Comparative Kinetic Analysis of Butyrylcholinesterase Activity in *Moroccan* and *Oasis Locusts* (*Gampsocleis Buergeri*)

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ABSTRACT
Cholinesterases are essential neuroenzymes involved in synaptic signal transmission in insects and represent primary molecular targets of organophosphate and carbamate insecticides. However, comparative kinetic data on butyrylcholinesterase (BChE) activity among locust populations adapted to different ecological conditions remain limited. In the present study, BChE activity and kinetic parameters were comparatively investigated in Moroccan and Oasis locusts (Gampsocleis buergeri). Enzyme activity was determined spectrophotometrically using the Ellman method with butyrylthiocholine as substrate. Enzyme kinetics were analyzed by Lineweaver-Burk double reciprocal plots to calculate Michaelis-Menten constants. The results demonstrated pronounced interpopulation differences. Oasis locusts exhibited a lower Michaelis-Menten constant ($Km = 6.71 \times 10^{-5}$ M) and a higher maximum reaction velocity ($Vmax = 4.037 \times 10^{-6}$ mol·L^{-1}·min^{-1}) compared to Moroccan locusts ($Km = 8.27 \times 10^{-5}$ M; $Vmax = 2.936 \times 10^{-6}$ mol·L^{-1}·min^{-1}). These findings indicate higher substrate affinity and catalytic efficiency of BChE in Oasis locusts. Enhanced enzymatic performance suggests population-specific neuroenzymatic adaptation associated with environmental stress tolerance and differential sensitivity to neurotoxic compounds. The study provides new insights into insect neurophysiology and biochemical adaptation mechanisms relevant to pest management strategies.

Keywords: butyrylcholinesterase; enzyme kinetics; Ellman method; locust neurophysiology; *Gampsocleis buergeri*; insect adaptation

Introduction

Cholinesterases are essential enzymes in insect neurophysiology, playing a pivotal role in the rapid termination of synaptic transmission through the hydrolysis of choline esters. By regulating the concentration of acetylcholine in synaptic clefts, these enzymes ensure precise neural signaling and prevent prolonged neuronal excitation. Two major forms of cholinesterases have been identified in animals and insects: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Acetylcholinesterase is classically recognized as the primary enzyme responsible for acetylcholine hydrolysis at synaptic junctions, whereas BChE has long been considered a secondary or auxiliary enzyme. However, increasing evidence indicates that BChE contributes significantly to neural regulation, detoxification processes, and physiological adaptation, particularly under conditions of environmental stress (Čolović et al., 2013; Bajgar, 2004).

In insects, cholinesterases are not only involved in neurotransmission but also play an important role in maintaining neural homeostasis and enabling rapid behavioral responses to environmental stimuli. Structural and functional studies of AChE have revealed an exceptionally high catalytic efficiency, which is essential for the rapid clearance of acetylcholine following synaptic transmission (Lawler, 1961; Sussman et al., 1991). While AChE has been extensively characterized at the molecular and kinetic levels, BChE has received comparatively less attention, particularly in insects. Nevertheless, several studies suggest that BChE may act as a protective or compensatory enzyme, contributing to the regulation of cholinergic signaling when AChE activity is compromised or inhibited (Shafferman et al., 1994; Gilson et al., 1994).

Organophosphate and carbamate insecticides exert their toxic effects primarily through inhibition of cholinesterases, leading to accumulation of acetylcholine in synaptic clefts and subsequent overstimulation of the nervous system. This disruption of cholinergic signaling ultimately results in neuromuscular paralysis and death of insects, making cholinesterases key molecular targets in pest control strategies (Bajgar, 2004; Akdur et al., 2010). Although the majority of toxicological studies have focused on AChE inhibition, growing evidence indicates that BChE activity may also play an important role in insect survival under chemical stress. In particular, BChE has been shown to be more sensitive than AChE to certain organophosphate

compounds, suggesting its potential utility as an early biochemical marker of pesticide exposure (Kushiev et al., 1994, Brahmi et al., 2006; Dewan et al., 2008).

