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Bioremediation of Chlorpyrifos Contaminated Soil Using Biomass of Fruit-vegetable Waste

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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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Short Research Article

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ABSTRACT

India is a country where more than 70% of its population relies on its agricultural production. Excess production of crops leads to contamination by pests and to prevent these pesticides are in wide use. Chlorpyrifos is a pesticide commonly used due to its broad-spectrum activity. It is highly toxic and does not get degraded easily leading to irreversible hazards to the environment. The process of bioremediation involves the all-encompassing action of microbes, including aerobic and anaerobic bacteria and fungi. Most of the earlier studies involved the direct use of microbes or their enzymes in the bioremediation processes. In the present study we have attempted bioremediation of soil samples harnessed with chlorpyrifos at two different concentrations (10% & 20%) using crude fruit-vegetable waste biomass. The results of the study proved an exemplary reduction in the COD values as well as an enhancement in the essential nutrients (N,P,K) in the soil. Chlorpyrifos was completely degraded into its intermediary compounds phosphorothioic acid and1-methyl dodecyl benzene within a span of 30 days.

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1. INTRODUCTION

For achieving self-sufficiency in food crop production, wastage of crops should be primarily controlled. Since 1950, for preventing crop losses, a wide range of pesticides have been used. It is noted that only about 3-5% of the applied pesticides have actually been utilized for the purpose. The rest become residues and degrade the soil quality. Sub-surface run-offs from agricultural fields contain harmful pesticides in them. When they flow into rivers and other water bodies, they cause harm to the aquatic fauna and also to the human beings.

Chlorpyrifos (O, O-diethyl O-3, 5, 6-trichloro-2pyridinyl phosphorothioate) is an organophosphorothionate pesticide that is most widely used due to its broad-spectrum activity. Chlorpyrifos is highly toxic and has been reported as the second most commonly detected pesticide in food and water (Wołejko et al., 2022). In the soil, it is broken down by UV light and its complete degradation takes many weeks to years. Rampant growth of pesticide industries, their waste generation and non-judicious use of pesticides lead to irreversible hazards to the At present, there are several environment. physical, chemical and biological methods for treating pesticide wastes but they are not completely effective.

Bioremediation is a promising technology to control soil pollution in an efficient way (Mokrani et al., 2024; Ezekiel, Osuji, & Onojake, 2021). The process involves numerous microbes. including aerobic, anaerobic bacteria and fungus. Through the all-encompassing active action of microorganisms. bioremediation is heavily in the involved degradation, eradication, immobilisation, or detoxification of various chemical wastes and physically dangerous elements from the environment. Bioremediation of pesticide in soil with simple techniques can be easily done by anyone. Farmers can easily adopt this method to improve the soil quality in their lands. Several biological compounds have been used to quench these harmful soil pesticides either in the crude form or by extraction (Dua & Joshi, 2020). Most of the earlier studies on bioremediation of soil pesticides have been performed using isolated strains of bacteria (Fulekar & Geetha, 2008; Lakshmi, Kumar, & Khanna, 2008; Chen et al., 2012), fungi (Silambarasan & Abraham, 2013;

Russo et al., 2019) from the location. Apart from this, monocultures (Sumit, 2011) as well as enzyme preparations from microbial populations (Liu et al., 2023; Jaiswal, Bara, Soni, & Shrivastava, 2017) have also been used.

In the present study we have attempted to explore the possible role of fruit-vegetable waste biomass in the bioremediation of soil samples harnessed with a commonly used agricultural pesticide, Chlorpyrifos at two different concentrations. The study also tries to derive the efficacy of fruit-vegetable waste in improving the nutrient contents of pesticide infused soil.

2. MATERIALS AND METHODS

Alluvial soil was collected from an uncultivated agricultural field located at Kovilambakkam, a southern suburb of Chennai, Tamilnadu for the Collected soil was experimental study. thoroughly air dried and grounded in a mixer (particle size~0.004mm). It was stored in sealed glass containers at room temperature. Decayed and rotten vegetables (onions, tomatoes. pumpkins, lady's finger, potatoes, beans, carrots, radishes, brinials, drumsticks, cucurbits) and fruits (bananas, oranges, pomegranate, grapes, apples, guavas, pears, chickoos, pineapples, figs) were collected from the kitchen and the markets and were stored in plastic bottles 15 days prior to the experiment to ensure complete decomposition of the fruit and vegetable waste.

