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Promising Biological Agents Isolated for Metabolization of Lethal Pesticides- Lindane and Dieldrin

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ABSTRACT
Pesticides are not only lethal to human beings but have adversely affected the environmental factors and quality of soil environment as well. These recalcitrant chemical compounds are foreign to most of the microbes thus interfering the biogeochemical cycles. In this pesticide utilization-screening programme, many fungal strains isolated from the rhizosphere of Juglans regia L. showed the potential to grow on the media containing Lindane and Dieldrin like pesticides. Isolated strains were characterized on the basis of ITS1, ITS2 and 5.8s DNA sequences. BLASTN was used to search ten closely related sequences in the Gen Bank database. ClustalW was used to do pairwise alignment and UPGMA in MEGA5 software was used to carry out phylogenetic analysis. The strains like Trichoderma koningii k132, Penicillium notatum k840, Aspergillus terricola k850 and A. niger mtc 872 were successful in degrading the supplemented pesticides and exhibited the optimal growth in the temperature range of 20-30^{0}C. The expansion and utilization rate of pesticides on the Potato Dextrose Agar media (Hi Media) supplemented with Lindane and Dieldrin as a sole source of carbon and nitrogen were recorded to be different. The metabolization of Lindane as a sole source of carbon and Dieldrin as carbon and nitrogen font was exposed competently by Trichoderma koningii k132 and Aspergillus terricola k850.
***Key words**: Lindane, Dieldrin, Biogeochemical cycle, Biological Agents, Metabolization, Gene Bank Database.*

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INTRODUCTION

Environmental pollution is the main challenge to the scientific world and has been one of the largest concern to the general public as well. The industrialized world is confronted with the contamination of soils, water sources and toxic xenobiotics. Though regulatory steps have been taken to reduce or eliminate the production of pesticides and release to the environment of these chemicals, significant environmental contamination has occurred in the past and will probably continue to occur in the future. The industrialization of agriculture in relation to higher yield and quality production, rapid growth in the chemical industry and the need to generate cheap forms of energy have all caused the continuous release of pesticides into the environment. Bioremediation is a process by which living organisms degrade or transform hazardous organic contaminants to less toxic compounds [1].

The use of fungi as biological agents to clean up the environment from the pollution provides an option of bioremediation as microfungi have the ability to degrade the organic pollutants [2]. In the past little attention was given to the bioremediation using fungi since most bioremediation research has focused mainly on the use of bacteria. Though, some researchers have used the bioremediation technique in oil contaminated soils by using

fungal strains like *Trichoderma species* [3], degradation of fruit wastes by fungi [4], garbage biodegradation by white-rot fungi [5], sludge degradation [6]. In recent times fungi have received significant attention for their bioremediation potential which is endorsed to the enzymes they produce. Cosgrove *et al.,* have also studied the biodegradation of polyester-polyurethane (PU) in which *Geomyces pannorum* and a *Phoma* species were the dominant species in soil fungal communities involved in the biodegradation of PU [7]. In addition, fungi have advantages over bacteria such as fungal hyphae can penetrate the soil to reach the pollutants [8] and have potential to degrade the agro-industrial wastes [9,10,11,12,13,14,15,16].

The green revolution has led to the production of more chemical compounds which has consequently increased the number of compounds identified as being potential environmental threats to living organisms and are purported to be pollutants. These pose challenges to the designers of future treatment plants and related ethodology for their eradication [17].

Fungi are prominent source of enzymes and the power of fungal enzymes is janus- faced [18]. Filamentous fungi are suitable microorganisms to grow on the solid substrate because their morphological features allow them to colonize and penetrate the solid media [19].

