IAU Journal of Entomological Research

Volume 14, Issue 2, pages: 26-40

Islamic Azad University, Arak Branch
ISSN 2008-4668
http://jer.iau-arak.ac.ir

STRESS EFFECTS ON BEES, POLLINATORS & PARASITIC INSECTS

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Abstract

Stressors in bees affect them individually as well as at the colony level. Changes in beekeeping practice, such as inspection on a warm day or heating hives on cool days can be stressful to bee colonies. Hive transport and inspection also may cause stress. In addition, warm humid air conditions or sudden change of temperature by pulling air through the base of the hive, as well as the presence of natural or newly introduced enemies (e.g. *Vespa velutina*, or hive beetles), or sudden alterations of signals around the hives such as constructions or posters may be stressful to bees. Stress signals are received in the brain and free radical formation in cells affects cell metabolism and may cause a reaction to the stressors. The cell endoplasmic reticulum (ER) in response to stress can cause cell apoptosis. Gene expression may respond through transcription factors in cells and may repair DNA by antioxidant pathways. Epigenetic responses by DNA methylation may affect cells and the germ line in reaction to stress and their effects in queen reproduction can produce adaptation in their next generation. Following is a description of general effects of stress on *Apis mellifera*, *Apis florea*, *Bombus terrestris, Blastophaga psenes* and parasitic insects.

Key words: Bee-Wasp-Stress-Pollinators

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Received: 10 June.2022 – Accepted: 27 Aug.2022

Introduction

Various stressors such as heat, pesticides and pathogens have different effects on insects (Kristensen et al, 2018) and are all considered to be stressors (Beasley, 2013). The main stressors that affect insect and are referred in various publications are grouped as: (1) **Climate change** or variation that in an evolutionary time scale has produced the present insect diversity. Local heat or cold stress can induce dispersal of insects to elevations or low lands. Climate stress can increase the capacity of insects to migrate or adopt by selection of resistant strains (Kellermann & van Heerwaarden, 2019; Holsch, et al, 2021; Jamieson, 2912). (2) **Stress of toxins and toxicants** such as pesticides at sub-lethal doses produce toxic effects and provoke the immunity system to detoxify the poison. Plant secondary metabolites (PSMs) play an important role in producing secondary metabolites and kairomones that benefit the insect herbivores (Murali-Baskaran et al, 2018). Viral diseases, food deficiency and diesel oil exposure or *Varroa* parasites can provoke similar physiological reaction in bee to keep away from the stress sources. Stress significantly reduces the normal longevity of bees by affecting learning ability, rate of foraging, pollination or activity (Reitmayer, 2019). (3) **Environmental stressors** are various other factors that harm an insect such as injuries, the presence of natural enemies, climate and competition. They can change fitness and shift the mean character of an organism that may cause directional selection. Insect traits are affected by ecological, symbiosis, development, phenotypic plasticity and other environmental factors that eventually they evolve to a new species. Rapid shift in insect morphological trait by mutation, selection or hybridization caused changes in their characters and enabled them to produce new sibling species (Hofmann and Hercus, 2000; Sgro et al, 2016.).

Phenotypic plasticity in wasps and bees is affecting individuals in different castes. Stressors cannot change colonies to new species or new phenotypes which may be possible for solitary insects by selection, adaptation or mutation. Honey plays an important role in thermal stabilization of the hive. Removal of the honey comb through harvesting also causes a loss of colony thermal stability and food shortage for the brood (Bordier et al 2017). Nutritional stress and environmental stimuli primarily affect the worker and nurse casts. Lack of sufficient food affects the queen that produces resistant brood or new genotypes for future colonies (Kumar & Mall, 2018; Perez & Aron, 2020). The colony collapse disorders (CCD) is a syndrome caused by a combination of various stressors that results in loss of the worker population of a colony.

