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DEGRADATION OF DIMETHOATE AND PYRETHROID BY USING FUNGAL STRAINS ISOLATED FROM THE RHIZOSPHERE OF *JUGLANS REGIA L.* IN THE NORTHERN REGION OF JAMMU AND KASHMIR, INDIA

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ABSTRACT

Indiscriminate use of chemicals regardless of climate, soil vigor and other factors has adversely affected the environmental quality and soil ecosystem. Fungi play a key role in maintaining the biogeochemical cycles in the soil by utilizing many recalcitrant chemical compounds and some common insecticides. In the present study, many fungal species were isolated from the rhizosphere of *Juglans regia L*. in the Northern regions of Jammu and Kashmir, India and tested in this insecticide utilization-screening programme. Among the isolated strains, only *Trichoderma koningii, Penicillium notatum, Aspergillus terricola and A. niger* were successful in degrading the supplemented insecticides viz., dimethoate and pyrethroid. The strains exhibited the optimal growth in the temperature range of 20-30^{0}C. The two insecticides were provided as the sole source of carbon and nitrogen in the Potato Dextrose Agar (Hi Media). *Trichoderma koningii* used dimethoate as carbon source at a faster rate compared to that of pyrethroid as the nitrogen source. However, *Penicillium notatum* and *Aspergillus niger* used dimethoate both as carbon and nitrogen sources. *Trichoderma koningii* and *Aspergillus niger* utilized pyrethroid as carbon source more vigorously than *Penicillium notatum* and *Aspergillus terricola.*

Key words: Dimethoate, Pyrethroid, *Juglans regia,* Carbon, Nitrogen, Xenobiotics, Biodegradation, Growth pattern, *Trichoderma koningii, Penicillium notatum, Aspergillus terricola and A. niger.*

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INTRODUCTION

Biological decomposition of pesticides is the most important and effective way to remove these compounds from the environment. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule[1,2]. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders[3]. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it nontoxic. The biotransformed pesticide is released into the soil, where it is susceptible to further degradation by bacteria[4]. Synthetic pyrethroid (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate) having molecular formula $C_{22}H_{19}Cl_2NO_3$ and dimethoate *(O,O*-Dimethyl-*S*- (*N*-methyl carbamoyl methyl phosphorodithioate; 2-Dimethoxy phosphinothioylthio-*N*-methylacetamide) having molecular formula $C_5H_{12}NO_3PS_2$, have been widely used for controlling many pests in agriculture, forestry, horticulture, animal and public health[5]. Extensive applications not only result in pest resistance to these insecticides, but also may lead to environmental issues and human exposure. Fungi are the key drivers to maintain the ecological balance of the nature. Some fungi may grow in environmental conditions that are too extreme for survival. The stresses include extreme osmotic potential, heat, temperature and lack of organic compounds. A huge array of organic molecules and other xenobiotics are amenable to digestion by one or other fungus, acting alone or in unison[6,7]. With the increasing practice of applying various types of chemicals in the soil to increase the productivity, there has been a trend in losing the fertility of soil in turn. One of the reasons for this decline is the accumulation of recalcitrant xenobiotics in the soil. To remove such xenobiotics, application of microbes with unique enzyme system capable of decomposing such genotypes has been felt very important. Pesticide contamination of ground and surface waters has been well documented worldwide (Table 1) and constitutes a major issue that give rise to concerns at local, regional, national and global scales[8,9,10].

Table 1

Residues of pesticides in ground and superficial waters found in some countries according to the available data (Country wise detected pesticides Levels)

Country	Detected pesticides	Levels	Water	Reference
Portugal	Lindane,atrazine, simazine,dimethoate, metribuzin,endosulfan, prometryn,metolachlor	0.22–17 µg L-1	Ground water	Barcelo [11]
Netherlands	Atrazine, simazine, dieldrin, propazine and lindane (+ -HCH)	00-200 ng L-1	Ground water	Maanen *et al*.,[12]
Greece	Lindane (γ-BHC), chlorpyrifos,propachlor	0.005-0.01 µg L-1	Ground water	Karasali *et al*.,[13]
Canada	Alachlor, metalaclhor, atrazine, metribuzin, cianazina.	0.17–0.34 µg L-	Superficial	Goss *et al*.,[14]
England	Lindane (α+β-HCH),	5.5-160	Superficial	Fatoki and

	heptachlor, Aldrin, γ-Chlordane, endosulfan, dieldrin, endrin, 2,4'-DDT, etc.	ng L-1		Awofolu [15]
South Africa	Lindane (α+β-HCH), heptachlor, aldrin, γ-Chlordane, endosulfan, dieldrin, endrin, 2,4'-DDT, etc.	6–80 ng L-1	Superficial	Fatoki and Awofolu[15]
Brazil	Alachlor,atrazine, chlorothalonil,endosulfan, simazine, metribuzin, monocrotofos, malathion, chlorpyrifos, metribuzin, etc.	0.001-0.174 µg L-1	Superficial, River, Lakes	Laabs *et al*.[16]
Hungary	Acetochlor,atrazine, carbofuran, diazinon, fenoxycarb, metribuzin, phorate, prometryn, terbutryn, and trifluralin	*59%	Superficial	Maloschik *et al*.,[17]

