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A Study of Hygienic and Grooming Behaviors in the Iranian Honeybee (*Apis mellifera meda*) Colonies Against *Varroa destructor*

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Abstract

This study evaluated the grooming and uncapping & removing hygienic behaviors of the honeybee colonies of the West Azerbaijan province of Iran from April 2021 to October 2022. Eighty colonies of Iranian honeybees infected with *Varroa* mite from Mahabad, Urmia, Oshnavieh, and Khoy cities of West Azerbaijan province were selected and studied regarding grooming and uncapping & removing hygienic behaviors. The results showed that there is no significant difference between the studied cities in terms of grooming behavior. The results showed that the season affects the grooming behavior of honeybee colonies in the studied cities. Therefore, the grooming behavior of the studied honeybee population in summer was significantly higher than that of colonies in spring ($P < 0.05$). Comparing the means of uncapping & removing hygienic behaviors after 48 hours showed that the honeybee colonies of the studied cities significantly differ in terms of these behaviors ($P < 0.05$). So, the highest and lowest averages of uncapping & removing hygienic behaviors after 48 hours were observed in the honeybee colonies of Khoy and Oshnavieh cities, respectively. The results showed a positive correlation between hygienic behaviors and all the functional-behavioral characteristics of honeybee colonies in this research. Our finding showed that the Iranian honeybee colonies of West Azerbaijan province of Iran can defend themselves against the *Varroa* mite by performing both grooming and uncapping & removing hygienic behaviors. Therefore, it is possible to improve the level of these behaviors in the honeybee colonies of this province by implementing breeding programs.

Introduction

The *Varroa* mite (*Varroa destructor* Anderson & Trueman, 2000) is the most important pathological threat to the western honeybee (*Apis mellifera* Linnaeus, 1758) colonies (Dietemann et al., 2012). This mite damages honeybee colonies by feeding on fat body tissues of honeybees' different developmental stages (larvae, pupae, and adult) and the transmission of pathogenic viral agents (Anderson & Trueman, 2000; Ramsey et al., 2021). This mite is related to high rates of honeybee colony mortality, causing the loss of billions of dollars in crops (Beaurepaire et al., 2019). *Varroa destructor* was observed and reported for the first time in Iran in 1983, and it is currently the biggest problem in the beekeeping industry in Iran (Rahimi et al., 2014; Rahimi et al., 2017; Ghasemi et al., 2022; Rahimi et al., 2022, 2023). Beekeepers use synthetic acaricides such as Apistan, Coumaphos, and Amitraz to control this parasite in their apiaries (Ghasemi et al., 2022). Using synthetic acaricides has been the primary effective method of controlling *Varroa* mites (Bahreini et al., 2020; Ghasemi et al., 2022). Although these compounds have desirable results in controlling this pest, recent evidence of acaricide resistance and infestation of the hive products, especially honey and beeswax, has

been reported in Italy, France, the USA, and Iran (Elzen et al., 1999; Milani, 1999; Ghasemi et al., 2016; Ghasemi et al., 2022). Therefore, infestation of hive products and *Varroa* mite resistance to synthetic acaricides brought about the idea of finding new and safer methods of controlling *Varroa* mites.

Honeybees have developed behavioral mechanisms against parasitic mites like *V. destructor*, including grooming and uncapping & removing hygienic behaviors (Hamiduzzaman et al., 2017). Hygienic behaviors are known as a behavioral response of honeybee workers to spreading infections in the colony. These behaviors lead to bees' resistance to hive pests and diseases. Hygienic workers can detect the presence of an infected larva or pupa and react by uncapping the cell cap & removing the diseased or parasitized individual (Morfin et al., 2019). Grooming behavior involves physical removal. This behavior consists of using legs, mandibles, and abdomen shaking to attempt to remove mites from their bodies (Morfin et al., 2020). Honeybees can restrain the growth of *V. destructor* population in honeybee colonies by expressing grooming and uncapping & removing hygienic behaviors. Due to the adverse effects of chemical acaricides in *Varroa* control, grooming and uncapping & removing hygienic behaviors are the mechanisms of interest for studies of social immunity and breeding purposes (Guzman-Novoa et al., 2012). These behaviors are among the most important ones of resistance against mites in the Asiatic honeybee (*Apis cerana* Fabricius, 1793). These behaviors have also been observed in *Apis mellifera* but are expressed at a lower frequency than in *A. cerana* (Fries et al., 1996).

