Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0502.12**

BIODEGRADATION OF PESTICIDES FROM THE ISOLATED MICROBIAL FLORA OF CROP FIELD CONTAMINATED SOIL

L. Krishnasamy[1], C. Shanmuga Sundaram[2*], J. Sivakumar[1]

1. PG & Research Department of Biotechnology, Hindustan College of Arts & Science, Padur, Chennai, India.

2. PG & Research Department of Microbiology, Hindustan College of Arts & Science, Padur, Chennai, India.

ABSTRACT: Biodegradation of three pesticides: Endosulfon, Carbofuron and Chlorpyrifos were studied. Five pesticide contaminated soil samples were collected from Agricultural crop field places in Thiruporur Town Panchayat. As a result of spread plate technique the microbial colonies were enumerated and three different organisms such as bacteria namely *Bacillus sp, Pseudomonas sp*, and *Azotobacter sp; Actinomycetes* namely *Streptomyces sp*; fungi namely *Aspergillus flavus* and *Penicillium citrinum* were identified. The isolated microbial organisms were identified through cultural and biochemical characterization. The isolated microbial strains were used in studying the biodegradation rate of Endosulfon, Carbofuron and Chlorpyrifos on liquid media. The isolated strains were inoculated with each of the three pesticides at a concentration of 100 ppm for 20 days. The biodegradation rate of the three pesticides on liquid media was determined using UV spectrophotometer. Also the remaining concentrations of the tested pesticides were chromatographically measured using TLC after optimization of solid phase extraction conditions. The results showed that among the bacteria *Bacillus* sp had a high efficiency to degrade Endosulfon with rate 88% and rate 76% with Carbofuron and less efficiency for Chlorpyrifos with degradation rate 40%. *Penicillium citrinum* showed moderate rate of degradation of the three pesticides; Carbofuron 53%, Endosulfon 47% and 39% for Chlorpyrifos respectively, while the *Streptomyces* sp showed the best efficiency for Chlorpyrifos with rate 87%, and moderate efficiency for Endosulfon with rate 67%, and the least for Carbofuron with rate 37%.

KEYWORDS: *Azotobacter sp, Aspergillus flavus, Bacillussp, Pseudomonas sp, Streptomycetes*, and pesticide degradation.

Corresponding Author: Dr. C. Shanmuga Sundaram* Ph.D.

PG & Research Department of Microbiology, Hindustan College of Arts & Science,

Padur, Chennai, India. Email Address: amudhashanmugam1977@gmail.com

1.INTRODUCTION

Present agriculture is readily related through the utilization of diverse chemical contribution. Alternative classes of pesticides are used in managing dissimilar grouping of pests to make the most of crop production and congregate the demands for higher provisions of food of the fast-growing human population. An idyllic pesticide has to be poisonous only to the target organism, recyclable and should not percolate into ground water. Unfortunately, this is hardly ever the case and the extensive use of pesticides in recent agriculture is of concern [1]. Due to the incessant use of pesticides in agriculture, considerable amount of herbicides and their tainted products may build up in the ecosystem leading to serious trouble to man and the surroundings. Consequently, it is necessary to learn the residue and deprivation pattern of herbicides in crops, soils and water scientifically in order to create significant information from the point of view of plant fortification, public health and ecological protection. The dilapidation of herbicides in soil and their cause on microbes should be studied so that their use can be appropriately synchronized [2]. Quan [3] noticed that a bacterial isolate accomplished of quickly debasing di-2-ethylhexyl phthalate (DEHP) was secluded from soil and known as *Bacillus subtilis*. The organism also make use of diethyl phthalate, dibutyl phthalate, dipropyl phthalate, dipentylphthalate and phthalic acid and their biodegradation proportion was more than 99%, when the incubation was carried out for 5 days at 30Â°C. The microorganism tainted dibutyl phthalate and di-2-ethylhexyl phthalate in the course of the transitional configuration of mono-2ethylhexyl phthalate and monobutylphthalate, which were then metabolized to phthalic acid and additional by a protocatechuate pathway, as evidenced by oxygen uptake studies and GCMS investigation. The refinement of soil spoiled with di2ethylhexyl phthalate by *B. Subtilis* was investigated. Investigational results showed that the damage could mortify about 80% of 5 mM DEHP just by adding 8% culture medium to soil, representing that the deprivation can happen still when other organisms are present. The contamination of the surroundings by means of anthropogenic crude composite has become such an obvious concern that it wants no additional prologue. Microbes take part in a key position in the breakdown and mineralization of these contaminants [4]. Chemical herbicides are additional perhaps the largely significant constituent of weed management scheme for most of the major crops. The eventual purpose of herbicidal chemicals is the soil where they get nearer in drop a line to with diverse microflora which is accountable for different biochemical alterations connected to mineral nourishment to vegetations. Diverse information predicted that herbicidal application has unfavourable causes on bacterial, fungal [5] and actinomycetes inhabitants [6]. The common pesticides used in the tea cultivation are endosulfan, dicofol, fenazaquin, glyphosate, 2, 4-D, paraquat dichloride, etc. These pesticides are belonging to cyclodiene family. They are highly toxic and an endocrine disruptor. These chemicals control a broad variety of sucking and chewing insect pests. Its remains have been noticed in the environment, soils, sediments, surface water and foods. The recommended dose is 1:400 (HV). Microbes take part in a vital role in the mineral cycles on earth.

