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Dual Functionality of Soil Microbes: Chlorpyrifos Biodegradation and Enhancement of Plant Growth

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SHORT TITLE: Chlorpyrifos Biodegrading Formulations for Agricultural Use.

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Key Highlights

- 99% chlorpyrifos degradation in 5 days with biofertilizer synergy
- 15-20% crop yield boost via a dual-action microbial consortium
- Microbial enzymatic cascade degrades chlorpyrifos completely
- Field-ready solution for contaminated vegetable farmlands
- Eco-friendly alternative to chemical-intensive practices

Abstract

The present investigation aimed to formulate and evaluate a bacterial consortium capable of metabolizing chlorpyrifos under in vivo conditions. This consortium was also assessed for its potential to promote the growth of vegetables such as cauliflower, spinach, and radish. Bacterial consortia capable of degrading chlorpyrifos were isolated from pesticide-contaminated agricultural soil found within and around the National Capital Region (NCR), New Delhi, India. Subsequently, a compatible and efficient bacterial consortium was combined into an optimized formulation for further evaluation. The consortium was tested under controlled pot culture conditions using chlorpyrifos-contaminated soil and water as the growth media. Moreover, the crop seeds were treated using the bacterial formulation before sowing in the experimental setup. Chlorpyrifos residue levels in soil and water were monitored over time using gas chromatography for quantitative analysis. In addition, a phosphate-solubilizing biofertilizer (PSB) was incorporated into the bacterial mixture for its supplementary effect on plant growth. According to the results, 98–99% degradation of chlorpyrifos occurred at the centralized time point of the bacterial consortium within 10 days. This high degradation rate was consistent in both contaminated water and soil environments. Notably, when the PSB was included in the formulation, 98–99% degradation was achieved within just 5 days. Furthermore, shoot height and biomass increased by about 15% to 20% across all three crops tested in the study. These improvements indicate a significant enhancement in plant growth performance with the bacterial treatment. Therefore, this approach demonstrates dual promise: rapid pesticide degradation and enhanced crop yield. It offers a sustainable and eco-friendly strategy to mitigate chlorpyrifos contamination in agricultural systems.

Keywords: Chlorpyrifos biodegradation; Microbial consortium; Plant growthpromotion; Biofertilizer augmentation; Sustainable agriculture; Pesticide remediation

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Introduction

Chlorpyrifos [O, O-diethyl O-(3,5,6-trichloro-2-pyridyl phosphonothioate)] is a widely used potent organophosphate pesticide in agriculture that is highly resistant to decomposition. Chlorpyrifos has been known to be used in about 100 countries around the world to prevent losses due to insect pests on agricultural and residential areas (Egelko, 2019) and is known to be annually applied over approximately 8.5 million acres of agricultural land. The resistance to decomposition, along with modern agricultural practices, has aided in considerably increasing the pesticide pollution index in several regions (Sharma et al., 2019). Chlorpyrifos, while effective in pest control, tends to accumulate in ecosystems as residues, raising long-term contamination issues. The environmental half-life of chlorpyrifos is estimated to extend from 7 to 120 days based on several environmental situations and soil characteristics. Furthermore, the primary degradation product of chlorpyrifos, TCP (3,5,6-trichloro-2-pyridinol), aggravates the ecological damage in the contaminated soils. TCP inhibits almost all the beneficial soil microorganisms that are responsible for maintaining soil health and fertility in agricultural ecosystems (Akbar and Sultan, 2016; Supreeth et al., 2016). In India, vegetable crops such as cauliflower, spinach, and radish receive an estimated 13–14 per cent of total pesticide use. Such pollution is anticipated to jeopardize both ecosystem health and food safety within these production systems (Kodandaram et al., 2013).

Yet, the key limitation of the present-day approach to restoring chlorpyrifos is that numerous challenges limit their application in actual fields. First, most studies on microbial degradation have been carried out under controlled laboratory settings and within artificial media. Such investigations have thus generated a significant knowledge gap that questions the efficacy of these microbes under on-site field conditions. Second, the majority of investigations evaluate exclusively the degradation of the pesticide without considering its effects on the productivity and growth of crops (Arias-Castro et al., 2025; Kollah et al., 2020; Yadav et al., 2020).This raises concerns because of ample evidence showing that chlorpyrifos contamination negatively impacts overall plant growth and development.Third, degradation is a function of several genera of bacteria, including *Pseudomonas*, *Bacillus*, and *Arthrobacter* (Srinivasan et al., 2020). Studies targeting entire and functional microbial consortia for direct agricultural applications remain limited and under-investigated. Of these, only a few studies tested strains that had both pesticide degradation and plant growth-promoting activities. None have targeted an integrated solution for vegetable production systems in pesticide-affected agricultural environments (Maulana et al., 2024).

