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Microbial preparation for pesticide residues (chlorpyrifos ethyl) treatment in agricultural soil

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Abstract. Chlorpyrifos ethyl (CPF), a widely used organophosphate pesticide, poses significant environmental risks because of its excessive application beyond recommended levels. This study presents the development of a microbial preparation (MP) for the treatment of CPF residues in soil using indigenous strains *Methylobacterium populi* (CNN2) and *Ensifer adhaerens* (VNN3), which were selected on the basis of their demonstrated CPF-degrading capability in prior research. The objective was to identify optimal conditions for biomass production to formulate a stable and effective MP. Laboratory-scale experiments were conducted to evaluate biosafety and determine ideal parameters, including pH, temperature, fermentation media, and inoculum ratio for microbial growth. The selected strains met European biosafety standards, exhibited mutual compatibility, and showed no antagonism toward beneficial microorganisms. Optimal biomass production was achieved at 30 °C and pH 6.5–7.0, in an SX1 medium with a 3% inoculum ratio. The strains demonstrated impressive *in vitro* CPF degradation capabilities, achieving up to 81% and 95% degradation efficiency for CNN2 and VNN3, respectively, after three days of cultivation. The developed MP maintained high viability with cell counts of 10^9 CFU/g after one month, 10^8 CFU/g after six months, and 10^7 CFU/g after twelve months. These results demonstrate the potential of native, eco-friendly microbial agents for effective bioremediation of pesticide-contaminated agricultural soils.

Keywords: pesticide, microbial preparation, chlorpyrifos ethyl, organophosphate, residue treatment

1 Introduction

Plant protection products based on organic phosphorus, particularly chlorpyrifos ethyl (CPF), are extensively utilised in modern agriculture because of their remarkable effectiveness and versatility in controlling a broad spectrum of agricultural pests [1, 2]. As one of the most widely applied organophosphate pesticides globally, CPF has become an integral component of contemporary farming practices, contributing significantly to crop protection and agricultural productivity.

However, the widespread and intensive use of CPF has resulted in serious environmental consequences, leading to extensive pollution of ecosystems and subsequently affecting human health through bioaccumulation in the food chain [3]. The environmental persistence and mobility of CPF have resulted in its ubiquitous presence across multiple ecological compartments. Alarmingly, CPF contamination has been

documented in groundwater systems, surface water bodies, agricultural soils, sediments, and has even been detected in human breast milk, indicating the extent of its environmental penetration and potential for human exposure [2, 4, 5, 6]. The severity of this contamination is exemplified by findings in Vietnam's tea-growing regions, where CPF residues have reached concentrations as high as 28 mg/kg in soil [7] – a level that exceeds the maximum allowable limit established by Vietnamese national standards (TCVN 15: 2008/BTNMT) by 280 times, which sets the permissible threshold at 0.1 mg/kg soil. This dramatic exceedance of safety standards underscores the urgent need for effective remediation strategies.

Given these alarming contamination levels and their potential impacts on food safety and public health, the removal of CPF residues from the environment, particularly from agricultural lands where food crops are cultivated, has become a critical priority for protecting human health and ensuring sustainable farming practices.

Traditional approaches to CPF remediation have relied on various physicochemical methods for treating contaminated environments, including deep burial in oceanic sites, incineration in open pit systems, and advanced oxidation processes. However, these conventional treatment methods encounter significant limitations, as they frequently lead to the formation of secondary pollutants with potentially higher toxicity levels than the original contaminants. Additionally, these processes often result in the accumulation of recalcitrant substances that are difficult to degrade, requiring expensive, environmentally unfriendly, and technically challenging treatment approaches [8]. In response to these limitations, the scientific community has increasingly turned towards biological remediation strategies. The utilisation of indigenous microorganisms for CPF removal from contaminated environments has emerged as the preferred approach among researchers because of its demonstrated effectiveness, cost-efficiency, and environmentally sustainable nature [2, 9, 10]. This bioremediation approach offers the advantage of complete mineralisation of the pesticide without generating harmful byproducts, while being economically viable for large-scale applications.