They are concerned in the biodegradation of a lot of compounds; these processes take place not only in the soil atmosphere, but also in symbiosis through other organisms (eg. lichens, intestinal and rumen bacteria) [7]. Soil feature does not depend just on the physical, physico-chemical and chemical belongings of soil but strongly concurrent to the soil microbiological characters [8]. Microbes are crucial for soil richness and for the deprivation of organic substance and contaminants in soils. Microbial biomass in soil is measured as significant feature of soil quality [9]. It serves as a measure of possible natural activity and its energetic transforms would assist in considerate the procedures concerned in nutrient cycling and ecosystem functioning [10]. Since the concerns about the environment, the side effects of pesticides on soil microorganisms were studied expansively [11, 12, 13, 14]. Spectrophotometric determinations engross the response of paraquat with 1% aqueous sodium dithionite in 0.1N NaOH. The paraquat concentration was determined at 620 nm as a resultant of blue cation complex. For residue level determinations the optimum absorption at 396 nm for the paraquat radical are more commonly used. [15, 16]. The objectives of this study were to evaluate the ability of microbial consortium to pesticide degradations under the variation of media compositions, incubation temperature and initial pH.

2. MATERIALS AND METHODS

Sample Collection

The current research was performed in Thiruporur one of the suburban area, located on the OMR road, Kanchipuram District, Tamil Nadu 43 Km away from Chennai city. In order to find out the biodegradation of pesticide, five soil samples associated with pesticide were collected at a depth of 1-5 cm. Contaminated soil samples were collected from different locations such as Thandalam, Kannagapattu, Kalavakkam, Madaiyathur and Sembakkam. Samples were collected in screw caped sterile plastic container then it was taken to the laboratory. Pesticides such as Endosulfon, Carbofuron and Chlorpyrifos were procured from the local Pesticide Store, Kelambakkam with the authenticated approval letter.

Identification and characterization of pesticide contaminated soil microbes

The collected sample was analyzed for isolation of microbes. 1 gram of pesticide oil contaminated soil sample was taken in a clean conical flask with 10ml of sterile distilled water. The mixture was shaken and serially diluted and from 10^{-1} to 10^{-7} range [17].The aliquot (0.1ml) of the dilution was poured on (MSM) mineral salt medium by spread plate method. Potato Dextrose Agar for fungi and Actinomycetes agar for Actinomycetes was prepared and screening was made by spread plate technique. For the entire sample, three replica plates were preserved and kept for the incubation at 37ºC for bacteria (24hrs); actinomycetes (2-5 days) and for fungi at room temperature (3-4 days). After the incubation the growth of microorganisms were seen on the culture plate [18]. The isolated colonies were sub cultured in agar slants and conserved under preservation temperature. Bergey's Manual of Determinative Bacteriology was referred to identify the bacteria based on the macroscopic and

microscopic examination [19]. The fungus was identified bylacto phenol cotton blue staining methodwith the key characters [20]. Guidelines were followed to determine the features of actinomycetes[21].

Biodegradation activity

The growth of pesticide degrading isolates (*Bacillus sp, Pseudomonas sp*, and *Azotobacter sp*; *Streptomyces sp*; *Aspergillus flavus* and *Penicillium citrinum*) was determined by using Minimal Salt Broth. For this, 1 ml of the bacterial inoculum was inoculated into 50 ml of Mineral salt broth containing 1ml of pesticide. The flasks were then incubated at 27°C for 20 days in a microbial shaker at 150 rpm. Five ml of culture was drawn and centrifuged at 5000rpm for 10 minutes. The pellet was discarded and the supernatant was collected to evaluate the growth of pesticide degrading microbes. The growth of the pesticide degrading microbes was assessed by using UV Spectrophotometer at 640nmafter 0, 4, 8, 12, 16 and 20 days of treatment with UV spectrophotometer in the culture medium for every four days.

Thin Layer Chromatography

The silica gel was applied onto the plate uniformly and then allowed to dry and stabilized. Activated TLC plates were kept in hot air oven at 105^0 C for 30 mins. Samples were applied onto the TLC plate and air dried. The mobile phase was poured into the TLC chamber to a level few centimeters above the chamber bottom. Then the plate was prepared with sample spotting was placed in TLC chamber such that the side of the plate with sample line was towards the mobile phase. Then the chamber was closed with a lid. The TLC plates were allowed for sufficient time for the development of spots. Then the plates were removed and allowed to dry. The sample spots were visualized in suitable UV light chamber or using developing reagent.

Isolation of Genomic DNA from the isolated strains

1.5 ml of isolated microbial culture was transferred to a micro centrifuge tube and spin 2 min. supernatant was removed. The pellet was resuspended in 467 µl TE buffer by repeated pipetting. 30 µl of 10% SDS and 3 µl of 20 mg/ml were added to proteinase K, mixed, and incubated for 1 hr at 37°C. An equal volume of phenol/chloroform was added and mixed well by inverting the tube until the phases are completely mixed. The tubes were spun for 2 min. The upper aqueous phase was transferred to a new tube and an equal volume of phenol/chloroform was added. Once again the mixture was well and spun for 2 min. The upper aqueous phase was transferred to a new tube. 1/10 volume of sodium acetate was added along with 0.6 volumes of isopropanol and mixed gently until the DNA precipitation was formed. The DNA was washed by adding 1 ml of 70% ethanol for 30 sec. DNA was resuspended in 100-200 µl of TE buffer. The isolated sample was electrophoresised in 1% agarose gel and the bands were observed under UV transilluminator.

