Review paper

FACTORS AFFECTING IMMUNE RESPONSES IN HONEY BEES: AN INSIGHT

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Abstract

Honey bees pollinate various crops and wild plants which ensures food security. However, in this modern world, bees are suffering. Decreasing global domesticated and wild bee populations while the demand for agricultural pollination services is increasing is a matter of concern. Colony losses are driven by such factors or interactions as changing environmental conditions, exposure to agrochemicals, parasite and pathogens attack and decreased flower abundance and diversity. All these stresses individually or together affect the immune system of bees and consequently bee fitness. Poor nutrition is the key stressor as it directly suppresses the immune system and reduces host resistance to other stressors. To cope with these stresses, honey bees have well-developed individual and social immune systems which initiate several defence reactions, but its activation, maintenance and use are detrimental for bee survival as it occurs at the cost of bee health. This review summarizes the causal factors and their possible interaction responsible for immune suppression in honey bees.

Keywords: honey bee, immune suppression, interactions, nutrition, oxidative stress, stressors

INTRODUCTION

Honey bees are of great ecological, economic and scientific value. They provide various hive products of great significance with respect to nutrition, immunity and medication (Bhatnagar et al., 2020; Sharma et al., 2020) and are also essential for crop pollination (Jivan, 2013). Their contribution in the pollination of more than 80% crops and wild plants make them an important component of food security and biodiversity maintenance (Fontaine et al., 2005; Bascompte et al., 2006; Klein et al., 2007; Ollerton et al., 2011). Their value in pollination is fifty-four times the value of the honey they produce (Sanjerehei, 2014).

Despite their importance, the domesticated and wild bee populations have been reported to be declining globally at alarming rates in the United States, Europe, India, China, Israel and Turkey (Gross, 2007; Currie et al., 2010; van der Zee et al., 2012; Sihag, 2014). Currently available honey bee populations are not keeping pace with the growing demands for agricultural pollination services, which puts stress on the global pollination capacity (Aizen & Harder, 2009). A number of factors have been documented for the honey bee colony losses including parasitic and pathogenic infections, pesticidal toxicities, nutrient deficiencies and changing environmental conditions (vanEngelsdorp & Meixner, 2010;

Goulson et al., 2015; Stanimirović et al., 2019). Recently, the honey bee colony losses have been linked to an impaired immune system (Alaux et al., 2014; Steinmann et al., 2015). The immune system consists of certain organs and mechanisms which protect the organism from such threats as parasites, pathogens and foreign particles by recognizing them and then responding against them (Larsen et al., 2019). Insects have well developed innate immune systems which protect them from different stresses (Strand, 2008). Currently, the most studied insect is the fruit fly, *Drosophila melanogaster*, and its immunological studies are used as a reference not only for insects but for humans as well (Larsen et al., 2019). Honey bees possess a similar immune system to that of fruit flies and mosquitoes except that the bees have nearly one third genes for immunity as compared to such solitary insects as flies, moths and mosquitoes (Evans et al., 2006). Instead, honey bees have more genes related to their behavior and social organization skills including for odour receptors and food collection (HGSC, 2006). So, predictably the lower number of immunity related genes in honey bees is a consequence of well-developed social immunity which lowers the pressure on individual immunity. Thus, such social insects as honey bees defend themselves through both social as well as individual immunities. Social immunity mainly relies on behavioral responses (Simone-Finstrom, 2017) whereas individual immunity consists of humoral and cellular defense responses (Schmid-Hempel, 2005).

Immunocompetence depends upon several internal as well as external factors including diet, age, caste, pesticides, parasites, pathogens and environment (Jones, 1962; Wilson-Rich et al., 2008; Mao et al., 2013; Negri et al., 2015). These factors individually or together led to immune suppression (Alaux et al., 2010a; Pettis et al., 2012; Boncristiani et al., 2012; Pettis et al., 2013). Evidences of a link between reduced bee immunity and stress factors have been provided by Yoshida (1988); Vandame & Belzunces (1998); Szymaś & Jędruszek (2003); Shen et al. (2005); Higes et al. (2007); Gregorc et al. (2012);

Steinmann et al. (2015); Brandt et al. (2016). Recent studies associated with honey bee immunity have been receiving more attention as faded immunity increase the probability of disease occurrence and progress (Pamminger et al., 2018).

The present review describes the current knowledge on immunity types in honey bees, factors affecting these immunities and the result of possible interaction among such factors on overall bee health.

