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Usage of Detoxification Enzyme Glutathione-S-Transferases over *Galleria mellonella* L. (Lepidoptera: Pyralidae) as Biomarkers of Insecticide Resistance

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Abstract

Insecticides are preferred because of their easy applicability in combating pest insects and their short-term results. Insecticides used over dose and unconsciously in agricultural areas cause ecological damage. Also; the use of over dose insecticides in agricultural areas has led to the development of insect resistance. Since the resistance mechanism developed is genetic, it is transmitted to later fertilizers. For this reason, it is important to apply the insecticides in the restricted areas and in the optimal amount. Insects increase their detoxification capacities to protected themselves from insecticides. Detoxification mechanisms are based on the breakdown of insecticides before reaching the target area. The most important detoxification enzyme in insects are Glutathion-S-Transferases (GST). GST enzyme in insects has a role in protection of cellular membranes against oxidative degradation as well as detoxification mechanism. *Galleria mellonella* may be used as a model organism for understanding the insecticide resistance mechanism.

Key words: *Galleria mellonella*, detoxification, oxidation, antioxidant, toxicology.

1. Introduction

The use of insecticides management pest insects in agricultural areas is increasing day by day. The insecticides used against these harms are toxic to the environment and to non-target organisms. Therefore, it is important to investigate new chemical agents that are alternative to insecticides. The use of model insect is very important in the search for alternative chemicals. *Galleria mellonella* is an important model organism causing damage in agricultural areas. *G. mellonella* is frequently used in recent years in the biological contorol studies. It is also used in the fields of pharmacy, medicine and veterinary medicine as it is easy to produce and easy to cultivate (Büyükgüzel 2001, Büyükgüzel and Kalender 2009, Büyükgüzel et al 2013, Sugeçti et al 2016, Sertçelik et al 2018).

1.1 Insecticide resistance

Today, the unconscious use of synthetic insecticides has been proven by scientific studies, such as resistance to ending pests, negative health effects such as human health and environmental toxicity. (Miller and Uetz 1998, Sugeçti et al 2016, Sertçelik et al 2018). For many years, the resistance against synthetic insecticides in beetles has been increasing, and this negative effect has been increased. Studies conducted with different concentrations of insecticides have also indicated that insects have negative effects on both physiology and biochemical activities. (Uçkan and Sak 2010, Çetin et al. 2010).

1.2 Detoxification enzyme Glutathion S transferase (GST)

GST enzyme is present in nature in aerobic microorganisms, plants, insects, fish, birds, vertebrate and invertebrate animals. (Konanz and Nauen, 2004). GST are enzymes that play an important role in the detoxification of foreign compounds taken in the body. The prominence of GSTs in insecticide metabolism has been noted for the first time in the elimination of toxicity of organic phosphorus compounds. The GSTs were later studied on insects against organic chlorinated and cyclodiened insecticides. GSTs play an important role in resistance to insecticides against organophosphorus in insects (Susurluk, 2008). The GST enzyme was purified from the insects of Lepidoptera, Diptera, Coleoptera and Hymeneoptera. The GST enzyme found in insects has a molecular weight of 19,000-35,000 Da and consists of 2 parts, homodimer and heterodimer. GST enzyme insecticide detoxification in insects as well as protection of cellular membranes against oxidative degradation (Yu, 2008). GSTs are known as functional enzymes responsible for the conjugation of glutathione (GSH). This enzyme catalyzes the conjugation of glutathione with electrophilic substrates. GSTs carry many events, such as the replacement of some groups in glutathione, the addition and removal of new groups. Genes responsible for more than 40 GST enzymes have been identified in mammals, in plants and in insects. (Hollingworth and Dong, 2008). GSTs found in insects were collected in two categories as class I (Delta) and class II (Sigma). Recently, the GST class, called Epsilon, has been identified in some insect species such as Anopheles gambiae (Diptera: Culicidae) (Kranthi, 2005). The GST enzyme found in insects catalyzes insecticide detoxification after phase I. The insect GST level is associated with organic phosphorus and chlorinated hydrocarbon insecticide resistance. The fact that GST is dissolved and stable in tissues allows characterization and purification of many insect species. The most information about GST biochemistry found in insects was obtained from studies conducted on housefly. Similar GST purification studies have also been done on some insect species within the Diptera and Lepidoptera 143 sets. It has been determined that the electrophoretic variability, catalytic activities and substrate activities of the purified GST enzymes were different in the studies performed (Soderlund, 1997).

