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Biodegradation of Chlorpyrifos Insecticide by *Bacillus cereus ST06* and *Chryseobacterium sp 6024* Isolated from Agricultural Soil, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Indigenous soil bacteria have the potential to degrade the harmful chlorpyrifos insecticide, this identifies the importance of biodegradation as an eco-friendly method for chemical pollutant cleanup.
Aims: To compare the potential of Bacillus cereus ST06 and Chryseobacterium sp 6024 in biodegrading chlorpyrifos insecticide singly or as a consortium in a liquid medium.
Study Design: Enrichment culture technique was used to evaluate the bacterial potential in biodegrading chlorpyrifos insecticide.
Place and Duration of Study: Agricultural soil sample containing chlorpyrifos degrading bacteria was obtained from Ukukwa village Amansea Nigeria (6o16' 30" N and 7o 07'30"E) from depths of 15cm. Experiment was conducted from January till March 2022.

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Methodology: In this study, previously isolated and characterized Bacillus cereus ST06 and *Chryseobacterium sp* 6024 by standard microbiological method based on their phenotypic test, biochemical test, cultural morphology and 16S rRNA sequencing was used for the experiment. Their growth response to 20mg/l and 60mg/l chlorpyrifos in mineral salts medium singly and as a consortium was compared and determined by monitoring the optical density at 600nm at the optimum condition of pH 6.5 and 30oC temperature for 28 days. The residual chlorpyrifos concentration after 28 days was also compared and determined using Gas Chromatography-Electron Cathode Detector (GC-ECD).

Results: The result showed a significant difference (P< .001) as Bacillus cereus ST06 and *Chryseobacterium sp* 6024 responded differently to different concentration of chlorpyrifos. Bacillus cereus ST06 and *Chryseobacterium sp* 6024 reached maximum growth in medium containing 20mg/l chlorpyrifos with a mean OD of 0.23±0.20 and 0.42±0.02 respectively on 16th day than 60mg/l chlorpyrifos with a mean OD of 0.47±0.02 and 0.81±0.02 respectively on 20th day. The bacterial consortium also reached maximum growth on 20mg/l and 60mg/l of chlorpyrifos with mean OD of 0.21±0.31 and 0.29±0.02 on 20th day respectively. The result of residual chlorpyrifos concentration shows that the bacteria consortium degraded 79 per cent and 78 per cent of 20mg/l and 60mg/l chlorpyrifos respectively, while Bacillus cereus ST06 and *Chryseobacterium sp* 6024 degraded 63 per cent and 57 per cent of 20mg/l chlorpyrifos and 61 per cent and 37 per cent of 60mg/l chlorpyrifos.

Conclusion: The study shows that bacteria consortium possessed potential to be used in biodegradation of 20mg/l and 60mg/l Chlorpyrifos than the individual isolates. It is therefore recommended that further studies on RNA profiling of each bacterium and synergistic interaction of the bacterial consortium be studied to better understand regulation of genes and individual bacterial roles in degradation chlorpyrifos efficiently.

Keywords: Isolation; characterization; bacteria; biodegradation; chlorpyrifos; Nigeria.