**** Percentage of the samples containing pesticide***

MATERIALS AND METHODS

Northern regions of Jammu and Kashmir, India, was air dried and sieved. 1 gram of the soil was taken from each sample and was serially diluted up to 10^{-6} dilutions. After completion of serial dilutions, 0.5 ml of soil solution from each test tube was inoculated on three types of petri plates containing Potato Dextrose Agar (Hi Media), Malt Agar Medium (Hi Media) and Czapek-Dox Agar Medium (Hi Media). Chloramphenicol was added to the media as 250mg/100ml to check the growth of bacteria. Inoculated petri plates were kept in incubator (adjusted at a temperature of 25^0C) for a period of 3-5 days for fungal culturing. Sub culturing was done in order to get the pure colonies. Pure cultures were periodically transferred onto fresh medium to allow continuous growth and viability of fungi. Many fungal species were isolated, identified under the research microscope (Olympus; CH 20i) and tested to degrade the insecticides-pyrethroid and dimethoate at different temperatures and pH values for a period of 7 days. These strains were transferred on the PDA plates supplemented with dimethoate and pyrethroid as a sole source of carbon and nitrogen. The fungal strains used were observed for utilizing the insecticides as sole nitrogen or carbon source at a temperature of 25-28^{0}C and having a pH range of 7-8. A control was also maintained in which neither dimethoate nor pyrethroid was supplemented as a source of carbon or nitrogen. The strains of *Trichoderma koningii, Penicillium notatum, Aspergillus terricola and A. niger were* successful during the screening programme for insecticide degradation which was monitored by observing the growth and color patterns of isolates on the PDA medium supplemented with pyrethroid and dimethoate as carbon and nitrogen sources. The ultimate breakdown of organic compounds is mainly the result of microbial action. In contrast to naturally occurring compounds, synthetic compounds often have structures not generally found in natural chemicals, and therefore are poorly

The soil collected from the rhizosphere of *Juglans regia L.* from different sites of

degradable. However, many microbes (bacteria and fungi) have been isolated by utilizing selective pressure under batch and continuous culture enrichments, which are able to utilize these xenobiotic compounds as their sole source of carbon and energy[18,19].

RESULTS AND DISCUSSION

In the present study, 26 different types of fungal colony isolates were obtained. Preliminary identification of the isolated fungi was done under the research microscope (Olympus; CH 20i) on the morphological basis and growth patterns[20,21,22,23,24,25]. Isolated and identified fungi were used in the screening programme where pyrethroid and dimethoate were supplemented as a sole source of carbon and nitrogen. The results revealed that strains used can't tolerate high temperature and low and high pH. The strains were able to grow well in the temperature range of 20-30^{o}C but could not tolerate temperature higher than 40^{o}C. The trend of pyrethroid and dimethoate degradation was consistent with the growth of fungi. In the temperature range of 25-28^{o}C, the strains showed better growth within 7 days which was measured by adhesive tape sample preparation for light microscopy involving staining of slides with lactophenol cotton-blue to highlight fungal structures. Slides were examined under the microscope to determine the number of identifiable fungal mycelia and spores [26,27,28]. Samples were examined and placed into one of 4 categories: Category I or no growth category (< 10 fungal structures/ cm^2) indicates a clean surface; Category II or slow growth category (10-100 fungal structures/ cm^2) indicates light deposition of fungal structures including hyphal fragments and spores; Category III or moderate growth category (100-1,000 fungal structures/ cm^2) indicates accumulation of fungal structures; Category IV or high growth category (greater than 1,000 spores/ cm^2) indicates heavy accumulation of fungal structures and possible amplification. The presence of conidiophores and hyphae were used as indicators for the growth pattern. The results indicate that the adaptive pH value for fungal growth was between 6 and 9. The higher pyrethroid and dimethoate degradation was observed between pH 7.0 and 8.0, with the optimum value at pH 7.5. Non significant degradation was observed at extreme pH values of 5.5 and 10.0. The insecticide dimethoate has been found to be better utilized as nitrogen or carbon sources preferably as carbon source than pyrethroid by some of the fungi viz., *Trichoderma koningii, Penicillium notatum, Aspergillus terricola and A. niger* as shown in Plate 1, while *T. koningii* can use dimethoate as carbon source much promptly compared to that of pyrethroid as the nitrogen source. *P. notatum* and *A. niger* can use dimethoate both as carbon and nitrogen sources though preferably as carbon source. Plate 2 indicates that *T. koningii* and *A. niger* can use pyrethroid as carbon source more vigorously than *P. notatum* and *A. terricola.* The growth pattern and mycelia color of the tested fungi varies in two different insecticides- pyrethroid and dimethoate as shown in table 2 and 3. In general, *T. koningii* and *A. niger* can use both pyrethoid and dimethoate as carbon source much efficiently (bar graph 1and 2) and point out that rhizosphere fungi may contribute to insecticide degradation. This is supported by the ability of certain fungal genera to apparently utilize pyrethroid and dimethoate as a sole source of carbon and nitrogen.