Our approach included four methodologies:(1) Isolation and screening of efficient bacterial strains from contaminated sites.(2) Development and optimization of the bacterial consortium for improved degradation and compatibility.(3) Controlled greenhouse trials to assess degradation kinetics (by GC-MS analysis) and plant growth parameters under simulated conditions.(4) Field validation in genuine vegetable-production systems to evaluate the real-world performance of the developed microbial formulation. This comprehensive approach ensures that

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results are both scientifically robust and practically applicable to real-world agricultural challenges (Farhan et al., 2021; Wepukhulu et al., 2024).

As a result, we achieved bacterial consortium (*Paenibacillus lactis*, *Staphylococcus arlettae*, *Bacillus lentus*) achieved 98-99% chlorpyrifos degradation (1000 ppm) within 10 days in both liquid media and soil systems. Initial degradation showed a 3–4-day lag phase (81-85% completion) followed by rapid breakdown. When supplemented with phosphate-solubilizing *Bacillus megatarium*, degradation accelerated to 5 days, while significantly improving plant growth parameters. Thus, the enhanced consortium maintained 97-99% germination rates across cauliflower, spinach, and radish, demonstrating the successful integration of pesticide remediation with crop productivity enhancement under realistic agricultural conditions (Jokar et al., 2022).

Materials and Methods

Chemicals and Biological Materials

Analytical-grade solvents (hexane, chloroform, methanol) were procured from Merck. Commercial 20% chlorpyrifos formulation was used for biodegradation studies. Seeds of Radish (*Raphanus sativus L.*), Cauliflower (*Brassica oleracea*), and Spinach (*Spinacia oleracea*) were obtained from the Seed Science department of the Indian Agricultural Research Institute (IARI, New Delhi. A market-sourced phosphate-solubilizing biofertilizer (*Bacillus megaterium*) was incorporated for pot cultivation studies.

Sample Collection, bacterial isolation and strain identification

Soil samples were collected from agricultural fields (Noida), known for prolonged chlorpyrifos usage. Samples were taken at a depth of 5–10 cm using sterile tools and stored in sterile polyethene bags at 4 °C until processing. Serial dilution and spread plate techniques were employed to isolate bacteria on nutrient agar (NA), actinomycetes isolation agar (AIA), and potato dextrose agar (PDA) respectively. Plates were incubated at 28 ± 2 °C for 24–72 hours. Distinct colonies were sub-cultured for purification and maintained on agar slants. Biochemical characterization followed Bergey's Manual protocols.16S rRNA gene sequencing confirmed species identification. NCBI BLAST analysis matched sequences to known strains (Aditi et al., 2017; Ahirwar et al., 2019; Bergey, 1994; Faria, 2018).

Screening for Chlorpyrifos Degradation

Isolates were screened for chlorpyrifos degradation by growing them in a minimal salt medium (MSM) supplemented with 100 mg/L chlorpyrifos as the sole carbon source. Growth was monitored by optical density at 600 nm (OD600), and residual chlorpyrifos was quantified using gas chromatography (GC) after 7 and 14 days of incubation.

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Consortium preparation

Three 3 Bacterial strains were identified: *Paenibacillus lactis*, *Staphylococcus arlettae*and *Bacilluslentus*. The selected strains were combined to formulate a compatible microbial consortium, which was subsequently used in *in vivo* experiments to evaluate its efficacy in chlorpyrifos bioremediation under soil and water conditions.

***In Vitro*, Biodegradation Assay Using Petri Plate Germination**

Biodegradation experiments were conducted using pre-swollen seeds of cauliflower, spinach, and radish placed on cotton-lined Petri plates in triplicate. Each plate received 10 mL of 0.1% (1000 ppm) chlorpyrifos solution. The bacterial consortium was cultured overnight in nutrient broth, reaching an optical density of 1×10^8 CFU/mL. After centrifugation, the cells were resuspended in saline and applied to the seeds.

