليميراز المتسلسل (Real-Time PCR) وذلك بهدف مقارنة الثلاث حالات من طوائف النحل المختبرة وهي طوائف ناتجة من التطريد الطبيعي، وطوائف ناتجة من التقسيم (التطريد الصناعي بها ملكات) وطوائف ناتجة من التقسيم بدون ملكات (خلايا يتيمة) مع توحيد الأصل الوراثي وقوة المستعمرات والعمليات النحلية للأنواع الثلاثة من طوائف النحل محل الدراسة وبالتالي إعادة الإختلاف في السلوك الصحي إلى حالة الطائفة. كشفت نتائجنا أن معدل إزالة الحضنة الميتة في طوائف التطريد كانت أعلى بكثير منها في كل من طوائف التقسيمات التي بها ملكات وطوائف التقسيمات بدون ملكات وذلك باستخدام اختبار القتل بالدبوس للحضنة (HB) ٪. كما أن طوائف التطريد الطبيعي أظهرت معدلات أعلي في السلوك الصحي ومختلفة عن الأخري باستخدام التعبير الجيني. لذا نوصي بأخذ هذه الصفة بعين الإعتبار في برامج تربية الملكات، كما نوصي بترك خلايا النحل للتطريد الطبيعي والذي يعد منبهًا ويعزز من الجينات المسؤلة عن السلوك الصحي.

الكلمات المفتاحية: طوائف النحل، السلوك الصحي، التطريد الطبيعي، التقسيمات، عدم وجود ملكة، الشغالات الواضعة للبيض، اختبار قتل الحضنة بالدبوس، التعبير الجيني.