

Study Of The Seasonal Occurrence Of Varroa Jacobsoni Oudemans On Apis Mellifera Honey Bees In Basra Province

Amira Jassim Mohammed* and Khaled Abdul Razzaq Fhad

Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq

Abstract

The study aimed to calculate the rates of infection and the seasonal presence of the ectoparasite *Varroa jacobsoni* Oudemans, one of the most important pests of honey bees, *Apis mellifera*. During three months, which included the months of September and October for the 2020 season and February of the 2021 season, for three different locations in Basra province: Al-Haritha, Shatt Al-Arab, and Abi Al-Khasib districts. The results of the study showed that there were significant differences in the percentage of infection of adult honey bees, as Abi Al-Khasib district recorded the highest infection rate of 15.00% during the month of September, while the lowest infection percentage was in Shatt Al-Arab district, which amounted to 2.86% during the month of February. The results also achieved significant differences in the rate of infection percentage for honeybee brood, as the highest infection rate reached 23.83% in Abi Al-Khasib area during the month of February, and the lowest infection percentage reached 7.67% in Shatt Al-Arab district during the month of September. As for the seasonal presence of the *Varroa* parasite, the experiment was conducted in the Al-Haritha area within the hives of the bee project of the Plant Protection Department in the Basra Agriculture Directorate. The results of the study showed significant differences between phases and months, the highest rate of *Varroa* mites being 28.00 mites/100 male broods during February, and the lowest rate reaching 2.33 *Varroa* mites/100 adult bees during January.

Keywords: *Varroa jacobsoni*, *Apis mellifera*, Basrah

1- Introduction

The honey bee *Apis mellifera* L. is one of the most widespread types of bees around the world and in Iraqi apiaries due to its economic importance, its suitability to the natural environment, and its ease of handling in modern breeding methods (Al-Hasnawi, 2016). The number of beekeepers in Basra province for the year 2021 reached 193 beekeepers, with a total of 7,635 beehives, distributed proportionally among the agricultural incisors (Ministry of Agriculture, 2021). Breeding honey bees is one of the important agricultural activities that contribute to increasing agricultural production because of the food products it provides. It has received a lot of attention from ancient times to the present because of its high nutritional value (Hanley et al. 2015). It is also one of the most important pollinators in the cross-pollination of plants (Maggi et al. 2013 and Hung et al. 2018).

2- Materials and Methods

2-1: Study location

The study was conducted in wooden Lancaster hives, each hive containing ten tires of honey bees for three separate location, the first site is in the bee project of the Plant Protection Department in the Basra Agriculture Directorate, located in Al-Haritha district, the second location is in Abi Al-Khasib district at a beekeeper in Al-Mahila area, and the third location is at a beekeeper in Shatt Al-Arab district, with twenty beehives for each location, then divided five groups (treatments) and each treatment included three cells (replicates), each cell represents a repeat in the experiment in addition to the control treatment.

2-2: Calculation of the percentage of Varroa mite infection in adult honey bees

A survey and calculation of the percentage of *Varroa jacobsoni* infection of the local honey bee *Apis mellifera* was conducted in three agricultural areas of Basra province, including Al-Haritha, Shatt Al-Arab and Abi Al-Khasib during the season 2020-2021, and for a period of three months (September, October and February). The infection rate was calculated by randomly taking one of the bees-containing tires from one cell for every five cells inside the apiary after examining it well to ensure that it is free of the queen, then a container was taken and a white paper A4 size was placed inside it. The bees were dropped into the pot from the bee-containing frame using a soft brush with approximately 100 male and female bees, then the fallen bees were collected and placed inside a 1 kg tightly packed glass bottle with a clip cap with small holes of up to 10msh that was modified for this purpose. Allows the varroa parasite and sugar powder to escape through it and prevents the bees from passing through. Then three tablespoons of powdered sugar were added and the bottle was shaken and sieved for one minute to ensure that the bees' body was covered with sugar powder and all the varroa fell out through the process of cleaning the bees' parts of their body from sugar and then leaving the bottle aside On the ground for five minutes and then inverting and shaking the bottle, which leads to the varroa falling from the back and belly rings of the workers and males inside the bottle and then falling on the white paper with powdered sugar. With a measure of 0.5 mm, Varroa is then collected and calculated according to the following equation: (Allen, 1996):