TYPES OF IMMUNITY

Being eusocial, honey bees are highly prone to infections and other diseases as they live in closed environment and share food with one another (Cremer et al., 2007). Honey bees lower the risk of disease outbreaks through a combination of their well-built behaviours at a social level (social immunity) and immune responses at individual levels (individual immunity) (DeGrandi-Hoffman & Chen, 2015). The social and individual immunities together provide a defence against various stresses. The social and innate defence responses against the various foreign bodies are summarized in Tab. 1.

Social immunity due to behavioural cooperation among members of a colony defends against invading pathogens and combats disease transmission risks that arise from group living (Cremer et al., 2007). It is provided through the following behavioural defences: (1) nest building and guarding i.e. disinfection of nesting material with antimicrobial products (propolis) collected from outside and guarding of nest entrance by guard bees (Cremer et al., 2007), (2) grooming i.e. removal of foreign particle, pollen or pathogen from oneself (auto-grooming) or from another adult hive mate (allo-grooming) (Simone-Finstrom, 2017), (3) hygienic behaviour via removal of diseased or parasitized brood by adult bees (Wilson-rich et al., 2009; Larsen et al., 2019), (4) undertaking i.e. removal of dead adults (Evans & Spivak, 2010), (5) altruistic behaviour i.e. voluntary leaving of colonies by sick adults and dying outside the colony (DeGrandi-Hoffman & Chen, 2015; Larsen et al., 2019), (6) thermoregulatory behaviour i.e. raising tempera-

ture (social fever) of hive against heat sensitive pathogens (DeGrandi-Hoffman & Chen, 2015; Simone-Finstrom, 2017), (7) offspring cannibalism i.e. feeding of nurse bees on brood which died due to food scarcity or extreme temperatures (Cremer et al., 2007; Larsen et al., 2019) and (8) polyethism i.e. age-related task allocation reduces interaction between different age groups (Simone-Finstrom, 2017). Individual immunity consists of mechanisms which make an individual capable of resisting, tolerating and eliminating a foreign particle. It has basically two components, innate and adaptive, but the latter is found in higher vertebrates and lacking in insects (Larsen et al., 2019). Honey bees' innate immune system,

the first line of defence, restricts invading pathogens or other foreign particles with physical or mechanical barriers- exoskeleton, trachea and peritrophic membrane lining the gut of insect (Gliński & Jarosz, 1995; Evans & Spivak, 2010). If foreign particles break the barriers and enter the insect's body, then there is second line of defence with constitutive and induced defence responses (Schmid-Hempel, 2005; Laughton et al., 2011). Constitutive defence consisting cellular and humoral responses is always present, non-specific and instant in effect (Gillespie et al., 1997; Schmid-Hempel, 2005). Induced responses occur only after the recognition of a specific pathogen, and thus require more production time with specific

Table 1.

Summarizes the social and innate defence responses against the various foreign bodies

Immune Parameter	Foreign Body
Social Immunity	
Social fever	Fungi (Starks et al., 2000)
Plant Resins	Bacteria (Antúnez et al., 2008) Fungi (Simone-Finstrom & Spivak, 2012)
Grooming	Tracheal mite (Danka & Villa, 1998) <i>Varroa</i> (Guzman-Novoa et al., 2012)
Hygienic behaviour	Fungi (Gilliam et al., 1983) Bacteria (Spivak & Reuter, 2001) <i>Varroa</i> (Ibrahim et al., 2007)
Division of labour	Pathogen transmission (Naug & Camazine, 2002)
Innate Immunity	
Phagocytosis	Small bacteria and virus (James & Xu, 2012; Larsen et al., 2019)
Nodulation	Fungi (James & Xu, 2012; Larsen et al., 2019)
Encapsulation	Large parasites (James & Xu, 2012; Larsen et al., 2019)
Antibacterial activity	Neonicotinoids and bacteria (Dickel et al., 2018) <i>Varroa</i> and Virus (Gregorc et al., 2012)
Antimicrobial peptides	Pesticides (Garrido et al., 2013) Bacteria, fungi, protozoa (Danilčík et al., 2015)
RNA interference, dsRNA	Virus (Brutscher et al., 2015)
Prophenoloxidase activation	Microbial infection (Li et al., 2018)

and long-lasting effects. Induced responses produce antimicrobial peptides and proteins which are absent in healthy bees (Boman & Hultmark, 1987). Haemocytes or blood cells are an essential component of cellular immunity and provide defence through phagocytosis, nodulation and encapsulation responses (Strand, 2008). Humoral defence includes the production of antibacterial immune proteins and enzymatic systems which regulate wound healing, melanin biosynthesis, phagocytosis, nodulation and encapsulation (Kaaya, 1993; Cerenius et al., 2008).

