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The *in vitro* Effect of Neonicotinoid Insecticides Imidacloprid and Thiamethoxam on Glutathione Reductase Enzyme Activity from Baker's Yeast *(Saccharomyces cerevisiae)*

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Abstract

In this study, glutathione reductase from baker's yeast *(Saccharomyces cerevisiae)* (GR) exposed to 0, 25, 50, 100, 250 and 500 mg/L neonicotinoid insecticides imidacloprid (IMI) and thiamethoxam (TMX). Under the exposure of 25, 50, 100, 250 and 500 mg/L concentrations, % GR activity alterations were calculated as -1.01; +0.50; 0.00; 0.00 and -1.01 in IMI applications, while these alterations were calculated as -2.91; -1.46; -0.97; -1.46 and -0.97 in TMX applications, respectively. According to control activity, no statistical changes were observed in GR activities (p>0.05). The present study showed that the tested neonicotinoid insecticides did not cause significant changes in GR enzyme activities.

Keywords: Glutathione reductase, Neonicotinoid insecticides, Imidacloprid, Thiamethoxam

Neonikotinoid İnsektisitler İmidakloprid ve Tiyamethoksam'ın Ekmek Mayası *(Saccharomyces cerevisiae)* Glutatyon Redüktaz Enzim Aktivitesi Üzerine *in vitro* Etkisi

Öz

Bu çalışmada, ekmek mayası (*Saccharomyces cerevisiae*) glutatyon redüktazı (GR), 0, 25, 50, 100, 250 ve 500 mg/L neonikotinoid insektisit imidakloprid (IMI) ve

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tiyamethoksam'a (TMX) maruz bırakılmıştır. 25, 50, 100, 250 ve 500 mg/L konsantrasyonlara maruz bırakılma ile, % GR aktivitesindeki değişimler, IMI uygulamalarında sırasıyla; -1.01; +0.50; 0.00; 0.00 ve -1.01 olarak hesaplanırken, TMX uygulamalarında sırasıyla -2.91; -1.46; -0.97; -1.46 ve -0.97 olarak hesaplanmıştır. Kontrol aktivitesine göre GR aktivitelerinde istatistiksel bir değişiklik gözlemlenmemiştir (p>0.05). Bu çalışma, test edilen neonikotinoid insektisitlerin GR enzim aktivitelerinde önemli değişikliklere neden olmadığını göstermiştir.

Anahtar Kelimeler: Glutatyon redüktaz, Neonikotinoid insektisit, İmidakloprid, Tiyamethoksam

1. Introduction

Glutathione reductase (EC 1.8.1.7) is an antioxidant defense enzyme. Glutathione reductase converts oxidized glutathione (GSSG) into reduced glutathione (GSH) in the presence of NADPH (β-nicotinamide adenine dinucleotide 2'-phosphate reduced) [1]. Glutathione reductases include two subunits [2]. Each subunit contains Flavin Adenine Dinucleotide (FAD) at active site. Seemingly, NADPH reduces the FAD and transfers its electrons to the disulfide bridge (-S-S-) between two cysteine residues in the active site, two -SH groups form and react with GSSG and reduce GSSG to 2 GSH and again form disulfide bridge between cysteines.

Imidacloprid (*N*-{1-[(6-Chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl}nitramide) is belonging to the nitroguanidine subgroup of the neonicotinoid insecticides. IMI blocks nicotinic acetylcholine receptor (nAChR) and interfere with the transmission of nerve impulses in insects. It is used in agriculture against insects [3]. This insecticide is more toxic to insects than mammals. At the same time, IMI is more strongly bound to insect neuron receptors than mammalian neuron receptors [4].

Thiamethoxam (3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine) is a neonicotinoid insecticide. TMX also blocks nicotinic acetylcholine receptor (nAChR) of the nervous system in insects. It is used in many countries on many crops [5]. TMX is also more selective against the acetylcholine receptors of insects than mammals [6].

IMI and TMX neonicotinoid insecticides are widely used in agriculture. But, in February 2018, the European Food Safety Authority issued a new report stating that neonicotinoids constituted a serious threat to honeybees and wild bees [7]. In April 2018, European Union member states decided to ban three main neonicotinoids (clothianidin, imidacloprid and thiamethoxam) for all outdoor uses [8]. Bees provide pollination. In case of continued use of neonicotinoids, the bees will not pollinate and will have great ecological problems. At the same time, neonicotinoids have residual problems. They cause oxidative stress in living organisms. Therefore, we investigated the effects of IMI and TMX neonicotinoid insecticides on glutathione reductase.

2. Materials and Methods

2.1. Chemicals

Glutathione reductase from baker's yeast *(Saccharomyces cerevisiae)*, L-Glutathione oxidized, β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate, Imidacloprid (Fig. 1a), were bought from Sigma-Aldrich. Thiamethoxam (Fig. 1b) was bought from Fluorochem. All other chemicals used were analytical grade.

