MELLIFERA REVIEW ARTICLE

Pesticide and Pathogen Induced Oxidative Stress in Honey Bees (*Apis mellifera* L.),

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A B S T R A C T

Honey bees represent an important cultural and economic benefit for humans by pollinating wildflowers and crops. Honey bees, like other organisms, face a wide range of environmental stressors throughout their lives. These stress factors disrupt the physiological balance of the organism. During the use and metabolism of oxygen taken into the organism, aggressive molecules known as free radicals are formed and the organism cannot keep these radicals under control and oxidative stress occurs. In such a situation, free radicals attack, oxidize, and degrade healthy cells. This degradation is characterized by the increased production of reactive oxygen species (ROS), by the simultaneous degradation of waste systems. Exceeding the oxidative stress threshold in honey bees causes bee losses on an individual or colony level. Colony losses, which have been increasing day by day due to environmental factors, reveal the importance of studies on the formation, physiology, and prevention of oxidative stress. The most important antioxidant enzymes identified in honey bees are glutathione S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD). This review examines the mechanism of oxidative stress and the effects of pesticides and pathogens on oxidative stress in honey bees.

Keywords: Honey bee, *Apis mellifera* L., oxidative stress, reactive oxygen species (ROS), pesticide, pathogen

Introduction

Pollination is a vital ecosystem service required for 76% of global crops and about 87,5% of all flowering plants [1, 2]. The most important pollinator species in worldwide are honey bees (*Apis mellifera*), providing about 50% of the pollination of global products [3]. Honey bees have a long history with humans and are the most domesticated pollinator species globally [4]. Unfortunately, a significant loss in bee population has been observed in recent years. Both bee population loss and colony collapse are caused by climate change, habitat loss, environmental stress factors, the availability and diversity of feeds, as well as particularly insecticide use, parasites, and pathogens [5-13].

Various chemicals (insecticides, drugs, metals, smoke, the abnormal concentration of oxygen, etc.), physical (radiation,

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temperature, noise, vibration, etc.), and physiological (diseases, physical injury, aging, etc.), stressors may cause stress that can disrupt homeostasis. Such a situation is called oxidative stress. Oxidative stress occurs when excessive oxygen radicals are produced in cells that may exceed normal antioxidant capacity, and reactive oxygen species (ROS), are produced simultaneously enhanced by waste degradation. Increasing ROS concentrations cause oxidative damage to proteins, lipids, and nucleic acids; therefore, the functions of cells, organs, or the whole organism by being severely impaired lead to death [14].

The Mechanism of Oxidative Stress

Oxidative stress causes an abnormal ROS (Reactive Oxygen Species), level such as free radicals (hydroxyl, nitric acid, superoxide, etc.), or non-radicals (hydrogen peroxide, lipid peroxide), by damaging cells or tissue, is a general term used to describe the effect of oxidation by damaging specific molecules. Oxidative stress results from an imbalance between the ability to easily detoxify reactive intermediates of a biological system with reactive oxygen production or to repair the damage that has occurred. In other words, oxidative stress is a defect in the balance between ROS (free radicals), production and antioxidant defenses that can cause tissue injury. Oxidative stress occurs when ROS production in a system exceeds the system's ability to neutralize and eliminate them. The imbalance can be caused by production, distribution and/or environmental or behavioral stressors and lack of antioxidant capacity caused by excessive production of ROS. This damage can affect a specific molecule or an entire organism. If not regulated properly, excess ROS can inhibit normal function by damaging a cell's proteins, lipids, carbohydrates, and DNA, which can lead to cytotoxicity, genotoxicity, and even carcinogenesis when damaged cells proliferate; therefore, oxidative stress plays a role in the aging process as well as increasing diseases [15-17].

Reactive Oxygen Species (ROS), Oxidative Damage and Cell Singal

Reactive oxygen species (ROS), are highly reactive molecules composed of various chemical types such as superoxide anion (O2−), hydroxyl radical (•OH), and hydrogen peroxide (H2O2). ROS are

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produced as byproducts of aerobic respiration and various other catabolic and anabolic processes. Mitochondria are the largest producers of ROS in cells, and most part of the mitochondrial ROS is produced in the electron transport chain. Electrons infiltrate directly into oxygen through the electron transport chain and generate short-lived free radicals such as O2−. O2− can be converted into non-radical derivatives such as H2O2, either spontaneously or catalyzed by superoxide dismutase (SOD). H2O2 is relatively stable and membrane-permeable; besides, it can diffuse into the cell and be removed by cytosolic antioxidant systems such as catalase, glutathione peroxidase, and thioredoxin peroxidase. In addition to being produced during cellular metabolism in mitochondria, ROS can be produced in response to different environmental stimuli such as growth factors, inflammatory cytokines, ionizing radiation, UV, chemical oxidants, chemotherapeutics, hyperoxia, toxins, and transition metals. Apart from mitochondrial respiration, a number of cytosolic enzymes can produce ROS. Nicotinamide adenine dinucleotide phosphate (NADPH), oxidases are a group of enzymes associated with the plasma membrane found in various cell types. The function of NADPH oxidases is to produce superoxide from oxygen using electrons from NADPH [18].