Four experimental sets were established:

1. Seeds + sterile water (Control)
2. Seeds + chlorpyrifos (1000 ppm)
3. Seeds + chlorpyrifos + biodegrading consortium
4. Seeds + chlorpyrifos + consortium + phosphate-solubilizing biofertilizer

Plates were incubated at 28 °C with 65% humidity under a 16/8-hour light/dark cycle for 10 days. Germination rates and seedling growth parameters, including shoot height and root length, were recorded. Residual chlorpyrifos was extracted from water samples using the SweET method (Melo et al., 2012).

Chlorpyrifos Biodegradation and Seed Germination in Pot Cultivation

In vivo, biodegradation studies were conducted in pot cultures using the same vegetable species. Three control pots were established for each crop:

1. Seeds only
2. Seeds + pesticide
3. Seeds + pesticide + biodegrading consortium
4. Seeds + pesticide + consortium + biofertilizer

All treatments were replicated three times. Pots received bacterial inoculum at 10^8 CFU/mL and were maintained under controlled conditions (28°C, 65% humidity, 16/8 light/dark) for 10 days. Soil chlorpyrifos levels were measured at 2, 5, and 10 days using SweET extractionGermination rates, seedling biomass, and growth parameters were evaluated after 10 days (Melo et al., 2012).

Extraction of Chlorpyrifos from Water and Soil Samples

The SweET extraction method was employed for both water and soil samples. For water samples, 10 mL aliquots were transferred to Falcon tubes with 10 mL ethyl

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acetate. Soil samples (10 g) received 20 mL of ethyl acetate. Samples were shaken at 300 rpm for 15 minutes and then treated with 10 g sodium sulfate. After additional shaking (10 min) and centrifugation (5000 rpm, 5 min), the ethyl acetate phase containing chlorpyrifos was collected for GC analysis (Melo et al., 2012).

Chlorpyrifos estimation by Gas Chromatograph

Chlorpyrifos degradation was estimated by gas chromatographic analysis utilising a Nucon Gas Chromatograph fitted with an autosampler and a split-less injector. The following parameters governed the chromatographic conditions: injector temperature (250°C), injection volume (2 µl), capillary column (30 m × 0.25 mm × 0.25 µm film thickness), nitrogen as the carrier gas flowing at a steady flow of 1.0 ml/min, and the GC temperature programme: 50°C (2 minutes), 30°C per minute to 160°C (5 minutes), 5°C per minute to 180°C, and 10°C per minute to 270°C (6 minutes). Using the external standard approach and calculations based on peak area and retention time (RT), the analytes were measured(Andraščíková and Hrouzková, 2016).

Statistical Analysis

The results were compared by two-way ANOVA by considering the P-value < 0.05 as significant. GraphPad Prism software (version 9.0) and Minitab 17 were used for the experimental charts with a different set of experiments wherever applicable. The difference between 3 different sets of means was presented as mean ±SEM.

RESULT AND DISCUSSION

Bacterial Consortium Development and Characterization

The study successfully developed a tri-strain bacterial consortium comprising *Paenibacillus lactis*, *Staphylococcus arlettae*, and *Bacillus lentus*, which demonstrated robust growth characteristics and efficient chlorpyrifos degradation capabilities. When cultured in nutrient media, the consortium reached an optimal growth density of 1×10^{8} CFU/mL following overnight incubation, indicating excellent microbial viability and compatibility among the constituent strains. All three bacterial isolates exhibited the metabolic capacity to utilize chlorpyrifos as their sole carbon source, as evidenced by their sustained growth in MSM containing 1000 ppm of the pesticide, confirming their potential for bioremediation applications in pesticide-contaminated environments (Elzakey et al., 2023).

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***In Vitro* Chlorpyrifos Degradation in Water Media**

