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RESEARCH ARTICLE

Biodegradation of pesticide, Endosulfan from synthetic and wastewater by isolated bacterial strains

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SUMMARY: Pesticides cause pollution by running off from agricultural fields, horticultural land and domestic gardens. Pesticides decrease biodiversity in the soil because they do not just kill the intended pest, they often kill many of the other small organisms. In order to prevent the ecosystem from the ill effects of pesticides, they are necessary to be removed. Endosulfan is an organochlorine pesticide under the cyclodiene group, a derivative of hexachlorocyclopentadieneand chemically similar to aldrin, chlordane and heptachlor. Present research was carried out for the development of treatment technology for the isolation of soil micro-organisms having affinity for the biodegradation of endosulfan contaminated wastewater.

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Pesticides are toxic and are potentially hazardous to human, animals, other organisms and the environment. Pesticide pollution was reported to have killed fishes and resulted in reproductive failure in birds. Chronic effects from exposure to certain pesticides include birth defects, toxicology to a foetus, production benign or malignant tumors, nerve disorders, blood disorders, genetic changes, endocrine disruption and reproductive effect. The symptoms of pesticides poisoning can range from a wild skin irritation, permanent blindness, to coma or even death. Micro- organisms can metabolize pesticides if they are bioavailable and if they have chemical structure compatible with the organisms' enzymes that catalyze the biodegradation. Mechanisms of degradation include mineralization, partial degradation to secondary compounds, adsorption, humiliation and volatilization. In this study, pesticide tolerant bacterial strains were isolated for bioremoval of endosulfan.

EXPERIMENTAL METHODOLOGY

***Initial characterization of wastewater* :**

Wastewater was procured from *Buddah Nullah* sewage of Ludhiana where waste water from agricultural fields using pesticides and effluent from industries is disposed off into the water bodies. pH of waste water sample was 5.4, total solids 11800mg/l, total dissolved solids 5600mg/l, total suspended solids 6200 mg/l, alkalinity 480mg/l, hardness 650mg/l, turbidity 42 NTU, biological oxygen demand 220mg/l, chemical oxygen demand 450mg/l and nickel 2ppm and chromium 4ppm (APHA, 1995).

Isolation and characterization of pesticide tolerant strains :

In the present study,bacteria which can remove endosulfan from aqueous solution by using endosulfan as carbon source were isolated by enrichment method. The isolated strains were characterized for various biochemical, growth and morphological characteristics (Harley and Prescott, 1993).

Procurement of synthetic endosulfan :

Synthetic pesticide was procured from Bharat Insecticides Limited. The net content of the bottle of synthetic endosulfanwas 100 ml.

Concentration of synthetic endosulfan is 35 per cent (Endocid-40).

Estimation of endosulfan in test samples :

For preparation of standard graph for estimation of endosulfan concentration, rapid spectrophotometric method was used. This method was based upon the reaction of endosulfan with 4-(4-nitrobenzyl) pyridine (4, 4-NBP) in an alkaline medium to form a pink complex with an absorption maximum at 520 nm. The relationship between the absorbance and concentration of endosulfan was linear in the range of 2 to 100µg/ml (Sowbhagya*et al*., 2006).

Standarad graph for estimation ofendosulfan concentration :

Sample solution of standard concentration of endosulfan, 5.0 ppm, 10.0 ppm, 15.0 ppm, 20.0 ppm, 25.0 ppm, 30.0 ppm, 35.0 ppm, 40.0 ppm, 45.0 ppm and 50.0 ppm were prepared. 5ml of diluted samples were taken in serial 25 ml flasks. Blank was also prepared (5ml distilled water +1.0 ml 4, 4-NBP+ alkaline solution) to set zero. The absorbance was measured at 520 nm. From Fig.A, it was found that with increase in concentration there was increase in the absorption at 520 nm (Sowbhagya *et al*., 2006).

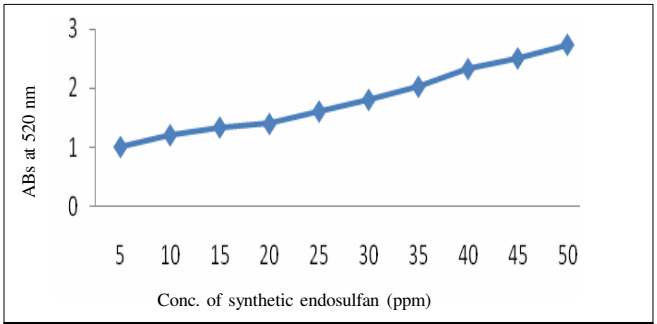

Fig A : Standard graph for known concentrations of endosulfan

EXPERIMENTAL FINDINGS AND DISCUSSION

Strain M1 was white in colour while strain M2 was yellow in colour. Both strains were motile, possessing gram negative characteristics and were rod shaped bacteria. When colonies were grown on Nutrient agar plates, strain M1 colonies were moderate and convex in appearance and colonies of strain M2 were moderate and had elevated appearance. The characteristics of the isolated bacterial strains seem to be comparable to *Pseudomonas* sp. (Lee *et al*., 2006, Kumar *et al*., 2008). The morphological and biochemical characters of both the isolated strains are given in Table 1 and 2.

Selection of more tolerant strain for maximum removal of endosulfan :

It was found that bioremoval of endosulfan (500 ppm) by strain I was 43 per cent and by strain II was 31 per cent. Bioremoval of synthetic endosulfan was more by strain I as compared to strain II, so, strain I was selected for further study.