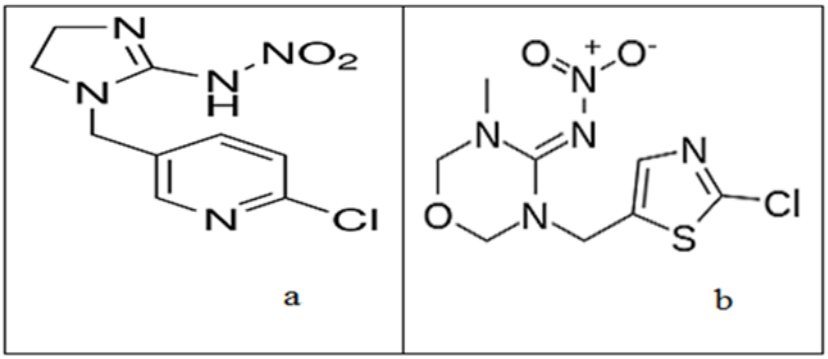

Figure 1. Structures of imidacloprid (a) and thiamethoxam (b)

2.2. Protein Determination

The protein content of GR was determined spectrophotometrically at 750 nm according to the method of Lowry et al. [9]. Bovine serum albumin was used as standard for the measurement of GR protein concentration. For determination of protein concentration, four solutions were prepared. Solution (A): 0.5 g $CuSO_4$ .5 H_2O and 1 g

sodium citrate dihydrate were dissolved at distilled water and completed to 100 mL by distilled water. Solution (B): 2 g Na_2CO_3 and 0.4 g NaOH were dissolved at distilled water and completed to 100 mL by distilled water. Solution (C): 2 mL solution A was added to 100 mL solution B. Solution (D): 10 mL Folin-Ciocalteu was added to 10 mL distilled water. After preparation of these four solutions, 2.5 mL solution C was added to 0.5 mL of GR solution, shaken and waited for 10 minutes at room temperature, then added 0.25 mL solution D, shaken and waited for 30 minutes and read at 750 nm for determination of GR concentration.

2.3. GR Activity

GR activity was determined spectrophotometrically by reading the changes in absorbance at 340 nm during oxidation of NADPH to $NADP^+$ by GSSG at 37 °C [10]. The reaction system of 1 ml contained: 1.0 mM GSSG, 0.1 mM NADPH, 0.10 M potassium phosphate buffer (pH 7.6). The oxidation of 1 μmol of NADPH/minute under these conditions was used as a unit (U) of GR activity. Millimolar extinction coefficient of β- NADPH at 340 nm was used as 6.22. The activity of GR was calculated as U/mL, then the specific activity of GR was expressed as U/mg of protein.

2.4. Effect of Neonicotinoid Insecticides on Enzyme Activity

5000 mg/L IMI in 2 mL 90% ethyl alcohol and 5000 mg/L TMX in 2 mL 50% ethyl alcohol was prepared. Because of little solubility of IMI and TMX in water, IMI and TMX were dissolved in different percentage of ethyl alcohol according to their polarities. After that, preparation of 25, 50, 100, 250 and 500 mg/L IMI and TMX with ethyl alcohol plus 700 μL GR solution were done [11]. At control or 0 ppm, 300 μL 90% ethyl alcohol for IMI and 300 μL 50% ethyl alcohol for TMX plus 700 μL GR solution were used. The solution volume of enzyme and ethyl alcohol solution and neonicotinoid insecticide was 1 mL. The preparations were waited at room temperature for 10 minutes. After that, activities of GR were measured.

2.5. Data Analysis

The data was shown as mean ± standard deviation. For the statistical analyses, one-way analysis of variance (ANOVA) was used, followed by the Student Newman-Keul's

test using the IBM SPSS version 22 statistical software (SPSS Inc. Chicago, IL, USA). Differences were considered as significant if $p<0.05$.

3. Results and Discussion

3.1. Effect of Imidacloprid on GR Activity

The effects of IMI prepared from 0 to 500 mg/L on GR were measured. Enzyme activity and standard deviation data was given in Table 1. Activity-concentration graph was shown in Fig. 2. When Table 1 and Fig. 2 were examined, the increasing concentration of IMI did not change GR enzyme activity statistically significant compared to the control activity ($p>0.05$, n=3). IMI's 25, 50, 100, 250 and 500 mg/L effects on the GR enzyme changes in the percentage changes -1.01; +0.50; 0.00; 0.00 and -1.01 were calculated respectively.

Table 1. Effect of imidacloprid and thiamethoxam neonicotinoid insecticide concentrations on glutathione reductase activity. The data was given as mean ± standard deviation. The letter "a" in the table shown that there was no statistical distinction between activity levels according to the control activity ($p>0.05$, n=3)

Neonicotinoid Concentration (mg/L)	GR activity ± standard deviation (U/mg) for IMI	GR activity ± standard deviation (U/mg) for TMX
0	199±8a	206±0a
25	197±12a	200±3a
50	200±2a	203±2a
100	199±8a	204±4a
250	199±3a	203±1a
500	197±1a	204±3a

3.2. Effect of thiamethoxam on GR activity

The effects of TMX prepared from 0 to 500 mg/L on GR were measured. Enzyme activity and standard deviation data was given in Table 1. Activity-concentration graph was shown in Fig. 3. When Table 1 and Fig. 3 were examined, it was seen that the increasing concentration of TMX did not change GR enzyme activity statistically significant according to control activity ($p>0.05$, n=3). TMX's 25, 50, 100, 250 and 500 mg/L effects on the GR enzyme changes in the percentage changes -2.91; -1.46; -0.97; -1.46 and -0.97 were calculated respectively.