Several studies have shown that certain honeybee genotypes, such as the so-called Africanized bees (hybrids of *Apis mellifera scutellata*), are more resistant to *V. destructor* than European bees. The main reason for this difference is due to the difference in expressing the level of grooming and uncapping & removing hygiene behaviors (Moretto et al., 1993; Guzman-Novoa et al., 1999; Aumeier, 2001; Invernizzi et al., 2015; Morfin et at., 2020). The expression of these behaviors is also observed in the Iranian honeybee, although many variations have been documented between its populations. Elmi et al. (2009) investigated the honeybee population in the East Azerbaijan province of Iran in terms of grooming and uncapping & removing hygienic behaviors. Their study showed that the honeybee population in this province can defend itself against the *Varroa* mite by expressing these behaviors. Kosha (1991) also investigated 50 colonies of honeybees in the Isfahan province of Iran for four years without any *Varroa* mite control method. In the third year, 18 out of 50 colonies survived, and their *Varroa* mite infection rate varied from zero to 10%. As a result of this study, he reported that the Iranian honeybee can defend itself well against the *Varroa* mite by expressing grooming and uncapping & removing behaviors. In another study conducted by Hosseini et al. (2013) on the honeybee population of the Zanjan province of Iran, similar results were observed and reported in line with the results of Kosha (1991) and Elmi et al. (2013) studies. Based on the results of the studies, Iranian honeybees exhibit desirable grooming and uncapping & removing hygienic behaviors. So, Iranian honeybees can defend themselves against the *Varroa* mite by expressing these behaviors. Therefore, identifying and selecting honeybees for resistance to *Varroa* mites produces colonies with high expression of grooming and uncapping & removing hygienic behaviors.

Iran is one of the poles of beekeeping in Asia and ranks third and eighth in the world regarding the number of colonies and honey production, respectively. Therefore, the honeybee and beekeeping industry have a high economic value in Iran regarding agriculture, medicine, and natural resources (Rahimi et al., 2023, 2022). Applying chemical acaricides has led to mite resistance and infestation of colony products and the environment in recent years. For this reason, using low-risk, non-chemical methods for bees, colony products, humans, and the environment, such as improving hygienic behaviors (including grooming and uncapping & removing hygienic behaviors) for honeybees against *Varroa* mites has been considered. Therefore, the main objective of this study was to investigate the grooming and uncapping & removing hygienic behaviors in honeybee colonies of the West Azerbaijan province of Iran against *Varroa destructor*.

Materials and Methods

Date and location of the experiment

The present study was conducted on the native honeybee population of the West Azerbaijan province of Iran from 2021 to 2022. This experiment was performed on honeybee colonies of four cities of the West Azerbaijan province (including Khoy, Urmia, Mahabad, and Oshnavieh cities). This way, two apiaries (apiaries with more than 300 colonies) from each city and ten colonies from each apiary (colonies with more than 10% infestation) were randomly selected and examined. This study was carried out on 80 native honeybee colonies (colonies with nine frames of bees) infected with *Varroa* mite.

The method of the experiment

The colonies were homogenized in terms of queen age, population (adults and brood), and honey storage according to the instructions of Delaplane et al. (2005) before the start of the experiments. During the experimental period, the studied colonies did not receive any acaricide. Also, the infection rate of colonies with *Varroa* mite was evaluated using the instructions of Dietemann et al. (2013) for adult bees and based on the guidelines of Zemene et al. (2013) for brood three times a year (spring, summer, and autumn months). Then, uncapping and removing hygienic behaviors, grooming behavior, aggressive behavior, honey production, and population (brood and adults) traits were evaluated in honeybee colonies in this study.