1. INTRODUCTION

Chlorpyrifos, identified as o, o-diethyl o-3,5,6-trichloro-2-pyridyl phosphorothioate, stands out as a broad-spectrum organophosphate insecticide lethal against various pests infesting grains, fruits, and vegetables [1,2]. Its use has significantly contributed to enhanced crop productivity and the control of disease vectors like malaria [3]. While pesticides play a crucial role in addressing global food security challenges posed by an expanding population, their widespread application has led to concerning issues such as microbial imbalances, environmental pollution, and health hazards [1]. The consistent use of chlorpyrifos in agriculture and industrial activities has resulted in neurological diseases and contamination of water and soil [4]. Research has pointed to chlorpyrifos as a potential cause of gene mutations and adverse effects on human mental and physical health [5,6]. Classified as a moderately toxic pesticide (Class II) by the World Health Organization (WHO) and Globally Harmonized System (GHS), addressing the problems associated with chlorpyrifos becomes crucial to developing safe and effective remediation methods [7,8].Numerous studies have explored the degradation of chlorpyrifos in water and soil, employing methods such as photochemical degradation, nanometal, and UV catalytic degradation [9]. Biodegradation, a method utilizing microorganisms to break down organic compounds into simple inorganic molecules, has proven to be an efficient and environmentally friendly approach for decontaminating soil and water polluted by chlorpyrifos [10]. Noteworthy research efforts have reported the successful use of biodegradation in cleaning up chlorpyrifos-contaminated soil [11]. Isolates like Enterobacter b-14 [11], Bacillus cereus mcas02 [12,13], Stenotrophomonas sp., and *Sphingomonas sp.* [14] have demonstrated their ability to use chlorpyrifos as a source of energy for growth. Given chlorpyrifos's role in insect control, identifying bacteria with the potential to break down this insecticide becomes imperative. Understanding the intricate interplay between bacteria and chlorpyrifos is not only crucial for environmental assessment but also for devising effective bioremediation strategies. The current study aims to evaluate and compare the biodegradation potentials of Bacillus cereus ST06 and *Chryseobacterium sp* 6024. The assessment involves exposing these bacteria to different concentrations of chlorpyrifos, both individually and as a consortium, in a liquid

medium. The knowledge gained from this study will provide researchers and governmental bodies with insights for optimizing bioremediation practices and predicting bacterial behavior in contaminated environments. This comprehensive approach will contribute to addressing the challenges posed by chlorpyrifos and promoting sustainable environmental practices.

2. MATERIALS AND METHODS

2.1 Insecticide

Commercial grade of chlorpyrifos commonly known as Perfect Killer® (containing 20g active ingredient/L, Emulsifiable concentrate 20%) and manufactured by Nantong Jinling Agrochemical co, limited China was purchased from Eke-Awka Market Anambra State, Nigeria.

2.2 Media

The mineral salts medium (MSM) described by [15] containing(g/l) 1.5g of KH_2PO_4, 0.5g of $NaCl_2$, 0.6g of Na_2HPO_4, 2g NH_4SO_4, 0.2g $MgSO_47H_2O$, 0.01 $CaCl_2$ and 0.001g $FeSO_4.7H_2O$ was used for the isolation of bacteria and biodegradation experiment.

2.3 Sample Collection

Soil sample was collected aseptically January 15, 2022 from Ukukwa Amansea, Awka North Local Government Area of Anambra State, Nigeria by using auger. The soil sample (1kg) was collected from the rhizosphere at a depth of 15cm from all corners of the farm. The location of the soil sampling was recorded by Geographical information system Laboratory, Department of Geography, and meteorology Nnamdi Azikiwe University Awka as latitude 6º16' 30" N and longitude 7º 07'30"E.The soil was sorted, mixed and put into a sterile polyethylene bag and then conveyed immediately to the Laboratory of the Department of Applied Microbiology and Brewing Nnamdi Azikiwe University Awka for analysis.

2.4 Culture and Isolation of Bacteria

Bacillus cereus ST06 and *Chryseobacterium sp* 6024 with potential of degrading chlorpyrifos which was previously isolated and characterized by [16] based on modified methods of [17-19] was used for the study.