3. RESULTS AND DISCUSSION

The isolated microbial colonies were observed in the culture plate. Highest bacterial load was observed in Thandalam 258 X 10^{-7}CFU/ml and Sembakkam 235 X 10^{-7}CFU/ml. White coloured; margined and elevated colonies were observed in all the locations. In the Gram's staining one of the purified strains was positive and the other produced negative results. Endospore staining was performed for *Bacillus sp*. Based on the Bergey's manual's reference, the screened organisms were confirmed as *Bacillus sp. Pseudomonas sp*and *Azatobacter sp* (Figure. No.1, 2 and Table No. 1). Maximum number of *Actinomycetes* colonies 12x10^{-5} CFU/ml was observed in Sembakkam and only two colony 2x10^{-5} CFU/ml was shown in Thandalam and Kalavakkam. No *Actinomycetes* were seen in Kannagapattu and Madaiyathur (Figure. No.1 and Table No.1). Two fungal colonies were observed 2 X 10^{-4}CFU/ml in Thandalam, Kannagapattu, and Madaiyathur. Whereas no fungal colony was formed in Kalavakkam and Sembakkam (Figure.No.1 and Table No.1).

Table No 1: Pesticide contaminated soil microbial count in CFU/ml.

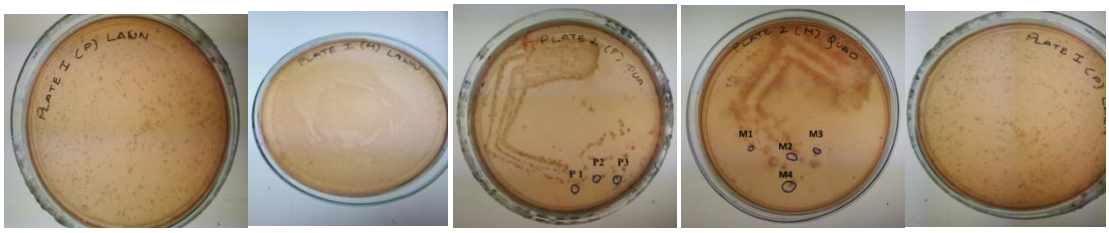
S.No	Sample Collection Site	Bacteria	Fungi	Actinomycetes
1	Thandalam	258X10^{-7}	2X10^{-4}	2X10^{-5}
2	Kannagapattu	126 X10^{-7}	2X10^{-4}	Absent
3	Kalavakkam	91 X10^{-6}	Absent	2 X 10^{-5}
4	Madaiyathur	108 X10^{-6}	2 X 10^{-4}	Absent
5	Sembakkam	235 X10^{-7}	Absent	12 X 10^{-5}

Table No 2: Characterization of the isolated bacterial strain from the pesticide contaminated soil

S. No	Colony Morphology & Preliminary Tests	*Pseudomonas sp*	*Bacillus sp*	*Azatobacter sp*	*Streptomyces sp*
1	Colony colour	White	Pale-white	Creamish-White	White-dull
2	Margin	Entire	Circular	Entire	Wavy
3	Elevation	Convex	Flat	Raised	Umbonate
4	Opaque / Translucent	Opaque / Translucent	Opaque	Translucent	Opaque
5	Shape	Circular	Round	Irregular	Irregular
6	Size	Medium	Medium	Large	Large

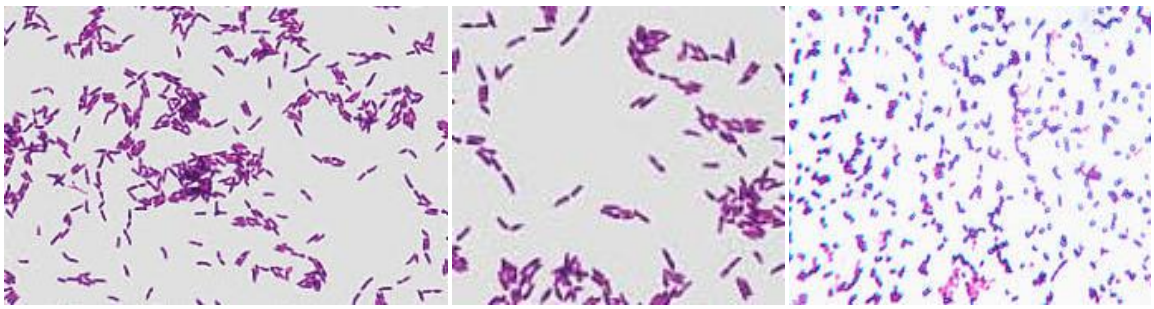
Table 3.Biochemical test for *Pseudomonas sp*, *Bacillus sp* and *Azatobacter sp*

S. No	Test	*Pseudomonas sp*	*Bacillus sp*	*Azatobacter sp*	*Streptomyces sp*
1	Mineral salt medium	Light yellow colonies	Creamy colour colonies	Creamish White	Brown
2	Gram staining	Gram negative rod	Gram positive rods	Gram positive rods	Gram positive rods
3	Motility	Motile	Motile	Motile	Non-Motile
4	Endospore staining	-	+	-	-
5	Catalase	+	-	+	+
6	Oxidase	+	-	+	+
7	Indole	-	-	+	-
8	Methyl red	-	-	+	-
9	VogesProskauer	-	-	+	-
10	Citrate	+	+	+	-
11	Urease	+	-	+	+
12	TSI	-	-	+	-