Evaluation of traits

Grooming Behavior

We evaluated the grooming behavior by placing a plastic sheet coated with odorless grease on each colony bottom board and separated by a screen mesh to prevent *Varroa* mites from escaping or climbing back to the brood frames. The plastic sheets were removed from the bottom board of the colonies and examined every 24 hours. Next, the number of dead and damaged mites on plastic sheets was counted and recorded. This evaluation was done for one week in the spring (June) and one week in the summer (July) of 2022.

Uncapping and removing hygiene behaviors

Liquid nitrogen was used to kill the pupa to evaluate the hygienic behaviors of uncapping the cell cap and removing the cell contents of the studied honeybee colonies (Spivak and Reuter, 1998). The studied colonies were visited before the experiment. Then, each was given the frame coded to have pupa of the same age when using the liquid nitrogen. Then, all the studied colonies were fed for three days to stimulate the queens to lay eggs. Then, the queen's oviposition area was marked on the frame of each colony. Fifteen days after the queen laid eggs, liquid nitrogen was used to kill the pupa. All colonies were visited to ensure the presence of pupa one day before using liquid nitrogen. The marked pupa frames were removed from the colonies (two from each colony), and the rest of the work steps were performed on them in the field chamber. A hollow tubular cylinder made of galvanized materials with a diameter of 6 cm and a height of 15 cm was used to pour liquid nitrogen on the pupae (Fig 1). About 300 ml of liquid nitrogen was used for each frame to kill about 160 pupa cells. A circle with a diameter of 6 cm was created by the cylinder on the marked pupa on the surface of the frame. Then, the cylinder was slowly rotated to reach the center wire of the frame. Next, the number of empty cells inside the cylinder was counted and recorded. Then, we poured 300 ml of liquid nitrogen into the cylinder and waited until the nitrogen evaporated completely. Subsequently, frames containing dead pupa were transferred to the mother colonies.

Fig 1. Pouring liquid nitrogen inside the cylinder on the frame.

The number of dead pupa cells in the mentioned area was counted and recorded 24 and 48 hours after pouring nitrogen twice daily (morning and afternoon). In this study, hygiene behaviors assessment was done in spring and summer (June and July). According to the definition of a hygiene colony, colonies that removed more than 95% of dead pupae from their cells within 48 hours after pouring liquid nitrogen were considered hygiene colonies.

Honey production

The weight of the harvested honey and the remaining in each colony was calculated to evaluate the amount of honey produced by the colonies in the honey harvesting season in the studied cities. The amount of honey produced by each colony was obtained from the difference in the weight of each colony before and after honey extraction. The method of remaining honey level on the frames is used to evaluate the exact weight of remaining honey in each colony. A special frame designed with galvanized wire into 10 × 5 cm rectangles was used to evaluate the exact amount of remaining honey in each colony (Fig 2). Thus, each decimeter square of honey on both sides of the special frame was considered equal to 304 grams. In this method, the total of remaining honey in each colony was calculated by placing a special frame on both sides of the remaining honey frames in the colonies. Therefore, the total honey production of each colony in the year was obtained from the sum of the remaining honey and the harvested honey from each colony.

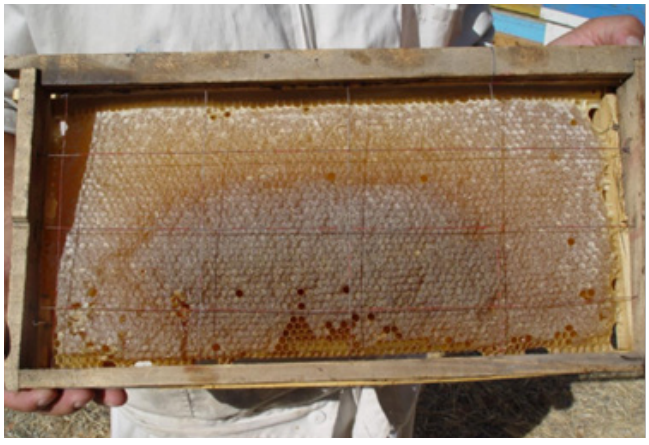

Fig 2. Evaluation of the remaining honey in the colony using a special frame.