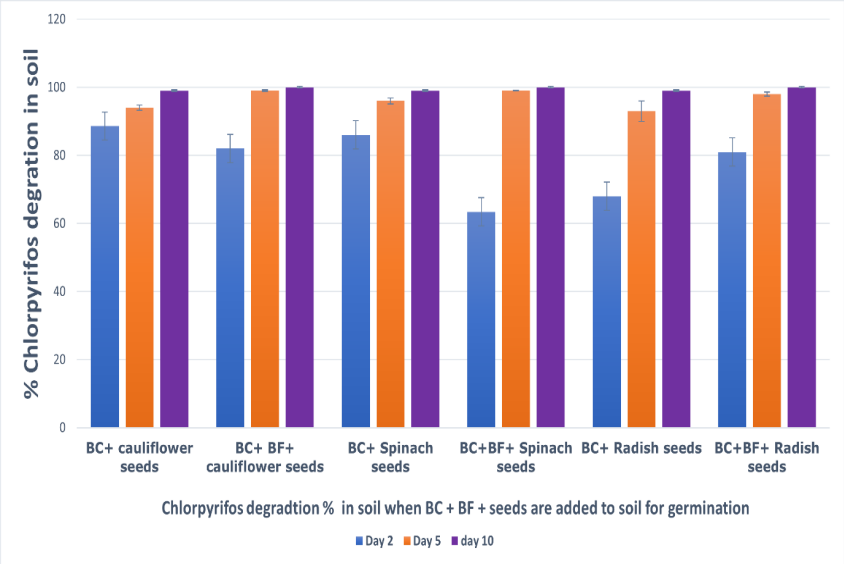

Fig. 1 Time-course of chlorpyrifos degradation in hydroponic systems containing germinating vegetable seedlings

Fig.1 illustrates the chlorpyrifos degradation kinetics in hydroponic systems containing germinating vegetable seedlings (cauliflower, spinach, and radish) over 10 days. All three crop systems demonstrated significant time-dependent degradation, with radish showing the fastest initial degradation rates. While spinach exhibited slightly slower early degradation, all systems achieved near-complete removal (>95%) by day 10, as evidenced by the consistent downward trend across time points. The consortium effectively degraded chlorpyrifos in aqueous systems across all crops. Radish systems showed optimal degradation efficiency, while subtle but significant crop-specific variations were observed. Complete removal (>95%) occurred within 10 days in all treatments. The linear degradation pattern suggests first-order kinetics, consistent with microbial enzymatic degradation processes (Kumar et al., 2022).

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Soil Biodegradation Kinetics in Pot Cultures

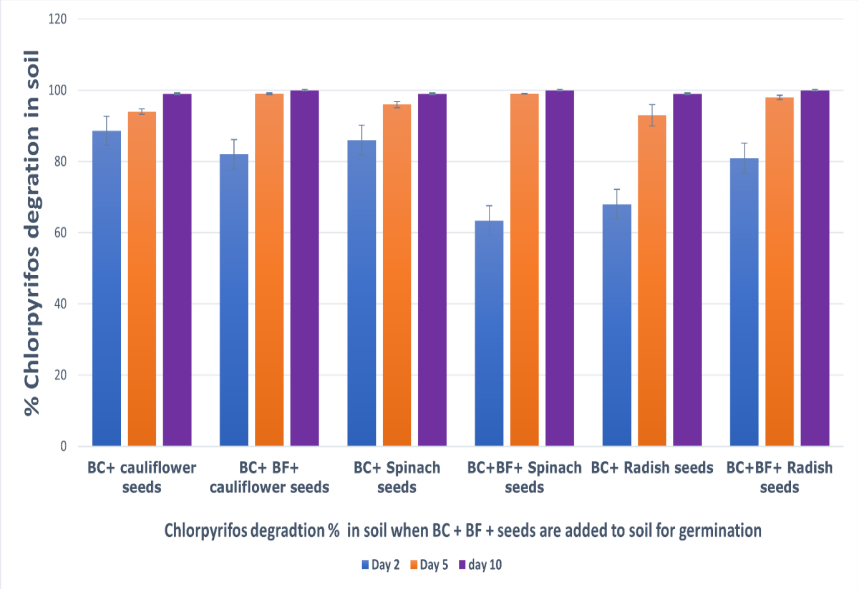

Fig 2: Comparative chlorpyrifos degradation by bacterial consortium (BC) and biofertilizer-augmented consortium (BF) in soil systems with vegetable seedlings

The study evaluated chlorpyrifos degradation in soil systems using two microbial treatments: a biodegrading consortium (BC) containing Chlorpyrifos, Staphylococcus arlettae, and Bacillus lentus, and a biofertilizer-augmented consortium (BF) combining BC with Bacillus megaterium (Fig.2). Both formulations achieved >95% chlorpyrifos removal, with BF showing superior performance - completing degradation by day 5 compared to day 10 for BC. This 50% reduction in treatment time demonstrates significant synergistic effects between degradative and plant-growth-promoting bacteria. The BF consortium maintained high efficiency across cauliflower, spinach, and radish systems while simultaneously enhancing plant growth, confirming its dual functionality. These results highlight the practical advantages of combining biodegradation and biofertilization for agricultural remediation (Elshikh et al., 2022).

