Middle East Journal of Agriculture Research
Volume: 13 | Issue: 04| Oct. – Dec.| 2024

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2024.13.4.52
Journal homepage: www.curresweb.com
Pages: 903-914

# Effect of Different Time and hybrid on the Amount of Blood Cells in Honeybee Workers

**Sherif A.S.F., Mohamed G.M. Ghazy, Ahmed R. Mazeed, Zienb A.E. Hassanein and Konper H.M.A**

Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt.

**Received:** 20 August 2024 **Accepted:** 30 Sept. 2024 **Published:** 10 Oct. 2024

## ABSTRACT

Insects have an innate immune system consisting of humoral and cellular defense mechanisms. Hemocytes, the main cellular component, play a significant role in immunity, with their numbers varying in response to different factors. This study examined the effect of different durations (one, two, three, and eleven days) and hybrid types (Carniolan, Italian, and Buckfast) on the number of various blood cells in honeybee (*Apis mellifera*) workers larvae raised under normal conditions during both winter and spring seasons. Three replicates (experimental colonies) were used for each treatment, and all colonies were provided with pollen. Significant differences in blood cell numbers between winter and spring were observed among the three honeybee hybrids. In winter, prohemocytes were most numerous in all hybrids after one day, whereas spherulocytes peaked after two, three, and eleven days. In spring, spherulocytes consistently had the highest numbers at all time points across the three hybrids. Coagulocytes without granules consistently showed the lowest numbers throughout both winter and spring in all three honeybee hybrids

***Keywords:*** Honeybee (*Apis mellifera*), immune system, Hemocytes, hybrid types

## 1. Introduction

Hemocytes (different blood cells) are the most important component of the insect haemolymph and represent the cornerstone of the insect's innate immune system that is their sole line of defense (Papadopoulou *et al.,* 1993; Abd El-Wahab *et al.,* 2016). Insects' immune system utilizes two types of reactions, humoral and cellular reactions (Armstrong *et al.,* 1996; Kavanagh; Reeves, 2004). Cellular immunity is provided by hemocytes (Armstrong, 1996; Cruz-Landim, 2009) which are responsible for many functions, like phagocytosis, nodulation, encapsulation, cytotoxicity - through the production of reactive oxygen intermediaries -, enzymes secretion, and hemolymph coagulation after the break in the cuticle and epidermis (Gillespie *et al.,* 1997; Lavine; Strand, 2002; Cruz-Landim, 2009, Butolo *et al.,* 2021). Several studies have shown changes in the number of circulating hemocytes of honeybees in response to many external factors such as exposure to contaminants including insecticides, fungicides, and pathogens (Alaux *et al.,* 2010; Brandt *et al.,* 2016; Domingues *et al.,* 2017), elimination of natural vegetation, changes in diets (Gilliam; Shimanuki, 1970; Szymás; Jedruszuk, 2003), and changes in temperature (Butolo *et al.,* 2021). Many internal factors can also influence the amount of circulating hemocytes in the hemolymph such as caste, sex and the developmental stage (age difference, instar) (Gilliam and Shimanuki, 1967; Mahmood and Yousuf, 1985, Schmid *et al.,* 2008; Mason *et al.,* 2013; Hystad *et al.,* 2017 Butolo *et al.,* 2021). Comparative studies across several species revealed that hemocytes are produced during two stages of insect development: in the embryonic stage from the head or dorsal mesoderm and in the larval or nymphal stage in hematopoietic organs derived from mesoderm (Strand michaelr, 2008). Researches demonstrate that the number of hemocytes reduces with the increase in age and the caste, with records of 21,000 hemocytes/mL for workers and drones (Snodgrass, 1956), 3824 hemocytes/mL for queens (Cruz-Landim, 2009), 10,640 hemocytes/mL for fifth days larvae, and 9020 hemocytes/mL for pupae

**Corresponding Author:** Sherif. A. S. F., Plant Protection Research Institute, Agricultural Research Centre, Giza, Egypt.

(Gilliam; Shimanuki, 1966). The proteome and cellular aspects of honey bee immunology are still mostly unknown to us. Although estimates of the total number of circulating hemocytes over the life span of honey bees have been made (Schmid *et al.,* 2008; Wilson-Rich *et al.,* 2008), surprisingly little is known about the distinct immune cell subpopulations that exist within honey bees, or about the differences between these subpopulations and how they change along the different developmental stages (Richardson *et al.,* 2018). Given the agricultural and ecological significance of honey bees, filling these knowledge gaps is an important priority for researchers seeking to understand the factors affecting bee health.

## 2. Materials and Methods

The Effect of different honeybee worker hybrid on number of the fifth instar larval blood cells at different time intervals were evaluated. Three honeybee hybrids (Carniolan, Italian and Buckfast) were used during two study periods (1st at January 2022 and 2nd at April 2022). Three colonies were used for each hybrid as a replicate. All the experimental colonies were deprived of sugar nutrition and were fed only with a dietary supplement consists mainly of: 6 k-grams of pollen + 50 k-grams of sugar powder + 5-kilogram cotton honey. Each colony has fid with 100 gm every 15 days.

### 2.1. Collection of honeybee workers larvae

Larvae of the fifth instar were collected from the experimental colonies at two different time intervals. The first collection was made at January (after1, 2, 3, 4, 5 and 11 days post supplemental feeding) and the second collection was made at April (after 1,2, 3 and 11 days post supplemental feeding).

### 2.2. Collecting of larval blood samples

The abdomen of the collected honeybee workers larvae was punctured with a fine scissor. The haemolymph drops were received on a glass slide, and then a smear was done (Shapiro, 1968). The smears were left to dry and then the slides were stained using wright's blood stain by putting them into a jar containing Wright's stain (composed of 25 ml methyl alcohol + 100 ml ethyl alcohol + g powder Wright's stain) for 2-5 minutes. The slides were then transferred directly to a jar containing a buffer solution (composed of 3.315 monobasic potassium phosphate + 1.28 dibasic sodium phosphate + 500 ml distilled water) for 2-5 minutes. After that the slides were then washed with distilled water and then left to dry (Salinger 1963 and Arnold and Hinks 1976). The smears were investigated under oil immersion at 1250x and maximum of 100 haemocytes /slide were differentiated based on the classification of Jones (1962) and Akai and Sato (1973).

## 3. Results

### 3.1. Larval differential haemocytes count (DHC) in winter

Fig. (1) indicates the blood cells in three worker honeybee hybrids after one day from fed. The highest prohaemocytes cells number was (31.75) recorded in the Buckfast hybrid as the lowest was (30.5) recorded in Carniolan. The plasmatocytes cells highest number was (17) recorded in the Carniolan as the Italian hybrid recorded the lowest number (12.75). The highest Oenocytes cells number was (22) recorded in the Italian hybrid as the lowest was (13) recorded in Buckfast. The spherulocytes cells highest number were (31.75) recorded in the Buckfast as the Carniolan hybrid recorded the lowest number (29). The highest Coagulocytes with granules cells number was (2.5) recorded in the Italian hybrid as the lowest were (1.75) recorded in Carniolan and Buckfast hybrid. The Coagulocytes without granules cells highest number was (1.5) recorded in the Buckfast as the Carniolan hybrid recorded the lowest number (0.5).