ROS after produced, react with lipids, proteins, and nucleic acids that cause oxidative damage to macromolecules. ROS readily attack DNA and produce various DNA lesions such as oxidized DNA bases, abasic regions, and DNA strand breaks, leading to genomic instability [19, 20]. 7,8-dihydro-8-oxo-deoxyguanosine (8-oxo-dG), is one of DNA lesions good characterized and the most common caused by ROS. It is a mutagenic lesion resulting in G: C to T: A transversions. To limit cellular damage caused by ROS, the cells have developed amount of enhancement defense mechanism. DNA lesions produced by ROS are mainly repaired by base excision repair and other DNA repair pathways such as nucleotide excision repair, double-strand rupture repair, and mismatch repair. Additionally, the detrimental effects of ROS can be neutralized through high antioxidant defense pathways that include SOD, catalase, and glutathione peroxidase [18]. Depending on the cell types, ROS has been found to function as signaling molecules in cell proliferation, cellular

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aging or cell death. Many cellular processes being different effects of ROS mediate not only harmful byproducts of ROS but also various signaling pathways [18].

Increased oxidative stress causes many diseases in humans: cardiovascular diseases, cancer, diabetes, neurodegenerative diseases (Parkinson, Alzheimer's disease, paralysis, dementia, epilepsy, etc.), and psychiatric diseases (attention deficit, hyperactivity disorder, autistic disorder, anxiety disorder, bipolar disorder, depression and mood disorders, history of suicide attempt, psychosis, schizophrenia, and sleep disorders). In addition to these diseases, the role of oxidative stress has been well specified for diseases such as alcohol and drug abuse, asthma, chronic obstructive pulmonary disease (COPD), various seizures, hepatitis and liver diseases, rheumatoid arthritis, kidney diseases, and various eye disorders [21].

Oxidative stress can be classified according to density with density scales ranging from physiological oxidative stress (eustress), to toxic oxidative load that damages biomolecules. Various oxidants are produced by endogenous or exogenous sources. Low exposure of oxidant cells and organisms allows specific targets in the use of the redox signal (oxidative eustress; beneficial stress), while high exposure results in disruption of the redox signal and/or damage to biomolecules (oxidative distress). In the 21st century, as Paracelsus addressed in his dictionary, the paradigm "*dose creates poison*" is a viable paradigm for oxidative stress [22, 23] (Figure 1).

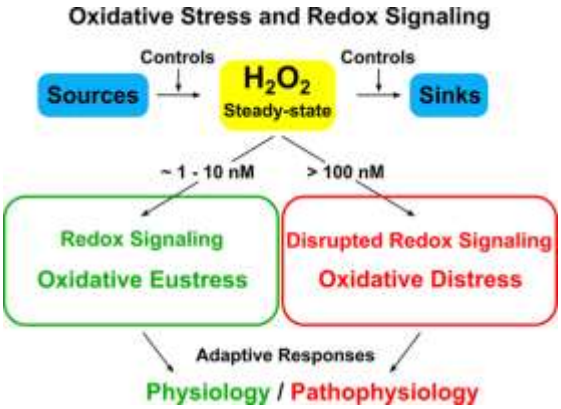

Figure 1. Role of hydrogen peroxide in oxidative stress [24].

Oxidative equivalents used in redox signaling target directly or indirectly regulatory pathways, particularly those addressed by transcription factors. Hydrogen peroxide has emerged as a major redox metabolite that is effective in redox detection, signaling, and redox

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regulation [23-26] (Figure 1). However, nitric oxide, hydrogen sulfide, and peroxynitrite play an important role as superoxide anion radical and single molecular oxygen redox metabolites. In the short term, it acts on the activation of pre-existing enzymes or ion channels, while in the longer-term (hours/day), it is mediated by the activation of gene transcription resulting in changes in enzyme patterns [23].