2-3: Calculation of the percentage of Varroa mite infection for honeybee brood

The infection percentage was calculated in bee brood by opening 100 sealed hexagonal eyes from worker brood and 100 eyes from male brood, extracting it with forceps, and placing it in a glass bottle containing 70% ethyl alcohol. Write down the area information and cell number. After bringing it to the laboratory, shake the vial well for several minutes; To separate the varroa stuck to the body of the brood, taken from the sealed hexagonal eye, then empty the contents of the vial into a container using a wire mesh strainer with 3 x 3 mm holes, allowing only the passage of varroa, and the process is repeated; To ensure that all broods are separated from the brood and then calculated according to the equation:

-4: The annual presence of Varroa mites on adults, brood of workers and male honeybees

To study the seasonal occurrence of Varroa mites, the Al-Haritha location of the Basra Agriculture Directorate was chosen, with twenty cells. The population density of Varroa mites in the experimental cells was calculated once during a month and for a period of six months, starting from September 2020 until February 2021, as in the previous two paragraphs (2-2 and 2-3).

2-5: Statistical Analysis:

The field experiment was conducted according to the randomized complete block design (R.C.B.D) and factorial design with three replications. Also, all means were compared using the L.S.D method and at a probability level of 0.05 for the field experiment (Al-Rawi and Khalaf Allah, 1980). The results were analyzed according to Gen Stat discovery edition 3.

Results and discussion:

3-1: Calculation of the percentage of Varroa mite infection of adult honey bees

A field survey was conducted and the percentage of infection with Varroa mites in adults and honey bee brood was conducted in three areas of Basra province, namely Al-Haritha, Shatt Al-Arab and Abi Al-Khasib districts, as shown in Table (1). Where significant differences were found for the effect of the regions, the district of Abi Al-Khasib recorded the highest infection rate, which amounted to 10.64%, while the Shatt Al-Arab district recorded the lowest infection rate, which amounted to 4.45%. As for the average time, the month of September recorded the highest rate of infection, which amounted to 9.92%, and the lowest rate recorded during the month of February, with a average of 4.76%. The results of the interaction between the months and the surveyed areas showed that there were significant differences in the incidence rates, as the Abi Al-Khasib district recorded the highest infection rate of 15.00% during the month of September, while the lowest infection rate was in the Shatt Al-Arab district, which amounted to 2.86% during the month of February.

Table 1: Calculation of the percentage of Varroa mite infection of adult honey bees during the season 2020-2021

Months	Regions			
	Al-Haritha	Shatt Al-Arab	Abi Al-Khasib	months average
September	8.93	5.83	15.00	9.92
October	7.76	4.66	9.50	7.31
February	4.00	2.86	7.43	4.76
Regions average	6.90	4.45	10.64	7.33
L.S.D 0.05	Months=0.47	Regions=0.47	Interaction= 0.82	

3-2: Calculation of the percentage of Varroa mite infection for honeybee brood

The results of (Table 2) showed that there were significant differences in the rates of infection with Varroa mites for honeybee brood between regions and time, and the interaction between them. The highest infection rate was 20.53% for Abi Al-Khasib district, while Shatt Al-Arab district recorded the lowest infection rate, which

amounted to 9.22%.With regard to the time factor, it had a significant effect on the rate of Varroa mite infection on honey bee brood. The infection rate reached during February the highest average of 16.22%, while the lowest rate was during the month of September with an infection rate of 12.28%.The results of the interaction also showed that there were significant differences between regions and time in the incidence of infection, as the highest infection rate reached 23.83% in Abi Al-Khasib area during the month of February, and the lowest infection rate reached 7.67% in Shatt Al-Arab district during the month of September.

