

NATURAL RESISTANCE TRAITS IN APIS MELLIFERA SAHARIENSIS: A FOCUS ON HYGIENIC BEHAVIOR AND SEASONAL PERFORMANCE

Adjlane Noureddine*, Kaouche Soumeiya*, Diffalah Hocine**,
Haddad Nizar***,
Hatjina Fani****

*University of Boumerdes, Faculty of Sciences, Department of Agronomy, Algeria

**National Association of Professional Beekeepers (NAPB), Algeria

***Innovations & Business Development, Fresh Del Monte, De L'Ora Bio, Amman, Jordan

****Division of Apiculture, Inst. of Animal Science, ELGO 'DIMITRA', Greece

Citation: Adjlane N., Kaouche S., Diffalah H., Haddad N., Hatjina F., 2025, NATURAL RESISTANCE TRAITS IN APIS MELLIFERA SAHARIENSIS: A FOCUS ON HYGIENIC BEHAVIOR AND SEASONAL PERFORMANCE. *Natural Resources and Sustainable Development*, vol. 15, is. 2, pp: 225-232. <https://doi:10.31924/nrsd.v15i2.188>

Abstract

Honey bees (*Apis mellifera*) are crucial pollinators that contribute to ecological stability and agricultural productivity. However, in recent years, a significant global decline in bee colonies has been reported, primarily due to multiple stressors including *Varroa destructor*, an ectoparasitic mite that weakens bees and transmits deadly viruses. Chemical treatments have become less effective due to the emergence of resistant mite populations and residue contamination in hive products. As a sustainable alternative, the selection of bees with natural resistance traits, particularly hygienic behavior, has gained attention. This study aimed to assess the hygienic behavior of the Saharan honey bee (*Apis mellifera sahariensis*) as a potential defense mechanism against *Varroa destructor*. The research was conducted during 2019–2020 on 30 colonies in the Béchar region. Hygienic behavior was evaluated using the freeze-killed brood assay. Results revealed significant seasonal variation, with the highest cleaning rate observed in spring (94.74%), followed by summer (89.78%), autumn (80.44%), and the lowest in winter (70.69%). These findings highlight the influence of environmental factors on behavioral expression and confirm the strong hygienic potential of *A. m. sahariensis*. Previous studies support the role of hygienic behavior as a heritable trait and a key component of social immunity, providing protection against pathogens such as *Paenibacillus larvae*, *Ascosphaera apis*, and *Varroa destructor*. This study suggests that *Apis mellifera sahariensis* is a promising candidate for breeding programs aimed at enhancing colony resistance under arid and semi-arid conditions.

Key words: *Apis mellifera sahariensis*, hygienic behaviour, freeze – killed brood assay, natural resistance

INTRODUCTION

Honey bees play a vital role in maintaining global ecological balance through the pollination of numerous plant species. They also produce natural products of significant interest, such as honey, propolis, royal jelly, and beeswax. However, in recent years, many countries have reported a significant decline in bee colonies, with abnormally high winter mortality and decreasing populations throughout the year. This phenomenon is known as Colony Collapse Disorder (CCD) (Adjlane et al., 2016).

Corresponding author

Among the main factors contributing to this decline is varroosis, an ectoparasitic disease caused by the mite *Varroa destructor* (Adjlane and Haddad, 2017).

This parasite feeds on the hemolymph of bees, causing detrimental effects on their morphology and physiology, including reduced emergence weight (De Jong et al., 1984), decreased hypopharyngeal gland volume in adult worker bees (Schneider and Drescher, 1987), and shortened lifespan. *Varroa* also serves as a vector for the deformed wing virus, as well as other viruses that can ultimately lead to total colony collapse (Adjlane and Haddad, 2014; Annoscia et al., 2019).

Chemical control using acaricides such as Apistan (fluvalinate), Bayvarol (flumethrin), or Apivar (amitraz) has led to the emergence of resistant mite strains and the presence of chemical residues in hive products (Adjlane, 2017; Rinkevich, 2020; Hernández-Rodríguez et al., 2021). This has prompted growing interest in breeding *Varroa*-resistant bees, particularly by selecting for hygienic behavior.