Oxidative Stress in Honey Bees

While there is a balance between ROS production and antioxidant process under normal conditions, exogenous stress factors (pesticides, heavy metals, biotic infections, etc.), can disrupt this balance and cause more than normal ROS production [27]. There are enzymatic and non-enzymatic defense systems to prevent damages caused by ROS [27, 28]. The most important antioxidant enzymes identified in honey bees include glutathione S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), while non-enzymatic components are glutathione, NAD(P)H, vitamins C and E, albumin, uric acid, and keratin [29-33]. GST is one of the important oxidative stress enzymes related to insecticide resistance. The damage of insecticides entered into the body on the redox balance, oxidative stress, as well as the production of lipid hyroperoxides, can be decreased by GST activities [34].

In *Apis mellifera*, vitellogenin and juvenile hormone are proteins involved in oxidative stress [35]. The life span and oxidative stress levels of bees depend on the levels of hexamerins (Hex), and vitellogenin (Vg), which are the main storage proteins [36,37]. Vg being the reproductive protein plays an important role as an antioxidant, which can explain the resilience in the aging of the worker bees and the queen. [35, 36, 38, 39].

In particular, the hemolymph titer of Vg is directly linked to the survival of acute oxidative stress [36]; the classical antioxidant defenses may be less important to explain the differences in life expectancy between honey bee casts [40]. In contrast, drones may be more susceptible to oxidative stress because they have much lower Vg levels and are haploid [41,42]. The correlation of resistance with the expression of Vg to

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oxidative stress in worker honey bees reveals that Vg can extend the lifespan of the queen and worker bees [43].

Pesticide induced oxidative stress in honey bees

The main causes of honey bee losses are pesticides, poor reared and feeding conditions, weather conditions, and bee diseases. Sudden and intense deaths in bees are usually seen due to poisoning. The cause of poisoning in bees is the agricultural drugs used in plant production as we call pesticides. The areas where plant production and pesticides are used are a common use for bees too. Beekeepers and farmers using pesticides do not have sufficient knowledge and awareness about the effects of pesticides on bees. Farmers using pesticides and beekeepers in the same region are not in contact with relevant public officials who have the necessary knowledge and experience. An inadequate control and legal practices, insufficient knowledge and guidance of the central beekeepers' association and beekeeper unions on pesticide use, the taking insufficient control of the current use of pesticides without considering their toxicity on bees by scientists and experts in universities and research institutes can be listed as the main poisoning reasons with pesticides [44].

In recent years, with the increase of insecticide use in agricultural areas, bees are exposed to these insecticides through direct contact or during the collection of contaminated pollen and nectar. Insecticides cause toxic effects both at the individual level and at the entire colony-level due to carry them to their colonies [31, 33, 45] (Figure 2). In particular, it is observed that the performance of bees is negatively affected, such as the decrease in the foraging instinct and success of bees, lethargy, dizziness, paralysis, abnormal bee behavior, loss of balance, uncontrolled movements, difficulty in finding the hive entrance, as well as incubation production and decrease in disease resistance. On the other hand, the presence of a large number of dead forager bees (500-1000 or more), in front of the hives within an hour (s), or day (s), as a result of the poisoning of the forager bees in the healthy and strong hives is one of the main negative effects of pesticide-induced bee poisoning [31, 46-48].

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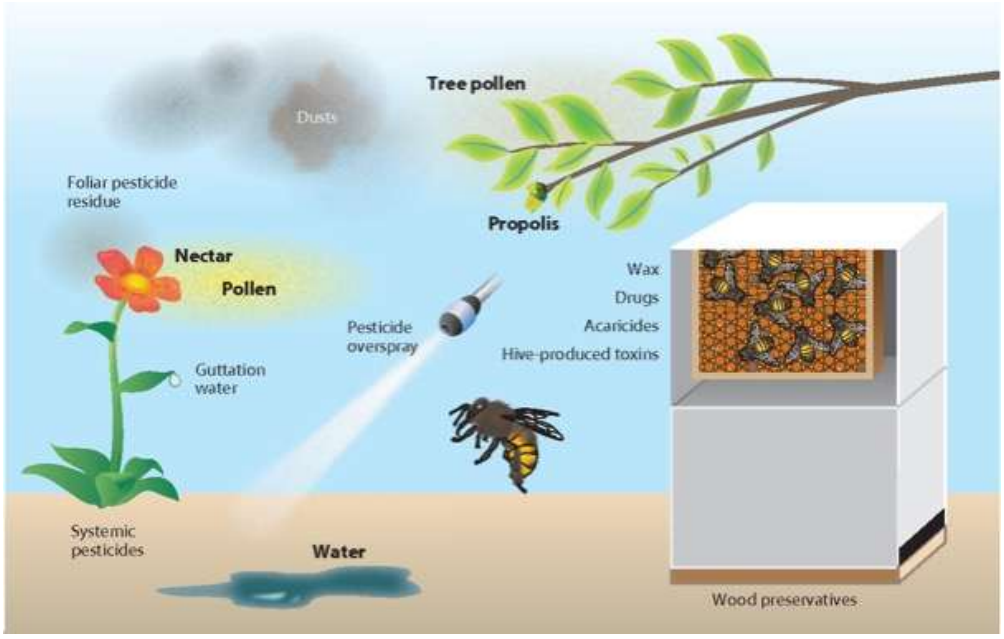