FACTORS CAUSING IMMUNE SUPPRESSION

Honey bee colony losses are linked to parasites, pathogens, pesticides, poor nutrition, climate change and changing management practices (van Engelsdorp & Meixner, 2010; Generch et al., 2010; Potts et al., 2010; Goulson et al., 2015). The causal links between these factors and immune-suppression has been established (Shen

et al., 2005; Steinmann et al., 2015). Several factors influencing bee immunity are illustrated in Fig. 1.

Parasitic infestations

Bees suffer from a wide range of parasitic mites. More than hundred mite species are associated with honey bees, but *Varroa* spp. and *Tropilaelaps* spp. (both ectoparasites) and *Acarapis* spp. (endoparasite) most threaten bee survival (Sammataro et al., 2000).

Ectoparasitic mite, *Varroa destructor*, a major threat to honey bees throughout the world, infests and kills a European honey bee colony within one to three years if no control measures are taken (Strauss et al., 2015). Additionally, *Varroa* further act as a vector to various bacteria (Gliński & Jarosz, 1992), viruses (Allen & Ball, 1996) and fungi (Liu, 1996). Immature and adult stages of this mite feed on the haemolymph of developing and adult honey bees through a single

Fig. 1. Various factors influencing bee immunity.

puncture hole. During feeding, mite's salivary secretions damage haemocytes and suppresses the wound healing responses, keeping wound open for days to ensure continuous feeding of its immature stages (Richards et al., 2011). Recent research has shown that *Varroa* feed primarily on fat bodies rather than haemolymph and these damaged fat tissues may cause immune suppression by altering the production of vitellogenins and antimicrobial peptides (Ramsey et al., 2019). *Varroa* infestation interferes with both cellular and humoral immunity and suppresses the cellular immune system by reducing total haemocyte count (THC), differential haemocyte count (DHC), surface area of haemocytes and clotting (Salem et al., 2006; Richards et al., 2011). Whereas, the altered expression of immune-responsive antibacterial peptides suppresses humoral immunity in *Varroa*-infested bees (Gregorc et al., 2012). *Tropilaelaps* spp. in Asia, another major ectoparasitic mite infesting European honey bees, has similar lifecycle as *Varroa*. They disturb the regulations of phagocytosis receptor gene and antimicrobial peptide encoding genes in infested honey bees (Khongphinitbunjong et al., 2015). On the other hand, endoparasitic mite, *Acarapis woodi*, enters the trachea of young bees and punctures the tracheal wall to suck haemolymph causing nutrient loss and damaged wing muscles. They also affect air flow and allow secondary infections through punctured wounds (Eischen, 1987). Feeding on haemolymph may reduce the number of haemocytes as reported in *Varroa* infested bees (Salem et al., 2006). Colonies infested with this mite have reduced social immunity as the bees become unable to thermoregulate due to damaged wing muscles (McMullan & Brown, 2009).

Pathogenic infections

Like other organisms, honey bees are naturally infected with disease-causing pathogens including bacteria, viruses, fungi and protozoans, which along with other pests are considered as a reason behind the global honey bee decline (Ratnieks & Carreck, 2010). Haemocytic sensitivity of honey bee immature and mature

stages to American foul brood (AFB) infection was reported by Zakaria (2007). The bacterial pathogen *Paenibacillus larvae*, causative agent of AFB disease, suppress larval honey bees' innate immunity as the antibacterial peptide encoding gene's transcript levels are negatively correlated with the disease levels (Evans & Pettis, 2005). They directly suppress the cellular immunity by damaging haemocytes in larvae (Gregorc & Bowen, 1998).

Expression of genes encoding immunity-related enzymes and antibacterial peptides vary after infection with *Nosema apis* and *Nosema ceranae* (Antúnez et al., 2009). *N. ceranae* suppress both cellular and humoral immunities by degrading or hypertrophying host cells (Higes et al., 2007) and by reducing the expression of immunity related genes (Chaimanee et al., 2012). *Nosema* also causes energy stress in the host probably by competing for nutrients (Mayack & Naug, 2009). *Nosema* lacks mitochondria and develop only after direct contact with the cytoplasm of its host cell which fulfils the requirement of an external energy source for reproduction of this microsporidia (Fries et al., 1996; Weidner et al., 1999; Martín-Hernández et al., 2011). Such heavily infested epithelial cells lining the bee gut assure poor nutrient absorption resulting in bees dying early death due to starvation (Liu, 1984). The *Nosema*-caused energetic stress reduces the trehalose levels in haemolymph (Mayack & Naug, 2010) and affects the flight efficiency and thermoregulatory ability of bees (Campbell et al., 2010). *Nosema* directly interfere with the innate immune responses by reducing apoptosis in host bees through enhanced transcription of apoptosis protein-(iap)-2 inhibitor gene (Kurze et al., 2015).

