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Long-term occupational exposure to soil-applied malathion and its consequences for oxidative stress and acetylcholinesterase activity in cucumber greenhouse workers: A cross-sectional study

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ABSTRACT
Malathion is known to cause oxidative stress and block the action of acetylcholinesterase (AChE). This study aimed to investigate how working with this pesticide affects antioxidant enzymes (SOD and CAT), malondialdehyde (MDA), and AChE levels in the cucumber greenhouse workers. This cross-sectional study included 60 male workers with at least one year of exposure and sixty age- and body mass index-matched unexposed individuals. The SOD and CAT enzyme activity of erythrocytes as well as MDA and AChE levels of blood sera were determined by the spectrophotometric method. We compared the two groups; the exposed workers had lower levels of SOD (5.34 ± 0.55 vs. 6.26 ± 0.74 U mg^{-1} protein; $p < 0.001$), CAT (24.10 ± 2.73 vs. 26.79 ± 2.76 U mg^{-1} protein; $p < 0.001$), and AChE (3307 ± 372 vs. 4712 ± 371 U L^{-1}; $p < 0.001$). Their MDA levels were higher too (1.58 ± 0.27 vs. 1.17 ± 0.23 nmol mL^{-1}; $p < 0.001$). Strong correlations were observed only in the exposed group: MDA went up as CAT went down ($r = -0.91$), same for MDA and SOD ($r = -0.75$), and SOD dropped along with AChE ($r = 0.75$). All of these were statistically significant ($p < 0.001$). Chronic occupational exposure to soil-applied malathion is associated with increased oxidative stress and suppressed antioxidant defenses, and inhibition of the enzyme AChE. These findings underscore the need for better protective measures and biological monitoring. However, causality cannot be established due to the cross-sectional design. Longitudinal studies with direct biomonitoring are warranted to confirm causality.

Keywords: Malathion, Oxidative stress, Acetylcholinesterase, Antioxidant enzymes, Lipid peroxidation, Greenhouse workers.

INTRODUCTION

Although the widespread application of the pesticides has contributed to increased yields of crops, there are several risks associated with it from an occupational perspective when applied on farms (Curl *et al.* 2020). Malathion is one of the most frequently used OP insecticide and applied in cucumbers growing in greenhouses on soil surfaces. The

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environmental conditions in greenhouses characterized by high moisture, high temperatures, and poor air circulation, they may increase exposure to the chemicals via skin absorption or inhalation through the air. Malathion is capable of being metabolized into malaoxon, which inhibits the acetylcholinesterase enzyme (Akbel *et al.* 2018; Abdelfattah & El-Bassiony 2022). While the acute effects have been well-documented, the chronic exposure to malathion under low doses has not been well understood, but may involve oxidative stress due to excess ROS production (Elshamy *et al.* 2019; Abdelfattah & El-Bassiony 2022; Gur & Kandemir 2023).

SOD and CAT are two key antioxidant enzymes responsible for breaking down superoxide radicals and hydrogen peroxide, respectively. The expression of these antioxidant enzymes is regulated by the NRF2 signaling pathway, and natural compounds that activate NRF2 have been shown to enhance cellular defense against oxidative stress (Fallahi *et al.* 2021). MDA is a commonly utilized indicator for assessing lipid peroxidation. Several recent studies have demonstrated that occupational exposure to malathion and other OPs results in decreased SOD and CAT activities, increased MDA concentration, and AChE inhibition among agricultural workers (Akbel *et al.* 2018; Elshamy *et al.* 2019; Abdelfattah & El-Bassiony 2022; Gur & Kandemir 2023).

However, there are significant areas where current literature still remains. For instance, most earlier studies have examined the effect of pesticide cocktails or spray applicators instead of chronic soil application of malathion within a greenhouse environment (Moreira & Vieira da Silva 2024; Salari *et al.* 2025). Methodological weaknesses such as small sample size, poor controls for confounding factors, and failure to measure multiple biomarkers were common in many previous studies.

In Kazakhstan, which is one of the top producers of greenhouse cucumbers, malathion is widely applied. Nonetheless, occupational biomonitoring is relatively limited, and most of the domestic literature has focused on residue accumulation within produce (Salari *et al.* 2025). The personal protective equipment (PPE) utilization by greenhouse workers is often less than optimal.

