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ANALYSIS OF ENDRIN DEGRADATION BY *Kocuria* sp. USING GC AND FTIR

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ABSTRACT

Pesticides are chemical substances that are meant to kill pests. The use of pesticides is so common that the term pesticide is often treated as synonymous with plant protection products. It is commonly used to eliminate or control a variety of agricultural pests that can damage crops and livestock and reduce farm productivity. Organochlorine (OC) pesticides, a group of chlorinated hydrocarbon derivatives are the class of synthetic pesticides used extensively throughout. OCs are extensively used in the chemical industry and in agriculture. OCs are highly toxic due to their persistence and severe ill effects on the ecosystem. These compounds show very slow degradation and bioaccumulation but are known for their high toxicity in the environment. In spite of a ban on OCs in many developed and developing countries, the use of these agents is still being continued. It was proved that the *Kocuria* species was capable of degrading OC pesticide, endrin. The present study was aimed at analyzing its ability in degrading endrin using Gas Chromatography (GC) and Fourier Transform Infrared (FTIR) techniques.

Keywords: Endrin, Fourier transform infrared, Gas chromatography, *Kocuria* sp.

INTRODUCTION

Pesticides are meant for destroying and killing pest species. These include insecticides, herbicides, fungicides, or other pest control compounds. These are highly toxic and show adverse health impact on non-target organisms. The impact of synthetic pesticides is uncertain; contaminating the land surface that is far off

from the site where they were originally utilized wherein these organic chemicals enter down in water bodies at detectable level **[1]**. Organochlorines (OC) are persistent organic pollutants (POPs) with high persistence in the environment widely used as pesticides. OCs is banned in most of the developed countries which were successfully used in the control of malaria and typhus **[2]**. Eventhough the consumption of OC in India is still very low, about 0.5 kg/ha of pesticides compared to 6.60 and 12.0 kg/ha in Korea and Japan. Non-judicious use of pesticides results in rapid contamination of food commodities with pesticide residues. In India, 51% of foods commodities are contaminated with pesticide residues and out of these, 20% have pesticides residues above the maximum residue level values on a worldwide basis. Issues on human health such as suppression of the immune system, irregularities in hormone regulation, diminished intelligence, reproductive abnormalities, and cancer may be induced upon that their long-term, low-dose exposure **[3, 4]**. Due to their low cost and the need against various pests, most widely used OC pesticides in developing countries of Asia are DDT, hexachlorocyclohexane (HCH), aldrin and dieldrin are among the **[3-5]**. It has been shown that OCs had any effects on germination or growth rate of plants but high doses of OCs had a slight toxic effect on the resting cell and caused a delay in the early stages of prophase and a shortening of chromosomes in metaphase and a slight delay in metaphase **[6]**. Endrin is a highly toxic organochlorine pesticide which does not accumulate in the tissues of man or animals, and which is not persistent in the environment **[7]**. Endrin is a solid, white, almost odorless substance that was used as a pesticide to control insects, rodents, and birds **[8]**. In light of its importance in agriculture and a need to degrade it in the environment, the present study has been taken up to analyze the ability of *Kocuria* species to degrade OC *in vitro* using GC and FTIR techniques.

MATERIALS AND METHODS

Endrin degrading *Kocuria* sp.

Soil samples were collected from agricultural fields where commercial crops like cotton was extensively grown in the Guntur District of Andhra Pradesh, India lies in the Latitude 16.3 North and Longitude 80.4 East and endrin (United Chemicals, Gujarat, India) pesticide was used intensively, bycontemplating such soil would contain pesticide contamination and natural micro-flora experiencing pesticide stress. The samples were brought together and collected into a sterile polythene bag and enriched the soil by adding 1ml endrin and subjected for serial dilution. These samples were subjected to microbial, biochemical and molecular level screening

closely resembled *Micrococcus* by phylogenetic analysis was identified as *Kocuria* sp. This culture was procured from Acharya Nagarjuna University **[9]**.