Fig. (2) indicated the blood cells in three worker honeybee hybrid after two days from fed. The highest prohaemocytes cells number were (31) recorded in the Italian as the lowest was (29) recorded in the Buckfast hybrid. The plasmatocytes cells highest number was (16) recorded in the Buckfast as Italian hybrid recorded the lowest number (12.25). The highest Oenocytes cells number was (20.75) recorded in the Carniolan hybrid as the lowest were (19.25) recorded in Italian. The spherulocytes cells highest number was (34.5) recorded in the Italian as the Carniolan hybrid recorded the lowest number (29.75). The highest Coagulocytes with granules cells number was (2.75) recorded in the Carniolan hybrid as the lowest was (1.26) recorded in the Italian hybrid. The Coagulocytes without

granules cells highest number was (1.75) recorded in the Carniolan as Italian hybrid recorded the lowest number (0.96).

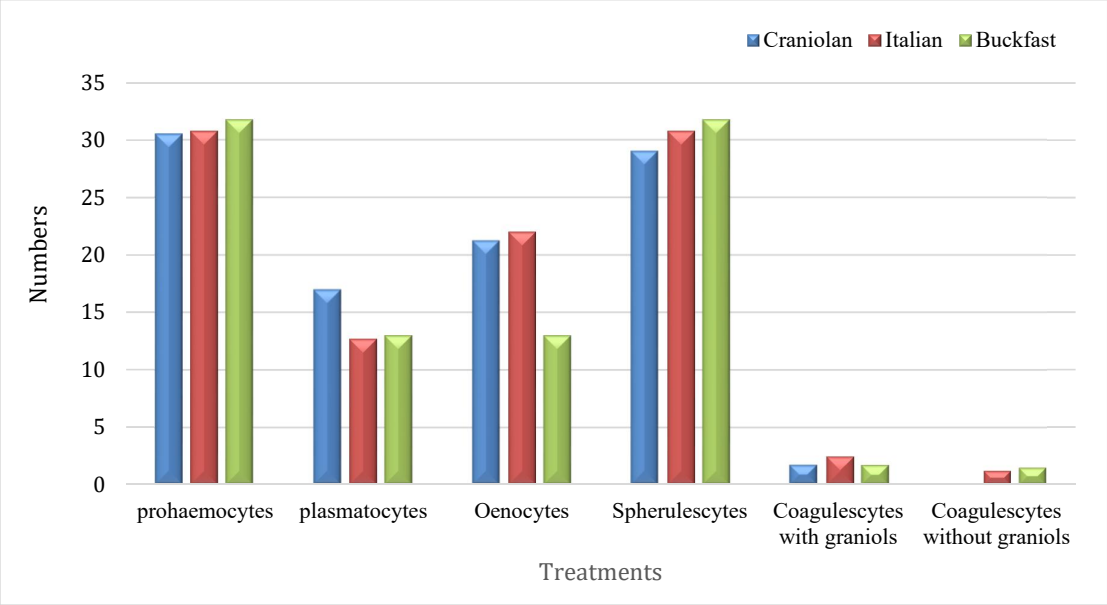


**Fig. 1:** Blood cell in three honeybee hybrids after one day

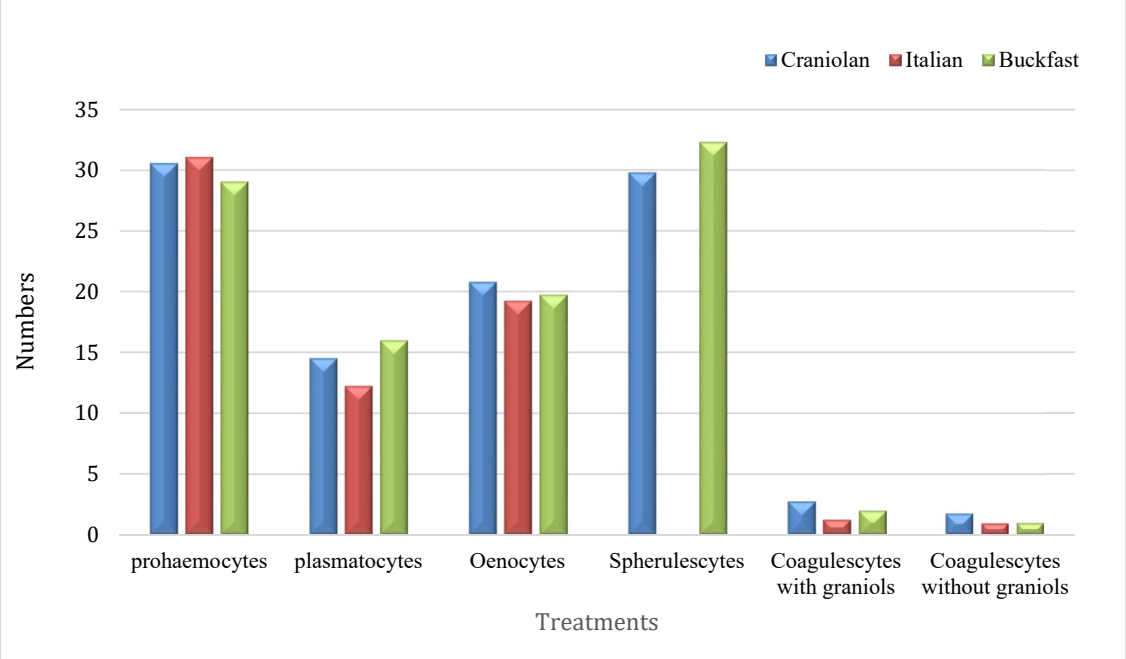


**Fig. 2:** Blood cell in three honeybee hybrids after two days

Fig. (3) indicated the blood cells in three worker honeybee hybrid after three days from fed. The highest prohaemocytes cells number was (32.5) recorded in the Buckfast as the lowest was (30.25) recorded in the Italian hybrid. The plasmatocytes cells highest number was (12.75) recorded in the Italian as Buckfast hybrid recorded the lowest number (10.75). The highest Oenocytes cells number was (22.5) recorded in the Buckfast hybrid as the lowest was (21. 5) recorded in Carniolan. The spherulocytes cells highest number was (34.5) recorded in the Carniolan as the Buckfast hybrid recorded the lowest number (32.5). The highest Coagulocytes with granules cells number was (1.5) recorded in the Italian hybrid as the lowest was (0.75) recorded in the Carniolan hybrid. The

Coagulocytes without granules cells highest number was (0.5) recorded in the Italian and Buckfast whereas the Carniolan hybrid recorded the lowest number (0.25).