Bee keepers of America and Europe were faced by a sudden death of the worker bees in their hives in 2010. Collapsed colonies had sufficient food reserve, yet, the workers died within five days. Food stores in the colony were not rubbed and the queen was alive. The colony did not have damaging population of *Varroa destructor* and were not seriously affected by *Nosemia* spp. In Europe and America beekeepers from healthy hives usually extract about 33 kg honey from each hive (Ferrier et al, 2018; Dainat et al, 2012). The financial loss was great and the last explanation is that the collective stress effects by unusual factors might be the cause was not satisfactory.

The mechanism of learning and changes in behavior for learning is affecting brain in bees and *Bombus terrestris* (Klein et al, 2017). Adaptation to continuous environmental stressors is regulated by the brain secretions of substances such as octopamine, dopamine and serotonin (Harrisan & Woodrring, 1992). Collective functioning of colony as a unit affected by stress produce behavioral response to queen. Effects of hypopharyngeal glands disproportionally affect colony health and interactions among individuals. In a stressful environmental selection increases the survival chance of some genotypes. Subpopulations may have different morphology and physiology for adaptation to variable environmental conditions (Brenebaum & Liao, 2019). One of the traits evolved by phenotypic plasticity in ants, wasps (Vespidae), and bees or bumble bees (Apidae) is producing different castes and living in new colonies (Manfredini et al, 2019; Jones and Robinson,2018). Molecular mechanisms for the change in characters are triggered by nutritional and environmental stimuli (Branchiccela et al, 2019).

A few genotypes of normal bees, wasps and ants have the potential to change their behavior and produce new Africanized genotypes that can attack man or domestic animals. A list of invasive species in Australia shows many ants have obtained invasive characters and bite humans

(Invasion of wasp, bees and ants in Australia; Monash University, 2020: Manfredini et al, 2019). The ability of eusocial insects to respond adaptively to their environment through phenotypic plasticity may lead them to change their behavior that can be screened by natural selection. If changes increase their fitness then genetic accommodation can lead to allele frequency changes, increasing the expression of related genotypes. The key factors for appearance of invasive forms are through epigenetic and genetic accommodation (Jones and Robinson, 2018). Molecular mechanisms for the change are triggered by nutritional and environmental stimuli (Corona et al, 2016). In this review I discuss the following species' reactions to stress:

1-General stress effects on bees
2-Stress effects on *Apis mellifera* and *Apis florea*
3-Stress effects on pollinators
4-Stress effects on pest parasitoids
5-Stress effect on *Nasonia vitripennis*

1-General effects of stress on bees

1-1: Starvation stress

Bees must gather large volumes of dispersed pollen from flowers. By visual and olfactory stimuli they sense the environment and pollen or nectar availability around the hive. Their brain structure gives them capacities needed to increase their capabilities for foraging. Bees can learn localities and memorize position of plants and buildings around the hive. Hive displacement increases various stress incidents among workers that they search flowers. Stress effects on the pathway for each cell and their biochemical changes are not known. On one hand stress can change the brain hormones and consequently affect the normal health of the bees and on the other hand it affects the normal protein function of cells. Protein machinery in ER also can affect the metabolism and immunity system (Mckinstry et al, 2016; Klein et al, 2016).

Shortage of food such as pollen, which supplies essential nutrients to developing workers, can increase death. Pollen shortage, poor nutrition and viral infection are suspected to be the main cause of worker sudden death and bee paralysis (Ribiere et al, 2007). Deformed wing virus (DWV), acute bee paralysis virus (ABPV), Israeli acute bee paralysis virus (IAPV), Kashmir bee virus (KBV), slow paralysis virus (SPV), and chronic bee paralysis virus (CBPV) are known to cause death in workers. They do not produce mass sudden killing of workers. *Varroa* mite is also known to be the main carrier of virus to bees and damage colony extensively. All of the symptoms that make up the cause definition for CCD are unique and each kind of viral problem provoke their specific root for activating the immune system (Dainat et al, 2012). Gene ontology analysis studies describe the linkage of genes and brain during worker development. Genes according to worker tasks are differentially transcribed to regulate functions between casts. CNS gene set restructuring the cell shape and cell-to-cell signaling. Studies show there is some relation between the number of genes involved with various tasks in worker bees and their relation to task performance for pollen gathering (Vleurinck et al, 2016).