Analysis of the culture filtrates for endrin degradation by GasChromatography

Sample preparation

The *Kocuria* sp. containing 10^5 cells per inoculum incubated for 48 h in the presence of 3.84 g/l Endrin, where the degradation activity was maximum were filtered through pre-weighed Whatmann No.1 filter paper to separate the biomass. The supernatant was collected and was subjected for degradation studies by Gas Liquid Chromatography. The flasks without inoculants served as control **[10]**.

Extraction of pesticide residues

The culture filtrate was taken in a bottle and was added with 100ml of acetone and n-hexane in the ratio1:1. The culture filtrate and the solvents were mixed thoroughly and filtered through a filter paper with the help of a suction pump. The filtrate was allowed for evaporation and then the culture extract was concentrated by adding 50ml of 15% Sodium Chloride solution and 50ml of Di-Chloro Methane (DCM), shaken thoroughly and was allowed to settle for 15-20 minutes. The DCM layer was collected into a bottle through a funnel arranged with cotton and Sodium sulphate (Na_2SO_4), then 50ml of DCM was added to it again and that layer was collected through cotton and Na_2SO_4 funnel. Then the filtrate was allowed to evaporate completely. Then 10ml of acetone or n- hexane was added to the bottle and the residue was collected in to a vial. This step was repeated twice. Then the amount of pesticide in the samples was estimated through GC analysis **[11, 12]**.

The samples were subjected for quantification by drawing out comparison of peak heights with standards of known concentration. The percentages of recoveries are given in the respective data tables with corrections for internal standards were appropriate.

Instrumentation employed

The amount of the pesticide residue in the samples was analyzed through GC technique. Varian CP 3800 model Gas Liquid Chromatography was employed with VF-1 MS column of length 15 meters, thickness of 0.25 µm and 0.25 mm internal diameter. The carrier gas used was Nitrogen wherein the injection temperature was 260 ^{0}C. The detector system used was electron capture detector (ECD) tritium source electron capture detector and a Tracor model MT-220. Electron capture GLC determinations employed two columns (0.64 cm by 1.8 m): one contained 2% OV-101 and 100/120-mesh Gas-Chrom Q; the other contained equal parts of 0.75% OV-17 and 0.85% OV-210 on 100/120 Gas-Chrom Q. Column, detector, and inlet temperatures were 185,

250, and 250°C, respectively. The carrier gas was nitrogen, used at a flow rate of 25 ml/min. Respective columns, detectors (ignited), and injector temperatures were 180, 160, and 220°C, and gas flow rates for 0_2, air, H_2, and N_2 (carrier) were 20, 50, 200, and 60 ml/min, respectively.

Calculation of percentage of degradation of the pesticide

- **Amount degraded = original amountadded-amount recovered**
- **% degradation = amount degraded×100÷amount added**
- **% recovered = amount recovered in the control ÷original amount added ×100**

Recovery data for GLC analyses are given in the respective tables. All calculations for GLC data are based on known standards.

Fourier Transform Infrared (FTIR) Spectroscopic analysis of Endrin degradation

To analyze the qualitative bond and functional group information of endrin samples, FTIR Spectroscopy was used **[13]**. The non-destructive nature of FTIR additionally allows sample analysis by alternative techniques that were not explored in this particular study. This protocol, was developed with consideration of possible future adaptation to include both quantification and coupled analytical techniques.

RESULTS AND DISCUSSION

Analysis of the culture filtrates for Endrin degradation by GC

In the present investigation, bioremediation of the pesticide, endrin was carried out using pure culture of *Kocuria* sp. in a shake flask under controlled environmental conditions. The GLC data showed that endrin degraded upto 75% in the *Kocuria* sp. culture containing 3.84 g/l endrin. It is assumed that the microorganism *Kocuria* sp. was found to be well adapted to endrin. The percentage of degradation was much more than the previous observations **[14-16]** in both the test organisms with reference to the concentration of Endrin used. This evinced that *Kocuria* sp. was able to degrade OC pesticides more rapidly **(Figure 1)**. 1869-9391

