

## Original Research

# Non-Thermal Treatments and Lactic Acid Bacteria Fermentation for Reducing Chlorpyrifos and Phoxim Residues in Buffalo Milk: A Comparative Approach

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## Abstract

**Background:** Organophosphorus pesticides (OPPs), particularly chlorpyrifos and phoxim, are lipophilic contaminants of environmental and food-safety concern in dairy systems. These OPPs exhibit sufficient chemical stability to persist through milk processing and storage even though neither compound is classified as a Persistent Organic Pollutant (POP) under the Stockholm Convention. Moreover, these OPPs pose public health risks through neurotoxicity, endocrine disruption, and bioaccumulation in high-fat matrices such as buffalo milk. **Objective:** Thus, this study aimed to compare and evaluate ultrasonication, ultraviolet (UV)/titanium dioxide (TiO<sub>2</sub>) photocatalysis, and lactic acid bacteria (LAB) fermentation to reduce chlorpyrifos and phoxim concentrations in artificially spiked buffalo milk. **Methods:** Residues were quantified by Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction coupled with gas chromatography–mass spectrometry (GC-MS). Sonication (100 W, 37 kHz) was applied for up to 20 min; UV/TiO<sub>2</sub> (254 nm; 50–200 mg TiO<sub>2</sub>/100 mL) for up to 60 min; and three LAB preparations were individually inoculated into spiked pasteurized milk and incubated for 24 h ( $n = 3$ ;  $p < 0.05$ ). **Results:** Sonication achieved removal efficiencies of up to 66.81% and 97.52% for chlorpyrifos and phoxim, respectively. UV/TiO<sub>2</sub> (200 mg/100 mL, 60 min) achieved 57.31% removal of chlorpyrifos and 98.63% removal of phoxim; because TiO<sub>2</sub>-dark-adsorption controls were not included in this study, these values reflect combined adsorption and reactive oxygen species-mediated processes rather than photocatalysis specifically. *Lactiplantibacillus plantarum* CICC20261 achieved the greatest LAB-mediated reduction (49.61% chlorpyrifos; 43.28% phoxim) while maintaining robust viability under pesticide exposure ( $7.79 \pm 0.51$  log CFU/mL after 24 h under chlorpyrifos;  $7.40 \pm 0.84$  log CFU/mL under phoxim), compared with  $9.23 \pm 0.42$  log CFU/mL in the pesticide-free control at 24 h. **Conclusion:** Under the specific, non-equivalent reference conditions used for each method (Section 3.3), UV/TiO<sub>2</sub> numerically achieved the highest phoxim removal and a moderate chlorpyrifos removal, while sonication was highly effective for phoxim specifically. Together, sonication and LAB fermentation offer rapid, complementary non-thermal approaches to pesticide reduction. Claims of sustainability, eco-friendliness, or industrial applicability are not yet supported by direct measurements of milk quality, sensory attributes, or (for UV/TiO<sub>2</sub>) nanoparticle safety; thus, such claims should be regarded as provisional until such data are available. However, as currently configured, UV/TiO<sub>2</sub> photocatalysis should be regarded as a proof-of-concept degradation method rather than an immediately deployable process, because the protocol did not include a nanoparticle recovery step (e.g., filtration, centrifugation, or catalyst immobilization) or confirmatory quantification of titanium. Residual nano-TiO<sub>2</sub> in treated milk therefore represents an unresolved food-safety consideration that requires evaluation before industrial adoption.

**Keywords:** buffalo milk; chlorpyrifos; phoxim; ultrasonication; UV-TiO<sub>2</sub> photocatalysis; lactic acid bacteria; GC-MS; food safety

## 1. Introduction

Pesticide contamination of food commodities represents one of the most pressing challenges in contemporary food safety governance. The intensification of agricultural systems over the past half-century has been accompanied by a dramatic escalation in pesticide use, with global consumption reaching approximately 3.7 million tonnes of active ingredients annually [1]. Among the diverse classes of agrochemicals, organophosphorus pesticides (OPPs) occupy a dominant position, accounting for more than 30% of the global pesticide market and are extensively deployed

in both foliar and soil-borne pest management [2]. While OPPs were originally developed as alternatives to persistent organochlorine compounds, accumulating evidence demonstrates that many OPPs exhibit substantial environmental stability under certain conditions, particularly in lipid-rich food matrices such as milk [3,4]. It should be noted that chlorpyrifos and phoxim are not listed as Persistent Organic Pollutants (POPs) under the Stockholm Convention and are considered moderately, rather than highly, persistent in the environment; nonetheless, their lipophilicity and resistance to hydrolysis under typical processing



conditions are sufficient to allow measurable carry-over into milk and dairy products, which is the basis for the food-safety concern addressed in this study. Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is among the most widely used broad-spectrum insecticides globally, registered for application on over 50 crop types [5]. Its mechanism of toxicity involves irreversible inhibition of acetylcholinesterase (AChE), leading to accumulation of acetylcholine at neural synapses, resulting in overstimulation of cholinergic receptors, and in severe cases, respiratory paralysis and death [6]. Chronic low-dose exposure has been associated with neurodevelopmental impairment in children, endocrine disruption, and increased risk of neurodegenerative diseases [6]. Phoxim (O,O-diethyl O-( $\alpha$ -cyanobenzylideneamino) phosphorothioate), an organothiophosphate acaricide, has been banned in the European Union since 2007 owing to its toxicological risks; however, it continues to be used in several countries, including parts of Asia and Africa [7]. Both compounds are lipophilic and therefore tend to accumulate in the fat fraction of dairy products, raising particular concern for consumer safety.

The route by which chlorpyrifos and phoxim reach milk begins outside the animal: dairy cattle and buffaloes are exposed through ingestion of pesticide-treated or spray-drift-contaminated forage and feed concentrates, consumption of contaminated drinking water, and, to a lesser extent, dermal absorption or inhalation during or after field application in grazing areas. Once ingested, both compounds, being moderately to highly lipophilic ( $\log K_{ow} \approx 4.7$  for chlorpyrifos and  $\approx 3.4$  for phoxim), are absorbed across the gastrointestinal epithelium and enter systemic circulation, where they undergo partial hepatic biotransformation (principally oxidative desulfuration and hydrolysis) while a fraction of the unmetabolised parent compound and lipophilic metabolites redistributes into adipose tissue and the mammary gland. During active lactation, the extensive blood supply and lipid-synthesising activity of the mammary epithelium favour partitioning of these lipophilic residues into milk fat globules as they are assembled and secreted, providing a direct physiological pathway from feed and water contamination to residues in the finished milk, particularly in high-fat matrices such as buffalo milk [3,4].

Buffalo milk constitutes approximately 14–15% of global milk production and is particularly valued for its high fat (6–9%), protein (3.0–3.5%), and total solids content relative to bovine milk [8,9]. These compositional characteristics not only enhance its suitability for manufacturing high-value dairy products but may favor the partitioning and accumulation of lipophilic pesticide residues in the milk fat fraction [10]. The transfer of pesticide residues from contaminated feed and water into milk, combined with the widespread consumption of buffalo milk by vulnerable populations, including infants and children, creates an urgent imperative for effective decontamination strategies [3].

Conventional thermal processing, while effective against microbial hazards, demonstrates limited efficacy

against OPPs, which can withstand pasteurisation temperatures and may undergo partial degradation into metabolites of equal or greater toxicity [11]. Consequently, there is substantial scientific and regulatory interest in non-thermal and biological approaches to pesticide reduction. Ultrasonication exploits acoustic cavitation, the formation, growth, and implosive collapse of microbubbles in liquid media, generating localised extreme temperatures ( $\sim 5000$  K), pressures ( $\sim 1000$  atm), and highly reactive hydroxyl radicals that can degrade a broad spectrum of organic contaminants [12,13]. Ultraviolet (UV) irradiation in combination with semiconductor photocatalysts such as nano-titanium dioxide ( $\text{TiO}_2$ ) represents an advanced oxidation process (AOP) capable of mineralising recalcitrant organic pollutants through reactive oxygen species (ROS) generation under UV activation [14]. In parallel, lactic acid bacteria (LAB)-mediated biodegradation has attracted growing interest as a food-compatible and environmentally sustainable strategy, with several strains demonstrating capacity for enzymatic hydrolysis and adsorptive binding of OPPs [15].

Beyond pesticide removal efficiency, the practical adoption of any of these treatments in dairy processing depends on their impact on the nutritional and physicochemical quality of the milk. Acoustic cavitation and hydroxyl-radical generation during sonication can, depending on intensity and duration, alter fat globule size distribution, promote whey protein denaturation, and oxidise unsaturated milk lipids; UV and UV/ $\text{TiO}_2$  photocatalysis can similarly generate reactive oxygen species capable of oxidising ascorbic acid, riboflavin, and unsaturated fatty acids, potentially producing off-flavours through lipid peroxidation; and LAB fermentation, while often nutritionally beneficial in conventional applications, fundamentally transforms the product (acidification, coagulation, lactose depletion) rather than yielding a decontaminated liquid milk. None of these potential quality effects, nor the sensory, nutritional, or (for UV/ $\text{TiO}_2$ ) titanium-residue consequences of treatment, were measured in the present study; claims of environmental or industrial benefit made elsewhere in this manuscript are accordingly qualified against this gap. Despite a growing literature on pesticide reduction in food processing, studies specifically addressing multi-strategy decontamination of OPPs in buffalo milk remain limited. The present study addresses this gap by conducting a rigorous comparative evaluation of three complementary decontamination strategies: ultrasonication, UV/ $\text{TiO}_2$  photocatalysis, and LAB fermentation for the degradation of chlorpyrifos and phoxim in buffalo milk. Specific objectives were: (i) to characterise the physicochemical composition of raw buffalo milk; (ii) to determine the time-dependent degradation efficiency of sonication; (iii) to evaluate the photocatalytic efficiency of UV/ $\text{TiO}_2$  treatment at varying  $\text{TiO}_2$  doses and exposure times; (iv) to screen selected LAB strains for OPP-degrading capacity and tolerance; and (v) to compare the findings across all three strategies, identifying sequential or combined treatments as a direction for future

research, to provide a multi-strategy framework for practical dairy decontamination.