1-2: Pesticides and gene expression.

Honey bees are under the stress of sublethal pesticide or toxins that are called hormesis (dose-response phenomenon). They show some response to various concentrations of neonicotinoids. The infected bees had population decline in their colony. Detoxification is caused by reaction to stress activating genes that their expression produces related enzymes. Stressful events evoke long term changes in bee's nervous system and metabolism is changed during pesticide detoxification (De Smet, 2017). The effects of various pesticides on median lethal dose (LD50) of adult and larvae of honey bee is measured and evaluated in 93 publications. In general exposure to sublethal doses of pesticides can diminish honey bee performance and cause population reduction. Probiotic supplementation is suggested to be a promising strategy that can be easily incorporated along the current agricultural and apicultural practices (Chmiel et al, 2020).

Functional gene products such as proteins are expressed by gene expressions. Transcriptome by small nuclear RNAs (snRNAs) via markers produce useful substances during bee development. Some of the gene expression biochemical pathways for bee immunity and defense detoxification against sublethal doses of pesticides are given by De Smet et al, 2017 and Hu et al, 2017 are:

- Detoxification genes encoding CYP9Q3 and CYTP450.
- Imidicaloon that decreases hypopharyngeal gland size (HGs).
- A general immune suppression.
- No detoxification mechanism for some poisons.
- Sublethal exposure of neonicotinoids.
- Thymal coumaphos and some other miticides.
- Chlorothalonil, Myclobutanil and some other fungicides.

Sublethal dose of pesticide upregulated AChE2, CYP9Q1, CYP9Q3 and CYT P450

1-3: Pathogens and gene expression

Virus was suspected to be the main cause of worker sudden death and bee paralysis (Ribiere et al, 2007). Deformed wing virus (DWV), acute bee paralysis virus (ABPV), Israeli acute bee paralysis virus (IAPV), Kashmir bee virus (KBV), slow paralysis virus (SPV), and chronic bee paralysis virus (CBPV) are known to cause death in workers. They do not produce mass sudden killing of workers. *Varroa* mite is also known to be the main carrier of virus to bees and damage colony extensively. All of the symptoms that make up the cause definition for CCD are unique and each kind of viral problem provoke their specific root for activating the immune system (Dainat et al, 2012). Gene ontology analysis studies describe the linkage of genes and brain during worker development. Genes according to worker tasks are differentially transcribed to regulate functions between casts. CNS gene set restructure the cell shape and cell-to-cell signaling. Studies show there is some relation between the number of genes involved with various tasks in bees and their relation to task performance (Vleurinck et al, 2016).

Honey bee larvae are resistant to relatively low doses of black queen cell virus (BQCV). Although a dose of 1.4 × 109 BQCV per larva may seem rather high, the number of RNA copies of a virus is most likely an overestimate of the number of infectious virus particles. Most of the viral RNA present at any one time in a cell increases or will be unpackaged. Nicotinoid (Thiacloprid) and BQCV were individually injected to larva and adult honey bees in the laboratory. The pesticide effect was significantly increased by the synergic action of the virus (Doublet et al, 2015).

1-4: Genome and Transcriptome Studies

How is the genome of a bee structured to transfer environmental plasticity to the next generation? The plasticity genes in bee genome are recorded in collocated form. The assemblage of series of genes in the bee genome were triggered in an evolutionary time period since 80 MY ago. They were marked by histone modification that prefigures the gene expression. Changes occur in the linear sequence in the genome region of the ovary. The bee genome is organized to coordinate with gene expression changes in response to environmental stress. Chromatin organization in these regions is responding to various signals (Duncan et al, 2020). *Bombus terrestris* genetic diversity in Britain also responds similarly to environmental stress. The genetic diversity in genome of this species affecting nervous system and wing development in different populations and show resilience of the bumblebee to selection pressure. The research helps us to understand the adaptive potential and conservation strategies for pollinators (Colgan et al, 2021).