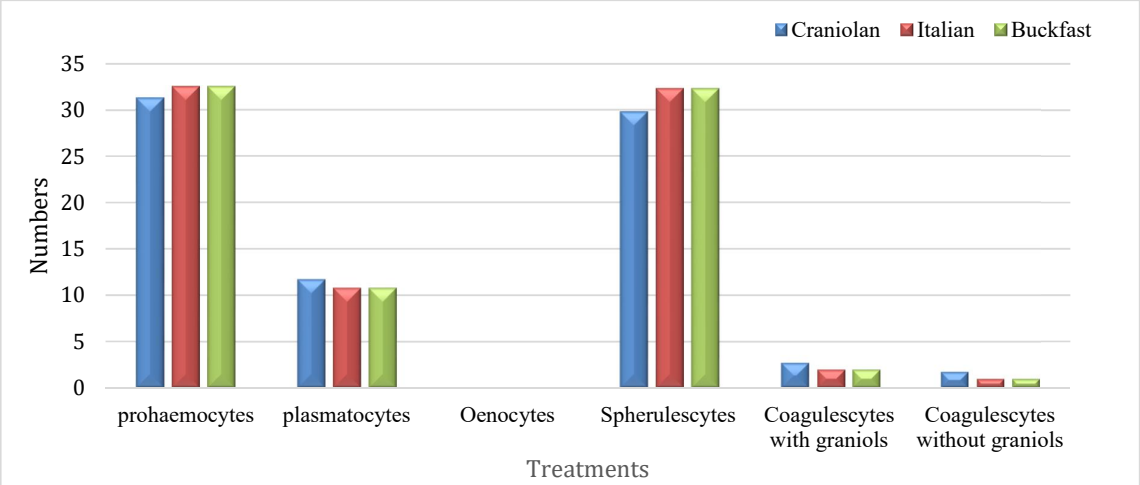


**Fig. 3:** Blood cell in three honeybee hybrids after three days

Fig. (4) indicated the blood cells in three worker honeybee hybrid after eleven days from fed. The highest prohaemocytes cells number was (31.75) recorded in the Carniolan. As the lowest were (11.25) recorded in the Italian hybrid. The plasmatocytes cells highest number were (12.5) recorded in the Italian and Buckfast hybrid. Whereas the Craniolan hybrid recorded the lowest number (11.82). The highest Oenocytes cells number were (23.5) recorded in the Carniolan hybrid as the lowest were (21.75) recorded in the Italian. The spherulocytes cells highest number were (33.5) recorded in the Buckfast as the Carniolan hybrid recorded the lowest number (31.75). The highest Coagulocytes with granules cells number were (1.5) recorded in the Carniolan hybrid as the lowest were (0.75) recorded in the Buckfast hybrid. The Coagulocytes without granules cells highest number were (0.5) recorded in the Carniolan and Italian hybrid whereas the Buckfast recorded zero number (0).

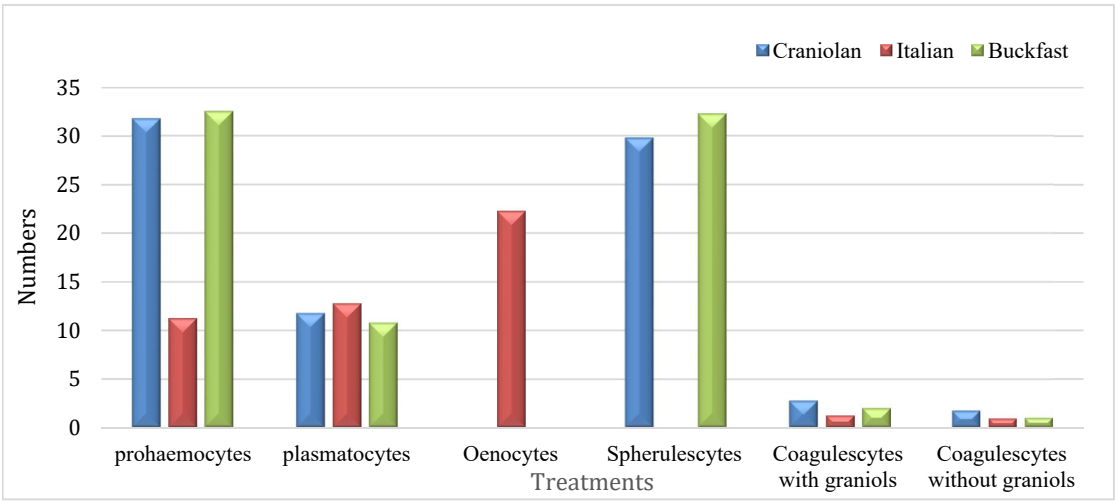


**Fig. 4:** Blood cell in three honeybee hybrids after eleven days

Fig. (5) indicated the blood cells in Carniolan honeybee worker hybrid after three different time from fed. The highest prohaemocytes cells number were (31.75) recorded in the eleventh day. As the lowest was (30.5) recorded in the first and second days. The plasmatocytes cells highest number was (17) recorded in the first day. Whereas the third day recorded the lowest number (11.82). The highest Oenocytes cells number were (23.5) recorded in the eleventh day as the lowest was (20.75) recorded in the second day. The spherulocytes cells highest number were (34.5) recorded in the third day as the lowest number (29) recorded in the second day. The highest Coagulocytes with granules cells number

were (2.75) recorded in the second day as the lowest were (0.75) recorded in the third day. The Coagulocytes without granules cells highest number were (1.75) recorded in the second day whereas the lowest number recorded in the third day (0.25).

