MELLIFERA REVIEW ARTICLE

Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests

Murat GENÇ[1*], Ferat GENÇ[2]

[1] Department of Animal Science, Faculty of Veterinary Medicine, Atatürk University, Erzurum 25240, Turkey

[2] Department of Animal Science, Faculty of Agriculture, Atatürk University, Erzurum 25240, Turkey

* *Corresponding author e-mail: vet.murat.genc@gmail.com*

Received: 30th May, 2019; accepted: 10th July, 2019; published: 18th September, 2019

A B S T R A C T

Animal health is an important factor that may limit efficiency in beekeeping, as in other fields yielding animal products. After all, the diseases, parasites, and pests that pose a risk for honey bees do not only affect their health, but can decrease productivity, as well. The colony may collapse eventually unless measures are taken to improve the situation. Occasional epidemics of diseases, parasites, and pests cause producers to suffer great losses and harm beekeeping on a national scale in Turkey.

As with all living organisms, honey bees have a strong multicomponent defense (resistance, immunity) mechanism against diseases, parasites, and pests that threaten their health. However, various stress factors from those rooted in their genetic makeup to others resulting from the environment collapse their defense system and with the decline of bodily resistance, honey bees start getting ill and eventually die. Moreover, these stress factors or stressors interact with one another, with pathogenic microorganisms, parasites, and pests to create a synergic effect leading to exhaustion in bees, their offspring, and whole colonies. Therefore, it is critically important to eliminate stress factors that impair and collapse the defense system in honey bees.

Keywords: Honey bee, stress factors, components of defense system.

Introduction

I: Stress Factors on Honey Bees

1. Genetic Deficiency

Honey bee breeds show a wide variety in their genetic predisposition or resistance to certain diseases and parasites. Disease resistance of larvae is also a hereditary feature wildly varying across various breeds. In other words, each genotype has some specific inherited characteristics [1,2].

The hygienic behavior of various races differs wildly when it comes to practices like clearing honeycomb cells off dead brood after a disease such as American foulbrood, chalkbrood, or parasitic infection with the *Varroa destructor*. The heritability of hygienic behavior is $h^2 = 0.40$ to 0.67. Races with genetically poor hygienic behavior are more susceptible to certain diseases and experience more health problems [3,4]. For example, Caucasian and Anatolian honey bees carry more propolis to their nests to disinfect honeycomb cells as a protection against disease. However, Caucasian bees are more susceptible to Nosema disease than other races. On the other hand, Anatolian bees are susceptible to paralysis virus, but more resistant against acariasis and Nosema than Caucasian bees. Resistant lines developed against the parasite *Varroa destructor*

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

through breeding and selection can also develop resistance against American foulbrood, tracheal mites, chalkbrood, Nosema, and even the wax moth [1,2].

African bees display more hygienic behavior against the chalkbrood disease. While Italian bees are resistant against acariasis, wax moths, and European foulbrood, the breed called European dark bee is susceptible to these diseases. Carniolan honey bees protect their nests well against parasites such as hornets or wax moths and their brood is almost never affected with disease especially in their native land. They are highly adaptable to environmental conditions, but susceptible to bee paralysis [1,2]. A genetic characteristic of Japanese honey bees allows them to raise their body temperature up to 48-50 ^{o}C in the event of an attack on their nests to almost burn the intruder to death. European honey bees display a similar behavior in the presence of wasps, but they cannot possibly survive a temperature rise at the same level [5].

Another important genetic deficiency negatively affecting bee survival is a result of continued inbreeding in apiculture. In nature, it is unlikely for a queen to mate with drones of her kin. However, kinship increases with inbreeding and systematic indoor beekeeping. In particular, using drones of the same breeding colony for artificial insemination and breeding a large number of sisters to distribute them as queens among colonies of the same apiary will rapidly increase kinship in future generations, thus narrowing down genetic variation and giving rise to inbreeding degeneration. As the queen mates with drones that share her sex alleles, the colony has more diploid eggs to turn into males with a low survival chance. Worker bees notice this type of genetically flawed broods at the beginning of the larval stage and eat them. This leaves the brood comb with a large number of empty cells, which in turn slows down colony development and makes the colony vulnerable to diseases [1,6,7,8,9].

Using old, disabled, or poorly queen bees also affects the development and strength of the colony, thus disrupts its dynamics. Weak colonies have low resistance against diseases [10].

2. Unfavorable Climate and Environmental Conditions

Since honey bees are diverse in their adaptability and will to survive, they respond differently to different environmental conditions. Temperature, wind, precipitation, humidity, altitude and other environmental factors are extremely important for the well-being and genetic efficiency of bees. As mentioned above, not all races thrive and realize their full genetic potential under the same circumstances. Some races thrive in high altitudes while others in low-altitude coastal areas, some have a healthier existence in humid regions with high levels of precipitation while others are better off in the desert. Therefore, it is important to provide the environmental conditions suitable for each race or, failing that, it is recommended to work with genotypes that do not have adaptation problems [1,7,8,9].

Since honey bees are cold-blooded insects, temperature is a crucially important factor in all their activities. Body temperature of honey bees changes according to ambient temperature and for survival they can get adapted to a range of 0±40 ^{o}C. However, the optimal ambient temperature for their regular activities is between 21-35°C. The farther the temperature moves away from this range, the more stressed and

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

uncomfortable they get. Decrease in temperature slows down the metabolism, while increased temperature increases the respiratory rate and accelerates the metabolism. High metabolic rate increases energy consumption and speeds up physiological aging [1,2,7,8,9].