This behavior, known for its effectiveness against American foulbrood (Facchini et al., 2019), enables hygienic bees to detect, uncap, and remove brood infested by *Varroa destructor* (Gerdt et al., 2018), thereby disrupting the mite's reproductive cycle (Morfin et al., 2019).

The present study aims to quantify the hygienic behavior of the Saharan honeybee (*Apis mellifera sahariensis*) in view of selecting this subspecies for *Varroa* tolerance.

MATERIAL AND METHOD

The study was conducted during 2019/2020 in an apiary located in the Béchar region, using 30 colonies of *Apis mellifera sahariensis*, all equalized and headed by one-year-old queens. Hygienic behavior was evaluated using the method described by Spivak and Gilliam, 1998, based on the freeze-killed brood assay.

To assess the hygienic behavior of the bee colonies, we used the liquid nitrogen freeze-killed brood method, recognized for its reliability and ease of application.

A frame containing capped brood of uniform age, without empty cells or pollen, was selected from each hive. An area of approximately 44 cm² (equivalent to a 7.5 cm diameter circle, or about 100 cells) was marked in the center of the brood. A metal cylinder of the same diameter was placed vertically over the selected area to contain the liquid nitrogen. About 100 ml of liquid nitrogen was slowly poured into the cylinder, instantly freezing the targeted brood. After complete evaporation of the nitrogen, the cylinder was removed, and the frame was returned to the hive (Fig. 1).

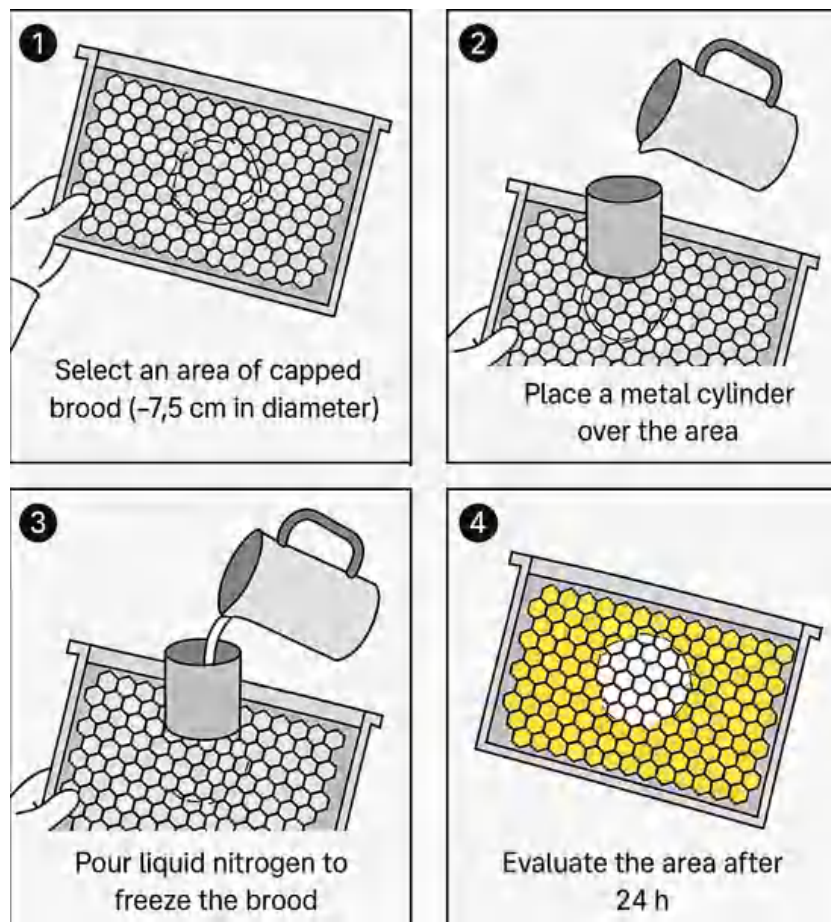


Fig. 1. Frozen brood assay with liquid nitrogen

After 24 to 48 hours, the frame was re-examined, and the number of cleaned (emptied) cells was counted. The hygienic behavior score was calculated as the percentage of cleaned cells relative to the total number of treated cells. A rate above 95% within 24 hours is considered indicative of very high hygienic behavior, while a rate below 80% reflects low hygienic performance. Data were analyzed using ANOVA with Statistica v5.0 software.