Figure 2. A summary of the different routes by which honey bees may be exposed to potentially toxic pesticides. Materials collected by foraging honey bees are in bold letters [49].

Pesticides are known to cause significant oxidative stress on all insects such as honey bees. Honey bees, on the other hand, can use various mechanisms as a defense against pesticides. A high level of antioxidant enzyme activity is an important marker for honey bees in pesticide-loaded environments [31]. In a study, in honey bees (*Apis dorsata* and *A. cerana*), exposed to pesticides (organophosphorus (OP), pesticide, a synthetic pyrethroid (SP)- cypermethrin and an organochlorinated pesticide (ES)-endosulfan), whether there was a change in SOD, CAT and xanthine oxidase (XOX), antioxidant levels were examined and as a result, it was found that the SOD and CAT levels in honey bee samples collected from regions with high pesticide density were higher than those with low pesticide density [31]. In another study, it was found that chlorpyrifosa, being one of pesticides, exposed by *Apis mellifera* increases lipid peroxidation known to cause oxidative stress/damage in the nervous system [50].

One of the most frequently used pesticides for insect control on plants in the world is imidacloprid, one of the neonicotinoid insecticides [33, 51]. Imidacloprid is a neurotoxin that acts as an antagonist of nicotinic acetylcholine receptors and causes paralysis and death. Old bees with higher antioxidant protection have been reported to be less susceptible to imidacloprid toxicity, so the toxic effect of the pesticide is dangerous in the early stages of honey bees' lives [52]. Nicodemo et al. [53] demonstrated that imidacloprid affects energy production by bees' mitochondria; besides, Nicodemo et al. [54] showed that imidacloprid reduced the level of lipoforin in hemolymph of honey bees. Balieira et al. [33] investigated the effects of imidacloprid on the cellular antioxidant system of honey bees and the potential antioxidant activity of caffeine. They found that imidacloprid increases the activity of GPX and CAT antioxidant enzymes in bee thorax as an indicator of oxidative stress induction. In addition, it is known that the use of caffeine, which affects the antioxidant systems and lifespan of worker bees, in bee nutrition increases the activity of GPX, CAT, SOD and GTS. Caffeine acting as an antioxidant has a preventive effect on the damage caused by insecticides [33]. As in Strachecka et al. [55], Balieira et al. [33] observed that caffeine promotes an increase in the activity of GPX and CAT enzymes. Besides, the addition of 2.0 ng/mL caffeine to imidacloprid has been found to reduce the formation of MDA, which shows antioxidant activity, although the insecticide only causes an increase in GPX activity.

The responses of acetylcholinesterase (AChE), carboxylesterases (CaEs-1-3), glutathione-S-transferase (GST), catalase (CAT), and alkaline phosphatase (ALP), were evaluated in bees exposed to insecticides such as deltamethrin, fipronil, and spinosad and it was determined that fipronil and spinosad induce CAT activity; deltametrine modulates CaE-1 and CaE-2 with opposite effects; spinosad exhibits an induction profile for most biomarkers other than AChE; fipronil does not modulate AChE, CaE-2, or GST and does not increase CAT and CaE-1, but it decreses ALP [56].

Acetylcholinesterase inhibitors, both organophosphate (OP), and methylcarbamate (MC), insecticides act on the nervous system of honey bees by

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inhibiting the activity of acetylcholine esterase (AChE), the enzyme that inactivates the neurotransmitter acetylcholine in central nervous synapses [57]. Both classes of AChE-inhibiting insecticides have an extremely broad toxicity to bees (topical LD50 = 0.018¬¬¬–31.2 µg/bee), [58, 59]. However, highly toxic OPs and subsets of MHs also pose a significant hazard to bees [60]. Coumaphos, a subset of OP, has such low acute toxicity (LD50 = 31.2 µg/bee), that it is used by beekeepers to control Varroa mites [61]. With repeated use, coumaphos reaches concentrations as high as 90 ppm in wax of colonies [62, 63]. The use of coumaphos in colonies is thought to cause increased larval mortality in both queens and workers [64, 65]. It has been determined that larvae reared with a diet containing 8 mg/L coumaphos have a significantly higher mortality rate during development than control larvae [66].

