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Original article (Orijinal araştırma)

Responses of acetylcholinesterase and glutathione S-transferases activities to thiamethoxam toxicity in *Blattella germanica* (L., 1767) (Blattodea: Ectobiidae)[1]

Blattella germanica (L., 1767) (Blattodea: Ectobiidae)*'*da thiamethoxam toksisitesine karşı asetilkolinesteraz ve glutatyon S-transferaz enzim aktivitelerinin tepkileri

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Abstract

Cockroaches are significant pests that act as vectors for many disease agents, and the indiscriminate use of insecticides has led to widespread resistance development in these species. This study, conducted at Karamanoğlu Mehmetbey University between 2023 and 2024, aimed to determine the toxic effects of thiamethoxam on German cockroach, *Blattella germanica* (L., 1767) (Blattodea: Ectobiidae) populations and to investigate the responses of acetylcholinesterase (AChE) and glutathione S-transferase (GST). The field populations (Dokuma, Konyaaltı, and Ahatlı) generally exhibited low levels of resistance to thiamethoxam at a range of 1.88-3.24-fold compared to the susceptible World Health Organization (WHO) strain. The WHO group showed the lowest AChE activity before and after thiamethoxam exposure compared to the field groups ($p<0.05$). The most significant decrease in AChE activity was observed in the WHO group at 25%, while the decrease in the Ahatlı group was more limited, remaining at 13%. GST activity in the susceptible WHO strain was lower than that of field strains, and the difference was significant. Thiamethoxam-treated Dokuma and Konyaaltı groups exhibited the highest GST activity alterations (44%). However, Ahatlı showed the highest GST activity after the thiamethoxam administration. Principal component analysis (PCA) and Hierarchical clustering analysis (HCA) confirmed the WHO groups were most biochemically differentiated. Analyzing enzymatic biomarkers contributes to more comprehensive insights into the toxicodynamic characteristics of thiamethoxam.

Keywords: Acetylcholinesterase, German cockroach, glutathione-S-transferase, resistance, thiamethoxam

Öz

Hamam böcekleri birçok hastalık etkeninin vektörü olan önemli zararlılar olup, insektisitlerin bilinçsiz kullanımı bu türlerde geniş çaplı direnç gelişimine neden olmaktadır. Bu çalışma, 2023-2024 yılları arasında at Karamanoğlu Mehmetbey Üniversitesi'nde yürütülmüş olup, thiamethoxam'ın Alman hamamböceği, *Blattella germanica* (L., 1767) (Blattodea: Ectobiidae) popülasyonları üzerindeki toksik etkilerini belirlemeyi ve asetilkolinesteraz (AChE) ile glutatyon S-transferaz (GST) enzimlerinin tepkilerini araştırmayı amaçlamıştır. Saha popülasyonları (Dokuma, Konyaaltı ve Ahatlı), duyarlı WHO suşuna kıyasla thiamethoxam'a karşı 1.88-3.24 kat arasında değişen düşük düzeyde direnç göstermiştir. WHO grubu, thiamethoxam maruziyeti öncesi ve sonrası AChE aktivitesi bakımından saha gruplarına göre en düşük değeri sergilemiştir ($p<0.05$). AChE aktivitesinde en belirgin azalma %25 ile WHO grubunda gözlenirken, Ahatlı grubunda bu azalma daha sınırlı olup %13 seviyesinde kalmıştır. Duyarlı WHO suşunda GST aktivitesi saha suşlarına göre daha düşük bulunmuş ve fark anlamlıdır. Thiamethoxam uygulanan Dokuma ve Konyaaltı gruplarında GST aktivitesinde en yüksek değişim (%44) gözlenmiştir. Bununla birlikte, thiamethoxam uygulaması sonrasında en yüksek GST aktivitesi Ahatlı grubunda tespit edilmiştir. Temel bileşen analizi (PCA) ve Hiyerarşik kümeleme analizi (HCA), WHO grubunun biyokimyasal olarak en farklı grup olduğunu doğrulamıştır. Enzimatik biyobelirteçlerin analiz edilmesi, thiamethoxam'ın toksikodinamik özelliklerine dair daha kapsamlı bir anlayışa katkı sağlamaktadır.

Anahtar sözcükler: Asetilkolinesteraz, Alman hamam böceği, glutatyon-S-transferaz, direnç, thiamethoxam

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Introduction

The order Blattodea comprises more than 4600 species of cockroaches (Guzman & Vilcinskas, 2020). However, approximately 40 species are considered pests, as they inhabit areas where human interaction occurs, including homes, basements and cafes. Among these, *Blattella germanica* (L., 1767) (Blattodea: Ectobiidae), is recognized as one of the most prevalent urban pests globally (Tang et al., 2019). These insects serve as mechanical vectors for a diverse range of disease-causing pathogens, including bacteria, viruses, and fungi. Additionally, their faeces, saliva, and shed body parts can elicit allergic reactions in humans (Abbasi, 2025).

A variety of methods, including cultural, physical, chemical, biological, and similar approaches, are employed to fight cockroaches, but the predominant method utilized against these pests remains chemical control. Although some have been restricted or banned in many countries, the main classes of insecticides used against these pests include neonicotinoids, synthetic pyrethroids, carbamates, organochlorines, organophosphates, and insect growth regulators. In modern pest management, however, the use of older compounds such as organochlorines and certain organophosphates has significantly declined due to their high environmental persistence, neurotoxicity, and non-target effects. Instead, newer-generation insecticides, particularly neonicotinoids and synthetic pyrethroids, have become more prevalent due to their relatively selective action and improved safety profiles. Nonetheless, resistance development and ecological concerns continue to drive interest in integrated pest management strategies and safer alternatives (Keswani et al., 2022; Araújo et al., 2023). In Türkiye, the most commonly used insecticide group against cockroaches is the synthetic pyrethroids. Nevertheless, the widespread use of these insecticides has led to strong resistance in cockroach populations globally (Fardisi et al., 2019; Oz et al., 2021; Tisgratog et al., 2023). Consequently, research efforts are increasingly focused on identifying alternative active ingredients.