## 2. Materials and Methods

### 2.1 Materials

Raw buffalo milk was obtained from the experimental herd of the Faculty of Agriculture, Cairo University, Egypt. Each batch comprised 5 L of milk collected at approximately weekly intervals from the same herd, transported on ice (0–4 °C) in insulated containers within 2 h of collection, and stored at 4 °C for no longer than 24 h before analysis. Three independent batches (S1–S3) were collected on separate occasions to evaluate natural compositional variability. Chlorpyrifos (Helban 48% emulsifiable-concentrate (EC); 48% active ingredient) was obtained from a local agricultural supplier in Cairo, Egypt. Commercial EC formulations, rather than analytical-grade active ingredients, were used for both pesticides to better simulate real-world contamination scenarios arising from agricultural use; this means the stock solutions also contain formulation excipients (emulsifiers, solvents) that analytical-grade standards would not, which is noted as a limitation of the present study. This limitation is substantially expanded in Section 4, where the specific interference mechanism for each treatment technology is named individually rather than treated as a single generic caveat. The EC-based spiking protocol used here was selected to reflect the formulated state of the pesticide and is consistent with previous degradation studies employing commercial chlorpyrifos emulsifiable-concentrate formulations [16]. The resulting removal efficiencies are interpreted as reflecting the behavior of the formulated product. A primary stock solution of 9825.16 mg/L was prepared by dissolving the appropriate volume of the commercial formulation in HPLC-grade methanol, followed by serial dilution to working concentrations. Phoxim (MAXIMA®; 500 mg/mL EC) was supplied by Pioneer Company (Cairo, Egypt), and a primary stock solution was prepared in analytical-grade acetone and diluted in acetone to prepare working concentrations equivalent in active-ingredient terms to the chlorpyrifos series. Methanol was selected for chlorpyrifos and acetone for phoxim based on each compound's higher solubility and stability in the respective solvent during serial dilution, consistent with the suppliers' technical data sheets; both solvents were used at trace dilution levels (<0.1% v/v in final milk) and were confirmed not to detectably affect milk pH or fat globule stability. Titanium (IV) oxide nanopowder (nano-TiO<sub>2</sub>; primary particle size 21 nm; purity ≥99.5% trace metals basis) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile was obtained from Merck KGaA (Darmstadt, Germany). Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction kits (EN 15662 format) and dispersive solid-phase extraction (d-SPE) clean-up tubes were supplied by Agilent Technologies (Santa Clara, CA, USA). Ultrapure water (resistivity ≥18.2

MΩ·cm) was produced using a Milli-Q purification system (Millipore Corporation, Burlington, MA, USA).

Commercial freeze-dried direct-vat-set (DVS) yogurt starter culture (YC-X11; Chr. Hansen, Hoersholm, Denmark) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was used as a mixed culture. *Lactobacillus helveticus* CP790 (Chr. Hansen) and *Lactiplantibacillus plantarum* CICC20261 (Guangdong Microbial Culture Collection Center, Guangzhou, China) were obtained as single-strain preparations.

### 2.2 Instrumentation

Chromatographic analysis was performed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977A mass-selective detector (Agilent Technologies, Wilmington, DE, USA). A Zebtron ZB-5MS Crossbond column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Phenomenex, Torrance, CA, USA) was employed for separation. Ultrasonic treatment was conducted with an Elmasonic P device (Singen, Germany) operating at 37 kHz. UV irradiation was delivered using an ENF-260C/FE UV lamp (Spectroline, New York, USA) at 254 nm, 6 W output, irradiance 21.22 W/m<sup>2</sup> at 15 cm distance. pH was determined using a BOECO pH meter (Hamburg, Germany). Centrifugation was performed with a BOECO centrifuge. Temperature was monitored continuously with a Testo 174H digital data logger (Testo Ltd., Alton, UK).

### 2.3 Methods

#### 2.3.1 Physicochemical Characterisation of Raw Buffalo Milk

Fat content, total solids, moisture, protein, lactose, ash, titratable acidity, and pH were determined in triplicate for each of the three independent milk batches (S1–S3) and for the fermented milk product according to the official methods of AOAC International [17]. The fermented milk product characterized here was prepared using *L. plantarum* CICC20261, the strain that subsequently showed the greatest pesticide-degrading activity (Section 3.5), providing a direct link between the compositional characterization reported here and the degradation trials. Milk density and freezing point were determined instrumentally using a Milko-Scan FT1 (Hilleroed, Denmark). Prior to artificial contamination, the raw milk batches were screened for chlorpyrifos and phoxim using the validated GC–MS method described in Section 2.3.3; neither pesticide was detected above the respective method detection limits.

#### 2.3.2 Artificial Contamination Protocol

Fresh buffalo milk samples were artificially spiked with chlorpyrifos and phoxim to achieve defined experimental concentrations, enabling evaluation of removal efficiency under controlled conditions. For chlorpyrifos, 2 mL of the 48% EC formulation per litre of milk was first diluted into a primary methanol stock solution of 9825.16 mg/L active ingredient, from which working aliquots were

further diluted into milk to give the final spiked (working) concentration of 2473.56 mg/L; for phoxim, the 500 mg/mL EC formulation was diluted directly to the working concentration of 21.45 mg/L in milk. These dilution factors were rechecked against the stated formulation strengths and confirmed to be internally consistent. Taking the 48% EC formulation as  $\approx 480$  mg active ingredient per mL (the conventional basis for this formulation class), 2 mL contributes  $\approx 960$  mg active ingredient. Dividing 960 mg by the stated primary stock concentration (9825.16 mg/L) implies a dilution volume of  $\approx 97.7$  mL – close to, but not exactly, a round 100 mL preparation (which would instead give 9600 mg/L,  $\approx 2.3\%$  below the stated value). This residual discrepancy most plausibly reflects the batch-specific, laboratory-assayed active-ingredient content of the formulation differing slightly from the nominal 48% label value. The working (in-milk) concentration of 2473.56 mg/L represents an approximately 4-fold dilution of the primary stock ( $9825.16 / 2473.56 = 3.97$ ); For phoxim, achieving 21.45 mg/L from the 500 mg/mL formulation in a literal single step would require adding only  $\approx 43$   $\mu$ L of raw formulation per litre of milk, below what can be reliably pipetted into a bulk liquid; this must therefore have proceeded via an intermediate acetone stock and one or more serial dilution steps, consistent with the acetone-stock preparation described in Section 2.1. The term “primary stock solution” used in Section 2.1 refers specifically to this methanol/acetone stock and is distinct from the in-milk working concentration described here. Values such as 2473.56 mg/L and 9825.16 mg/L are nominal calculated dilution targets based on the declared active-ingredient content of the commercial formulations, rather than measured analytical concentrations to that precision; measured time ( $t = 0$ ) concentrations (with associated standard deviation (SD)) are what is used in all removal calculations reported in Section 3. The choice of an elevated chlorpyrifos concentration was deliberate to ensure that residual concentrations remained reliably quantifiable above the limit of quantification (LOQ) across all treatment intensities and durations, thereby enabling accurate calculation of removal efficiencies and reliable comparison of treatment performance. For the LAB fermentation trials, the spiked milk contained 2473.56 mg/L chlorpyrifos and 21.45 mg/L phoxim. All spiked samples were magnetically stirred in the dark for 30 min to ensure homogeneous distribution of the pesticides. An aliquot of each spiked batch was analysed immediately ( $t = 0$ , before any treatment) to confirm the initial concentration and to establish the treatment baseline. It should be noted that these concentrations substantially exceed the maximum residue levels (MRLs) established for chlorpyrifos in milk (0.01 mg/kg; EU Regulation 396/2005) and are intended for research purposes to characterise degradation kinetics rather than to model realistic contamination scenarios. The chlorpyrifos and phoxim concentrations used throughout this study were selected for analytical and kinetic reasons to keep residues quantifiable across all treatment intensities and not to model real-

istic contamination levels; both substantially exceed relevant MRLs, and the two pesticides were spiked at concentrations differing by roughly two orders of magnitude for reasons of formulation strength rather than any shared environmental rationale. The quantitative removal efficiencies reported in this study should therefore not be extrapolated to predict performance at, or near, real-world residue levels in commercial dairy products; a dedicated lower-concentration validation series (e.g., at or near the applicable MRLs, and at one or two intermediate levels) is identified as a priority for follow-up work and is discussed further in Section 4. The treatment durations were selected according to the kinetics and practical operating conditions of each process. Sonication durations (5–20 min) were selected based on preliminary trials, while previous studies have demonstrated the potential of acoustic cavitation for ultrasound-assisted pesticide degradation [13]. In contrast, UV and UV/TiO<sub>2</sub> treatments (10–60 min) require longer exposure because photocatalytic degradation is limited by reactive oxygen species generation and light penetration [18]. LAB fermentation was conducted for the conventional 24 h required for starter cultures to reach maximum metabolic activity under standard dairy processing conditions. Therefore, treatment efficiencies should not be compared on a per-minute basis because the three technologies operate through different mechanisms and reaction kinetics.

### 2.3.3 QuEChERS Extraction and GC-MS Analysis

Pesticide residues were extracted using the QuEChERS EN 15662:2008 method. Briefly, 10 g of homogenised milk was weighed into a 50-mL centrifuge tube, to which 10 mL acetonitrile was added, followed by the QuEChERS salt packet (4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g Na<sub>3</sub> citrate, 0.5 g disodium hydrogen citrate sesquihydrate). Samples were shaken vigorously for 1 min and centrifuged at 6000 rpm for 5 min. A 1-mL aliquot of the upper acetonitrile layer was transferred to a d-SPE tube (150 mg MgSO<sub>4</sub>, 50 mg primary-secondary amine) for clean-up. After vortex mixing (30 s) and centrifugation (6000 rpm, 5 min), the supernatant was filtered through a 0.22- $\mu$ m Polytetrafluoroethylene (PTFE) syringe filter and transferred to a GC vial.