Aggressive behavior

The evaluation of this behavior in the apiaries under study used the theoretical method. In this method, the honeybees were evaluated in terms of their behavior on the frame during the visit to the colony and received a score according to their behavior. In this way, the assessment of aggressive behavior according to bees' behavior was recorded for each colony as follows:

Hives that were calm without smoking (score 4)
Hives that are calmed by smoking a little (score 3)
Hives that did not calm down with smoking average (score 2)
Hives that did not calm down Smoking a lot (score 1)

Population (adults and brood)

During the implementation of this project, the evaluation of the adult bee population was done visually in two stages, the first in June and the second in September 2021. When visiting the colony, a frame covered with adult bees on both sides was considered a complete bee frame. If adult bees cover a part of the frame, a fraction of the frame is regarded as the population of adult bees. The total population of frames was considered the colony population and recorded for each colony. The brood population was evaluated in two stages in June and September in all the studied colonies. The population of brood includes frames containing eggs, larvae, and pupa. Evaluation of the brood's population in June is similar to the adult bees' population assessment. In September, we used an empty frame framed into 5 x 5 cm squares to calculate the population of brood accurately. The number of squares containing brood in each frame was counted by applying the special frame to every frame of brood in each colony. In this study, the coverage area of the brood was calculated in square centimeters (Fig 3).

Fig 3. Evaluation of colony brood using a special frame.

Statistical analysis

Hygiene behaviors for all bees of each colony were calculated through the formula of Palacio et al. (2005) as follows:

THB = X-Y-Z/X×100

THB = Colony Hygiene behaviors

X = The number of pupa cells (160 cells)

Y = The number of dead pupal cells of the unremoved cap after 48 hours

Z = The number of dead pupae cells with uncapping cell caps but not removing cell contents after 48 hours

This study analyzed the data as a nested design (Province (A), A city within the province (B), Apiaries within the city of each province (A(B(C))), A colony inside an apiary within a city of each province(A(B(C(D)))) using SAS V. 9.4 software. The comparison and correlation between variables were compared with Duncan's test and Pearson's correlation method, respectively.

Results

Grooming behavior

In this study, grooming and uncapping & removing hygiene behaviors were investigated in the honeybee population of West Azerbaijan province, Iran. The results of the comparison of means of grooming behavior in the studied cities of the West Azerbaijan province of Iran are shown in Table 1. The results showed that there is no significant difference between the studied cities in terms of grooming behavior ($P > 0.05$).

Table 1. The results of the comparison of means of grooming behavior in different cities of West Azerbaijan province, Iran.

Province	City	Grooming behavior
Azerbaijan province	Urmia	66.1[a]
	Mahabad	65.7[a]
	Khoy	67.9[a]
	Oshnavieh	66.3[a]
SEM		2.79
P-value		0.18

Season effect on grooming behavior

The current study examined the effect of season on grooming behavior. The results showed that the season affects the grooming behavior of honeybee colonies in the studied cities. Therefore, the grooming behavior of the studied honeybee population in summer (July), with an average of 69 mites, was significantly higher than the grooming behavior of colonies in spring (June), with an average of 64 mites ($P < 0.05$) (Table 2).

Table 2. The average grooming behavior of the studied honeybee population in the spring and summer seasons in the current study.

Season	Month	The average grooming behavior of the studied honeybee population
Spring	June	64[a]
Summer	July	69[b]
SEM		1.46
P-value		0.02

Uncapping and Removing Hygiene Behaviors

The comparison of means for the hygienic behavior of uncapping the cell cap and removing cell content after 48 hours in the studied cities of the West Azerbaijan province of Iran is given in Table 3. The results showed that the honeybee colonies of the studied cities significantly differ regarding the uncapping cell cap and removing cell contents hygiene behaviors 48 hours after pouring liquid nitrogen ($P<0.05$). Therefore, the highest percentage of uncapping cell caps and removing cell contents hygiene behaviors after 48 hours with an average of 91.3 and 87.6 % t were related to honeybee colonies in Khoy city, and the lowest percentage of uncapping cell caps and removing cell contents hygiene behaviors with an average of 79.8 and 75.8 % were observed in the honeybee colonies of Oshnavieh city, respectively (Table 3).

Table 3. The average of uncapping cell caps and removing cell contents hygiene behaviors 48 hours after pouring liquid nitrogen in cities of West Azerbaijan province, Iran.