To compensate for the disturbed balance inside the hive due to suboptimal temperature, humidity, and CO_2 level, bees shrink the flight hole, cluster together and ventilate. However, for this purpose, there must be a sufficient number of bees and the hive should be suitable for airflow. Therefore, wooden beehives made of natural material that allows a better balance of temperature, humidity, and CO_2 should be preferred. Too high, too low, or continuously fluctuating body and hive temperatures, as well as excessive CO_2 levels in the hive stress out bees, negatively affect their lives, and accelerate physiological aging due to increased energy consumption. Rising hive temperatures cause eggs to dry and die, whereas insufficient incubation temperature will cause breeding to stop [11,12].

Especially in poorly ventilated hives honey absorbs the moisture in the atmosphere and this excess moisture reduces its acidity. Bees cannot compensate for this loss of acidity and high water content in honey activates yeast spores. Moisture accumulated in the hive starts fermentation in honey. The disrupted acidity balance, high water ratio, and fermentation of the honey weaken the natural defense mechanism of the bees. The moist environment in the hive not only causes digestive disorders in honey bees, but the rapid proliferation of microorganisms renders them vulnerable to fungal diseases, as well [1,2,10,13].

Unfavorable environmental conditions directly affect the life and health of bees. Continued exposure to wind, excessive cold, excessive rainfall and moist hinder the flight of bees. Not being able to fly for a long time is a strong stress factor on bees. Under circumstances of constant wind bees hardly fly and get disoriented, thus they cannot go on foraging. In regions with extreme heat, excessive precipitation or simoom, flowers stop secreting nectar and this gives rise to malnutrition. All these unfavorable environmental factors stress bees out, weaken their resistance, and leaves them vulnerable to illness [1,2,10,13,14,15].

3. Malnutrition

Bees obtain the nutrients they need to survive and thrive from nectar, honey, pollen, and water. They provide their carbohydrate and energy primarily from nectar and honey, while pollen is their only natural source of protein. And water is obviously essential for their life. Honey bees provide the energy they need for vital activities from carbohydrates. The nectar and/or pollen of some plants may contain compounds that are toxic or harmful for bees. Bees are not interested in some carbohydrates at all, but some of them shorten their lifespan, even have a toxic effect. A medium-sized colony consumes about 75 kg honey and 20-50 kg pollen per year. A honey stock in the hive under 9-10 kg (about 3 frames) any time of the year causes bees to suffer hunger stress. Malnutrition and starvation because of nutritional mistakes are important reasons leading to collapse of colonies. Therefore, it is essential to keep colonies well stocked above the stress line with sufficient and quality food at all times [1,2,16].

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

Stationary beekeeping where colonies always remain in the same place, too many colonies in the same apiary, and closely spaced apiaries push bees into extreme competition to access food sources, resulting in malnutrition, hunger stress, and eventually poor resistance against diseases. Inadequate pollen accelerates physiological aging and shortens bee lives. The amount of pollen transported to the hive is closely linked to the queen's rate of spawning and worker bees' inclination to rear the young, to make wax and build honeycombs, to raise and feed the drones. Bees can accomplish these tasks only if they consume enough pollen to renew their fat tissue, which in turn increases their blood protein levels [12,13].

Pollen contains many fatty acids. Decanoic (capric), dodecanoic (lauric), myristic, linoleic, and linolenic fatty acids protect bees against bacterial and fungal diseases with their antimicrobial effects. For example, linoleic acid kills *Paenibacillus larvae*, the agent of American foulbrood disease; linoleic and lauric acids protect against *Bacillus cereus*, the soil bacteria whose toxins cause food poisoning; and lauric acid inhibits the development of *Helicobacter pylori* bacteria. Moreover, the fatty acids in pollens inhibit the development of other bacteria such as *Staphylococcus*, *Salmonella*, *E. coli* and *Bacillus anthracis* [1,2,16,17].

Nurse worker bees consume pollen to produce the royal jelly they feed the queen bee and larvae with. Royal jelly contains 10-hydroxy-2-decanoic acid (10-HDA), which has an antibiotic effect with very important lipid compounds and immunizes the larvae against infections. Bees process pollen to produce bee bread which helps larvae develop rapidly, protects the broods against disease, and extends the lifespan of bees. It is a natural probiotic with its content of lactic acid bacteria and bifidobacteria and is 3 times more nutritious than pollen, with a 6 times higher lactic acid content [1,2,16,17]. In the spring, colonies with an insufficient stock of pollen, bees must use the fat tissue in their bodies to compensate for the protein deficit, but this shortens their lifespan and diminishes incubation activity. Worker bees that lost 40% of their body protein reserve can live only for 20-25 days. Tissue renewal is disturbed unless the bee gets the necessary essential and antimicrobial fatty acids by consuming sufficient amounts of quality pollen, which again weakens immunity and shortens lifespan [1,2,18].

Vitellogenin is the protein found in the blood of honey bees. Juvenile hormone (JH) secreted by the corpus allatum gland on the rear part of the brain induces vitellogenin production. Vitellogenin is produced and stored in fat tissue cells covering the entire surface of the abdomen, and is delivered directly to blood from these cells [1,2,19].

The activity of the corpus allatum (i.e. production of secretions) and vitellogenin production are at lower levels in newly mated queen bees. In the first weeks the queen bee matured into adulthood, the blood vitellogenin level increases parallel with the JH level to reach its normal amount. Queen bees have a higher level of vitellogenin than worker bees. On the other hand, the blood vitellogenin levels of worker bees start to increase right after hatching and reach maximum in 7-10 days. From among the worker bees, foragers have less vitellogenin than nurse bees. Blood vitellogenin in the lowest in drones [1,2].