Pathogenic viruses also cause immune suppression. Mites act as a vector or activator to turn the asymptomatic presence of viruses into lethal infections (Shen et al., 2005). The occurrence of such pathogenic viruses as deformed-wing virus (DWV) (Shen et al., 2005; Ryabov et al., 2014), Kashmir-bee virus (KBV) (Shen et al., 2005) and acute bee paralysis virus (ABPV) (Ball & Allen, 1988) are correlated with the presence of mites, and together they suppress the immunity of host

bees. DWV vectored by *Varroa* suppresses host immunity by interfering with the nuclear factor-kappa B (NF- κ B) protein family and reducing the Toll pathway controlled antiviral response (Nazzi et al., 2012). Presence of *Tropilaelaps*, another mite, is also linked to infections of DWV (Forsgren et al., 2009). Such immune responses as melanisation and encapsulation correlate negatively with DWV levels in a host (Di Prisco et al., 2016). Threats posed by DWV-*Varroa* association become more severe in the presence of additional stresses including poor diet and pesticides which further promote virus replication (Nazzi & Pennacchio, 2014; Goulson et al., 2015). The interference of neonicotinoids with the NF- κ B immune signalling reduces immune responses and promotes the DWV replication in honey bees bearing covert infections (Di Prisco et al., 2013). DWV replication in several honey bee body tissues including fat bodies (Fievet et al., 2006) regulate various physiological processes including immune defences through vitellogenin (Trenczek & Faye, 1988). The interference of DWV replication with the expression of vitellogenin may be responsible for its lower levels in collapsed colonies in comparison to healthy colonies (Dainat et al., 2012b).

Temperature variations

For the well-being of the entire colony including immature and mature stages, the hive temperature is maintained between 32-35°C by the resident bees (Kronenberg & Heller, 1982; Seeley, 2014). They use fanning and evaporative cooling mechanisms to combat higher temperature whereas cluster formation or metabolic heat generation through contraction and relaxation of flight muscles combat lower temperature (Southwick & Heldmaier, 1987). Elevated temperatures induce in honey bees a heat shock response with an increased expression of heat shock proteins. At the same time, heat shock suppresses the multiple immunity-related genes (McKinstry et al., 2017). Compared to summer bees, winter bees exhibit a more reduced expression of immunity related genes and higher DWV loads (Steinmann et al., 2015), and the occurrence of *Varroa* and DWV

reduces their life span (Dainat et al., 2012a). Temperature also affects the flight muscles of bees, which either by shivering or through heat loss maintain a temperature range for efficient working of muscles. Any temperature below this range is responsible for the failure of wing, leg and body muscles (Esch, 1988) which may affect social immunity.

Relatively high and low temperatures cause oxidative stress in arthropods (Lopez-Martinez et al., 2008), which occurs when there is imbalance of free radical production and antioxidant activity (Sies, 2000). Its interference with such cell components as DNA (increased mutations due to breakdown of single-strand, base deletion or degradation) (Jena, 2012), protein (reduced enzymatic activity due to modification of amino acids and rupturing of peptides) (Davies, 2016) and lipids (destruction of cell membrane by lipid peroxidation) (Cheeseman, 1993) affect the physiology of an organism (Simone-Finstrom et al., 2016). Exposure to pesticides, heat, cold, ultraviolet radiations and hydrogen peroxide induces oxidative stress in honey bees (Li et al., 2016) and bees respond to these stresses through varying expression levels of detoxifying or immunity related genes (Zhu et al., 2016). Modified environmental conditions in greenhouses induce oxidative stress and thus adversely affect the cellular and humoral immune systems of honey bees (Morimoto et al., 2011). Simulated heat waves alter the vitellogenin level which protects against oxidative stress (Bordier et al., 2017). Glutathione S-transferases (GSTs) play an important role in oxidative stress management and insecticide resistance, but variations in temperature change GST-related gene expression in a time dependent manner (Yan et al., 2013). Due to oxidative damage, bees face severe survival (Perry et al., 2015; Chakrabarti et al., 2020) and memory issues (Farooqui, 2008).