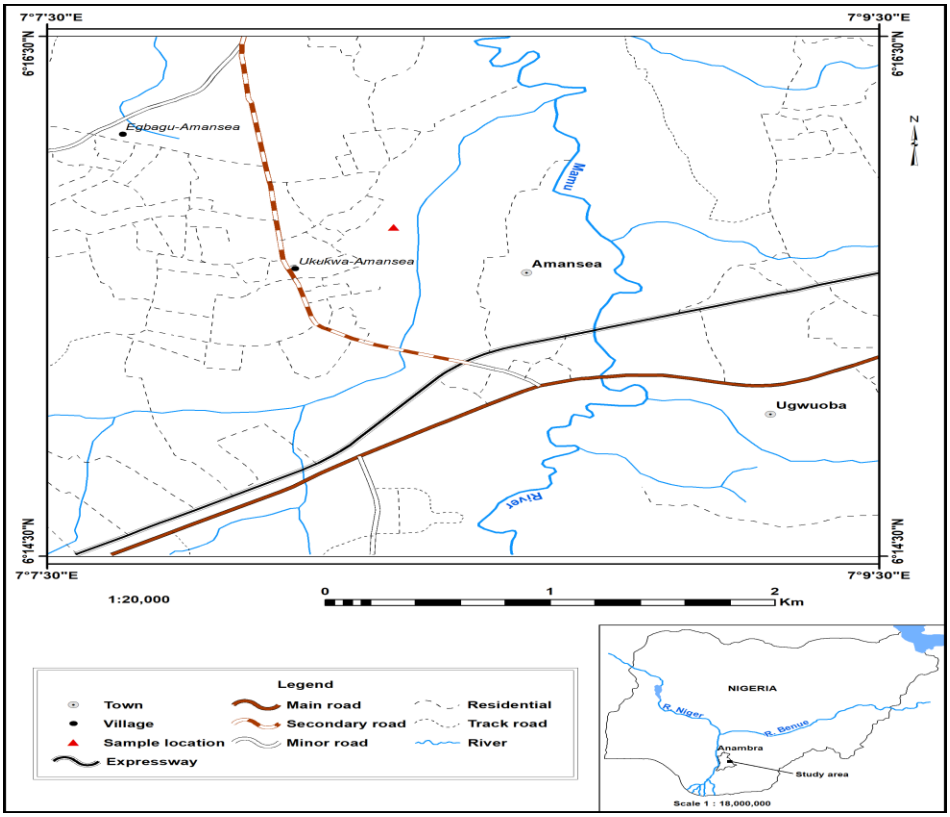

Fig. 1. Geographic map Anambra state showing Amansea

Source: GIS Lab, Department of Geography and meterology Nnamdi Azikiwe University Awka Anambra State, Nigeria

2.5 Biodegradation Experiment

2.5.1 Inoculum standard Preparation

The inoculum used for all the experiments was prepared using viable count method [20].1ml of the 24 hours culture containing 1.3X10^4 CFU/ml (determined by viable count method) was used as inoculum for the biodegradation proper.

2.5.2 Assessing the growth response of the isolated bacteria to varying the initial concentration of chlorpyrifos in mineral salts medium singly and as a consortium during biodegradation process by monitoring the optical density (OD) at optimum condition of 600nm, pH 6.5 and 30°C for 28 days

One milliliter of the 24 hours culture (1.3 X 10^4) individually and as a consortium were used to inoculate 250ml flasks containing 100ml MSM and Chlorpyrifos in the concentration of 20mg/l and 60mg/l, respectively in triplicates. The chlorpyrifos was added as a source of carbon. The flasks were incubated on a rotary shaker at 150 rpm at 30℃ for 28 days . The optical density of the isolates were determined at 4 days intervals for 28 days using a Spectrophotometer (OD 600nm) as described by [20].

2.5.3 Determination of residual Chlorpyrifos after biodegradation and Percentage degradation

After 28 days incubation the method of Pszczolińska and Miche [21] was used to determine residual chlorpyrifos. 5ml each of the culture were taken from each flask and placed in centrifuge tubes. These portions of the culture were extracted with equal volume of ethyl acetate as the extracting reagent by centrifuging at 150rpm for 20minutes. The ethyl acetate with residual Chlorpyrifos was filtered through Whatman No 1 filter paper. The final extracts were analyzed by Gas Chromatograph-Buck M910 scientific gas chromatography equipped with Electron capture detector (GC-ECD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/g and µg/kg range) from the matrix to which other detectors do not respond. [22]