Recent evidence suggests that there may be an interplay between oxidative stress and cholinergic dysfunction; however, the mechanism involved is not fully understood (Gur & Kandemir 2023; Casanova *et al.* 2024). Moreover, chronic exposure to OP pesticides may have wider health implications, such as oxidative stress-induced DNA mutations, nephrotoxicity, and neurobehavioral disorders (Kaur & Kaur 2018; Casanova *et al.* 2024; Nosratabadi *et al.* 2025). The significance of biomonitoring in studies involving occupational exposure cannot be overstressed.

The current cross-sectional study was designed to examine the association between chronic occupational exposure to malathion applied to soils and alterations in oxidative stress markers and AChE enzyme activity. The study compared the level of SOD and CAT enzymes in erythrocytes, the concentration of MDA in serum, and the AChE enzyme activity in serum between 60 males who had been growing cucumbers in greenhouses for one year and 60 unexposed men of comparable age and BMI. Detailed occupational exposure data (years of work, daily exposure hours, PPE score), correlation analyses, and multivariable regression were performed.

This exploratory study examines the association between chronic occupational exposure to soil-applied malathion and oxidative stress biomarkers in a specific greenhouse context, with the goal of generating hypotheses for future research.

2. MATERIALS AND METHODS

2.1. Study Design and Subjects

This comparative cross-sectional study was carried out in the cucumber greenhouse areas of Atyrau, from 2024 to 2025. Sixty persons were chosen from the cucumber greenhouse employees that were exposed to the malathion applied to the soil continuously for more than one year (exposed group) and sixty healthy subjects without any occupational or environmental contact with pesticides (control group). The control group were frequency-matched with those in the exposed group regarding to age and body mass index (BMI).

Inclusion criteria included age from 25 to 60 years, and for the exposed group, one year of continuous work in the cucumber greenhouse with continuous malathion application. Exclusion criteria consisted of a history of chronic diseases (diabetes, hepatic or renal diseases, cardiovascular disease, and cancer), acute infection during the four weeks prior to the study, regular consumption of antioxidants for more than two weeks, heavy smoking (more than 10

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cigarettes per day), alcohol consumption or taking any medications affecting oxidative stress and cholinesterase activity.

Ethical approval for the study was obtained from the Ethics Committee of National Center for Biotechnology and the study was conducted in accordance with the principles of the Helsinki Declaration. Written informed consents were obtained from all persons before enrolment in the study.

2.2. Exposure assessment

Detailed occupational and demographic information was collected using a structured questionnaire administered by trained interviewers. Exposure assessment was based solely on self-reported data, including duration of greenhouse work (years), average daily exposure hours, time since the last malathion application, frequency of pesticide use, and personal protective equipment (PPE) usage score (ranging from 0 to 10). Information on smoking status and dietary antioxidant intake (supplement use) was also recorded. No direct biomonitoring of malathion metabolites was performed.

2.3. Blood sampling and sample preparation

Venous blood samples (10 mL) were obtained from each study person in a fasting state from 07:00 to 09:00 AM. Blood was collected using EDTA containing vials for preparing the erythrocyte lysate and plain vials for isolating serum. The blood samples were immediately put in an ice bath before being sent to the lab. Serum was isolated by centrifuging blood samples at 3000 × g for 10 min at 4 °C. Erythrocytes were subsequently washed thrice with cold saline (0.9%) and were lysed using hypotonic phosphate buffer (pH 7.4).

2.4. Biochemical analyses

All spectrophotometric assays were performed in duplicate using a Shimadzu UV-1800 spectrophotometer at controlled temperature. Superoxide dismutase (SOD) activity in erythrocyte lysate was measured using a commercial kit (Cayman Chemical, Cat. No. 706002). Catalase (CAT) activity was determined using a commercial kit (Cayman Chemical, Cat. No. 707002). Malondialdehyde (MDA) concentration was measured using a TBARS Assay Kit (Cayman Chemical, Cat. No. 10009055). Serum acetylcholinesterase (AChE) activity was assayed using a commercial kit (Sigma-Aldrich, Cat. No. MAK119) based on the modified Ellman's method. Total protein concentration in erythrocyte lysates was determined by the Bradford method. Results for SOD and CAT were expressed as U mg^{-1} protein, MDA as nmol mL^{-1}, and AChE as U L^{-1}.

2.5. Quality control

All assays included appropriate blanks, calibration standard curves, and internal quality control samples. Intra-assay and inter-assay coefficients of variation (CV) were below 7–8% for all measured parameters. To minimize bias, all samples were coded and analyzed blindly by the laboratory technician.