Lindane, also known as *gamma*-hexachlorocyclohexane (γ-HCH), and dieldrin are organochlorine chemicals. The World Health Organization classifies lindane as "moderately hazardous", and its international trade is restricted and regulated under the Rotterdam Convention on Prior Informed Consent. It was first applied in the form of technical HCH-a mixture of α-HCH (53–70 %), β-HCH (3–14 %), γ -HCH (10–18 %), δ-HCH (6–10 %), ε -HCH (1–5 %), and also traces of other isomers [20]. Later it was used as purified form in the name of Lindane. Between 1950 and 2000, an estimated 600,000 tonnes of lindane were produced globally, and the vast majority of which was used in agriculture and 4.8 million tones of HCH residues are still present worldwide [21]. It has been manufactured by several countries, including the United States, China, Brazil, and several European countries, but as of 2007, only India and possibly Russia are still producing it. The production and agricultural use of lindane are the primary causes of environmental contamination. Dieldrin is an extremely persistent organic pollutant; it does not easily break down. Furthermore, it tends to biomagnify as it is passed along the food chain [22]. These pesticides are unaffected by conventional secondary treatment systems at municipal wastewater treatment facilities. They appear in the effluent, usually at levels lethal to invertebrates [23]. These pesticides are potent carcinogens and mutagenic compounds [24,25]. Enormous studies have been conducted for degradation of such harmful pesticides by using the potentiality of soil microorganisms [26]. Scientists all over the world are busy to find out the remedial measures for the degradation of pesticides like lindane [27,28,29]. In this study rhizosphere microfungi were screened for biodegradation programme.

MATERIAL AND METHODS

Soil samples were collected 20 to 40 cm deep from the rhizosphere of different plants of *Juglans regia* L. from Nothern India- Jammu and Kashmir which lies between 32° 15' to 37° 05' North latitude and 72°35' to 83° 20' East longitude. The selection of plants was based on environmental and microclimate conditions and the samples collected were air dried and sieved. Samples were carried in sterilized cryo-bags to the laboratory and treated under disinfected conditions and ultimately 1 gram of the soil was taken from each sample and was serially diluted from 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. After completion of serial dilutions, 0.5 ml of soil solution from each test tube was inoculated on petri plates containing Potato Dextrose Agar (Hi Media) and Malt Agar Medium (Hi Media). Chloramphenicol was added to the media as 250mg/100ml to check the growth of bacteria. Inoculated petri plates were incubated at wide range of temperatures 10^0C, 15^0C, 20^0C, 25^0C, 30^0C, 35^0C, 40^0C and 50^0C) for a period of 5-7 days and pH was also maintained between 7-8 for fungal culturing. Sub culturing and mono culturing was made in order to get the pure colonies. Pure cultures were identified by using manual of soil fungi - Joseph C. Gilman and have also been characterized on the basis of ITS1, ITS2 and 5.8s DNA sequences. BLASTN was used to search ten closely related sequences in the Gen Bank database. ClustalW was used to do pairwise alignment and UPGMA in MEGA5 software was used to carry out phylogenetic analysis [30]. Strains were periodically transferred onto fresh sterilized Czapek-Dox Agar Medium (Hi Media) to allow continuous growth and viability of fungi. As the microbial

community perform multiple complex chemical and physiological processes which allows them to survive [31]. Selected fungi were tested in complex order on the same plate to degrade the pesticides lindane and dieldrin at wide range of temperatures 10^0C to 50^0C and pH range of 7-8 for a period of 14 days. PDA plates were supplemented with 1% (w/v) dieldrin and lindane as a sole source of carbon and nitrogen. The fungal strains inoculated were screened for utilizing the pesticides as sole nitrogen or carbon source. A control was also maintained in which neither dieldrin nor lindane was supplemented as a source of carbon or nitrogen. Carbon and nitrogen are essential elements for the growth of mircroorganisms. At low concentrations of these elements expression of laccase and other enzymes occur which degrade xenobiotic compounds [32]. The strains of *Trichoderma koningii* k132*, Penicillium notatum* k840*, Aspergillus terricola* k850 *and A. niger mtc 872 were* successful during the screening programme for pesticide degradation which was monitored by observing the filamentous growth and colour pattern of isolates on the PDA medium supplemented with the pesticides as carbon and nitrogen sources. The ultimate breakdown of pesticides is mainly the result of microbial action like dehalogenation [33], key reaction, which enables them to grow on the pesticide supplemented growth media and is considered as the most important step for degradation.