When the insecticides enter the insect body, their toxicity is quickly eliminated. The elimination of toxicity of these substances takes place in two phases, phase I and phase II (Yu, 2008). Phase I involves the stages of oxidation, hydrolysis and reduction. In the Phase I reaction, the poisonous molecules in the non-polar structure are converted into intermediates which are less toxic by the addition of some functional groups. These functional groups are divided into 2 parts:

- ✓ Electrophilic substances: Epoxidase functions and α, β carbonyl groups are electrophilic carbonaceous structures. Some insecticides enter the insect body and are then converted into inactive substances by electrophilic functional groups.
- ✓ Nucleophilic substances: Alcoholic or phenolic hydroxyl groups, amino and carboxyl groups are nucleophilic substances. Thanks to the nucleophilic materials, the insecticides in the non-polar structure are transformed into materials with less toxicity (Soderlund, 1997).

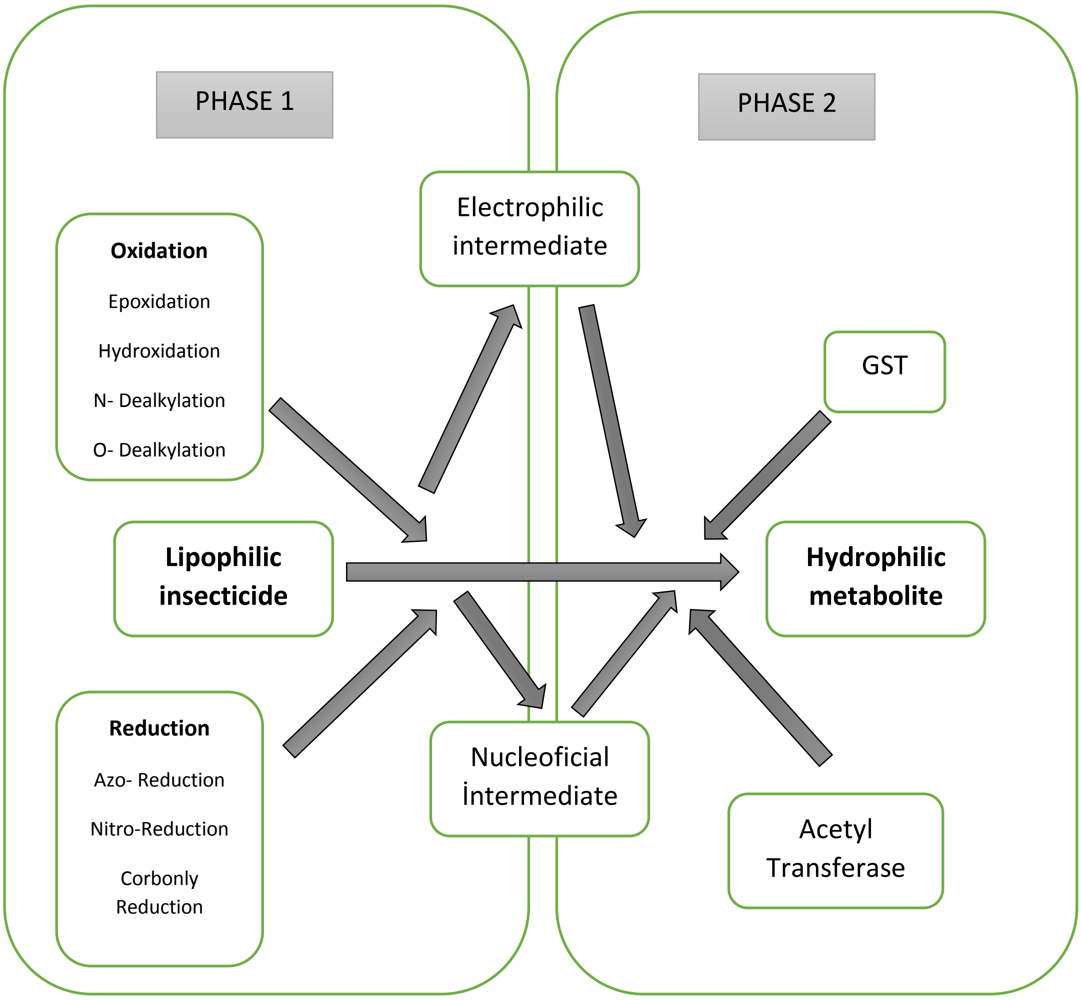


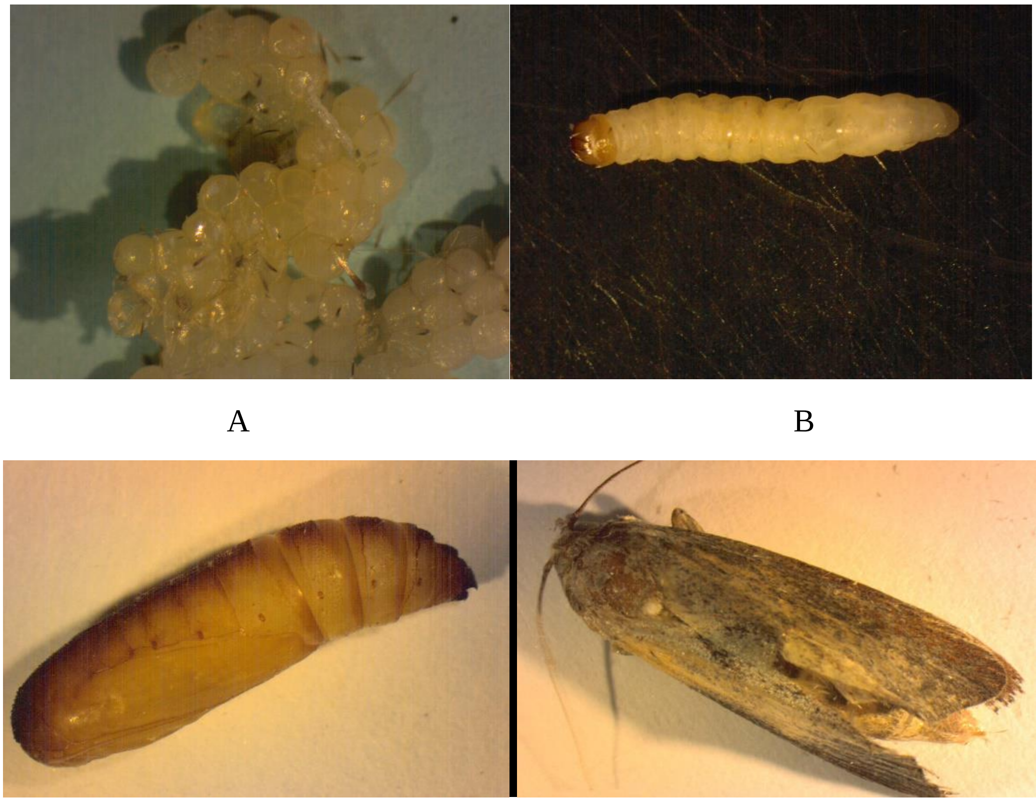
Figure 1. Biochemical pathways of insecticide metabolism (Kranthi, 2005, Yorulmaz and Ay 2010)

In Phase I, insecticides are converted to intermediates by the addition of electrophilic or nucleophilic substances through the P450 and esterase enzymes found in the insect locus. In phase II, the substances formed in phase I are generally converted to hydrophilic substances by the GST enzyme and are thrown off the body (Yu, 2008) (Figure 1).

1.3 The use of model insect *G. mellonella* in culture and biological control

Various researches have resulted in different nutrient media. Beck (1960) investigated the nutritional requirements of *G. mellonella*, and Bronskill (1961) experimented with different mixtures of honey, bran, honey and water to bring artificial nutrients to the laboratory environment. With the development of these nutrients, various biochemical, physiological and toxicological studies have started to be made on cultivated insects. House (1972) investigated the effects of various nutrients on the longevity and egg production. Vinson (1976, 1981) investigated the efficacy of parasitoids fed on hosts fed with different foods. *G. mellonella* holometabol is an insect and consists of the life cycle, egg larvae, pup, and adult stages (Figure 2).