Table 2: Calculation of the percentages of Varroa mite infection for honeybee brood during the season 2020-2021

Months	Regions			
	Al-Haritha	Shatt Al-Arab	Abi Al-Khasib	months average
September	12.67	7.67	16.50	12.28
October	16.00	9.33	21.27	15.53
February	14.17	10.67	23.83	16.22
Regions average	14.28	9.22	20.53	14.68
L.S.D 0.05	Months=0.66	Regions=0.66	Interaction= 1.14	

The possible reason for the difference in the level of infection between locations may be due to environmental factors and the difference in temperature between months and regions, which are among the main determinants of the growth of the Varroa parasite and its natural spread and influence the methods of combating it.It was found that the most suitable temperature for the growth of the parasite ranges between (15-30 °C), while growth and spread decrease at a temperature of (14 °C). As well as the grooming behavior and hygienic behavior of the bees which may vary between sites and also between cells based on the strength of the hive.The results of the study agreed with the findings of Banawas and Khanbash (2020) about the variation in the population density of Varroa mites on brood in experimental cells during the months of the year. Varroa mites on the brood during the months of October and November, as it ranged between 0-24 nipples / cell and between Al-Hasnawi, (2019) the percentage of infection with Varroa parasite in honey bees through two readings, the first in the month of April recorded an infection rate in the brood reached (3%) and (1%) in adult bees, It also showed the discrepancy in the rates of infection between districts. The district of Abi Al-Khasib recorded the highest rate of infection, which reached (4%) in the brood and (3%) in the adult bees. While the Zubair district recorded the lowest infection rate (1%) in brood and 0% for adult bees. As for the second reading in September, the infection rate was (2.6%) in adult bees and (6%) in brood, and the highest rate recorded in Abi Al-Khasib district, the infection rate was (6%) on adult bees and (23%) in brood, Zubair district recorded the lowest infection rate of 0% in brood and (3%) for adult bees.Although the ideal temperatures for the honeybee hive are around 33°C, the average temperatures in the brood infected with

Varroa mites are higher than those in the low-infested brood, which reached (34.73 and 32.99°C), respectively. The infection of Varroa mites in honey bee colonies is positively correlated with temperature and negatively correlated with humidity, which amounted to (73.87 and .77.73%) respectively (Hou et al., 2016). The population density of Varroa mites increases in honeybee hives at the beginning of September to reach its highest levels during the months of November and December, respectively, and may reach 23 times the number after several months (Hajeej, 2009) and Gao (2002) showed that temperatures between 34 - 35 degrees Celsius are more beneficial for the reproduction of Varroa mites, and that they are negatively affected at the temperature below 33 degrees Celsius, as the average number of mites was 2.4 at (35 ° C), while the average number of mites was 0.9 at temperature (33°C). Mustafa (2011) stated that honey bee colonies provide in their hives a suitable environment for the growth and reproduction of Varroa mites, especially in the brood, which made them immune to the influence of external factors, but this is not completely isolated from the climate element that most affects the behavior of the Varroa parasite and its reproduction, especially temperature. The behavior of bees is directly related to the climatic elements surrounding the hives, which clearly affect their overall vital activities. What prompts bees to create different behaviors and behaviors for the purpose of maintaining appropriate conditions for the sustainability of life according to their needs, as they use solar energy as a means to determine the reproduction of Varroa mites, by exposing beehives to them by placing glass panels that raise temperatures and thus reduce the tendency of the bee colony to The laying of fertilized eggs produced by the males during the active seasons of honey bees and is one of the main elements for the growth of Varroa mites. This explains the cause of infection with Varroa at high temperature in honeybee hives. This may also indicate that honeybees are adopting a kind of defensive response that lowers the temperature, resulting in environmental conditions that are not suitable for the breeding of Varroa mites. This is in line with what was indicated (vanEngelsdorp et al., 2008) that regions with relatively lower average temperatures have lower Varroa infections in honeybee hives. In this context, Rosenkranz et al. (2010) report that honeybees exhibit a variety of behavioral traits associated with social interactions. One of these behaviors involves ventilation of the wings as a behavior that hinders the survival and reproductive processes of Varroa, however, ventilation of the wing would consume honeybee energy and increase the temperature. This observation indirectly supports the fact that there is a significant correlation between the intensity of Varroa mite infection and environmental factors (Temperature and Humidity) which were induced to fluctuate by the behavior of honey bees (Bonoan et al., 2014).