While several studies have made significant contributions to understanding microbial degradation of CPF, including evaluations of various microbial groups' capacity to degrade CPF in contaminated soil environments [11–13], elucidation of degradation mechanisms [14], and assessment of the activity and application potential of individual or combined microorganisms in CPF residue treatment [12, 15], there remains a significant gap in the literature regarding the practical production and application of microbial preparations derived from CPF-degrading strains. This paper addresses this critical knowledge gap by presenting comprehensive results on the development and production of microbial preparations (MP) from carefully selected microbial strains with demonstrated CPF degradation capacities. The ultimate goal of this work is to provide a practical, scalable solution to treating CPF residues in agricultural lands, thereby contributing to the establishment of safe and sustainable farming practices in Vietnam and potentially serving as a model for similar applications globally.

2 Materials and methods

2.1 Research materials

Three strains, identified as *Methylobacterium populi* (CNN2), *Ensifer adhaerens* (VNN3), and *Acinetobacter pittii* (CNN4), were selected for the production of MP because of their high degradation activity [1].

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Two strains *Azotobacter chroococcum* and *Bacillus polymyxa* were obtained from the microbial culture collection of the Environmental Biology Department, Institute of Agricultural Environment.

All experiments were conducted by using equipment from the Experimental Biology Laboratory and specialised laboratories of the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology. Analytical-grade chemicals with a purity of 99.5%, sourced from Dr. Ehrenstorfer, were employed in this study.

2.2 Research methods

Quantitative determination of microbial density

Microbial density was determined according to TCVN 4884-1:2015 – Part 1 [16] as follows: Microorganisms were cultured on media SX1, SX2, and SX3, with their compositions detailed in the following section, then preserved and stored for designated periods. Colony-forming units (CFU) were counted on agar plate media to calculate cell density, expressed as CFU/mL. For phosphate-solubilising bacteria, microbial density was determined according to the method of Pikovskaya (1948), as modified by Brick et al. [17]. The ability to decompose insoluble phosphate was assessed by measuring the $Ca_3(PO_4)_2$ solubilisation zone on the solid medium that appeared as a clear halo surrounding bacterial colonies or agar wells.

Biosafety assessment

The relative safety of microbial strains was evaluated on the basis of their taxonomic classification according to European Community Directive 90/679/EEC (November 26, 1990) on biosafety guidelines. Safety assessment was conducted according to the method of Carter (1984) [18].

Bacterial suspensions of CNN2, VNN3, and CNN4 at densities $>10^8$ CFU/mL were administered to white mice (Mus musculus, 50–60 g body weight) via intravenous and intraperitoneal injection at doses of 0.2 and 0.5 mL per mouse. The mice were monitored for morbidity and mortality over the observation period. The control groups received sterile distilled water instead of bacterial suspensions.

Optimisation of fermentation conditions

Fermentation parameters were optimised through single-factor experiments to determine optimal conditions for microbial biomass production. Cell density (measured as described in the above section) served as the primary response variable.

- Temperature optimisation: Fermentation temperatures were tested at 20, 25, 30, 35, 40, and 45 °C.
- pH optimisation: Initial medium pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0.
- Culture medium selection: Three different growth media were evaluated. Medium SX1: Sugar (20 g), yeast extract (10 g), K_2HPO_4 (0.2 g), and distilled water (1,000 mL); medium SX2: Mung bean sprout extract (40 g), glucose (5 g), yeast extract (5 g), and distilled water (1,000 mL); medium SX3: peptone (20 g), yeast extract (10 g), glucose (1 g), and distilled water (1,000 mL); all media were sterilised by autoclaving at 121 °C for 15 minutes at 1 atm pressure.
- Inoculum size optimisation: The starter culture inoculum was added at concentrations ranging from 0.5 to 5% (v/v). These cultures were prepared in a liquid medium on a rotary shaker at 150 rpm until reaching cell densities of more than 10^8 CFU/mL after 48 hours, with purity confirmed before use.

- Aeration rate optimisation: Air supply rates during fermentation were tested at 0.5, 0.6, 0.7, 0.8, and 0.9 vvm (volume of air per volume of medium per minute). The effect of aeration on final cell density was evaluated at each air flow rate.