Soil spiking: Experimental soil was treated with pesticides chlorpyrifos at varying concentrations (20%, 10%), normally used by farmers. One kilogram and two hundred grams of soil was weighed and partitioned into six parts each with 200 grams. They were then transferred into six glass containers. Of the total 6 parts of soil, 2 parts were amended with the pesticide 5 ml each (10% or 20% chlorpyrifos) which served as negative controls and 2 parts with pesticide and fruit vegetable biomass (50 gms biomass to each part) that served as experimental samples. All samples were thoroughly mixed with a metal spatula. The remaining two parts were maintained as control without any pesticide or biomass (Fig. 1).

Mechanical stirring of the soil samples with a spatula was done for 5 minutes, once in two days throughout the experiment to facilitate thorough mixing and aeration. The time period of the

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Fig. 1. Experimental set up

experiment is 30 days and soil samples were drawn for analysis on Day 1, day 15 and day 30.

Analysis of nutrients and estimation of COD in soil samples: Analysis of physico-chemical parameters and nutrients (Nitogen, Phosphorus, Potassium) in the samples were performed following the standard protocols published in the Practical Manual of ICAR - IIPR, Kanpur, Uttar Pradesh (Singh & Praharaj, 2017). Accordingly total nitrogen in soil samples were estimated using Kjeldahl method (Kjeldahl, 1883) by acid digestion of the samples followed by distillation with boric acid and titration against a standard acid and estimation by flame photometry. Quantification of phosphorus in the samples were determined by extraction of the soil samples using 0.5N NaHCO3 (pH 8.5) and estimating phosphorous from the filtrate by spectrometric analysis (Olsen, Cole, Watanabe, & Dean, 1954). Potassium levels in the samples were analysed following Jackson, (1973) using 1N ammonium acetate extractant of the soil and estimating using a flame photometer. Chemical Oxygen Demand (COD) in the samples were analysed using USEPA approved Dichromate reactor digestion method (Hach Company 2007) in which the acid digested samples were determined for COD by colorimetric analysis (HACH, 1980).

GC- MS Analysis: Soil samples were drawn on day 1, day 15 and day 30 and were treated with acetone. It is incubated for 3-4 hours and the

extract was collected from the soil using a filter paper. Soil sample extract was analysed by Gas chromatographic - mass spectroscopy (GC-MS) for pesticides and its intermediates. The Column Agilent 889 hp_5ms ($30m \times 250 \ \mu m \times 2.5 \ \mu m$) was unlocked with a temperature range -60 °C – 325 °C ($350 \ ^{\circ}$ C) and 11.367 psi ($75 \ ^{\circ}$ C) pressure. Helium was used as the carrier gas. The flow rate was 1.2mL/min and the average velocity was 40.402 cm/sec. The injection volume was 1 µl and it was held for 1.2376 minute and was run for 53.5 minutes. The mass spectra of metabolites were obtained in Scan_B.mx. The data was analysed at 4.pmx.

Statistical Analysis: The given data were expressed in terms of the mean of triplicates \pm SD. Data regarding the nutrient and COD levels due to quenching of 10% and 20% Chlorpyrifos by fruit vegetable waste biomass with respect to positive and negative controls were analysed by one-way ANOVA.

3. RESULTS

The physico chemical characteristics of soil and fruit- vegetable waste is presented in Table 1. The analysis showed that fruit vegetable waste biomass was extremely rich in primary nutrients and low COD. Analysis of soil spiked with 10% chlorpyrifos and treated with fruit-vegetable waste mixture revealed that there has been a significant increase in the levels of nutrients especially with regard to potassium, followed by phosphorus and nitrogen (Table 2). The Nitrogen, Phosphorus and Potassium levels were 0.3%, 8.5mg/kg and 29mg/kg in the soil sample before adding biomass. After adding fruitvegetable waste biomass the Nitrogen level increased to 1.52% on day 15 and to 2.36% on day 30, the Phosphorus level increased to 9.9mg/kg on day 15 and 14.3mg/kg on day 30; the Potassium level increased to 49mg/kg on day 15 and 58 mg/kg on day 30. Initially upon the addition of pesticide the COD of the soil sample was estimated as 4.0%. After bioactivation by adding biomass to the samples, the COD was reduced to3.4% in 15 days in samples with fruitvegetable waste biomass and was further reduced to 1.63% on day30. The COD remained the same in soil samples that had no biomass.