The insecticide market has been dominated by just three chemical classes since the 1970s: organophosphates, carbamates and synthetic pyrethroids (Maienfisch et al., 2001). However, a number of new chemical insecticides still continue to be introduced to the market. Among these insecticides, neonicotinoids have been widely utilized for the management of pests in both agricultural and public health settings. Thiamethoxam, a second-generation neonicotinoid was launched in 1998 by, has been employed for the control of a variety of pests, including *Musca domestica* De Geer, 1776 (Diptera: Muscidae), *Lygus lineolaris* (Palisot de Beauvois, 1818) (Hemiptera: Miridae) (Polat & Cetin, 2020; Du et al., 2024). The European Union completely banned the outdoor use of three nitroguanidine-type: neonicotinoids-imidacloprid, clothianidin, and thiamethoxam in 2018. However, this ban only covered outdoor areas, with exceptions initially granted for certain special circumstances (such as use in greenhouses). Meanwhile, in the United States and other countries, the use of these chemicals has been regulated more restrictively and is still widely used in some areas (Dentzman et al., 2025). Thiamethoxam binds to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system through both direct contact and ingestion. This binding replaces acetylcholine (ACh), causing an agonistic effect, and continuous nerve signal transmission. Continuously stimulated nerve cells experience neurotransmitter imbalance, ACh accumulation and synaptic fatigue. ACh accumulation may cause compensatory changes (increase or inhibition) in acetylcholinesterase (AChE) enzyme activity in some insects (Lu et al., 2022). A review of the extant literature reveals an absence of studies conducted on the toxic effects of thiamethoxam on cockroaches in Türkiye. Furthermore, a paucity of studies worldwide is evident (Sims & Appel, 2007; Fardisi et al., 2017).

Current insecticides mainly affect four nerve targets found in animals but not in plants: acetylcholinesterase, voltage-gated chloride channel, acetylcholine receptor and γ-aminobutyric acid receptor systems. However, the organism continues its struggle for survival with various biochemical and molecular mechanisms such as detoxification mechanisms against toxicological effects of pesticides. The most valuable data in defence against pesticides are the glutathione S-transferase (GST), acetylcholinesterase

(AChE), butyrylcholinesterase (BChE), carboxyl esterase (CarE) and cytochrome P450 enzyme families. Numerous researchers have conducted biochemical studies on enzymes involved in thiamethoxam toxicity in pests of public health and agricultural significance (Abdallah et al., 2016; Yang et al., 2016; Yasoob et al., 2018; Balkan & Kara, 2020).

In the study conducted, the toxic effect of thiamethoxam on German cockroaches was aimed to be revealed by evaluating AChE and GST activities. Studies on the biochemical evaluations of thiamethoxam on cockroaches are limited in the literature. Therefore, the comparison of the current study results with the literature was made with other organisms. On the other hand, the findings obtained with the study conducted will make a significant contribution to the limited studies in the literature.

Materials and Methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade and provided from Sigma and Merck (Germany). Thiamethoxam and reduced-GSH (glutathione) were purchased from Sigma-Aldrich (Darmstadt, Germany). 5,5'-Dithiobis(2-Nitro Benzoic Acid) (DTNB), Ach iodit, and 1-Chloro-2,4-dinitrobenzene (CDNB) were purchased from Merck (Darmstadt, Germany).

Insect

The cockroaches used in this study were collected from Ahatlı, Konyaaltı and Dokuma regions of Antalya, Türkiye, which are resistant to synthetic pyrethroids (Öz et al., 2021). These populations have been cultured in the Vector Control and Ecology Laboratory of Akdeniz University since 2014. The susceptible population of *B. germanica*, originating from Denmark, was obtained from the Pesticide Testing Laboratory of Hacettepe University Ankara, Türkiye. It has been reared since 2008 under laboratory conditions at a temperature of 25±2°C and 70±10% RH in incubators with a photoperiod of 12:12 (L:D) h without any exposure to insecticides. The cockroaches were fed ad libitum with a mixture of flour-honey and water.

Resistance tests

One performed resistance tests using the standard method described by Öz et al. (2021). For the tests, one obtained stock solutions of thiamethoxam in acetone. The insecticidal solutions were applied to the inner surfaces of glass jars with a capacity of 250 mL and a total inner surface area of 260 cm^2. Applications were performed using 1 mL of solution per jar according to the designated application doses (0.0001, 0.0005, 0.001, 0.01, and 0.1 g a.i./m^2), which corresponded to 0.0026, 0.013, 0.026, 0.26, and 2.6 mg of active ingredient per jar, respectively. The jars were then rotated horizontally until the solvent completely evaporated, ensuring uniform coverage of the inner surfaces with the insecticide. One applied only acetone to the control group. Following 24 hours, one placed ten adult male cockroaches (15-30 days old) in the jars and exposed to the insecticide for one hour. After the exposure period, one put the cockroaches into clean jars, each containing a cotton ball saturated with water.

One conducted a preliminary series of tests using five doses (0.0001, 0.0005, 0.001, 0.01, 0.1 g a.i./m^2) to determine the lethal dose (LD_{50}) values, resulting in mortality rates ranging from 5% to 100%. Three replicates were used for each tested dose and the control group, and mortality was assessed 24 hours after treatment. Cockroaches were considered dead if they were unable to return to their normal position when prodded on the abdomen with forceps.