2.6 Data Analysis

Statistical Package for Social Sciences (SPSS) version 23.0 was used to perform data calculation and statistical analysis in order to show mean significant differences between two treatments on isolated bacteria. Two-ways Analysis of variance (ANOVA) was used. The rate of degradation was calculated from the following equation:

Rate of degradation = ($ConI_0$ – ConI) / ($ConI_0$) X 100 (1) [23]
$ConI_0$ = Initial Concentration
ConI = Final Concentration

3. RESULTS

3.1 Isolation and Identification of Bacteria

Bacillus cereus ST06 and *Chryseobacterium sp* 6024 were identified by 16s rRNA gene amplification using thermocycler as sequenced in previous research [16]. The partial 16s rRNA gene sequences were compared with that of referred strains gene sequences in the GenBank. [22]

The Accession No of the isolated bacteria represented in Table 1 shows that the two isolates are identical to *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024.

3.2 Growth Response of the Isolated Bacteria to 20mg/l of Chlorpyrifos at Optimum Condition of 600nm, pH 6.5 and 30^{0}C for 28 Days

The results, shown in Fig. 2, indicates that *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024 exhibited varying responses to 20mg/l chlorpyrifos at 4-day intervals over a 28-day period. Specifically, *Bacillus cereus* ST06 had a mean OD of 0.55, while *Chryseobacterium sp* 6024 had a mean OD of 0.67. The bacterial consortium also had mean OD of 0.47 for the same period. Also, from the graph *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024 had maximum growth on day 16 with mean OD of 0.23 and 0.42 respectively. The bacteria consortium had maximum growth on day 20 with mean OD of 0.21. This means that each bacterium responded differently to 20mg/l of chlorpyrifos. The ANOVA result revealed that there is a significant difference in the mean optical density (OD). This confirms that the three isolates responded differently to 20 mg/L of chlorpyrifos. The bacterial consortium tolerated 20mg/l of CP most and *chryseobacterium sp* 6024 tolerated the least. This suggests that the bacterial consortium may be more effective at bioremediation of chlorpyrifos-contaminated environments than *Bacillus cereus* ST06 or

Chryseobacterium sp 6024 individually. The control also showed variable and nonlinear pattern in the decrease and increase in the OD value. This can be attributed to some biotic and abiotic factors that contribute to reaction in the control.

3.3 Growth Response of the Isolated Bacteria to 60mg/l of Chloropyrifos at Optimium Condition of 600nm, pH 6.5 and 30^{0}C for 28 Days

Result reveals bacterial response to 60mg/l chlorpyrifos (Fig. 3). *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024 reached maximum growth on 60mg/l chlorpyrifos with a mean OD of 0.47±0.02 and 0.81±0.02 respectively on day 20. The bacterial consortium also reached maximum growth on 60mg/l of chlorpyrifos with a mean OD of 0.29±0.02 on day 20 too. This means that each bacterium responded differently to 60mg/l of chlorpyrifos. The ANOVA result also revealed that there is a significant difference in the mean optical density (OD). This means that there is an inverse relationship between the concentration of chlorpyrifos and the bacterial growth response. Generally, for both graphs there is a significant difference (P<.001) in the mean OD after 28 days implying that at different concentration of chlorpyrifos bacterial isolates behaviors differently.

The growth response to chlorpyrifos by *Bacillus cereus* ST06 decreased slowly with increase in chlorpyrifos concentration and metabolite accumulation.

Table 1. Bacterial strain identification by I6S rRNA

Isolates	Accession No	Identity	Confirmed Name
A1	MH475925.1	97%	*Bacillus cereus* ST06
A2	KY056237.1	99%	*Chryseobacterium sp.* 6024