Adaptation to Israel Active Paralysis Virus (IAPV) in honey bees is through transcription and DNA methylation by gene expression. By injecting the virus immune system reaction was recorded 5 hours (prereplicative), 20 hours (Replicative) and 48 hours (Termina). During this period profound responses and distinct molecular process accompanied lethal progression of the virus. The temporal dynamics of the transcriptomic responses by genes that expressed differentially and the number of differentially methylated regions that decreased dramatically was recorded. They suggest that response to viral infection changes cell growth and proliferation (Li-Byarlay et al, 2020).

1-5: Physiological stress

Biogenic amines in insects such as adipokinetic neurohormones, ecdysidropin, allato regulatory neurohormones control carbohydrate and lipid metabolism as a primery response to stress (Peric-Matruga et al 2006). Learning in bees and stress are closely related to brain secretions. Bee brain biogenic amines are dopamine, serotonin and octopamine. Newly emerged worker bee show an increase in these biogenic amines by aging (Harris and Woodring, 1992). Octopamine (OA) increases the waggling in bees and modulates aspects of reporting pollen and nectar location to other worker bees by dance (Barron et al, 2007). Hunger stress or malnourishment of larva also affect their weight, longevity and dance (Scofield et al, 2015; Peric-Matruga et al, 2006; Branchiccela et al, 2019).

Psychological stress may lead to depression by influencing the metabolism of the monoamine neurotransmitter system. The monoamine hypothesis, describing deficiency or imbalance of the monoamine systems can cause memory loss and learning ability in bees (Reitmayer et al, 2019). Displacement activities around the hives affect waggle dance that after foragers return they cannot show the locality of pollen. The behavior occurs during the waggle dance to excite the bees to forage (Root-Bernstein, 2010).

1-6: Stress and epigenetic effects

Epigenetic by DNA modification affect cast polymorphism and have connection with the social environment of the colony by queen worker and other castes differentiation (Weiner and Toth, 2012). In insects Euchromatin and Heterochromatin of DNA can be activated by transcription are methylated differentially in histone tail. Promoter activates gene expression by tetramer or octamer histone element (Glastad et al, 2019). Epigenetic information provides instruction on how genetic information should be used. Epigenetic information can regulate gene expression and nucleobases in the genome. Gene expression in different types of cells and tissues is modulated by two major mechanisms. (1) DNA methylation at the cytosine bases is involved in both initiation of transcription and silencing of the gene. Gene expression, when the methyl groups inhibit the transcription factors from binding to the promoter region, is inactivated. In this way, DNA expression with the use of other chromatin modifying factors, bind to methylated CpGs. The methylation is promoter of cytosines in repetitive dinucleotide sequences of cytosines and guanines (CpG). (2) Histone proteins pack DNA into structural units called nucleosomes. Nucleosome is composed of two copies of each of the four core histones, H2A, H2B, H3 and H4 wrapped around to form an octamer.

DNA methylation can regulate genome at CG sets on chromosomes. They can also regulate transcription at CpG nucleotide. Epigenetic also regulates DNA response to environmental stress by methylation and reaction is transferred to organ by transcriptions. Trans-generational inheritance through DNA methylations is affecting the egg, sperm or the germline. Environmental stressors can promote epigenetic alterations (Burgrenn, 2016).

How social experiences are represented in bee brain that by learning they can change their behavior to profit the colony? It is known that intruders to colony provoke an immediate attack to intruder and last about one hour by affecting the whole brain transcriptomic responses as well as mushroom bodies (MBs). Genes related to hormones provoke stress respond to stimuli by transcriptional factor (TFs). They promote differentially expression related to genes (DEGs). The response to TFs in MBs changes is chromatin accessibility response showing how biology can affect social life of colony regulate their social activity (Shpigler et al, 2017; Biergans et al, 2017).