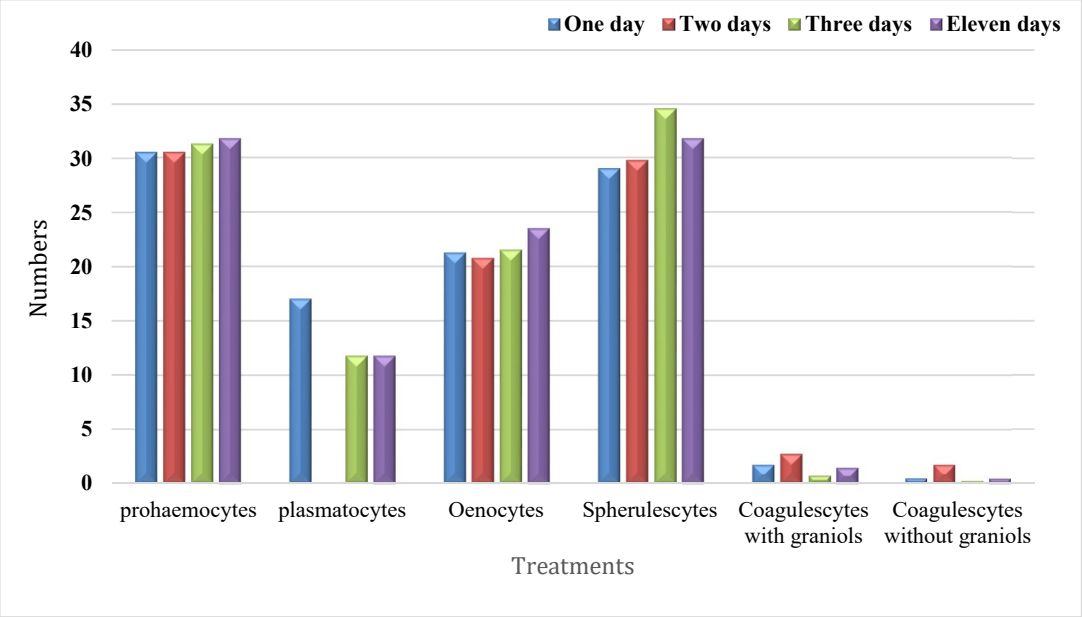


**Fig. 5:** Blood cell in Craniolan honeybee hybrids after three different time

Fig. (6) indicated the blood cells in the Italian's honeybee worker hybrid after three different times from fed. The highest prohaemocytes cells number were (30.75) recorded in the first day. As the lowest were (11.25) recorded in the eleventh day. The plasmatocytes cells highest number were (12.75) recorded in the first and third days. Whereas the second day recorded the lowest number (12.25). The highest Oenocytes cells number were (22.25) recorded in the third day as the lowest were (19.25) recorded in the second day. The spherulocytes cells highest number were (34.5) recorded in the second day as the lowest number (30.75) recorded in the first day. The highest Coagulocytes with granules cells number were (2.5) recorded in the first day as the lowest were (1.25) recorded in the eleventh day. The Coagulocytes without granules cells highest number were (1.25) recorded in the first day whereas the lowest number recorded in the third and eleventh days (0.5).

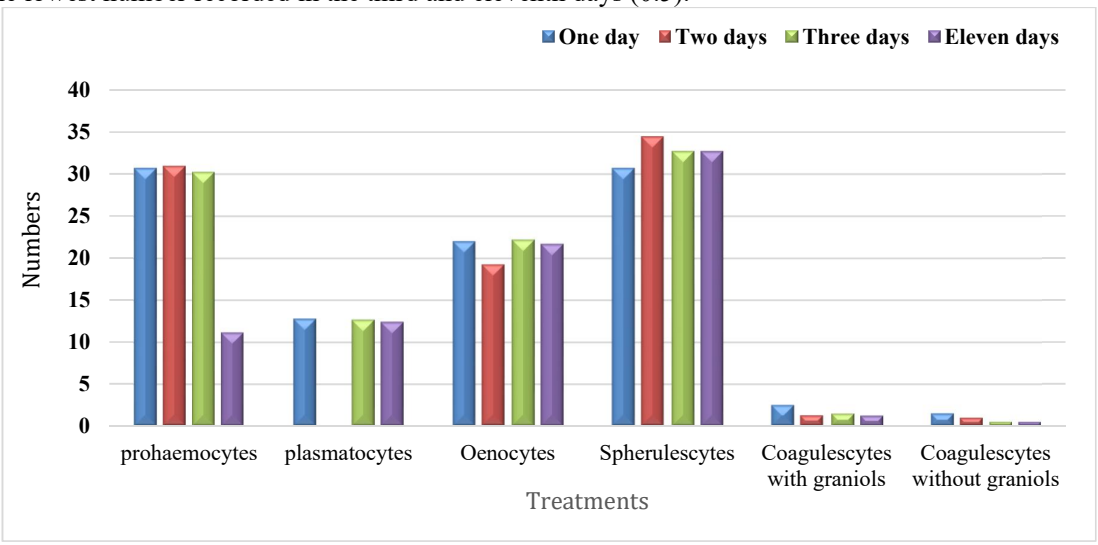


**Fig. 6:** Blood cell in Italian honeybee hybrids after three different time

Fig. (7) indicated the blood cells in the Buckfast's honeybee worker hybrid after different times from fed. The highest prohaemocytes cells number were (32.5) recorded in the third day. As the lowest were (29) recorded in the second day. The plasmatocytes cells highest number were (16) recorded in the second day. Whereas the third day recorded the lowest number (10.75). The highest Oenocytes cells number were (22.25) recorded in the third and eleventh days as the lowest were (13) recorded in the first day. The spherulocytes cells highest number were (33.5) recorded in the eleventh day as the lowest number (31.75) recorded in the first day. The highest Coagulocytes with granules cells number were (2) recorded in the second day as the lowest were (0.75) recorded in the eleventh day. The Coagulocytes without granules cells highest number were (1.5) recorded in the first day whereas the eleventh day recorded no Coagulocytes without granules (0).

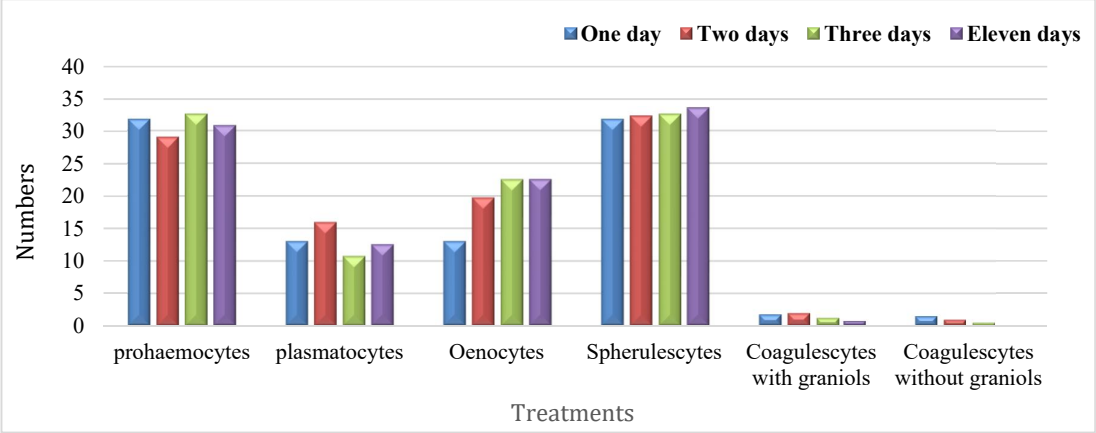


**Fig. 7:** Blood cell in Buckfast honeybee hybrids after three different time

### 3.2. Larval differential haemocytes count (DHC) in spring

Fig. (8) indicated the blood cells in three worker honeybee hybrids after one day from fed. The highest prohaemocytes cells number was (31) recorded in the Buckfast hybrid as the lowest was (28.5) recorded in Carniolan. The plasmatocytes cells highest´s number was (15.75) recorded in the Carniolan as the Italian and Buckfast hybrid recorded the lowest number (12.75). The highest Oenocytes cells number was (22) recorded in the Carniolan and Italian hybrid as the lowest was (21) recorded in Buckfast. The spherulocytes cells highest number were (33.75) recorded in the Buckfast as the Italian hybrid recorded the lowest number (30.75). The highest Coagulocytes with granules cells number was (2.5) recorded in the Italian hybrid as the lowest were (1) recorded in Buckfast hybrid. The Coagulocytes without granules cells highest number was (1.25) recorded in the Italian as the Buckfast hybrid recorded the lowest number (0.5).