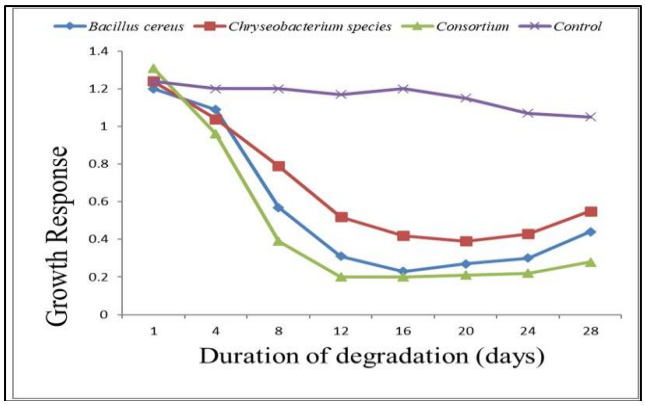

Fig. 2. Growth Response of bacteria isolate on 20mg/l Chlorpyrifos

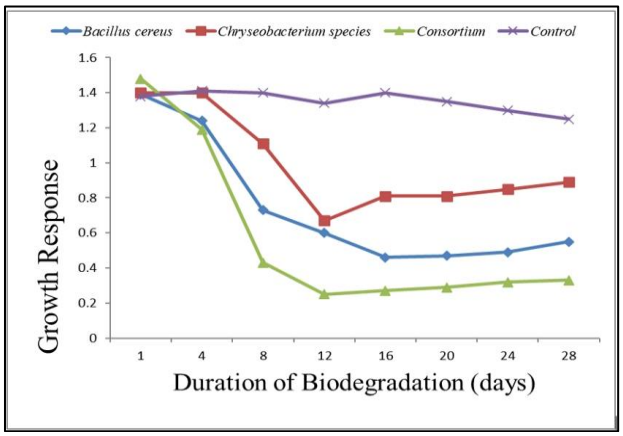

Fig. 3 Growth Response of bacteria isolate on 60mg/L Chlorpyrifos

3.4 Determination of Residual Chlorpyrifos after Biodegradation and Percentage Degradation

The result of residual Chlorpyrifos determination (Table 2) shows that all treatments, including the control, resulted in decreased chlorpyrifos concentration. The chlorpyrifos-degrading bacteria isolated singly and in consortium has the ability to catabolize 20mg/L and 60mg/L of chlorpyrifos in mineral salts medium, in vitro. The two organisms showed variation in their ability to degrade 1ml volume of 20g/L and 3ml vol of 60mg/L Chlorpyrifos after 28 days. *B. cereus* ST06 reduced 20mg/L Chlorpyrifos to 7.41mg/L while *Chryseobacterium sp* 6024 reduced 20mg/L chlorpyrifos to 8.80mg/L. B. cereus reduced 60mg/L Chlorpyrifos to 23.40mg/L and *Chryseobacterium sp* 6024 reduced 60mg/L chlorpyrifos to 37.82mg/L. The consortium showed highest reduction of both 20mg/L and 60mg/L at 4.20mg/L and 13.20mg/L respectively. This indicates that degradation occurred under the experimental conditions. The isolates *Bacillus cereus* ST06, *Chryseobacterium sp* 6024, and the consortium showed higher degradation rates than the control. This suggests that these bacterial isolates have the potential to enhance chlorpyrifos degradation. The degradation rates were generally higher at the lower initial concentration of 20 mg/l compared to the higher initial concentration of 60 mg/l showing a significant different of (P<.001). The Control showed some degradation, likely due to abiotic factors or the activity of naturally occurring microorganisms. The rate of degradation as shown in (Fig 4,5) shows that *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024 showed 63% and 56% Chlorpyrifos degrading capacity at 20mg/l respectively, and 61% and 37% at 60mg/l respectively within a time period of 28days. The consortium showed 79% and 78% degradation of chlorpyrifos at 20mg/l and 60mg/l respectively. This means that chlorpyrifos degradation is concentration dependent. The Two-way ANOVA result shows it significantly different (P < .001) meaning that as chlorpyrifos concentration in liquid medium increases, the bacteria response or growth to it decreases.