In another study, it was determined that exposure of honey bees to the herbicide atrazine, which is widely used in the laboratory and hive, leads to oxidative stress responses that can endanger the health of bee colonies; in addition, having a general decrease in antioxidant enzyme activities, and changing the relative expression levels of some antioxidant encoding genes after exposure to atrazine, differently were specified [67].

In a study on the development of acetylcholinesterase (AChE), carboxylesterases (CaE1, CaE2, CaE3), glutathione-S-transferase (GST), alkaline phosphatase (ALP), and catalase (CAT), as enzyme biomarkers of exposure to xenobiotics such as thiamethoxam in honey bees, it had been determined that exposure to thiamethoxam has non-lethal effects and alter the activity of CaEs, GST, CAT, and ALP (There was no response for AChE; however, an increase for GST, CAT, and CaE2 and a decrease in CaE1 and CaE3 had been observed. Besides, ALP and CaE3. showed opposite variations in 2.56 ng bee only), [68].

Malondialdehyde (MDA), is a general biomarker for measuring oxidative stress in honey bees. By Simone-Finstom et al. [69], between stationary colonies and migratory beekeeping was carried out among agricultural lands where the probability of exposure to insecticides is high, the life span and oxidative stress levels of honey bees were affected and

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MDA levels of honey bees were measured. It was observed that the level of oxidative damage was lower in adult worker bees reared in the migratory colony environment in the early period of the season and then placed in a stationary environment compared to the worker bees in the migratory colonies throughout their lifespans. While the MDA level increased throughout the season for bees in the stationary colonies, it was observed to remain at a constant level for bees in migratory colonies. The increased exposure of bees to pesticides in agricultural landscapes may explain why an increase in MDA levels.

Abdelkader et al. [70], studied the effects of insecticides on oxidative stress on the sperm of drones. It has been stated that clothianidin shows significant increases in SOD, GP, CAT, and MDA levels. Since the protein content in the sperm of drones exposed to clothianidin is significantly reduced, it has been thought that drones can cause oxidative stress in the spermatozoa, which can affect the semen quality and hence queen fertility.

Oxidative stress, the copes with it of the organism or time to cause its death is associated with how strong the immune system is. Pesticides also cause rapid damage on the honey bee immune system. The immune response can be divided into a humoral response and cellular response. The humoral response generates antimicrobial peptides (AMPs), through activation of the four immune pathways: Toll, immune deficiency pathway (IMD), c-Jun N-terminal kinase (JNK), and Janus kinase/signal transducers and activators of transcription (JAK/STAT). Sublethal pesticide exposure impairs the humoral immune response by reducing the production of AMPs. The cellular immune response is orchestrated through hemocyte function. Hemocytes can facilitate the melanization of pathogens and wounds through activation of prophenoloxidase (PPO), to phenoloxidase (PO), and reactive oxygen species (ROS), as a by-product. In addition, hemocytes can phagocytosis and clear invading pathogens, as well as differentiation into other immune cells. Multiple aspects of the cellular immune response are impaired by sublethal pesticide exposure [71] (Figure 3).

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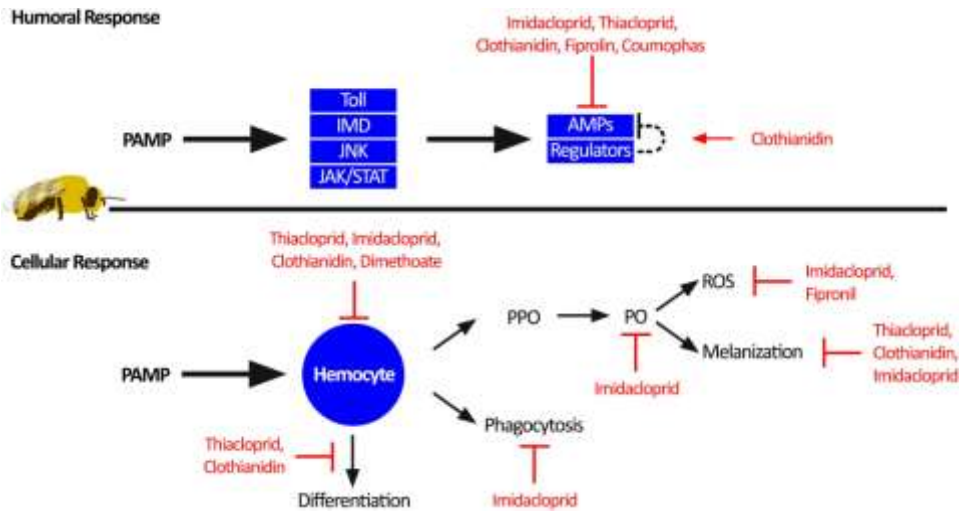