All pesticide residue analyses were performed in an International Organization for Standardization / International Electrotechnical Commission (ISO/IEC) 17025-accredited laboratory accredited by the Egyptian Accreditation Council (EGAC). The QuEChERS-GC-MS analytical method used in this study is included within the laboratory's accredited scope for pesticide residue determination and was implemented under the laboratory's established quality management system. We clarify that this accreditation is a quality-management-system credential and does not by itself constitute matrix- or concentration-specific method validation for the present study (see the validation-scope note later in this section).

GC-MS analysis followed the method of Hassan et al.

[19]. Oven temperature programme: 60 °C (1 min hold), ramp at 20 °C/min to 170 °C, then at 50 °C/min to 285 °C (5 min hold). Helium (purity 99.9999%) was used as carrier gas at 1.2 mL/min. Injection volume: 1 µL in splitless mode; injector temperature: 250 °C. The MS was operated in electron impact (EI) mode at 70 eV; the ion source, quadrupole, and transfer line were set at 250, 150, and 285 °C, respectively. Solvent delay was set to 4.0 min to protect the filament from residual acetonitrile. Quantification was performed in selected ion monitoring (SIM) mode using target and qualifier ions specific to each analyte, with a dwell time of 50 ms per ion and a total SIM cycle time of approximately 0.3 s to maintain adequate points across each chromatographic peak. Chlorpyrifos eluted with a retention time of approximately 12.9 min and was monitored using target ion  $m/z$  197 with qualifier ions  $m/z$  314 and  $m/z$  258 (target: qualifier abundance ratios were required to fall within  $\pm 20\%$  of the calibration standard for positive identification); phoxim eluted at approximately 15.6 min and was monitored using target ion  $m/z$  129 with qualifier ions  $m/z$  77 and  $m/z$  105, applying the same  $\pm 20\%$  ion-ratio acceptance criterion. The analytical performance characteristics of the validated method implemented within the laboratory's accredited scope were as follows: linearity  $R^2 > 0.999$  over the calibration range for both analytes; limit of detection (LOD) of 0.002 mg/kg and LOQ of 0.006 mg/kg for chlorpyrifos, and LOD of 0.003 mg/kg and LOQ of 0.009 mg/kg for phoxim, determined from signal-to-noise ratios of spiked blank milk; recovery of 75–110%, mean 91.4% for chlorpyrifos and 88.7% for phoxim, at three fortification levels ( $n = 3$  each); and matrix-matched calibration used throughout to control for matrix effects, with matrix-effect values of  $-5\%$  to  $+8\%$  relative to solvent-only standards. A seven-point matrix-matched calibration curve was used for quantification, with calibration verified by a mid-range check standard analysed after every 10 samples (accepted within  $\pm 15\%$  of nominal). Terminology note: throughout this manuscript, 'removal', 'reduction', and 'parent-compound loss' are used interchangeably to mean loss of the parent analyte as measured by GC-MS-SIM, and do not by themselves imply confirmed detoxification or a specific loss mechanism (e.g., true chemical degradation, adsorption to a surface, volatilisation, or redistribution within the matrix). Mechanistic terms such as 'photocatalytic' or 'sonochemical' are used only in paragraphs that explicitly discuss mechanism and carry the corresponding caveats (see Sections 3.2–3.3 and Section 4). Removal efficiency was calculated as:

$$\text{Removal}(\%) = [(C_0 - C_t) / C_0] \times 100$$

where  $C_0$  is the initial pesticide concentration immediately before treatment and  $C_t$  is the residual pesticide concentration at the specified treatment time. The calculated removal

efficiency represents the percentage reduction of the parent pesticide relative to its initial concentration and is used solely to compare treatment performance.

#### 2.3.4 Ultrasonication Treatment

Sonication was conducted following the procedure described by Yuan et al. [18], with modifications. A 100-mL aliquot of spiked buffalo milk was placed in a 150-mL round-bottom flask immersed in an HX-0508 thermostatic circulator (Kenfan Electronic Technology Co. Ltd., Nanjing, China) to maintain temperature stability. The ultrasonic probe was positioned 10 mm below the liquid surface. Sonication was performed using a 750 W ultrasonic processor (VCX750, Sonics & Materials Inc., Newtown, CT, USA) equipped with a 0.5-in. probe. The processor was operated at 100 W for 5, 10, 15, and 20 min. All samples were maintained at  $\leq 20$  °C during treatment to minimize thermal effects. Non-sonicated spiked milk samples maintained under identical conditions served as controls. All treatments were performed in triplicate ( $n = 3$ ).

#### 2.3.5 UV Irradiation and UV/Nano-TiO<sub>2</sub> Photocatalytic Treatment

UV irradiation experiments were conducted following the procedure of Crook et al. [20], with modifications. Artificially contaminated milk (200 mL per replicate) was placed in a transparent quartz vessel positioned 15 cm below the UV lamp (ENF-260C/FE; 254 nm; 6 W; irradiance 21.22 W/m<sup>2</sup>). Three treatment conditions were evaluated: (i) dark control (spiked milk held in opaque vessels under identical conditions with no UV exposure); (ii) UV irradiation alone; and (iii) UV irradiation combined with nano-TiO<sub>2</sub> at doses of 50 or 200 mg/100 mL. For UV/TiO<sub>2</sub> treatments, nano-TiO<sub>2</sub> powder was dispersed in 5 mL of ultra-pure water by probe sonication (2 min) before addition to the milk. Treatments were applied for 10, 30, and 60 min. Room temperature was monitored continuously ( $25 \pm 1$  °C), and all experiments were performed in triplicate ( $n = 3$ ). The dark control demonstrated that spontaneous reduction of the pesticides during the experimental period was negligible under the experimental conditions. See Section 3.3 and Section 4 for the strengthened dark-adsorption-control caveat and the critical discussion of TiO<sub>2</sub>'s marginal contribution for phoxim relative to its food-safety cost. Removal efficiency relative to the dark control was calculated as:

$$\begin{aligned} \text{Removal Efficiency}(\%) \\ = [(C_{\text{dark}} - C_{\text{treated}}) / C_{\text{dark}}] \times 100 \end{aligned}$$

where  $C_{\text{dark}}$  is the pesticide concentration in the dark control at the same time point, and  $C_{\text{treated}}$  is the concentration in the UV-treated or UV/TiO<sub>2</sub>-treated sample.

### 2.3.6 Activation and Inoculation of LAB Starter Cultures

Frozen DVS starter cultures were activated by inoculation of 0.2% (w/v) into sterilised reconstituted skim milk (10% w/v), followed by incubation under standard (non-sparged, ambient air) incubation conditions. *S. thermophilus*/Lb. *bulgaricus* (YC-X11) and *Lb. helveticus* CP790 were incubated at 42 °C for 18 h; *L. plantarum* CICC20261 was incubated at 37 °C for 18 h. Activated cultures were adjusted to  $\sim 10^7$  CFU/mL before inoculation into experimental milk. No sterile (uninoculated), chemically acidified, heat-inactivated-cell, or cell-free-supernatant controls were included alongside the live-strain treatments; see Section 4 for the mechanistic implications of this.

Raw buffalo milk was pasteurized at 72 °C for 15 s (high-temperature short-time, HTST) and cooled to 40 °C. Pasteurized milk was cooled and inoculated under a laminar-flow biosafety cabinet using flame-sterilised equipment, with the sterility of the pasteurized milk confirmed by plating an uninoculated aliquot on non-selective agar prior to inoculation. The initial pesticide concentrations were confirmed by GC-MS analysis at this stage (post-pasteurisation, pre-inoculation,  $t = 0$ ) to account for any thermal reduction during pasteurisation. LAB strains were inoculated individually at  $\sim 10^7$  CFU/mL into pesticide-spiked pasteurized milk (chlorpyrifos: 2473.56 mg/L; phoxim: 21.45 mg/L) in triplicate. Mixed starter culture (YC-X11) was inoculated at the manufacturer-recommended rate. Incubation was at 42 °C for 24 h for all strains except *L. plantarum* CICC20261 (37 °C, 24 h). Residual pesticide concentrations and viable cell counts were determined at 0 h and 24 h.

### 2.3.7 Microbiological Analysis

Viable cell counts were determined in triplicate by serial decimal dilution in sterile 0.9% NaCl, followed by surface plating on selective media. *S. thermophilus* was enumerated on M17 agar (37 °C, 48 h, aerobic). *Lb. bulgaricus* was enumerated on MRS agar (42 °C, 72 h, anaerobic). *Lb. helveticus* CP790 was enumerated on de Man, Rogosa and Sharpe (MRS) agar adjusted to pH 5.4 (42 °C, 72 h, anaerobic). *L. plantarum* CICC20261 was enumerated on MRS agar supplemented with vancomycin (20 mg/L) (37 °C, 48 h, anaerobic). Results were expressed as log CFU/mL.

### 2.3.8 Statistical Analysis

Data are presented as mean  $\pm$  SD of three independent biological replicates ( $n = 3$ ).  $n = 3$  throughout the treatment-efficacy experiments (Sections 3.2–3.5) refers to three independently prepared and processed replicate aliquots run in parallel for each treatment condition. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test for pairwise comparisons, using a significance threshold of  $p < 0.05$ . One-way ANOVA

was applied separately within each fixed factor combination (e.g., within each TiO<sub>2</sub> dose across time, or within each strain across condition), because the experimental design did not include full factorial replication suitable for repeated-measures modelling across all factors simultaneously; two-way/repeated-measures ANOVA would be more powerful and is flagged here as a methodological limitation and a recommendation for future studies. All analyses were conducted using SPSS Statistics v.26 (IBM Corp., Armonk, NY, USA).