1-7: Cells stress pathways

ER regulates genes encoding proteins and prevents polypeptide aggregation folding assembly. It also interferes with the rate of protein synthesis. PERK and IRE1 are trans-membrane protein kinase that in ER they respond to stress (Bertolotti et al, 2000). Biotic factors influencing honey bee health and colony productivity are commonly measured by MDA biomarker. Reactive oxygen species (ROS) are the main cause of oxidative stress and bees suffer from diminished redox homeostasis. Cells usually remove excess of ROS, or in case of severe stress the protein management in ER is disrupted. PERK is an ER resident protein that mediates signal transduction during ER stress. PERK, IRE1 and ATF6 transducers can shift to larger complexes by unfolded protein response related to ER (Malhorta & Kaufman, 2007; Hetz, 2012; Kazek et al, 2020).

Generally cellular and humeral immune system in bee are activated by mitogenic protein kinase (MAPK) that produce immunity against heat or wounds in bees. Stress is sensed by nervous system and its long term pathway reactions is by inhibition of proteasome caused during the built up of badly folded proteins in the cytoplasmic compartment of cells. Protease complex in cells are designed to hydrolise selective proteins. Proteasome are protein complexes that degrade damaged or unneeded proteins. In this way proteasome keeps the homeostasis of folding proteins processed in the endoplasmic reticulum (ER). Wound and pathogens can lead to disruption of cell septic infection with heat shock targets. Humeral immune system secretion by plasma cells mediates with antibody molecules to control antigen. Antigens are linked to B-cell's antigen receptors and are changed to peptides by mitogen activated protein kinase (MAPK). To determine the effects of heat shock stress the UPR responding to the proteostatic perturbation in the ER and the mitochondrial UPR (UPR^{mit}) perturbation responding in HSR is measured. Stress may lead to multiple processes and pathways but HSR built-up of mis-folded proteins show that the proteases network is conserved network of cellular stress. Heat shock responses in multiple immune genes in the abdomen, by wounding the cuticle of abdomen, resulted in decreased expression of many HSR genes in bee tissues. (Mckinstry et al, 2017).

2- **Stress effects on *Apis mellifera* and *A. florea***

Factors causing CCD may be various environmental stressors (Dadgostar, 2015). Severe or chronic stressors, like prolonged sublethal pesticide exposure, changes in beekeeping practice, hive inspection on a warm day or preheating hive in cool days can be stressful to bee colonies and affect the colony in various ways (Wang et al, 2016; Reitmayer et al, 2019). Reactive oxygen species (ROS) may be induced by exogenous sources such as pesticides and the environment. Oxidative stress can lead to apoptosis and cellular damage and is linked to aging. The MDA (Malone di Aldehyde) biomarker is a common measure of oxidative stress in honey bees. It is the main organic compound produced from lipid peroxidation of polyunsaturated fatty acids in cellular membranes (Simone-Finstorm et al, 2016; Perez & Aron, 2020). Excessive amounts of denatured proteins in cells might be the reactions to intracellular stress (Even et al, 2012). Cellular stress responses described by pathogen attacks or poisons increases production or activation of antioxidant proteins. Bee metabolic rate increases as a consequence to stressors. The general responses of insects and mammals to stressors are comparable for their ATPase ions in their malpighian tubes of insects and kidneys of mammals (Anstee et al, 1980).

Unfolded protein response of ER (UPRER) biomarkers contributes to the maintenance of cellular proteostrases. Heat shock response (HSR) activation impacts other processes involved in proteostrases communicating to other compartments in cell-wide responses (McKinstry et al. 2017). Cellular and humeral immune in bee body are the mitogenic activated proteins kinase (MAPK) that produce humeral immunity in bee body. Transcriptome analysis has revealed the relative antibody in their hemolymph. While AB-cells encounter its matching antigen, toll-like receptor (TRL) proteins play a key role in the innate immune system. Their signaling pathways antigen can negatively affect transduction pathways (Li et al, 2018). The flow of information from RNA to proteome complexity is destined to affect the bee metabolism. Metabolites can change the final downstream product of the gene by stress biomarkers that may reach the genome affecting phenotype in biological system (Manfredini et al, 2019; Wang et al, 2019).