Hive transportation provides pollination services to different crops, and transported bees underwent transport stress due variable temperatures, humidity, pressures and vibrations

(Melicher et al., 2019). Migratory bees have more oxidative damage than stationary bees (Simone-Finstorm et al., 2016). Transport stress affects both, individual immunity by downregulating the genes associated with immunity as well as social immunity by decreased thermoregulation (Melicher et al., 2019). Migratory bees have a short life span compared to stationary bees (Simone-Finstorm et al., 2016). Transportation also reduces the hypopharyngeal gland size in nurse bees which is essential for brood food production (Ahn et al., 2012).

Apart from direct effects, varying temperature in different seasons create conditions suitable for infestation by various parasites and pathogens (Le Conte & Navajas, 2008). The death of honey bee colonies in winters is closely associated with mite attack (van Dooremalen et al., 2012). DWV titres increase between summer and fall when mite incidences increase as well (Gauthier et al., 2007; Dainat et al., 2012b). The expression of immunity related gene (*eater*) decreases from summer to fall and then increases from fall to winter (Dainat et al., 2012b). High relative humidity combined with low temperatures enhances the chalkbrood development (Flores et al., 1996). Suboptimal brood temperatures increase the susceptibility of adults to pesticides (Medrzycki et al., 2010).

Pesticidal toxicity

Pesticides especially insecticides as well as fungicides and herbicides are reported to harm honey bees (Belzunces et al., 2012) either individually or synergistically with other stresses (Vadame & Belzunces, 1998). Honey bees may be directly exposed to pesticides when visiting crop flowers during pesticide application or indirectly when coming in contact with a crop after pesticide application (May et al., 2015). They also become exposed when pesticides are directly applied to hives for the control of various bee enemies or diseases (Martel et al., 2007). Short-term effects of pesticides include instant bee death and long-term effects include morphological deformities, compromised foraging, impaired learning, weakened thermoregulation, exploitation of nutritional reserves and

reduced immunities (Alaux et al., 2010a; Henry et al., 2012; Sánchez-Bayo et al., 2016; Meikle et al., 2016). Neonicotinoid (imidacloprid, thiacloprid, clothianidin etc.) and pyrethroid (ethofenprox and bifenthrin) insecticides interfere with haemocyte densities and affect cellular immunity (Brandt et al., 2016; Perveen & Ahmad, 2017). They reduce antimicrobial activity of haemolymph and responses including wound healing, melanisation and encapsulation (Brandt et al., 2016; Brandt et al., 2017) and induce such cell deformities as de-nucleation, nuclei side displacement, ruptured cell wall, distorted cell shape and agglutination (Perveen & Ahmad, 2017). Fungicides increase the transcript levels of enzyme related to melanisation, while insecticides and herbicides decrease them (Gregorc et al., 2012). Thymol and coumaphos acaricides downregulate genes involved in detoxification pathways and those genes whose products are involved in humoral (Basket) and cellular (Dscam) defence responses (Boncristiani et al., 2012), whereas flumethrin upregulate the expression of genes that encode antimicrobial peptides and immunity-related proteins (Garrido et al., 2013). Also, direct interferences of azole fungicides and glyphosate herbicide with Cytochrome P450 enzyme system, known to play a role in pesticide resistance and detoxification (Claudianos et al., 2006), affects the innate immunity of honey bees (Yoshida, 1988; Gregorc et al., 2012). Glyphosate also alters midgut microbial communities which defend honey bees against various pathogens (Dai et al., 2018; Motta et al., 2018). Apart from directly affecting cellular immunity (Cousin et al., 2013), herbicides cause indirectly food shortages and nutritional stress by removing alternative food source for bees (Albrecht, 2005; May et al., 2015).

Further, honey bees respond to pesticide induced oxidative stress (Chakrabarti et al., 2015) by increasing the activity of such antioxidant enzymes glutathione peroxidase and catalase (Balieira et al., 2018). In addition to pesticides, antibiotics cause immune suppression by downregulating the expression of immune-related genes (Li et al., 2019).

Nutritional deficiency

Proper nutrition is imperative for the maintenance of an individual's health and disease prevention (Sharma & Prajapati, 2014). Pollen and honey/nectar serve as key components in the honey bee's diet. Pollen, being a source of protein, provides many essential amino acids to bees for peptide synthesis in immune pathways (DeGrandi-Hoffman & Chen, 2015). On the other hand, honey is a source of carbohydrates and provides energy for various metabolic processes associated with cellular and humoral immune responses (DeGrandi-Hoffman & Chen, 2015). Thus, proper nutrition plays a role in the maintenance of the immune system. Short-term nutritional deprivation downregulates the immune system, whereas food access rapidly upregulates the immune system of insects (Siva-Jothy & Thompson, 2002). Both individual and social immunities are modified by protein consumption. Glucose oxidase (GOX) activity, a parameter of social immunity, greatly increases with pollen consumption in bees (Alaux et al., 2010b). GOX act as a catalyst in the oxidation process of β -D-glucose into gluconic acid and hydrogen peroxide. The antiseptic nature of hydrogen peroxide helps in colony food sterilization (White Jr et al., 1963).