## 3. Results and Discussion

### 3.1 Physicochemical and Chemical Composition of Buffalo Milk

The physicochemical properties of the three independent raw buffalo milk batches (S1–S3) and the fermented milk product are presented in Tables 1,2. All compositional parameters for raw milk were within the accepted reference ranges for Egyptian buffalo milk. Mean fat content ranged from  $6.06 \pm 0.15\%$  (S2) to  $8.95 \pm 0.21\%$  (S3), and mean protein content ranged from  $3.07 \pm 0.06\%$  (S2) to  $3.49 \pm 0.14\%$  (S3). Moisture content ranged from  $82.28 \pm 0.49\%$  (S3) to  $85.47 \pm 0.53\%$  (S2), with corresponding solids-not-fat (SNF) values of 8.36–8.93%. Mean pH values of 6.59–6.65 and titratable acidity of 0.17–0.18% were consistent with the normal range for fresh buffalo milk [21]. Milk density ranged from 1.033 to 1.034 g/cm<sup>3</sup>, and freezing points of  $-0.56$  to  $-0.57$  °C indicated no adulteration. The inter-batch variability observed across S1–S3 reflects the natural biological variation inherent to milk produced by different animals under field conditions [9].

Fermented milk differed from raw milk in several key parameters: pH declined markedly to  $4.81 \pm 0.12$ , reflecting lactic acid accumulation during fermentation; titratable acidity increased substantially to  $0.64 \pm 0.04\%$ ; lactose content decreased from a mean of  $\sim 4.5\%$  in raw milk to  $3.57 \pm 0.12\%$ , consistent with microbial conversion of lactose to lactic acid [22]. Moisture content in the fermented product ( $80.16 \pm 0.59\%$ ) was lower than in raw milk batches, attributable to gel formation during coagulation. These compositional changes are consistent with published data for LAB-fermented buffalo milk and confirm successful fermentation before pesticide analysis [9]. Importantly, no chlorpyrifos or phoxim residues were detected in any of the raw milk batches by GC-MS, confirming that the concentrations observed in treated samples were attributable exclusively to the artificial contamination protocol.

### 3.2 Effect of Ultrasonication on the Reduction of Chlorpyrifos and Phoxim

The effect of sonication duration (5–20 min) on the concentrations of chlorpyrifos and phoxim in spiked buffalo milk is illustrated in Fig. 1. Chlorpyrifos concentration progressively decreased from 2473.56 mg/L ( $t = 0$ , pre-treatment) to approximately 820.79 mg/L after 20 min, cor-

**Table 1. Physical and physicochemical properties of buffalo milk samples.**

| Milk sample           | Freezing point (°C) | Density (g/cm <sup>3</sup> ) | pH          | Titrateable acidity (%) | Chlorpyrifos & phoxim (mg/kg) |
|-----------------------|---------------------|------------------------------|-------------|-------------------------|-------------------------------|
| Whole buffalo milk S1 | -0.57 ± 0.014       | 1.033 ± 0.004                | 6.62 ± 0.21 | 0.17 ± 0.02             | ND                            |
| Whole buffalo milk S2 | -0.56 ± 0.001       | 1.034 ± 0.005                | 6.65 ± 0.13 | 0.18 ± 0.01             | ND                            |
| Whole buffalo milk S3 | -0.57 ± 0.011       | 1.033 ± 0.002                | 6.59 ± 0.20 | 0.18 ± 0.02             | ND                            |
| Fermented milk*       | -0.54 ± 0.006       | 1.030 ± 0.002                | 4.81 ± 0.12 | 0.64 ± 0.04             | ND                            |

S1–S3: independent raw buffalo milk batches. \*Fermented milk prepared by inoculation with selected LAB under controlled fermentation conditions until coagulation. Values are mean ± SD of three independent replicates. ND: not detected; LAB, lactic acid bacteria; SD, standard deviation.

**Table 2. Chemical composition of buffalo milk samples (mean ± SD, n = 3).**

| Milk sample           | Moisture (%) | Fat (%)     | SNF (%)     | Protein (%) | Lactose (%) | Ash (%)     |
|-----------------------|--------------|-------------|-------------|-------------|-------------|-------------|
| Whole buffalo milk S1 | 85.16 ± 0.42 | 6.46 ± 0.13 | 8.93 ± 0.25 | 3.24 ± 0.14 | 4.21 ± 0.18 | 1.03 ± 0.14 |
| Whole buffalo milk S2 | 85.47 ± 0.53 | 6.06 ± 0.15 | 8.36 ± 0.09 | 3.07 ± 0.06 | 4.61 ± 0.17 | 0.69 ± 0.02 |
| Whole buffalo milk S3 | 82.28 ± 0.49 | 8.95 ± 0.21 | 8.82 ± 0.24 | 3.49 ± 0.14 | 4.86 ± 0.16 | 0.72 ± 0.02 |
| Fermented milk*       | 84.36 ± 0.59 | 6.77 ± 0.14 | 8.82 ± 0.18 | 3.43 ± 0.08 | 3.57 ± 0.12 | 0.85 ± 0.03 |

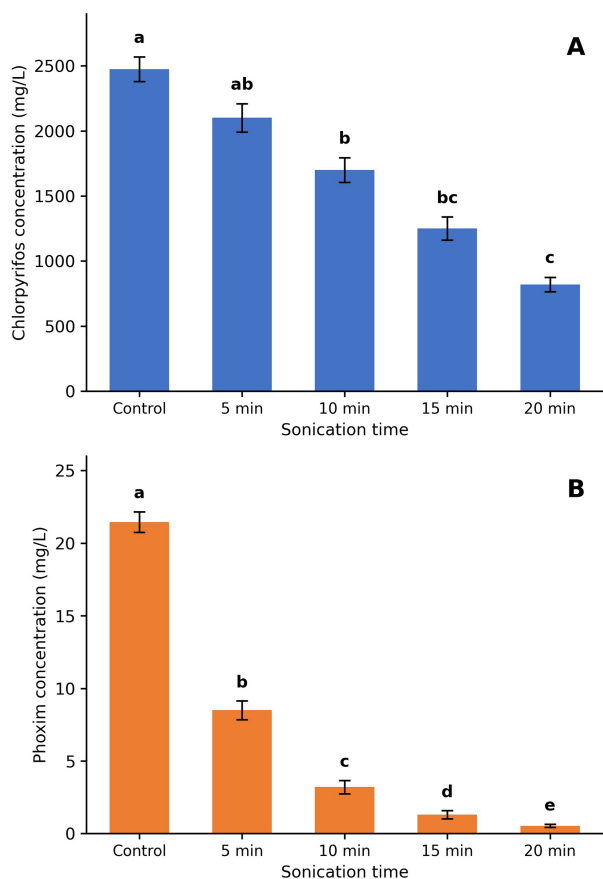
\*Fermented milk: produced by inoculation with selected LAB strains. The reduced lactose content (3.57%) relative to raw milk reflects microbial conversion during fermentation. Values are mean ± SD of three independent replicates. SNF, solids-not-fat.

responding to a maximum removal efficiency of 66.81%. According to Tukey's HSD test, the chlorpyrifos concentration after 5 min was not significantly different from the non-sonicated control, whereas significant reductions were observed from 10 min onward ( $p < 0.05$ ) (Fig. 1A). Phoxim exhibited markedly greater susceptibility to acoustic cavitation, with its concentration declining from 21.45 mg/L to 0.534 mg/L after 20 min, corresponding to a removal efficiency of 97.52%. For phoxim, all sonication durations differed significantly from the non-sonicated control and from each other ( $p < 0.05$ ) (Fig. 1B). Overall, the concentrations of both parent pesticides decreased with increasing sonication duration.

The differential susceptibility of the two pesticides to ultrasonic treatment is consistent with differences in their physicochemical properties. Phoxim ( $\log K_{ow} \approx 3.4$ ; vapour pressure 0.18 mPa) is more volatile and relatively less hydrophobic than chlorpyrifos ( $\log K_{ow} \approx 4.7$ ; vapour pressure 2.5 mPa at 25 °C). These differences extend beyond hydrophobicity and volatility alone: the P=S and C–Cl bonds in phoxim and chlorpyrifos, respectively, differ in bond dissociation energy, influencing their relative susceptibility to thermal and radical-mediated cleavage within and around collapsing cavitation bubbles; diffusion-limited transport toward the cavitation-bubble interface is also faster for the smaller, more volatile phoxim molecule; aqueous solubility differs between the two compounds (chlorpyrifos ~1.4 mg/L vs. phoxim ~2 mg/L at 25 °C), favouring greater aqueous-phase radical exposure for phoxim; and the milk fat/protein matrix differentially partitions and sequesters each compound, with consequences for both substrate availability at the bubble interface and competitive radical scavenging by matrix components. Acoustic cavitation promotes the transformation and reduction of organic

contaminants through two primary mechanisms: (i) pyrolysis within the high-temperature core of cavitation bubbles, which predominantly affects hydrophobic, volatile compounds that partition into the bubble–liquid interface; and (ii) hydroxyl radical ( $\bullet OH$ ) generation in the aqueous phase surrounding collapsing bubbles, which targets hydrophilic and non-volatile compounds [12,13]. The pronounced reduction in phoxim concentration observed in the present study may therefore reflect its susceptibility to a combination of interfacial/pyrolytic and radical-mediated processes under the applied sonication conditions. These findings align with Agarwal et al. [23], who reported increasing azinphos-methyl degradation with extended sonication from 20 to 60 min in aqueous systems, and with Ali et al. [13], who demonstrated >90% reduction of chlorothalonil in spinach juice after extended ultrasonic treatment.

Critically, the milk matrix may influence cavitation dynamics relative to aqueous systems. The fat globules, proteins, and dissolved minerals present in buffalo milk can act as nucleation sites for cavitation bubbles, potentially enhancing cavitation intensity, while simultaneously offering hydrophobic microenvironments that could sequester lipophilic pesticides from radical attack [11]. There are, in effect, two competing hydrophobic partitioning sinks in the milk system: the transient vapour–liquid cavitation-bubble interface, where pyrolytic degradation occurs, and the dispersed milk-fat globules, which are present at far greater volume than the transient bubble interface. The bulk milk fat phase is considered the dominant sink for highly hydrophobic chlorpyrifos, sequestering it away from both the bubble interface and the aqueous radical field; this is offered as the reason for its lower degradation relative to the less strongly fat-partitioning phoxim, rather than implying that the same partitioning event simultaneously enhances



**Fig. 1. Effect of sonication duration (5, 10, 15, and 20 min; 100 W, 37 kHz) on pesticide residues in artificially spiked buffalo milk.** (A) Concentration (mg/L) of chlorpyrifos following sonication. (B) Concentration (mg/L) of phoxim following sonication. Control indicates the initial pesticide concentration at time (t) = 0 prior to treatment. Different letters above bars indicate statistically significant differences between time points ( $p < 0.05$ , Tukey's HSD). Values are mean  $\pm$  SD of three independent replicates ( $n = 3$ ). HSD, honestly significant difference.