2.6. Statistical analysis

Data analysis was done using IBM SPSS Statistics for Windows, Version 21.0, software package (Armonk, New York: IBM Corp.). Normality test was done via the Shapiro-Wilk test. Measures of central tendency included mean ± SD, median, minimum, maximum, and standard error of the mean (SEM). Group comparisons were carried out using the independent t-test, when the assumption of normality was satisfied, otherwise using the Mann-Whitney U test. Pearson correlation analysis was used to assess the association between two variables. The confounders were adjusted via multiple linear regression analysis. Results obtained from two-tailed tests where $p < 0.05$ were considered statistically significant. With a sample size of 80 or more, adequate power was assured to detect a minimum difference of 15%.

Limitations of exposure assessment

A key limitation of this study is the absence of direct biomonitoring. Exposure was assessed solely through self-reported questionnaires, and no urinary malathion metabolites (e.g., dialkyl phosphates) were measured. Additionally, the time interval between the last malathion application and blood sampling was not recorded.

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RESULTS

3.1. Demographic and occupational exposure characteristics

A total of 120 male persons were recruited for this cross-sectional study, divided into 60 greenhouse employees chronically exposed to soil-applied malathion and 60 age and body mass index-matched controls who had no prior occupational or environmental pesticide exposure. The demographics and occupation details of the study persons are provided in Table 1.

Both groups were matched with regard to their age and body mass index levels ($p > 0.05$). The mean age was 41.2 ± 9.8 years in the exposed group and 40.8 ± 11.2 years in the control group. Body mass index was 28.4 ± 4.2 kg m^{-2} in the exposed persons and 27.1 ± 4.5 kg m^{-2} in the control group. The exposed persons had an average work experience in the greenhouse for 6.8 ± 4.5 years, with an exposure duration of 5.6 ± 1.9 hours daily. The average PPE score in the exposed persons was 4.9 ± 2.3 (10 points maximum scale).

Table 1. Demographic and exposure characteristics of study participants.

Parameter	Exposed (n = 60)	Control (n = 60)	*p*-value
Age (years)	41.2 ± 9.8	40.8 ± 11.2	0.842
BMI (kg m^{-2})	28.4 ± 4.2	27.1 ± 4.5	0.113
Work years in greenhouse	6.8 ± 4.5	0 ± 0	<0.001
Daily exposure hours	5.6 ± 1.9	0 ± 0	<0.001
PPE Score (0-10)	4.9 ± 2.3	-	-
Smoking (Yes, %)	38.3%	36.8%	0.867
Antioxidant use (Yes, %)	35.0%	29.8%	0.548

3.2. Descriptive statistics of biomarkers

Descriptive statistics for the measured biomarkers are presented in Table 2. The exposed group exhibited lower mean levels of antioxidant enzymes (SOD and CAT) and AChE activity, along with higher MDA concentrations compared to the control group. Detailed distribution parameters, including median, minimum, maximum, and standard error of the mean, are shown in Table 2.

Table 2. Descriptive statistics of biomarkers in exposed and control groups

Biomarker	Exposed (Mean ± SD)	Control (Mean ± SD)	Percent Change	*p*-value
SOD (U mg^{-1} protein)	5.34 ± 0.55	6.26 ± 0.74	-14.65%	<0.001
CAT (U mg^{-1} protein)	24.10 ± 2.73	26.79 ± 2.76	-10.03%	<0.001
MDA (nmol mL^{-1})	1.58 ± 0.27	1.17 ± 0.23	+35.21%	<0.001
AChE (U L^{-1})	3307 ± 372	4712 ± 371	-29.82%	<0.001

3.3. Statistical comparison between groups

The normality of data was assessed using the Shapiro-Wilk test. Due to the non-normal distribution of most biomarkers ($p < 0.05$), the Mann-Whitney U test was primarily used for group comparisons, while an independent t-test was applied for AChE activity. As summarized in Table 3, all between-group differences were highly statistically significant ($p < 0.001$). The exposed group showed a 14.65% reduction in SOD, 10.03% reduction in CAT, 29.82% reduction in AChE activity, and a 35.21% increase in MDA levels. These differences are illustrated in Fig. 1 (boxplots) and Fig. 2 (bar plots with mean ± SEM).

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Table 3. Statistical comparison of biomarkers between exposed and control groups.