Province	City	Average uncapping cell caps hygiene behavior after 48 h	Average removing cell contents hygiene behaviors after 48 h
Azerbaijan province	Urmia	85.6[b]	84.3[b]
	Mahabad	83.9[b]	78.5[a]
	Khoy	91.3[b]	87.6[b]
	Oshnavieh	79.8[a]	75.8[a]
SEM		2.27	1.74
P-value		0.01	0.03

Season effect on uncapping and removing hygiene behaviors

Season effect on hygiene behaviors of uncapping cell caps and removing cell contents after 48 hours is shown in Table 5. Based on the obtained results, the effect of season was not significant on the hygiene behaviors of uncapping cell caps and removing cell contents studies honeybee colonies. However, the mean of hygiene behaviors of uncapping cell caps and removing cell contents in the studied honeybee colonies in the spring was statistically higher than in the summer (Table 4).

Correlation of Uncapping and Removing hygiene behaviors with functional traits

In this study, correlation analysis (Pearson) was used to obtain the relationship between the uncapping and removing hygiene behaviors and the traits of honeybee colonies, and its results are presented in Table (5). The results showed a positive correlation between the uncapping and removing hygiene behaviors with all the functional-behavioral traits of the honeybee colonies in this research. Based on the results,

Table 4. The effect of the season on hygiene behaviors of uncapping cell caps and removing cell contents studied honeybee colonies in the current research.

Season	Month	The average hygiene behavior of uncapping cell caps after 48 h	The average hygiene behavior of removing cell contents after 48 h
Spring	June	86.27[a]	83.15[a]
Summer	July	84.03[a]	79.95[a]
SEM		3.19	3.67
P-value		0.37	0.07

Table 5. Correlation between the Uncapping and Removing hygiene behaviors of dead brood with the functional-behavioral traits of honeybee colonies in this study.

Traits	The hygiene behavior of Uncapping the cell cap (%) 48 hours after pouring nitrogen (1)	The hygiene behavior of Removing the cell contents (%) 48 hours after pouring nitrogen (2)	Honey production (3)	Population (4)	Aggressive behavior (5)
1	1				
2	0.521**	1			
3	0.283**	0.104**	1		
4	0.187	0.052	0.305*	1	
5	0.54**	0.84**	0.026	0.087	1

a positive and significant correlation was observed between the percentage of uncapping of cell cap and the removal of cell contents 48 hours after pouring liquid nitrogen ($P < 0.01$). Also, a positive and significant correlation was observed between the uncapping & removing hygiene behaviors 48 hours after pouring liquid nitrogen with honey production ($P<0.05$) and aggressive behavior ($P<0.01$).

Discussion

The *Varroa* mite is the biggest problem in the world's beekeeping industry. It causes a lot of damage to beekeepers every year by feeding on the fat bodies of bees, transferring pathogens, and finally destroying colonies. Due to the adverse effects of chemical acaricides used to control the *Varroa* mite in the colonies on honeybees, colony products, and the environment, nowadays, more focus is placed on non-chemical methods, including the genetic resistance of bees against the *Varroa* mite. The grooming and uncapping & removing hygienic behaviors are considered the most important genetic defense mechanisms of honeybees against *Varroa* mites. Therefore, the present study evaluated the grooming and uncapping & removing hygienic behaviors of the Iranian honeybee population in the West Azerbaijan province of Iran. The results of the present study showed that the honeybee population in West Azerbaijan province showed desirable hygienic behaviors against the *Varroa* mite. Previous studies conducted on other Iranian honeybee populations in different regions of Iran also confirm this subject. Elmi et al. (2009) investigated the honeybee population of East Azarbaijan province of Iran regarding hygienic behavior. These researchers reported that the hygienic behavior in the honeybees of this province is expressed at a high level, and the bees of this province were able to defend themselves against the *Varroa* mite by expressing these behaviors. In another study, Bahraini (2001) in the honeybee population of Alborz province, Kosha (1991) in the honeybee population of Isfahan province, and Akef (2001) in the honeybee population of Tehran province mentioned similar results for the Iranian honeybee located in these provinces, which is consistent with the results of the present study. According to the results of this study, the grooming behavior observed in the honeybee colonies of the four cities was not different among them but was higher than those reported by previous studies. Elmi and coauthors reported the average grooming behavior of honeybee colonies in East Azerbaijan province to be 52.4 mites per colony (Elmi et al., 2007). Meshaikhi et al. (2001) reported the average grooming behavior of honeybee colonies in Tehran province as 54.9% and in Khorasan province as 53.9% per colony. The large number of mites observed on the floor of the hives in the present study is in line with the results of Bahraini (2001), Elmi et al. (2007), and Mashayekhi et al. (2001). Our finding indicates that the honeybees of West Azerbaijan province can defend themselves against *Varroa* mites by identifying mites on their bodies and other bees and removing them from themselves.