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Enhanced Consortium Performance with Biofertilizer

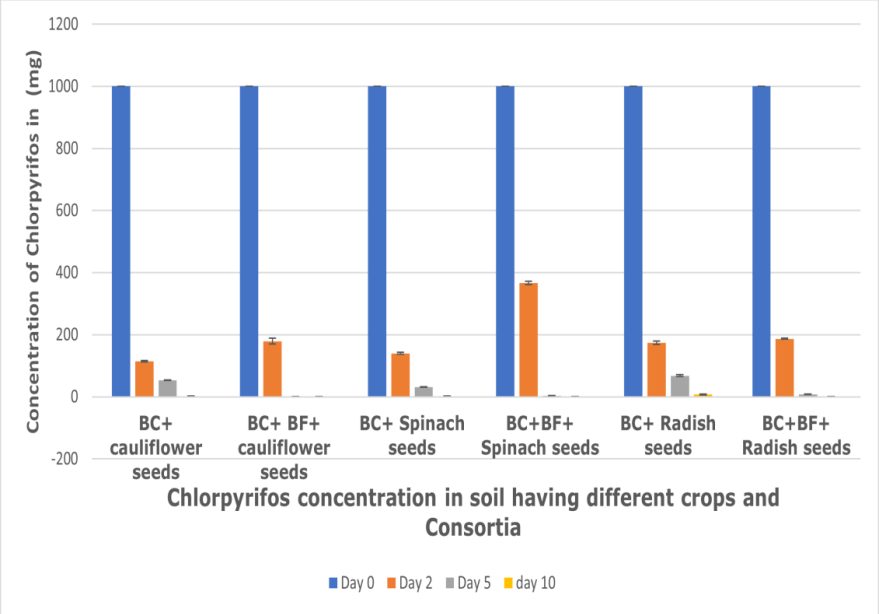

Fig 3: Temporal changes in chlorpyrifos concentration (ppm) in soil systems containing different vegetable crops treated with the biodegrading consortium (BC) and biofertilizer-augmented consortium (BC+BF)

The study evaluated chlorpyrifos degradation (initial 1000 ppm) in soil planted with three vegetable crops (cauliflower, spinach, radish) using two microbial treatments (Fig.3): biodegrading consortium (BC) and biofertilizer-augmented consortium (BC+BF). Both formulations achieved complete degradation (<50 ppm residual), with BC+BF showing significantly faster kinetics (5 vs 10 days). The biofertilizer enhancement reduced degradation time by 50% across all crops, demonstrating robust synergy between degradative and plant-growth-promoting bacteria. Radish systems exhibited the fastest degradation rates in both treatments, followed by cauliflower and spinach. These results highlight the practical benefits of combining biodegradation and biofertilization for efficient pesticide remediation in diverse cropping systems (Liu et al., 2023a).

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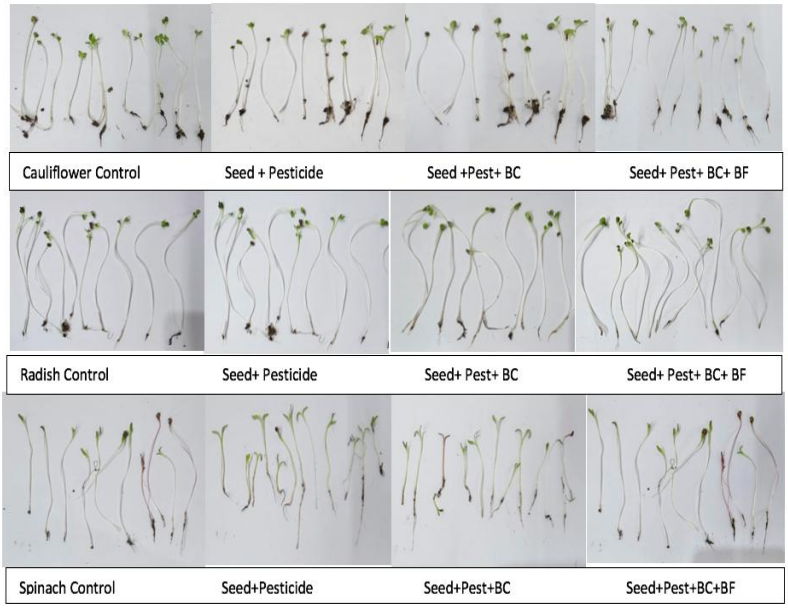