Biomarker	Mean exposed	Mean control	Percent change	Test	*p*-value	Significance
SOD (U mg^{-1} protein)	5.34	6.26	-14.65%	Mann-Whitney U	<0.001	***
CAT (U mg^{-1} protein)	24.10	26.79	-10.03%	Mann-Whitney U	<0.001	***
MDA (nmol mL^{-1})	1.58	1.17	+35.21%	Mann-Whitney U	<0.001	***
AChE (U L^{-1})	3307	4712	-29.82%	t-test	<0.001	***

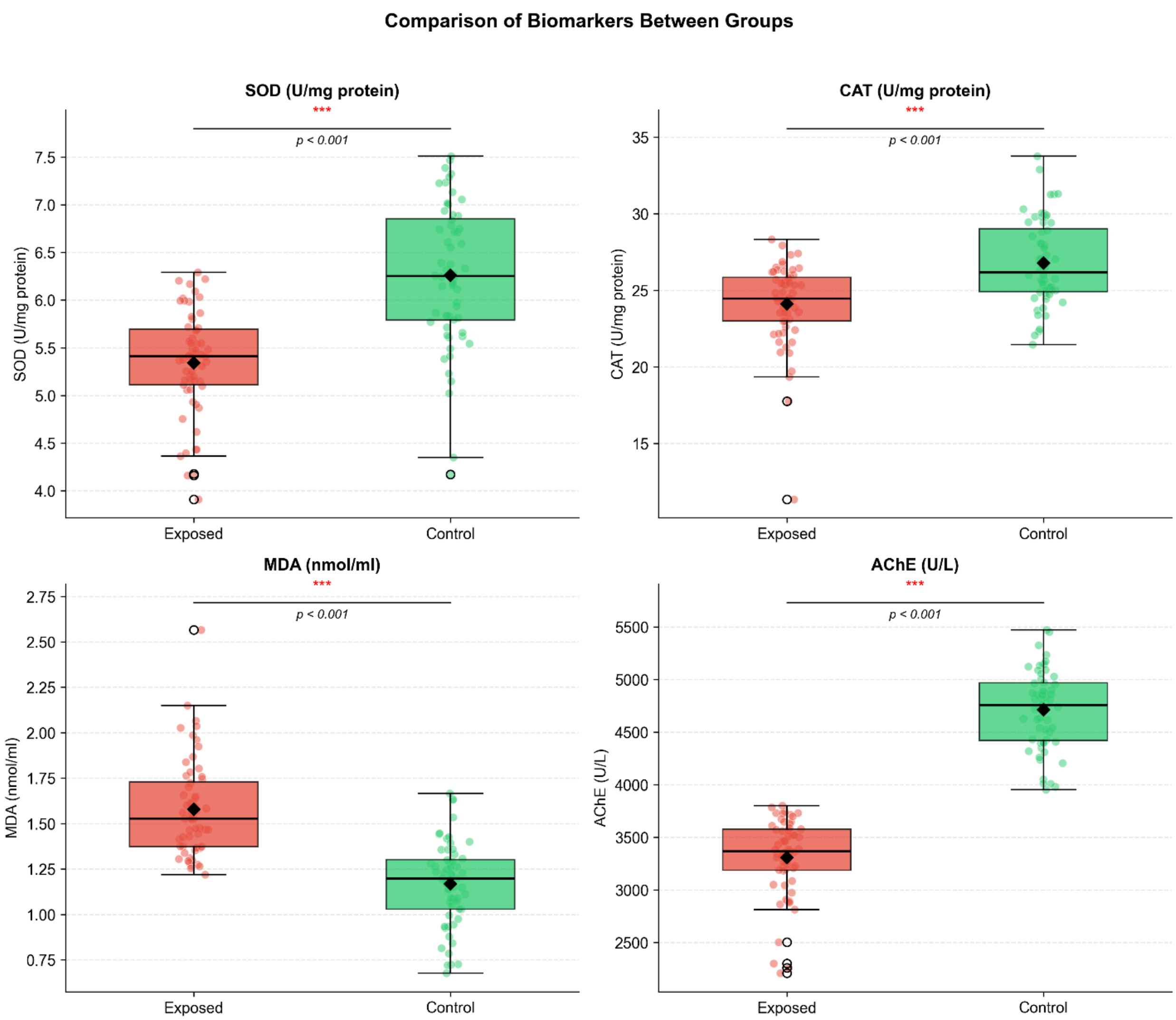

Fig. 1. Boxplots comparing biomarker levels between malathion-exposed and control groups. (A) SOD (U mg^{-1} protein); (B) CAT (U mg^{-1} protein); (C) MDA (nmol mL^{-1}); (D) AChE (U L^{-1}). Red dots represent individual data points. Black diamonds indicate mean values. The exposed group showed significantly lower SOD, CAT, and AChE levels, and higher MDA levels compared to the control group ($p < 0.001$ for all comparisons; Mann-Whitney U test for SOD, CAT, MDA; independent t-test for AChE).