Evaluation of CPF degradation with the isolated bacterial strains

The isolates were cultured in the MSM medium supplemented with 100 mg/L CPF until the OD620 value of 0.6 was reached. After 24, 48 and 72 h, the CPF concentration of the samples was determined, and the CPF degradation ability of the isolated strains in the cultured medium was evaluated [1]. The determination of CPF concentration was conducted as follows: the CPF samples were shaken with 5 mL of acetonitrile and filtered using a 0.22 μm syringe filter. CPF concentration was measured using HPLC at 300 nm [1]. In HPLC analysis, the UV-Vis detector (integrated within the HPLC system) records the signal intensity at a wavelength of 300 nm, from which the concentration of CPF is determined based on the calibration curve.

The experiments were arranged in a randomised complete block design with three replicates.

Data analysis

Microsoft Excel was employed for data management, graph plotting, calculation, and comparison of treatment means.

3 Results and discussion

3.1 Assessment of microbial biosafety

Prior to developing microbial preparations, comprehensive safety evaluations of the selected bacterial strains were conducted. The biosafety assessment involved acute toxicity testing using white mice as an animal model.

Table 1. Safety assessment results of microbial strains on experimental mice

	CNN2		VNN3		CNN4		Control	
Number of mice injected	5	5	5	5	5	5	5	5
Dosage of injection (mL)	0.2	0.5	0.2	0.5	0.2	0.5	0.2	0.5
Route of injection	V	S	V	S	V	S	V	S
Tracking time (day)	10	10	10	10	10	10	10	10
Number of sick/dead mice	0	0	0	0	1/5	1/5	0	0
Time of sick/dead mice (h)	0	0	0	0	16	16	0	0
Assessment	SF	SF	SF	SF	NSF	NSF	SF	SF

V: Vein; S: Sinus; SF: Safe; NSF: Not safe

The results presented in Table 1 demonstrate that the two strains, CNN2 and VNN3, exhibited no acute toxicity, as evidenced by the absence of mortality in test animals. Cross-referencing these strains against the European Community's biosafety classification list (Directive 90/679/EEC, November 26, 1990) confirmed that *M. populi* and *E. adhaerens* are not classified as restricted microorganisms. According to these findings, both the strains were deemed suitable for further development of chlorpyrifos (CPF)-degrading microbial products because of

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their high biosafety profile and minimal risk to organisms and the environment.

3.2 Strain compatibility assessment

To maximise the efficacy of a multi-strain formulation, the compatibility between *M. populi* and *E. adhaerens* was investigated. The strains were co-inoculated on an LB agar medium that uses intersecting inoculation lines to create contact zones. The absence of inhibition zones at these contact points would indicate compatibility between the two strains.

Table 2. Relationship between strains *M. populi* and *E. adharens*

Strains	*A. chroococcum*	*B. polymyxa*	CNN2	VNN3
A. chroococcum		+	+	+
B. polymyxa	+		+	+
CNN2	+	+		+
VNN3	+	+	+	

Additionally, the potential antagonistic effects of these strains on beneficial soil microorganisms were also evaluated. Specifically, interactions with *Azotobacter chroococcum* (a nitrogen-fixing bacterium) and *Bacillus polymyxa* (a phosphate-solubilising bacterium) were assessed to ensure that the final product would not negatively affect soil microbial communities.

As shown in Table 2, both *M. populi* and *E. adhaerens* demonstrated successful co-cultivation with *A. chroococcum* and *B. polymyxa* on the LB medium without exhibiting mutual inhibition. These results indicate that the selected strains are compatible with each other and with beneficial soil microorganisms, making them suitable candidates for combined use in microbial product formulations.

The compatibility findings are essential for practical applications, as they suggest that a multi-strain formulation could potentially provide enhanced CPF degradation while maintaining beneficial interactions with the existing soil microbiome. This compatibility is crucial for developing environmentally sustainable bioremediation products that can be safely integrated into agricultural systems.

3.3 Optimisation of biomass fermentation conditions

The fermentation parameters for both *M. populi* and *E. adhaerens* were systematically optimised by using a Bioexcel L automated fermentation system (Centrion, South Korea) to maximise biomass production for industrial-scale microbial product development.