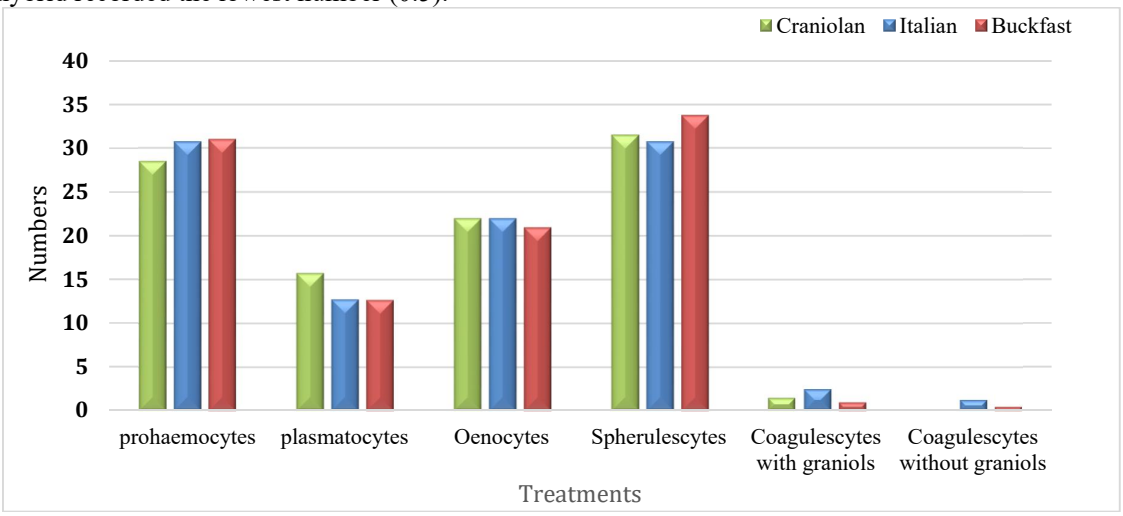


**Fig. 8:** Blood cell in three honeybee hybrids after one day in spring

Fig. (9) indicated the blood cells in three worker honeybee hybrid after two days from fed. The highest prohaemocytes cells number were (30) recorded in the Carniolan and Italian as the lowest was (29.5) recorded in the Buckfast hybrid. The plasmatocytes cells highest number was (13) recorded in the Buckfast as Italian hybrid recorded the lowest number (11.25). The highest Oenocytes cells number was (24.25) recorded in the Buckfast hybrid as the lowest were (21.75) recorded in Italian. The spherulocytes cells highest number was (35.25) recorded in the Italian as the Buckfast hybrid recorded the lowest number (31.75). The highest Coagulocytes with granules cells number was (1.5) recorded in the Carniolan hybrid as the lowest was (1) recorded in the Buckfast hybrid. The Coagulocytes without granules cells highest number was (0.75) recorded in the Carniolan as Italian and Buckfast hybrid recorded the lowest number (0.5).

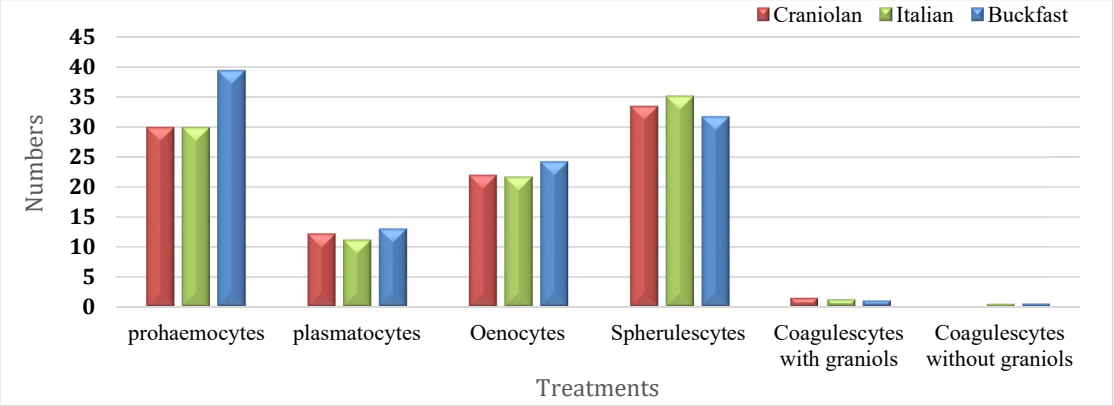


**Fig. 9:** Blood cell in three honeybee hybrids after two days in spring

Fig. (10) indicated the blood cells in three worker honeybee hybrid after three days from fed. The highest prohaemocytes cells number was (31) recorded in the Buckfast as the lowest was (29.25) recorded in the Italian hybrid. The plasmatocytes cells highest number was (12.25) recorded in the Italian as Carniolan and Buckfast hybrid recorded the lowest number (11.75). The highest Oenocytes cells number was (26.5) recorded in the Italian hybrid as the lowest was (24.75) recorded in Buckfast. The spherulocytes cells highest number was (31.75) recorded in the Carniolan as the Buckfast hybrid recorded the lowest number (30). The highest Coagulocytes with granules cells number was (1) recorded in all hybrid. The Coagulocytes without granules cells number recorded in all hybrid was (0.5).