Importantly, cholinesterase activity is not uniform across insect species or populations. Variability in enzyme activity and kinetic properties has been reported as a consequence of genetic differences, environmental conditions, and exposure to xenobiotics. Such variability reflects adaptive responses that enhance insect tolerance to chemical stressors and environmental challenges (Tulyabaev, Kushiev et al. (1995), Worek et al., 1999; Holas et al., 2012). In this context, BChE is increasingly recognized as a key component of insect detoxification systems, complementing classical metabolic enzymes and contributing to physiological plasticity.

Locusts are among the most destructive agricultural pests worldwide and are characterized by remarkable ecological and physiological plasticity. Their ability to survive, migrate, and reproduce under diverse climatic and ecological conditions makes them an excellent model for studying biochemical adaptation mechanisms. Locust populations inhabiting different ecological niches are exposed to distinct environmental pressures, including temperature fluctuations, salinity, water availability, and varying levels of agrochemical application. These factors are likely to influence neuroenzymatic regulation and cholinesterase activity. Despite the economic importance of locusts, comparative biochemical studies focusing on cholinesterase kinetics among populations from distinct ecological habitats remain scarce. In particular, data on BChE activity and kinetic behavior in different locust populations are limited (Kushiev et al., 1994,).

Understanding population-specific differences in cholinesterase activity is essential not only for elucidating mechanisms of insect adaptation but also for improving pest management strategies. Enzyme kinetic parameters, such as the Michaelis–Menten constant (Km) and maximum reaction velocity (Vmax), provide valuable insights into substrate affinity and catalytic efficiency, which may reflect adaptive neurophysiological regulation. Comparative kinetic analysis of BChE can therefore contribute to a more comprehensive understanding of insect neurobiology and the biochemical basis of pesticide sensitivity or resistance.

Therefore, the present study aims to comparatively analyze BChE activity and kinetic parameters in Moroccan and Oasis locust populations (*Gampsocleis buergeri*). By applying classical spectrophotometric assays and enzyme kinetic analysis, this research seeks to elucidate population-specific differences in neuroenzymatic regulation and to contribute to a deeper understanding of insect physiological adaptation under diverse ecological conditions.

Materials And Methods

Insect Material and Experimental Design

Moroccan and Oasis locusts (*Gampsocleis buergeri*) were collected from agricultural areas of the Syrdarya region, Uzbekistan, during the active vegetation period. To minimize variability associated with developmental stage, only adult individuals were selected for the study. After collection, insects were transported to the laboratory under cooled conditions and processed immediately to preserve enzymatic activity.

All experimental procedures were designed to ensure comparability between the two locust populations. Thoracic and abdominal tissues were selected as target material because these regions contain metabolically active tissues associated with neuromuscular function. Dissections were performed on ice to prevent enzymatic degradation. Immediately after dissection, tissues were frozen in liquid nitrogen and stored briefly until homogenization.

Preparation of Enzyme Extracts

Frozen thoracic and abdominal tissues were homogenized thoroughly using a pre-chilled mortar and pestle in phosphate buffer (pH 7.4). The use of physiological pH conditions is critical for preserving the native structure and activity of cholinesterases (Kushiev et al., 1994, Čolović et al., 2013). Homogenization was carried out at a constant tissue-to-buffer ratio to ensure uniform extraction efficiency across all samples.

The resulting homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C using a refrigerated centrifuge. This step allowed effective separation of cellular debris and insoluble material from the soluble enzyme fraction. The supernatants were carefully collected without disturbing the pellet and served as the enzyme source for all subsequent assays. All enzyme extracts were kept on ice during experimental procedures to prevent activity loss.