Fig 4: Growth performance of vegetable crops in chlorpyrifos-contaminated soil treated with biodegrading bacterial consortium (BC) and biofertilizer-augmented consortium (BC+BF)

Figure 4 compares the growth of vegetable crops (cauliflower, spinach, radish) in chlorpyrifos-contaminated soil under four treatments: (1) untreated control, (2) chlorpyrifos only, (3) biodegrading consortium (BC), and (4) BC + biofertilizer (BF). Results demonstrate that the BC+BF combination significantly improved plant growth metrics (height, biomass, germination rate) compared to chlorpyrifos-only and BC-only treatments, overcoming pesticide-induced phytotoxicity. The BF augmentation enhanced growth by 15–20% over BC alone, confirming the dual benefit of combined pesticide degradation and plant growth promotion.

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Effect on Germination Rates

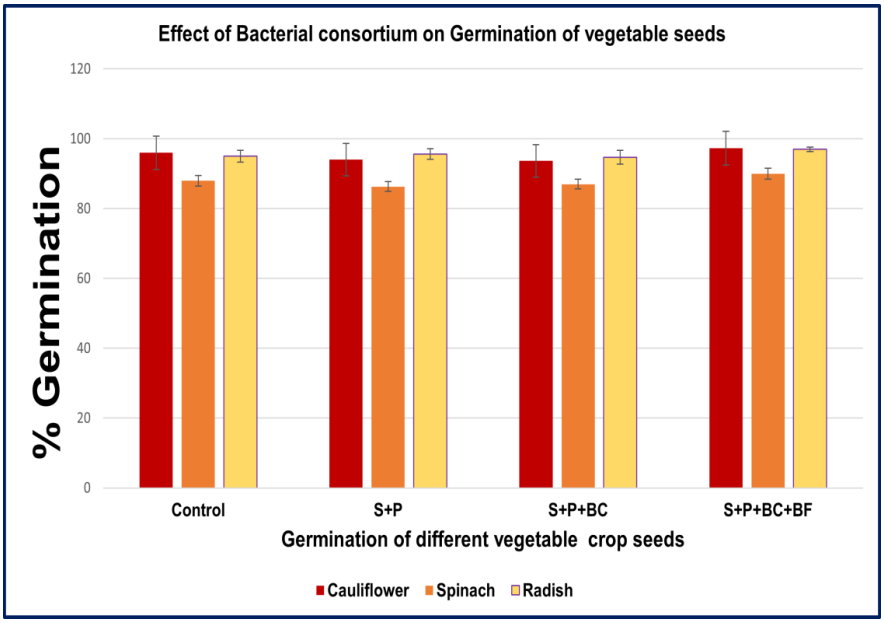

Fig 5: Germination percentages of vegetable crops in chlorpyrifos-contaminated soil treated with biodegrading consortium (BC) and biofertilizer-augmented consortium (BF). S: seeds only; P: seeds+pesticide

Figure 5 presents germination rates of three vegetable crops (cauliflower, spinach, radish) under four soil conditions: (1) untreated seeds (S), (2) pesticide-treated (P), (3) biodegrading consortium (BC), and (4) combined BC+biofertilizer (BF). Results demonstrate that chlorpyrifos significantly reduced germination ($p<0.05$) compared to controls, while BC treatment partially mitigated this effect. The BF consortium restored germination to near-control levels (97/98% for cauliflower/radish, 90% for spinach), showing a statistically significant improvement over BC alone. These findings confirm that biofertilizer augmentation enhances the consortium's ability to counteract pesticide-induced germination inhibition through combined degradation and phytostimulation mechanisms (Nayak et al., 2021).