During transportation, colonies are challenged by a variety of stressors. The condition of a hive prior to transportation is often locally acclimated to ecological conditions which often differ greatly from those of the destination. They are moved without secured rapping and jolt during transport. Changes in temperature, day length, and nutrient supplementation that bees experience after transportation can increase foraging activity and brood production earlier than would have occurred before relocation and in cooler green environment. Bees keep the temperature in hive at 32-35 C. They may shiver in cold or fan the interior of hive at high temperatures. Relocating hives also causes some loss of foragers which typically require time and an obstruction or landmark to reorient them to the new location. Transportation stress has received less attention because of the difficulty of collecting data during shipping. Even though transportation lasts only a few days, colonies experience confinement, increased variation in temperature, air pressure, and vibration. During shipping, colonies experience a rapid progression of changing elevation and latitude. Ventilation is a primary concern because poorly ventilated colonies often die from overheating. The consequences of low-temperature stress are less obvious. A healthy colony will maintain an internal temperature between 32 and 35°C which is necessary for the development of brood, flight, and efficient worker activity (Melicher et al, 2015).

Apis florea is another of the nine species of *Apis* that live in tropical areas of the world and produce honey. They are recorded from Persian Gulf costs and found in Pakistan, Iraq, India, Bangladesh, Indonesia, Thailand and Africa (Mossadegh, 2014). Their brood rearing starts in February and continues until June. During this time the mass of bees are found on tree branches at 3-14 meter height. Sometimes their colonies are built in tree trunks or inside buildings with easy access to fields for pollen gathering. Their cluster generates sufficient heat for their survival in the winter. Bees on the outer layer of their colony mass protect the colony from cold or intrusion of foreign bees. At the center of the cluster the temperature is about 35°C. The main stress for bees is starvation. Honeydew is an important supplementary food and the main source of honey production. At high temperatures honeydew evaporate quickly. The honeydew of aphids also is often collected by foragers. In high temperature aphids and plant dews are not available and bees become starved and there are little flowers to feed their brood by pollen. Their only choice is to consume reserved food or search pollen by longer distance flying.

2 -1: Flight activity and stress

Three weeks after working in hives bees usually leave their interior tasks and go to foraging behavior. They may fly several hours and up to 8 km per day. Senescence accelerates after the transition to foraging, leading to nearly 100% mortality within 14 days. The flight capacity of foraging bees decreases with age, an effect likely due to oxidative damage in flight muscle. A total of 60 foragers that carry pollen of unknown age collected at the entrance of the colony in desert region with dry environment but plenty of water and dead honey bees were counted daily. Frequent and highly aerobic behaviors likely contribute to naturally occurring stress, accelerate senescence and limit lifespan. For understanding how the physiological and cellular mechanisms determine the onset and duration of senescence and shape new behavioral development refer to Segel et al, 2013.

Most studies relate the onset and duration of flight to senescence in honey bees, and an accelerated transition to foraging leads to functional senescence. By measuring and manipulating the frequency time, distance and actual intensity of each individual flight in addition to days spent flying will yield a far more comprehensive view of flight-associated oxidative damage. However, the effects of these variables on senescence and oxidative damage in a natural environment have not been well studied. Experiments suggest that brain tissue and flight muscle respond differently to cellular stress. Association of reactive oxygen species (ROS) and accumulation of antioxidant activities in bees with varying amounts of flight show while age and flight activity are correlated the rate and duration of bee flight is related to oxidative damage of wing muscles and the accumulation of antioxidant also affect the flight duration (Margotta et al, 2018).

2-2: Royal jelly production and stress

Royal jelly is a special food for young larvae and the queen. It extends the life span of the queen relative to worker. It regulates oxidative stress effects in bees and inflammations in bee digestive tracts. An extensive research for increasing royal jelly production is published by studying bee proteins. In these studies the genetic way to select queens and bee strains that produce higher amount of royal jelly are described. (Altaye et al, 2019). Proteins are made by promoter of DNA sequence and its transcription by single strand RNA. The promoter is translating mRNA into proteins. Entire set of cell, organ or body proteins are proteomes. The study of these proteins or their function is called proteomics. The molecular basis of different organs and tissues that allow bees to perform their biological tasks is initiating from the brain. The worker bee changes its work from cleaning the colony to feeding the larvae or collecting pollen outside the hive. More than 300 genes are involved in expressing signals to regulate caste duties and sex signals. In a study of gene and brain signals the molecular processes for reconstructing the cell shape or cell to cell signaling are involved in worker tasks (Veleurinck et al., 2016).