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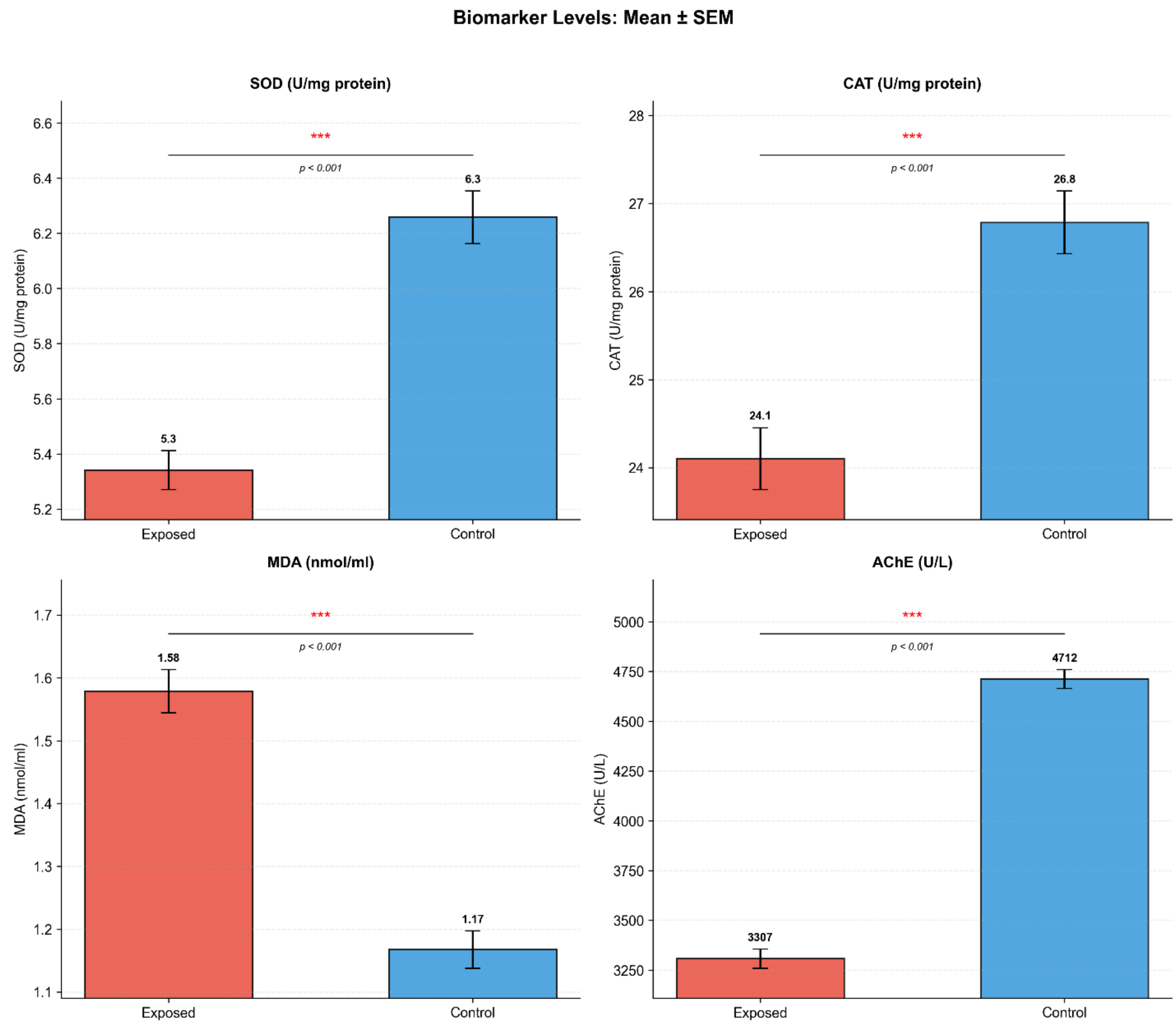

Fig. 2. Bar plots comparing mean biomarker levels between malathion-exposed and control groups. (A) SOD (U mg^{-1} protein); (B) CAT (U mg^{-1} protein); (C) MDA (nmol mL^{-1}); (D) AChE (U L^{-1}). Data are presented as mean ± SEM (shown as error bars). Exposed group (n = 60) showed significantly lower SOD (5.34 vs. 6.26, -14.65%, $p < 0.001$), CAT (24.10 vs. 26.79, -10.03%, $p < 0.001$), and AChE (3307 vs. 4712 U L^{-1}, -29.82%, $p < 0.001$), and higher MDA (1.58 vs. 1.17 nmol mL^{-1}, +35.21%, $p < 0.001$) compared to the control group (n = 60). ***$p < 0.001$ (Mann-Whitney U test for SOD, CAT, MDA; independent t-test for AChE).