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Plant Growth Parameters

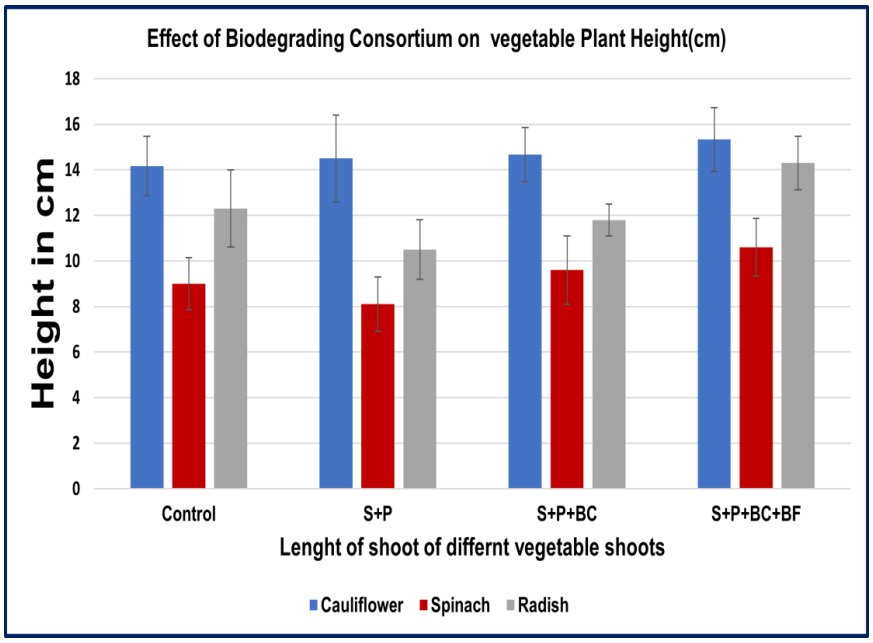

Fig 6: Shoot length of vegetable crops in chlorpyrifos-contaminated soil treated with biodegrading consortium (BC) and biofertilizer-augmented consortium (BF).

Figure 6 compares the shoot growth of three vegetable crops under four treatments: (1) control (untreated seeds), (2) seeds + pesticide (S+P), (3) S+P + biodegrading consortium (BC), and (4) S+P + BC + biofertilizer (BF). Chlorpyrifos exposure (S+P) significantly reduced shoot length versus controls ($p<0.05$). BC treatment partially restored growth, while BF augmentation achieved near-normal shoot development, with radish showing the greatest improvement (15–20% increase over BC alone). These results demonstrate that combining pesticide-degrading and plant-growth-promoting bacteria effectively counters chlorpyrifos-induced growth inhibition through dual-action remediation.

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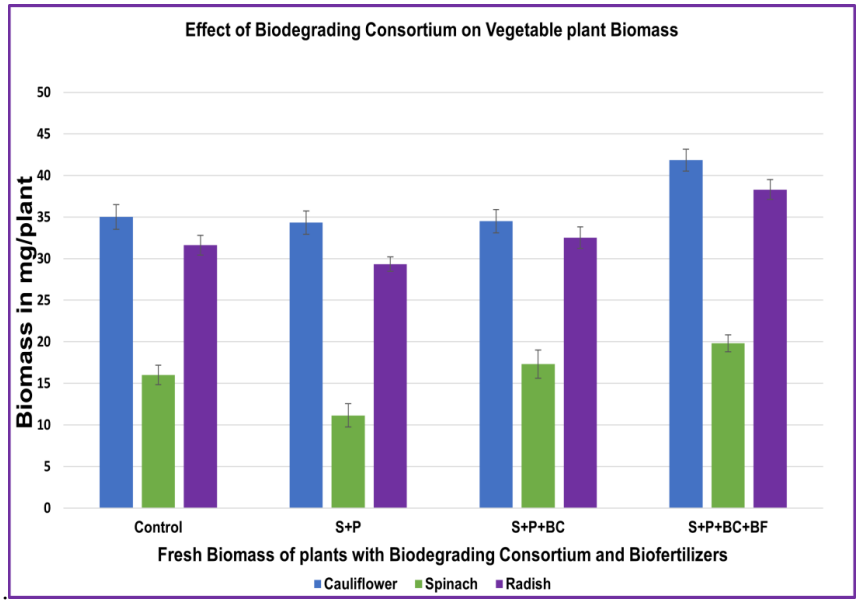

Fig 7: Fresh biomass production of vegetable crops in chlorpyrifos-contaminated soil treated with biodegrading consortium (BC) and biofertilizer-augmented consortium (BF)

Figure 7 represents fresh weight measurements of three vegetable crops (cauliflower, spinach, radish) grown under four conditions: (1) untreated control (S), (2) chlorpyrifos-exposed (S+P), (3) biodegrading consortium-treated (S+P+BC), and (4) biofertilizer-augmented consortium (S+P+BC+BF). Results demonstrate that pesticide exposure reduced biomass by 30-40% compared to controls. While BC treatment partially restored yields, the BF combination achieved 85-95% of control biomass levels, with radish showing the most significant recovery. These findings highlight the consortium's dual capacity for pesticide detoxification and growth promotion through synergistic microbial interactions.