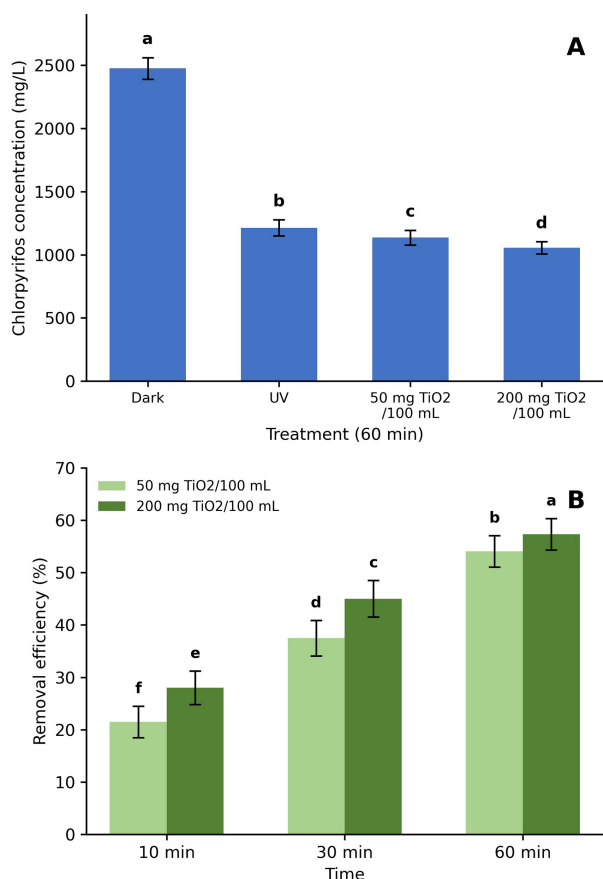
and protects against degradation. The relatively lower removal of chlorpyrifos (66.81%) compared with phoxim (97.52%) at identical treatment conditions is consistent with chlorpyrifos exhibiting stronger partitioning into the milk lipid phase, rendering it less accessible to aqueous-phase radical attack. These findings indicate that sonication can effectively reduce organophosphorus pesticide residues in buffalo milk under the experimental conditions employed, and the temperature was carefully controlled during sonication.

### 3.3 Effect of UV Irradiation and UV/Nano-TiO<sub>2</sub> Photocatalysis on the Reduction of Chlorpyrifos and Phoxim

The photocatalytic reduction of chlorpyrifos and phoxim under three treatment conditions (dark control, UV irradiation alone, and UV combined with nano-TiO<sub>2</sub>) is pre-

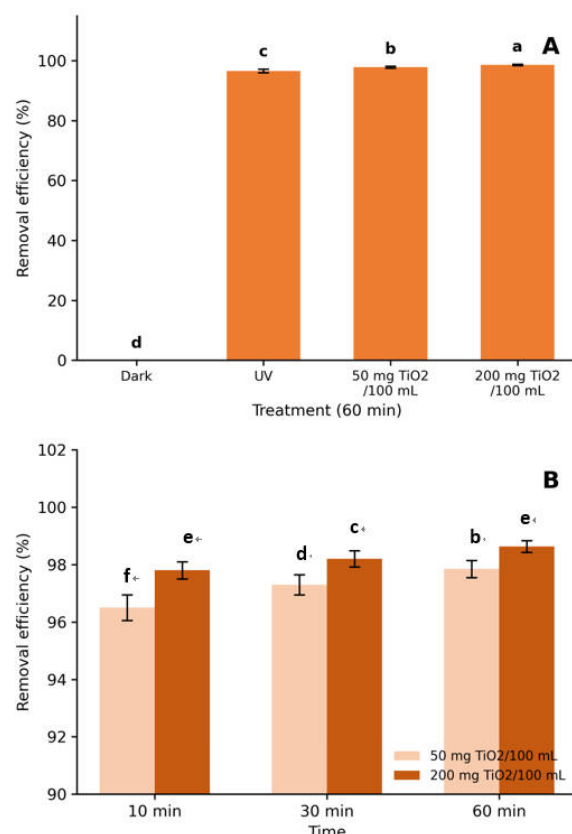
sented in Figs. 2,3. For chlorpyrifos (Fig. 2A), pesticide concentration under dark control conditions remained relatively stable at  $2473.56 \pm 84.3$  mg/L after 60 min, confirming that spontaneous reduction in the absence of UV was negligible. UV irradiation alone reduced chlorpyrifos concentration to  $1213.23 \pm 62.7$  mg/L after 60 min, representing a 50.97% reduction relative to the dark control. The addition of nano-TiO<sub>2</sub> at 200 mg/100 mL further enhanced removal efficiency, reducing chlorpyrifos to  $1055.9 \pm 48.2$  mg/L, a removal efficiency of 57.31% relative to the dark control at 60 min (Fig. 2B). At 50 mg TiO<sub>2</sub>/100 mL, chlorpyrifos was reduced to 1135.9 mg/L, a removal efficiency of 54.08% at 60 min. Across all time points tested (10, 30, and 60 min), higher TiO<sub>2</sub> loading consistently outperformed the lower dose, with removal efficiencies increasing progressively with exposure time at all catalyst concentrations (Fig. 2B). Statistically significant differences were observed among the treatment conditions at each time point ( $p < 0.05$ ).

The marked reduction in parent chlorpyrifos residues observed at 60 min with 200 mg/100 mL TiO<sub>2</sub> is attributed to the photocatalytic generation of ROS, principally hydroxyl radicals ( $\bullet$ OH), superoxide anion radicals ( $O_2^{\bullet-}$ ), and hydrogen peroxide ( $H_2O_2$ ), upon UV activation of the TiO<sub>2</sub> semiconductor. TiO<sub>2</sub> photocatalysis proceeds via the generation of electron-hole pairs, with electrons in the conduction band and holes in the valence band, with valence band holes oxidising surface-adsorbed water and hydroxide ions to yield  $\bullet$ OH radicals, which are among the most powerful non-selective oxidants known ( $k^{\bullet}OH \approx 10^9-10^{10} M^{-1} s^{-1}$ ) [24]. The dose-dependent effect of TiO<sub>2</sub> loading reflects the greater surface area and photon absorption capacity available at higher catalyst concentrations, thereby increasing the flux of ROS generation. El-Saeid et al. [14] similarly reported enhanced degradation of diazinon and other OPPs under UV/TiO<sub>2</sub> systems in wastewater, and Budarz et al. [24] demonstrated 80% chlorpyrifos reduction in solution after 24 h under photocatalytic conditions, with bacterial presence exerting minimal influence on pesticide removal rates. It should be noted that dark-adsorption controls with TiO<sub>2</sub> present but no UV exposure were not included in this study, so the reported removal-efficiency values for the UV + TiO<sub>2</sub> treatments may include a contribution from surface adsorption of pesticide onto TiO<sub>2</sub> particles in addition to true photocatalytic degradation; future studies should include TiO<sub>2</sub>-dark controls and, where possible, quantify TiO<sub>2</sub>-bound pesticide directly. Because TiO<sub>2</sub>-dark controls were not performed, the 57.31% (chlorpyrifos) and 98.63% (phoxim, reported below) removal-efficiency values represent removal attributable to the combined UV + TiO<sub>2</sub> system, not photocatalysis specifically – the photocatalysis-specific contribution cannot currently be isolated from surface adsorption onto the TiO<sub>2</sub> particles, and TiO<sub>2</sub>-dark controls plus direct quantification of TiO<sub>2</sub>-bound pesticide.



**Fig. 2. Effect of UV irradiation (254 nm, 6 W, 21.22 W/m<sup>2</sup>) and nano-TiO<sub>2</sub> on chlorpyrifos degradation in spiked buffalo milk.** (A) Residual chlorpyrifos concentration (mg/L) after 60 min under dark control, UV alone, and UV + nano-TiO<sub>2</sub> (50 or 200 mg/100 mL). (B) Removal efficiency (%) of chlorpyrifos relative to the time-matched dark control at 10, 30, and 60 min for each TiO<sub>2</sub> dose. Different letters indicate statistically significant differences between treatments/time points ( $p < 0.05$ , Tukey's HSD). Values are mean  $\pm$  SD,  $n = 3$ . TiO<sub>2</sub>, titanium dioxide.

