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EFFECT OF ORGANOCHLORINE PESTICIDE ON GROWTH OF ACTINOMYCETES

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ABSTRACT

The present study aims to see the effect of organochlorine pesticides on growth actinomycetes. Most of the isolates recovered from parboiled rice mill effluent belonged to genus *Streptomyces* followed by *Micromonospora, Streptosporangium* and *Nocardia*. Growth was found to be impaired by organochlorine pesticide toxicity. However, two isolates belonging to genus Streptomyces could resist particular concentration of pesticides tested – Lindane, endosulfan and chlordane. The amount of pesticides used was 100µg/l, 200µg/l, 300µg/l and 500 µg/l. The results indicate that pesticides can be metabolized by Actinomycetes.

Key words: Organochlorine Pesticides, Actinomycetes

Actinomycetes population has been identified as one of the major group of soil population (Kuster 1968; Philip *et al.*, 2012), which may vary with the soil type. These organisms participate in the turn over of the soil components, especially in the transformation of organic compounds (Kononova 1966; Kuster 1967; Kuzner 1968; Huntzens 1972). In soil major function of actinomycetes is decomposition and mineralization cycles with the production of extracellular enzymes, such as cellulases, amylases (Philip *et al.*, 2012), chitinases, and lignin peroxidases. Similar studies were done by Philip *et al.,* 2011a; Philip *et al.,* 2011b from Gelatin factory. Actinomycetes were found to be potential biodegraders as tested in their studies. For quite some time, actinomycetes also known as actinobacteria, have been categorized as extremely fascinating organisms with an unexhausted reserve of bioactive compounds that are being exploited for various commercial applications which includes environmental, biomedical and industrial applications. They have possessed a very important position as potential producers of unique metabolites, antibiotics and enzymes exhibiting the capacity to metabolize recalcitrant molecules. Among the diverse microbial communities, these may also be adversely affected by organochlorine pesticides which are known to be highly persistent in the environment. Even though pesticides and toxic compounds in the ecosystem are successfully degraded by several microbes (Chetna and Madhuri, 2012) at sufficiently high concentrations, the pesticides in turn are known to modify the growth and activities of micro-organisms. These alterations are mediated through cytological and biochemical changes at the cellular level (Ruplal and Shivaji, 1984). Actinomycetes are one among the important group which effectively disintegrate and bioremediate the pesticides and other xenobiotics in the environment. Actinomycetes play important roles in the environmental fate of toxic metals with a multiplicity of physico-chemical and biological mechanisms effecting transformations between soluble and insoluble phases and produces significant levels of bio-surfactants (Subhajit, 2012). Although their use has been banned in many countries, they are still used in developing countries. In the last few decades, highly toxic organic compounds like the organochlorine pesticide lindane/hexachlorocyclohexane have been released into the environment. All hexachlorocyclohexane isomers are acutely toxic to all animals as well as plants. Along with other diverse microbial communities, these may also be adversely affected by organochlorine pesticides. Few actinomycetes group have been found to degrade pesticides with widely different chemical structures. (Fuentes *et al.,* 2010). Although nowadays its use is restricted or completely banned in most countries, it continues to pose serious environmental and health concerns.

MATERIALS AND METHOD

Isolation of actinomycetes

Isolation of Actinomycetes was done by using dilution and pour plate method on asparagine glycerol media. Since glycerol is the

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changed carbon source which Actinomycetes are able to metabolize, the media becomes selective. Also antifungal and antibacterial agents were used in minimal quantities. This helps in retarding fungal and bacterial growth. (Philip *et al.*, 2012).

Culture maintenance

Isolated actinomycetes will be maintained on slants at 4ºC.

Identification

Identification at was done by studying:-

a. Colony characters will be studied on AGA medium.
b. Morphology by simple cover slip method using Asparagine glycerol agar media. (Kawato *et al.,* 1959).
c. Measurement of mycelial width, sporulation structures pattern of fragmentation & branching criteria for assigning the genus. For characterization, description given in Bergy's manual of determinative bacteriology (2000) was followed.

Pesticide Effect on Actinomycetes

a. Organochlorine pesticide used are Lindane, chlordane and endosulfan.

b. Increasing concentration of these pesticides were added to medium.

c. Effect of pesticides on growth of actinomycetes will be seen on agar plates by supplementing the sterile medium with 100 µg/lit, 200 µg /lit, 300 µg /lit and 400 µg /lit. Spot inoculation was done with actinomycete isolates and incubated for 14 days.