RESULTS AND DISCUSSION

The hygienic behavior of *Apis mellifera sahariensis* colonies, measured as the percentage of cleaned (emptied) brood cells within 24 hours after freeze-killing, showed significant seasonal variation.

After 24 hours, the highest cleaning rate was observed in spring at 94.74%, followed by summer with 89.78%, and autumn at 80.44%. The lowest rate was recorded in winter, with an average of 70.69%. These differences suggest that environmental conditions, particularly temperature and colony activity levels, may influence the expression of hygienic behavior (Fig. 2 and 3).

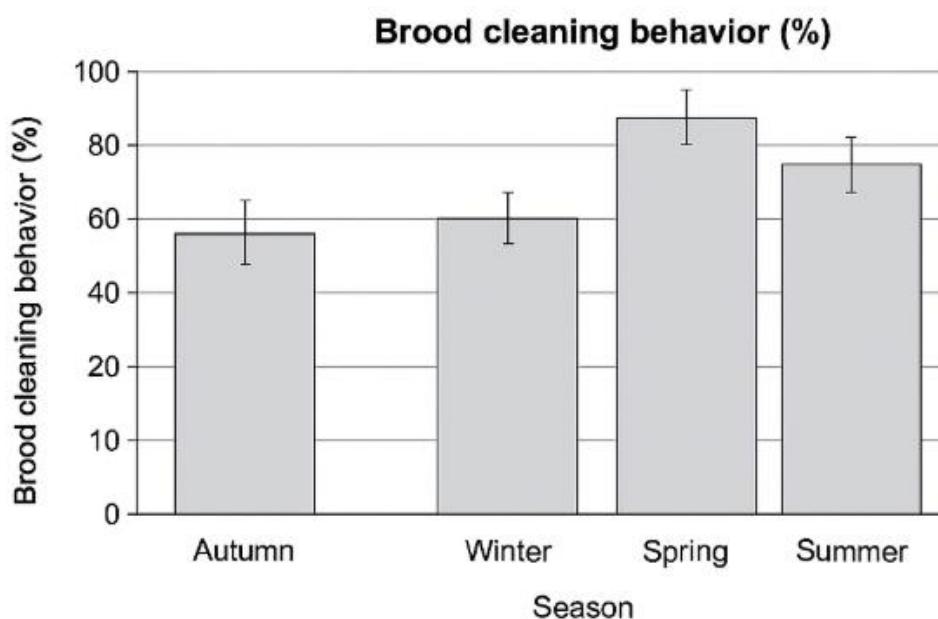


Fig. 2. Seasonal variation in brood cleaning behavior in *Apis mellifera sahariensis*

A significant difference between seasons was observed ($p < 0.05$), indicating that hygienic behavior varies according to environmental conditions. These rates are considered highly satisfactory compared to those reported for other subspecies.