Analysis of Endrin degradation using FTIR

FTIR was used to study the biodegradation process of OC pesticides. The residual pesticides were extracted by solvents, and the solution was then concentrated after degradation. The concentrated solution was scanned and analyzed by FT-IR. During the biodegradation of Endrin, the IR peaks of C-Cl with 600–800 cm^{-1} and C-H with 2991 cm^{-1} were obviously dropped. It can be inferred that the cleavages of C-Cl and C-H were bonded, and chlorine was further converted into chloride. The IR spectrum for pure endrin showed five

major peaks at 767.58, 829.78, 1658.33, 1588.96 and 1486.06 cm^{-1}, respectively. The first and second peaks were with reference to C-Cl and 1, 4- benzene substitution, and the last three peaks were in relevance to the benzene special vibration. There was a decrease in intensity of the major peaks and disappeared after the UV irradiation, which indicates that the aromatic ring of endrin was destroyed in the photocatalytic degradation **(Figure 2)**.

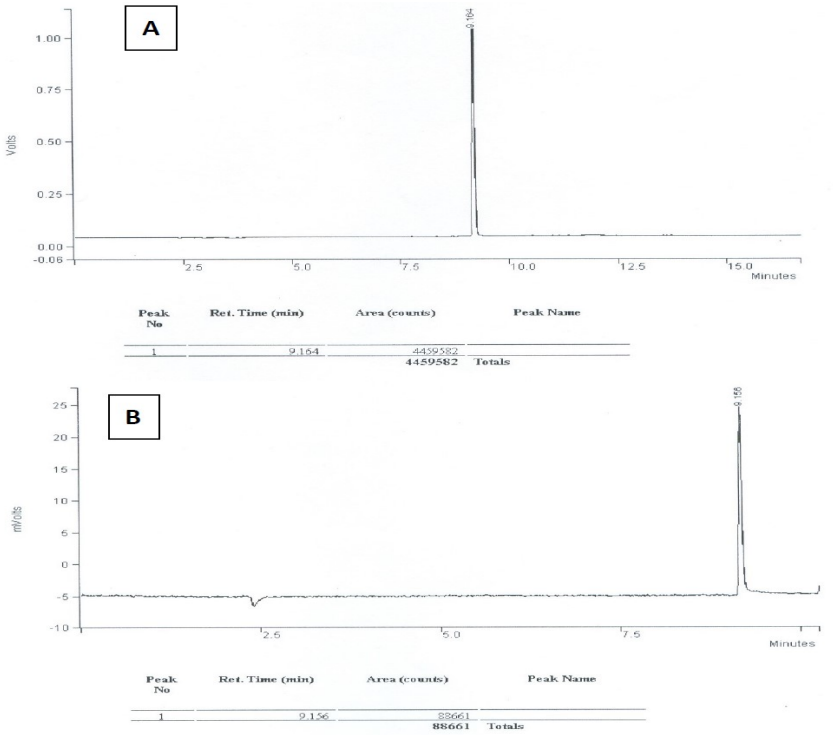

Figure 1: A: Control: Endrin without Inoculum; B: *Kocuria* sp. culture embedded with Endrin