Table 1: Morphological characteristics of isolated strains

Sr.No.	Morphological character	Result	
		Strain M1	M2
On agar petriplates			
1.	Mode of nutrition	heterotrophy	Heterotrophy
2.	Color of colonies	White	Yellow
3.	Growth on agar	Moderate	Moderate
4.	Shape of colony	Rod shaped	Rod shaped
5.	Form of colony	Mucoid	Elliptical
6.	Margin	entire	Smooth
7.	Elevation	convex	Elevated
8.	Size	0.25-2.0 mm	0.5-2.0 mm
On broth			
9.	Clouding	slight	Heavy
10.	Sediment	flocculent	Scanty

Table 2 : Biochemical characterization of the two isolated strains

Sr. No.	Tests	Strain M1	Strain M2
1.	Hydrolysis of gelatin	Positive	Positive
2.	Casein hydrolysis	Positive	Positive
3.	Urease test	Positive	Negative
4.	Citrate utilization test	Positive	Positive
5.	Hydrogen sulphide production	Negative	Negative
6.	Indole production	Negative	Negative
7.	MR test	Negative	Negative
8.	VP test	Negative	Negative
9.	Starch hydrolysis	Positive	Positive
10.	Catalase test	Positive	Positive
11.	Litmus milk reaction	Negative	Negative

Effect of pH and temperature :

Various pH values *i.e.* (4, 7 and 9) and temperature ranges *i.e.* (28^{0}C, 33^0 C and 37^0 C) were optimized for bioremoval of endosulfan (500 ppm) by strain I. It was found that at 4, 7 and 9 pH, bioremoval of endosulfan were 14 per cent, 56 per cent and 24 per cent, respectively. The optimum pH for maximum bioremoval of endosulfan by isolated strain I was observed at pH 7.0. The maximum removal of endosulfan by *Pseudomonas aeruginosa* was observed at pH 7 (Arshad*et al*., 2008). At different temperatures of 28^0 C, 33^{0}C and 37^{0}C, per cent bioremoval of endosulfan were 51 per cent, 35 per cent and 22.8 per cent, respectively.

From this it was observed that optimum temperature for maximum bioremoval of endosulfanis was 28^0C. The highest bioremoval of endosulfan by degradation with *Pseudomonas aeruginosa* was at temperature 28-30^0 C (Arshad *et al*., 2008).

Effect of inoculum and agitation speed :

Various percentages of 2, 5 and 7 per cent inoculums and agitation speed *i.e.* (static, 130 rpm and 160 rpm) were optimized for maximum bioremoval of endosulfan (500 ppm) by strain I. It was observed that maximum per cent removal of endosulfan by strain I was 62, 48 and 38 per cent when 2 per cent, 5 per cent and 7 per cent inoculum were added, respectively. It was observed that maximum bioremoval of endosulfan was 49 per cent when agitation speed was 160 rpm. When agitation speed increases or decreases than 160 rpm then per cent bioremoval of endosulfan by isolated strain I was decreased.

Effect of concentration of endosulfan :

Different concentrations of endosulfan 200 ppm, 300 ppm, 400 ppm and 500 ppm were taken in different conical flasks in media and added 2 per cent inoculum of strain I. Incubated at 28^0 C, for 24 hours at shaker (160 rpm). Maximum bioremoval of endosulfan was 74 per cent at 400 ppm. Percentage removal of parameters TS, TDS, TSS, alkalinity, hardness, turbidity, BOD, COD, chromium, nickel and endosulfan concentration from the procured waste water sample were 11per cent, 46 per cent, 49 per cent, 42 per cent, 31 per cent, 57 per cent, 52 per cent, 48 per cent, 47 per cent, 37.5,50 and 44 per cent, respectively.

Conclusion :

Isolated bacterial strains can be successfully used for bioremoval of synthetic pesticide endosufan and wastewater treatment for reducing various contaminants.

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REFERENCES

APHA (1995). *Standarad methods for examination of water and wastewater* (American Public Health Association, Washington, D.C., USA).

Arshad, M., Hussain, S. and Saleem,M.(2008). Optimisation of environmental parameters for biodegradation of alpha and beta endosulfan in soil slurry by *Pseudomonas aeruginosa*. *J. Appl. Microbiol.*, **104**(2):364-370.

Harley, J.P. and Prescott, L.M. (1993). Basic laboratory and culture techniques In: *Laboratory exercises in microbiology,* 2nd Ed. W.C Brown Publishers, pp: 14-46.

Kumar, M.,Vidyalakshmi, C. and Khanna, S. (2008). Microbial biodiversity and *in situ* bioremediation of endosulfan contaminated soils. *Indian J. Microbiol.*, **48**(1): 128-133.

Lee, J.B., Sohn, H.Y., Shin, K.S., Jo, M.S., Kim, J.E., Lee, S.W., Shin, J.W., Kum, E.J. and Kwon, G.S. (2006). Isolation of a soil bacterium capable of biodegradation and detoxification of endosulfan and endosulfansulphate. *J. Agric.& Food Chem.*, **54**(23): 8824-8828.

Sowbhagya, H.B., Visweswaraiah, K.M.and Suvendu, K. (2006). A rapid spectrophotometric method for the estimation of endosulfan, *Pesticide Sci. J.*, **15**(6): 571-573.

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