Figure 3. Humoral and cellular response of honey bee immune response against pesticide-associated molecular models (PAMPs), [71].

Considering the destructive effect of pesticides on bees, studies have been initiated on new pesticides and insecticides whose damages are reduced in order to cope up with these effects. Flupyradifurone is the active ingredient of Transform® in Sivanto ™ and sulfoxaflor. Both are relatively new insecticides that have been developed to reduce negative effects on bees when applied to plants. This study was conducted to better understand the potential non-lethal adverse effects of these pesticides on bees. In the experiment, the effects of two pesticides which were applied by a Potter Tower sprayer on nutrient consumption and oxidative stress levels in bees that were exposed to certain application dosages in the field were investigated. In both pesticide applications, a significant difference was observed between the treatment groups and control groups in the amount of sugar syrup and water consumption. The highest mortality rate was observed in bees exposed to Transform® followed by Sivanto ™ exposed bees. Estimates of reactive oxygen/nitrogen species showed significantly increased oxidative stress in both pesticide application groups compared to controls. In addition, caspase-3 protein tests, which are an indicator of

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the onset of apoptosis, were found to be significantly higher in pesticide administration groups [72].

Pathogen induced oxidative stress in honey bees

Many stressors such as poor nutrition, loss of natural habitat, pesticide exposure, pathogens (i.e., bacteria, fungi, protozoans, viruses), and parasites have caused the big concern for global bee decline and potential economic losses to insect-dependent agricultural crops. Especially pathogens caused colony losses to come in sight due to wrong beekeeping practices such as not doing the control and treatment of *Varroa*, regularly, having an insufficient knowledge on pathogens and their struggle methods, and not following the disinfectant rule in their colonies, etc. Thus, pathogens have been coming big concern on oxidative stress which has an effect on honey bees` lifespan.

The effect of fungus pathogens on oxidative stress

CAT, one of the antioxidant enzymes, is the primary defense against the overproduction of reactive oxygen species (ROS), which can occur after *Nosema ceranae* infection (the obligate intracellular fungi), which is located in the branch of microsporidia, and proliferates in the midgut epithelial cells of honey bees. However, ROS is non-specific and when not sufficiently reduced, it can react with biologically important macromolecules such as lipids, proteins, nucleic acids, and carbohydrates, which can damage the organism and eventually lead to cell death [73]. In the midgut, which is the first barrier to parasite development and oral pesticide exposure, high CAT activity is detected in the queen bee in the presence of *N. ceranae* infection combined with imidacloprid, while a trend towards a decrease in GST activity, a detoxifying enzyme among antioxidant enzymes. This decrease can negatively affect metabolic and detoxification functions. However, the loss of the colony resistance necessary for the survival of the colony under adverse conditions can be observed, as well as the reduction of the queen's lifespan, the reduction of the queen's labor production, and the death of the queen and the workers [74]. Taric et al. [75] measured high CAT activity in caretaker bees collected from commercial colonies with high *Nosema* load and

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Dussaubat et al. [74] had supported the results obtained.

Antúnez et al. [76] stated that consistency was observed between the decrease in Vg expression in worker bees infected with *N. ceranae* [73, 77] and the shortening of the life span of workers whose Vg expression was suppressed. Dussaubat et al. [74] observed that GST activity increased in worker bees with *N. ceranae* infection to tackle with oxidative stress. It has been determined that the titers and antioxidant capacity of Vg, an egg yolk protein that can reduce oxidative stress, increase in infected queen bees.

In a recent study on the differences of commercial and traditional colonies in the parameters of oxidative stress and the prevalence of *N. ceranae*, higher *N. ceranae* prevalence in commercial colonies could be the reflection of observation significantly higher activity of CAT which has an important protective role in insects having high intestinal parasites [76]. At the same time, significantly higher activity of GST was observed in commercial colonies probably due to higher pathogen prevalence measured in the study of Vidau et al. [78].