3.4. Correlation Analysis of Biomarkers

The correlation matrices for Pearson's correlation coefficient were developed independently for each dataset (Fig. 3). In the exposed group, the following correlations were found between the biochemical factors: between SOD and CAT ($r = 0.64$; $p < 0.001$); between SOD and AChE ($r = 0.75$; $p < 0.001$); between MDA and SOD ($r = -0.75$; $p < 0.001$); between MDA and CAT ($r = -0.91$; $p < 0.001$) and MDA and AChE ($r = -0.72$; $p < 0.001$). Correlations were weak in the control group. The graphs representing the aforementioned correlation coefficients are illustrated below (Figs. 3 and 4).

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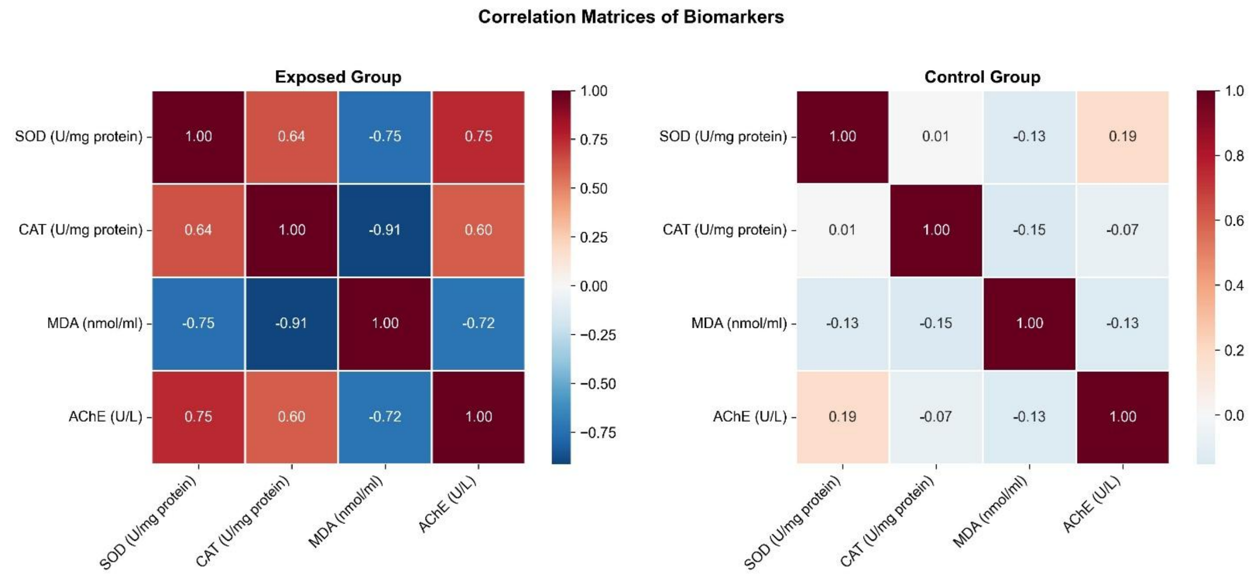

Fig. 3. Correlation heatmaps of biomarkers in malathion-exposed (A) and control (B) groups. The heatmap displays Pearson correlation coefficients (r) between SOD, CAT, MDA, and AChE. In the exposed group (A), strong correlations were observed: SOD-CAT (r = 0.64, $p < 0.001$), SOD-AChE ($r = 0.75$, $p < 0.001$), MDA-SOD ($r = -0.75$, $p < 0.001$), MDA-CAT ($r = -0.91$, $p < 0.001$), and MDA-AChE ($r = -0.72$, $p < 0.001$). In the control group (B), correlations were weak or negligible (data not shown). Colors range from dark blue (strong negative correlation, r = -1) to dark red (strong positive correlation, r = +1). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