Biochemical analysis

Cockroaches were exposed to the LD_{25} dose of thiamethoxam for 90 min to induce enzymes. After induction, cockroaches were crushed whole in ice-cold phosphate buffered saline (pH 7.4) in a crucible and homogenised for 3 min using a titanium probe homogeniser (Bandelin Sonopuls HD 2200, Germany). The homogenates were then centrifuged at 4000×g for 15 min at +4°C. The supernatants were used to evaluate enzyme activities and protein amounts. The protein content of the samples was determined using the Bradford (1976) assay. The basic principle is a quantitative method based on the binding of proteins to Coomassie dye in an acidic medium. Briefly, an aliquot (30 µL) of unknown sample or each standard (bovine serum albumin) was added into the test tubes. Then 1.5 mL of the Coomassie reagent was added to each tube and mixed well. The sample tubes were incubated for 10 min at room temperature. The absorbance was subsequently read against the blank at 595 nm wavelength. The protein concentration (mg/mL) in the samples was determined by using a standard curve.

Glutathione S-transferase activity was measured according to the method described by Habig et al. (1981). GST is based on catalyzing the conjugation of GSH with CDNB. Briefly, an aliquot (100 µL) of enzyme source was added to the tube containing 2.7 mL of phosphate buffer solution (100 mM, pH: 6.5). A 100 µL GSH (30 mM) as a cofactor was added to the tube. Then, 100 µL CDNB solution (30 mM) in ethanol was added and mixed to initiate the reaction. The absorbance was read at 340 nm wavelength at 30 sec intervals during 3 min. The specific enzyme activity was expressed as unit/mg protein per min.

Acetylcholine esterase activity was carried out by the method of Ellman et al. (1961) with minor modifications. The basic principle is based on the hydrolysis of acetylcholine by acetylcholinesterase to produce choline. The enzyme converts acetylthiocholine to thiocholine. Thiocholine reacts with Ellman's reagent (5,5-dithio-bis-2-nitrobenzoic acid, DTNB) to form yellow color TNB (5-thio-2-nitrobenzoic acid). The absorbance of the formed TNB is measured at a wavelength of 412 nm. Briefly, a 2700 µL of sodium buffer (50 mM, pH=8.00), 100 µL of DTNB (0.25 mM, pH=7.00) and 100 µL of supernatant were added to the tubes for analysis. The tubes were incubated at 37°C for 5 min. Subsequently, 100 µL of acetylthiocholine iodide (3 mM) prepared in sodium buffer was added to the tubes, followed by incubation at 37°C for 10 min. The absorbance of the reaction mixture was then measured at 412 nm. The specific activity was determined by expressing the enzyme activity relative to the amount of protein.

Following the application, the metabolic response (%) was calculated for understanding enzyme activity changes using Equation 1.

$$\text{Alteration (\%)}=(\text{Control}-\text{Thiamethoxam})/\text{Control} \times 100 \qquad \text{Eq.1}$$

Data analysis

The statistical analyses were performed using the SPSS 20.0 software package. Mortality data were analyzed using one-way analysis of variance (one-way ANOVA), and differences between means were determined by Tukey's HSD test at a significance level of $p<0.05$. Median lethal dose (LD_{50}) values for each population were estimated after 24 hours of exposure using probit analysis. Resistance ratios (RR) were calculated by dividing the LD_{50} values of field populations by that of the susceptible reference strain. The classification of resistance levels followed the criteria proposed by Chai & Lee (2010), which define five distinct resistance categories. These categories were defined as follows: ≤1, absence of resistance; >1 to ≤5, low resistance; >5 to ≤10, moderate resistance; >10 to ≤50, high resistance; and >50, very high resistance. The mean ($\bar{X}$) and standard deviation (SD) values of the biochemical enzyme analysis results were calculated. The differences among groups were evaluated using a one-way ANOVA, followed by the Tukey HSD test to compare pairwise group means. In addition, differences between control and thiamethoxam-treated groups within each population were analyzed using an independent samples Student's t-test, as the groups consisted of different individuals. Statistical significance was considered at a level of $p\leq0.05$.

Spearman's correlation coefficient was utilised to ascertain the relationship between AChE and GST activities. In addition, Principal component analysis (PCA) and Hierarchical clustering analysis (HCA) was performed to evaluate the parameters interaction of all groups. The data were standardized using z-scores and subjected to PCA using Origin Pro9 software (SAS). The graphs were prepared using Origin Pro9 software.

Results

To obtain LD_{50} values for thiamethoxam, five doses were subjected to analysis. The application of doses of 0.1 and 0.01 g ai/m^2 resulted in 100% mortality in all populations. Consequently, lower doses were subjected to testing. At a dose of 0.001 g ai/m^2, 86.67% mortality was observed in the WHO population. In the field populations, mortality rates were 66.67% in Konyaaltı, 76.67% in Dokuma, and 76.67% in Ahatlı. A general increase in mortality was observed in all populations as the dose increased, with a statistically significant difference between doses (Table 1).

Table 1. Comparative toxicity (%mean mortality±standard error) of different doses (gr ai/m2) of thiamethoxam on adult *Blattella germanica* populations, including LD_{50}, (95% Fiducial limits), RR, df (degree of freedom), and chi-square values

Populations	Doses (gr ai/m^2)												
	N	Control	0.0001	0.0005	0.001	0.01	0.1	LD_{50}	LD_{50} (95% FL)	RR	Chi-square‡	Df	*P*
WHO	180	3.33±3.33a†	30.00±10.00a	83.33±8.82b	86.67±13.3b	100b	100b	0.000186	0.000078-0.000310	1	188.28	13	0.0001
Ahatlı	180	0.00±0.00a	13.33±6.67a	23.33±12.02a	76.67±6.67b	100b	100b	0.000602	0.000377-0.001020	3.24	175.23	13	0.0001
Dokuma	180	0.00±0.00a	30.00±0.00b	46.67±13.33b	76.67±3.33c	100d	100d	0.000351	0.000224-0.000517	1.88	79.53	13	0.0001
Konyaaltı	180	3.33±3.33a	13.33±6.67a	30.00±17.32ab	66.67±8.82bc	100c	100c	0.000493	0.000353-0.000685	2.65	168.31	13	0.0001

†: The different lower-case letters indicate statistical difference in the same coloumn (Tukey, *p*≤0.05).