Vitellogenin has a role in the production of egg cells in the ovary and the transport of

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

nutrients required for embryonic development. An in worker bees, it provides the nutrients and amino acids needed for the production of royal jelly. It is crucial for the immune system and lifespan, not to mention all activities of bees inside and outside the hive [1,2,19].

Since the blood vitellogenin level is lower in forager bees as compared to nurse bees, their lifespan is also shorter. Before wintering, old bees in the colony consume excessive pollen and increase their blood protein levels to 10 times the amount in young bees. Rising vitellogenin levels in autumn help extend the lifespan. Thus bees survive until spring and the colony lives on. Research suggests that forager bees carry various bacteria and other pathogens from nature to the hive, which then pass to the royal jelly produced by nurse bees and eventually to the queen bee feeding on it. In the body of the queen bee these bacteria are broken down to be bound by the vitellogenin in the blood and transferred to eggs produced. Consequently, the larvae are vaccinated even before hatching [1,19].

Malnutrition, starvation, scarcity of nectar and pollen stress out bees, thereby facilitating the growth of fungi, which in turn lays the groundwork for the activation of *A. apis*, which is an opportunistic fungus and the agent of chalkbrood. For this reason, bees consume large amounts of pollen in autumn to renew fat tissue, grow body and blood protein reserves, and boost immunity. All this physiological transformation they undergo secures their survival until spring [20,21]. Water is another essential food for bees. Daily water consumption in a colony can reach up to 4 liters, especially in warmer regions and during periods of intensive brood rearing [1].

4. Nutritional Mistakes

Natural foods (nectar, honey and pollen) are essential in the diet of honey bees. Digestive system and enzymes of the bees are adapted to absorb these natural nutrients. However, insufficient nectar and pollen resources, dependence on the nectar and pollen of a single type of plant, and inability to meet nutritional needs directly from nature are causes of nutritional deficiency. In this case, the diet of the bees can be supplemented with carbohydrate and protein feeds, even vitamin and mineral combos can be provided [1,16].

The nectar of some plants yields poisonous or bitter honey. Some of them are toxic only to humans, but some may be toxic and harmful to bees, as well. Nectar containing poisonous compounds is a major cause of food poisoning and death in bees. Moreover, berries that often attract bees may bear large amounts of pathogens, the larvae of carnivorous beetles, or mites and this may attack bees, rendering them weak and vulnerable [16,22].

Honey has antimicrobial effects owing to its high sugar and low water content, acidic structure, and hydrogen peroxide, various flavonoids, and phenolic acids it contains. However, boiling honey-added bee feed for a source of carbohydrates or using heated honey in feed preparation largely eliminates the antimicrobial effects of the honey in feeds. Therefore, sugar syrups and carbohydrate feeds prepared for bees should never be boiled or directly exposed to a heat source. Furthermore, treatment with heat breaks down monosaccharides, in particular fructose, and furthers the formation of a toxic compound called hydroxymethylfurfural (HMF). Depending on the level of their HMF content, heat-treated honey and syrups may cause poisoning, even mass deaths of bees [1,2,16,23].

Be sure that honey and pollen to be used in feeding did not come from colonies with disease. If mold is found in the honeycomb or honey becomes sour or fermented, it must not be used as food. Bees should be fed mature and high-quality honey during winter. Especially in regions with harsh and long winters, it is important to avoid using honeydew honey with a high content of mineral salts and dextrin. This is because bees wintered on immature or poor-quality honeydew honey produce more feces. When the amount of fecal matter exceeds the bearing capacity of the rectum, bees are forced to defecate in the honeycombs. This leads to contamination and by extension to bacterial growth and intestinal infections in honeys. Moreover, old bees in wintering colonies struggle to access high-carbon sugars [1,22].

After the honey harvest, colonies start winter without much loss and with a younger bee population and then they thrive again during the springtime with a fast paced and more efficient breeding. Excessive feeding, adding honey and pollen from diseased colonies in their feed, or using sour or poor-quality honey or moldy pollen in feeds are all risk factors for the health of bees. In recent years, vitamin and mineral combos can be found in the market in addition to ready-made feeds for bees. However, they should not be preferred since their methods of preparation, content, duration and conditions of storage, and the way they are marketed are hardly reassuring most of the time. It is advised to keep bees in regions rich in flora, to allow them meet their nutritional requirements directly from the nature by feeding on nectar and pollens [1].

Artificial feeding can never be considered as a substitute to nectar and pollen. In fact, it is usually a cause of digestive disorders and other health problems in bees. Brewer's yeast, milk powder, soya bean flour and similar additives used in the bee feeds to provide protein actually alkalize the digestive environment in the body of the bee and promote fungal development with their high protein content. Such mistakes in nutrition and some other environmental factors damage the immune system in larvae and adult bees, leaving them vulnerable to pathogens [24,25].

Excessive syrup feeding also promotes fungal development by increasing relative humidity inside the hive. When excess fructose is loaded into colonies in order to produce more honey, bees cannot convert all of it into honey sugars and end up storing a significant amount of fructose. This is yet another stress factor on the bees and it shortens the lifespan of worker bees, increasing the wintering losses [22,25].

Fructose and glucose sugars are produced in the industry by processing the starch from corn, potato, or rice. These industrial sugars with a high fructose content are mostly obtained from GMO corns and used in bee feeds. They also contain very high levels of HMF. Invert sugar syrup with a high HMF value kills bees within two to three weeks. For this reason, starch-based industrial sugars should better be avoided in bee feeds and traditional kinds of sugar obtained from sugar beets or sugarcanes should be preferred instead. It is also worth noting that in agricultural areas where GMO sunflower seeds are used, the honey yield is initially very high, but as years pass by, colony losses up to 75% are observed and the surviving colonies experience a parallel decrease in population. This shows that bacterial toxins in GMOs have a negative impact on the health of bees [1,2,16].

