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Effects of *Varroa destructor* Anderson & Trueman Infestation on Antioxidant Enzymes of Adult Worker Honey Bee (*Apis mellifera* L.)

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One of the most important honeybee pathogens is the mite *Varroa destructor*, which threats honeybee health and incomes from bee keeping. *Varroa* mite, among other effects, can cause oxidative stress which is indicated by alterations in antioxidant enzyme activity and total protein levels. In this study, activities of catalase, glutathione S-transferase, superoxide dismutase enzymes and quantity of total protein levels were compared in mite infested and mite-free adult worker bees. Superoxide dismutase enzyme activity remarkably increased in mite infested bees as compared to uninfested ones. Catalase enzyme activity was found low in mite infested bees although no significant difference was observed in glutathione S-transferase enzyme activity. Total protein level in mite infested bees was detected lower than uninfested ones. Changes in enzyme activities and total protein levels are resulted from oxidative stress caused by mite infestation.

Keywords: *Varroa destructor*, Honey bees, Oxidative stress, Antioxidant enzymes.

INTRODUCTION

The ectoparasite mite, *Varroa destructor*, is obviously the most significant honeybee pathogen since it feeds on hemolymph of larvae, pupae and adult bees as well as transferring viruses and many other secondary infections. Feeding of the mite on integument of honeybees results in wounds which facilitates secondary pathogens to spread through honeybee colonies. In addition to cause to weaken honeybees by decreasing their hemolymph during feeding, mite also reduces low molecular weight proteins [1]. Therefore, it has various damages on honeybees and so causes serious economic losses on bee keeping industry.

Reactive oxygen species (ROS) are produced by all organisms through metabolic processes and by various mechanisms under aerobic conditions which have many functions in cells between normal values such as cell signaling, regulate hormone action, growth factors body's immune system [2,3]. However, excessive production of reactive oxygen species could be quite harmful to organisms because they are from a group of free radicals that have high oxidizing properties. Rahman [4] stated that they can easily initiate autocatalytic reactions so that molecules to which they react are themselves converted into free radicals to propagate the chain of damage. Moreover, excessive reactive oxygen species production can damage cellular macromolecules such as nucleic acids, proteins and lipids as well as causing mtDNA mutations, aging and cell death [3,5]. Various biotic and abiotic stress factors may induce excess production of reactive oxygen species in organisms. For example attack of pathogenic agents as viruses, bacteria and parasites are among biotic stress factors increasing reactive oxygen species production through immunological reactions [2,3].

Organisms have defense mechanisms to protect themselves from harmful effects of reactive oxygen species. Inability to detoxify excess amounts of reactive oxygen species is called as "oxidative stress" [3,6]. To overcome oxidative stress, organisms activate enzymatic defense systems as catalase (CAT), glutathione S-transferase (GST) and superoxide dismutase (SOD) [5,7]. Any increase or decrease in the activity of these antioxidant enzymes in organisms is an indication of adaptive response against damage [8]. Each of these enzymes perform unique functions in cells. For example, superoxide dismutase is a metallothionein that catalyzes dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide. Catalase, is a common enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen. Glutathione S-transferase catalyzes the conjugation of the reduced form of glutathione to xenobiotic substrates for the purpose of detoxification.

Any unfavourable situation that affects worker honeybees' health will affect the whole productivity as well as benefits from bee keeping. Therefore, it is important to know if *Varroa*

mite infested worker bees will expose to oxidative stress or not. Little is known about the results of mite infestation in cellular level and defense mechanisms of honeybees [9,10]. The aim of this study was to investigate activities of catalase, glutathione S-transferase, superoxide dismutase and total protein quantity in mite infested and non-infested adult worker bees as an indication of oxidative stress.

EXPERIMENTAL

Sample collection: Adult honeybees (*Apis mellifera* L.) were collected from hives in the apiary in Gaziosmanpasa University, Tokat, Turkey. Twenty *Varroa destructor* mite infested (one mite on each bee) and 20 non-infested adult worker bee samples were randomly selected for the experiments.

Enzyme extractions and assays: Bee samples were grounded with liquid nitrogen and homogenized in 50 mM KH_2PO_4 buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged 15000 × g for 15 min 4 °C and resulting supernatants were freshly used for determination of superoxide dismutase, catalase, glutathione S-transferase and total proteins. Enzyme activities were expressed per g fresh bodies.

