Global Scientific JOURNALS

GSJ: Volume 9, Issue 7, July 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

ISOLATION OF CHLORPYRIFOS DEGRADING BACTERIA FROM PESTICIDE CONTAMINATED SOIL

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ABSTRACT

The major goal is to isolate microorganisms that degrade chlorpyrifos from pesticide-contaminated soil. Using bacterial strains, test for chlorpyrifos tolerance. Characterize chlorpyrifos-degrading isolated strains on a morphological, biochemical, and molecular level, optimise chlorpyrifos degradation by bacterial isolates, and estimate chlorpyrifos degradation in both optimised and non-optimized conditions.

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INTRODUCTION

Agriculture is critical to the Indian economy's long-term growth and development. In India, agriculture employs 60-70 percent of the population. Pests waste between 30 percent to 35 percent of India's annual crop yield. Pesticides are poisonous chemicals that are used in particular situations to destroy pests that are specifically targeted. Herbicides are used to control weeds, insecticides are used to control insects, and fungicides are used to control plant diseases and fungi. In agriculture, pesticides have become a necessity. Organophosphates, carbamates, and pyrethroids are the most widely used pesticides in India. These three groups of compounds are hydrolyzed spontaneously and produce neurotoxicity in mammals (Sogorb and Vilanova 2002). Their use in agriculture for the control of weeds, insects, and rodent pests has grown increasingly essential. They are deadly, but they perform a vital part in feeding the world's population (Kurzel and Cetrulo 1981; Akhtar and Ahmed 2002). Pesticide overuse also inhibits plant growth and causes mutagenesis and carcinogenic effects in non-target microorganisms. Pesticides have acute and chronic health impacts, depending on the type of the exposure, the product's toxicity, and the amount of pesticide ingested, and can even result in death in extreme situations

(Singh et al 1987). Organochlorinated pesticides were phased out in India in favour of organophosphorus insecticides because to their extended environmental persistence (Adityachaudhury et al 1997). Tetraethyl pyrophosphate (TEPP), the first organophosphorus pesticide, was produced and employed in 1937 (Dragun et al 1984). During 1500 distinct organophosphate chemicals have been created over the last century (Kang et al 2006), making them the most diverse category of chemical pesticides utilised in plant protection worldwide (Hertel 1993). Poisoning by organophosphorus compounds is a global issue, with an estimated 3 million poisonings and 200,000 deaths per year (Karalliedde and Senanayake 1999; Sogorb et al 2004). The main method of action of organophosphate insecticides is to inhibit carboxyl ester hydrolases, particularly acetylcholinesterase (AChE) (Hertel 1993). Acetylcholine (ACh) is a neurotransmitter that is converted to choline and acetic acid by the enzyme AChE. The central and peripheral neural systems, neuromuscular junctions, and red blood cells all contain ACh (RBCs). Organophosphates phosphorylate the serine hydroxyl group at AChE's active site, rendering it inactive. Phosphorylation happens when an organophosphate leaving group is lost and a covalent link with AChE is formed. ACh accumulates throughout the nervous system once AChE is inactivated, leading in overstimulation of muscarinic and nicotinic receptors. The autonomic and central nervous systems, as well as nicotinic receptors on skeletal muscle, are activated, resulting in clinical consequences. Organophosphorus **pesticides** inhibit the **regular hobby** of the AChE **ensuing** in accumulation of acetylcholine **on the** synapses **degree** and **consequences** in convulsions, paralysis, **dying** of **bugs** and mammals (Ragnarsdottir 2000). Although **poisonous** to **better** organisms, organophosphorus **insecticides constitute** an **plentiful supply** of **nutrients** for bacteria (Horne et al 2002). Chlorpyrifos is an organophosphate insecticide. Pure chlorpyrifos is **made from** white or colorless crystals. It has a **barely** skunky odor, like rotten eggs or garlic. The **most effective felony** indoor use for chlorpyrifos is in **bins** with **handled** baits. Chlorpyrifos [O,O-diethyl-O-3,5,6-trichloropyridin 2-yl phosphorothioate] is a **extensive** spectrum systemic phosphorothioate ester insecticide patented and **brought through** Dow Chemical Company in United States of America in 1965 (Murray et al 2001). Chlorpyrifos is accessible in granules, wettable powder, dustable powder, emulsifiable concentrate (Swathi and Singh 2002) and utilized for the control of a wide scope of nuisances like cutworms, corn rootworms, cockroaches, grubs, bug creepy crawlies, flies, termites, fire subterranean insects, aphids, lice, and different bugs. It is applied to various yields including cotton, nuts, corn, organic products, vegetables, elaborate plants and is profoundly industrious in foliar application. Chlorpyrifos effectsly affects the climate and is additionally hurtful to people, causing cerebral pains, sickness, solid jerking, seizures, birth deserts, and even passing. After openness, chlorpyrifos spreads to all pieces of the casing. At the point when the edge attempts to close it down, it structures chlorpyrifos oxon, which is some other shape. The oxon ties for all time to catalysts which control the messages that movement between nerve cells. At the point

when chlorpyrifos ties to such a large number of the compounds, nerves and muscles don't work effectively. The body then, at that point should make more catalysts so typical nerve capacity can continue. The body can separate and discharge a large portion of the unbound chlorpyrifos in excrement and pee inside a couple of days. Chlorpyrifos that discovers its direction into the sensory system might remain there any longer. It is extremely risky among every living animal.

degree by microbial societies, either by use of the mixtures as wellsprings of energy or supplements, or by catabolize with different substrates supporting microbial development (An examination on pregnant ladies presented to chlorpyrifos through home bug spray utilize showed a connection between in utero openness to chlorpyrifos and diminished birth length and diminished birth weight. These consequences for size were at this point not huge in babies brought into the world after 2001, when indoor private utilization of chlorpyrifos was eliminated. Chlorpyrifos is additionally a presumed endocrine-upsetting compound. Sex-explicit practices in mice can be changed by chlorpyrifos openness, with these neuroendocrine-disturbing impacts influencing mice contrastingly relying upon their sex. Moderate dosages have been displayed to change chemical levels in other creature contemplates. When chlorpyrifos is in the dirt, it adheres unequivocally to soil particles. Plant roots will not ordinarily get it, and it will not effectively get into groundwater. Chlorpyrifos might wash into waterways or streams if disintegration moves the treated soil. Chlorpyrifos or the synthetic compounds it breaks into may get into the environment and travel significant distances. Analysts discovered chlorpyrifos in indoor air, residue, rugs, and on kids' toys in homes where items with chlorpyrifos in them had been utilized. Pesticide corruption in soil relies upon populace densities, movement of pesticide debasing microorganisms, pesticide bioavailability and soil boundaries like pH, water content and temperature (Parkin and Daniel 1994). Debasement of pesticides is the separating of harmful synthetic substances into non-poisonous mixtures and, now and again, moves in to their orig-in all components. The debasement or breakdown of pesticides can happen in plants, creatures, and in the dirt water; or it can happens upon openness bright (UV) radiation. There are some stringently substance responses which happens in the dirt that guide in the corruption, However, the most well-known kind of debasement there happens through the movement of microorganisms, particularly the microscopic organisms and parasites. There isn't anything baffling about microbial debasement of pesticides. Bioremediation is an alternative that offers the likelihood to annihilate different pollutants utilizing regular organic movement. Since bioremediation is by all accounts a decent option in contrast to traditional tidy up innovations, research in this field is expanding quickly. Bioremediation is characterized as the cycle whereby natural squanders are organically debased under controlled conditions. Bioremediation includes the