The result of analysis of soil spiked with 20% chlorpyrifos and treated with fruit-yegetable waste mixture is presented in (Table 3). The Nitrogen, Phosphorus and Potassium levels were 0.7%, 9mg/kg and 33mg/kg in the soil sample before adding biomass. After adding fruitvegetable waste biomass the Nitrogen level increased to 1.5% on day 15 and to 2.6% on day 30, the Phosphorus level increased to 10.2mg/kg on day 15 and 15 mg/kg on day 30, the Potassium level increased to 50 mg/kg on day 15 and 57 mg/kg on day 30. After bio-activation by adding biomass to the samples, the COD was reduced to 3.5% in 15 days in samples with fruitvegetable waste biomass and was further reduced to 2% on day 30. The COD remained the same in soil samples that had no biomass.

 Table 1. Analysis of physico - chemical characteristics of soil and fruit- vegetable waste

 biomass

Parameter	Soil	Fruit vegetable waste
Colour	Black	Brown-Black
рН	7	6
Moisture	5	9
Dissolved Oxygen	6.8 mg/kg	12 mg/kg
Temperature	28	28
Electrical Conductivity	0.12 µs/cm	0.08 µs/cm
Nitrogen	0.031 %	4 %
Phosphorus	8.5 mg/kg	560 mg/kg
Potassium	29 mg/kg	837 mg/kg
COD	4.9	1.9

Table 2. Analysis of soil samples spiked with 10% Chlorpyrifos and treated with fruit ve	getable
waste	

Parameters checked	+ve Control (soil)	-ve Control (soil + 10% chlorpyrifos)	Experiment (soil + 10%chlor + FV - biomass)	Comparison between groups (P value)
Nitrogen (%)				
Day1	0.31±0.01	0.23±0.008	0.6±0.025	0.032*
Day 15	0.3±0.01	0.23±0.008	1.52±0.04	
Day 30	0.3±0.008	0.23±0.003	2.36±0.06	
Phosphorus (m	g/g)			
Day1	8.5±0.03	4.1±0.09	8.9±0.08	0.011*
Day 15	8.5±0.07	4.1±0.04	9.9±0.07	
Day 30	8.5±0.03	4.1±0.06	14.3±0.03	
Potassium (mg/	/g)			
Day1	29±0.05	18±0.04	31±0.11	0.019*
Day 15	29±0.11	18±0.02	49±0.13	
Day 30	29±0.05	18±0.04	58±0.07	
COD (%)				
Day1	4.9±0.06	4.5±0.01	4±0.12	0.042*
Day 15	4.9±0.04	4.5±0.03	3.4±0.05	
Day 30	4.9±0.08	4.5±0.03	1.63±0.03	
-				

*-denotes significance

Parameters checked	+ve Control (soil)	-ve Control (soil + 20% chlorpyrifos)	Experiment (soil + 20% chlor + FV - biomass)	Comparison between groups (P value)
Nitrogen (%)				
Day1	0.25±0.01	0.1±0.04	0.7±0.02	0.05*
Day 15	0.3±0.01	0.1±0.02	1.5±0.09	
Day 30	0.29±0.01	0.2±0.02	2.6±0.15	
Phosphorus (mg/g)				
Day1	8±0.15	3.9±0.12	9±0.15	0.007**
Day 15	7.9±0.13	4±0.06	10.2±0.15	
Day 30	8±0.10	3.9±0.06	15±0.15	
Potassium (mg/g)				
Day1	25±0.65	15±0.36	33±0.76	0.007**
Day 15	26±0.60	15.2±0.18	50±0.93	
Day 30	26.5±0.61	14.9±0.26	57±0.60	
COD (%)				
Day1	4.6±0.06	4.4±0.10	4.9±0.21	0.06*
Day 15	4.5±0.09	4.5±0.09	3.5±0.12	
Day 30	4.6±0.10	4.7±0.03	2±0.09	

Table 3. Analysis of soil samples spiked with 20% Chlorpyrifos and treated with fruit vegetable waste

*-denotes significance, **-denotes high significance

Degradation Of Chlorpyrifos - GC-MS Analysis: Analysis of chlorpyrifos spiked soil treated with fruit-vegetable waste by GC-MS revealed that the pesticide was completely degraded into its intermediary compounds within 30 days (Graphs 1-7). Phosphorothioic acid was isolated in the GC-MS analysis (Graphs 4,5) along with 1methyl dodecyl Benzene as an additional product. The presence of DETP along with phosphorothioic acid was detected within 15 days and then it was further metabolized into other sample compounds such as 1-methyl dodecyl benzene (Graphs 6,7). The data indicates that both phosphorothioic acid and benzene were present in the fruit-vegetable waste treated soil till the end of the experimental study i.e 30 days.