Temperature optimisation: Temperature significantly influenced the growth kinetics of both bacterial strains over the 72-hour cultivation period (Table 3). *M. populi* demonstrated optimal growth within the temperature range of 25–30 °C, while *E. adhaerens* showed a peak performance at 30 °C. Both the strains achieved cell densities of 10^8 CFU/mL at 30 °C, which was selected as the optimal temperature for subsequent experiments.

Table 3. Effect of temperature on growth of microorganisms (CFU/mL)

t, °C	**CNN2**	**VNN3**
20	$(3.4 \pm 0.9) \times 10^5$	$(4.1 \pm 1.2) \times 10^6$
25	$(2.3 \pm 1.1) \times 10^8$	$(2.2 \pm 0.8) \times 10^7$
30	$(3.1 \pm 0.7) \times 10^8$	$(6.2 \pm 0.9) \times 10^8$
35	$(3.7 \pm 1.2) \times 10^7$	$(4.3 \pm 1.4) \times 10^7$
40	$(4.1 \pm 1.0) \times 10^6$	$(3.3 \pm 1.3) \times 10^6$
45	$(3.3 \pm 0.6) \times 10^4$	$(2.4 \pm 0.8) \times 10^3$

Temperatures below 20 °C and above 35 °C proved unsuitable for biomass fermentation of both the strains, likely because of reduced metabolic activity at low temperatures and protein denaturation at elevated temperatures. These findings align with those of previous

studies on chlorpyrifos-degrading bacteria, including work of Zhao et al. (2014), who reported an optimal growth of *Acinetobacter calcoaceticus* at 30 °C [19], and Singh et al. (2004), who observed a peak performance of Enterobacter strain B-14 at similar temperatures [12].

***pH optimisation*:** Initial medium pH substantially affected bacterial growth, with strain-specific optima observed (Table 4). *M. populi* achieved maximum biomass production at pH 6.5, while *E. adhaerens* performed optimally at pH 7.0. These slightly acidic to neutral conditions are consistent with the natural soil environments from which these strains were originally isolated and support findings of Farhan et al. (2021), who reported enhanced degradation activity at near-neutral pH for CPF-degrading strains [12].

***Culture medium selection and fermentation duration*:** Three production media (SX1, SX2, and SX3) were evaluated for biomass yield and cultivation efficiency (Table 5). For *M. populi*, both the SX1 and SX2 media supported high biomass yields, with optimal harvesting at 72 hours. *E. adhaerens* showed superior growth on the SX1 medium with a shorter cultivation time, allowing biomass harvest after 48 hours.

A faster growth of *E. adhaerens* than that of *M. populi* suggests different metabolic efficiencies and nutrient utilisation patterns between the two strains. This information is crucial for industrial production scheduling and cost optimisation.

Table 4. Effect of pH on growth of microorganisms (CFU/mL)

pH	CNN2	VNN3
5.0	$(4.5 \pm 1.7) \times 10^4$	$(3.9 \pm 1.2) \times 10^4$
5.5	$(4.7 \pm 1.4) \times 10^4$	$(3.8 \pm 1.8) \times 10^5$
6.0	$(5.1 \pm 1.2) \times 10^6$	$(6.3 \pm 1.5) \times 10^5$
6.5	$(3.4 \pm 1.4) \times 10^8$	$(4.5 \pm 1.7) \times 10^7$
7.0	$(3.4 \pm 1.6) \times 10^7$	$(5.6 \pm 1.6) \times 10^8$
7.5	$(7.9 \pm 1.7) \times 10^7$	$(3.2 \pm 1.0) \times 10^7$
8.0	$(4.1 \pm 1.9) \times 10^7$	$(3.2 \pm 1.3) \times 10^6$