utilization of living beings to debase the ecological pollutants into less poisonous structures. It utilizes normally happening microscopic organisms and growths or plants to corrupt or detoxify that are substances dangerous to human wellbeing and the climate. The microorganisms might be native to a tainted region or they might be secluded from somewhere else and brought to the sullied site. Foreign substance compounds are changed by living beings through responses that happen as a piece of their metabolic cycles. Biodegradation of a compound is regularly a consequence of the activities of different life forms. At the point when microorganisms are imported to a defiled site to upgrade corruption we have a cycle known as bioaugmentation. Microorganisms assume a critical part in the change and debasement of pesticides. Indeed, even the most determined pesticides can be processed to some Castillo et al., 2006). Quantities of bacterial species are accounted for biodegradation of various pesticides as, Pseudomonas stutzeri strain S1 for beta-cyfluthrin debasement (Saikia et al., 2005), Leifsonia strain PC-21 and Pseudomonas sp. 1G for imidacloprid corruption (Anhalt et al., 2007). Diverse contagious societies are additionally answered to be the productive pesticide degraders, white decay growth Phanerochaete chrysosporium is one of them (Castillo et al., 2001). The most broadly portrayed compounds are phosphotriesterase (PTEs), organophosphate hydrolase (oph) and organophosphate corrupting protein (opda). The opda or oph chemical is fit for hydrolyzing a wide scope of organophosphorus pesticides (Shimazu et al 2001). The detailed half-existence of chlorpyrifos in soil fluctuates from 10 to 120 days (Getzin 1981; Racke et al 1988) with TCP as the major corrupting item (Singh and Walker 2006). The initially recorded organophosphorus biodegrading quality was recognized in Pseudomonas diminuta (Serdar et al 1982). There is an expanding need to foster new strategies to distinguish, disengage and portray the microbial strains that assume a significant part in corruption measures (Vallaeys et al 1996). Bigger microbial populaces will in general exist in rhizosphere soil than in mass soil and show expanded corruption of natural synthetic substances including pesticides (Reilley et al 1996). Bioremediation is by all accounts perhaps the most earth safe and practical strategies for debasing pesticides. The dirt microorganisms end up being the best and productive bioremedial reusing specialists because of their presented benefits like little size, omnipresent circulation, high particularity, possibly quick development rate and unmatched enzymatic and wholesome flexibility (Lal 1983; Alexander 1985). The objective of bioremediation is to decrease contamination levels to imperceptible, nontoxic or satisfactory levels or to mineralize them to carbon dioxide. According to a natural perspective this all out mineralization is alluring as it addresses total detoxification (Gan and Koskinen 1998).

MATERIALS AND METHODS

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SOIL SAMPLE COLLECTION

The Pesticide contaminated soil was collected from the pesticide manufacturing industry (The Scientific Fertilizer Co Pvt. Ltd) Gundur (Trichy).

PURCHASE OF CHLORPYRIFOS

Technical grade Chlorpyrifos (99% purity) were obtained from the Scientific Fertilizer Co Pvt. Ltd, Pesticide Division, Gundur, Tiruchirappalli, Tamilnadu, India.

ISOLATION OF CHLORPYRIFOS DEGRADING BACTERIA FROM SOIL

10 grams of soil sample was subjected to enrich in 100 ml minimal salt broth with 100 ppm chlorpyrifos and it is incubated at 30°C for 7 days in orbital shaker at 150 rpm. After 7 days of incubation 0.1 ml of enriched soil aliquots were spread into the minimal salt agar medium containing 100 ppm chlorpyrifos and incubated the plates at 37°C for 48 hours. After the incubation three different colonies were observed. The three different bacterial colonies were subsequently, separately inoculated on Minimal salt agar medium containing 100 ppm chlorpyrifos to obtain pure cultures.

MAINTANENCE OF CHLORPYRIFOS DEGRADING ISOLATES

The pure cultures of bacterial strains used in the present study were sub-cultured on enriched nutrient agar plates containing 200 ppm of chlorpyrifos. The purity of the isolates were checked with microscopic observation followed by Gram's staining.

ASSAY OF CHLORPYRIFOS TOLERANCE

The maximum concentration of Chlorpyrifos tolerated by the bacterial strains was determined by streaking the isolated strain on Minimal salt agar medium with different concentration of pesticide 100-1000 mg L^{-1}. All the plates were inoculated and incubated

at 37°C for 48 hours (Shafiani and Malik, 2003).

IDENTIFICATION OF CHLORPYRIFOS DEGRADING ISOLATES

Isolated strains were identified by using morphology, biochemical and molecular characteristics:

MORPHOLOGICAL CHARACTERISTICS

Isolated strains were examined for colony morphology in the Enriched Nutrient agar plates based on Colony size, colour, elevation, margin followed by Gram's staining and Motility test.

Gram staining (Baily and Scott, 1966)

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Bacterial cultures were placed on clean glass slide. A thin smear was made and cemented with heat. The smear was then stained with crystal violet for a minute and washed with distilled water. Gram's iodine solution was added to the sample and allowed for a minute. The slides were washed with decolorizing solution (alcohol) and washed again with distilled water. Later stained with safranine and allowed for a minute. The slides were washed with distilled water and air dried completely. The slides were observed under the microscope with 10X and 100X objectives and results were recorded.

Motility test (Baily and Scott, 1966)

Around the concavity of the depression slide, a ring of petroleum jelly was placed. Using sterile method, a loop-full of isolates was inserted in the centre of a clean cover slip. The depression slide was put over the cover slip with the concave surface facing down and gently pressed to produce a seal between the slide and the cover slip. The slide was then immediately shifted over to the right side. As a result, the drop sticks to the cover slip's inside surface. The slide was next examined under a light microscope.

BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

The bacterial isolates were characterized by using various biochemical tests.

Indole production test

Some bacteria oxidize tryptophan an essential amino acid by the tryptophanase enzyme resulting in the formation of indole, pyruvic acid and ammonia. A loopful of overnight grown culture was inoculated into the sterilized Tryptone broth (1%) and incubate at 37°C for 24 hours. After incubation Kovac's reagent was added in to the tubes. Formation of red layer indicate positive whereas the absence of red coloration shows negative result.

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Methyl red and Voges proskauer test

The methyl red (MR) and Voges Proskaur (VP) test are used to distinguish bacteria that produce organic acid and those that produce neutral product acetoin as an end product. Loopful of overnight culture was inoculated into the MRVP broth for 48 hours at 37°C. After incubation 5 drops of methyl red indicator was added to the test tube and change in red colour was observed. 12 drops of Barritt's reagent A and 2-3 drops of Barritt's reagent B were added to the test tube for VP test separately. The caps were taken off to expose the media to oxygen and shaken gently. Development of deep rose colour indicate positive result and absence of rose colouration indicate negative result.

Citrate utilization test

Citrate test is used to indicate weather an organism utilizes citrate as the sole car bon source. The cultures were inoculated in slants of Simmon's citrate agar medium for 48 hours at 37° C. Change of Slant colour into deep blue colour indicate positive whereas no colour change shows negative result.

Hydrogen sulfide production test

It is a test to know the ability of an organism to reduce sulfur compounds tohydrogen sulfide. The bacterial isolates were inoculated into the SIM agar slant tubes at 37°C for 48 hours. Formation of black colour indicate the positive result.

Catalase test

It is test to know the ability of the organism to produce catalase enzyme to convert the hydrogen peroxide into water and oxygen. A loopful of overnight grown culture was placed on a clean glass slide. Add 3 to 4 drops of 3% H_2O_2 over the culture. Brisk effervescence indicates positive reaction and absence of effervescence indicates negative result.

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Urease hydrolysis test

This test is perform to identify the bacterial isolates based on the ability to produce urease enzyme in the substrate urea. The bacterial isolates were inoculated in the christensen's agar medium and incubate at 37°C for 48 hours. The development of pink colour indicate the positive result.

Nitrate reduction test

The ability of the bacteria to produce nitrate reductase enzyme when nitrate consume as a sole source of energy and ability to reduce nitrate to nitrite. The bacterial strains were inoculated into the peptone nitrate broth for 24 hours at 37°C. After the incubation period, add 0.5 ml sulphalinic acid and then - napthylamine to see if the test tube develops a red colour. The creation of red colour indicates a happy outcome.

Starch hydrolysis test

Amylase is an exo-enzyme produced by the bacteria that hydrolyses starch into maltose and glucose. The bacterial isolates streaked on the starch agar medium and incubate at 37°C for 48 hours. After incubation the plates were flooded with iodine solution. Formation of clear zone around the colony indicate positive starch hydrolysis.

Gelatin hydrolysis test

The organism produce proteolytic exo-enzyme gelatinase to hydrolyze gelatin. The strains were streaked on the gelatin agar medium and incubate for 48 hours at 37°C. After incubation the plates were flooded with mercuric chloride for 10-15 min and clear zone around the colony shows positive result.

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MOLECULAR CHARACTERIZATION

DNA ISOLATION PROCEDURE

2 ml of overnight bacterial culture were taken into the 2ml centrifuge tube. The tubes were centrifuged for 10 minutes at 10000 rpm. Pellets were mixed in 200 µl TE buffer and 300 µl lysis buffer (SDS-0.5%, 1M Nacl). Incubate the tubes at 55˚C degree for 20 min. Phenol chloroform mixture (1:1) 1ml were added to the mixture and incubate for 15 minutes in ice. The above mixtures were centrifuged at 10,000 rpm for 10 minutes. After centrifuge Supernatant were collected into the fresh tube and equal volume of isopropanol was added. Pellet was washed with 75% ethanol after centrifugation. The Pellet was dried and dissolves in TE buffer. DNA is conformed after visualize into the gel documentation.

PCR amplification of 16S rRNA

The actinomycetes 16S rRNA was amplified by PCR using universal primer pair of 0.5µl forward primer (27f) (5'- AGAGTTTGATCCTGGCTCAG -3') and 0.5µl reverse

primer (1492r) (5'- GGTTACCTTGTTACGACTT -3'). The 50µl reaction mixture contained the following components: 5.0µl template DNA, 5.0µl 10X buffer, 2.0µl of Taq DNA polymerase, 19µl of ddNTP mixture and sterile water 34.0µl. The 50 µl reaction mixture was taken on 0.5ml micro centrifuge tube.

The total 50µl mixture in the tube was gently spin for 10 seconds and allowed to settle the contents. The samples were kept in PCR thermal cycler. The PCR program was carried out in the following manner of 35 cycles with an initial denaturation step at 94˚C for 2 min, followed by denaturation step at 94˚C for 45 sec, annealing at 52˚C for 30 sec, extension at 72˚C for 1 min and final extension at 72˚C for 2 min. 10µl of PCR product with 2 ml of loading dye were mixed and loaded on a 1% agarose gel and analyzed electrophoretically at 50V for 45 mins. The gel was observed on gel doc imaging system (UVP).

OPTIMIZATION STUDIES

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Effect of pH on chlorpyrifos degradation

The optimum pH for the isolate was determined by adjusting the pH values such as 5,6,7,8 and 9. The medium pH values were adjusted by 0.1 N NaOH and 0.1N HCL and sterilized. The culture was inoculated and incubated at 35˚C for 4 days. After 4 days the degradation rate was estimated at 290 nm in UV-Spectrophotometer.

Effect of temperature on chlorpyrifos degradation

The optimum temperature of the isolated strains were determined by inoculating the culture in liquid broth and kept it at different temperature (25, 30, 35, 45 and 55˚C) and incubate for 4 days. After incubation, the degradation was determined by taking OD value at 290 nm.

Effect of carbon source on chlorpyrifos degradation

The ability of the isolates for utilization of various carbon sources was observed. Carbon sources like Dextrose, Sucrose, Maltose, Lactose, Fructose were tested on carbon utilization supplemented with 2% carbon sources. The isolates were incubated in the broth containing various carbon sources and the degradation was determined by taking OD value at 290 nm.