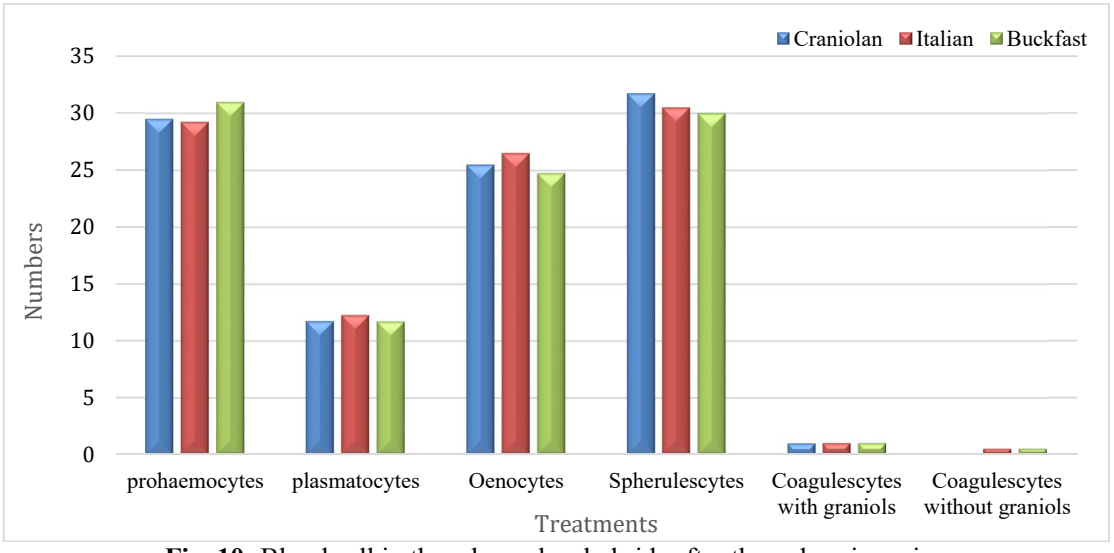


**Fig. 10:** Blood cell in three honeybee hybrids after three days in spring

Fig. (11) indicated the blood cells in three worker honeybee hybrid after eleven days from fed. The highest prohaemocytes cells number was (30) recorded in the Carniolan. As the lowest were (11.25) recorded in the Italian hybrid. The plasmatocytes cells highest number were (13.25) recorded in the Buckfast hybrid. Whereas the Carniolan hybrid recorded the lowest number (11.25). The highest Oenocytes cells number were (26) recorded in the Carniolan hybrid as the lowest were (21.75) recorded in the Italian. The spherulocytes cells highest number were (32.75) recorded in the Italian as the Carniolan hybrid recorded the lowest number (30.75). The highest Coagulocytes with granules cells number were (1.25) recorded in the Italian hybrid as the lowest were (0.25) recorded in the Buckfast hybrid. The Coagulocytes without granules cells highest number were (1) recorded in the Carniolan and hybrid whereas the Buckfast recorded the lowest number (0.25).

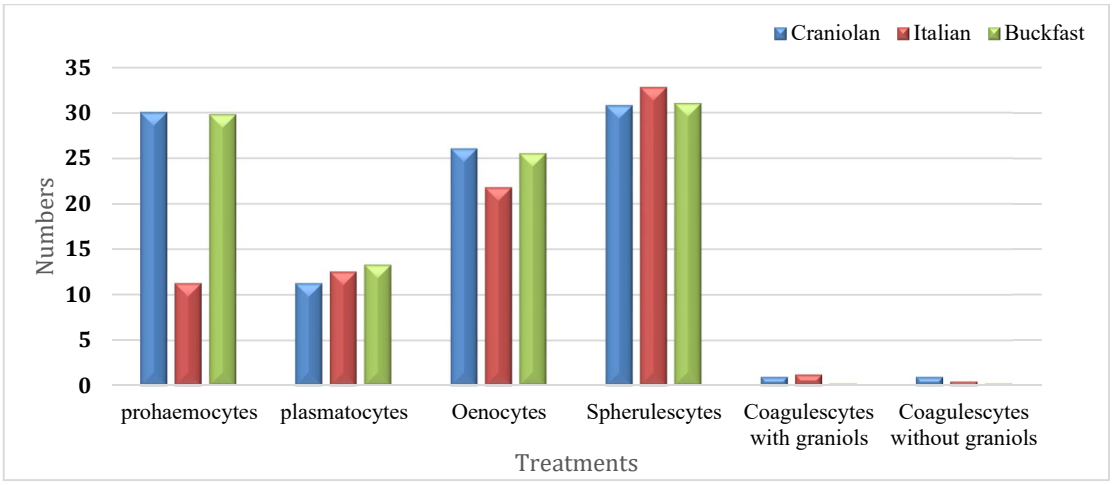


**Fig. 11:** blood cell in three honeybee hybrids after eleven days in spring

Fig. (12) indicated the blood cells in Carniolan honeybee worker hybrid after three different time from fed. The highest prohaemocytes cells number were (30) recorded in the two and eleventh days. As the lowest was (28.5) recorded in the first day. The plasmatocytes cells highest number was (15.75) recorded in the first day. Whereas the eleventh day recorded the lowest number (11.25). The highest Oenocytes cells number were (26) recorded in the eleventh day as the lowest was (22) recorded in the first and second days. The spherulocytes cells highest number were (33.5) recorded in the second day as the lowest number (30.75) recorded in the eleventh day from fed. The highest Coagulocytes with granules cells number were (1.5) recorded in the first and second day as the lowest were (1) recorded in the third and eleventh day. The Coagulocytes without granules cells highest number were (1) recorded in the eleventh day whereas the lowest number recorded in the third day (0.5).

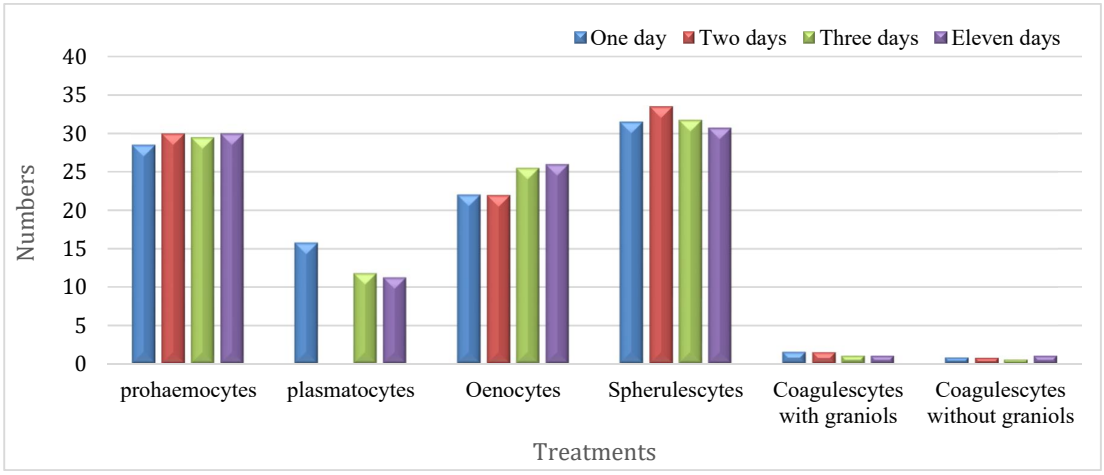


**Fig. 12:** Blood cell in Craniolan honeybee hybrids after three different time in spring

Fig. (13) indicated the blood cells in Italian honeybee worker hybrid after three different time from fed. The highest prohaemocytes cells number were (30.25) recorded in the first and eleventh days. As the lowest was (29.25) recorded in the third day. The plasmatocytes cells highest number was (12.75) recorded in the eleventh day. Whereas the second day recorded the lowest number (11.25). The highest Oenocytes cells number were (26.5) recorded in the third day as the lowest was (21.75) recorded in the second day. The spherulocytes cells highest number were (35.25) recorded in the second day as the lowest number (30.5) recorded in the third day. The highest Coagulocytes with granules cells number were (1.25) recorded in the second day as the lowest were (1) recorded in all the other times. The Coagulocytes without granules cells highest number were (0.5) recorded in the first, second and third days whereas the lowest number recorded in the eleventh day (0.25).

