

## Studies on pesticide degradation using isolated fungal cadidates

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### Abstract:

Pesticides play an important role in agriculturally dependant countries like India. Although they improve the quality and yield of the agricultural products but do have certain serious effects on the environment. This study focuses on development of a method to reduce the environmental burden of the pesticide by way of biodegradation. During this study Four fungal isolates were found to show significant ability to carry out the degradation of selected pesticides, these cultures were identified as *Aspergillus niger*, *Ganoderma austral*, *Trichosporon* , *Verticillium dahaliae*.

**Keywords:** Pesticides, Fungal Isolates, Malathion, Endosulfan, Biodegeradation, Environmental Pollution.

### Introduction:

Since before 2000 BC, humans have utilized pesticides to protect their crops. The first known pesticide was elemental sulfur dusting used in ancient Sumer about 4,500 years ago in ancient Mesopotamia. The Rig Veda, which is about 4,000 years old, mentions the use of poisonous plants for pest control. By the 15th century, toxic chemicals such as arsenic, mercury and lead were being applied to crops to kill pests. In the 17th century, nicotine sulfate was extracted from tobacco leaves for use as an insecticide. The 19th century saw the introduction of two more natural pesticides, pyrethrum, which is derived from chrysanthemums, and rotenone, which is derived from the roots of tropical vegetables [1]. Until the 1950s, arsenic-based pesticides were dominant. Paul Müller discovered that DDT was a very effective insecticide. Organochlorines such as DDT were dominant, but they were replaced in the U.S. by organophosphates and carbamates by 1975. Since then, pyrethrin compounds have become the dominant insecticide . Herbicides became common in the 1960s, led by "triazine and other nitrogen-based compounds, carboxylic acids such as 2,4-dichlorophenoxyacetic acid, and glyphosate" [2].

In the 1960s, it was discovered that DDT was preventing many fish-eating birds from reproducing, which was a serious threat to biodiversity. Rachel Carson wrote the best-selling book *Silent Spring* about biological magnification. The agricultural use of DDT is now banned under the Stockholm Convention on Persistent Organic Pollutants, but it is still used in some developing nations to prevent malaria and other tropical diseases by spraying on interior walls to kill or repel mosquitoes [3].

Pesticides can be classified by target organism (e.g. herbicides, insecticides, fungicides, rodenticides, and pediculicides<sup>l</sup>, chemical structure (e.g. organic, inorganic, synthetic, or biological (biopesticide), although the distinction can sometimes blur), and physical state (e.g. gaseous (fumigant)). Biopesticides include microbial pesticides and biochemical pesticides. Plant-derived pesticides, or "botanicals", have been developing quickly. These include the pyrethroids, rotenoids, nicotinoids, and a fourth group that includes strychnine and scilliroside [4,5].

During the past twenty years, concern has arisen as to the presence of pesticides in the environment and the threat they pose to wildlife and mankind. Certainly, pesticides have improved longevity and quality of life, chiefly in the area of public health [6].

Pesticide exposure inflicts chronic and acute threats to human health. For example, long term low dose exposure to pesticide causes immune suppression, hormonal disruption, diminished intelligence, reproductive abnormalities and carcinoma [7]. Amongst most of the important problems associated with pesticides application are their possible persistence in the environment and therefore, their possible incorporation into the food chain affects ecosystems and human beings [8]. Another problem is the conversion of pesticides into the obsolete form, which may even show more harmful effects than the former. When the pesticides are not used within the given time of their efficacy, they become obsolete. They are decomposed into their chemical components, which sometimes become even more toxic than the original pesticides. Most pesticides expire in two years after production, meaning they cannot be used unless they are tested and proved stable [9]. Therefore, these toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure [10].

The present study therefore is directed towards isolating the fungal species which are capable of carrying out the pesticide degradation.

### **Review of literature:**

These toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure [11].

On numerous occasions, mixed bacterial cultures with pesticide degradation ability are isolated but their individual components are unable to utilize the chemical as an energy source when purified [12,13,14]; an example is the organophosphorus nematicide fenamiphos [15]. Several other studies failed to obtain micro-organisms capable of growing on specific chemicals. However, this failure does not exclude biological involvement in degradation and could be attributed to the selection and composition of the liquid media under artificial environments,

strains requiring special growth factors, or a major role of non-culturable microorganisms [16]. A recent report of growing previously non-culturable bacteria in the laboratory with a simulated natural environment may lead to isolation and characterization of several new chemical-degrading bacteria [17].