The proteins are synthesized by hypopharyngeal gland (HG) and their acini are developed mainly during the nursing stage of the bee when they feed larvae with the royal jelly. More than eight proteins are involved in processing nectar to honey and royal jelly (Altaye et al, 2019). While worker bees undergo physiological changes from nursing to foraging nurse workers produce royal jelly proteins but foragers mainly produce Alfa glycoside that convert nectar to glucose and fructose (Ueno et al., 2015; Wang, 2019). Neurophysiological changes in bee brain through proteome and transcriptome make the proteins needed for the royal jelly production. The procedure is categorized for the honeybee brain proteomes (Hernandez et al, 2012).

3-Stress effects on pollinators

3-1: *Bombus terrestris* and stress;

Crucial period for plant flowering should be synchronized by bee activity but weather warming has changed this synchronization in some localities. The impact of temperature rise and water stress decreases the nectar volume and quantity of nectar sugars produced per flower. The relative percentage of amino acids in flowers under stress was changed. The modifications affected plant-pollinator interactions and affected *Bombus* nutritional needs (Greenop et al, 2020). Land-use and buildings also stress bumble bee and affect cuticular chemical surface and their morphology. *Bombus lapidarius* respond differently to land use and lack of grasslands in their vicinity and affect them seriously (Straub et al, 2022). Understanding the relationship between the effects of stressors on the nervous system, and factors affecting bee foraging and metabolic biomarkers, help us to monitor preserving health in *Bombus terrestris* and their colony survival (Wang et al, 2019; Klein et al, 2017).

3-2: Fig wasps

Fig wasp *Blastophaga psenes* (Hymenoptera, Agaonidae) is a solitary pollinator of *Ficus carica* (Moraceae) and wild figs. Its reproduction is inside the fig fruit where wingless males are found. They overwinter at the last larval stage in the male fruits that stay on trees from the summer. In the spring the last larval stages complete their development and females emerge in search for receptive figs. The female fly to different trees and search the fruits for wingless male and simultaneously pollinate seeds within the same fruit. About half of the *Ficus* species are monoecious. In Estahban (Northeast of Shiraz) more than 2,500,000 trees in 24,000 hectare are pollinated by female containing branches from fig trees hanged over edible fig trees (*Ficus carica*) (Kolahi et al, 2013).

Fig wasp's life is endangered by ozone stress. O_3 is known to disrupt chemical communications between plant and their pollinators. High concentrations of O_3 affect pollinator physiology and behavior. Experiments have shown that by increasing O_3 concentration from 40 to 80, 120 and 200 PPb for 60 minutes exposure the normal behavior of fig wasp is increasingly more disrupted. Doses of 120 to 200 PPb O_3 can increasingly kill more of the treated wasps (Vanderplank et al, 2021).

4- Stress effects on pest parasitoids

Stress effects on locust, insect pests and *Aphis gossypii* are previously described (Hodjat, 2016, 2022; Hodjat and Kamali, 2022; Hodjat and Soleyman Nejad, 2022; Hodjat and Saboori, 2021; Hodjat and Husemann, 2019). The effects of pollutants on parasitoids show 7.6% of them from 9 families, 35 genera and 37 species of Hymenoptera and Diptera orders are affected by general environmental pollutants. Pollutants included pesticide residues, exhaust fuel, dust and Ozone (O_{3}), Sulfur Oxides (SO_x), Nitrogen Oxides (NO_x), Carbon oxides (CO_x), and soil heavy metals. The detail effects of stress on parasitoids need further research but at low concentration pollutants increased activity while at higher dose they affected searching behavior of parasitoids (Butler et al, 2009). The effect of drought stress on brassica aphids differed for generalist pest (*Myzus persicae*) compared to specialist pest (*Brevicoryne brassicae*). Parasitoids (*Diaeretiella rapae*) in both cases only responded under medium and high stress by becoming less effective (Shehrzadet al, 2020). General stress effects of sublethal pesticides doses on beneficial insects have been previously briefly discussed (Desneux, 2007).