Effect of nitrogen sources on chlorpyrifos degradation

The ability of the isolates to utilize various nitrogen sources like Alanine, Tyrosine, Peptone, Glutamine, and urea were tested by supplementing with 2% nitrogen sources in broth. The isolates were inoculated in the broth containing various nitrogen sources and the degradation was determined by taking OD value at 290 nm.

Degradation study of chlorpyrifos in optimize and un-optimize condition

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When microorganisms are inoculated in a minimum salt medium with no carbon source, the microbes utilise an alternate carbon source provided by Chlorpyrifos, which is artificially incorporated in the mineral salt media (Sumit Kumar et al., 2011). The pesticide breakdown in the media was monitored for 7 days by measuring the optical density of the cell free extract of the minimum media supplemented with chlorpyrifos in both optimised and unoptimized conditions at the chlorpyrifos absorbance maxima of 290 nm (Zalat et al., 2014).

RESULT AND DISCUSSION

ISOLATION OF CHLORPYRIFOS DEGRADING BACTERIA FROM SOIL

Three different bacterial colonies were observed after 48 hours of incubation in the mineral salt agar medium contains 200 ppm of chlorpyrifos. The mineral salt medium contains trace amount of nutrient and addition of chlorpyrifos is a larger carbon and energy source to grow the bacteria in the medium. Similar results was found in MSM agar plate supplemented with chlorpyrifos (S.Anwar et al., 2009).

Plate 1: Isolation of chlorpyrifos degrading bacteria from soil

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MAINTANENCE OF CHLORPYRIFOS DEGRADING ISOLATES

The three bacterial isolates named as CDS-I, CDS-II and CDS-III was subculture in enrich nutrient agar plate. It is stored in 4°C for further analysis.

Plate 2: Chlorpyrifos degrading bacterial isolates

ASSAY OF CHLORPYRIFOS TOLERANCE

The maximum concentration of chlorpyrifos tolerance by the bacterial isolates were determined by streaking the isolates on mineral salt agar medium with different concentration of pesticide 100-1000 mg L^{-1}. The bacterial strain CDS-I shows maximum tolerance of chlorpyrifos than the CDS-II and CDS-III. CDS-I tolerate up to 800 ppm whereas CDS-II and CDS-III tolerate maximum of 500 and 700 ppm of chlorpyrifos.

IDENTIFICATION OF CHLORPYRIFOS DEGRADING ISOLATES

MORPHOLOGICAL CHARACTERIZATION

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The bacterial isolates were identified based on their morphology and biochemical characteristics. The CDS-I (Chlorpyrifos Degrading Strain) was withe in color, circular colony, 4 mm diameter in size with entire margin. The CDS-II was observed as yellow colored circular colony with 3 mm size and entire margin. The third isolate CDS-III was irregular in shape with 5 mm in size, transparent white greenish colored and the margin is undulate. The three bacterial isolates were gram positive non motile in nature (Table 1).

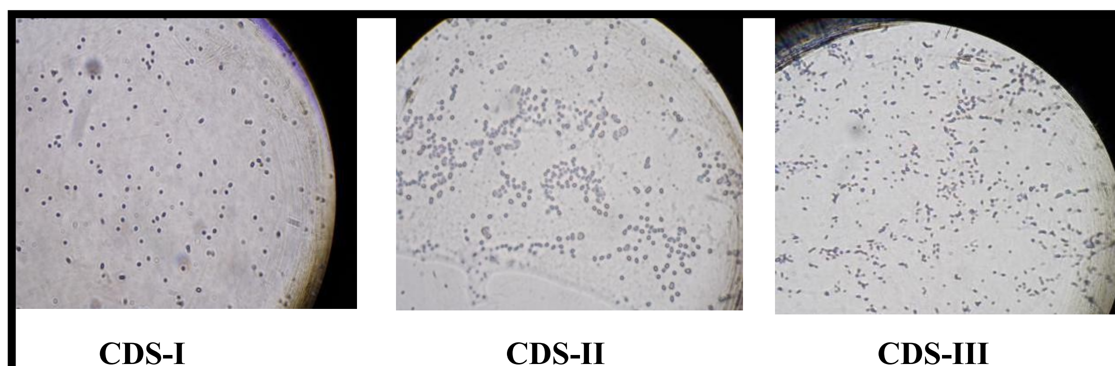


Plate 3 : Gram staining

BIOCHEMICAL CHARACTERIZATION

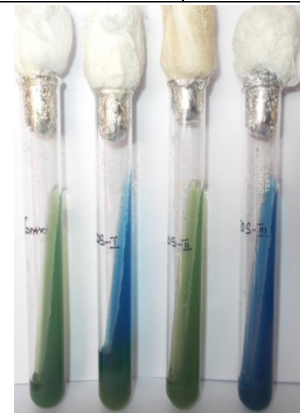
Based on the morphology and biochemical characterization the isolates were identified up to genus level. In the three bacterial isolates CDS-I shows positive results in methyl red, voges prouskauer, citrate utilization, and nitrate reduction whereas negative results was noticed in indole, catalase, urease, starch hydrolysis, gelatin hydrolysis, caesin hydrolysis, and hydrogen sulfide production. The another bacterial strain CDS-II was positive in methyl red, urease, nitrate test. The CDS-II shows negative results in indole, voges prouskauer, citrate, catalase, starch, gelatin hydrolysis, caesin hydrolysis, and hydrogen sulfide production. The third strain CDS-III was observed to be positive in methyl red, citrate and catalase test. Through morphological and biochemical test CDS-I was identified as Streptococci whereas the CDS-II was Enterococci and the CDS-III was identified as Staphylococci.

Table 1 : Morphology and Biochemical characterization

MORPHOLOGY AND BIOCHEMICAL TEST	**ISOLATED STRAINS**		
	CDS-I	**CDS-II**	**CDS-III**
Colony Shape	Circular	Circular	Irregular
Colony Size (mm) and color	5 - White	3 - Yellow	4 - Green
Elevation	Raised	Convex	Raised
Colony Margin	Entire	Entire	Undulate
Grams staining	Positive	Positive	Positive
Motility	Non motile	Non motile	Non motile
Indole	Negative	Negative	Negative
Methyl red	Positive	Positive	Positive
Voges-proskauer	Positive	Negative	Negative
Citrate Utilization	Positive	Negative	Positive
Urease	Negative	Positive	Negative
Catalase	Negative	Negative	Positive
Nitrate reduction	Positive	Positive	Negative
Starch hydrolysis	Negative	Negative	Negative

Gelatin hydrolysis	Negative	Negative	Negative
Caesin hydrolysis	Negative	Negative	Negative
Hydrogen sulfide production	Negative	Negative	Negative