Chemicals and Reagents

Butyrylthiocholine iodide, used as a specific substrate for butyrylcholinesterase (BChE), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; Ellman reagent) were obtained from Sigma-Aldrich (USA). Phosphate-buffered saline (PBS, pH 7.4) was used throughout all experiments. All reagents were of analytical grade and prepared freshly prior to use to ensure assay reliability and reproducibility.

Determination of Butyrylcholinesterase Activity

BChE activity was determined spectrophotometrically using the classical Ellman method, which remains one of the most widely accepted and sensitive approaches for cholinesterase activity determination (Ellman et al., 1961; Holas et al., 2012). This method is based on the enzymatic hydrolysis of butyrylthiocholine, resulting in the formation of thiocholine, which reacts with DTNB to produce the yellow-colored 5-thio-2-nitrobenzoate anion.

Reaction mixtures contained phosphate buffer (pH 7.4), enzyme extract, DTNB at a final concentration of 2×10^{-3} M, and butyrylthiocholine substrate at varying concentrations. The total reaction volume was kept constant for all assays. Reactions were initiated by addition of the substrate and incubated at 30°C, a temperature selected to approximate physiological conditions and maintain enzyme stability.

Absorbance changes were monitored continuously at 412 nm using a HACH DR3900 spectrophotometer. Initial reaction rates were calculated from the linear portion of absorbance–time curves to ensure that measurements were obtained under initial-rate conditions. BChE activity was calculated using the molar extinction coefficient $\varepsilon = 17{,}500\ M^{-1}\cdot cm^{-1}$, as originally described by Ellman et al. (1961). Enzyme activity was expressed as the rate of substrate hydrolysis per unit time.

Enzyme Kinetic Analysis

To characterize the kinetic properties of BChE, substrate concentrations were systematically varied over a range sufficient to achieve enzyme saturation. For each substrate concentration, initial reaction velocities (V_0) were determined from linear absorbance changes. Care was taken to avoid substrate depletion and product inhibition during measurements.

Michaelis–Menten kinetic parameters, including the Michaelis constant (Km) and maximum reaction velocity (Vmax), were calculated using Lineweaver–Burk double reciprocal plots (Lineweaver & Burk, 1934). Although nonlinear regression methods are increasingly used, the Lineweaver–Burk approach remains valuable for comparative enzymatic studies, particularly when comparing kinetic behavior between populations under identical experimental conditions (Kushiev et al., 1994, Tulyabaev, Kushiev et al. (1995), Worek et al., 1999; Holas et al., 2012).

Methodological Considerations and Reliability

The applied methodology is based on standardized biochemical protocols widely used in cholinesterase research. Use of physiological buffer conditions, controlled temperature, and established kinetic analysis ensures reproducibility and comparability with previous insect cholinesterase studies. All assays were performed under identical experimental conditions for both locust populations, allowing reliable comparative analysis of enzymatic activity and kinetic parameters.

Results

Butyrylcholinesterase Activity and Substrate Hydrolysis

Butyrylcholinesterase (BChE) activity was reliably detected in homogenates prepared from both Moroccan and Oasis locust populations (*Gampsocleis buergeri*). To ensure that measured activity reflected enzymatic catalysis rather than non-enzymatic processes, spontaneous hydrolysis of butyrylthiocholine was assessed separately. The contribution of spontaneous hydrolysis was minimal in comparison to enzyme-mediated hydrolysis, confirming that the observed changes in absorbance were predominantly due to BChE activity.

The time-dependent increase in absorbance at 412 nm demonstrated a linear relationship during the initial reaction phase, allowing accurate determination of initial reaction velocities (V_0). Across all experiments, reaction rates increased proportionally with substrate concentration until a saturation plateau was reached, indicating classical Michaelis–Menten behavior.

Effect of Substrate Concentration on BChE Activity

The dependence of BChE activity on butyrylthiocholine concentration was evaluated for both locust populations. As shown in table 1, increasing substrate concentration resulted in a progressive increase in reaction velocity for both Moroccan and Oasis locusts. However, at equivalent substrate concentrations, Oasis locusts consistently exhibited higher reaction velocities.