RESULTS AND DISCUSSION

On the basis of morphological features fungal species were identified by using the manual of soil fungi-Joseph C. Gilman and were also characterized on the basis of ITS1, ITS2 and 5.8s DNA sequences. BLASTN was used to search ten closely related sequences in the Gen Bank database. ClustalW was used to do pairwise alignment and UPGMA in MEGA5 software was used to carry out phylogenetic analysis. Many isolated strains were tested in this screening programme but only four fungal strains- *Trichoderma koningii* k132*, Penicillium notatum* k840*, Aspergillus terricola* k850 *and A. niger mtc 872* were successful in degrading the pesticide lindane and dieldrin supplemented to fresh sterilized PDA media in the form of 1% (w/v) per petri plate. Fungal species showed optimal growth rate in the temperature range of 20^0 to 30^0C having pH of 7 [34] and couldn't tolerate the temperature above 40^0C. However, increased concentration of pesticides showed decreased growth of strains. Similar results of lower degradation and growth rate at high concentration of hazardous organic compounds have also been shown by many other researchers [35,36,37].

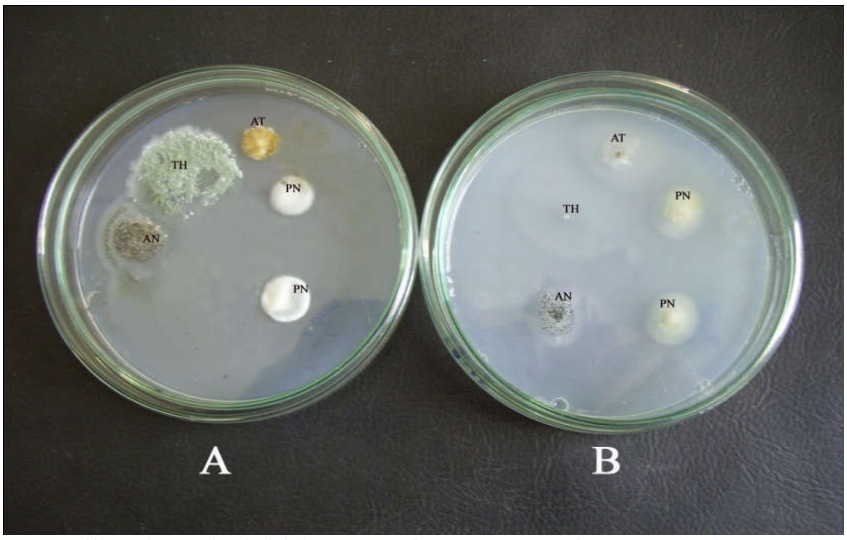

Figure 1. *Plate A: Utilization of Dieldrin as carbon source Plate B: Lindane as nitrogen sources AT: Aspergillus terricola k850, AN: A. niger mtc 872, PN: Penicillium notatum k840 TK: Trichoderma koningii k132*

Strains which showed better optimal growth were measured in regular interval of time by adhesive tape-sample preparation for light microscopy involving staining of slides with lactophenol cotton-blue to highlight fungal structures. Slides were also examined under the microscope to determine the number of identifiable fungal mycelia and spores [38,39,40].

Trichoderma koningii k132, *Penicillium notatum* k840, *Aspergillus terricola* k850 *and A. niger* mtc 872 utilized pesticide dieldrin when supplemented as nitrogen or carbon sources more efficiently than lindane while *T. koningii* k132 can use dieldrin as carbon source much promptly as compared to that of lindane when used as nitrogen source. The result revealed that microbial degraded lindane and dieldrin was eco-friendly and less cost effective technique to prevent the soil environment.