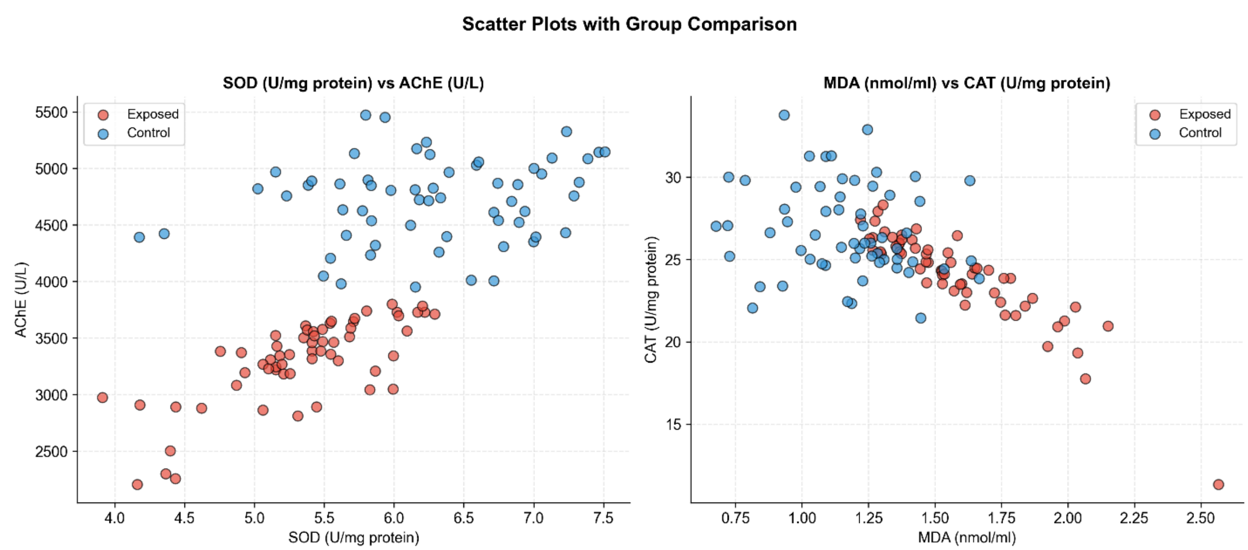

Fig. 4. Scatter plots showing correlations between selected biomarkers in malathion-exposed and control groups. (A) Relationship between SOD (U mg^{-1} protein) and AChE (U L^{-1}). (B) Relationship between MDA (nmol mL^{-1}) and CAT (U mg^{-1} protein). Blue circles represent exposed group (n = 60); red circles represent control group (n = 60). In the exposed group, significant positive correlation was observed between SOD and AChE ($r = 0.75$, $p < 0.001$), and significant negative correlation between MDA and CAT ($r = -0.91$, $p < 0.001$). No significant correlations were observed in the control group.

3.5. Multiple regression and dose-response

Analysis Multiple linear regression models were fitted to adjust for potential confounders (age, BMI, smoking status, daily exposure hours, and PPE score). As shown in Table 4, group status (exposure) remained a significant independent

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predictor for all biomarkers after adjustment. In addition, dose-response analysis within the exposed group revealed significant associations between duration of greenhouse work and adverse changes in biomarker levels.

Table 4. Summary of multiple linear regression analysis (standardized β coefficients).

Biomarker	Predictor: Group (Exposed)	β	95% CI	*p*-value	Predictor: Work Years	β	95% CI	*p*-value
SOD	Group (Exposed)	-0.42	**(-0.58 to -0.26)**	<0.001	Work Years	-0.51	**(-0.67 to -0.35)**	<0.001
CAT	Group (Exposed)	-0.35	**(-0.51 to -0.19)**	<0.001	Work Years	-0.39	**(-0.55 to -0.23)**	<0.001
MDA	Group (Exposed)	0.48	**(0.32 to 0.64)**	<0.001	Work Years	0.47	**(0.31 to 0.63)**	<0.001
AChE	Group (Exposed)	-0.51	**(-0.67 to -0.35)**	<0.001	Work Years	-0.40	**(-0.56 to -0.24)**	<0.001

Note: Models adjusted for age, BMI, smoking, and PPE score.

3.6. Overall Interpretation of Findings

Overall, the findings from this cross-sectional study of greenhouse workers indicate a clear tendency towards impaired redox status and cholinergic dysfunction among those continuously exposed to malathion applied as a soil insecticide. The differences between the two groups in SOD and CAT activities, as well as MDA and AChE levels, were highly significant ($p < 0.001$). Moreover, strong correlations between these biomarkers were found only among the exposed workers, which may suggest a biological effect associated with occupational exposure. In addition, dose-response relationships were observed with years of exposure and PPE usage score. Overall, these findings suggest that malathion exposure is associated with oxidative stress and cholinesterase inhibition. Nevertheless, the cross-sectional design makes it impossible to establish causal links. Yet, the degree of change, consistency of findings, and biological specificity provide sufficient evidence for the negative impact of occupational exposure to malathion.