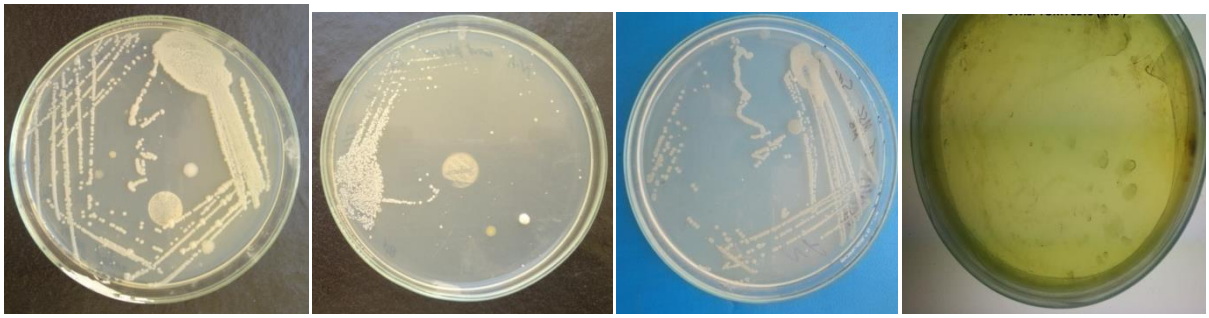
A.Thandalam B. Kannagapattu C. Kalavakkam D. Madaiyathur E. Sembakkam

Fig No: 1 Colony morphologyof microbial strains from different locations



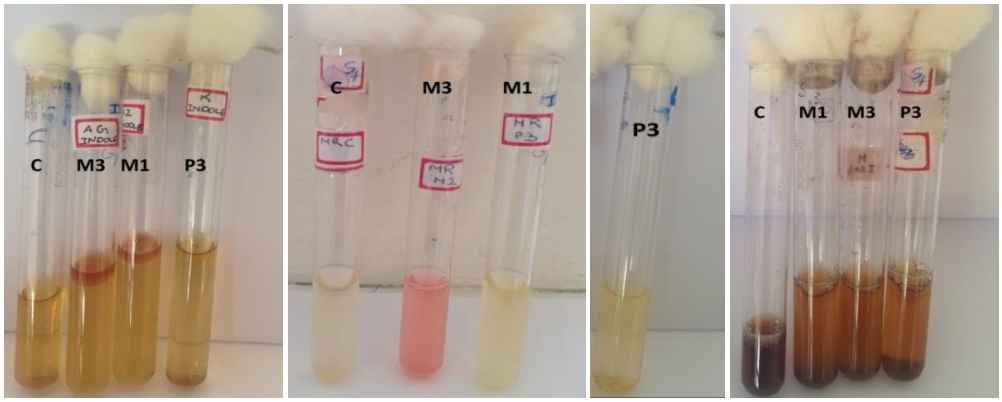
A.*Pseudomonas sp* B.*Bacillus sp* C.*Azatobacter sp*

Fig No: 2 Photomicrograph of isolated bacterial strains under 100X magnification

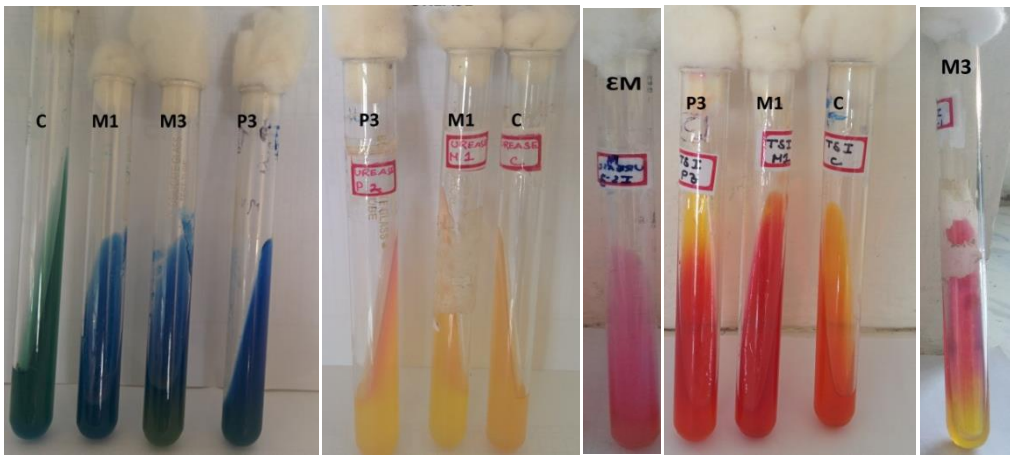


A.*Pseudomonas sp* **B.*Bacillus sp*** **C.*Azatobacter sp*** **D. *Streptomyces sp***

Fig No: 3 Colony morphology of identified microbial organisms from different locations