A



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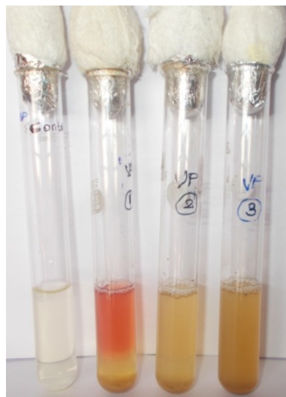

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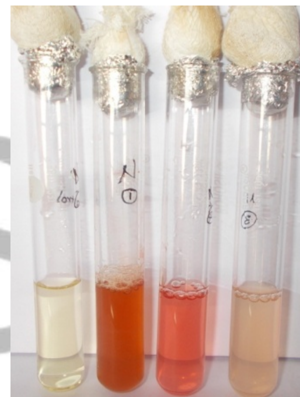

D

Plate 4 : Biochemical characterization (a-Methyl red, b-Citrate, c-Voges prouskeur, d-Nitrate reduction, e-Urease, f-Catalase)

MOLECULAR CHARACTERIZATION

Isolation of genomic dna from the bacterial isolates

The genomic DNA of the isolated strians were view under the gel documentation.

The isolated genomic DNA was 1 kb in size (Plate 5).

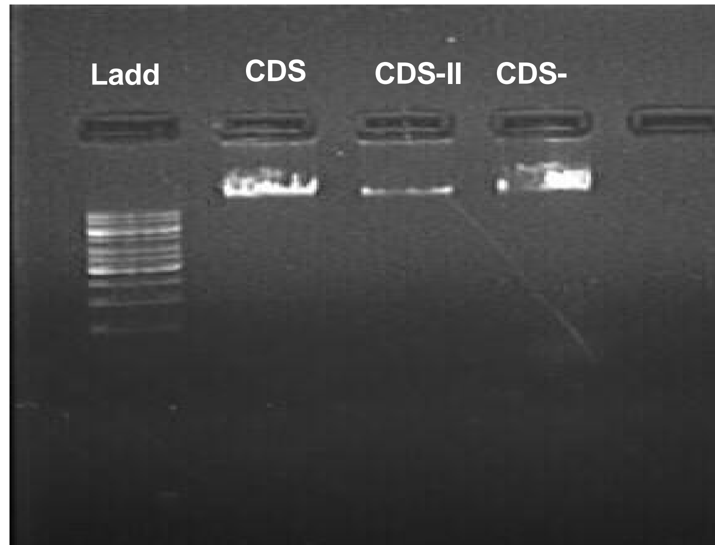

Plate 5: Genomic DNA of the isolates

AMPLIFICATION OF GENOMIC DNA FOR SPECIES IDENTIFICATION

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The amplified Genomic DNA was visualized under the gel documentation. The amplified DNA was 1500 base pairs in size.

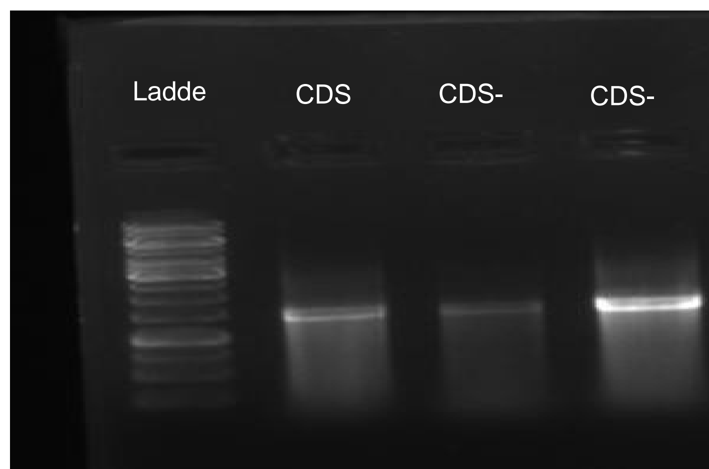

Plate 6: PCR amplified DNA of the bacterial isolates

OPTIMIZATION STUDIES

Effect of pH on degradation of chlorpyrifos

The effect of pH on degradation of pesticide by the bacterial isolates were evaluated by UV Spectrophotometer. The bacterial strain CDS-I and CDS-II was observed to degrade maximum of 30% and 25 % at pH 8 whereas the CDS-III strain at pH 7 shows 28% of degradation in the mineral salt media. The three bacterial strain was suspected to be Nuetrophilic in nature (Table 2 and Fig 1).

Effect of temperature on degradation of chlorpyrifos

Optimum temperature for the degradation of bacterial strain was observed at 290 nm in UV-Spectrophotometer. The optimum temperature for CDS-I was 35°C whereas CDS-II and CDS-III shows better degradation at 45°C. The three bacterial isolates was suspected to be mesophilic bacteria (Table 3 and Fig 2).

Effect of carbon source on degradation of chlorpyrifos

Carbon source is an important energy source to enhance the growth of bacteria and hence the effect of carbon source on the degradation studies was read at 290 nm after 7 days of incubation. The bacterial isolates were grown in MSM supplemented with 1% (w/v) of dextrose, sucrose, maltose, lactose, and fructose. The bacterial strain CDS-I utilize dextrose as a carbon source for their growth and degrade 58% of chlorpyrifos whereas the CDS-II and CDS-III was well grown in sucrose and dextrose shows possible percentage of degradation (Table 4 and Fig 3).

Effect of nitrogen source on degradation of chlorpyrifos

Nitrogen is an important growth component to enhance the growth of bacteria and hence the effect of nitrogen on pesticide degradation was studied at 290 nm after 7 days of incubation. From the result, it was clear that CDS-I and CDS-II had grown well in tyrosine and shows maximum of 45% and 48% degradation. The CDS-III had grown well in peptone and degrade 45% of chlorpyrifos (Table 5 and Fig 4).