Table 1. Effect of butyrylthiocholine concentration on BChE activity in Moroccan and Oasis locusts

Substrate concentration [S] (M)	Reaction velocity V ($mol\cdot L^{-1}\cdot min^{-1}$) – Moroccan	Reaction velocity V ($mol\cdot L^{-1}\cdot min^{-1}$) – Oasis
1.27×10^{-5}	6.11×10^{-7}	5.26×10^{-7}
2.50×10^{-5}	8.74×10^{-7}	1.01×10^{-6}
2.04×10^{-4}	2.35×10^{-6}	2.50×10^{-6}
2.78×10^{-4}	2.94×10^{-6}	3.21×10^{-6}

These results demonstrate a clear difference in enzymatic response between the two populations, with Oasis locusts showing enhanced BChE activity, particularly at higher substrate concentrations.

Kinetic Analysis of BChE Activity

To quantitatively characterize the observed differences, enzyme kinetics were analyzed using the Michaelis–Menten model. Reaction velocities (V) were calculated from absorbance changes according to the following relationship:

$$V = \frac{\Delta C}{\Delta t}$$

where ΔC represents the change in substrate concentration derived from absorbance values using the molar extinction coefficient (ε = 17,500 $M^{-1} \cdot cm^{-1}$), and Δt represents the reaction time.

The Michaelis–Menten equation was applied:

$$V = \frac{V_{max} * [S]}{K_m + [S]}$$

To obtain kinetic parameters, Lineweaver–Burk double reciprocal plots were constructed by linear transformation of the Michaelis–Menten equation:

$$\frac{1}{V} = \frac{K_m}{V_{max}} * \frac{1}{[S]} + \frac{1}{V_{max}}$$

Linear regression of the Lineweaver–Burk plots (Figure 1A, 1B) allowed determination of Km and Vmax values for both locust populations.

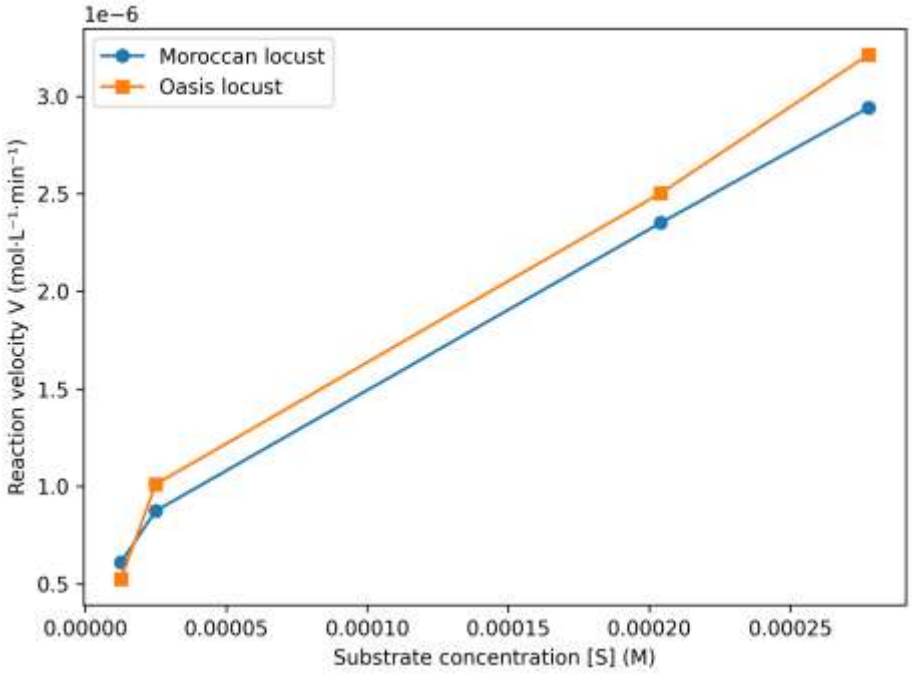

Figure 1A. Michaelis–Menten plots showing the dependence of butyrylcholinesterase activity on substrate concentration in Moroccan and Oasis locusts (*Gampsocleis buergeri*)