Indole **Methyl Red** **VogesProskauer**



Citrate **Urease** **TSI**

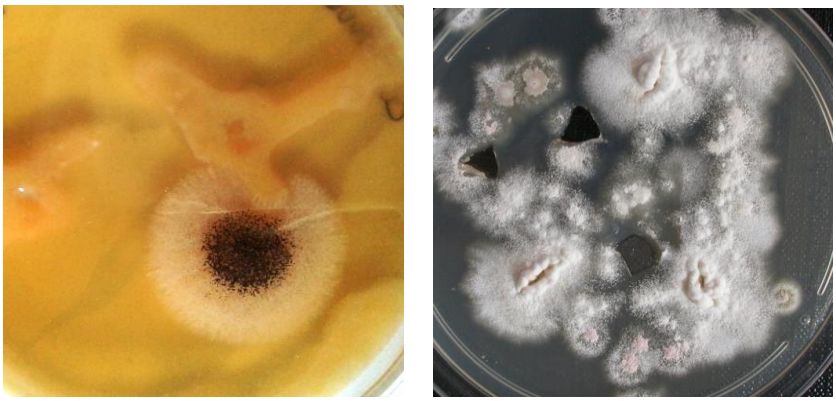
Fig No:4 Biochemical characterization of isolated bacterial strains

The sample collected from the Thandalam showed the colony morphology on the plates were pale-white, circular, flat, opaque, round, medium and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large. In the case of Kannagapattu white, entire, convex, opaque or translucent, circular, medium and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large colonies were observed. Whereas in Kalavakkam the produced colonies were seen like creamish-white, entire, raised, translucent, irregular, large and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large. The sample collected from the Madaiyathur showed the colony morphology on the plate was observed as white, circular, raised, opaque, round, large and the white-dull, entire, pulvinate or umbonate,

opaque, irregular, large. Whereas in Sembakkam showed white-dull, wavy, umbonate, opaque, irregular, large, motile and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large colonies (Figure. No. 2 and Table No. 2) Biochemical tests also showed some interesting facts (Figure. No. 3 and Table No. 3).

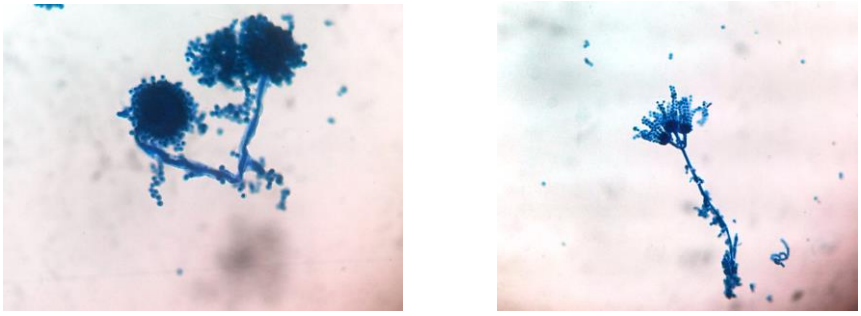
Table No 4: Macro and Microscopic features of isolated fungal strains from the pesticide contaminated soil

S.No	Colony Morphology &LPCB Staining	*Aspergillus sp*	*Pencillium sp*
1	Colony colour	Dark green, Brown	White
2	Size	300-600 µm	100-200 µm
3	Surface	Filamentous, elevated	Umbonate, elevated
4	Vesicle Serration	Biseriate	Branched sterigmata
5	Shape	Globose, ellipsoid	Granule, chain
6	Medulla Covering	Entirely	Partially
7	Conidia Surface	Smooth, finely roughened	Branched with conidiophores



A. *Aspergillus flavus* B.*Pencillium citrinum*

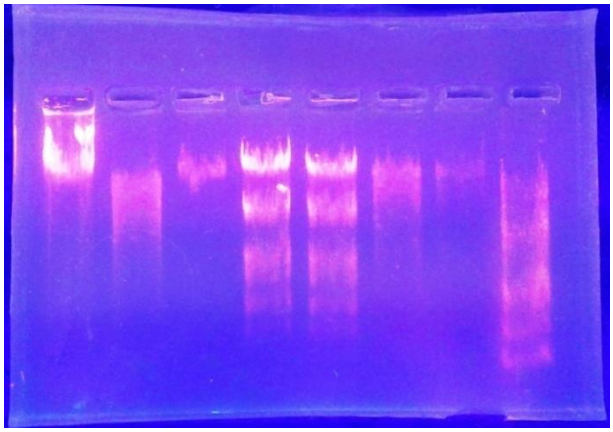
Fig No: 5 Colony morphology isolated fungal strains from the pesticide contaminated soil



A. *Aspergillus flavus* B. *Pencillium citrinum*

Fig No: 6 Photomicrograph of isolated fungal strains under 40X magnification

In the case of first fungal plate two different dark green and black spongy colonies were observed. Whereas in the second plate white spongy colonies were observed. Lacto phenol cotton blue staining showed some interesting results. The first plate showed stalk with cluster of sterigmata. In culture plate 2 three branched; brush shaped; conidiophores were observed (Figure No.5, 6 and Table No. 4).



Lane 1 – 500bp ladder

Lane 2 –*Pseudomonas sp*

Lane 3 & 4 – *Bacillus sp*

Lane 5 –*Azatobacter sp*

Lane 6 –*Streptomyces sp*

Lane 7 –*Aspergillus flavus*

Lane 8 –*Pencillium citrinum*

Fig No: 7 Isolation of Genomic DNA from the identified micro organisms

Further the isolation of genomic DNA for the identified organisms showed some interesting results. The first lane filled with 500bp ladder followed by *Pseudomonas sp*, third and fourth lane *Bacillus sp*; *Azatobacter sp*, *Streptomyces sp*, *Aspergillus flavus*, and *Pencillium citrinum* respectively. The DNA bands were observed in the agarose gel plates under the UV transilluminator. Among these *Bacillus sp*, *Azatobacter sp*, and *Pencillium citrinum* showed better results (Figure No. 7).