Table 2. Residual chlorpyrifos concentration after degradation

S/n	Isolates	Residual Chlorpyrifos concentration			
		Initial conc.(mg/l)	Final conc.(mg/l)	Initial conc.(mg/l)	Final conc.(mg/l)
1	*Bacillus cereus* ST06	20.00	7.41[b]	60.00	23.40[b]
2	*Chryseobacterium spp* 6024	20.00	8.80[c]	60.00	37.82[c]
3	Consortium	20.00	4.20[a]	60.00	13.20[a]
4	Control	20.00	13.46[d]	60.00	53.40[d]

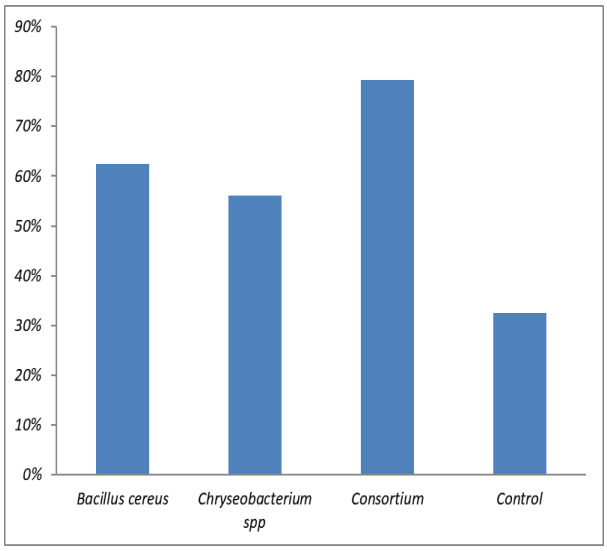

Fig. 4. Percentage degradation ability of the isolate at 20mg/L Chlorpyrifos

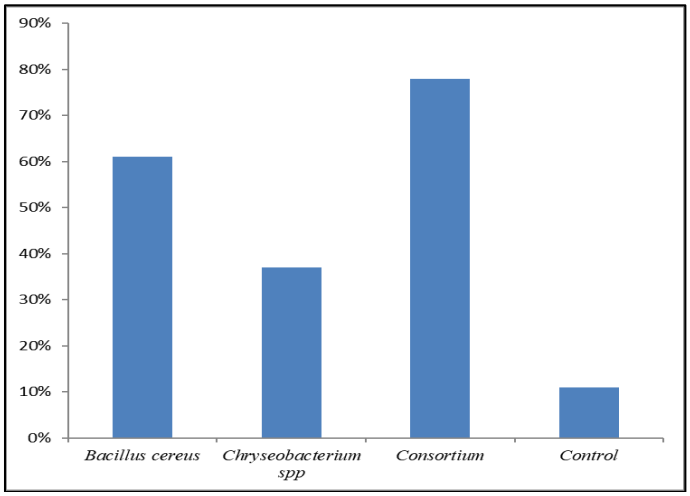

Fig. 5. Percentage degradation ability of isolates at 60mg/L Chlorpyrifos

Table 3. Degradation of other chlorpyrifos degraders

Microorganisms	Initial concentration (mg/l)	Rate of degradation(%)	Time (days)	references
Cupriavidus nantongensis X1T	200	100	2	[23]
Bacillus cereus ST06	20	63	28	This study
B. cereus ST06	60	61	28	This study
Chryseobacterium sp 6024	20	56	28	This study
C. sp 6024	60	37	28	This study
Consortia (B. cerus ST06 *and C. sp* 6024*)*	20	79	28	This study
Consortia (B. cerus ST06 *and C. sp* 6024*)*	60	78	28	This study
Pseudomonas putida T7, Pseudomonas aeruginosa M2, Klebsiella pneumoniae M6 and *Aspergillus sp*	500	100	30	[36]
Alcaligenes faecalis	100	98.6	20	[37]
Pseudomonas aeruginosa DKC2	100	71	2	[38]
Bacillus cereus Ct3	125	88	8	[24]
Staphylococcus aureus	50	82.06	14	[39]
Kocuria kristinae	50	30.78	14	[39]

4. DISCUSSION

In this study, we delved into the potential of two indigenous soil chlorpyrifos-degrading strains, namely *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024, along with their consortium, to biodegrade moderately harmful chlorpyrifos insecticide. Building on our previous research, which identified a variety of microorganisms capable of degrading chlorpyrifos [16], we focused on understanding the specific capabilities of *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024 in degrading chlorpyrifos within a certain concentration range.