The figure illustrates the relationship between substrate concentration [S] (M) and reaction velocity V ($mol \cdot L^{-1} \cdot min^{-1}$) for butyrylcholinesterase (BChE) activity in Moroccan and Oasis locust populations. In both populations, enzyme activity increases progressively with increasing substrate concentration, indicating that the reaction follows classical Michaelis–Menten kinetics.

Across nearly all substrate concentrations, the Oasis locust population (orange line) exhibits higher reaction velocities compared to the Moroccan locust population (blue line). This difference becomes more pronounced at intermediate and high substrate concentrations (approximately 2.0×10^{-4} to 2.8×10^{-4} M), suggesting a higher maximal catalytic capacity (Vmax) of BChE in the Oasis locusts.

At lower substrate concentrations, the consistently higher reaction velocity observed in the Oasis population indicates a greater substrate affinity, reflected by a lower apparent Michaelis–Menten constant (Km). This suggests that BChE in Oasis locusts binds and hydrolyzes the substrate more efficiently than in Moroccan locusts.

Overall, the figure clearly demonstrates that the catalytic efficiency of butyrylcholinesterase is higher in the Oasis locust population compared to the Moroccan population. These differences likely reflect population-specific neuroenzymatic adaptation, potentially associated with ecological conditions and enhanced tolerance to environmental or chemical stressors.

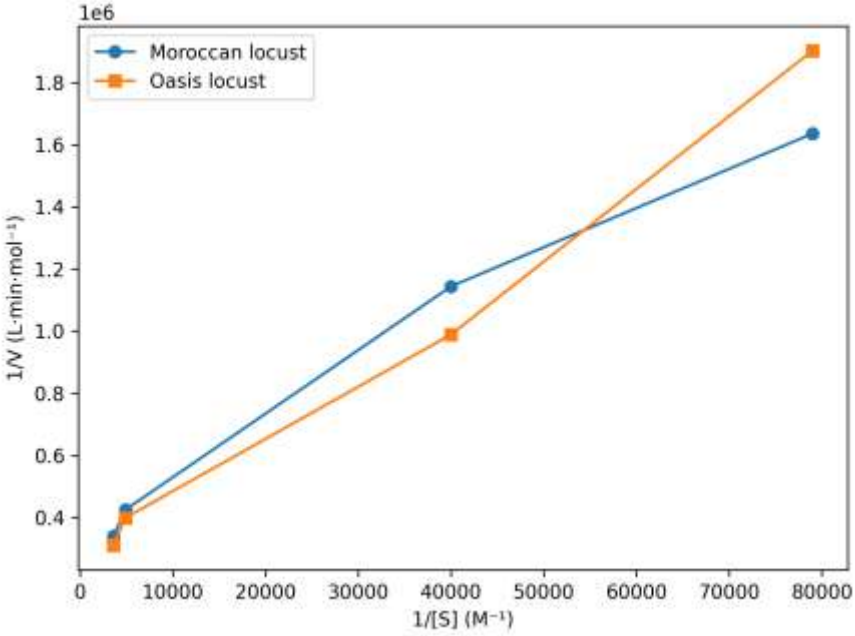

Figure 1B. Lineweaver–Burk plots used to determine Km and Vmax values of butyrylcholinesterase in Moroccan and Oasis locusts

The Oasis locust population (orange line) shows a lower y-intercept compared to the Moroccan locust population (blue line), indicating a higher maximum reaction velocity (Vmax) for BChE. In addition, the apparent x-intercept of the Oasis locust plot is shifted closer to the origin, corresponding to a lower Michaelis–Menten constant (Km) and thus higher substrate affinity.