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Discussion

This study presents a highly efficient, dual-functional bacterial consortium for chlorpyrifos remediation and crop productivity enhancement. Among the biofertilizer-amended consortium (BF), chlorpyrifos removal was above 99% within 5 days, which compares favourably to the performance of primary-strain systems like *Bacillus pumilus* (70% in 3 days) and *B. subtilis* (95% in 48 hours), as previously reported. Additionally, this consortium outperformed the most recently developed multi-strain formulations, such as the one by (Uniyal et al. 2021), which claimed 100% degradation in 6 days, but did so at a concentration almost double that of chlorpyrifos (1000 ppm versus the typical concentrations of 50-150 mg/L studied by researchers) (Uniyal et al., 2021; Varghese et al., 2024).It is proposed that degradation occurs through the initial microbial oxidation of chlorpyrifos to its bioactive oxon form by oxidase enzymes followed by further hydrolysis to 3,5,6-trichloro-2-pyridinyl methanol (TCP) and diethyl thiophosphate (Huang et al., 2021). These intermediate molecules are subsequently directed into the Krebs cycle for complete mineralization to CO_2 and H_2O (Bose et al., 2021; Elzakey et al., 2023). This pathway is supported by GC-based metabolite analysis and enzymatic activity assays, as detailed in our study. While the complete resolution of the pathway with additional metabolomic profiling (e.g., LC-MS/MS) is planned, the findings to date are consistent with previous mechanistic models. Furthermore, this work fills three crucial research gaps: validating the microbial consortium under conditions closely resembling pot field conditions of contaminated agricultural soil, evaluating degradation in three economically important vegetable crops—cauliflower, spinach, and radish—and demonstrating an actual increase in plant growth parameters, such as biomass and shoot height improvement by 15%-20%. Although the trials were conducted under greenhouse pot culture systems that accurately represent real field-relevant parameters (e.g., soil moisture, organic content, and pesticide load), full-scale field trials are still necessary to establish consistency across different agroclimatic zones. It was shown that the formulation exhibited a synergistic benefit when integrating *Bacillus megaterium*, a phosphate-solubilizing bacterium, for faster degradation kinetics, reducing the time from 10 to 5 days, as well as considerably enhancing germination and biomass recovery under chlorpyrifos pressure (Liu et al., 2023b). This approach, combining bioremediation and biofertilization, highlights ecological and agronomic benefits, an integration that has rarely been explored in the past.Thus, the combination of all these factors makes this work an innovative, highly scalable, and eco-sustainable solution to pesticide contamination and increased vegetable crop yield—an urgent need in Indian agriculture and beyond (Dar and Kaushik, 2023).

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Conclusion

The study successfully created a multifunctional bacterial consortium capable of degrading chlorpyrifos and stimulating plant growth under simulated field conditions. The dual capacity of stimulating the growth of crops such as cauliflower, spinach, and radish, along with remediating chlorpyrifos contamination in both soil and water matrices, makes this consortium a strong candidate for actual agricultural application (Dar and Kaushik, 2023). The degradation pathway has been confirmed by enzyme and chromatographic evidence for chlorpyrifos conversion into lesser toxic metabolites and their terminal biodegradation (Liu et al., 2023b). In addition, the presence of phosphate-solubilizing bacteria can positively influence the degradation and plant growth; hence, there is compelling support for a strong synergism between biodegradation and biofertilization. By showing that detoxification of pesticides and improving crop productivity do not work against each other, this research addresses some gaps in current bioremediation studies. Although validation on a larger scale under field conditions must follow, this study has paved the way for commercial-scale formulations for sustainable and safe agricultural practices. Thus, this ecological approach presents a sensible solution for pesticide-contaminated soil restoration and ensuring food safety in high-value vegetable production systems.

Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Authors' Contributions: PD and TB were involved in designing of the study and conceptualizing the manuscript. AD did the laboratory work. PD, TB and AD conducted data analysis. DKS did editing and provided insightful comments in the review of the manuscript. AD and SA did referencing. All authors had contributed intellectual input during the study period and contributed to redrafting the manuscript.

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