Several studies have explored bacterial strains capable of degrading chlorpyrifos, as summarized in Table 3. These studies have isolated and characterized various bacteria from local environments for potential biodegradation applications. Our research, on the other hand, not only evaluates bacterial growth responses to chlorpyrifos in a liquid medium at different concentrations but also explores the synergistic effects when bacteria are used in a consortium. Our findings highlight that *Bacillus cereus* ST06 demonstrated a degree of tolerance to chlorpyrifos, with degradation percentages of 63% and 61% at concentrations of 20mg/l and

60mg/l, respectively, over 28 days at 30°C and pH 6.5. These results contrast with a study by [24], where *Bacillus cereus* Ct3 resisted up to 125 mg/l of chlorpyrifos and successfully degraded 88% in just 8 days at pH 8 and a temperature range of 30–40°C.

Furthermore, [25] isolated another *Bacillus sp* that degraded 12 mg/l up to 79.5% in 35 days. [26] reported a resistant strain of *Bacillus pumilus* C2A1, which tolerated 50mg/l chlorpyrifos and achieved a degradation rate of up to 97% in 45 days. Notably, [27] isolated *B. cereus* CP6 and *Klebsiella pneumoniae* CP19, capable of degrading above 70% chlorpyrifos at initial concentrations ranging from 200-300 mg/L.

Additionally, [28] investigated the ability of *Bacillus cereus* to degrade chlorpyrifos under different culture conditions, such as pH, temperature, and chlorpyrifos concentration. Their results indicated that the optimum conditions for chlorpyrifos degradation were 30°C, pH 7.0, and concentrations less than 100 mg/l. Considering these diverse findings, it becomes evident that different strains of *Bacillus sp* exhibit varying levels of tolerance to a wide range of chlorpyrifos concentrations. The discrepancies in tolerance levels may be attributed to factors such as the accumulation of intermediate metabolites in the medium, inhibiting bacterial responses to chlorpyrifos, and effects on lag phase and inoculum size [29-31]. This underscores the importance of understanding the nuances of bacterial responses to chlorpyrifos under different conditions for effective biodegradation strategies.

Our study not only highlighted the chlorpyrifos-degrading capabilities of *Bacillus cereus* ST06 but also shed light on the susceptibility of *Chryseobacterium sp* 6024 to chlorpyrifos. In contrast to *Bacillus cereus* ST06, *Chryseobacterium sp* 6024 exhibited limited involvement in the biodegradation of chlorpyrifos in the soil [32]. Our findings revealed that *Chryseobacterium sp* 6024 displayed a degree of susceptibility to chlorpyrifos, particularly at concentrations of 20mg/l and 60mg/l. At an initial concentration of 20mg/l chlorpyrifos, *Chryseobacterium sp* 6024 demonstrated a degradation rate of 57% within 28 days, indicating a slight potential for chlorpyrifos degradation. However, as the concentration of chlorpyrifos increased to 60mg/l, the growth response of *Chryseobacterium sp* 6024 decreased significantly, resulting in a degradation rate of 37%. This concentration-dependent response was further supported by the Two-way ANOVA result, indicating a significant difference in the the mean OD of each bacterium and the consortium to varying chlorpyrifos concentrations. Contrary to our findings, existing research has shown limited documentation of *Chryseobacterium's* participation in chlorpyrifos biodegradation in the soil [32]. However, other studies have identified *Chryseobacterium* strains as degraders of organochlorine pesticides, poly lactic acid, and glyphosate [33-35]. For instance, *Chryseobacterium sp.* Y16C exhibited the capability to degrade up to 400mg/l of glyphosate in just 4 days, highlighting its potential in degrading diverse environmental contaminants [34].