Table 5. Growth potential of microorganisms on fermentation media (CFU/mL)

t, h	Medium SX1		Medium SX2		Medium SX3	
	CNN2	VNN3	CNN2	VNN3	CNN2	VNN3
0	$(5.3 \pm 1.6) \times 10^4$	$(3.3 \pm 1.9) \times 10^4$	$(3.6 \pm 1.2) \times 10^4$	$(3.5 \pm 1.8) \times 10^4$	$(3.8 \pm 1.0) \times 10^4$	$(4.6 \pm 1.9) \times 10^4$
24	$(3.7 \pm 1.4) \times 10^6$	$(5.5 \pm 1.7) \times 10^7$	$(5.7 \pm 1.7) \times 10^5$	$(3.8 \pm 1.9) \times 10^6$	$(3.5 \pm 1.7) \times 10^5$	$(4.2 \pm 1.2) \times 10^6$
48	$(2.4 \pm 1.6) \times 10^8$	$(5.2 \pm 1.2) \times 10^8$	$(5.8 \pm 1.3) \times 10^6$	$(6.5 \pm 1.62) \times 10^7$	$(4.4 \pm 1.5) \times 10^6$	$(5.1 \pm 1.4) \times 10^7$
72	$(5.4 \pm 1.5) \times 10^8$	$(4.3 \pm 1.0) \times 10^8$	$(6.2 \pm 1.4) \times 10^8$	$(5.3 \pm 1.1) \times 10^7$	$(6.5 \pm 1.9) \times 10^7$	$(7.4 \pm 1.5) \times 10^7$
96	$(5.5 \pm 1.9) \times 10^8$	$(4.7 \pm 1.6) \times 10^8$	$(6.4 \pm 1.8) \times 10^8$	$(6.8 \pm 1.7) \times 10^8$	$(2.2 \pm 1.3) \times 10^8$	$(6.1 \pm 1.6) \times 10^8$

Inoculum size optimisation: The effect of initial inoculum concentration (0.5–5% v/v) on the final biomass yield was investigated (Table 6). For *M. populi*, the 3% inoculum size yielded a cell density of 4.56×10^8 CFU/mL, with no significant improvement observed at 5% inoculum. Similarly, *E. adhaerens* showed optimal performance at the 3% inoculum size.

***Aeration rate optimisation*:** Oxygen supply significantly influenced bacterial growth, with an optimal performance achieved at 0.7 dm^3 air per litre medium per minute for both the strains (Table 7). This aeration rate likely provides sufficient dissolved oxygen for aerobic metabolism while avoiding excessive foam formation or cellular stress from over-aeration.

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Table 6. Effect of first-generation ratio on growth of microorganisms (CFU/mL)

First-generation ratio (%)	CNN2	VNN3
0.5	$(6.3 \pm 1.7) \times 10^7$	$(6.6 \pm 1.5) \times 10^7$
1	$(8.2 \pm 1.3) \times 10^7$	$(8.2 \pm 1.9) \times 10^7$
2	$(8.5 \pm 1.0) \times 10^7$	$(8.4 \pm 1.7) \times 10^7$
3	$(4.6 \pm 1.8) \times 10^8$	$(5.4 \pm 1.3) \times 10^8$
4	$(4.6 \pm 1.1) \times 10^8$	$(5.5 \pm 1.0) \times 10^8$
5	$(5.1 \pm 1.2) \times 10^8$	$(5.5 \pm 1.4) \times 10^8$

Table 7. Effect of aeration rate on growth of microorganisms (CFU/mL)

Aeration rate*	CNN2	VNN3
0.5	$(4.4 \pm 1.3) \times 10^6$	$(5.2 \pm 1.3) \times 10^6$
0.6	$(3.3 \pm 1.7) \times 10^7$	$(4.5 \pm 1.2) \times 10^7$
0.7	$(6.6 \pm 1.4) \times 10^8$	$(7.0 \pm 1.0) \times 10^8$
0.8	$(5.8 \pm 1.9) \times 10^8$	$(5.9 \pm 1.5) \times 10^8$
0.9	$(5.1 \pm 1.5) \times 10^8$	$(5.1 \pm 1.1) \times 10^8$

* dm^3 air/L of medium/min

Integrated optimisation results: The comprehensive optimisation study established the following optimal conditions for biomass production (Table 8): Temperature: 30 °C; pH: 6.5 (*M. populi*) and 7.0 (*E. adhaerens*); Medium: SX1 for both the strains; Inoculum size: 3% (v/v); Aeration rate: 0.7 dm^3 air/L medium/min; Harvest time: 48 hours (*E. adhaerens*) and 72 hours (*M. populi*).

According to the parameters provided in Table 8, the production flow of the microbial formulation is briefly illustrated in Diagram 1.