Honey bees obtain the lipid compounds they need from their feed and mostly from pollen. For this reason, fat is not used in bee feeding. In fact, the fat content of bee feed should not exceed 5%. Otherwise, bees may suffer from severe digestive problems. Excess mineral consumption is another factor that can shorten the lifespan of bees. The detrimental effects of excessive minerals are worse in long flightless periods. Salt rapidly decreases lifespan and water or feeds with a salt content higher than 1% causes bees to die within a few days. For this reason, honeydew honey with a high mineral content and salt must be avoided in bee feeds especially during long periods of wintering [1,2,16,22].

5. Using Poor Quality Honeycombs

It is best to use honeycomb made of pure wax. Since wax is a very important carrier vector for bacterial and fungal bee diseases, it should not be contaminated with disease pathogens and be completely free of naphthalene and any chemical residues. During the production of the honeycomb care should be taken to prevent wax from contamination with paraffin, ceresin, petroleum jelly, stearin, renin, lard or tallow, resins, mineral oils, or any other foreign matter. These substances neutralize the high acidity index of wax to prepare a suitable environment bacteria and fungi. Moreover, residual feces and pupae in old honeycombs provide favorable conditions for the development of fungi [1,2,16,22].

6. Mistakes in Colony Checks and Management

All kinds of practices to check and manage colonies must be organized in accordance with the biological needs of the bees. Colony checks, especially on cold, cloudy days in the early spring or autumn, weakens the larvae by bringing down the temperature in the incubation area and promotes fungal development. Unnecessarily frequent colony checks in bad weather conditions disturb the balance the bees struck between temperature, humidity, and CO_2 levels - especially if the hive is kept open for a prolonged time! Then, the bees have to spend a lot of time, consume even more energy, and stress out to restore the balance. In the hives with weak colonies, it is advisable to reduce the area in early spring and late autumn using a partition board [8,9,22].

Each colony forms its own population balance with groups of worker bees specialized in different tasks and their colony dynamics. Partitioning or joining, taking swarms, bringing in new brood or bees to colonies all disrupt this balance and stress the bees out. Once colony dynamics is disturbed through swarming, artificial swarming, or supplementing the brood, the number of nurse bees per colony declines. This leads part of the brood to be undernourished, while leaving others exposed to cold. As a result, larvae become more susceptible to chalkbrood disease [1,2,24,25].

The bees in a hive establish the optimum order and nest layout for themselves. The order they establish must be preserved and changing the location and direction of honeycombs during colony checks must be avoided unless there is an obligation to for technical reasons. Otherwise, bees spend days trying to adapt to the new layout deteriorating order and this waste of time and energy cause them extreme stress. Colony transfers in migratory beekeeping are always a major stressor for bees. In particular, transfers in extremely hot

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

weather and without adequate ventilation may lead to mass deaths [1].

The following are not advisable practices with regard to the health and efficiency of bees: keeping colonies always in the same apiary, keeping an excessive number of colonies in the same apiary, not allowing for enough distance between colonies and apiaries, overfeeding, underfeeding, using unnecessary and excessive antibiotics, swarming from diseased colonies, using the honey and pollen of colonies contaminated with pathogens in feeds, exchanging bees, broods, honey, and pollen between healthy and diseased colonies, merging, using hives and other materials from diseased colonies in healthy ones without disinfection, ignoring rules of hygiene during colony checks, dividing a honeycomb base into several frames or not providing a base at all, leaving the honeycomb building entirely to the bees or using a plastic honeycomb, using poor quality or very old honeycombs contaminated with chemicals and pathogens, wintering colonies with an aged or insufficient bee population and/or with poor quality or inadequate food stock, or not providing suitable wintering conditions in general. In summary, mismanagement of the colony causes a decline in efficiency and productivity, because it stresses bees out, affects their health adversely, and facilitates the transmission and spread of diseases and parasites [7,26,27].

7. Chemical Pollution

The bee digestive system naturally contains a plethora of microorganisms, such as bacteria, viruses, fungi, yeasts, and protozoa. They live together on a symbiotic basis without causing any disease. Owing to their antagonistic relationship, bacteria and fungi inhibit the growth of each other in the same environment, thus mutually keeping populations below the threshold that could cause disease [1,2].

Antibiotics used to prevent bacterial bee diseases eradicate the bacteria in the digestive system cause new generations build antibiotic resistance, and accelerate fungi growth, eventually disturbing the biological balance between various microorganisms and laying the ground for fungal diseases. Furthermore, antibiotics do not only kill the harmful bacteria in the bee’s system, they annihilate the good bacteria that produce digestive enzymes and lactic acid, as well. As a result of such unnecessary and excessive use of antibiotics, the biological balance in the bee’s body is disturbed and nutritional disorders develop, resistance against pathogens declines and bacterial and fungal diseases start to affect the bee [22,28].

In fields where crops are grown, in silos, in shelters and other habitats for humans and animals, tons of insecticides are used annually. These chemicals cause serious pollution in natural resources such as soil, plants, and water. Biological or chemical substances that pollute the air, soil, water resources, and plants in and around apiaries create a serious problem for bees [1,2,29,30].

Chemicals contaminating nectar, pollen, propolis, and water are a huge adverse effect on nutrition and living of bees. In particular, systemic insecticides, mostly used for oilseed plants, can lead to food contamination and bee deaths. The chemicals forager bees carry to the hive on their bodies cause orientation and memory problems, as a result of which bees cannot find their way back home. They also affect nurse bees, their glands diminish because of these chemicals and they cannot feed the young, or the chemicals pass to the

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

digestive system through honey, pollen, propolis, brood feeds, and honeycombs eventually to poison and kill both the bees and their offspring en masse [1,16].