For phoxim, dark control concentrations remained stable at  $21.45 \pm 0.7$  mg/L over 60 min, confirming negligible spontaneous degradation. UV irradiation alone reduced parent phoxim residues by 96.60% after 60 min (residual fraction 3.40%) (Fig. 3A). Both 50 and 200 mg/100 mL TiO<sub>2</sub> treatments further reduced phoxim by 60 min, with removal efficiencies of 97.85% and 98.63%, respectively (Fig. 3B), representing only a modest improvement over UV irradiation alone (+1.25–2.03 percentage points), indicating that UV irradiation itself accounted for the majority of parent phoxim residue reduction, though still significantly greater than UV alone ( $p < 0.05$ ). The greater susceptibility of phoxim to UV photolysis, even without TiO<sub>2</sub>, compared with chlorpyrifos, is consistent with the higher UV absorptivity of phoxim, attributable to its benzaldoxime chromophoric moiety, which facilitates direct UV-mediated bond cleavage [25]. For phoxim, direct UV photolysis is



**Fig. 3. Effect of UV irradiation and nano-TiO<sub>2</sub> on phoxim removal in spiked buffalo milk.** (A) Removal efficiency (%) of phoxim at 60 min under dark control (reference, 0%), UV alone, and UV + nano-TiO<sub>2</sub> (50 or 200 mg/100 mL), calculated relative to the dark control at the same time point, replacing the residual-concentration axis used previously so that all four treatments are directly comparable on a common 0–100% scale. (B) Removal efficiency (%) of phoxim relative to the time-matched dark control at 10, 30, and 60 min for each TiO<sub>2</sub> dose. Different letters indicate statistically significant differences between treatments/time points ( $p < 0.05$ , Tukey's HSD). Values are mean  $\pm$  SD,  $n = 3$ .

already highly effective, and nano-TiO<sub>2</sub> addition provides only a marginal additional benefit. Because nano-TiO<sub>2</sub> improves phoxim removal by only  $\approx 2$  percentage points over UV alone (96.60%  $\rightarrow$  97.85–98.63%) and given the unresolved nanoparticle-clearance and food-safety questions discussed in Section 4, the practical case for including TiO<sub>2</sub> specifically to treat phoxim is weak relative to UV alone. TiO<sub>2</sub> contributes a proportionally larger increment for chlorpyrifos (50.97% for UV alone  $\rightarrow$  57.31% at 200 mg/100 mL, a  $\approx 6$  percentage-point gain), so any process-design decision should treat UV alone and UV/TiO<sub>2</sub> as pesticide-specific options rather than assuming TiO<sub>2</sub> addition is equally justified for both compounds. The progressive accumulation of ROS during extended UV/TiO<sub>2</sub> exposure likely promoted continued oxidation of the parent pesticide molecules, resulting in the time-dependent increase

in removal efficiency observed for both pesticides. Tabasum et al. [26] reported similar UV-time-dependent kinetics for organophosphate photocatalysis, with catalysts retaining activity for up to five reaction cycles. Collectively, under the specific, non-equivalent reference conditions used for each method (see the methodological-asymmetries discussion below), UV/TiO<sub>2</sub> numerically achieved the highest parent-pesticide removal among the treatments tested in this study; this describes the results obtained under these particular conditions rather than a general ranking of treatment effectiveness.

Several methodological asymmetries limit direct cross-treatment comparison among sonication, UV/TiO<sub>2</sub>, and LAB fermentation in this study: (i) chlorpyrifos and phoxim were spiked at very different concentrations, with no shared rationale beyond commercial formulation strength; (ii) sonication removal is referenced to the initial ( $t = 0$ ) concentration, whereas UV/TiO<sub>2</sub> removal efficiency is referenced to the time-matched dark control, meaning the two percentages are not strictly the same metric; and (iii) *L. plantarum* CICC20261 was incubated at 37 °C versus 42 °C for the other LAB preparations, confounding strain-intrinsic tolerance with temperature-dependent growth and chemical hydrolysis. Comparative statements made elsewhere in this manuscript (e.g., describing a treatment as the most efficient single-treatment strategy) should accordingly be read as indicative rather than strictly quantitative, given these differing reference frames.

### 3.4 Effect of Chlorpyrifos and Phoxim on the Growth of LAB Strains

The effects of chlorpyrifos and phoxim on the viable cell counts of four LAB preparations at 0 h and 24 h are presented in Table 3. All strains achieved robust growth in pesticide-free control conditions over 24 h. *L. plantarum* CICC20261 and *Lb. helveticus* CP790 demonstrated active growth even in the presence of the pesticides, with *L. plantarum* counts increasing from  $8.76 \pm 0.65$  (0 h) to  $9.23 \pm 0.42$  log CFU/mL (24 h) under control conditions, and maintaining  $7.79 \pm 0.51$  log CFU/mL after 24 h of exposure to chlorpyrifos, and 7.40 log CFU/mL after 24 h of exposure to phoxim. For clarity, all three 24-h values are therefore:  $9.23 \pm 0.42$  log CFU/mL (pesticide-free control),  $7.79 \pm 0.51$  log CFU/mL (chlorpyrifos exposure), and 7.40 log CFU/mL (phoxim exposure). In contrast, *S. thermophilus* and *Lb. bulgaricus* (YC-X11 mixed culture; see the mixed-culture interpretation caution in Section 3.5) exhibited significantly reduced counts ( $p < 0.05$ ) after 24 h exposure to both pesticides, declining from  $\sim 7.5$  to 6.6–6.8 log CFU/mL in pesticide-containing media compared with 6.85–6.91 log CFU/mL in the control (Table 3). All pairwise comparisons among control, chlorpyrifos, and phoxim conditions at 24 h were significant at  $p < 0.05$  (one-way ANOVA with Tukey's HSD); F-statistics for each strain's 24-h comparison across the three conditions ranged from 9.8 to 34.6 (all  $p < 0.05$ ,  $df = 2, 6$ ), as reported in the Table 3 footnote.

The differential sensitivity of LAB strains to OPPs aligns with the known variability in pesticide tolerance among lactic acid bacteria, which can be attributable to differences in membrane lipid composition, efflux pump activity, and enzymatic detoxification capacity [15,27]. The ability of *L. plantarum* to proliferate in the presence of both chlorpyrifos and phoxim suggests the existence of intrinsic resistance mechanisms, potentially including expression of organophosphorus hydrolase (OPH) and phosphatase enzymes capable of catalysing OPP hydrolysis [28]. The maintenance of viable populations throughout the 24 h incubation period is a prerequisite for sustained enzymatic activity that may contribute to the reduction of parent pesticide residues, and the data in Table 3 confirm that all strains, including those showing reduced growth rates, remained above 6.2 log CFU/mL, a threshold considered sufficient for active metabolic activity in fermented dairy products [18].

It should be noted that the initial viable counts ( $t = 0$ ) differed slightly between the control and pesticide-containing treatments for some strains, with differences exceeding 1 log CFU/mL in a few cases (e.g., *L. plantarum*: 8.76 vs. 7.06 log CFU/mL; Table 3). These differences most likely reflect minor variations in inoculation and sample handling prior to incubation rather than the effects of pesticide exposure and should therefore be considered when interpreting the strain tolerance results. The pesticide removal efficiencies reported in Section 3.5 were calculated from the measured initial ( $t = 0$ ) pesticide concentrations and are therefore independent of the observed differences in viable cell counts. In addition, the YC-X11 preparation represents a mixed starter culture inoculated according to the manufacturer's recommendation, whereas the other preparations were single-strain cultures standardised to approximately  $10^7$  CFU/mL. Consequently, comparisons between the mixed culture and the individual strains should be interpreted with appropriate caution.

### 3.5 LAB-Mediated Reduction of Chlorpyrifos and Phoxim

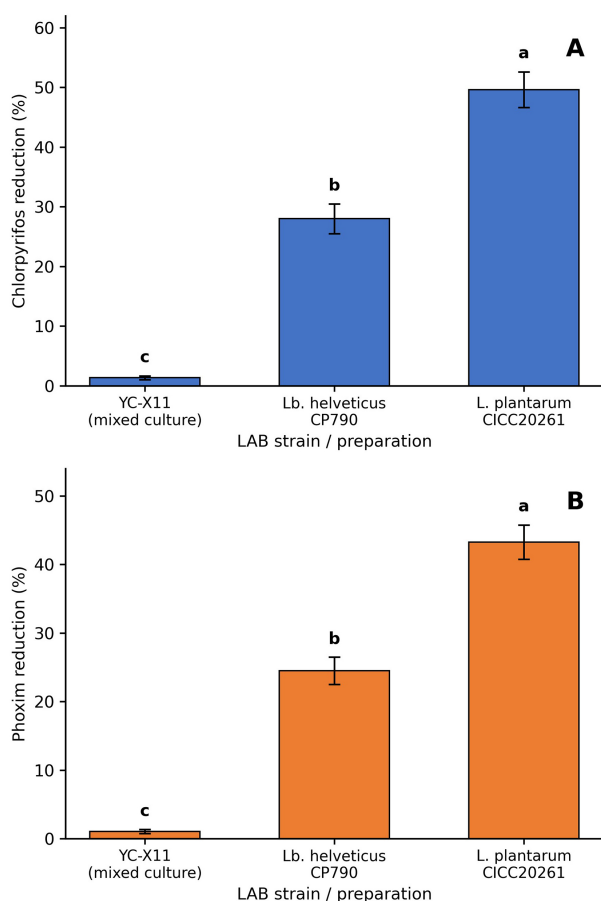
The reduction capabilities of the four LAB strains/preparations against chlorpyrifos (initial concentration: 2473.56 mg/L post-pasteurisation) and phoxim (21.45 mg/L) following 24 h fermentation at their respective incubation temperatures are illustrated in Fig. 4. All four LAB preparations exhibited measurable reduction of both pesticides, with percentage reduction ranging from 1.04 to 49.61% across strains and pesticides. *L. plantarum* CICC20261 achieved the highest reduction of both chlorpyrifos (49.61%) and phoxim (43.28%), significantly greater than all other strains ( $p < 0.05$ ). *Lb. helveticus* CP790 demonstrated the second-highest reduction capacity for both pesticides. The mixed YC-X11 culture (*S. thermophilus*/*Lb. bulgaricus*) exhibited the lowest reduction of both OPPs among the preparations tested. Therefore, direct comparison with individual strains should be interpreted