Based on established methodologies from the Institute of Agricultural Environment, Vietnam Academy of Agricultural Sciences [7, 20–22], a practical formulation process was developed by using anhydrous dextrose as a carrier. The optimised biomass (verified for purity and minimum cell density of 10^8 CFU/mL) was processed through centrifugation at 6,000 rpm for 30 minutes, followed by mixing with dextrose carrier at a 1:100 ratio with a rotary drum mixing machine. The final product achieved uniform distribution with a 12% moisture content and was sealed in foil bags and stored in dry areas away from direct sunlight.

Table 8. Technical parameters suitable for biomass production of microbial strains

Technical parameters	CNN2	VNN3
pH	6.5	7.0
Temperature (°C)	30	30
Time (h)	72	48
First-generation ratio (%)	3	3
Fermentation media	SX1	SX1
Aeration rate (dm^3 air/L of medium/min)	0.7	0.7

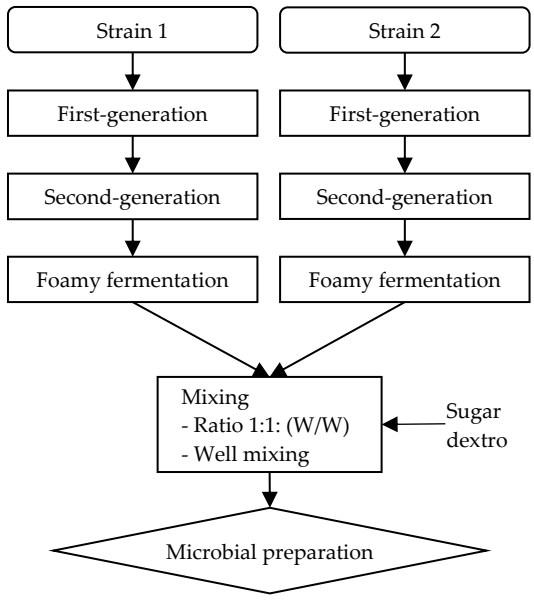

Diagram 1. Microbial product formulation

This formulation approach differs from that of previous studies by emphasising long-term microbial viability in the final product rather than focusing solely on growth and degradation activity. The integration of controlled aeration parameters (0.7 dm^3/L/min) with dextrose carrier

technology provides a robust foundation for industrial-scale production.

The optimised conditions established in this study are consistent with those in the literature for similar CPF-degrading microorganisms, validating the reliability of these parameters for practical applications. The systematic optimisation approach ensures both technical feasibility and economic viability for large-scale microbial product manufacturing.

3.4 Evaluation of biological activity of microorganisms

***Chlorpyrifos degradation efficiency*:** The CPF degradation capabilities of the selected strains were evaluated under controlled laboratory conditions (Table 9). Both *M. populi* and *E. adhaerens* demonstrated exceptional biodegradation performance, achieving 81.4 and 95.2% CPF removal, respectively, within 3 days of treatment at an initial concentration of 100 mg/L.

These results represent a significant improvement over natural degradation processes. According to Barles (1979), CPF exhibits a half-life of 16–120 days under natural environmental conditions [23]. The microbial treatment accelerated this process dramatically, achieving substantial degradation within 72 hours, representing a degradation rate several orders of magnitude higher than natural attenuation.

Comparative analysis with published literature further highlights the superior performance of the selected strains. Zhao et al. (2014) reported 60% CPF degradation with Acinetobacter calcoaceticus over 4 days under similar conditions (100 mg/L initial concentration) [22]. Singh et al. (2004) achieved 75% degradation with Enterobacter strain B-14 in 7 days [12], while Islam and Iyer (2021) reported 65–70% degradation with a three-strain consortium after 7 days [15]. The current study's results demonstrate notably higher efficiency in a shorter timeframe, indicating the exceptional biodegradation potential of the indigenous CNN2 and VNN3 strains.

***Phosphate solubilisation activity*:** Beyond CPF degradation, both strains were evaluated for their ability to solubilise insoluble phosphate compounds, representing an additional plant-beneficial trait (Table 9). This dual functionality enhances the value proposition of the microbial product by potentially improving soil phosphorus availability while simultaneously degrading pesticide residues.