Another harmful chemical is the naphthalene used against pests and especially wax moth to protect the honeycombs stored for reuse after harvesting, honeycomb bases, and recycled wax. The residue of naphthalene and other chemicals used for similar purposes are a serious threat not only for the bees, but for humans, as well [1,2,22].

Detergent and sewage wastes that cause chemical pollution in the soil, plants, and water sources, toxic waste and flue gases of industrial plants, exhaust gas, and agricultural fertilizers also affect bee life and health adversely, causing stress, poisoning, and mass death. Bees should be protected against these stressors that weaken them and leave them vulnerable to parasites and pests [24,25].

8. Electronic Pollution

Bee habitats are under increasing pressure from electronic pollution or radio frequency pollution created by base stations, electromagnetic fields, TV signals, radars, high-voltage lines, wireless internet access, and the like. Unfortunately, this type of pollution is underestimated as it is not visible. However, electronic pollution represents a serious stressor for bees causing them to get disoriented [1].

Because of the electromagnetic radiation sources in the vicinity of an apiary, 70% of the bees flying out cannot find their way back to the hives. Cell phones placed near a beehive emit a radiation of 900-1.800 megahertz and drives the bees away. Furthermore, the vibration frequency (220 vibrations/sec) generated by mobile phones is very close to the vibration frequency (190-250 vibrations/sec) bees use to communicate with each other and to find directions: yet another factor causing disorientation. Electromagnetic pollution affects the resistance and strength in bees and makes them vulnerable to diseases. It was demonstrated that a mobile phone receiver at a distance of 300 m to the apiary increased restlessness and belligerence by 38%, the tendency to cluster by 25% and eventually caused 63% of the colonies to collapse. Therefore, electronic pollution sources, known to have serious adverse effects also on human health, should be kept at least 500 m away from apiaries, as well as all human settlements [1,31].

All the unfavorable circumstances explained above are stressors for honey bees either on their own right or in interaction with one another and the stress bees experience results with the burnout syndrome, collapse of the immune system, illness, and eventually death. Like all other living beings, honey bees have a defense system against factors that may adversely affect their health and life in general. The collapse of this defense system is ultimately detrimental to their health. Therefore, an adequate knowledge of the bee defense system and a focus on protective medicine to maintain this system in a strong and effective state are of vital importance [1,2].

II: Components of the Honey Bee Defense System

1. Endurance of Worker Bees

Honey bees are genetically coded to instinctively display certain hygienic defense behaviors.

a. For instance, nurse bees decrease the pH value and increase the acidity of the nutrients they store in their stomachs to feed

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

the larvae. This is a behavior to hinder bacteria and fungi growth, thus protect the larvae against pathogenic microorganisms [1,2,22].

b. Worker bees drive sick bees out and clear the hive off residue material, destroy parasites or groom each other to get rid of parasites, identify diseased larvae and young and clear the cells of the honeycomb off them and display other hygiene-oriented instinctual behavior [1,32,33,34,35,36,37].

c. Bees also cover the hatched areas of the honeycomb, narrow the flight hole, cluster together and air the hive to establish the ideal balance between temperature, humidity and CO_2 for hatching. This ensures that their offspring develop in healthy conditions and prevents the formation of an environment where fungi, yeast, or other pathogens could easily develop and reproduce [1].

d. Worker bees also effectively safeguard their nests against the possible raids or looting by robber bees and other pests. This keeps away any pathogenic microorganisms and parasites the robber bees might carry to colonies, as well as protecting the colony life against obvious dangers of predators, wasps, and other pests. A genetic characteristic of Japanese honey bees allow them to raise their body temperature up to 50 ^{o}C in the event of an attack on their nests to almost burn or suffocate the intruder to death. European honey bees display a similar behavior in the presence of wasps, but they cannot possibly survive a temperature rise at the same level [1,5,35].

2. Strength of the Larvae

The larvae have the following individual resistance mechanisms which are genetically coded and subject to change with age:

a. In the bee larvae, the midgut (stomach) and malpighian tubules are not connected to the hindgut. For this reason, digestive and nitrogenous metabolic residues are stored in the body and the food around the larvae is thus protected from fecal contamination. Once larvae complete their feeding period and start weaving the cocoon (before the prepupal stage), the stomach and malpighian tubules form a connection with the anus to dispose of the fecal material, which is then stored on the cell floor. This anatomical change in the digestive system of larvae ensures hygiene and prevents the food from turning into a breeding ground for microorganisms [1,2,16].

b. Before the prepupal stage, owing to the anaerobic conditions in the digestive tract due to lack of connection between the stomach and the hindgut, younger larvae are far more resistant against disease than older larvae [1,22].

c. The larval digestive system is not suitable for the growth of vegetative forms of bacteria. As the larva develops, the peritrophic membrane lining the inner surface of the ventriculus thickens and this prevents vegetative forms of bacteria to turn infective for older larvae [1,22].

d. Honey bee larvae are vaccinated against bacteria before hatching with the help of yolk protein (vitellogenin). The bacteria forager bees carry into the hive on their bodies pass to royal jelly and to the queen bee who feeds on it. The queen bee breaks down the bacteria in the royal jelly and the protein vitellogenin in her blood binds these bacterial fragments and transports them to the eggs she lays. Thus, the larvae are vaccinated against these bacteria even

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

before hatching. Based on this scientific process, it seems possible that a renewable vaccine can be developed in the future, for example against the spores of *P. larvae*, the bacterium causing American foulbrood disease [19,38,39].

e. The venom and hemolymph of many species contain various peptides (e.g. secropins, defensins, abaecins, gomesins, attacins, sarcotoxins, diptericins, and coleoptericins). Most of these are of low molecular weight and rich in amino acids such as proline, arginine, glycine and cysteine. They are effective on antibiotic-resistant gram-positive and gram-negative bacteria and protect the organism against bacterial infections. Some antimicrobial peptides do not only work well against bacteria, but they are also highly effective against viruses, fungi, protozoa, yeasts, and even parasites [1,2].