Table 2: Effect of pH on chlorpyrifos degradation

pH	ISOLATED STRAINS (% of Degradation)		
	CDS-I	CDS-II	CDS-III
5	10	9	11
6	16	15	18
7	25	19	28
8	32	26	22
9	27	16	19

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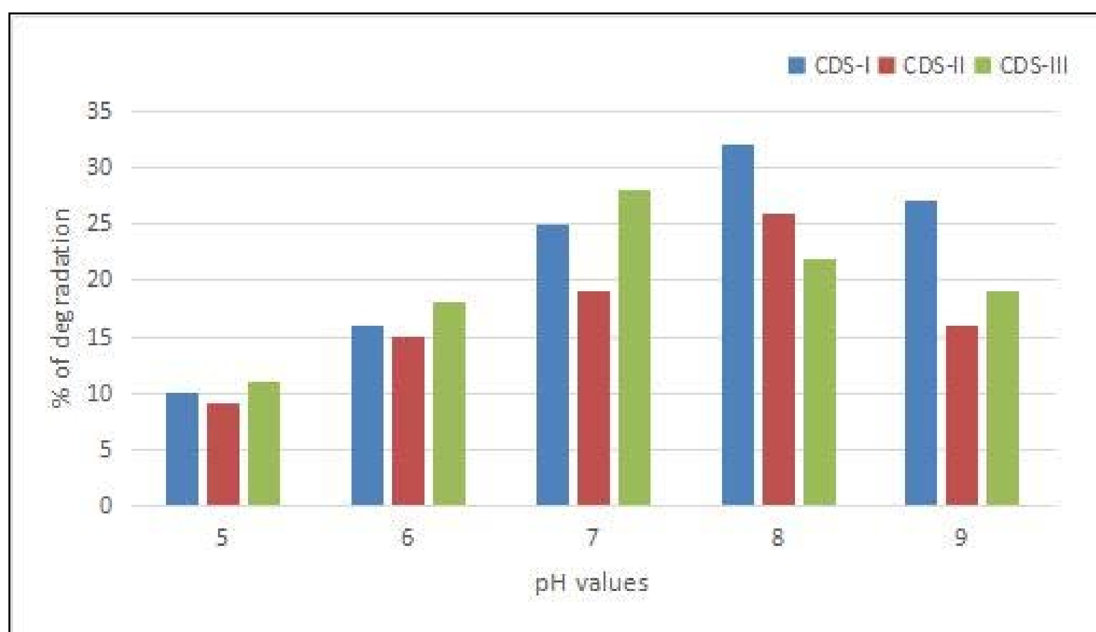

Figure 1 : Effect of pH on degradation of pesticide

Table 3: Effect of Temperature on chlorpyrifos degradation

Temperature (°C)	ISOLATED STRAINS (% OF DEGRADATION)		
	CDS-I	CDS-II	CDS-III
25	20	18	19
35	35	23	25
45	28	30	40
55	25	25	32
65	18	15	20

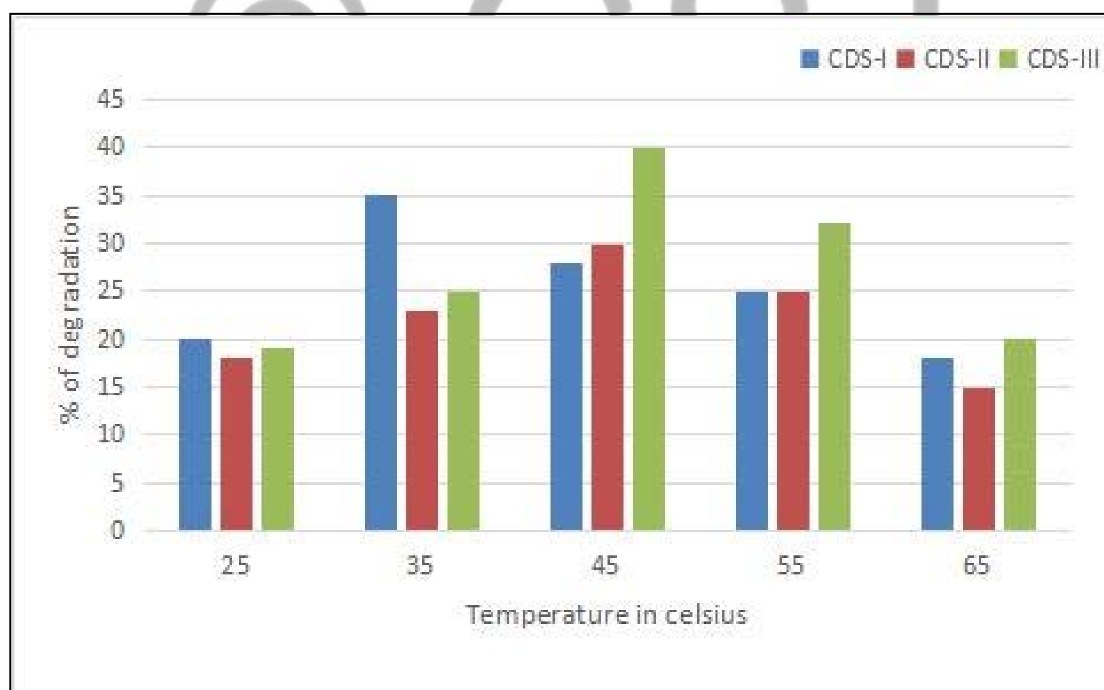

Figure 2 : Effect of temperature on degradation of pesticide

Table 4: Effect of carbon source on chlorpyrifos degradation

CARBON SOURCE	ISOLATEDE STRAINS (% OF DEGRADATION)		
	CDS-I	CDS-II	CDS-III
Dextrose	58	35	51
Sucrose	30	50	34
Maltose	33	33	35
Lactose	28	31	28
Fructose	25	28	21