**Table 3. Viable counts (log CFU/mL) of LAB strains at 0 h and 24 h in control, chlorpyrifos (2473.56 mg/L), and phoxim (21.45 mg/L) conditions.**

| Strain                        | Control                   |                          | Chlorpyrifos             |                           | Phoxim                   |                          |
|-------------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
|                               | 0 h                       | 24 h                     | 0 h                      | 24 h                      | 0 h                      | 24 h                     |
| <i>S. thermophilus</i>        | 7.55 ± 0.71 <sup>a</sup>  | 6.85 ± 0.33 <sup>c</sup> | 7.25 ± 0.72 <sup>b</sup> | 6.62 ± 0.40 <sup>de</sup> | 7.55 ± 0.55 <sup>a</sup> | 6.65 ± 0.62 <sup>d</sup> |
| <i>Lb. bulgaricus</i>         | 7.42 ± 0.46 <sup>ab</sup> | 6.91 ± 0.26 <sup>c</sup> | 7.48 ± 0.41 <sup>a</sup> | 6.20 ± 0.35 <sup>e</sup>  | 7.31 ± 0.38 <sup>b</sup> | 6.45 ± 0.24 <sup>d</sup> |
| <i>Lb. helveticus</i> CP790   | 8.22 ± 0.54 <sup>b</sup>  | 8.56 ± 0.50 <sup>a</sup> | 7.64 ± 0.47 <sup>c</sup> | 7.22 ± 0.45 <sup>e</sup>  | 7.37 ± 0.96 <sup>d</sup> | 6.37 ± 0.28 <sup>f</sup> |
| <i>L. plantarum</i> CICC20261 | 8.76 ± 0.65 <sup>b</sup>  | 9.23 ± 0.42 <sup>a</sup> | 7.39 ± 0.44 <sup>d</sup> | 7.79 ± 0.51 <sup>c</sup>  | 7.06 ± 0.62 <sup>e</sup> | 7.40 ± 0.84 <sup>d</sup> |

Values are mean ± SD of three independent replicates. Different superscript letters within the same row indicate statistically significant differences ( $p < 0.05$ , Tukey's HSD). *S. thermophilus* and *Lb. bulgaricus* were inoculated as a mixed culture (YC-X11). Initial counts at 0 h reflect viable counts immediately after inoculation and prior to incubation.

with caution because microbial interactions within mixed cultures may influence growth behaviour and pesticide reduction.



**Fig. 4. Reduction (%) of (A) chlorpyrifos and (B) phoxim residues in buffalo milk following 24 h fermentation with three LAB preparations: YC-X11 mixed culture, *Lb. helveticus* CP790, and *L. plantarum* CICC20261.** Bars represent mean ± SD. Different lowercase letters (a–c) indicate significant differences among LAB treatments within each pesticide ( $p < 0.05$ ).

The superior removal capacity of *L. plantarum* CICC20261 is consistent with its phylogenetically diverse

metabolic repertoire and the documented expression of phosphatase and OPH-like enzymes in *Lactiplantibacillus* species [27,28]. Phosphatase-mediated hydrolysis of the phosphoester bond is considered the primary biochemical mechanism for OPP detoxification, which has been reported to yield metabolites such as 3,5,6-trichloro-2-pyridinol TCP and diethyl phosphate in previous studies [29]. Whether these metabolites accumulate in fermented milk at biologically significant concentrations requires further investigation. The lower reduction activity of the mixed YC-X11 culture relative to single strains may reflect interspecies competition, metabolic cross-inhibition, and the partial suppression of individual enzymatic activities in complex microbial ecosystems [15].

The fermentation process may also contribute to the reduction of parent pesticide residues through several physical and biochemical mechanisms. The progressive acidification of the milk matrix (pH declining to ≤4.8) during LAB fermentation may accelerate acid-catalysed hydrolysis of organophosphorus pesticide phosphate ester bonds [30]. Additionally, the negatively charged surface of LAB cell walls, particularly those of *L. plantarum*, may facilitate the electrostatic and hydrophobic absorption of pesticide molecules, thereby contributing to the observed reduction in parent pesticide residues [31]. Disentangling the relative contributions of enzymatic transformation, acid-catalysed hydrolysis, and adsorption to the overall reduction of parent pesticide residues requires further mechanistic investigation, including metabolite profiling. Because sterile (uninoculated), chemically acidified (pH-matched, non-biological), heat-inactivated-cell, and cell-free-supernatant controls were not included in this design (Section 2.3.6). The relative contributions of enzymatic transformation, acid-catalysed hydrolysis, and adsorption discussed above are inferential rather than experimentally demonstrated.

From a practical standpoint, the ability of pesticide-tolerant LAB strains such as *L. plantarum* CICC20261 and *Lb. helveticus* CP790 to maintain both viability and degradation activity in OPP-contaminated milk suggests their potential as functional starter cultures capable of simultaneously fermenting milk and reducing pesticide load. This dual functionality represents a compelling value proposi-

tion for the dairy industry, particularly in contexts where non-thermal physical treatments are logistically challenging to implement on a scale [32,33].

#### 4. Limitations

A further consideration is that nano-TiO<sub>2</sub> itself remains the subject of ongoing food-safety review, and the present protocol did not include a post-treatment recovery or removal step (e.g., centrifugation, membrane filtration, or magnetic/immobilised-catalyst configurations) to confirm clearance of residual nanoparticles from the treated milk. Without such a recovery step and confirmatory titanium quantification (e.g., by inductively coupled plasma mass spectrometry (ICP-MS)), UV/TiO<sub>2</sub> as configured here should be regarded as a proof-of-concept degradation method rather than an immediately deployable food-processing step; catalyst immobilization and recovery are identified as priorities for future work.

It should be noted that dark-adsorption controls with TiO<sub>2</sub> present but no UV exposure were not included in this study, so the reported removal-efficiency values for the UV + TiO<sub>2</sub> treatments may include a contribution from surface adsorption of pesticide onto TiO<sub>2</sub> particles in addition to true photocatalytic degradation; future studies should include TiO<sub>2</sub>-dark controls and, where possible, quantify TiO<sub>2</sub>-bound pesticide directly.

Buffalo milk's high turbidity and fat content strongly attenuate UV-C penetration at 254 nm. The milk layer depth used in this study (200 mL in the quartz vessel, layer depth ~2 cm) was not formally optimised for UV path length, and the degradation reported therefore likely reflects a combination of surface-layer photolysis/photocatalysis and convective mixing during treatment rather than spatially uniform UV exposure. Thin-film or flow-through UV reactor configurations, which improve light penetration in turbid dairy matrices, are identified as a priority for scale-up studies.

Because YC-X11 was inoculated and analysed as a mixed culture, the degradation and viability values reported for this preparation reflect the combined community; individual-strain contributions within the mixed culture were not separately quantified, which is noted here as a limitation. Future work should use strain-specific quantitative Polymerase Chain Reaction (qPCR) or selective plating within the co-culture to apportion individual contributions.

It is further noted that only parent-compound loss was measured across all three treatments evaluated in this study (sonication, UV/TiO<sub>2</sub>, and LAB fermentation); transformation products (e.g., 3,5,6-trichloro-2-pyridinol and diethyl phosphate for chlorpyrifos, and sonolysis/photolysis intermediates for both compounds) were not quantified for any treatment. Some of these products may retain comparable or greater toxicity than the parent compound, and the term "degradation" as used throughout the Results and Discussion should accordingly be interpreted as parent-compound reduction, based on GC-MS-SIM quantification of the par-

ent analyte, rather than confirmed detoxification. Metabolite identification and toxicological screening are essential next steps before any of the three approaches can be regarded as improving the net safety of the milk.

The use of commercial EC formulations, rather than analytical-grade active ingredients, introduces co-formulant solvents and emulsifiers that could plausibly bias each treatment technology by a different mechanism: altered pesticide partitioning into milk fat and cavitation dynamics for sonication; competitive surface adsorption onto TiO<sub>2</sub> particles for UV/TiO<sub>2</sub> photocatalysis; UV-absorbing excipients competing for incident photons; and solvent/emulsifier effects on LAB growth or membrane integrity for fermentation. These effects were not isolated experimentally. Confirmatory trials using analytical-grade standards and/or blank-formulation (excipient-only) controls are recommended before the reported removal efficiencies are generalised beyond the specific formulated products tested here.

#### 5. Conclusions

This study demonstrates that ultrasonication, UV/TiO<sub>2</sub> photocatalysis, and LAB fermentation reduced, to varying degrees and under non-equivalent reference conditions, chlorpyrifos and phoxim residues in buffalo milk. Among the evaluated treatments, the combined UV/TiO<sub>2</sub> treatment numerically achieved the highest parent-pesticide removal for phoxim (98.63%) and a moderate removal efficiency for chlorpyrifos (57.31%), whereas ultrasonication was particularly effective for phoxim (97.52% removal efficiency) and provided rapid reduction of parent pesticide residues. Because TiO<sub>2</sub>-dark-adsorption controls were not performed, these UV/TiO<sub>2</sub> values reflect combined adsorption and photocatalysis rather than photocatalysis specifically, and because nano-TiO<sub>2</sub> improved phoxim removal by only ~2 percentage points over UV alone, its practical necessity for phoxim specifically is questionable given its unresolved food-safety profile. LAB fermentation also reduced pesticide residues and represents a promising biological treatment approach, though the relative contributions of enzymatic, acid-catalysed, and adsorptive mechanisms remain unconfirmed pending the control experiments identified in Section 4. These comparative findings should be interpreted within the experimental conditions of the present study because different treatment systems and reference conditions were employed, including differing treatment durations, pesticide concentrations that substantially exceed realistic residue levels and MRLs, and the use of commercial EC pesticide formulations rather than authentic incurred residues. Only the reduction of the parent pesticide compounds was quantified, whereas transformation products and their potential toxicological significance were not investigated. No milk-quality, sensory, or (for UV/TiO<sub>2</sub>) nanoparticle-safety data were collected; accordingly, descriptions of these approaches

as sustainable, eco-friendly, or industrially applicable should be regarded as provisional. Future studies should therefore identify transformation products, evaluate their toxicity, perform the mechanistic and matrix-specific validation controls identified in Section 4, test concentrations closer to realistic contamination levels, optimise treatment conditions for industrial application, and assess the sensory, physicochemical, and nutritional quality of treated milk. Overall, this study provides a basis for the further development and optimisation of environmentally friendly technologies for reducing organophosphorus pesticide residues in dairy products.

### Availability of Data and Materials

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

### Author Contributions

EME: investigation, supervision, validation, writing—review and editing. HM: supervision, writing—review and editing, interpretation of data. AMH: methodology, validation, writing—review and editing. SG: conceptualisation, formal analysis. AAEE: methodology, resources, writing—review and editing. AAH: data curation, methodology, resources, project administration, writing—original draft, review and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

### Ethics Approval and Consent to Participate

Not applicable.