Some of these antimicrobial peptides are found in the bee venom and hemolymph. Bees are protected against infections through synthesizing peptides such as apidaecins, abaecins, or defensins. These peptides with antimicrobial action play an important role in the immunity of honey bee larvae against bacterial infections and *P. larvae*, the agent of the American foulbrood disease [1,2,16].

3. Life of Symbiotic Microorganisms and Spore Filtering

a. Microorganisms naturally occurring in the digestive system of bees are in a symbiotic relationship, preventing one another from reproducing beyond a limit and causing disease [1,2,22].

b. The digestive tract of bees filters out spores that bear disease agents. Useful probiotic bacteria in the intestinal flora of honey bees protect the intestinal wall against pathogens and harmful substances, remove pathogens from the body. The spores expelled with the feces cannot survive in the sun [1,2,22].

c. Useful lactic acid bacteria that live in the ventriculus and produce lactic acid inhibit the reproduction of *Paenibacillus larvae* and other pathogenic bacteria, help toxins removed from the body, and reinforce the immune system [1,2,22].

4. Strength Based on Nutrition and Produce

The structure, content, and antimicrobial properties of propolis and natural foods bees consume, such as honey, pollen, bee bread, and royal jelly are important components of the immune systems of bees and larvae.

a. Honey has an antimicrobial effect owing to its high sugar content, low water content, acidic structure, and hydrogen peroxide and various flavonoids and phenolic acids it contains. It has an important part to play in the bee defense system, preventing the development of bacteria and other pathogenic microorganisms [1,2,40].

b. Pollen protects bees against bacterial and fungal diseases with the antimicrobial effects of capric, lauric, myristic, linoleic, and linolenic acids it contains. For example, linoleic acid has an inhibitory effect on the development of the bacterium *Paenibacillus larvae*. Bee bread bees obtain by processing pollen is a food for larvae helping them develop better. It also protects the offspring against diseases and increases the lifespan of bees [17,40.41].

c. Especially during autumn, bees expand their protein reserves by renewing their fat tissues. Vitellogenin is the protein found in the blood of honey bees. It is crucial for the immune system and lifespan, not to mention the production of royal jelly and all

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

other activities of bees inside and outside the hive. Bees that renew their fat tissues and increase blood vitellogenin levels before wintering have better immunity and live longer [1,2,19].

d. Royal jelly contains 10-hydroxy-2-decanoic acid (10-HDA) which protects larvae against infections and is very important as an antibiotic. An environment of approximately pH=6.6 is required for bacterial sporulation. However, the royal jelly used by nurses to feed the larvae is acidic (pH=3.6-4.2). Thus, the acidic nature and antimicrobial properties of royal jelly make larvae and bees resistant to bacterial and fungal disease agents [2,22].

e. Bees use propolis to narrow the flight hole as a protection against cold, robber bees and other natural enemies, to cover up holes and cracks in the hive, and to polish and disinfect the inner surfaces of the hive and honeycomb cells. Bees thus protect their nests from exposure to adverse external conditions, provide heat insulation, establish a balance between temperature and humidity in the beehive, and have protection against pathogens such as bacteria, viruses, and fungi. Moreover, they cover the remains of intruders that they killed, but could not remove from the hive. Organic remains buried under propolis do not decompose to stink or spread disease [30,42,43,44].

Conclusion

Honey bees developed a rather complex and strong defense system against pathogens, parasites, and pests through the structure of the digestive tract in larvae and adults. Microflora and probiotics in a symbiotic relation in their digestive system, vitellogenin and antimicrobial peptides in their blood, various hygienic and defensive behaviors of adult bees, and finally the structure, content, and antimicrobial properties of the natural food and propolis they consume.

However, bees are subjected to stress because of genetic insufficiency, unfavorable climate and environmental conditions, malnutrition, mistakes in nutrition and colony management in apiculture, as well as chemical and electronic pollution in their natural habitat. The result of such stress is the collapse of their defense system and rapid deterioration of their health. Therefore, it is of vital importance to eliminate these stressors and maintain the effectiveness of the immune system in order to protect the health and well-being of bees.

Bal Arılarında (*Apis mellifera* L.) Stres Faktörleri ile Hastalık, Parazit ve Zararlılara Karşı Savunma Sisteminin Bileşenleri

Öz: Hayvan sağlığı, diğer hayvansal üretim dallarında olduğu gibi, arıcılıkta da üretim etkinliğini sınırlayan önemli bir faktördür. Çünkü bal arısı hastalık, parazit ve zararlıları arıların sadece sağlığını olumsuz olarak etkilemekle kalmayıp, verimi düşürmekte ve hatta önlem alınmadığı taktirde koloni yaşamına son verebilmektedir. Zaman zaman salgın şeklinde ortaya çıkabilen hastalık, parazit ve zararlılar üreticilerin büyük kayıplara uğramasına yol açarak ülke arıcılığını çıkmaza sokmaktadır.