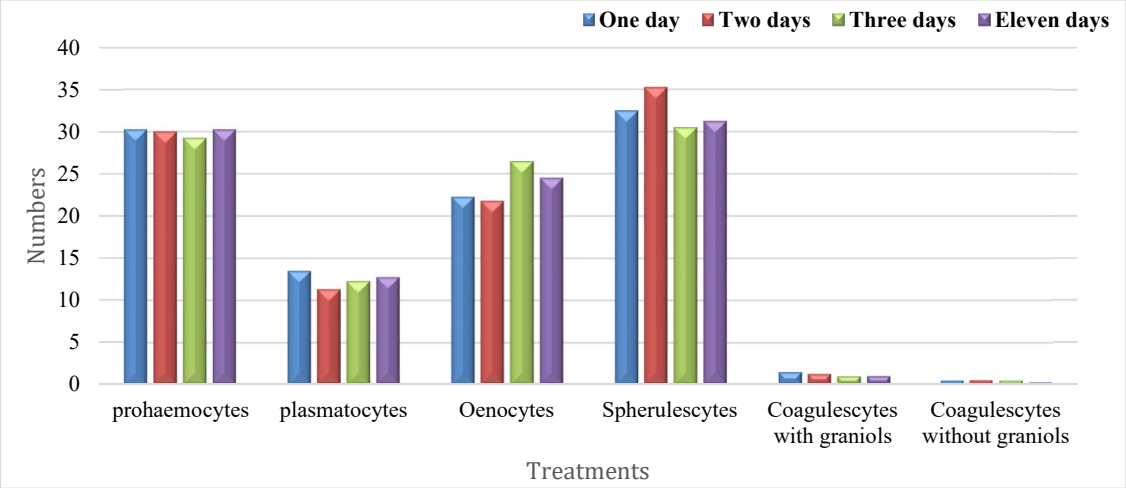


**Fig. 13:** Blood cell in Italian honeybee hybrids after three different time in spring

Fig. (14) indicated the blood cells in Buckfast honeybee worker hybrid after three different time from fed. The highest prohaemocytes cells number were (31) recorded in the first and third days. As the lowest was (29.5) recorded in the second day. The plasmatocytes cells highest number was (13.25) recorded in the eleventh day. Whereas the third day recorded the lowest number (11.75). The highest Oenocytes cells number were (25.5) recorded in the eleventh day as the lowest was (21) recorded in the first day. The spherulocytes cells highest number were (33.75) recorded in the first day as the lowest number (30) recorded in the third day. The highest Coagulocytes with granules cells number were (1) recorded in the first, second and third days as the lowest were (0.25) recorded in the eleventh day. The Coagulocytes without granules cells highest number were (0.5) recorded in the first, second and third days whereas the lowest number recorded in the eleventh day (0.25).

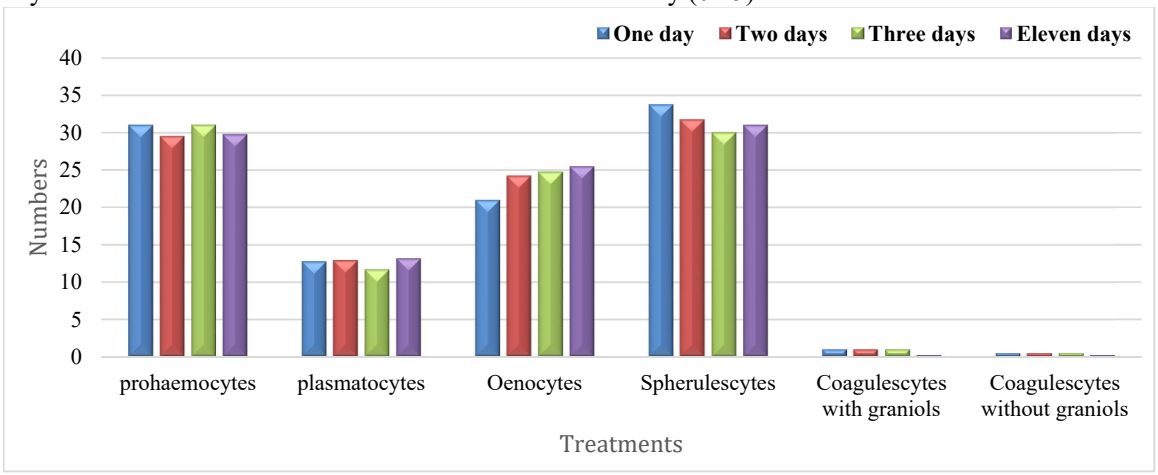


**Fig 14:** Blood cell in Buckfast honeybee hybrids after three different time in spring

## 4. Discussion

The recent decline in managed honey bee populations has raised widespread concerns across scientific, ecological, and economic fields. This reduction is linked to various factors, including parasites, diseases (caused by viruses and bacteria), pesticide exposure, and nutritional shortages resulting from habitat destruction (Martin *et al.,* 2012; Neumann *et al.,* 2012; Wagoner *et al.,* 2013; Alaux *et al.,* 2014; Steinmann *et al.,* 2015).

It has also been suggested that diseases caused by infectious agents exert significant selective pressure on honey bees. This can lead to increased mortality and illness, which may either directly cause hive collapse or gradually reduce hive quality due to decreased productivity (Jefferson *et al.,* 2013). Recently, rising honey bee mortality and colony losses have been associated with weakened immune systems in bees (Gätschenberger *et al.,* 2013; Alaux *et al.,* 2014; Steinmann *et al.,* 2015).

In recent years, the immune system of *Apis mellifera* has become a focal point for researchers seeking to understand how honey bees defend themselves against various parasites and pathogens. Despite this growing interest, the area of cellular immunity in honey bees has received relatively little attention (Marringa *et al.,* 2014). While substantial research has explored humoral and molecular immunity (Evans *et al.,* 2006) and social immunity (Wilson-Rich *et al.,* 2009), investigations into the cellular immune responses of A. mellifera have largely stagnated over the years. Only a handful of studies have focused on the characterization of honey bee hemocytes and the effects of Varroa destructor parasitism on the blood cells of honey bees (Price and Ratcliffe, 1974; Fluri *et al.,* 1977; Van Steenkiste *et al.,* 1988; Wienand and Madel, 1988; Beisser *et al.,* 1990; de Graaf *et al.,* 2002; Sapcaliu *et al.,* 2009).

Consequently, there is an urgent need for strategies that monitor hemocyte subsets, which could enhance our understanding of how pathogens contribute to colony failure (Marringa *et al.,* 2014). Prioritizing hemocyte analysis in honey bees could address critical questions related to the ongoing decline of these vital pollinators. Such research could provide insights into the immune responses of honey bees and their ability to cope with various stressors, ultimately aiding in the conservation of bee populations and improving their health (Gätschenberger *et al.,* 2013; Alaux *et al.,* 2014). Our study aimed at evaluating the number of circulating hemocytes in different hybrids (Italian, Cniolan and Buckfast) of honeybee (*A. mellifera*), in different times of the 5th larval instar. Th obtained results indicated that the number of circulating hemocytes in the hemolymph show a great variation which can affect the honeybee immune system.

Our results are in agreement with that of Wilson-Rich *et al.* (2008) who measured the number of hemocytes in hemolymph and the encapsulation response, across four developmental stages: larvae, pupae, nurses and foragers. The authors found that honey bee brood (larvae and pupae) has the highest total hemocyte counts than the adults.