‡: Chi-square values are significant at *p*≤0.05 levels. (N: total number of individuals; WHO: Susceptible population; Ahatlı, Konyaaltı, and Dokuma: Field population; df: Degree of freedom, RR: Resistance ratio, FL: Fiducial limits).

LD_{50} values for the WHO, Dokuma, Konyaaltı, and Ahatlı populations were listed in Table 1. The resistance ratios were found to be 1.88-fold, 2.65-fold, and 3.24-fold for the Dokuma, Konyaaltı, and Ahatlı populations, respectively. Consequently, all field populations were classified as exhibiting low resistance category.

The value of the enzyme activities of the control and thiamethoxam treatment groups is shown in Table 2. Furthermore, data is presented by way of visualisation in Figures 1&2, with a view to facilitating a more profound comprehension of statistical comparisons within and between groups.

Table 2. Enzyme activity values of control and thiamethoxam-induced *Blattella germanica* groups

	AChE*	GST*
WHO Control	21.52±0.99^{C†}	8.43±1.49^{C†}
WHO Thmx	16.23±1.32^{c‡}	11.43±1.05^{c‡}
Dokuma Control	24.75±1.86^{B†}	10.30±1.66^{BC†}
Dokuma Thmx	20.25±1.21^{b‡}	14.84±2.20^{b‡}
Konyaaltı Control	24.72±1.21^{B†}	11.85±1.99^{AB†}
Konyaaltı Thmx	21.46±1.40^{b‡}	17.07±1.94^{ab‡}
Ahatlı Control	27.87±1.00^{A†}	13.98±1.26^{A†}
Ahatlı Thmx	24.39±1.21^{a‡}	19.17±1.65^{a‡}

Cockroaches were exposed to the LD_{25} dose of thiamethoxam for 90 min to induce enzymes. (AChE: Acetylcholinesterase; GST: Glutathione S-transferase; Control: Sham group; Thmx: Thiamethoxam administered group; WHO: Susceptible population; Ahatlı, Konyaaltı, and Dokuma: Field population).

*: Enzyme activities are given in enzyme unit/min/mg protein; Data were expressed and shown as mean±standard deviation. A, B: Different capital letters in the column indicate statistical significance between control groups (using one-way ANOVA, followed by TUKEY HSD). a, b: Different lowercase letters in the column indicate statistical significance between thiamethoxam groups. (using one-way ANOVA, followed by TUKEY HSD).

†, ‡: Different daggers in the column indicate statistical significance within each group (using student's t-test).. *p*<0.05 accepted significantly different.

Figure 1 presents the acetylcholine esterase (AChE) activities of the control and thiamethoxam-induced groups. When the control groups of the populations were evaluated among themselves, a statistically significant difference was observed between the WHO group and other groups ($p<0.05$). Besides, the AChE activity of the Dokuma and Konyaaltı population had a significant difference compared to that of Ahatlı population ($p<0.05$). In the thiamethoxam-treated groups, AChE activity was significantly higher in the field population groups compared to the WHO group ($p<0.05$). Moreover, the AChE activity in the Ahatlı population was significantly higher than that observed in the other populations ($p<0.05$). On the other hand, there was a statistical difference between the control groups and thiamethoxam groups in all populations ($p<0.05$). Following thiamethoxam administration, AChE activity was observed to decrease by 13-25% in all populations (Figure 3). The greatest decrease was seen in the WHO population, with a reduction of 25%, followed by Dokuma (18%), Konyaaltı (13%), and Ahatlı population (13%).

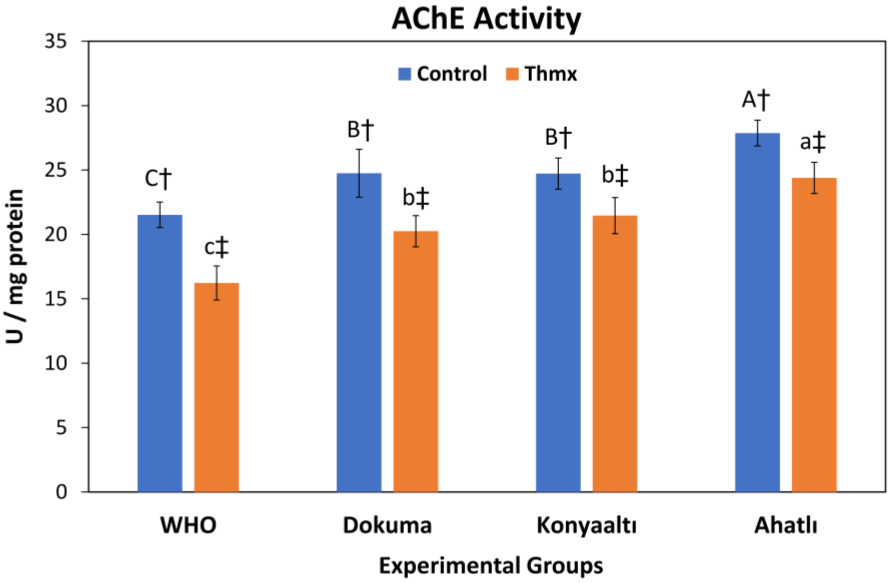

Figure 1. Acetylcholine esterase activities of control and thiamethoxam-induced *Blattella germanica* groups. Cockroaches were exposed to the LD_{25} dose of thiamethoxam for 90 min to induce enzymes. Data expressed as mean±standard deviation. Bar and error bar indicate mean and standard deviation, respectively. Acetylcholine esterase activities are given in enzyme unit/min/mg protein. (Control: Sham group. Thmx: Thiamethoxam administered group. WHO: Susceptible population. Ahatlı, Konyaaltı, and Dokuma: Field population). A, B: Different capital letters indicate statistical significance between control groups (using one-way ANOVA, followed by TUKEY HSD).

a, b: Different lowercase letters indicate statistical significance between thiamethoxam groups (using one-way ANOVA, followed by TUKEY HSD).