Solutions were prepared as follows:

1. Preparation of stock solution: To 100 ml of distilled water 10 gm of pesticide was dissolved.
2. For dilution of stock solution to 100 µg/lit, 0.01 ml was micropipetted to 999.99 ml of sterilized medium.
3. For dilution of stock solution to 200 µg/lit, 0.02 ml was micropipetted to 999.98 ml of sterilized medium.
4. For dilution of stock solution to 300 µg/lit, 0.03 ml was micropipetted to 999.97 ml of sterilized medium
5. For dilution of stock solution to 400 µg/lit, 0.04 ml was micropipetted to 999.96 ml of sterilized medium.
6. For dilution of stock solution to 500 µg/lit, 0.05 ml was micropipetted to 999.95 ml of sterilized medium.

RESULTS

Following characters were found according to which the isolates were identified as *Streptomyces.*

Isolate no. CP – 5. *Streptomyces* sp. recovered form parboiled rice mill effluent.

Temperature: C, Date: 12-09-11

Colony characteristics: Colony colour whitish yellow, rough as observed on AGA medium. With flat margins 1.7 cm diameter.

Morphology - Branched, slender, coenocytic mycelium, 1.0 µm in diameter, aerial mycelium at maturity forms straight conidiophores on which 10- 15 spherical conidia are present in straight , unbranched chains. Size of conidia was 1µm.

Isolate no. CP – 12. *Streptomyces* sp. recovered form parboiled rice mill effluent

C, Date: 12-09-11.

Streptomyces - Colony colour white, white initially, concentric alternating zones of black conidia and white mycelium appeared after 6 days of incubation . With flat margins 1.7 cm diameter as observed on AGA medium.

Morphology - Branched, slender, coenocytic mycelium, 1.0 µm in diameter, aerial mycelium at maturity forms straight conidiophores on which 10- 15 spherical conidia are present in straight , unbranched chains. Size of conidia was 1µm in diameter Broom shaped structure appeared due to clumping.

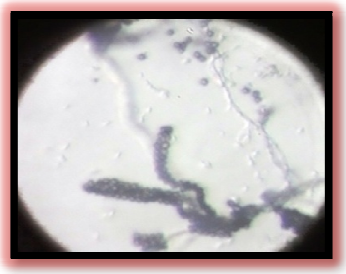
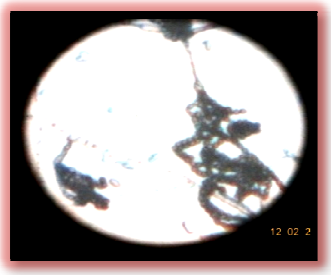
a.  b. 

Fig ; Morphological structure of *Streptomyces* sp. as observed, with conidia present in chains.

BIOCHEMICAL PROPERTIES OF THE ISOLATES TESTED

ISOLATE No.	Catalase Prod.	H_2s Prod.	Amylase Prod.	Cellulase Prod.	Gelatinase Production	Melanin Production	Indole Test	Urease Test
CP-5	+	+	+	-	+	+	+	-
CP-12	+	+	+	+	-	+	+	+

EFFECT OF ORGANOCHLORINE PESTICIDES ON GROWTH OF ACTINOMYCETE ISOLATE No. CP- 5, *Streptomyces*

A. Effect of **lindane**

S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.6 cm x 1.6 cm
2.	100 µg	1.5 cm x 1.5 cm
3.	200 µg	1 cm x .8 cm
4.	300 µg	0.8 cm x 0.8 cm
5.	400 µg	Very less growth observed
6.	500 µg	No growth observed

B. Effect of **endosulfan**

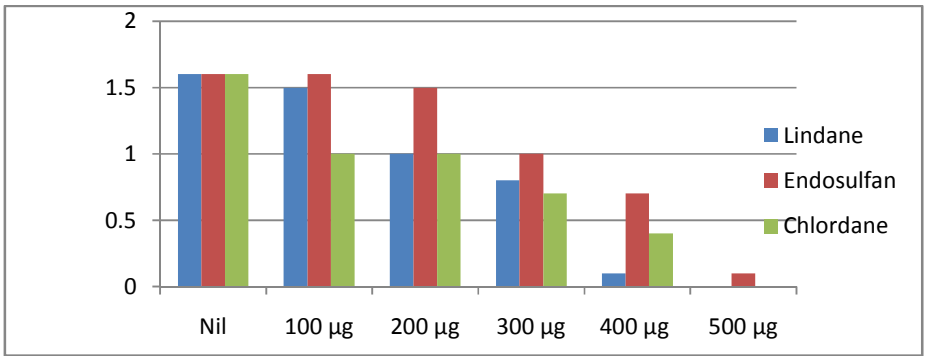
S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.6 cm x 1.6 cm
2.	100 µg	1.6 cm
3.	200 µg	1.5 cm
4.	300 µg	1 cm
5.	400 µg	0.7 cm
6.	500 µg	0.1 cm

C. Effect of **chlordane**

S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.6 cm x 1.6 cm
2.	100 µg	1 cm
3.	200 µg	1 cm
4.	300 µg	0.7 cm
5.	400 µg	0.4 cm
6.	500 µg	No growth observed

GRAPHICAL REPRESENTATION OF ABOVE DATA

A.