3-4: Annual presence of Varroa mites on adults, brood of workers and male honeybees

It was noted from the results of the statistical analysis of Table 3 that there were significant differences in the annual presence of Varroa mites on the different stages of honey bees during six months of the year. As for the interaction between phases and time, significant differences were recorded in the annual presence, as the highest rate was 28.00 nipple/100 male broods during February, and the lowest rate was 2.33 Varroa/100 adult bees during January. The reason may be attributed to the availability of brood during the month of February in the study area due to the appropriate environmental conditions, while it decreases during the month of September, which is the end of the honey overflow season, during which brood levels decrease before entering the autumn season, while February represents the most important month for the breeding of honey bees in Basra province. This is because brood size is at its highest. The growth of the honeybee brood can continue during the winter season, and this is one of the natural methods that determine the reproduction of the varroa

mite, while the production of the brood decreases during the summer as a result of high temperatures, and this difference leads to a difference in the dates of the natural transition of honeybees, the natural behavior of the spread of *Varroa*, where it is the swarming season. Male production begins during the month of February. Poorna et al., (2021) mentioned that despite the different environmental conditions of three different regions, the rate of infection of *V. jacobsoni* on *Apis cerana* brood was recorded at different levels during seven months and that the highest rate of infection during January was 14.00% and the lowest rate was 0.0 % during the month of September, and the reason was due to the peak infection period coinciding with the breeding season of honey bees and the presence of brood, and therefore a higher infection can be expected during that period. The infection of *Varroa* mites continues throughout the year until it reaches its peak population density during the period from January to February (Mathialagan et al., 2017). (Banwas and Khanbash, 2020) indicated that the number of *Varroa* mites on brood and adults of honey bees varied between the experiment cells during the months of the year, as well as their difference from one hive to another. most cells, While the lowest brood rate was recorded during October and November, ranging between 0-24 nipples/hive, the highest brood density was recorded on bees of 129.7 nipples/hive during February, and the lowest rate was 37.0 nipple/hive during October. However, Sakofski et al., (1990) showed that the incidence of *Varroa* infection decreases during the spring months and increases significantly during the summer months And the *V. jacobsoni* mites shows a much higher preference towards the male brood than the infected worker brood probably because the male brood contains methyl and ethyl esters of straight chain fatty acids, especially methyl palmitate, which is responsible for the attraction of *V. jacobsoni* males to the bees of *V. jacobsoni*. Honey. (Le Conte et al., 1989). Also, the reason may be due to the presence of Kairomonal components in the male brood, ranging from 5-6 times their quantity in the brood of the worker bees, in addition to the wideness of the space, the appropriate temperature and the duration of reproduction. Therefore, the *Varroa* mites multiplies in the male brood by about 8-10 times its reproduction within the brood of the worker bees, and that the *Varroa* mites is preferred reproduction in the sealed male brood, as the shingles cannot open the hexagonal eye when the pupa dies and remove it from the cell (Oudemans, 1904, Koeniger et al., 1983 and Rosenkranz et al., 2010). In addition, the *Varroa* parasite may be attracted to the brood of males instead of the brood of workers, because the brood of males takes a day or two longer to appear as full males when compared to the brood of workers, which leads to a longer period of *Varroa* feeding and therefore better development. Honey bees are infected with many pests and pathogens that negatively affect their behavior, life and production, the most important of these pests is the *Varroa* parasite (Hunt et al., 2016 and Evans and Schwarz (2011). *Varroa jacobsoni* is one of the most dangerous pests that leads to the deterioration of infected bee colonies Notable (Oudedams, 1904, and Figen et al. 2012). *Varroa* mites feed on the hemolymph of pupae and adults of honeybees or may feed on the fatty bodies of honeybees, causing severe physiological disturbances including a decrease in weight, body fluids, protein and carbohydrate level (Maggi et al., 2009 and Ramsey et al., 2019). *Varroa* parasite appeared and spread in Iraq after its spread to some neighboring countries, including Syria, Turkey, Iran, and Jordan. It was noticed by many beekeepers in different areas in 1985 (Al-Mashhadani, 1997). Then it was officially registered as an epidemic pest in 1987 in the city of Dohuk in northern Iraq (Mahdi et al., 2020 and Ministry of Higher Education and Scientific Research, 1987). After that, it spread throughout the province of Iraq, and its spread had a devastating effect on bee colonies in Iraq. The collapse in hives reached 90%, or nearly 500,000 beehives throughout Iraq (Al-Hasnawi and Al-Sami`, 2020). In view of the importance of *Varroa* nipple pest on honey bees and the losses it causes estimated at billions of dollars annually, and the urgent need to estimate