Collectively, these results suggest that *Chryseobacterium sp* possesses a novel capacity for chlorpyrifos biodegradation, hinting at the need for further research to comprehensively understand its role in environmental remediation. The concentration-dependent response observed in our study underscores the importance of considering varying concentrations of pollutants in assessing the potential of microbial strains for biodegradation

Our investigation revealed a notable decline in the utilization of chlorpyrifos by the isolates, both individually and in consortium, starting from day 16 (Fig.2,3). This decrease could be attributed to environmental factors, specifically the release and accumulation of 3,5,6-trichloro-2-pyridinol (TCP), an intermediate of chlorpyrifos degradation, into the liquid medium. This accumulation may render chlorpyrifos resistant to microbial attack. This observation aligns with the findings of [40], who reported that TCP exhibits antimicrobial effects against bacteria. Additionally, the work of [41] confirmed that TCP can limit the biodegradation of chlorpyrifos by microorganisms. Our study further demonstrated that the consortium of the two bacterial strains exhibited an enhanced capacity for chlorpyrifos degradation when compared to individual isolates. The medium containing the bacterial consortium at concentrations of 20mg/l and 60mg/l chlorpyrifos showed significantly higher growth ($P < .001$) than the medium with individual isolates. The maximum growth, observed at day 20, reached 0.29 (OD at 600nm). Moreover, the percentage utilization by the bacterial consortium in the medium

containing 20mg/l and 60mg/l chlorpyrifos was significantly (P < .001) higher than that in the medium with individual isolates. The maximum percentage utilization, achieved after 28 days of biodegradation, reached 78% and 79%, respectively. In contrast, a previous study by [36] involving bacterial consortia of *Pseudomonas putida* T7, *Pseudomonas aeruginosa* M2, and *Klebsiella pneumoniae* M6, along with *Aspergillus terreus* TF1, demonstrated the greatest potential in degrading chlorpyrifos in various environments, achieving up to 100% degradation. This implies that the development of bacterial consortia may offer more effective insecticide degradation compared to individual isolates.

Additionally, the results of our study indicated that the concentration of the uninoculated control decreased from 20mg/l to 13.40mg/l (a 33% reduction) and from 60mg/l to 53.40mg/l (an 11% reduction). This reduction could be attributed to the fact that once chlorpyrifos is introduced into a reaction, it may undergo volatilization and photodegradative conditions, either directly or indirectly, as proposed by [41]. These findings underscore the complexity of chlorpyrifos degradation and emphasize the potential benefits of bacterial consortia in enhancing the degradation process under certain conditions. Further research is warranted to explore the dynamics of chlorpyrifos degradation and its intermediates in more detail.

5. CONCLUSION

In the present study, two Chlorpyrifos- degrading bacteria were identified. They were *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024.The biodegradation study of the bacterial isolates was done singly and as a consortium to determine the response to chlorpyrifos. The result showed that *Chryseobacterium sp* 6024 is a novel bacterium in biodegradation studies with minimal potential to degrade Chlorpyrifos. *Bacillus cereus* ST06 can remove up to 63% and 61% of 20mg/l and 60mg/l chlorpyrifos from the liquid medium, indicating it can be employed to degrade chlorpyrifos. Result of this study also showed that the consortium of the isolates (*Bacillus cereus* ST06 and *Chryseobacterium sp* 6024) can remove up to 79% and 78% of 20mg/l and 60mg/l Chlorpyrifos after 28 days better than the individual isolates; hence they can be used for the degradation of Chlorpyrifos for sustainable agriculture.

Further studies must focus on sequencing chlorpyrifos degrading genes from *Bacillus cereus* ST06 and *Chryseobacterium sp* 6024, gene profiling of both bacteria to know the downregulated and upregulated genes during biodegradation, in order to harness the use of the two bacterial isolates to properly degrade Chlorpyrifos and its intermediate TCP. It is also important to study the mechanism of action of the bacterial consortia to understand their roles in degrading chlorpyrifos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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