Graph-1

Graph-2

Peak @ 8.913 Area 61311.157 Area % 14.78



Ion Table

81.0 999 · 109.0 748 · 29.0 742 · 111.0 651 · 47.0 650 · 93.0 647

Compound Name	Score	Rev. Score	Prob. %	Library Name	CASN	Library Id
Phosphorothioic acid, 0,0-diethyl S-methyl ester	703	840	80.09	mainlib	2404-05-9	55098





Ion Table

110.9 999 · 80.9 778 · 92.9 711 · 108.9 666 · 139.9 642 · 127.9 598

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS#	Library Id
Phosphorothioic acid, O,O-diethyl S-methyl ester	703	840	80.09	mainlib	2404-05-9	55098





Graph-4

Ion Table

111.0 999 • 138.0 992 • 81.0 714 • 109.0 664 • 82.0 566 • 93.0 483

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS#	Library Id
Phosphorothioic acid, S- [2-{(1-cyano-1- methylethyl)amino]-2	827	853	79.89	mainlib	3734-95-0	96792



Graph-5

138.0 999 • 111.0 888 • 81.0 692 • 96.9 639 • 109.0 599 • 82.0 498

Summary Hit Table

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS#	Library Id
Phosphorothioic acid, S- [2-[(1-cyano-1- methylethyl)amino]-2	827	853	79.89	mainlib	3734-95-0	96792

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5.90



Graph-6

Area %

Ion Table

Peak @

25.098

Area

24472.926

105.0 999 • 106.0 122 • 91.0 93 • 43.0 44 • 104.0 43 • 41.0 39

Compound Name	Score	Rev. Score	Prob. %	Library Name	CAS#	Library Id
Benzene, (1- methyldodecvi)-	660	737	14.94	mainlib	4534-53-6	88276





4. DISCUSSION

In the technique of bioremediation, microbes are involved in the biodegradation process transforming pesticides into degradation Earlier studies have shown that products. bioremediation of chlorpyrifos from contaminated soil is more viable through microbial degradation which is more safe and sustainable (Bosu et al., 2024). The microbes involved in the process are mostly bacteria or fungi which generate intra or extra cellular enzymes. In the present study during the bioremediation process, chlorpyrifos effectively and quickly reduced was to phosphorothioic acid within a span of 30 days. The metabolites identified during the degradation of chlorpyrifos were Phosphorothioic acid, O,Odiethyl S-methyl ester, Phosphorothioic acid [2-[{1-cyano-1-methylethyl}amino]-2 and Benzene, (1-methyldodecyl)- Phosphorothioic acid is the final product of chlorpyrifos degradation pathway without addition of water (George et al., 2014).

Fruit-vegetable waste is rich in fungi and hence in the present study we have attempted to use this as a bioremediation resource. Analysis of the physicochemical characteristics physico chemical characteristics of the soil, and fruitvegetable waste mixture indicated the presence of most of the vital nutrients N, P, K, which was comparatively more in fruit -vegetable waste. Significant increase in the levels of Potassium, Nitrogen and Phosphorus was observed in pesticide spiked soil upon treatment with fruit vegetable waste biomass in a span of 30 days. In this study, the exemplary reduction in the COD values of fruit-vegetable waste treated soil spiked with pesticides co-related with the degradation of parent (chlorpyrifos) compound to its intermediates (Geetha & Fulekar, 2008; Orts et al., 2017)) after a period of 30 days as observed from GC-MS values. The results from the present study regarding reduction in COD is in accordance with earlier studies which have also reported COD to be a direct indicator of bioremediation (Singh & Fulekar, 2007; Dar, Kaushik, & Villarreal-Chiu, 2019). This study showed that the COD dropped drastically within 30 days of treatment of pesticide spiked soil with fruit vegetable waste.