Isolate No. CP- 5. *Streptomyces sp.* showing growth in presence of high conc. of organochlorine pesticides used. This isolate was able to metabolize lindane upto 400 µg/lit. minimal growth was observed at this conc.

EFFECT OF ORGANOCHLORINE PESTICIDES ON GROWTH OF ACTINOMYCETE ISOLATE No. CP- 12, *Streptomyces*

A. Effect of **lindane**

S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.7 cm x 1.2 cm
2.	100 µg	No growth observed
3.	200 µg	No growth observed
4.	300 µg	No growth observed
5.	400 µg	No growth observed
6.	500 µg	No growth observed

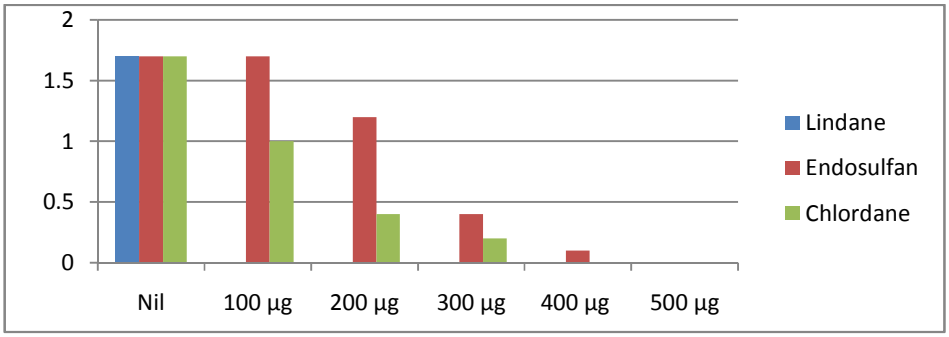
B. Effect of **endosulfan**

S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.7 cm x 1.2 cm
2.	100 µg	1.7 cm x 1.2 cm
3.	200 µg	1.2 cm
4.	300 µg	0.4 cm
5.	400 µg	Very less growth observed
6.	500 µg	No growth observed

C. Effect of **chlordane**

S .no	Conc of pesticide used/litre	Size of colony
1.	Nil	1.7 cm x 1.2 cm
2.	100 µg	1 cm
3.	200 µg	0.4 cm
4.	300 µg	No growth observed
5.	400 µg	No growth observed
6.	500 µg	No growth observed

B.



Isolate No. CP-12. *Streptomyces sp.* showing growth in presence of high conc. of organochlorine pesticides, endosulfan and chlordane used. Very sensitive to lindane - no growth was observed.

CONCLUSION

Isolate no. CP-5 has shown maximum resistance towards toxicity of all the pesticides tested. Minimal growth was observed at 400 µg/lit of lindane. Also growth was observed at 500 µg/lit conc. of endosulfan and 400 µg/lit conc of chlordane indicating the production of dechlorinase enzyme. Isolate no. CP -12 is very sensitive to lindane toxicity. However, growth was observed at 400 µg/lit of endosulfan and 300 µg/lit of chlordane. Growth was found to be impaired with increasing concentration of pesticides showing toxic effect on actinomycetes. According to the above studies it was found that *Streptomyces* sp. were able to adapt to the changed toxic conditions due to the presence of organochlorine pesticides. Such adaptable organisms can be used as biocontrol agents in indigenous conditions. Organochlorine pesticides are an inexpensive, quick and comfortable solution for controlling insect pests and weeds in urban landscapes and crop fields. However, pesticide use comes at a substantial price. Pesticides have contaminated almost every portion of our surroundings. Pesticide contamination poses significant dangers to the environment and non-target organisms ranging from beneficial soil micro-organisms, to insects, plants and to animals. Their use must be minimize even in the developing nations. Also more methods of soil treatment for removal from soil should be implemented for persistent pesticides as organochlorine pesticides. Contribution of microbes is significant towards primary production, nutrient cycling and decomposition in ecosystems, hence detrimental effects of pesticides on microbial species may have subsequent impacts on higher trophic levels.

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