Table 9. Ability of microorganisms to dissolve CPF and insoluble phosphates

	CNN2	VNN3	Control
% decrease of CPF in the culture medium containing a 100mg/L CPF solution (after 3 days)	81.4 ± 2.8	95.2 ± 2.2	0 ± 0.0
The resolution diameter of $Ca_3(PO_4)_2$ (D-d), mm	22 ± 1	18 ± 1	0 ± 0.0

3.5 Microbial viability and shelf-life stability

Long-term viability of microorganisms in the formulated product is critical for commercial application. The stability study monitored cell density over 12 months under standard storage conditions (Table 10).

Table 10. Density of *M. populi* and *E. adharens* strains in product over time – (CFU/g)

	CNN2	VNN3
0 h	7.2×10^9	8.4×10^9
1 month	5.9×10^9	5.1×10^9
3 months	7.5×10^8	6.3×10^8
6 months	6.5×10^8	7.2×10^8
9 months	5.2×10^8	5.6×10^8
12 months	2.4×10^7	9.0×10^7

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The initial microbial density exceeded 10^9 CFU/g for both the strains in the mixed formulation. After one month of storage, the cell density remained above 10^9 CFU/g, indicating excellent short-term stability. After three months, viable cell counts decreased to above 10^8 CFU/g for both *M. populi* and *E. adhaerens*, and this density remained stable through nine months of storage.

After 12 months, the cell density decreased to above 10^7 CFU/g for both the strains. While this represents a two-log reduction from initial levels, the final concentration remains within the range typically considered effective for biological products. This stability profile compares favourably with that of numerous commercial microbial formulations and demonstrates the practical viability of the dextrose-based carrier system. The sustained viability observed in this study represents a significant advantage over several research reports that focus primarily on degradation efficiency without evaluating long-term formulation stability. This aspect is crucial for commercial product development and field application success.

3.6 Field efficacy evaluation in tea cultivation systems

The microbial product demonstrated robust efficacy in remediating chlorpyrifos residues in tea cultivation systems under field conditions. In mature plantations with baseline contamination levels of 0.042 mg/kg, MP application resulted in reductions to 0.011–0.012 mg/kg, corresponding to a 72–76% decrease [24]. These outcomes notably exceeded the reductions achieved through natural attenuation processes such as photodegradation and leaching, which provided only limited remediation over the same period [25]. Moreover, experimental plots treated with MP further reduced CPF concentrations to 0.002 mg/kg, equivalent to remediation levels 11–16 times as high as untreated controls and over 76% relative to initial contamination [24]. In comparison with controlled laboratory experiments of Farhan et al. (2021), which reported 73% CPF degradation over 10 days [11], the current field trial confirms the product's effective translation from lab-scale efficacy to practical, in-situ performance. When combined with its demonstrated formulation stability (10^7 CFU/g viability after 12 months), MP stands out as a commercially promising biotechnological solution for CPF mitigation in tea-growing agroecosystems. These findings support its integration into sustainable agrochemical management strategies in high-risk agricultural zones.

4 Conclusion

These research findings indicated that *M. populi* and *E. adhaerens* strains are capable of degrading CPF by up to 81% and 95%, respectively, after three days of cultivation. They also exhibited the ability to decompose insoluble phosphate, with degradation zone diameters of 18 and 22 cm. These strains are biologically safe, capable of coexisting, and do not inhibit the growth of beneficial microorganisms. These two strains are indigenous bacteria initially isolated from tea-growing soil environments contaminated with CPF residues. They hold potential for use in the production of MP to treat CPF residues in soil environments. Optimal conditions for biomass production of these strains have been established, including a temperature of 30 °C, pH of 6.5 and 7, SX1 fermentation medium, and biomass harvest times of 48 and 72 hours. The first-generation ratio is 3%, and the survival rate of the strains in the product is maintained at 10^9 CFU/g after one month, 10^8 CFU/g after six months, and 10^7 CFU/g after 12 months. The product has been successfully applied, suggesting that it holds promise for developing a sustainable and safe

biological solution for treating pesticide residues, particularly CPF, in agricultural soil.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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