Diğer bütün canlılar gibi bal arıları da sağlıklarını olumsuz etkileyen hastalık, parazit ve zararlılara karşı çok bileşenli

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

güçlü bir savunma (direnç, bağışıklık) mekanizmasına sahiptir. Ancak bal arılarının sahip olduğu savunma sistemi, genetik yapı kaynaklı ve çevresel birçok faktörün arılar üzerinde oluşturduğu stresin etkisiyle yetersiz kılıp çökmekte ve vücut direnci düşen arılar sağlıkları bozularak hastalanıp ölmektedir. Üstelik söz konusu stres faktörleri birbirleriyle, patojen mikroorganizmalarla, parazit ve zararlılarla sinerjik etki oluşturarak arılar, yavrular ve kolonilerde tükenmişliğe yol açmaktadır. Bu nedenle öncelikle bal arılarında savunma sisteminin etkisiz kalıp çökmesine neden olan bu stres faktörlerinin elimine edilmesi şarttır.

Anahtar kelimeler: Bal arısı, stress faktörleri, savunma sisteminin bileşenleri.

R E F E RE N C E S

[1] GENC F; DODOLOGLU A (2012) Arıcılığın Temel Esasları. Atatürk Üniv. Ziraat Fak. Ofset Tesisi, Erzurum, 467s.

[2] GULER A (2017) Bal Arısı (*Apis mellifera* L.) Yetiştiriciliği, Hastalıkları ve Ürünleri Kitabı. Bereket Akademi Yayınları, Azim Matbaacılık, Büyük San. 1. Cad. Alibey İşhanı, 99/33, İskitler/Ankara, 419s.

[3] BIENEFIELD K; PIRCHNER, F (1990) Heritebilities for several colony traits in the honeybee (*Apis mellifera carnica*). Apidologie, 21:175-183.

[4] GENC F; CENGIZ M M (2019) Bal Arsı (*Apis mellifera* L.) Anatomisi, Genetik ve Islahı ile Ana Arı Yetiştiriciliği Kitabı. Birinci Basım, Gece Akademi, Gece Kitaplığı Yayını, Kızılay Mah., Fevzi Çakmak 1. Sok., Ümit Apt., No:22/A, Çankaya/Ankara, 201s.

[5] SUGAHARA M; SAKAMOTO F (2009) Heat and carbon dioxide generated by honeybees jointly act to kill hornets.. Naturwissenschaften, 96 (9): 1133–1136.

[6] KAFTANOGLU O (1986) Balarısı Kolonilerinde Akrabalık Derecesi. Teknik Arıcılık, 8: 10-13.

[7] GENC F (1990) Bal Arılarında Koloni Performansını Etkileyen Faktörler. Teknik Arıcılık, 27: 18-26.

[8] GENC F (1991a) Koloni performansının temel bileşenleri I. Teknik Tavukçuluk Derg., 73:40-43.

[9] GENÇ F (1991b) Koloni performansının temel bileşenleri II. Teknik Tavukçuluk Derg., 74:43-47.

[10] GENC F (1992) Bal Arısı (*A. mellifera* L.) Kolonilerinde Farklı Yaşta Ana Arı Kullanımının Koloni Performansına Etkileri. Doğu Anadolu Böl. I. Arıcılık Semineri (3-4 Haziran, 1992) Bildirileri, Atatürk Üniv., Zir. Fak. Ofset Tesisleri, Erzurum, s76-95.

[11] YILMAZ B (1998) Kolonide Isı Kontrolü ve Kışlama. Teknik Arıcılık, Sayı 62:10-13.

[12] GENC F (2016a) Türkiye Arıcılığının Nitelikleri, Sorunları ve Çözüm Önerileri. 2023-2071 Vizyonuyla Tarım Kongresi, (8-10 Nisan 2016), Toç-Bir Sen., Çam Hotel, Kızılcahamam-ANKARA, Cilt: 2:70-89.

[13] GENC F (2016b) Koloni Yönetimi ve Verimlilik. 5. Uluslararası Muğla Arıcılık ve Çam Balı Kongresi, 1-5 Kasım 2016, Ölüdeniz-Muğla-Türkiye.

[14] GENC F; DODOLOGLU A (2012) Arıcılıkta İklim, Bitki Örtüsü, Mer'a Seçimi ve Arılık Düzeni. Arıcının Sesi, Sayı: 8:18-19.

[15] KACMAZ N (2005) Tükenmişlik (Burnout) sendromu. İst. Tıp Fak. Derg., 68:29-32.

[16] SILICI S (2009) Bal Arısı Biyolojisi ve Yetiştiriciliği. Eflatun Yayınevi, Gazi Mustafa Kemal Bulvarı, 118/4, Maltepe-İstanbul, 236s.

[17] MANNING, R (2001) Fatty acids in pollen: a review of their importance for honey bees. Bee Wld., 82(2):60-75.

[18] GENC F; KAFTANOGLU O (1996) Determination of a suitable wintering method for honeybee, *A. mellifera* L., colonies in cold climates. Apiacta, 31(4): 116-122.

[19] SALMELA H; AMDAM G.V; FREITAK D (2015) Transfer of Immunity from Mother to Offspring Is Mediated via Egg-Yolk Protein Vitellogenin. PLoS Pathog, 11(7):e1005015. Doi:10.1371/jornal.ppat. 1005015.

[20] COHEN S; JANICKI-DEVERTS D; MILLER G.E (2007) Psychologal stress and diseasae. VAMA, 298:1685-1687.

[21] KANDEMIR I (2010) Bal arılarında koloni çökme bozukluğu konusunda yapılan son çalışmalar üzerine mini derleme. Uludağ Arıcılık Derg., 10(1):23-27.

[22] AYDIN L; DOGANAY A; ORUC H.H; YESILBAG K; BAKIRCI S; GIRISGIN O.A; GUNES N; MUZ M.N; BORUM A.E; GUNES M.E (2017) Bal Arısı Yetiştiriciliği, Ürünleri, Hastalıkları Kitabı. (1. Baskı), Editörler: Doğanay, A., Aydın, L., Dora Basım-Yayın Dağıtım Ltd. Şti., Altıparmak Mah., Bozkurt Cad., Avdan Apt., 10/1, Osmangazi/Bursa, 470s.