Superoxide dismutase activity: Total superoxide dismutase activity was measured by the modified method of Beyer and Fridovich [11]. 3 mL of the reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 60 µM nitroblue tetrazolium (NBT), 0.1 mM EDTA and 100 µL enzyme extract. The reaction was started by adding 60 µM riboflavin and placing the tubes under a light white fluorescent lamp for 0.5 h. A complete reaction mixture without enzyme served as control. The absorbance was recorded at 560 nm and one unit of superoxide dismutase activity was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50 %.

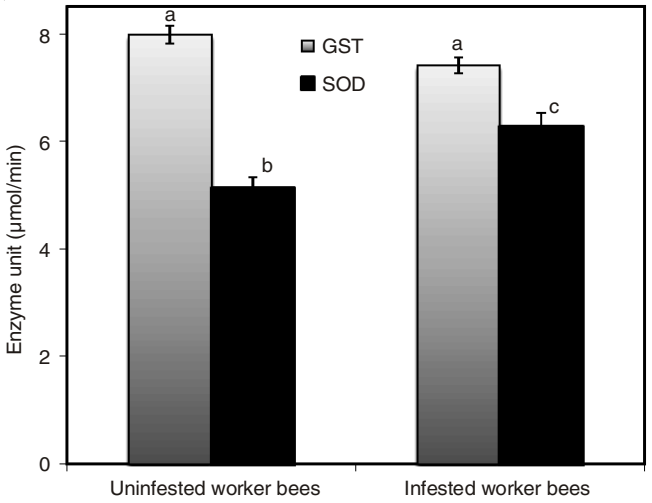
Catalase activity: Catalase activity was determined according to Aebi's method [12]. Briefly, the reaction mixture contained 50 mM KH_2PO_4 buffer (pH 7.0), 13 mM H_2O_2 and 30 µL enzyme extract. The decrease of H_2O_2 was recorded at 240 nm for 2 min. The activity of the enzyme was defined the amount of enzyme catalyzing 1 µmol H_2O_2 in a minute, calculated from the extinction coefficient (0.036 cm^2 μmol^{-1}) for H_2O_2.

Glutathione S-transferase activity: Glutathione S-transferase activity was determined according to Habig *et al.* [13]. The reaction volume contained 50 mM KH_2PO_4 buffer (pH 6.5), 0.1 mM GSH, 30 mM 1-chloro-2,4-dinitrobenzene and 20 µL supernatant. The increase of absorbance reads at 340 nm (ε = 9.6 mM^{-1} cm^{-1}).

Total protein amount: Protein amount in the supernatant was determined according to Bradford's method [14] by using bovine serum albumin as standard. All experiments were repeated three times. Statistical analyses were done by using the software package SPSS 17.0. Differences in mean values between infested and non-infested cohorts were tested with Student's *t* test.

RESULTS AND DISCUSSION

In this study, activities of defense enzymes and total protein level in infested and uninfested worker honeybees were compared (Figs. 1-3). Superoxide dismutase activity in mite infested bees is found higher than uninfested ones, which is statistically significant ($p < 0.05$) (Fig. 1). Catalase activity (Fig. 2) is slightly low but there is no significant difference in glutathione S-transferase activity (Fig. 1) in mite infested bees when compared to uninfested ones. Total protein quantity was found lower in infested bees than mite-free bees (Fig. 3).



nFig. 1. Glutathione S-transferase and superoxide dismutase activity in mite infested and uninfested adult worker bees. Data represent the mean value ± SE, n = 3, $p < 0.05$