The microbial consortium is abundant and varied in fruit vegetable waste than in normal soil. The fruit-vegetable waste used in the present study for bioremediation of pesticides consists of a consortium of bacteria and fungi. Studies on the identification of microbes involved in the spoilage of vegetables and fruits has shown that variety of

bacteria are associated with the rotting of vegetables. These include bacteria spp. such as Erwinia (carrots. carotovora onions). Pseudomonas aeruginosa (cucumbers). Corynebacterium michiganense (tomatoes), P. tolaasii (pumpkin), Aeromonas hydrophila (lady's finger), Pectobacterium carotovorum (potato), P. marsprunorum (beans), Agrobacterium (carrots). Similarly fruits are spoiled by bacteria such as Alicyclobacillus acidoterrestris (apples), Α. acidocaldarium (oranges). Α. pomorum (pomogrenate), A. herbarium(pears). Fungi that cause rotting and spoilage of vegetables have been identified as Bortrytis cinerra (pumpkin, onion), B.allii (onion), Aspergillus niger (onion, tomatoes), A. alliaceous (onion), Penicillium funiculosum(onion), Ρ. glabrum(onion). Colletotrichum coccodes (tomato, potato). Alternaria Rhizopus stolonifera (cabbage), gterwis (cucumber, tomato), Cladosporium cucurmenium (pumpkin, cucumber), Phytophtora nicotianae (onion, tomato). Fruits undergo spoilage by fungi such as *Penicillium expansum* (apple, pears), P. aurantiogriseum (grapes), **Byssochlamys** fulva (banana). Mucor piriformis(apple, Trichothecium pears) roseum(apple, grapes), and Coletotrichum gloesporoides (oranges, papaya) (Wiley, 1994; Tournas, 2005; Moss, 2008; Barth et al., 2009; Doyle & Sperber, 2009; Secor et al., 2016).

This is a clear indication that the consortium of bacteria and fungi are especially suited for the process of bioremediation as they are major decomposers and symbionts in soil as well as aquatic habitats due to their diverse metabolic activity (AbuQamar, Abd El-Fattah, & El-Saadony, 2023) Intracellular enzymes such as cytochrome P450 present in these genera of fungi are also important in the degradation of (Farhan chlorpyrifos et 2021).The al., extracellular enzymes released by the fungi predominant in the fruit-vegetable waste used in this study have been previously reported to degrade a wide range of organic and inorganic pollutants (George et al., 2014). Fundi are known to degrade a range of pesticides with their non-specific enzymes (Kavitha, Vajiha Banu, & Sumithra, 2023). The fungi present in the organic waste belong to the genera white rot fungi, brown rot fungi, filamentous fungi and These possess extracellular enzymes veast. such as manganese peroxide, laccase, lipain peroxidise and versatile peroxidase.

The occurrence of certain commonly occurring microbial consortia (Eg. *Aspergillus*, *Penicillium*, *Pseudomonas*, *Bacillus* and *Rhizopus*) identified

in fruit-vegetable waste in bioremediation of pesticide spiked soil under controlled condition showed that fruit-vegetable waste can be effectively used for the degradation of the tested soil pesticide (chlorpyrifos) at different concentrations besides enhancing the essential nutrient content

The analysis on the effect of fruit-vegetable waste in bioremediation of pesticide spiked soil under controlled condition in the present study proves that fruit-vegetable waste is an efficient natural alternative for bioremediation of pesticides from soil.

5. CONCLUSION

The extensive use of pesticides to prevent crop losses has resulted in hazardous effects including bioaccumulation and thereby posing a great threat to biodiversity. Microbial degradation of pesticides though a suitable and nature friendly option, is often hampered by nonavailability of required nutrients. In the present study we have attempted a natural strategy to improve the process of bioremediation of agricultural soil along with potential increase in the levels of primary soil nutrients. From the study we observed that crude fruit-vegetable waste could effectively degrade the tested pesticide into its intermediary compounds in a span of 30 days. Besides addition of fruitvegetable waste to pesticide spiked soil also enhanced the vital nutrient content of the soil significantly.

As indicated by the GC-MS analysis of the pesticide soil treated with fruit-vegetable waste the presence of intermediates such as DETP and phosphorothoic acid was detected within 15 days which was further metabolized into simpler compounds such as 1-methyl dodecyl benzene, which were present till the end of the experimental study of 30 days. Moreover, the steady reduction in COD in the treatment soil when compared to controls is a direct indicator of effective bioremediation of the pesticide used in the study. This study highlights the importance of fruit-vegetable effective waste as an bioremediator as well as a nutrient enhancer for pesticide treated agricultural soil.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative Al technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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