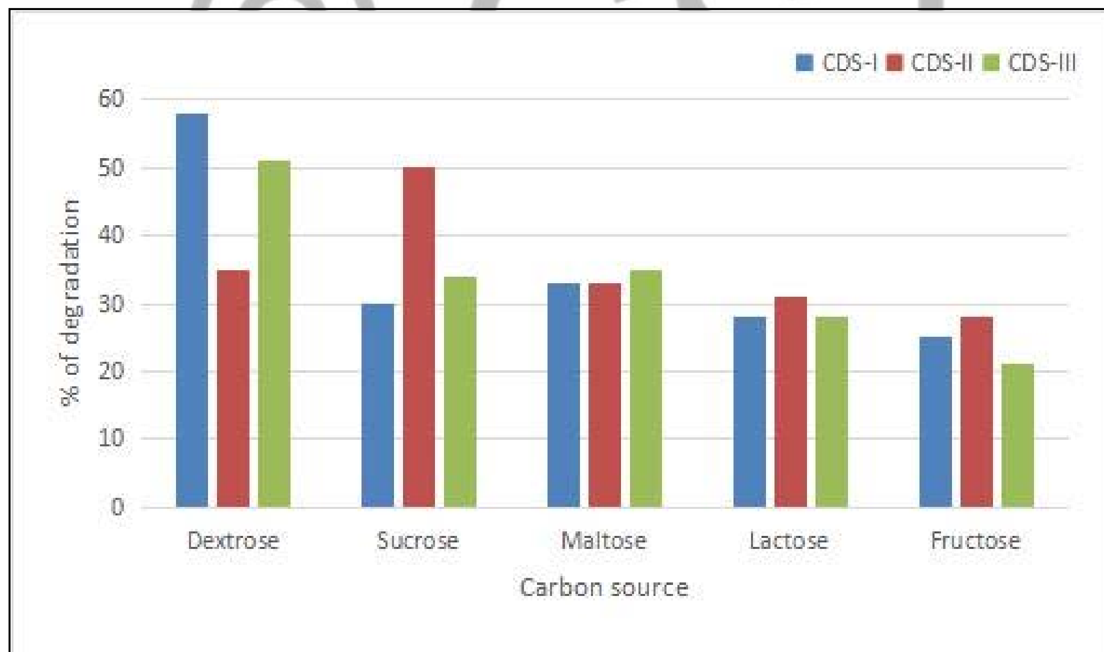

Figure 3: Effect of carbon source on chlorpyrifos degradation

Table 5: Effect of nitrogen source on chlorpyrifos degradation

NITROGEN SOURCE	ISOLATED STRAINS (% OF DEGRDATION)		
	CDS-I	CDS-II	CDS-III
Alanine	33	30	28
Tyrosine	45	48	34
Peptone	32	28	45
Glutamine	26	22	28
Urea	18	20	15

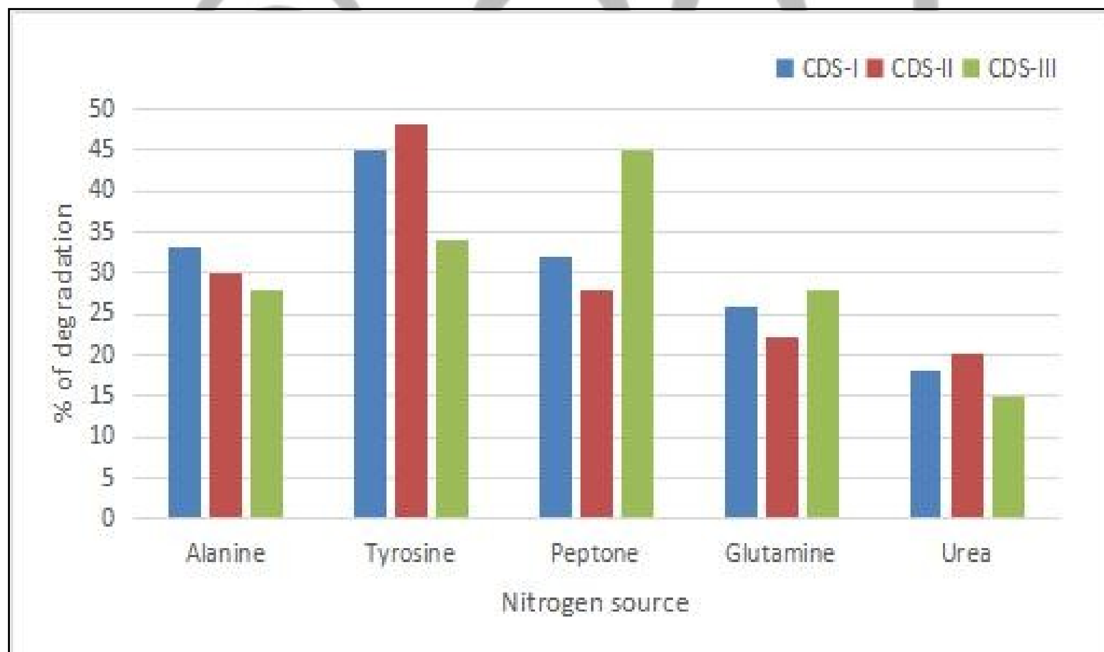

Figure 4: Effect of Nitrogen source on chlorpyrifos degradation

Degradation study of chlorpyrifos in optimize and un-optimize condition

The quantitative analysis of pesticide degradation was done by the help of simple UV-Spectrophotometric method (Zalat et al., 2014). This method is conventional and reliable method compared to TLC and HPLC method. The concentration of pesticide in the MSM with inoculated and uninoculated flask was determined at 290 nm in uv spectrophotometer for 7 days (Fig 5). The CDS-I shows 62.71% of degradation of chlorpyrifos in mineral salt medium in unoptimized condition. In controlled condition the degradation rate is 78% compared to unoptimize condition. The degradation rate was increased 15% in optimize condition because of the suitable condition for the growth. The CDS-II strain shows 49.59% degradation whereas in optimized condition 10% of degradation rate is increased. In controlled condition all parameters was suitable for the growth of bacteria and the degradation rate is increased compared to unoptimize condition. The CDS-III shows 58% of degradation in un-optimize condition. The increase in degradation rate of the bacteria was observed in CDS-III in optimize condition (Fig -5 and 6). Spectrophotometric analysis of chlorpyrifos degradation is also carried out in absorbence of 290 nm (Sharma et al., 2016).

Table 6: Spectrophotometric analysis of chlorpyrifos Degradation in un-optimized condition

Days	ISOLATED STRAINS (% OF DEGRADATION)		
	CDS-I	CDS-II	CDS-III
0	0	0	0
1	10	5.87	10.85
2	16.82	11.84	20.81
3	26.78	16.55	29.83
4	33.46	21.93	40.73