In contrast, the Moroccan locust population exhibits a steeper slope, which is characteristic of a higher Km/Vmax ratio, indicating lower substrate affinity and reduced catalytic efficiency. These kinetic differences demonstrate that BChE in Oasis locusts operates more efficiently across the tested substrate range.

Overall, the Lineweaver–Burk analysis provides quantitative confirmation of the higher catalytic efficiency of butyrylcholinesterase in Oasis locusts compared to Moroccan locusts. The observed differences likely reflect population-specific neuroenzymatic regulation and adaptive responses to distinct ecological conditions and environmental stressors.

Comparison of Kinetic Parameters

As summarized in **Table 2**, Oasis locusts exhibited a lower Michaelis–Menten constant (Km = 6.71×10^{-5} M) compared to Moroccan locusts (Km = 8.27×10^{-5} M), indicating higher substrate affinity of BChE in Oasis locusts. In addition, the maximum reaction velocity (Vmax) was significantly higher in Oasis locusts (4.037×10^{-6} mol·L^{-1}·min^{-1}) than in Moroccan locusts (2.936×10^{-6} mol·L^{-1}·min^{-1}).

Table 2. Kinetic parameters of BChE in Moroccan and Oasis locusts

Population	Km (M)	Vmax (mol·L^{-1}·min^{-1})
Moroccan	8.27×10^{-5}	2.936×10^{-6}
Oasis	6.71×10^{-5}	4.037×10^{-6}

Summary of Experimental Findings

Overall, the results demonstrate pronounced interpopulation differences in BChE activity and kinetic behavior. The lower Km and higher Vmax values observed in Oasis locusts indicate enhanced catalytic efficiency and substrate affinity, reflecting more effective enzymatic performance. These quantitative differences provide strong experimental evidence for population-specific variation in neuroenzymatic regulation.

Discussion

The present study demonstrates clear and significant interpopulation differences in butyrylcholinesterase (BChE) activity and kinetic properties between Moroccan and Oasis locust populations (*Gampsocleis buergeri*). The lower Michaelis–Menten constant (Km) and higher maximum reaction velocity (Vmax) observed in Oasis locusts provide strong evidence for enhanced substrate affinity and catalytic efficiency of

BChE in this population. These kinetic characteristics indicate more effective neuroenzymatic regulation and suggest population-specific biochemical adaptation.

From an enzymological perspective, a lower Km reflects a higher affinity of the enzyme for its substrate, while a higher Vmax indicates an increased catalytic turnover under saturating substrate conditions. Together, these parameters imply that BChE in Oasis locusts can hydrolyze butyrylthiocholine more efficiently across a wide range of substrate concentrations. This conclusion is supported by both the Michaelis–Menten and Lineweaver–Burk plots, which consistently show higher reaction velocities and favorable kinetic intercepts for the Oasis population. Such kinetic advantages are likely to have physiological relevance, particularly under conditions where rapid detoxification or neural signal termination is required.

Variability in cholinesterase activity has been widely reported as an adaptive mechanism in insects exposed to environmental and chemical stressors. Organophosphate and carbamate compounds exert their toxic effects primarily through inhibition of cholinesterases, leading to disruption of cholinergic neurotransmission (Kushiev, 1994, Bajgar, 2004; Čolović et al., 2013). While acetylcholinesterase (AChE) has traditionally been regarded as the principal toxicological target, increasing evidence suggests that BChE plays a complementary and, in some cases, protective role. Enhanced BChE activity has been proposed to act as a biochemical buffer, reducing the effective concentration of neurotoxic compounds and thereby mitigating their impact on neural function (Brahmi et al., 2006; Dewan et al., 2008).