Hosseini et al. (2013) reported that the expression of grooming behavior is affected by the season, and this behavior is more effective in summer than in spring. The results of the present study are also consistent with the results of Hosseini et al. (2013). In this way, the number of mites observed on the hive floor in June (69 mites) was significantly higher than in July (64 mites) in this study. The results showed that honeybee colonies of studied cities of the West Azerbaijan province of Iran significantly differ regarding the hygienic behaviors of uncapping cell caps and removing cell contents 48 hours after pouring liquid nitrogen ($P < 0.05$). Therefore, the highest percentage of hygienic behaviors of uncapping of cell cap and removing of cell contents 48 hours after pouring liquid nitrogen with an average of 91.3 and 87.6 %, respectively, are related to honeybee colonies in Khoy city, and the lowest these behaviors with an average of 79.8 and 75.8 % were observed in the honeybee colonies of Oshnavieh city. Akef (2001) investigated the hygienic behaviors of uncapping cell caps and removing cell contents in honeybee colonies in Khorasan, Tehran, Qazvin, Isfahan, and Central provinces of Iran. This researcher reported an average hygienic behavior of uncapping cell caps of 88% and removing cell contents of 67% after 48 hours after pouring liquid nitrogen into in the studied honeybee colony.. In another study, Elmi and coauthors reported the average hygienic behaviors of uncapping cell caps and removing cell contents 48 hours after pouring liquid nitrogen in the honeybee population of East Azerbaijan province to be 20% (Elmi et al., 2007). The results of this study are consistent with the results reported by Akef (2001), and Elmi et al. (2007) on different populations of Iranian honeybees, and they all indicate the desirable expression of hygienic behaviors of uncapping cell caps and removing cell contents in Iranian honeybee. The expression of these behaviors has also been reported in other subspecies of *Apis mellifera.* Oldroyd et al. (2008) investigated the hygienic behaviors of Australian honeybee colonies. These researchers reported that about 20% of Australian honeybee colonies show favorable hygienic behaviors. Another study was conducted on honeybee colonies in the United States of America in 1998. This study showed that about 10% of honeybee colonies in this country have desirable hygienic behavior (Spivak and Gary, 1998). The results of other studies on the Starline hybrid and the Italian subspecies honeybee showed that 26% of the bee colonies of the Starline hybrid and Italian subspecies honeybee were observed to have favorable hygienic behaviors (Spivak and Reuter, 2002). Kekecoglu et al. (2009) examined the hygienic behaviors of uncapping cell caps & removing cell contents of 250 colonies of the carnica subspecies honeybee. These researchers reported that 5% of colonies showed optimal hygienic behaviors. The study results of various researchers show that the hygienic behaviors of uncapping cell caps and removing cell contents are

expressed in *Apis mellifera,* and the percentage of expression of these behaviors is different between different subspecies of *A. mellifera* (Spivak and Downey, 1993; Boecking and Spivak, 1999; Spivak and Gary, 1998; Masterman et al., 2000; Gramacho and Spivak, 2003; Oldroyd et al., 2008; Palacio et al., 2010).

The results of the present study, in line with previous studies conducted in Iran, showed that the hygienic behaviors of uncapping cell caps and removing cell contents are also expressed in Iranian honeybees, and the percentage of expression of these behaviors is different among different populations of *A.m. meda* in different regions of Iran. However, the overall percentage of expression is higher than those recorded in other studies.