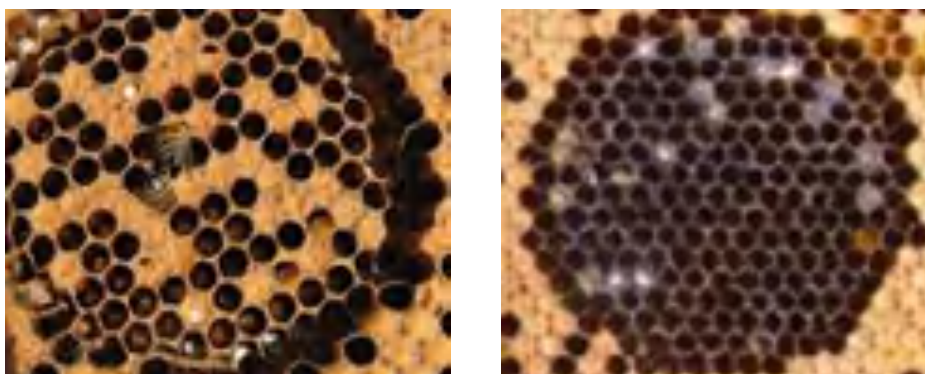


Fig. 3. Observation of the outcome of hygienic behavior

Previous studies (Balhareth et al., 2012; Kamel et al., 2003) have shown variable hygienic behavior depending on the race: *A. m. carnica* (35–44%), *A. m. jemenitica* (72–85%). According to Kefuss, 1995, *A. m. intermissa* (Tunisia) exhibits superior behavior compared to other subspecies. Other studies (Rodriguez et al., 1996) did not find significant differences among certain races (*A. m. mellifera*, *A. m. carnica*, “Buckfast”).

Hygienic behavior is a form of social immunity in bees, consisting of detecting, uncapping, and removing dead or diseased larvae or pupae from sealed brood cells. This mechanism helps reduce the spread of diseases such as American foulbrood, European foulbrood, chalkbrood, and infestations by the *Varroa destructor* mite and associated viruses.

The behaviors of *Apis mellifera* individuals within colonies determine the survival and development of those colonies. Indeed, certain bee species exhibit hygienic behavior, which involves the removal of mold and diseased larvae from the hive. This behavior is considered a defense mechanism providing protection against multiple parasites and other infections.

Hygienic colonies generally have less infected brood. Hygienic colonies eliminate about 70% of *Varroa* mites, compared to only 10% in non-hygienic colonies. Other studies revealed a negative correlation between the level of brood cleaning and increased *Varroa* levels. Hygienic behavior is therefore an important trait for evaluating colony resistance.

Hygienic behavior is a heritable trait, but its expression can vary depending on environmental factors. One study showed that neither the addition of food (sugar syrup) nor the amount of brood significantly affected hygiene levels, although an interaction between these factors could influence the behavior early in the season (Stanimirović et al., 2022).

Traits of interest for genetic selection related to *Varroa* resistance are primarily behavioral defenses, which are part of bees’ social immunity. For several years, researchers have been studying hygienic behavior, which, when expressed within a colony, reduces infection levels of several

pathogens among healthy brood (Sofi et al., 2023). In hygienic colonies, some worker bees are able to detect and remove dead or diseased brood infected by pathogens or parasites.

Several studies have demonstrated the effectiveness of hygienic behavior in resisting the bacterium *Paenibacillus larvae*, which causes American foulbrood (Spivak and Gilliam, 1998), and the fungus *Ascosphaera apis*, responsible for chalkbrood disease.

Hygienic bees detect diseased larvae through specific olfactory signals. Compounds such as phenylethyl acetate, 2-phenylethanol, and benzyl alcohol are associated with larvae infected by *Ascosphaera apis*, the agent of chalkbrood. Moreover, cuticular hydrocarbons such as (Z)-10-tritriacontene and (Z)-6-pentadecene have been identified as triggers of hygienic behavior, independently of the health status of the brood (Erez et al., 2022; Perez and Johnson, 2025).

At the neurobiological level, octopamine, a neuromodulator, plays a key role in modulating olfactory sensitivity. Hygienic bees show higher expression of octopamine-immunoreactive neurons in the deutocerebrum, enhancing their ability to detect olfactory signals from diseased brood.

CONCLUSIONS

The local Saharan honey bee (*Apis mellifera sahariensis*) stands out for its strong brood cleaning capacity, making it a valuable asset in the fight against *Varroa destructor* infestation. Further research is needed to deepen these findings, particularly by comparing this strain with *Apis mellifera intermissa* and studying behavior in response to naturally infested brood.

Acknowledgment

This study was funded by the PRIMA SafeAgroBee project, with support from the Directorate General for Scientific Research and Technological Development (DGRSDT), Algeria. The authors also wish to express their sincere thanks to the National Association of Professional Beekeepers (ANAP) for their valuable collaboration and field support.

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Received: May 06, 2025 Revised: June 13, 2025 Accepted: July 16, 2025 Published: November 30, 2025