The higher catalytic efficiency of BChE observed in Oasis locusts may therefore contribute to increased tolerance to neurotoxic agents and other environmental stressors. Oasis habitats are often characterized by complex ecological pressures, including temperature extremes, fluctuations in water availability, soil salinity, and variable exposure to agrochemicals (Yuldasheva et al., 2026) . These conditions may select for individuals with enhanced detoxification capacity and more robust neurophysiological regulation. In this context, the observed kinetic differences in BChE activity likely reflect long-term adaptive responses rather than transient physiological variation.

Population-specific differences in enzyme kinetics have been reported in other insect species and are frequently associated with local adaptation and ecological specialization. Kushiev et al. (1994), Tulyabaev, Kushiev et al. (1995), Worek et al. (1999) and Holas et al. (2012) demonstrated that cholinesterase activity and sensitivity to inhibitors can vary substantially depending on genetic background and environmental exposure history. Such variability underscores the importance of considering population-level biochemical traits when assessing insect physiology, toxicology, and resistance mechanisms.

The use of Lineweaver–Burk analysis in the present study enabled direct comparison with previously published cholinesterase studies and provided a clear visualization of kinetic differences between the two locust populations. Although nonlinear regression methods are increasingly employed for kinetic analysis, the classical double-reciprocal approach remains valuable for comparative studies, particularly when the objective is to highlight relative differences in Km and Vmax under standardized experimental conditions. The linearity of the Lineweaver–Burk plots obtained in this study further confirms that BChE activity in both populations follows Michaelis–Menten kinetics and supports the reliability of the derived parameters.

Importantly, the observed interpopulation differences in BChE kinetics should be interpreted within a broader physiological and ecological framework. Enhanced BChE activity may not only facilitate detoxification but also contribute to maintaining neural homeostasis under fluctuating environmental conditions. Efficient hydrolysis of choline esters ensures rapid termination of synaptic signaling, preventing prolonged neuronal excitation and potential neurotoxicity. Thus, higher BChE activity may provide a selective advantage in environments where insects are exposed to multiple stress factors simultaneously.

From an applied perspective, these findings have implications for pest management strategies. Population-specific differences in cholinesterase activity may influence the efficacy of cholinesterase-targeting insecticides and contribute to differential susceptibility or resistance. Understanding such biochemical variation is essential for developing more effective and sustainable control measures that account for local adaptation and physiological plasticity.

In conclusion, the present study provides compelling evidence that BChE activity and kinetic behavior differ significantly between Moroccan and Oasis locust populations. The lower Km and higher Vmax values observed in Oasis locusts indicate enhanced catalytic efficiency, reflecting population-specific neuroenzymatic adaptation. These results extend current knowledge of insect cholinesterase function and highlight the importance of integrating biochemical, physiological, and ecological perspectives in studies of insect adaptation and pest management.

Conclusion

This study provides a clear comparative kinetic characterization of butyrylcholinesterase (BChE) activity in Moroccan and Oasis locust populations (*Gampsocleis buergeri*). Quantitative enzyme kinetic analysis demonstrated that Oasis locusts exhibit a lower Michaelis–Menten constant ($Km = 6.71 \times 10^{-5}$ M) and a higher maximum reaction velocity ($Vmax = 4.037 \times 10^{-6}$ mol·L^{-1}·min^{-1}) compared to Moroccan locusts ($Km = 8.27 \times 10^{-5}$ M; $Vmax = 2.936 \times 10^{-6}$ mol·L^{-1}·min^{-1}), indicating increased substrate affinity and enhanced

catalytic efficiency of BChE. These results provide direct biochemical evidence for population-specific neuroenzymatic adaptation in Oasis locusts, which may contribute to improved neural regulation, greater tolerance to environmental stressors, and altered sensitivity to neurotoxic compounds. Overall, the findings advance current understanding of insect neurophysiology and highlight BChE as a robust biochemical marker for assessing physiological adaptation and population-level differences relevant to ecological studies and pest management strategies.

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