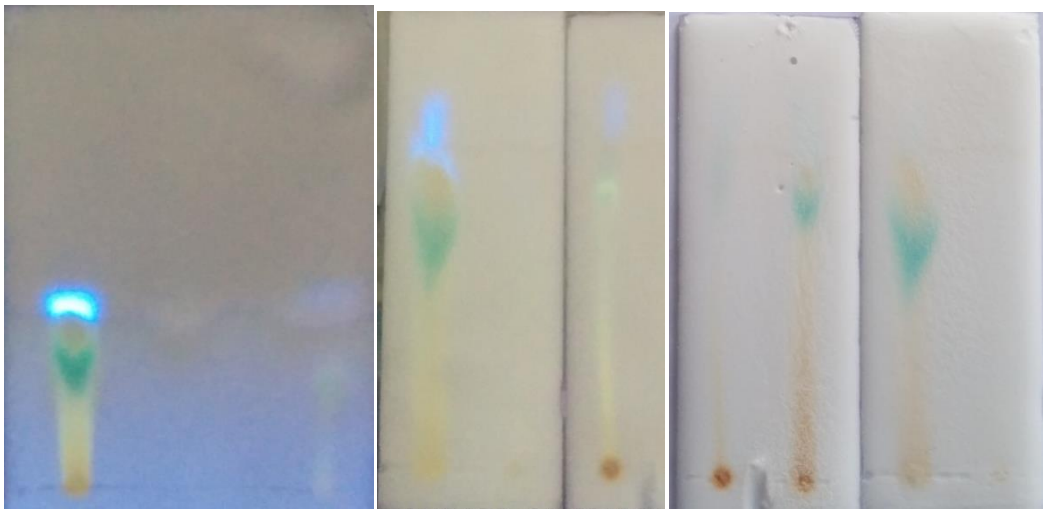


Fig No: 8 Thin Layer Chromatography showing the compound of degraded pesticides

As for as the Thin Layer Chromatography is concerned the bioactive compounds were identified based on the presence of pale bluish green colour spots on the silica gel plates. The TLC plates were observed under the UV transilluminator. The distance travelled from the beginning spot to the end were calculated. Rf value has been found to confirm the presence of degradable compounds from the degraded pesticide sample (Figure No. 8). The degradation of three different pesticides such as Endosulfon, Carbofuron and Chlorpyrifosis concerned the following results has been observed. Among the bacteria *Bacillus sp* had a high efficiency to degrade Endosulfon with rate 88% and rate 76% with Carbofuron and less efficiency for Chlorpyrifos with degradation rate 40%. *Penicillium citrinum* showed moderate rate of degradation of the three pesticides; Carbofuron 53%, Endosulfon 47% and 39% for Chlorpyrifos respectively, while the *Streptomyces sp* showed the best efficiency for Chlorpyrifos with rate 87%, and moderate efficiency for Endosulfon with rate 67%, and the least for Carbofuron with rate 37%. The degradation potential of other identified organisms against the pesticides were also noticed (Table No. 5). The composition and population of microbes in the rhizosphere microflora at the tea gardens located in Red-Yellow earth region of south-Anhui showed that there were various microbial groups in rhizosphere habitat of tea plant, and some of them which increased the soil fertility significantly, for example, Azotobacter, ammonifying bacteria, cellulose decomposing bacterium etc. [22]. The current results are coincided with Prabakaran [23] who studied on the deprivation of Endosulfan by a *Bacillus sp*. Yet another research work also matched our results; biodegradation of endosulfan into endosulfan sulfate with a top soil bacterium, *Bacillus sp* [24]. The bacterium tainted 60% of the composite within 4 days of incubation. A mixture of bacterial culture like *Staphylococcus sp*, *Bacillus circulans* was observed for deprivation of endosulfan in aerobic and facultative anaerobic circumstances through batch experiments among an initial endosulfan concentration of 60 mg/L. After 3 weeks of incubation, mixture of bacterial culture was capable to humiliate the endosulfan in aerobic and facultative anaerobic circumstances, correspondingly [25].

Table No. 5 Potential of biodegradation by isolated microbial strains on Pesticides

S. No	Degradation of Pesticides	Endosulfon				Carbofuron				Chlorpyrifos			
	Name of the isolated Organisms	Initial	Final	Difference	Degradation (in %)	Initial	Final	Difference	Degradation (in %)	Initial	Final	Difference	Degradation (in %)
1	*Pseudomonas sp*	100	42	58	58%	100	51	49	49%	100	56	44	44%
2	*Bacillus sp*	100	12	88	88%	100	24	76	76%	100	60	40	40%
3	*Azatobacter sp*	100	74	26	26%	100	60	40	40%	100	72	28	28%
4	*Streptomyces sp*	100	33	67	67%	100	63	37	37%	100	13	87	87%
5	*Aspergillus flavus*	100	68	32	32%	100	58	42	42%	100	59	41	41%
6	*Pencillium citrinum*	100	53	47	47%	100	47	53	53%	100	61	39	39%