In order to management the harmful effects of this insecticide, it is necessary to determine the effects on insecticide species to be used in the biological control, spreading, damage and fighting of the insecticide species for more effective research in the laboratory environment (Özer 2011). In this context, some antibacterial antibiotics and biological agent were added to the artificial nutrients and various effects on *G. mellonella* were investigated (Büyükgüzel and Kalender 2009, İçen 2003, Sugeçti et al. 2017) in order to prevent the various contaminants encountered during the growing of insects in laboratory environments and to prepare an experimental arrangement free of microorganisms. Antimicrobial agents added to synthetic nutrients have been found in a variety of studies that have affected the physiological standard of living of bugs. Therefore, the concentration of added antibiotics needs to be determined sensitively (İçen 2003).



A B

C D

Figure 2. *Galleria mellonella* life cycle (A:Eggs, B: Larvae, C:Pupae, D: Adult) (Photo: Cihat Çelik and Serkan Sugeçti)

2. Conclusion

Developing the resistance mechanism of insects; may lead to an increase in the dose of insecticide used in chemical management. Therefore; the use of intensive medicines in pesticides that have developed resistance may increase the cost and may also adversely affect the environment and the economy of the country. Studying the insecticide resistance and determining which chemical resistance the insects develop will allow alternative methods to be developed. The most important biochemical indicators for insecticide resistance are Glutathion-S-Transferase enzymes. The determination of the effects of chemicals on insect detoxification enzymes and the investigation of less toxic alternative chemicals are very important issues in terms of the ecological environment.

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