†, ‡: Different daggers indicate statistical significance within each group (using student's t-test). $p<0.05$ accepted significantly different.

Figure 2 illustrates that the lowest glutathione S-transferase (GST) activity was observed in the WHO population, whereas the highest GST activity was measured in the Ahatlı group. WHO control group had a statistically significant compared to the Konyaaltı and Ahatlı groups. Additionally, a significant difference in GST activity of control groups was measured between Dokuma and Ahatlı field populations. On the other hand, a significant increase in GST activity was detected in all populations following thiamethoxam exposure. Thiamethoxam-induced WHO population exhibited lower GST activity in comparison with the all-field populations. Although the highest GST activity was in the Ahatlı group, the highest induction was seen in the Dokuma and Konyaaltı groups. The Dokuma and Konyaaltı groups exhibited the highest increase in GST activity at 44%, followed by the Ahatlı with 37% and WHO with 36% (Figure 3). Negative altertaion was observed in ACHE activity, while positive alteration was observed in GST activity. Thiamethoxam was found to reduce AChE and induce GST. Statistically significant changes in GST activity were observed in all populations when comparing control and thiamethoxam-treated groups within each population ($p<0.05$).

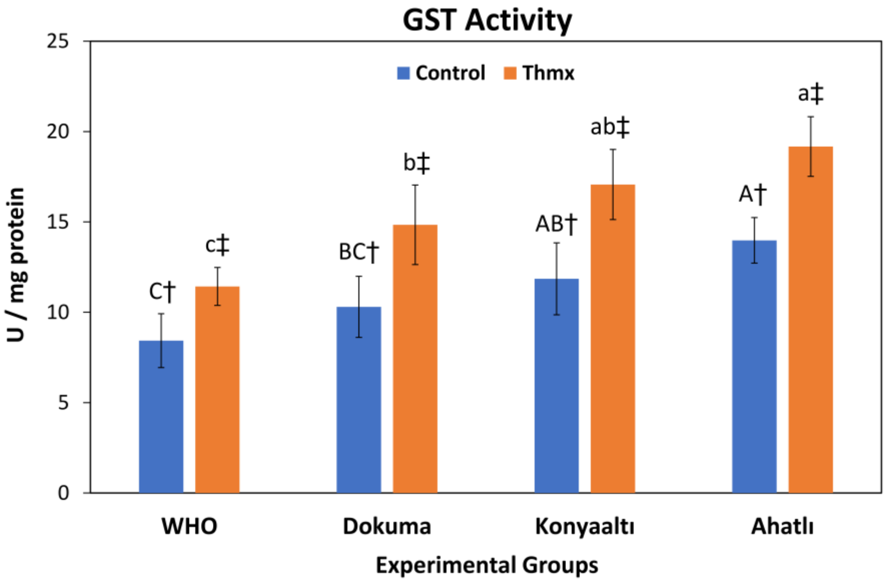

Figure 2. Glutathione S-transferase activities of control and thiamethoxam-induced *Blattella germanica* groups. Cockroaches were exposed to the LD_{25} dose of thiamethoxam for 90 min to induce enzymes. Data expressed as mean±standard deviation. Bar and error bar indicate mean and standard deviation, respectively. Glutathione S-transferase activities are given in enzyme unit/min/mg protein. (Control: Sham group. Thmx: Thiamethoxam administered group. WHO: Susceptible population. Ahatlı, Konyaaltı, and Dokuma: Field population.).

A, B: Different capital letters indicate statistical significance between control groups (using one-way ANOVA, followed by TUKEY HSD).

a, b: Different lowercase letters indicate statistical significance between thiamethoxam groups (using one-way ANOVA, followed by TUKEY HSD).

†, ‡: Different daggers indicate statistical significance within each group (using student’s t-test). $p<0.05$ accepted significantly different.

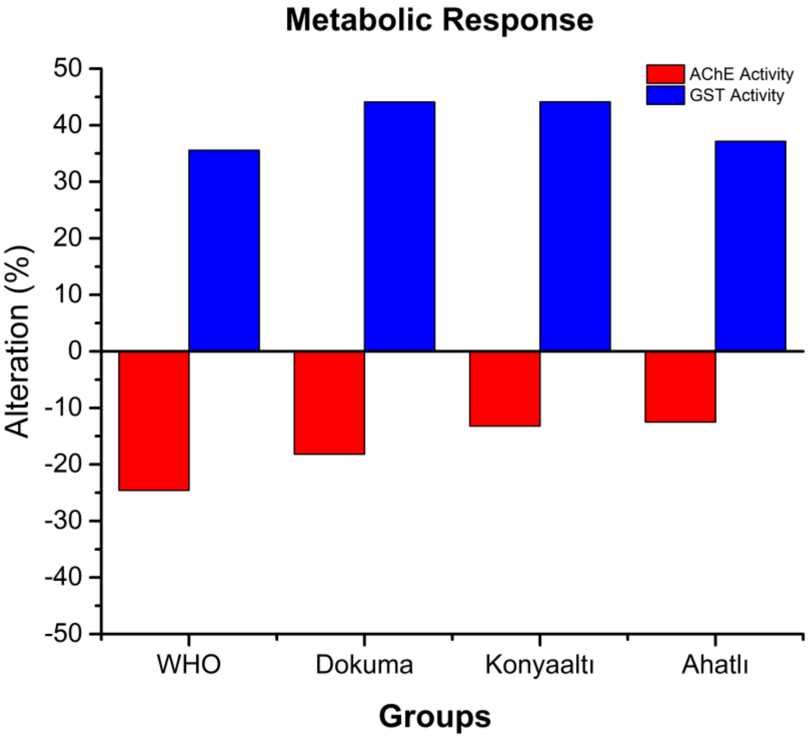

Figure 3. Metabolic response of the AChE and GST activities following thiamethoxam treatment.