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### Conflicts of Interest

The authors declare no conflicts of interest. Chr. Hansen A/S provided starter culture preparations for the study but had no role in the study design, data collection, analysis, interpretation of data, or preparation of the manuscript.

## References

- [1] Tang FHM, Wyckhuys KAG, Li Z, Maggi F, Silva V. Trans-boundary impacts of pesticide use in food production. *Nature Reviews Earth and Environment*. 2025;6:383–400. <https://doi.org/10.1038/s43017-025-00673-y>
- [2] FAO. Pesticide use and trade 1990–2023. Food and Agriculture Organization of the United Nations: Rome. 2025.
- [3] Shaker EM, Elsharkawy EE. Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt. *Environmental Toxicology and Pharmacology*. 2015; 39: 433–440. <https://doi.org/10.1016/j.etap.2014.12.005>
- [4] Saad H, Elfeky SA, El-Gamel NEA, Abo Dena AS. Organophosphate pesticides: a review on classification, synthesis, toxicity, remediation and analysis. *RSC Advances*. 2025; 15: 40802–40822. <https://doi.org/10.1039/d5ra05552k>
- [5] U.S. Environmental Protection Agency. Chlorpyrifos: Overview and uses. 2022. Available at: <https://www.epa.gov/ingredients-used-pesticide-products/chlorpyrifos> (Accessed: 13 July 2026).
- [6] Kalenik S, Zaczek A, Rodacka A. Chlorpyrifos and Chlorpyrifos-Oxon: A Widening Spectrum of Toxicity. *International Journal of Molecular Sciences*. 2026; 27: 3909. <https://doi.org/10.3390/ijms27093909>
- [7] 2007/442/EC: Commission Decision of 21 June 2007. Concerning the non-inclusion of certain active substances in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances. 2007. Available at: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32007D0442> (Accessed: 17 July 2026).
- [8] Food and Agriculture Organization of the United Nations (FAO). FAOSTAT: Milk production by species. 2023. Available at: <http://www.fao.org/faostat/en/#home> (Accessed: 12 July 2026).
- [9] Vargas-Ramella M, Pateiro M, Maggiolino A, Faccia M, Franco D, De Palo P, et al. Buffalo Milk as a Source of Probiotic Functional Products. *Microorganisms*. 2021; 9: 2303. <https://doi.org/10.3390/microorganisms9112303>
- [10] Mota-Rojas D, Napolitano F, Bettencourt AF, Bragaglio A, Nannoni E, Lanzoni L, et al. Water buffalo milk: physicochemical, nutritional properties, and potential benefits for human health. *Frontiers in Nutrition*. 2026; 13: 1742552. <https://doi.org/10.3389/fnut.2026.1742552>
- [11] Xiao O, Li M, Chen J, Dai X, Kong Z. A Comprehensive Analysis of the Fates of Pesticide Residues in Edible Agricultural Products during Food Processing. *Journal of Agricultural and Food Chemistry*. 2025; 73: 18525–18544. <https://doi.org/10.1021/acs.jafc.5c05285>
- [12] Azam SMR, Ma H, Xu B, Devi S, Siddique MAB, Stanley SL, et al. Efficacy of ultrasound treatment in the removal of pesticide residues from fresh vegetables: A review. *Trends in Food Science & Technology*. 2020; 97: 417–432. <https://doi.org/10.1016/j.tifs.2020.01.028>
- [13] Ali M, Manzoor MF, Goksen G, Aadil RM, Zeng XA, Iqbal MW, et al. High-intensity ultrasonication impact on the chlorothalonil fungicide and its reduction pathway in spinach juice. *Ultrasonics Sonochemistry*. 2023; 94: 106303. <https://doi.org/10.1016/j.ultrasonch.2023.106303>
- [14] El-Saeid MH, Alotaibi MO, Alshabanat M, Al-Anazy MM, Alharbi KR, Altowyan AS. Impact of photolysis and TiO<sub>2</sub> on pesticides degradation in wastewater. *Water*. 2021; 13: 655. <https://doi.org/10.3390/w13050655>
- [15] Armenova N, Tsigoriyna L, Arsov A, Petrov K, Petrova P. Microbial Detoxification of Residual Pesticides in Fermented Foods: Current Status and Prospects. *Foods (Basel, Switzerland)*. 2023; 12: 1163. <https://doi.org/10.3390/foods12061163>
- [16] Kundu S, Chanda A, Espinosa-Marvan L, Khetan SK, Collins TJ. Facile destruction of formulated chlorpyrifos through green

- oxidation catalysis. *Catal Sci Technol*. 2012; 2 :1165–1172. <https://doi.org/10.1039/C2CY00447J>
- [17] AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists. 20th edn. AOAC International: Rockville, MD. 2016.
- [18] Yuan S, Li C, Zhang Y, Yu H, Xie Y, Guo Y, et al. Ultrasound as an emerging technology for the elimination of chemical contaminants in food: A review. *Trends in Food Science & Technology*. 2021; 109: 374–385. <https://doi.org/10.1016/j.tifs.2021.01.048>
- [19] Hassan H, Elsayed E, El-Raouf AERA, Salman SN. Method validation and evaluation of household processing on reduction of pesticide residues in tomato. *Journal of Consumer Protection and Food Safety*. 2019; 14: 31–39. <https://doi.org/10.1007/s00003-018-1197-2>
- [20] Crook JA, Rossitto PV, Parko J, Koutchma T, Cullor JS. Efficacy of Ultraviolet (UV-C) Light in a Thin-Film Turbulent Flow for the Reduction of Milkborne Pathogens. *Foodborne Pathogens and Disease*. 2015; 12: 506–513. <https://doi.org/10.1089/fpd.2014.1843>
- [21] Abd El-Salam MH, El-Shibiny S. A comprehensive review on the composition and properties of buffalo milk. *Dairy Science & Technology*. 2011; 91: 663–699. <https://doi.org/10.1007/s13594-011-0029-2>
- [22] Akgun A, Yazici F, Gulec HA. The combined effect of probiotic cultures and incubation final pH on the quality of buffalo milk yogurt during cold storage. *Food Science & Nutrition*. 2018;6:492–502. <https://doi.org/10.1002/fsn3.580>
- [23] Agarwal S, Tyagi I, Gupta VK, Dehghani MH, Bagheri A, Yetilmezsoy K, et al. Degradation of azinphos-methyl and chlorpyrifos from aqueous solutions by ultrasound treatment. *Journal of Molecular Liquids*. 2016; 221: 1237–1242. <https://doi.org/10.1016/j.molliq.2016.04.076>
- [24] Budarz JF, Cooper EM, Gardner C, Hodzic E, Ferguson PL, Gunsch CK, et al. Chlorpyrifos degradation via photoreactive TiO<sub>2</sub> nanoparticles: Assessing the impact of a multi-component degradation scenario. *Journal of Hazardous Materials*. 2019; 372: 61–68. <https://doi.org/10.1016/j.jhazmat.2017.12.028>
- [25] Lin B, Yu Y, Hu X, Deng D, Zhu L, Wang W. Degradation mechanisms of phoxim in river water. *Journal of Agricultural and Food Chemistry*. 2011; 59: 312–321. <https://doi.org/10.1021/jf1029459>
- [26] Tabasum A, Alghuthaymi M, Qazi UY, Shahid I, Abbas Q, Javaid R, et al. UV-Accelerated Photocatalytic Degradation of Pesticide over Magnetite and Cobalt Ferrite Decorated Graphene Oxide Composite. *Plants (Basel, Switzerland)*. 2021; 10: 6. <https://doi.org/10.3390/plants10010006>
- [27] Li C, Ma Y, Mi Z, Huo R, Zhou T, Hai H, et al. Screening for *Lactobacillus plantarum* Strains That Possess Organophosphorus Pesticide-Degrading Activity and Metabolomic Analysis of Phorate Degradation. *Frontiers in Microbiology*. 2018; 9: 2048. <https://doi.org/10.3389/fmicb.2018.02048>
- [28] Kumari A, Ghosh C, Kannan N, Balaji S. *Lactiplantibacillus plantarum* as a sustainable solution for monocrotophos degradation and plant growth enhancement. *International Microbiology: the Official Journal of the Spanish Society for Microbiology*. 2025; 28: 1905–1917. <https://doi.org/10.1007/s10123-025-00671-6>
- [29] Pardini AA, Iurlina MO, Reynals Marcangeli A, Robles AD, Saiz AI. Lactic acid bacteria as bioremediation agents for chlorpyrifos degradation: a combined chemical and in silico approach. *Exploration of Foods and Foodomics*. 2026; 4: 1010127. <https://doi.org/10.37349/eff.2026.1010127>
- [30] Aziz A, Homayouni A. Bacterial degradation of pesticide residues in vegetables during fermentation. *Asian Journal of Chemistry*. 2009; 21: 6255–6264.
- [31] Li J, Hu K, Hu L, Hou X, Li Q, Liu A, et al. Adsorption behavior of 3-phenoxybenzoic acid by *Lactobacillus plantarum* and its potential application in simulated digestive juices. *International Journal of Molecular Sciences*. 2022;23:5809. <https://doi.org/10.3390/ijms23105809>
- [32] Kiruthika K, Suganthi A, Johnson Thangaraj Edward YS, Anandham R, Renukadevi P, Murugan M, et al. Role of Lactic Acid Bacteria in Insecticide Residue Degradation. *Probiotics and Antimicrobial Proteins*. 2025; 17: 81–102. <https://doi.org/10.1007/s12602-024-10298-0>
- [33] Abbasi A, Sheykhsaran E, Hosseinzadeh N, Bazdar M, Hamehjani M, Aghapour B, et al. Novel Approaches in Establishing Chemical Food Safety Based on the Detoxification Capacity of Probiotics and Postbiotics: A Critical Review. *Probiotics and Antimicrobial Proteins*. 2026; 18: 1641–1681. <https://doi.org/10.1007/s12602-025-10615-1>