the averages of infection and the annual presence of it, and as a result of not studying this aspect in Basra province, this study was proposed, which included a field and laboratory study.

Table 3: The annual presence of Varroa mites on honeybee Instars during the season 2020-2021

Months	Instars			
	adult	brood of workers	brood of male	months average
September	21.40	4.13	7.67	11.06
October	15.03	6.00	8.47	9.83
November	11.93	16.66	18.50	15.70
December	6.56	16.63	19.50	14.23
January	2.33	22.53	24.50	16.45
February	3.23	23.93	28.00	18.38
Instars average	10.08	14.98	17.77	14.28
L.S.D 0.05	Months=0.63	instars=0.44	Interaction= 1.09	

References

Banawas, Saeed Abdullah, Khanbash Muhammad Saeed. (2020). Study of the behavior of honey bees (*Apis mellifera jemenitica*) in cleaning against Varroa mite (*Varroa destructor*) in Wadi Dawan-Hadhramout-Yemen. *The Arab Journal of Scientific Research*, 8 (2): 1-9.

Hajjij, Noureddine Zahir, Al-Baraki, Ali, Al-Abed, Tammam. (2009). Efficacy of using oxalic acid by some methods in controlling the bee varroa parasite *V. destructor* (*Varroa Jacobsoni* Oud) and its effect on the bee colony, *Damascus University Journal of Agricultural Sciences*, Volume 25, Issue 2.

Al-Hasnawi Montaser Sabah Mahdi (2019). Spatial analysis of the spread of Varroa parasite and its effect on honey bees in Iraq. Ph.D. University of Kufa. college of Literature. Geographical department. 288 pages.

Al-Hasnawi, Montaser Sabbah (2016). Spatial analysis of honey bee breeding in the provinces of the Middle Euphrates. Master Thesis. University of Kufa. college of Literature. 223 pages.

Nat. Volatiles & Essent. Oils, 2021; 8(6): 2317-2325

Al-Hasnawi, Montaser Sabah, Al-Sami', Mahmoud Badr. (2020). The effect of temperature on the spread of Varroa parasite and methods of controlling it in Iraq. Journal of Geographical and Cartographic Research Center. 17(30): 153-185.

Alrawi, khashie Mahmoud and Khalaf Allah, Abdel Aziz Muhammad (1980). Design and analysis of agricultural experiments. House of wisdom for printing and publishing. University of Al Mosul . 488 pages.

Al-Mashhadani, Majeed Shehab Ahmed. (1997). Biology and control of Varroa jacobsoni Oud nipple parasitizing on honey bees mellifera L. Apis in Iraq, PhD thesis, College of Agriculture, University of Baghdad.

Mustafa, Abdel Rahim Omar. (2011). Effect of some physical techniques and some mite pesticides on the biological activity of Mellifra honey bees. A and Varroa nipple destructor V. in Erbil Governorate, PhD thesis, College of Agriculture and Forestry, University of Mosul.

Ministry of Higher Education and Scientific Research (1987). Mosul University, College of Agriculture and Forestry, Report, No. 9-3-6397.

Ministry Of Agriculture. (2021). Basra Governorate Agriculture Directorate, Plants Protection Department, Al-Nahl Division.

Allen, M., & Ball, B. (1996). The incidence and world distribution of honey bee viruses. Bee world, 77(3): 141-162.

Bonoan, R. E., Goldman, R. R., Wong, P. Y., & Starks, P. T. (2014). Vasculature of the hive: heat dissipation in the honey bee (*Apis mellifera*) hive. Naturwissenschaften, 101(6): 459-465.