As a result of the correlation between AChE and GST activities, Spearman correlation coefficient *ρ*=0.099 and *p*-value 0.504 were found. It shows that there is no statistically significant correlation between AChE and GST enzyme levels. In the scatter plot, the points do not show a general trend, which confirms the result of the correlation analysis (Figure 4).

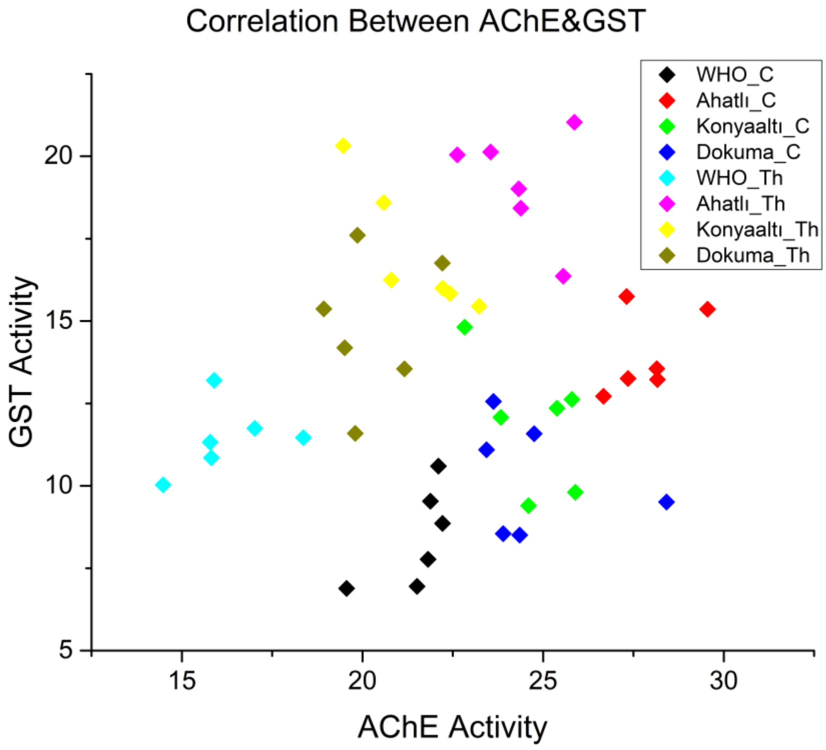

Figure 4. Correlation between AChE and GST enzyme levels.

The PCA Biplot demonstrated that thiamethoxam treatment clearly separated groups in terms of enzyme levels (Figure 5a). While AChE levels separated the samples in the positive PC1 direction, GST levels provided a strong separation in the negative PC2 direction. In particular, it is evident that groups such as WHO_Th (thiamethoxam-treated WHO group) and WHO_C (WHO control group) are distinctly differentiated, visually corroborating the assertion that insecticide application engenders alterations in the biochemical profile. The observation of similar vector lengths indicates that both enzymes make a substantial contribution to the PCA. The angular difference indicates that these two variables are independent of each other (low correlation), a finding that is also supported by the Spearman correlation (Figure 4). Clustering distances (vertical line length) in HCA dendrogram reflect the degree of difference between the groups (Figure 5b). HCA indicated that WHO Thiamethoxam group samples are strongly clustered in itself and separated from the others early. The WHO control group samples are also collected in a separate branch. On the other hand, there are more intertwined clusters between field groups (Dokuma, Konyaaltı, and Ahatlı). However, thiamethoxam applications clearly separated these groups from the control groups.

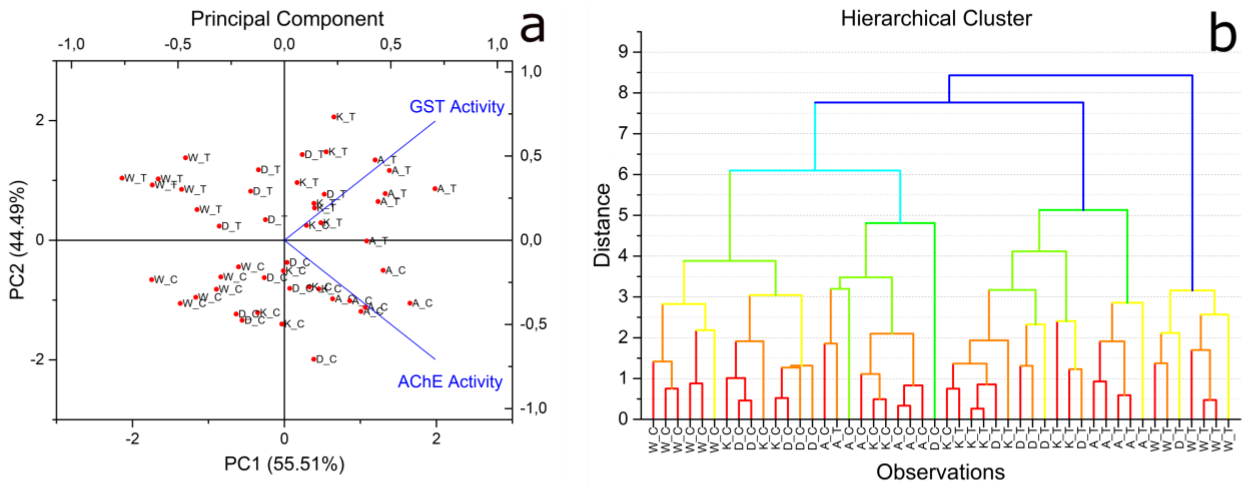

Figure 5. Principal component analysis a) biplot and b) hierarchical clustering dendrogram of the groups. (W: Who, D: Dokuma, K: Konyaaltı, A: Ahatlı, C: Control, T: Thiamethoxam).