Mathava [26] studied endosulfan mineralization by bacterial isolates and identified their possible degradation pathway. It was postulated that endosulfan was mineralized via hydrolysis pathway with the formation of carbenium ions and/or ethylcarboxylates, which later converted into simple hydrocarbons. Inoculation of *Pseudomonas fluorescence* and *P. aeruginosa* tainted 75 and 84% of chlorpyrifos in plots exclusive of cotton plants while 98% deprivation of chlorpyrifos was noticed in soil, where cotton plants were loaded with moreover *P. fluorescence* or *P. aeruginosa* as contrasted to un-loaded control soil [27]. Multiclass pesticide remains viz. endosulfan, monocrotophos, chlorpyriphos and cypermethrin have been approximated qualitatively and quantitatively in two vegetables, tomato (*Lycopersicom esculentum*) and radish (*Raphanus sativus*) by using high performance liquid chromatographic and gas liquid chromatographic methods [28]. In irrigates Endosulfon is dissolved on to elements and residues, with a half-life in European circumstances approximated to exist between 2 and 280 years depending on sunlight and intensity of water. It has been observed in surface waters, drinking water, and in groundwater. Carbofuron is highly acutely toxic and enters the body mainly by swallowing, or through damaged skin, but may also be inhaled. Common exposure symptoms include burns to the mouth, acute respiratory distress, loss of appetite, abdominal pain, thirst, nausea, vomiting, diarrhoea, giddiness, headache, fever, muscle pain, lethargy, shortness of breath and rapid heartbeat. There can be nosebleeds, skin fissures, peeling, burns and blistering, eye injuries, and nail damage including discolouration and temporary nail loss. Chlorpyrifos is described by US Environmental Protection Agency as “extremely biologically active and toxic to plants and animals”; and by the Environmental Risk Management Authority of New Zealand as “very ecotoxic to the aquatic environment”. It has caused teratogenic malformations in fish and amphibian,

disrupted hormones in frogs, and is genotoxic in tadpoles. Sunitha[29]reported about degradation of endosulfan upto 70% and endosulfan sulphate upto 100% by organisms isolated from endosulfan contaminated soils of South Indian States (Kerala and Karnataka) by the process of enrichment. Rainer Martens [30] isolated 16 fungi, 15 bacteria and 3 *actinomycetes* capable of metabolizing more than 30% of endosulfan. The major metabolites detected were endosulfate, formed by oxidation of the sulfite group, and endodiol, formed by hydrolysis of the ester bond. It is observed that almost 42 pesticidal mixes were tainted by a broad diversity of microbes. Mustafa [31] stated that *Rhizobium leguminosarum* and R. trifolii secluded from Egyptian soil be able to hydrolyse melathion by forming carboxy esterase. Comprehensive studies showed that 21 rhizobial isolates bear endosulfan, carboryl, melathion and carbofuran at the range of 30 to 115 ug/ml. isolated microbes from the nodules of *Indigofera echinata* and *I. duthei* tolerated melathion upto 125ug/ml. [32, 33]. Kothari [34] studied on the biodegradation of 2, 4-D by *Penicillium citrinum* and *P. oxalicum* isolated from paint coated teak wood.

4. CONCLUSION

In the present study, the microbes were inoculated into the pesticides in the concentration of 100 ppm. The gradually decreasing spectrophotometric readings of the tested samples clearly indicate that the Endosulfon, Carbofuron and Chlorpyrifos are getting degraded by the isolated micro organisms. From the spectrophotometric readings it can be concluded that the *Bacillus sp* degrades the pesticides faster than the *Pseudomonas sp*, *Azatobacter sp*, *Streptomyces sp, Aspergillus flavus* and *Penicillium citrinum.* Further the fungal isolate *Penicillium citrinum* also had the high potential to degrade the pesticides. The study can be carried out to find the combined effect of organic manure and pesticide degrading crop beneficial microorganisms for early degradation of pesticides and enhancement of crop productivity for the sustainable agriculture in future.

CONFLICT OF INTEREST

Authors have no any conflict of interest.

REFERENCES

1. Johansen A, Olsson S. Using Phospholipid Fatty Acid Technique to Study Short-Term Effects of the Biological Control Agent *Pseudomonas fluorescens* DR54 on the Microbial Microbiota in Barley Rhizosphere, Micro Ecol. 2005; 49: 272-281.
2. Lynch JM. Microorganisms and enzymes in the soil. In: Soil biotechnology, Microbiological Factors in Crop Productivity, Blackwell Sci. Publ., London. 1983.
3. Quan CS, Liu Q, Tian WJ, Kikuchi J. Biodegradation of an endocrine disrupting chemical, di-2-ethylhexyl phthalate, by *Bacillus subtilis*. App Microbiol & Biotech. 2005; 66: [6] 702-709.
4. Alexander M. Biodegradation of chemicals of environment concern. Sci. 1981; 211: 132-38.
5. Shukla AK. Effect of herbicides butachlor, fluchloralin, 2, 4-D and oxyfluorfen on microbial population and enzyme activities of rice field soil. Ind J of Ecol. 1997; 24: 189-192.