The results of this study showed that the hygiene behaviors of the uncapping of cell caps and removing cell contents are affected by the season, and the expression of these behaviors in spring is significantly higher than in summer ($P < 0.05$). The reason for this difference can be pointed to the increase of nectar entering the colony in spring. Various studies have already reported the same subject. For example, we can refer to the studies of Tahmasabi et al. (2019) on the improved Iranian honeybee population in Alborz province, Yahyaei and Karimi Dehkordi (2021) on the honeybee population in Lorestan province, and Taheri Imam Kandi et al. (2023) on the honeybee population of West Azerbaijan province of Iran. Other researchers have also investigated this subject in other subspecies of honeybees. Gramacho and Gonçalves (2009) reported that the hygiene behaviors of honeybees are affected by various factors such as genetics, nervous systems, and social and environmental conditions, and these factors can be effective in expressing the hygiene behaviors of colonies. Bigio et al. (2013) investigated the effect of season on the expression of hygiene behavior. These researchers reported that the effect of the season affects the expression of uncapping and removing hygiene behaviors. Due to more larvae in the spring season and more irritability in the colony, bees show hygienic behaviors faster and more in the spring than in the summer season. Genetic factors also play a major role in expressing uncapping and removing hygiene behaviors. Oxley et al. (2010) estimated the heritability of uncapping & removing hygiene behaviors to be 0.65. These researchers reported that the expression of these behaviors varies between different species and subspecies of honeybees.

The correlation analysis results showed a positive correlation between the uncapping and removing hygiene behaviors with all the functional-behavioral traits of the honeybee colonies in the current research. The results showed a positive and significant correlation between the percentage of uncapping cell caps and removing cell contents 48 hours after pouring liquid nitrogen ($P < 0.01$). The positive correlation between uncapping the cell cap and removing cell contents is obvious because the possibility of removing cell contents increases with the increase of the uncapping of the cell cap and pupa availability. Similar results were also reported for a positive and significant correlation between the uncapping of cell cap and the removing of cell contents in other Iranian honeybee populations by Banohosseini et al. (2013), Zalati et al. (2017), Tahmasabi et al. (2019) and Yahyaei and Karimi Dehkordi (2021). A positive and significant correlation was observed between the hygienic behaviors of uncapping & removing 48 hours after pouring liquid nitrogen with honey production and aggressive behavior. These findings were consistent with the results of studies by Afshari (2011), Banohosseini et al. (2013), Zalati et al. (2017), and Yahyaei and Karimi Dehkordi (2021). Rothenbuhler et al. (1964) showed that aggressiveness and hygienic behaviors have separate inheritance, and colonies with higher hygienic behavior are as aggressive as other colonies. Therefore, the results of Rothenbuhler et al. (1964) are not consistent with the results of the present study. The interaction of genotype and environment influences honeybee colonies. According to the results of the studies, the genetic correlation is reduced in most cases. Different environmental factors such as temperature, humidity, season, nectar flow, population size, and colony abilities affect the hygienic behavior of honeybee colonies (Bar-Cohen et al., 1978; Spivak & Giliam, 1993; Boecking & Spivak, 1999; Gramaco & Gonçalves, 2009).

Finally, based on the results of the current study and previous research on Iranian honeybee populations, it can be concluded that grooming and uncapping & removing hygienic behaviors are well-expressed in Iranian honeybee colonies of the apiaries located in the studied cities. Therefore, Iranian honeybees can defend themselves against the *Varroa* mite by performing grooming and uncapping & removing hygienic behaviors. So, we can target colonies of honeybees from apiaries from the studied cities as candidate lineages to improve the expression of these behaviors in different populations of Iranian honeybees by implementing breeding programs. Also, it is possible to reduce the number of synthetic chemical acaricides used in apiaries to control this parasite by creating genetic lines of honeybees resistant to the *Varroa* mite.

Authors' Contribution

AR and RT: conceptualization, methodology, software, formal analysis, validation, investigation, data curation, writing-original draft, project administration.

MG and AH: methodology, validation, supervision.

AR: Writing- review and editing.

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