[23] SAHINLER N; GUL A (2005) Effect of heating and storage on honey hydroxymethyl furfural and diastase activity. J. of Food Technology, 3(2):152-157.

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.

[24] ERKAN C; GOSTERIT A (2012) Bal arılarında stres. Uluslararası TÜRK Ve Akraba Topluluklar Zoot. Kongresi Kitabı, Isparta, Cilt 1:354-362.

[25] EVEN N; DEVAUD J.M; BARRON A.B (2012) General Stress Responses in Honey Bee. Insects, 3:1271-1298.

[26] YEAGENEHRAD H; HAMZEH R.K; SAJAD J (2015) Investigating the effect of high relative humidity and high carbon dioxide concentration in beehives on honeybees death rate in winter. 44.th APIMONDIA Int. Apic. Congress, Scientific Program Abstracts, Daejeon-Korea, p188.

[27] AKKAYA H (2010) Son yıllarda sıklıkla karşılaşılan nedeni bilinmeyen arı ölümleri ve bunlara karşı çözüm önerileri. II. Uluslararası Muğla Arıcılık ve Çam Balı Kongresi (5-8 Ekim, 2010), Muğla Üni. Arıcılık ve İpekböcekçliği Araştırma ve Uygulama Merkezi, Muğla, s119-126.

[28] SABANOGLU M (1991) Antibiyotikler ve Balarısı Hastalıklarına Karşı Kullanma Olanakları. Teknik Arıcılık, 32: 25-27.

[29] ÖZBEK H (1983) Arıların Zirai Mücadele İlâçlarından Etkilenmeleri ve Alınacak Önlemler. TOKB Zir. Müc. ve Zir. Kar. Gn. Md., Ofset Matbaası Tesisleri, Ankara, 46s.

[30] SHIMANUKI H; KNOX D; FURGALA A; CARON B; WILLIAMS J. L (1993) Diseases and Pests of Honey Bees. The Hive and Honey Bee, Dadant and Sons, Hamilton, Chapter 25, p 1083-1151.

[31] ERIKSSON P.S; WALLIN L (2004) Functional consequences of stressrelated suppression of adult hippocampal neurogenesis- a novel hypothesis on the neurobiology of burnout. Acta Neurol Scand, 110:275-280.,

[32] MILNE C. P. JR (1983) Laboratory measurement of honebee brood disease resistance. 2. Uncapping of freze-killed and live brood by nevly emerged workers in cages. J. of Apic. Res., 22(2):115-118.

[33] GENC F; AKSOY A (1992) The effects of infestation level of Varroa jacobsoni on wintering of honeybee (*Apis mellifera* L.) colonies. Apiacta, 27(2):33-38.

[34] DE GUZMAN I.L; RINDERER E.T; STELZER J.A (2001) Resistance to the parasite mite *Varroa destructor* in honey bees from far-eastern Russia. J. of Apic. Res., 32(4):381-399.

[35] ARECHAVALETA-VELASCO M.E; HUNT G.J (2003) Genotypic variation in the expression of guarding behavior and the role of guards in the defensive response of honey bee colonies. Apidologie, 34:439-447.

[36] IBRAHIM A; SPIVAK M (2006) The relationship between hygienic behavior and suppression of mite reproduction as honey bee (*Apis mellifera* L.) mechanisms of resistance to *Varroa destructor*. Apidologia, 37:31-40.

[37] OZBAKIR G. O (2013) İşçi Arılarda (*Apis mellifera* L.)Yumurta Denetleme Davranışı. Hayvansal Üretim, 54(2):24-29.

[38] AMDM G.V; NORBERG K; HAGEN A; OMHOLT S.W (2003) Social exploitation of vitellogenin. Proc. Natl. Acad. Sci., USA, 100:1799-1802.

[39] AMDM G.V; SIMÕES Z.L.P.; HAGEN A.; NORBERG K.; SCHRØDER K.; MIKKELSEN O.; KIRKWOOD T.B.L.; OMHOLT S.W (2004) Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honey bees. Exp. Gerontol., 39:767-773. ,

[40] DOGA H. (2014) Çiçek Ballarının Kimyasal, Fiziksel ve Antimikrobiyal Özelliklerinin Belirlenmesi. Atatürk Üni. Fen Bil. Enst., Gıda Müh. Anabilim Dalı, Yüksek Lisans Tezi, Erzurum, 72s.

[41] CUSHNIE T.P.T.; LAMB A.J (2005) Antimicrobial activity of flavonoids. Int. Joural Antimicrob. Apents, 26:343-356.

[42] DE CASTRO S.L (2001) Propolis: Biological and pharmacological activities. Therapeutic uses of this bee product. ARBS Ann. Rev. Biomed.. Sci., 3:49-83.

[43] KUTLUCA S; GENC F; KORKMAZ A (2006) Propolis. Tarım İl Müdürlüğü ÇEY Şubesi, Samsun, 61s.

[44] SCHIESSER A; OMUR G.C; ASLI O; NEVIN K (2015) In vitro evaluation of antimicrobial effect of propolis against *Paenibacillus larvae* genotypes in Turkey. 44.th APIMONDIA Int. Apic. Congress, Scientific Program Abstracts, Daejeon-Korea, p189.

Cite as: GENÇ, M; GENÇ, F (2019), Stress Factors on Honey Bees (*Apis mellifera* L.) and The Components of Their Defense System Against Diseases, Parasites, and Pests, Mellifera, 19(1):7-20.