Protein deficiency reduces metabolic cell activity but increases total haemocyte count and granulocyte count (Szymaś & Jędruszek, 2003). This may occur as a compensatory mechanism for protein deficiency or reduced metabolic cell activities. Diet diversity also affects immune competence levels because polyfloral diets have more GOX activity compared to monofloral diets (Alaux et al., 2010b).

The effectiveness of individual and social immunity is directly related to the nutritional status of a colony. Invasion by such outer parasites as *Varroa* disturbs this relationship and hence suppresses the immune system (DeGrandi-Hoffman & Chen, 2015). *Nosema* feeding patterns on honey bees elevates their hunger level, reduces nutrient absorption and causes energetic stress leading to downregulation of the immune system (Martín-Hernández et al., 2011). This type of energetic stress is further

enhanced by pesticides, and adverse effects could be seen in haemolymph sugar levels, foraging and thermoregulatory behaviours (Alaux et al., 2010a; Mayack & Naug, 2010). Starved bees might modulate the vitellogenin levels which induce early foraging in infected bees and these bees died during foraging due to the energetic stress (Amdam & Omholt, 2003; Nelson et al., 2007; Mayack & Naug, 2009). Compared to pollen restriction (nutritional stress), pollen ingestion upregulates the immunity-related genes (Corona et al., 2019). Furthermore, the treatment of nutrient-deficient honey bee colonies with antibiotics had more pronounced adverse effects - reduced lifespan, hypopharyngeal gland development, nutrient and immune gene expression and enhanced viral loads (Li et al., 2019).

Honey bees develop certain mechanisms to react to pollen shortage in their colony. They cannibalize their young brood to maintain the older brood but during a long-term pollen shortage they even stop rearing which affects colony strength and social immunity (Brodtschneider & Crailsheim, 2010). Stress related to transportation and habitat change may also occurs due to reduced diversity or nutrition. Polyfloral diets, good source of nutrition, enhance immunity-related enzymes and increase the resistance to such stresses (Huang, 2012). The use of pollen substitutes during dearth periods also reduces the total haemocyte count and metabolic activities of cells but the reductions are still lower than in protein free diets (Szymaś & Jędruszek, 2003).

Age-related impairments

Age-associated immune dysfunction, also known as "immune senescence", results in increased susceptibility to infections and impaired functioning of other systems (Ponnappan & Ponnappan, 2011; Sharma et al., 2019). Similarly, the expression of immunity-related genes also varies with the age of bees. Brood has a higher haemocyte density than adults with maximum THC in pupal stage. Nurse bees have a higher fat-body mass and foragers a higher phenoloxidase activity, which generally increases with

ontogeny (Wilson-Rich et al., 2008). In simple words, the development of an adult from nurse to forager is accompanied by both reduced haemocytes and fat body content and increased phenoloxidase and GOX activity (Schmid et al., 2008; Alaux et al., 2010a). Furthermore, the presence of a dominant haemocyte type varies with the age, from granulocyte dominance in European honey bee larvae to plasmatocyte dominance in adults (Richardson et al., 2018). Expression levels of a cytochrome P450 gene not only vary from highest in the egg stage to lowest in the adult stage but also within a single stage with maximum expression in the first instar larva and brown-eyed pupa (Zhu et al., 2016). Hence, minimum haemocyte densities and reduced cytochrome P450 expression in older bees indicates lower immunity in foragers as compared to younger stages. However, this age-dependent immune suppression is considered beneficial for bees as the foragers already have higher death rates due to foraging exhaustion. Because maintaining the immune system is a costly process, reduced immunities among foragers helps in the redistribution or saving of energy at colony levels (Schmid et al., 2008).