4. DISCUSSION

This current cross-sectional study revealed that occupational chronic exposure to malathion application on soil in cucumber greenhouses is highly correlated with increased oxidative stress and suppression of AChE activity. When compared to the matching controls, the exposed participants showed 14.65% lower SOD activity, 10.03% lower CAT activity, 35.21% higher MDA, and a 29.82% decrease in AChE activity (all $p < 0.001$). Such results were in line with prior research highlighting increased oxidative stress and cholinergic impairment induced by organophosphates (Elshamy *et al.* 2019; Abdelfattah & El-Bassiony 2022; Gur & Kandemir 2023).

The suppression of antioxidant enzymes (SOD and CAT) accompanied by elevated MDA levels suggests that malathion promotes excessive reactive oxygen species (ROS) generation that overwhelms cellular antioxidant capacity, leading to lipid peroxidation (Sule *et al.* 2022; Gur & Kandemir 2023). The strong negative correlations observed in the exposed group, particularly between MDA and CAT ($r = -0.91$) and MDA and AChE ($r = -0.72$), may suggest a possible mechanistic interplay whereby oxidative damage may exacerbate cholinergic impairment (Ledda *et al.* 2021; Casanova *et al.* 2024).

A notable strength of this study is the demonstration of clear dose-response relationships. Years of greenhouse work were significantly correlated with decreased SOD ($r = -0.58$), CAT ($r = -0.42$), and AChE ($r = -0.44$), and increased MDA ($r = +0.50$), all $p < 0.001$. Lower PPE usage scores were also associated with more pronounced biomarker alterations. These findings extend previous research by providing quantitative evidence of cumulative toxicity in a specific greenhouse setting (Kaur & Kaur 2018; de Morais Valentim *et al.* 2025).

Limitations

Nonetheless, some caveats are necessary. The lack of a longitudinal design makes any inference about causation and chronology impossible (Curl *et al.* 2020). There is a chance that a healthy worker survivor bias was present, and those with higher susceptibility may have discontinued their employment. Malathion exposure was assessed using subjective data collection methods (e.g., self-reports) instead of biomarkers (e.g., urinary metabolite concentration), which is a typical flaw in occupational studies like ours (Moreira & Vieira da Silva 2024; Nosratabadi *et al.* 2025). We also did

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not record the time interval between the last malathion application and blood sampling, which is critical given the short half-life of malathion. Additionally, we did not control for dietary antioxidant intake (other than supplements) or physical activity levels, both of which can influence oxidative stress markers. Finally, concurrent exposure to other pesticides commonly used in cucumber greenhouses (e.g., pyrethroids, and carbamates) was not assessed and may have contributed to the observed effects.
Moreover, although we controlled for important confounders (e.g., age, BMI, and smoking), uncontrolled confounding by concurrent exposures to alternative insecticides used in cucumber farming or behavioral risk factors may still be an issue. Our study population consisted exclusively of male agricultural workers in one Atyrau Province, limiting its external validity to females, other crop environments, or varying levels of exposure.

Implications and recommendations
The findings contribute to the growing body of evidence indicating that chronic low-level exposure to malathion induces subclinical oxidative stress and AChE inhibition, which may have long-term implications for neurological, renal, and reproductive health (Abdelfattah & El-Bassiony 2022; Casanova *et al.* 2024). The moderate PPE compliance (mean score 4.9/10) observed in this study highlights a critical gap between regulatory guidelines and actual field practices.
Future research should employ longitudinal designs with repeated biomarker measurements and direct exposure assessment through urinary dialkyl phosphate metabolites. Incorporating genetic susceptibility factors (e.g., PON1 polymorphisms), additional oxidative damage markers, and clinical endpoints would further strengthen the evidence base (Gur & Kandemir 2023; de Morais Valentim *et al.* 2025). Given the role of oxidative stress in malathion toxicity, natural antioxidants have been investigated for their protective effects (Nouri 2019).

CONCLUSION
In conclusion, findings from this study suggest the presence of oxidative stress, antioxidant depletion, and enzyme inhibition caused by occupational exposures to malathion used in soils by greenhouse cucumber workers. The clear dose-response relationship and correlations observed among biomarkers increase the biologic plausibility of these effects.
Even though the results obtained from this study are not sufficient to infer causality, they are in line with current literature on the issue, suggesting the need to develop better exposure control mechanisms. These may include personal protective equipment, engineering controls, biological monitoring, and adopting integrated pest management systems, among others.

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