Discussion

In this study, the effects of thiamethoxam application on AChE and GST enzyme activities in different German cockroach populations were investigated for the first time in Türkiye, and it was shown that field populations exhibited different biochemical responses to the pesticide compared to the susceptible group. In addition, the findings revealed that the field populations exhibited low resistance levels ranging from 1.88-fold to 3.24-fold according to the susceptible group WHO.

Thiamethoxam has not previously been used for the cockroach control in Türkiye. Consequently, it caused a high degree of mortality even in the Dokuma and Konyaaltı cockroach populations, which have been shown to have very high resistance (≥1000-fold) to synthetic pyrethroids (Öz et al., 2021). It is hypothesized that the low level of resistance to thiamethoxam in these synthetic pyrethroid-resistant German cockroach populations is due to the lack of selection pressure against this substance. This suggests that thiamethoxam has the potential to be employed as a novel and efficacious insecticide in the management of German cockroaches.

A review of the existing literature indicates a limited number of studies addressing cockroach resistance in Türkiye. In these studies, the resistance of German and American cockroaches to a number of insecticides from synthetic pyrethroid, organic phosphate and organic chlorinated groups was investigated (Garrett et al., 1968; Erdogan & Kocak, 1989; Öz et al., 2021). In a study by Öz et al. (2021), the effectiveness of four synthetic pyrethroids insecticides (alpha-cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin) was evaluated against German and American cockroach populations collected from the central districts of Antalya province, Türkiye. The results demonstrated that the WHO recommended doses were effective in American cockroach populations, while German cockroach field populations exhibited high resistance to insecticides in almost all populations.

Studies on the toxic effects of thiamethoxam insecticide on the German cockroach are limited. Fardisi et al. (2017) investigated the toxic effects of 14 insecticides, including thiamethoxam, on adult male German cockroaches. Similar to current results, thiamethoxam exhibited low resistance in field strains. They also reported the LC_{50} value as 0.8 µg/71.67 cm^2, the LC_{90} value as 5.4 µg/71.67 cm^2, and the LC_{99} value as 24.8 µg/71.67 cm^2 in the JWas-S susceptible population, with the diagnostic dose for field populations being 30 µg/71.67 cm^2. Additionally, field strains displayed resistance to some neonicotinoids, oxadiazines, phenylpyrazoles, and synthetic pyrethroids Sims & Appel (2007) investigated the toxic effect of thiamethoxam on adult male individuals of the German cockroach. They found that the LC_{50} value was 0.0038 mg.

In the studies, it has been clearly observed that thiamethoxam exposure leads to neurotoxic and oxidative stress responses in various insect species. In particular, changes in fundamental enzymes such as AChE and GST reflect the effects of thiamethoxam on both the nervous system and the ways of detoxification. Thiamethoxam administration suppressed AChE activity in all groups, but this decrease was more limited in field groups (Figure 1), indicating that field populations are less susceptible to the pesticide. Thiamethoxam exerts its neurotoxic effects by selectively binding to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system, where it mimics the action of acetylcholine as an agonist. This continuous stimulation disrupts normal synaptic transmission, leading to neurotransmitter imbalance, acetylcholine accumulation, and ultimately synaptic fatigue and neurological dysfunction. Although thiamethoxam does not directly inhibit AChE, prolonged receptor activation may impose an excessive physiological demand on the enzyme. Consequently, some insect species exhibit compensatory changes in AChE activity, such as upregulation or inhibition. Several biomarker-based studies have reported a measurable decrease in AChE activity following thiamethoxam exposure, which may reflect indirect effects of receptor overstimulation or secondary responses to metabolic stress. Thiamethoxam has demonstrated that earlier studies have suppressed esterase enzymes, including AChE and CarE. A recent study is consistent with the present study, reporting that thiamethoxam inhibited AChE, CarE, and adenosine triphosphatase activities after 96-h of acute toxicity exposure in the aquatic crayfish species *Astacus leptodactylus* Germar, 1827 (Decapoda: Astacidae) (Uçkun et al., 2021). In *Apis mellifera intermissa* Maa, 1953 (Hymenoptera: Apidae), 9-day exposure at a sublethal dose significantly suppressed AChE activity, confirming the neurotoxic potential of thiamethoxam (Benchaâbane et al., 2022). In contrast, exposure to a sublethal dose of thiamethoxam (0.0227 ng/µL) in the head tissue of Africanised *Apis mellifera* L., 1758 (Hymenoptera: Apidae) resulted in increased AChE activity and decreased CarE activity, which may be attributed to synaptic compensation or enzymatic induction mechanisms (Decio et al., 2021). In cowpea aphid, *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae), an increase in AChE activity was reported at the LC_{50} thiamethoxam dose, but at the same time a decrease in CarE and GST activities was recorded. This finding indicates that interspecific physiological variation is important (Abdelmoteleb et al., 2023). These findings suggest that AChE responds to thiamethoxam toxicity not only by inhibition but also by compensatory increases in activity. The direction of change in enzyme activity (i.e. increase or decrease) is likely to be contingent on the dose administered, the duration of exposure, and the tissue localization. In some cases, an increase in AChE activity was also observed, suggesting that metabolic adaptation strategies of the organism may be activated.