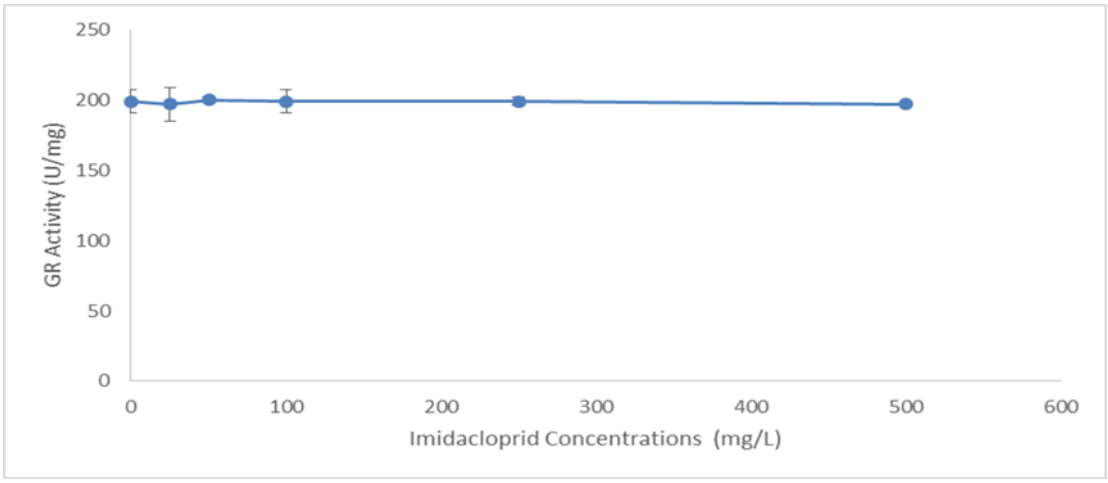

Figure 2. Effect of imidacloprid concentrations on glutathione reductase activity

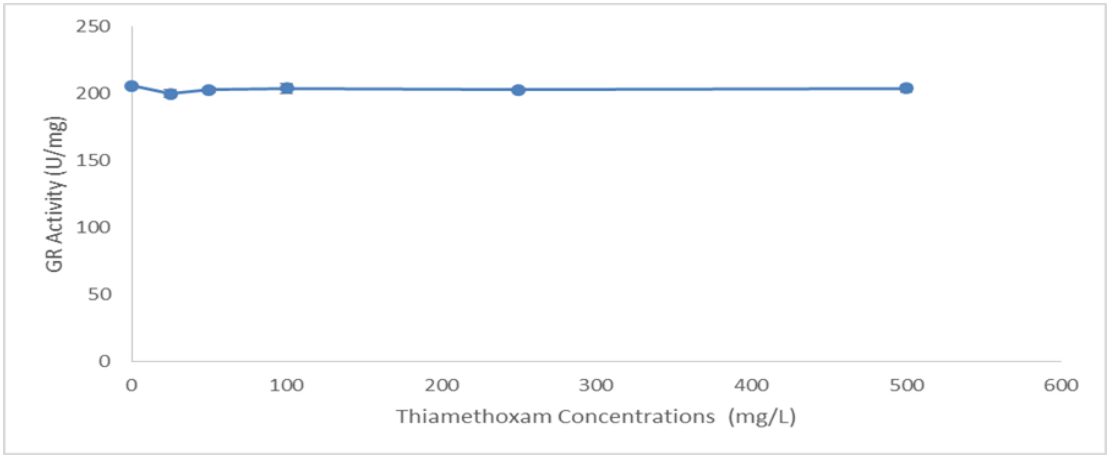

Figure 3. Effect of thiamethoxam concentrations on glutathione reductase activity

In the literature searches, it was observed that there were very few studies with IMI effect on GR activity. About this subject, study of Iturburu et al. has been found. These researchers reported that *Australoheros facetus* fishes were exposed to 0, 1, 10, 100 and 1000 µg L^{-1} IMI for a period of 48 h that GR enzyme activities were not statistically significant in livers and gills of these fishes (p>0.05) [12]. These results were consistent with our findings.

At the literature, it was found very few publications about the effect of TMX on GR activity. About this subject, study of Demirci et al. [13] has been found. They applied LC_{50}/100 concentrations of combinations of endosulfan, indoxacarb, thiamethoxam in combination with atrazine to *Gammarus kischineffensis* for 96 h. They found that level

of GR enzyme activities in the animals were not significantly different from the control groups [13]. These findings were similar to our results.

4. Conclusion

GR is an enzyme belonging to the class of oxidoreductase enzymes. GR catalyzes oxidation and reduction reactions. However, when GR interacted with IMI and TMX, no oxidation and reduction reactions were observed in the present study.

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