Stress effects of pathogens inflicted to parasitoids is mainly described for *Trichogramma* species and *T. chilonis*. *Trichogramma* and *Telenomus* are both parasites of the *Spilosoma oblique* (Arctiidae) eggs that are the pest of groundnut (*Arachis*: Fabaceae) and sunflower in India. Their competition is stressful for both parasitoids. Stress effects on predators (*Coccinella* and *Chrysopa carnea*), parasitic fungus on insects pests (*Pseudomonas*) and for *Bacillus* (T*richoderma*) are also described (Karuppiah et al, 2018).

Kairomones mediate interactions between plant and natural enemies. The use of hormones for bio-control of insect pests is for protecting plants against pests without pesticide toxins. High quantities of octadecanoic emitted from rice plants play an important role to attract *Trichogramma* spp and stimulating their ovipositor to lay eggs in the stem borer larvae. Volatile profiles of rice cultivar indicate the presence of natural harmonious relationships between the stem borer and its parasite population to attract pest parasites for the**ir** defense (Murali-Baskaran et al, 2018: Jameison et al, 2017). Drought stress changes the quality of kairomone metabolite and their attraction to parasitoid insects (Yi et al, 2021; Descampes, 2018). However studies on stress effects in insect parasites for biological control on crops research is mainly concentrated on *Tricjogramma*. Transcription response of *T. chilonis* by keeping prepupae at 10-25-and 40 C indicated that differentially expressed genes (DEGs) by heat stress can activate protein processing pathways in endoplasmic reticulum (ER) of the clls. The heat shock proteins (hsps) were highly upregulated. Gene pathways of starch and sucrose metabolism were down-regulated under cold stress (Yi et al, 2021).

5-Stress effects on *Nasonia vitripennis* (Walker)

Nasomia vitripennis (Hymenoptera; Pteromalidae) is parasite of flesh flies or blowflies that are regarded as forensic insects. As an ectoparasite of fly puparia they spend most of their life cycle (eggs, larvae, pupae, and early adult stage) inside the pupa of flies such as *Musca domestica, Lucilia seicat, Sarcophaga marshali*. Microbes are playing important roles in host defense and parasite development. The hosts are defended by bacterial communities and *N. vitripennis* is helped by *Wolbachia* (Duran et al, 2020). The parasitoid is escaping stress by producing Asym C, Cor and Oul genotypes with different traits. Oul males showed highest adjustment to heat stress by providing lost water needed for their metabolism while Asym C males were most vulnerable to stress after explosion of more than 10 hours at temperatures above 36 C. At the cellular level heat stress disrupt its water osmotic homeostatic conditions and accumulation of specific metabolites. Various metabolic traits exist in *Nosemia* populations that have resistance potentiality to various stressors (Kevin et al, 2019).

Male *Nosemia* when puparia are limited fight other males to possess food for mating and female oviposition. Fight and crowding of larvae inside puparia are stressful especially when *Mellitobia* also lay eggs in the same puparia occupied by *Nosemia*. *Mellitobia* is similar to *Nosemia* but is half the size of *Nosemia* and has a flattened elongated head. Head in *Nosemia* is rounded and spherical (Guinan et al, 2005).

Conclusion

Research on stress is stretched to all fields of biology as well as to define the concept of stress. In this article subjects related to stress is documented. For solving worker bee sudden death problem (CCD) a syndrome of problems was mentioned to cause their death. Stress can be the major cause for producing syndrome of response to reduce the worker resistance by parasite, disease, crowding and other problems. Scientists are now working to find out pathways to sudden death in their field of research to discover how to increase bee resistance mechanism to death by more investigation.

Acknowledgement

Dr. Martin Husemann, Leibniz Institute for the Analysis of Biodiversity Change and the Universität Hamburg, 20146 Hamburg, Germany; by reading the manuscript and writing many comments and suggestions for its improvement, is greatly appreciated.

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