GST is the major detoxification enzyme involved in the removal of xenobiotics and oxidative by-products from the cell (Pavlidi et al., 2018; Bk et al., 2022). Following the administration of thiamethoxam, an increase in GST activity was observed which is more pronounced in field populations, indicating that GST is activated as a potential detoxification response (Figure 2). In addition, Ahatlı already had higher GST activity among the control groups, so a 37% increase after thiamethoxam exposure gave the highest GST activity compared to Dokuma and Konyaaltı groups (44%). A significant increase in GST activity has been reported in association with thiamethoxam exposure (Decio et al., 2021; Uçkun et al., 2021). Du et al. (2024) reported that field-collected tarnished plant bug *L. lineolaris* showed moderate resistance (7.88) to thiamethoxam and after exposure, GST activity increased by 1.48-fold compared to the susceptible population. A significant increase in GST activity was observed in *Culex pipiens* L., 1758 (Diptera: Culicidae) larvae after 72 h LC_{50} exposure to thiamethoxam (Abdel-Haleem et al., 2020). A recent study (Maloni et al., 2025) has revealed that exposure to sublethal doses of thiamethoxam (LC_{50}/10 and LC_{50}/100) for six days resulted in an increase in GST activity in the head and abdomen of *Scaptotrigona postica* Latreille, 1807 (Hymenoptera: Apidae). Similarly, in *Frieseomelitta varia* (Lepeletier, 1836) (Hymenoptera: Apidae), a significant increase in GST activity was observed on days 1 and 5, especially in the abdomen, suggesting that abdominal tissues are under more detoxification pressure (de Souza et al., 2024). It has been previously reported that reactive oxygen species levels increased throughout the exposure period compared to control groups as a result of thiamethoxam toxicity (0.30, 1.25 and 5.00 mg/L) in zebrafish, *Danio rerio* (Hamilton, 1822) (Cypriniformes: Cyprinidae) on days 7, 14, 21 and 28. Additionally, GST activity was noted to increase only on day 28 (Yan et al., 2016). In a molecular study conducted with tobacco whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae), only GST was reported to have considerably more activity in the resistant population compared to the susceptible population, out of all the detoxifying enzymes (Yang et al., 2016). Furthermore, it was demonstrated that seven of the 23 GST genes were overexpressed in the resistant strain and that silencing the GST14 gene, in particular, increased the insect mortality rate against thiamethoxam (Yang et al., 2016). Thiamethoxam exposure results in significant changes in the activities of AChE, GST, CarE and other enzymes depending on species, tissue and exposure level. These changes are a reflection of both the neurotoxic effects of thiamethoxam and its burden on detoxification systems. Increases in GST generally indicate a detoxification response, whereas suppressions in AChE and CarE indicate synaptic transmission disorders and metabolic stress states. This evaluation of enzymatic biomarkers contributes to a more comprehensive understanding of the interspecies toxicodynamic properties and ecotoxicological profile of thiamethoxam.

The increase in GST activity against thiamethoxam treatment was clearly seen especially in Konyaaltı and Dokuma groups. The decrease in AChE usually indicates a neurotoxic effect and the decrease in AChE in thiamethoxam treated groups was also a significant discriminating factor in PCA. In the multivariate correlation analysis performed HCA confirmed that administration of thiamethoxam had a significant effect on AChE and GST profiles. In particular, the profile of the WHO group following administration of thiamethoxam clearly separated from the control group and formed an independent cluster. These findings indicate that heightened sensitivity to insecticide can be monitored at the biochemical level also, consistent with the PCA results. In the separation of the clusters, the left side of the graph is predominantly contained control (C) samples, while the right side is primarily populated by thiamethoxam (T) applied groups. These findings indicate that the application of insecticide exerts substantial influence on the enzyme profiles. WHO thiamethoxam groups located in an independent supercluster at the right end. This result shows that the WHO group exhibits the most sensitive response to insecticide, which may be indicative of the most significant biochemical differentiated. Conversely, Ahatlı, Konyaaltı, and Dokuma groups are more intertwined. This finding may suggest that the field groups have similar biochemical responses or are less differentiated.

Thiamethoxam resistance was found to vary between 5.98 and 2194.96 fold in 15 field cotton aphid *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) populations collected from China. Correlation analysis results showed that there was a significant positive correlation between cytochrome P450 enzyme activities and the logarithm of LC_{50} values, indicating that cytochrome P450 enzymes play a substantial role in thiamethoxam resistance (Shi et al., 2023). Similarly, thiamethoxam resistance in *Sogatella furcifera* (Horváth, 1899) (Hemiptera: Delphacidae), a plant pest, was found to be low to moderate across 18 populations. In this case, esterase activity in the field populations showed a substantial correlation with the LC_{50} values of thiamethoxam (Li et al., 2021). Whereas, Kaya et al. (2023) reported low to moderate resistance (1.1-20.8 fold) to thiamethoxam in nine *A. gossypii* field populations, with no correlation observed between resistance levels and GST or CarE enzyme activities. Besides, they reported that this resistance may be due to metabolic resistance mechanisms.

The findings of this study have important implications for both the control of *B. germanica* and the development of resistance management strategies. The first of them, the observed low resistance levels (1.88–3.24-fold) among field populations suggest that thiamethoxam retains its efficacy against German cockroach populations that are otherwise highly resistant to synthetic pyrethroids. This indicates that thiamethoxam could be employed as an effective alternative insecticide, especially in regions where pyrethroid resistance is widespread. Moreover, the differential enzymatic responses between the field and susceptible populations highlight the utility of AChE and GST as early biomarkers for resistance development. The limited decrease in AChE activity and the elevated GST induction in field populations may point to the early onset of metabolic resistance mechanisms. Therefore, routine biochemical monitoring of detoxification enzymes could support resistance surveillance and guide timely insecticide rotation. Finally, the integration of thiamethoxam into insecticide rotation schemes, alongside non-chemical methods, could delay resistance development and help maintain long-term efficacy within integrated pest management frameworks. These results underscore the need for region-specific, enzyme-informed control protocols and contribute valuable biochemical evidence toward the global discussion on sustainable cockroach management.

Conclusion

It was revealed that field populations of cockroaches exhibited low levels of resistance to thiamethoxam. Thiamethoxam application caused neurotoxic effects by decreasing AChE activity, while it triggered detoxification mechanisms by increasing GST activity. These effects were characterized by more limited AChE reduction and more pronounced GST induction in field populations, reflecting the enhanced resistance levels of these groups to insecticide. It is significant that these findings are included in the literature for the first time and that they shed light on future studies. The mechanisms by which thiamethoxam exerts its effects on these enzymes may be a focus for future studies.

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