6. Rajendran K, Lourdaraj AC. Residual effect of herbicides in Rice ecosystem a review. Agri, Rev. 1999; 20: 48-52.
7. Pratibha H, Sharmab GD. Online Int Interdis Res J. 2014; 4: 203-210.
8. Elliot LF, Lynch JM, Papendick RI. The Microbial Component of Soil Quality. In: Soil Biochemistry, Stotzky, G. and J.M. Bollag (Eds.). Marcel Dekker, Inc., New York, USA. 1996; 1-21.
9. Doran JW, Parkin TB. Defining and Assessing Soil Quality. In: Defining Soil Quality for a Sustainable Environment, Doran JW, DC. Coleman, DF. Bezdicek and B.A. Stewart (Eds.). Soil Science Society of America, Madison, WI, USA. 1994; 3-21.
10. Rath AK, Ramakrishnan B, Rath AK, Kumaraswamy S, Sethunathan N, Effect of pesticides on microbial biomass of flooded soil. Chemosphere. 1998; 37: 661-671.
11. Greaves MP, Davies HA, Marsh JA, Wing-Field GI. Herbicides and soil microorganisms. Crit. Rev. Microbiol. 1976; 5: 1-38.
12. Anderson JR, Drew EA. Growth characteristics of a species of Lipomyces and its degradation of paraquat. J of Gen Microbio. 1972; 70 [1]: 43-58.
13. Greaves MP. Effect of Pesticides on Soil Microorganisms. In: Experimental Microbial Ecology, Burns, R.G. and J.H. Slater (Eds.). Blackwell, Oxford. 1982; 613-630.
14. Gerbar HR, Anderson JP, Bugel-Mongensen B, Castle D, Domsch KH. Revision of recommended laboratory tests for assessing side effects of pesticides on soil microflora. Proceedings of the 4th International Workshop, Leverkusen. 1989.
15. Haney RL, Senseman SA, Hons FM. Effect of roundup ultra on microbial activity and biomass from selected soils. J. Environ. Qual. 2002; 31: 730-735.
16. Sparling GP. The Soil Biomass. In: Soil Organic Matter and Biological Activity, Vaughan, D. and R.E. Malcolm (Eds.). Martinus Nijoff Dr. W. Junk, Boston, Lanchester. 1985; 223-239.
17. Cappuccino JG, Sherman N. Microbiology - a Laboratory Manual, The Benjamin/Cummings Pub. Co. Inc. NewYork, USA. 1996; 137–49.
18. Gauri S, Ashok KS, Kalpana B. Biodegradation of Polyethenes by Bacteria Isolated From Soil, Inter J of Res and Dev in Phar and Life Sci. 2016; 5; [2] 2056-2062.
19. Holt JG, Krieg NR, Sneathm PH, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore, MD: Williams and Williams. 1994.
20. Raper KB, Fennell DI. The genus *Aspergillus*. Krieger RE (ed.) Huntington, New York. 1987; 686-695.
21. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* sp. Int J of Sys and Evol Microbio. 1966; 16: 313-340.
22. Zhenrui H, Yifu W, Yuezhen F, Jinpu D, Ningshu Li. Studies On Microflora of Rhizosphere Soils in Tea Garden. J of Tea Sci. 1985.

23. Prabakaran K, Allen P. Biodegradation of endosulfan by a novel gram positive soil bacterium. J of Ecobiol. 2005; 19: 235-238.
24. Shivaramaiah HM. Kennedy IR. Biodegradation of endosulfan by a soil bacterium. J of Env Sci and Health. 2006; 41:895-905.
25. Mathav K, Phylip L. Bioremediation of endosulfan contaminated soil and water—Optimization of operating conditions in laboratory scale reactors. J of Haz Mat. 2006; 136: 354–364.
26. Mathav K, Phylip L. Endosulfan mineralization by bacterial isolates and possible degradation pathway identification. Biorem J. 2006; 10: [4] 179– 190.
27. Vidya Lakshmi. *In situ* bioremediation of Chlorpyrifos in cotton fields: possible role of plant-microbe interaction. J of Pure and App Microbio. 2009; 3: [2]: 543-550.
28. Kumar D, Sharma RC. Chauhan P. Estimation of Multiclass Pesticide Residues in Tomato (*Lycopersicon esculentum*) and Radish (*Raphanus sativus*) Vegetables by Chromatographic Methods. Res J of Agri Sci. 2011; 2: [1] 40-43.
29. Sunitha S, Krishna Murthy V, Mahmood R. Degradation of Endosulfan by Mixed Bacterial Cultures Enriched from Endosulfan Contaminated Soils of Southern India. Int J of Biosci, Biochem and Bioinfo. 2012; 2: [1] 31-35.
30. Rainer M. Degradation of [8, 9-14C] Endosulfan by Soil Microorganisms. Appl. Environ. Microbiol. 1976; 31: [6] 853-858.
31. Mustafa IY, Fakhr IM, Bahig ME. Metabolism of organophophorus insecticides. XIII Degradation of melathion by Rhizobium sp. Arch. Environ. Microbiol. 1972; 86: 221.
32. Gangawane LV, Francis RP. Rhizobium from wild legumes and Pesticide degradation: the two in one. In: Microbial Biotechnology. Reddy *et al*., (eds). Scientific Publishers, Jodhpur, India. 1997; 59-63.
33. Reddy *et al.* Microbial Biotechnology. Scientific Publishers, Jodhpur, India. 1997; 266-250.
34. Kothari IL, Choksi PC, Patel HB, Udhaya J. Biodegradation of 2, 4-D by *Penicillium*. Rec Adv in the Ecobio Res. 1998; 85-86.