Synergistic interactions

Besides individually, these factors interact to suppress the immune system more negatively. Moreover, immune suppression by one factor creates conditions favourable for other factors and thus reduces the survival of infected bees. Interrelationships among colony's nutritional state, immunity, *Varroa* and viruses have been well elaborated by DeGrandi-Hoffman & Chen (2015). Colonies without *Varroa* parasitism have optimum brood rearing and colony growth which facilitate sufficient resource collection and proper nutrition, leading to a built-up immunity which reduces viral loads and ensures proper colony growth. However, the presence of *Varroa* can reverse this (DeGrandi-Hoffman & Chen, 2015). A *Varroa*-virus association becomes more dangerous in the presence of such additional stresses as pesticides and poor nutrition (Nazzi & Pennacchio, 2014; Goulson et

al., 2015). Pesticides especially neonicotinoids promote virus replication by interfering with the expression of the NF- κ B gene family (Di Prisco et al., 2013). Workers infested with *Varroa* have compromised protein levels which cannot be raised even if sufficient pollen is provided (van Dooremalen et al., 2013). *Nosema* feeding patterns also induce nutritional or energetic stress in bees and interfere with both individual and social immunities (Mayack & Naug 2009; Campbell et al., 2010; Chaimanee et al., 2012). Neonicotinoids and fipronil promote *Nosema* infections in colony by suppressing immunity-related genes (Aufauvre et al., 2014) or by affecting GOX activity (Alaux et al., 2010a). Inhibition of cytochrome P450 mediated ergosterol synthesis by azole fungicides increases insecticidal toxicity (Johnson et al., 2013).

Similar to insecticides, fungicidal residues also promote *Nosema* infections in colonies but their mechanisms are not known (Pettis et al., 2013). Unfavourable temperatures reduce the expression of immunity-related genes (Steinmann et al., 2015; McKinstry et al., 2017) and enhance *Varroa*, viral, fungal infections and susceptibility to pesticides (Flores et al., 1996; Medrzycki et al., 2010; Steinmann et al., 2015). High and low temperatures, pesticides and UV radiations cause oxidative stress in honey bees (Li et al., 2016) and this stress through cellular damages suppresses innate immunity. Natural compounds in the food of honey bees upregulate the immune system by detoxifying certain pesticides, but pollen and honey deficiency downregulates it (Mao et al., 2013; Negri et al., 2015; Corona et al., 2019).

Food collection by honey bees strongly depends on outside weather. Adverse climate conditions—rainfall, high winds and extreme temperature range restrict bee foraging (Riessberger & Crailsheim, 1997) and cause nutritional stress in the colonies. Improper nutrition thus plays a central role in immune suppression by reducing resistance to *Varroa*, *Nosema*, viruses and pesticides (Huang, 2012). Interrelationships between the various stress factors are shown in Fig. 2.

Fig. 2. Interrelationships between different stress factors downregulating immune system of honey bees. IRG, immunity related genes; GOX, glucose oxidase. Blue arrows show effects supported by literature. Red arrows show possible effects with less literature support. Numeric values indicate the references of supporting literature. 1 McKinstry et al. (2017); 2 Steinmann et al. (2015); 3 Lopez-Martinez et al. (2008); 4 Melicher et al. (2019); 5 Dainat et al. (2012b); 6 Gauthier et al. (2007); 7 Flores et al. (1996); 8 Szymaś & Jędruszek (2003); 9 Alaux et al. (2010a); 10 Martin-Hernandez et al. (2011); 11 Alaux et al. (2010b); 12 Mayack & Naug (2010); 13 DeGrandhi-Hoffmman & Chen (2015); 14 Huang (2012); 15 Brandt et al. (2016); 16 Perveen & Ahmad (2017); 17 Brandt et al. (2017); 18 Boncristiani et al. (2012); 19 Yoshida (1988); 20 Gregorc et al. (2012); 21 Motta et al. (2018); 22 Dai et al. (2018); 23 May et al. (2015); 24 Chakrabarti et al. (2015); 25 Shen et al. (2005); 26 Nazzi et al. (2012); 27 Goulson et al. (2015); 28 DiPrisco et al. (2013); 29 Evans & Pettis (2005); 30 Gregorc & Bowen (1998); 31 Zakaria (2007); 32 Higes et al. (2007); 33 Chaimanee et al. (2012); 34 Mayack & Naug (2009); 35 Campbell et al. (2010); 36 Salem et al. (2006); 37 Richards et al. (2011); 38 Esch (1988); 39 Vandame & Belzunces (1998); 40 Sánchez-Bayo et al. (2016); 41 Meikle et al. (2016).

USING IMMUNE SYSTEM ITSELF REDUCES HOST FITNESS

The maintenance of homeostasis is crucial to all forms of life, but environmental fluctuations and other stressors constantly threaten it (Baena-González, 2010; Clissold et al., 2013; Ahima, 2016). In order to neutralize the threat and to ensure survival under such situations, the stress response mobilizes the energy sources of an organism's body. Energy sources are shifted from growth or reproduction related biosynthetic processes to metabolic activities

that enhances stress tolerance resulting in accelerated senescence (Baena-González, 2010; Sharma & Martins, 2020). In this context, stress response is not helpful but relatively harmful, predisposing individuals to other problems (Rabasa & Dickson, 2016).