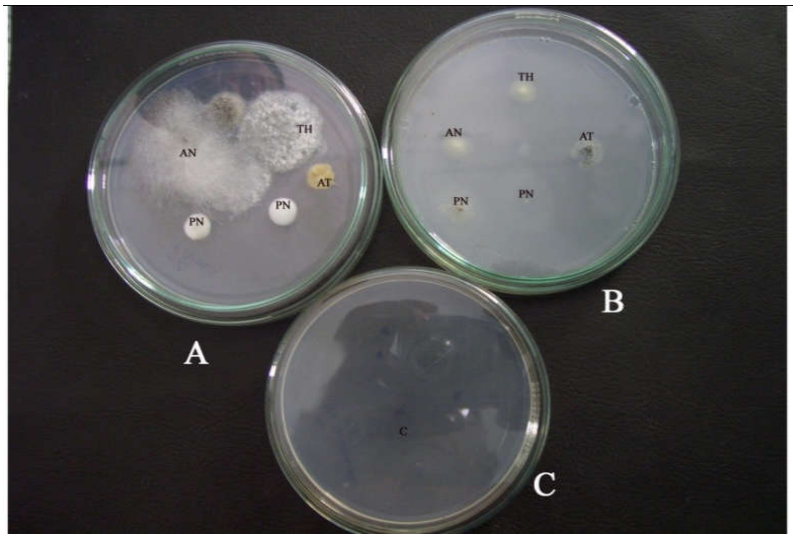

Figure 2. *Plate A: Utilization of Lindane as carbon source*
Plate B: Dieldrin as nitrogen source
Plate C: Control

Table 1. Growth rate of fungal isolates when dieldrin was used as C-source and lindane as N-source.

Fungal strains	**Pesticides**			
	Dieldrin as Carbon source		Lindane as Nitrogen source	
	Colour	Growth	Colour	Growth
1. *Trichoderma koningii k132*	Luster green	High	Light Green	Moderate
2. *Penicillium notatum k840*	Blue green	High	Light Green	Moderate
3. *Aspergillus terricola k850*	Golden Yellow	High	Light Yellow	Slow
4. *Aspergillus niger mtc 872*	Black	High	Black	Moderate

Table 2. Growth rate of fungal isolates when dieldrin was used as N-source and lindane as C-source.

Fungal strains	Pesticides			
	Dieldrin as Nitrogen source		Lindane as Carbon source	
	Colour	Growth	Colour	Growth
1. *Trichoderma koningii k132*	Dark green	High	Light Green	Slow
2. *Penicillium notatum k840*	Grey	Moderate	Blue green	Moderate
3. *Aspergillus terricola k850*	Yellow	Moderate	Yellow	Moderate
4. *Aspergillus niger mtc 872*	Grey	High	Grey	Slow

CONCLUSION

From these results it is concluded that the isolated fungal strains were effective for breakdown of toxic compounds like lindane and dieldrin which were supplemented to fresh PDA media as a source of carbon and nitrogen in the form of 1% (w/v) per plate. All the strains showed great potential to degrade dieldrin as carbon source but only two strains- *Trichoderma koningii k132* and *Aspergillus niger mtc 872* showed high growth in the medium when dieldrin was supplemented as a sole source of nitrogen. Similarly when strains were

grown on the PDA medium supplemented with lindane as carbon and nitrogen source they showed slow to moderate growth. The finding proved to be an important experiment as it paves way for the extensive research to explore the rhizosphere fungi in metabolizing some lethal pesticides and is in agreement with the findings of Sasek [41], who reported the ability of a white rot fungus *Phanerochaete chrysosporium* to metabolize a number of various important environmental pollutants, hence doable use of fungi isolated from the rhizosphere of *Juglans regia L.* may be an important aspect in this elucidation. The study gives a hint that lindane and dieldrin degrading microorganisms may be used both as models to understand how they have evolved in order to adapt to the environment and also as a biotechnological tool to remove deadly environmental pollutants.

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