Another fungus pathogen is *Ascosphaera apis*, an obligate fungal pathogen of honey bee brood and causes chalkbrood disease in honey bee larvae. *A. apis* infection may cause oxidative stress on honey bees larvae [79]. The upregulation of cellular defenses in resisting oxidative damage in honey bees can be provided by the increase in CAT activities as in the other insects such as *Drosophila melanogaster*. Besides the increase in SOD enzymatic activity play an important role to promote oxidative stress resistance [80]. In relation to these antioxidant enzymes activities, decreased CAT, GST, and SOD enzymatic activity in the guts of infected larvae with *A. apis* had been observed significantly by Li et al. [81] compared within the guts of control larvae not infected with *A. apis*.

The effect of Varroa on oxidative Stress

Lipiński and Żółtowska [82] found that antioxidant enzymes SOD, GPX, and ceruloplasmin (CP), were 4 times higher in activity than non-infected prepupae in their study on oxidative stress in drone prepupae infected with the parasitic mite *Varroa*. The other research supported the similar result that SOD and Catalase activities in *Varroa* infested worker pupae

were almost two times higher than non-infested worker pupae [83]. Gülmez et al. [84] observed that the SOD activity of bees infected with *Varroa* was higher than the non-infected bees. This increase in enzyme activity indicates that superoxidase radicals, which are formed as a result of *Varroa* invasion, activate the host defense mechanism.

The effect of viral diseases on oxidative stress

Łopieńska-Biernat et al. [85] observed that SOD activity was significantly lower in the honey bee group infested with *Varroa destructor* than that infected with Deformed Wing Virus (DWV). It was found that CAT activity was higher in the group infested with *V. destructor* and in the group infected with *N. ceranae* and lower in the group infected with DWV compared to the control group (non-infected group). In the group infected by both *V. destructor* and DWV at the same time, it was observed that CAT activity was lower compared to the groups infected with only one pathogen. GST activity was found to be higher in groups infected with *V. destructor*, *N. ceranae* and/or DWV compared to healthy bees.

The synergistic effect of pesticides and pathogens on oxidative stress

The combination of pesticides and parasites may cause strong colony losses. Firstly, Ladas [86] have been explained the possible interaction between *Nosema* and pesticides [78]. Alaux et al. showed the synergistic effect of *Nosema* and imidacloprid on the mortality of honey bees; however, any strong connection with the insect detoxification system was observed if did decrease or not [78, 87]. Though, low doses of imidacloprid, one of the common neonicotinoid pesticides, and *Nosema ceranae* infection alone or combined with pesticides caused the increased activity of GST and CAT in the head related to protective response to oxidative stress of honey bees [74]. In addition, the survivorship of queens and worker bees is strongly in dangerous due to exposure of the combination of pesticides and parasites [74]. Another study showed a similiar result that the combination of *N. ceranae* parasite with insecticide fibronil caused a disturbance in the production of ROS and increased oxidative stress; besides, in this combination, the parasite may trigger the increasing of fibronil toxicity on honey

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bees [88].

Several chemical substances as acaricides to eliminate negative impacts on hives by *Varroa* mites also have an impact on the oxidative stress on honey bees. The potential effects of two potent acaricides fluvalinate and oxalic acid on oxidative stress were tested by Rouibi et al. [89] on the adult stages of honey bees to determine the detoxification system for GST and AChE (Acetylcholinesterase). Since GST activity increased in emerged and nurse bees, AChE activity decreased for fluvalinate uses compared with the control group (untreated colonies with fluvalinate). However, in emerged and nurse bees, GST and AChE activity did not show significant differences for oxalic acid uses compared with the control group (untreated colonies with oxalic acid), [89]. A similar effect was reported on increase GST activity in emerged and nurse bees by Loucif-Ayad et al. for treatment with flumethrin and amitraz and by Nielson et al. for treatment with flumethrin [90, 91]. In the recent study, the effects of coumaphos that is the other most commonly used acaracide against *Varroa* mites in parameters of oxidative stress (CAT, SOD, and GST activities), were observed. Since normally in non-infested bees before coumaphos treatment, SOD activity was decreased, SOD activities significantly increased in non-infested untreated bees ($p<0.05$), and infested treated bees ($p<0.0001$), after coumaphos treatment (day 42). On the contrary, in all groups (non-infested untreated bees, non-infested treated bees, and infested untreated bees), except the infested treated bees group (where it declined), after treatment with coumaphos, both CAT and GST activities significantly increased (from $p<0.05$ to $p<0.0001$). Having a high infestation group showed efficacy against *Varroa* mites thanks to using coumaphos that is the reason why treatment decreased oxidative stress to contribute to increasing the colony health [92]. Moreover, a synergy between acaricides (coumaphos), and insecticides (imidacloprid), was demostrated that a mixture of coumaphos and imidacloprid were downregulation of CAT activities as well as inducing higher bee mortality [93].