Honey bees respond to any stress initially by recognition followed by the activation of signalling pathways and elimination of pathogen through humoral and cellular mechanisms (Larsen et al., 2019). Activation and use of immune systems against different stressors are costly; during

parasitism, such costs are usually masked by the host through compensatory resource intake (Moret & Schmid-Hempel, 2000). The restoration of immune systems through feeding demonstrates a strong association between energy costs and maintenance of an effective immune system (Siva-Jothy & Thompson, 2002). *Varroa* parasitism increases energy costs and reduces the survival of host honey bees (Aldea & Bozinovic, 2020). Another cost of immune activation could be the elimination of beneficial microflora from the gut of honey bees (Gilliam, 1997). Riessberger-Gallé et al. (2015) provided experimental evidence that the cost of using the immune system reduces survival in immune-challenged bees as compared to control bees. In response to endogenous bacteria, honey bees activate their immune system by upregulating immunity-related genes, but at the same time, the gene- encoding storage protein (Hexamerin 70b) is downregulated (Janashia & Alaux, 2016). This could be the reason behind increased food intake during parasitism. In addition to survival, the cost of activated immune system also affects the learning behaviour of bees (Mallon et al., 2003). Immuno-challenged workers and drones initially respond by increasing antimicrobial peptides (AMPs) but later AMP decreases because of its maintenance cost (Laughton et al., 2011). Those honey bees, which showed enhanced AMP production in response to bacteria, had a lower colony-production rate, possibly due to the higher production cost of AMPs (Evans & Pettis, 2005). Thus, it is predictable that the use of the immune system compromises bee health.

PERSPECTIVES AND FUTURE DIRECTIONS

Honey bees have well developed mechanisms at individual and social levels to naturally cope up with different stresses, and proper nutrition is of the utmost importance for their regulation. Major factors responsible for immunosuppression in honey bees are poor nutrition, unfavourable temperatures, pesticide toxicity, parasites and pathogens. Moreover, honey bee age also affects the immune system. Downregulation of the immune system with increasing age is

considered somewhat beneficial for bees as immune-system maintenance is costly and older bees already have higher mortalities due to foraging exhaustion (Schmid et al., 2008). All the stressors are directly or indirectly related to one another and together they harm bees as shown earlier in Fig 2. Occurrence of any one of the stressors makes conditions ideal for the co-occurrence of other stressors as well. The occurrence of nutritional stress in a colony apart from directly suppressing immunity also reduces resistance to other such stresses as *Nosema*, *Varroa*, viruses and pesticides (Huang, 2012). So, nutrition can be concluded to be the root of honey bees' defence system as it influences the expression of certain immunity-related genes and production of antimicrobial peptides (Alaux et al., 2011) and increases pesticide tolerance in bees (Mao et al., 2013; Negri et al., 2015). Furthermore, the high cost of defence responses to different stressors are detrimental for honey bee colony health as they affect their survival and productivity (Evans & Pettis, 2005; Riessberger-Gallé et al., 2015).

In addition to these major factors, we want to highlight the adverse effects of oxidative stress originated as a repercussion of different threatening situations. Since oxidative stress affects the overall well-being of a honey bee colony, future research should focus on the mechanisms by which oxidative stress affect the immunity or health of bees. One factor causing oxidative stress in bees is migratory beekeeping which has both many benefits and disadvantages as it affects the immunity and ultimately the survival of bees. Future research is needed to thoroughly understand the factors and mechanisms governing transportation stress and strategies to reduce it. It is now well understood that chances of occurrence and progress of a disease increases with diminished immunity so steps should be taken to combat this issue.

CONCLUSION

The present report provides comprehensive information on the types of immunity in honey

bees, factors affecting these immunities and their possible interaction responsible for immune suppression in honey bees. Honey-bee health suffers not only because of various stressors but also because of the activation and response of the high-cost immune system, so the focus should be on practices that actually save bees from facing stresses. Most of the stresses are anthropogenic in origin and could be managed through the adjustment of timings for pesticide applications, microclimate manipulation in case of domesticated bees, maintaining a strategic balance between migratory and stationary colony environment and the use of alternative flora for bees during dearth periods. In addition, sufficient and timely availability of high quality food, both pollen and honey, enables bees to avoid most stresses i.e. parasite or pathogen, pesticide, temperature and transport. Furthermore, those stresses which cannot be avoided, they are tolerated by mechanisms including compensatory resource intake if sufficient food is available.

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