Figen K., girisgin, A. O. aydin, L. (2012). Varroacidal efficacies of essential oils extracted from *Lavandula officinalis*, *Foeniculum vulgare*, and *Laurus nobilis* in naturally infested honeybee (*Apis mellifera* L.) colonies. Turk. J. Vet. Anim. Sci. 36, 554–559.

Gao, S. (2002). The effect of temperature and humidity of bee hive on the Varroa mite. Chin. J. Anim. Hub. Vet. Med, 4, 46-47.

Hanley, N., Breeze, T. D., Ellis, C., & Goulson, D. (2015). Measuring the economic value of pollination services: Principles, evidence and knowledge gaps. Ecosystem Services, 14, 124-132.

Hou, C. S., Li, B. B., Deng, S., & Diao, Q. Y. (2016). Effects of Varroa destructor on temperature and humidity conditions and expression of energy metabolism genes in infested honeybee colonies. Genetics and Molecular Research, 15(3): 1-13.

Hung, K. L. J., Kingston, J. M., Albrecht, M., Holway, D. A., & Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences, 285(1870): 2017-2140.

Hunt, G., Given, J. K., Tsuruda, J. M., & Andino, G. K. (2016). Breeding mite-biting bees to control Varroa. Bee Culture, 8, 41-47.

- Koeniger, N., Koeniger, G., & Delfinado-Baker, M. (1983).** Observations on mites of the Asian honeybee species (*Apis cerana*, *Apis dorsata*, *Apis florea*). *Apidologie*, 14(3): 197-204.
- Maggi, M. D., Sardella, N. H., Ruffinengo, S. R., & Eguaras, M. J. (2009).** Morphotypes of *Varroa destructor* collected in *Apis mellifera* colonies from different geographic locations of Argentina. *Parasitology research*, 105(6): 1629-1636.
- Maggi, M., Ruffinengo, S., Negri, P., Brasesco, C., Medici, S., Quintana, S., ... & Eguaras, M. J. (2013).** The status of bee health and colony losses in Argentina. *Honeybees: Foraging Behavior, Reproductive Biology and Diseases*. Ed. Cameron Molley. Nova Publishing Group, 212-234.
- Mahdi, M. S., Ali, M. B., & AL-abedy, A. N. (2020).** Genetic variability of the Mite *Varroa destructor* Isolated from Honey Bees in Iraq and Some Middle Eastern Countries. *Indian Journal of Forensic Medicine & Toxicology*, 14(1): 1-7.
- Mathialagan, M., Johnson, Y. S., Edward, T., David, P. M. M., Srinivasan, M. R., Mohankumar, S., & Senthilkumar, M. (2017).** A survey in Tamil Nadu of *Varroa jacobsoni* (Oudemans) ectoparasitic on Indian honey bees, *Apis cerana* F. *Journal of Entomology and Zoology Studies*, 5(6): 190-200.
- Oudemans, A. C. (1904).** On a new genus and species of parasitic Acari. *Notes from the Leyden Museum*. 24, 216 -222.
- Ramsey, S. D., Ochoa, R., Bauchan, G., Gulbranson, C., Mowery, J. D., Cohen, A., Lim, D., Joklik J., Cicero J., Ellis J., vanEngelsdorp D. & Hawthorne, D. (2019).** *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proceedings of the National Academy of Sciences*, 116(5): 1792-1801.
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010).** Biology and control of *Varroa destructor*. *Journal of invertebrate pathology*, 103, S96-S119.
- Rosenkranz, P., Aumeier, P., & Ziegelmann, B. (2010).** Biology and control of *Varroa destructor*. *Journal of invertebrate pathology*, 103, S96-S119.
- Sakofski, F., Koeniger, N., & Fuchs, S. (1990).** Seasonality of honey bee colony invasion by *Varroa jacobsoni* Oud. *Apidologie*, 21(6): 547-550.
- VanEngelsdorp, D., Hayes Jr, J., Underwood, R. M., & Pettis, J. (2008).** A survey of honey bee colony losses in the US, fall 2007 to spring 2008. *PloS one*, 3(12): e4071.