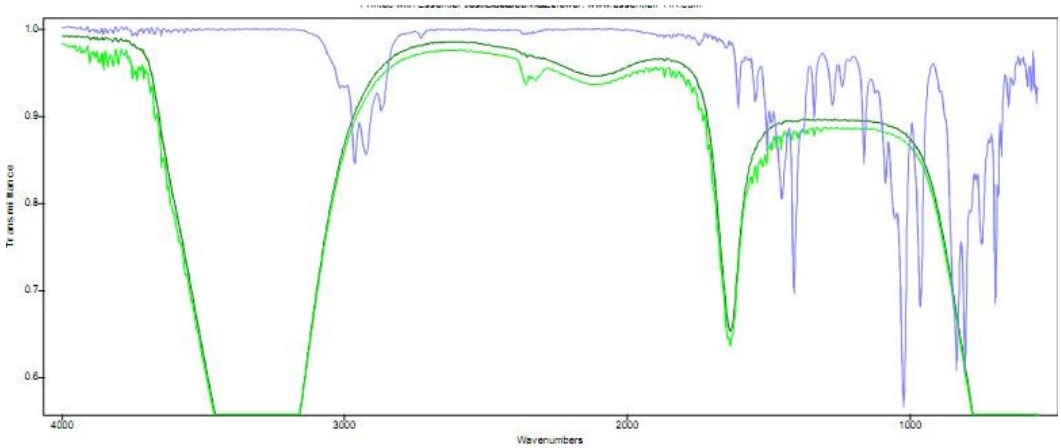

Figure 2: FTIR spectra of Pesticide Degradation
Blue : Pesticide– Endrin; Light Green – Degraded Wild type

The results evinced through the GC and FTIR analysis were in accordance with the previous studies on endrin degradation. A bacterium capable of degrading endrin was isolated and it was *Alcaligenes faecalis* DSP3. The primary metabolite was found to be POP. *Enterobacter* strain B-14 biotransformed endrin upto POP only **[17-20]**.

The mechanism of endrin degradation in bacteria and fungi is fairly understood and a number of degradation products such as chlorodihydro-2-pyridone, dihydroxy pyridine, tetrahydro-2pyridone, and maleamide semialdehyde have been identified. The combined experiments of photolysis and microbial degradation, the degradation of POP firstly into chlorodihydro2-pyridone by reductive dechlorination followed by its degradation into tetrahydro-2-pyridone and then to maleamide semialdehyde, which ultimately got mineralized into water, CO, and ammonium **[21]**. The occurrence of unidentified peaks in the chromatogram of present study may be byproducts of POP, which needs further investigation.

The pesticide level in the environment determines the dose and time at which an organism is exposed. Due to the mobility and persistence of these semi-volatile compounds, they can represent a hazard for the wider environment and can travel to remote locations. Hence, their persistence in the environment leads to a risk for life. Pesticide persistence in the environment is caused by either the physico-chemical properties of the pesticide or the lack of organisms able to degrade it. The bioremediation is a process of microbial degradation of pesticides is a cost-effective method of removing pollutants from the environment. To optimize the conditions, it serves as an important parameter for ensuring complete biodegradation and bioremediation of polycyclic aromatic hydrocarbons (PAHs) on site. Working conditions, namely, substrate concentration, bacteria concentration, pH, and temperature, have to be optimized. To degrade pesticides, bacteria must possess enzymes that cleave the ring of the aromatic halogen compound (PCP) **[22-25]**. The bacterial species such as *Actinobacteria, Firmicutes,* and *Gammaproteobacteria* that can degrade hydrocarbons seem to possess the degradation of pesticides. These reported microbiodegraders are gamma-proteobacteria (*Pseudomonas, Aerobacter, Acinetobacter, Moraxella, Plesiomonas*), β-proteobacteria (*Burkholderia, Neisseria*), α-proteobacteria (*Sphingomonas*), actinobacteria (M*icrococcus*), and Flavobacteria (*Flavobacterium*) **[26, 27]**. Bacteria belonging

to the genera Pseudomonas, Neisseria, Moraxella, and Acinetobacter are able to degrade the pesticide DDT almost

completely **[28]**, bacteria of the genera *Pseudomonas, Bacillus, Aerobacter, Micrococcus, and Burkholderiaa* have shown to biodegrade dieldrin and endrin **[29-31]**.

CONCLUSION

The present study reports that the strain of *Kocuria* species procured from Acharya Nagarjuna University is very efficient in degrading endrin, a OC pesticide. The same was proved to be effective against OP pesticides. Therefore this can be used for bioremediation of pesticide contaminated soil, thus cleaning the environment. Microorganisms are extensively used for the development of technologies that can be used for the environmental pollution control. The same can be employed in pollution control from the environment as it is a cost effective and more eco - friendly approach for sustainable environmental quality.

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Conflicts of Interest

The author declares no conflict of interest.

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