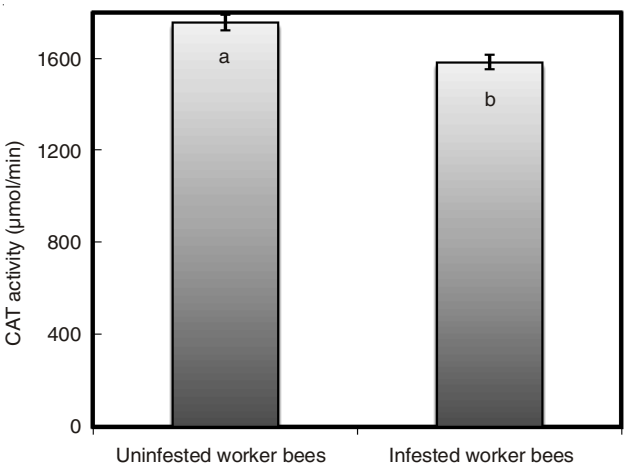


Fig. 2. Catalase activity in mite infested and uninfested adult worker bees. Data represent the mean value ± SE, n = 3, $p < 0.05$

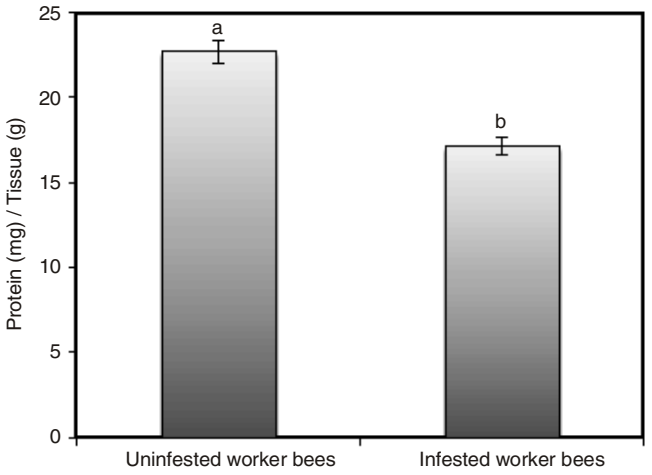


Fig. 3. Total protein level in mite infested and uninfested adult worker bees. Data represent the mean value ± SE, n=3, $p < 0.05$

Antioxidant enzymes are known to participate in defense of host organisms during parasite infections [3,8,9,15,16]. Organisms restrict oxidative radicals and other oxidants originated from parasite invasion in metabolically active tissues in these situations [17]. Therefore antioxidant enzyme activities may increase or decrease depending on the time and intensity of parasitism [7].

In this study, superoxide dismutase activity was determined to be high in mite infested bee samples compared to uninfested ones. The increase in the level of the enzyme activity indicates that host defense system is activated to scavenge superoxide radicals generated as a result of mite infestation. In similar studies, superoxide dismutase activity has been reported to be increased due to mite infestation in pupae of drones and worker bees Badotra *et al.* [9] and Lipinski and Zóltowska [10].

On the other hand, catalase activity in mite infested bees was found low compared to mite free bees. This can be explained as 1) catalase activity might have been inhibited by the accumulation of superoxide anion [18]; 2) catalase activity is directly regulated by the concentration of hydrogen peroxide. Ahmad *et al.* [19] stated that responses to oxidative stress vary according to animal species and catalase is inefficient at low hydrogen peroxide levels. In our study the level of H_2O_2 generated by honeybees as a result of mite infestation may not be enough to induce catalase activity. Some reports revealed that insects possess antioxidant enzymes other than catalase, such as ascorbate peroxidase (APOX) and dehydroascorbate reductase (DHAR) which can act in very low levels of hydrogen peroxide [20,21]. Therefore hydrogen peroxide could have been controlled by another enzyme in the mite infested bee samples.

According to our results, glutathione S-transferase activity was less affected by mite infestation so that no significant differences were found between infected and mite-free bees. This enzyme is related to glutathione mechanism and is known to play a vital role in xenobiotic detoxification and inactivation of toxic products of oxygen metabolism [22]. In the same way, Badotra *et al.* [9] reported no significant differences between mite infested worker bee pupae and control group in terms of glutathione S-transferase activity.

In this study, the total protein level in mite infested bee samples was found low as compared to uninfested ones. This is an expected result since mite feeds with host's hemolymph [8,23,24].

Conclusion

Consequently changes in the levels of antioxidant enzymes and total protein in infested worker bees is thought to result from oxidative stress. *V. destructor* mite is known to spread many pathogens and diseases among bee colonies which are responsible for yield loss, mass bee deaths and unexplained colony losses. Oxidative stress and decrease of total protein level due to mite infestation can also reveal similar effects and loss of economic income in bee keeping.

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