Conclusion

Oxidative stress is manifested by an imbalance between the production of reactive oxygen species (ROS), and cellular antioxidant defense systems. Free

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oxygen radicals, which are synthesized in small amounts in the organism during normal metabolism and do not harm the organism is overproduced and causes oxidative stress due to some cases such as climate change, habitat loss, environmental stress factors, insecticide use, exposure to parasites and pathogens, exposure to ionizing radiation, and environmental pollution.

In order to prevent pesticide-related deaths in honey bees, it is necessary to gather experts, gather reliable information, scan the literature, examine the crime scene, take the necessary and correct samples, if possible, determine the target pesticides well, send samples to the correct laboratory. In addition, it is an indispensable requirement to have a laboratory that specializes in this field and can follow up-to-date innovations, and have experts working in these laboratories. However, due to the lack of laboratories in Turkey to analyze each group of pesticides and especially clothianidin, imidacloprid, and thiamethoxam from the neonicotinoid group and agricultural products, the analysis in Turkey has not yet been clarified. In addition, in preventing the toxic effects of insecticides that cause oxidative stress, the inclusion of caffeine, which acts as an antioxidant, in the diet which is provided for bees, especially when hives are installed near crops that are attractive to bees, can be an important strategy and can be applied by beekeepers.

Zinc (Zn), the most important ingredient of the antioxidant enzyme Cu/Zn-SOD, may have an important effect on the increase of the concentration of royal jelly thanks to providing the increase of Zn content especially in the hemolymphs of nurse bees. By given it in honey bees` diets, the negative effect of oxidative stress caused by pathogens on the lifespan of bees can be reduced. For this purpose, it is recommended to supplement 30 mg kg-1 Zn in 50% sugar syrup and/or 60 to 75 mg kg-1 Zn into the pollen diet [94].

In national beekeeping, commercial beekeepers tend to transport their honey bee colonies among regional for many months of the year. With this migratory beekeeping, honey bee colonies among agricultural, especially monocultural, landscapes may increase to impose the potential of exposure of colonies with pesticides and pathogens due to high interaction; thus, those reared in a

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commercial operation of migratory beekeeping by increasing oxidative stress on honey bees significantly cause the reduction of lifespans of bees.

Bal Arılarında (*Apis mellifera* L.), Pestisit ve Patojen Kaynaklı Oksidatif Stres

Öz: Bal arıları, yabani çiçek ve mahsullerin tozlaşmasını sağlayarak insanlar için önemli bir kültürel ve ekonomik fayda sağlar. Bal arıları, diğer organizmalar gibi, yaşamları boyunca çok çeşitli çevresel stres faktörleri ile karşı karşıya kalmaktadır. Bu stres faktörleri organizmanın fizyolojik dengesini bozar. Organizmaya alınan oksijenin kullanımı ve metabolizması sırasında serbest radikaller olarak bilinen agresif moleküller oluşur ve organizma bu radikalleri kontrol altında tutamaz ve oksidatif stres oluşur. Böyle bir durumda, serbest radikaller sağlıklı hücrelere saldırır, okside eder ve bozarlar. Bu bozulma, reaktif oksijen türlerinin (ROS), atık sistemlerinin aynı anda bozulması ile arttırılmış üretimi ile karakterize edilir. Bal arılarında oksidatif stres eşiğinin aşılması, bireysel ya da koloni bazında arı kayıplarına neden olmaktadır. Her geçen gün çevresel faktörlere bağlı olarak, artarak oluşan koloni kayıpları oksidatif stresin oluşumu, fizyoloisi ve önlenmesi üzerine yapılan çalışmaların önemini gözler önüne sermektedir. Bal arılarında tanımlanan en önemli antioksidan enzimler glutatyon S-transferaz (GST), glutatyon peroksidaz (GPX), katalaz (CAT), ve süperoksit dismutaz (SOD)'dır. Bu derleme oksidatif stresin bal arılarındaki mekanizmasını ve pestisit ve patojenlerin bal arılarında oksidatif stres üzerindeki etkilerini incelemektedir.

Anahtar kelimeler: Bal arısı, *Apis mellifera* L., oksidatif stres, reaktif oksijen türleri (ROS), pestisit, patojen

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