The results are also similar to previous research by Schmid *et al.* (2008) who observed a reduction in the number of hemocytes with the increase in the bees' age and associated this fact with the replacement of cellular defense by the humoral defense to reduce energy costs for the colony.

## References

Abd El-Wahab, T.E., A.M.M. Ghania, and E.W. Zidan, 2016. Evaluation of a new pollen supplement diet for honey bee colonies and its impact on various biological activities. Journal of Agricultural Technology, 12(1): 55-62.

Alaux, C.D., F.O. Ducloz, D. Crauser, and Y.L. Conte, 2010. The influence of diet on the immunocompetence of honeybees. Biology Letters, 6: 562–565.

Alaux, C., D. Crauser, M. Pioz, C. Saulnier, and Y. Le Conte, 2014. The effects of parasitism and immune modulation on flight activity in honey bees, monitored using optical counters. Journal of Experimental Biology, 217: 3416–3424.

Armstrong, P.B., R. Melchior, and J.P. Quigley, 1996. The role of humoral immunity in long-lived arthropods. Journal of Insect Physiology, 42(1): 53-64.

Beisser, K., E. Munz, M. Reimann, and I.C.E. Renner-Muller, 1990. Experimental studies on the in vitro cultivation of cells from Carinthian honeybees (*Apis mellifera* carnica Pollmann). Journal of Veterinary Medicine, 37: 509–511.

Brandt, A., A. Gorenflo, R. Siede, M. Meixner, and R. Büchler, 2016. The neonicotinoids thiacloprid, imidacloprid, and clothianidin impair the immune competence of honey bees (*Apis mellifera* L.). Journal of Insect Physiology, 86: 40-47.

Butolo, N.P., P. Azevedo, L.D. Alencar, O. Malaspina, and R.C.F. Nocelli, 2021. The effects of low temperatures on the immune system of honeybees. Journal of Thermal Biology, 101: 103082.

Cruz-Landim, C.D., 2009. Bees: Morphology and function of systems.

Domingues, C.E.C., F.C. Abdalla, P.J. Balsamo, B.V.R. Pereira, M. de Alencar Hausen, M.J. Costa, and E.C.M. Silva-Zacarin, 2017. The impact of thiamethoxam and picoxystrobin on the survival and strain of the hepatonephric system in Africanized honeybees. Chemosphere, 186: 994-1005.

Evans, J.D., K. Aronstein, Y.P. Chen, C. Hetru, J.L. Imler, H. Jiang, *et al.,* 2006. Immune pathways and defense mechanisms in honey bees (*Apis mellifera*). Insect Molecular Biology, 15: 645–656.

Fluri, P., H. Wille, L. Gerig, and M. Lüscher, 1977. The relationship between juvenile hormone, vitellogenin, and hemocyte composition in winter worker honeybees (*Apis mellifera*). Experientia, 33: 1240–1241.

Gätschenberger, H., K. Azzami, J. Tautz, and H. Beier, 2013. The antibacterial immune competence of honey bees (*Apis mellifera*) adapts to various life stages and environmental threats. PLoS ONE, 8(6): e66415. https://doi.org/10.1371/journal.pone.0066415

Gillespie, J.P., M.R. Kanost, and T. Trenczek, 1997. Biological mediators of insect immunity. Annual Review of Entomology, 42: 611–643.

Gilliam, M., and H. Shimanuki, 1966. Total hemocyte counts in the hemolymph of immature honeybees. American Bee Journal, 106(10): 376.

Gilliam, M., and H. Shimanuki, 1967. In vitro phagocytosis of Nosema apis spores by honey bee hemocytes. Journal of Invertebrate Pathology, 3(9): 387-389.

Gilliam, M., and H. Shimanuki, 1971. Blood cells of the worker honeybee. Journal of Apicultural Research, 10(2): 79–85.

Graaf, D., D.C. Dauwe, R. Walravens, and K. Jacobs, 2002. Flow cytometric analysis of lectin-stained hemocytes in honey bees (*Apis mellifera*). Apidologie, 33: 571–579.

Hystad, E.M., H. Salmela, G.V. Amdam, and D. Münch, 2017. Variations in hemocyte-mediated phagocytosis among different castes of honey bee (*Apis mellifera*). PLoS ONE, 12: e0184108.

Jefferson, J., H. Dolstad, M. Sivalingam, and J. Snow, 2013. Barrier immune effectors remain active during the transition from nurse to forager in honey bees. PLoS ONE, 8(1): e54097.

Kavanagh, K., and E.P. Reeves, 2004. The potential of insects for in vivo pathogenicity testing of microbial pathogens. FEMS Microbiology Reviews, 28(1): 101-112.

Lavine, M.D., and M.R. Strand, 2002. The role of insect hemocytes in immunity. Insect Biochemistry and Molecular Biology, 32: 1295–1309.

Mahmood, A., and M. Yousuf, 1985. The effects of certain insecticides on the hemocytes of Gryllus bimaculatus de Geer. Pakistan Journal of Zoology, 17: 71-84.

Marringa, W.J., M.L. Krueger, N.L. Burritt, and J.B. Burritt, 2014. Hemocyte profiling of honey bees using flow cytometry. PLoS ONE, 9, e108486.

Martin, S.J., A.C. Highfield, L. Brettell, E.M. Villalobos, G.E. Budge, Powell, *et al.,* 2012. A parasitic mite alters the global viral landscape of honey bees. Science, 336: 1304–1306.

Neumann, P., O. Yanez, I. Fries, and J.R. de Miranda, 2012. The dynamics of Varroa invasion and adaptation of viruses. Trends in Parasitology, 28: 353–354.

Papadopoulou-Karabela, K., N. Iliadis, and V. Liakos, 1993. Changes in hemocyte profiles of honey bees (*Apis mellifera* L) after artificial infection with Pseudomonas aeruginosa. Apidologie, 24: 81-86.

Price, C.D., and N.A. Ratcliffe, 1974. A reconsideration of insect hemocyte classification based on blood from fifteen insect orders. Z. Zellforsch, 147: 537–549. [PubMed: 4407658].

Reeves, E.P., C.G.M. Messina, S. Doyle, and K. Kavanagh, 2004. The relationship between gliotoxin production and the virulence of Aspergillus fumigatus in Galleria mellonella. Mycopathologia, 158: 73–79.

Richardson, R.T., M.N. Ballinger, F. Qian, J.W. Christman, and R.M. Johnson, 2018. Characterization of hemocyte cell communities in honey bees (*Apis mellifera*). Apidologie, 49: 397–410.

Sapcaliu, A., I. Radoi, C. Pavel, N. Tudor, E. Cauia, A. Siceanu and F. Meiu, 2009. Research regarding haemocyte profile from *Apis mellifera carpatica* bee haemolymph originated in the south of Romania. Lucrări stiinłifice medicină veterinară vol. XLII, 2009, Timisoara.