There is an increasing interest and need to developing safe, convenient and economically feasible methods for pesticides remediation [18,19]. For this reason, several biological techniques, such as bioremediation and phytoremediation, have been developed [20]. Currently, bioremediation is one of the most environmentally safe and cost-effective methods of decontamination and detoxification of a pesticide-contaminated environment [21]

### **Materials and Method:**

#### **Collection of soil samples**

Soil samples were collected from 2 agricultural areas of Beed District, Maharashtra, with history of continued farming activities for more than 30 years. Surface soil from 0-15 cm were collected, placed in plastic bags, transported on ice to the laboratory and stored at 4°C until analysis. Soil samples were air-dried and sieved through a 10 mm mesh prior to bacterial screening.

#### **Isolation, Identification and Screening of fungus:**

The collected soil samples were amended with 25 ppm of each pesticide mixed thoroughly and this mixture is directly incubated at room temperature for about two weeks. The pretreated soil samples were washed with distilled water and allowed to stand still for 30 minutes. After all the soil debris has settled down, the supernatant was decanted into a sterile test tube and serially diluted. Dilutions below  $10^{-6}$  were plated in Potato Dextrose Agar (PDA) +pesticide medium. After 6-7 days of incubation a number of fungal strains were observed on the plate. The most prominent fungus was selected and identified as *Aspergillus* sp. The isolated fungal colonies were transferred on to the PDA slants and afterwards once again tested to grow in presence of pesticides by culturing them in PDA + pesticide medium, cultures showing highest degree of degradation were selected for further studies.

#### **Bioremediation Assay:**

To study the bioremediation of pesticide using *Aspergillus* sp., two different culture media were prepared in triplicate – medium containing PDA and 0.5 percent (w/w) of pesticide and medium containing PDA, 0.5 percent (w/w) of pesticide inoculated with the spores of *Aspergillus* sp. The plates were then maintained for about 8 days at 28°C in an incubator.

#### **Preparation of sample for GC-MS analysis:**

An aliquot of 15 g of the culture medium (both control and experimental) was mixed with 50 ml of ethyl acetate for 2 minutes. Then 50 g of anhydrous sodium sulfate was slowly added

with agitation. The mixture was filtered through a Whatman’s filter paper No. 1 and the liquid was suspended in a separating funnel. This process was repeated again with another 10 ml of the same solvent. The extract was condensed in an evaporator and redissolved in 10 ml of cyclohexane to obtain the sample ready to be injected into the gas chromatograph unit.

### Result and Discussion:

During this study four (04) fungal isolates were found to show significant ability to carry out the degradation of selected pesticides, these cultures were identified as *Aspergillus niger*, *Ganoderma austral*, *Trichosporon* , *Verticillium dahaliae* .

*Aspergillus niger*, has shown maximum 59% degradation of Endosulfan, and it has degradaed 29% of Lindane, it was not effective against other pesticides.

*Ganoderma austral*, has shown highest 61% degradation for Lindane whereas for Chlorpyrifos degradation was only 16%.

*Trichosporon* sp., has given highest degradation of Chlorpyrifos at 55%, and for Endosulfan and , Lindane it was 10% and 7% respectively.

For *Verticillium dahaliae*, maximum degradation of 64% was seen for Chlorpyrifos, whereas for Endosulfan it was 8%, , for Malathion it was 10% and for Lindane it was 10%.

**Table 1 : Isolation of Microorganisms from the pesticide contaminated soil samples**

S.No.	Isolates	Identified cultures
1	F24	<i>Aspergillus niger</i>
2	F43	<i>Ganoderma austral</i>
3	F79	<i>Trichosporon</i> sp.
4	F102	<i>Verticillium dahaliae</i>

**Table 2 : % Pesticide degradation by the isolated cultures**

S. No .	Microorganism	Methyl parathion	Endosulfan	Isoproturon	Chlorpyrifos	Igepal CO-210	Dimet oate,	Malat hion	Diazi non	Lind ane
1	<i>Aspergillus niger</i>	--	59	--	--	--		--		29
2	<i>Ganoderma austral</i>	--	--	--	16	--		--		65
3	<i>Trichosporon</i>	--	10	--	55	--		--		07
4	<i>Verticillium</i>	--	08	--	64	--		10		10

dahaliae									
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