5	44.72	30.77	49.21
6	54.10	35.75	55.80
7	62.67	44.72	65.67

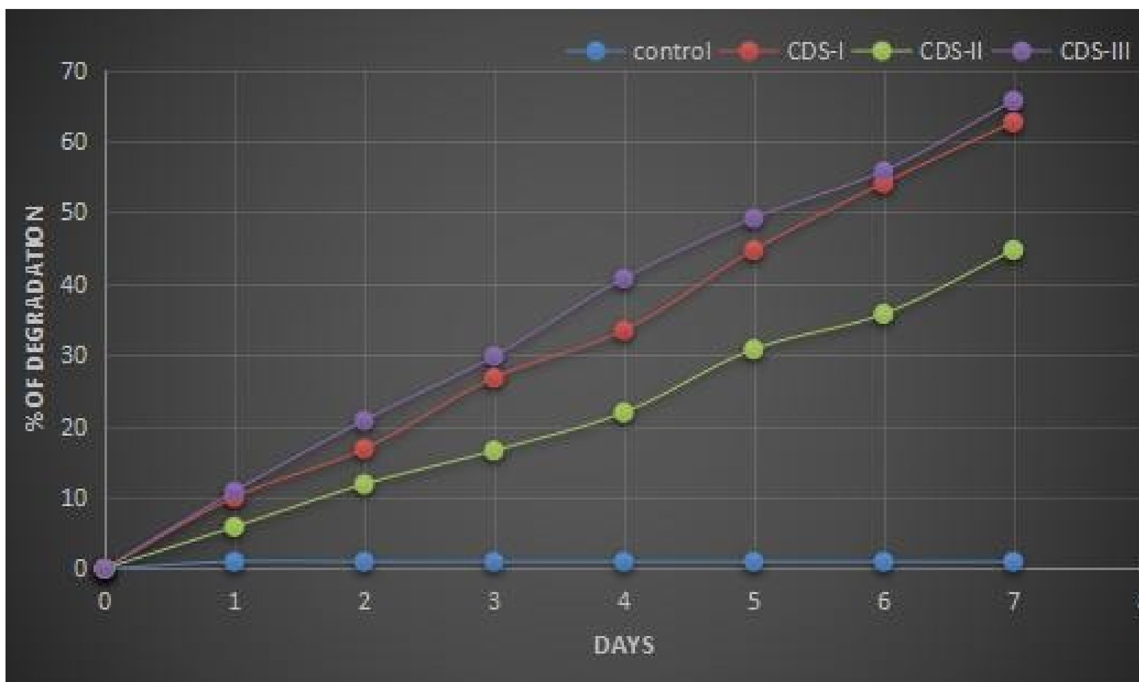

Figure 5 : Spectrophotometric analysis of chlorpyrifos degradation in unoptimized condition

Table 7: Spectrophotometric analysis of chlorpyrifos Degradation in optimized condition

Days	**ISOLATED STRAINS (% OF DEGRADATION)**		
	CDS-I	**CDS-II**	**CDS-III**
0	0	0	0
1	25	14	20
2	35.72	25	29
3	44.79	31	38
4	49.68	37	45
5	62.65	41	59
6	70.69	50	65

7	8.14	9.13	75

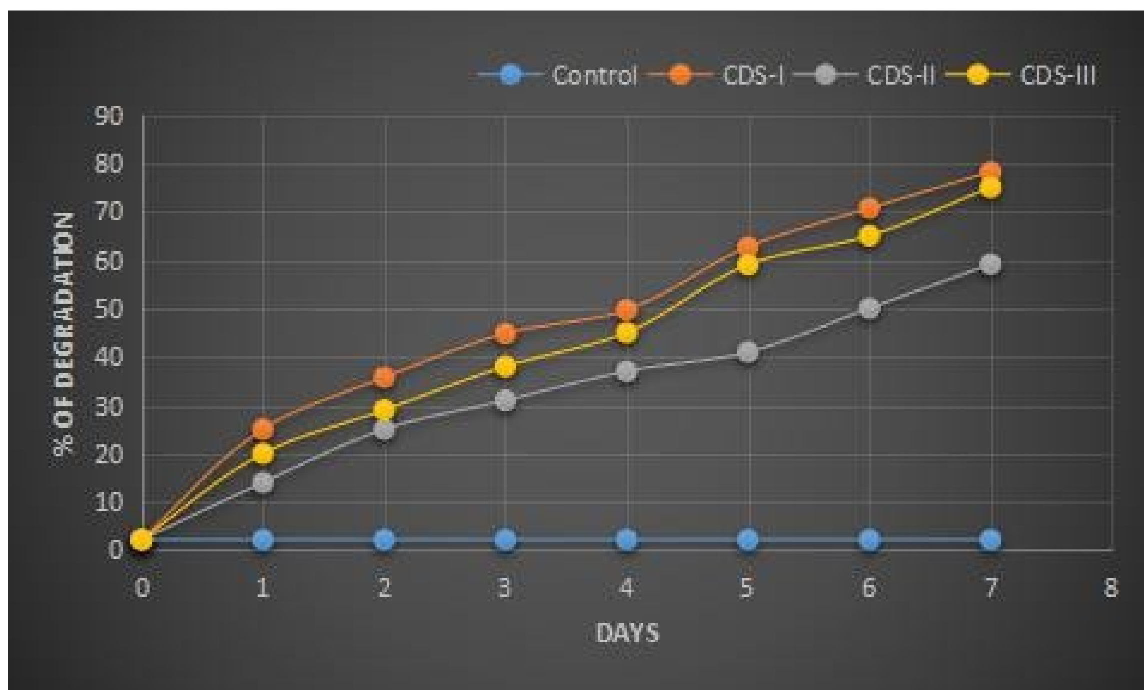

Figure 6 : Spectrophotometric analysis of pesticide degradation in optimize condition

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SUMMARY

Synthetic organic pesticides contaminate the environment, cause ill effect to animals and plants, alter the ecosystem and pose serious health problems in human being. However, there have been much interest in bioremediation technologies which use microorganisms to degrade toxic contaminant in soil into less toxic and/or non-toxic form. Microbial degradation of organophosphorous pesticides are of particular interest because of high mammalian toxicity of such compounds and their widespread and extensive use. In the present study, an attempt was made to isolate the chlorpyrifos degrading bacteria from pesticide contaminated soil in Thiruchirappalli. Three bacterial strain was isolated through spread plate method. All the isolates tolerate maximum concentration of chlorpyrifos. A simple spectrophotometric method was followed for quantitative analysis of chlorpyrifos degradation in the medium. Through the morphology and biochemical test the isolates were identified as Streptococci, Enterococci and Staphylococci. The optimization study shows that the three isolates shows better degradation in the mineral salt medium. The optimization results indicate that at pH 8 both CDS-I and CDS-II Enterococci degrade 30% and 25% whereas CDS-III shows 28% degradation of

chlorpyrifos. The suitable temperature for chlorpyrifos degradation was 35°C for CDS-I and 45°C for CDS-II and CDS-III. Dextrose is the suitable carbon source for better degradation of chlorpyrifos for CDS-I and CDS-III. In sucrose 50% of degradation was observed in CDS-II. Tyrosine is a suitable nitrogen source for CDS-I and CDS-II which shows 45% and 48% of chlorpyrifos degradation. The biodegradation of chlorpyrifos was observed in both optimize and unoptimize condition. Compared to unoptimize condition the degradation rate was increased in optimized condition. From the above observation and results it is concluded that the isolated bacterial strains were potentially degrade the chlorpyrifos. The three bacterial strains were possibly exploit to decontaminate the chlorpyrifos contaminated soil. The degradation of the isolate was determined by simple spectrophotometric method instead of HPLC. The is method is simple, reliable for quantitative analysis of chlorpyrifos degradation.

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