Plate 1

Utilization of Dimethoate as carbon (A) and pyrethroid as nitrogen (B) sources by A. terricola (AT), A. niger (AN) P. notatum (PN) and T. koningii (TK).

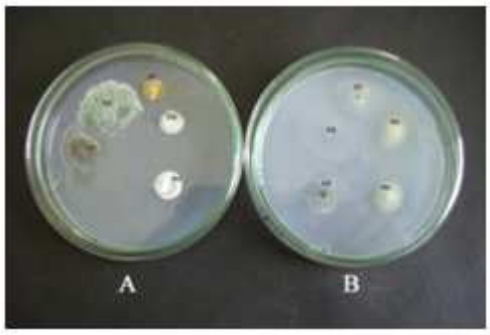


Plate 2

Utilization of Pyrethroid as carbon (A), Dimethoate as nitrogen (B) sources and control (C) by A.terricola (AT), A. niger (AN) P. notatum (PN) and T. koningii (TK).

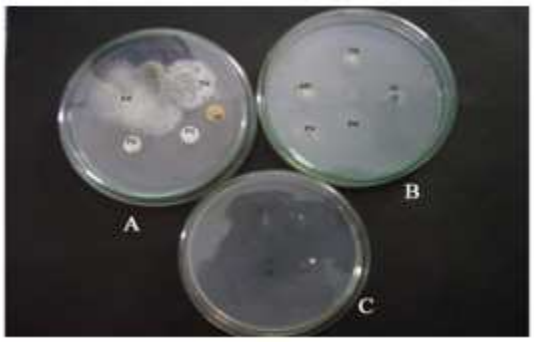


Table 2

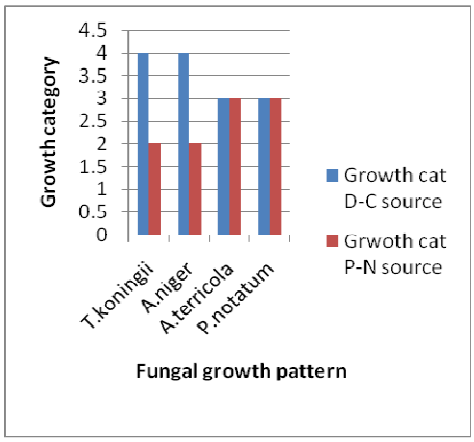
The color and growth pattern of fungal isolates when dimethoate was used as C-source and pyrethroid as N-source.

Fungi	Insecticides			
	Dimethoate as C- source		Pyrethroid as N-source	
	Color	Growth	Color	Growth
Trichoderma koningii	Luster green	High	Light green	Moderate
Aspergillus niger	Black	High	Black	Moderate
Aspergillus terricola	Golden yellow	High	Light yellow	Slow
Penicillium notatum	Blue green	High	Light green	Moderate

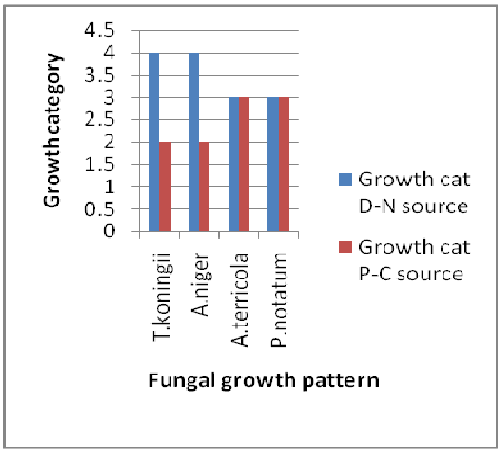
Table 3
The color and growth pattern of fungal isolates when dimethoate was used as N-source and pyrethroid as C-source.

Fungi	Insecticides			
	Dimethoate as N- source		Pyrethroid as C-source	
	Color	Growth	Color	Growth
Trichoderma koningii	Dark green	High	Light green	Slow
Aspergillus niger	White	High	White	Slow
Aspergillus terricola	Yellow	Moderate	yellow	Moderate
Penicillium notatum	White	Moderate	Blue green	Moderate

Bar Graph *1*
Dimethoate as carbon source and pyrethroid as nitrogen source.



Bar Graph 2
Dimethoate as nitrogen source and pyrethroid as carbon source.



CONCLUSION

The fate of pyrethroid and dimethoate pesticides in the environment is associated with both abiotic and biotic processes, including evaporation, photo-oxidation, chemical oxidation, bioaccumulation and microbial transformation[29]. Microbial degradation has been deemed the most influential and significant cause of pesticide removal. Therefore, biodegradation is considered to be a reliable cost-effective technique for pesticide abatement and a major factor determining the fate of pyrethroid pesticides in the environment[30]. To understand thoroughly the role of synthetic pesticides and biodegradation by microbes, it is necessary to carry out studies on isolation of fungi with metabolic mechanism, to evaluate objectively their role. The current study indicates the potentiality of *T. koningii* to use pyrethroid as well as dimethoate as carbon source along with *A. niger*. *Trichoderma koningii* is rather more vigorous than *A. niger* and hence has more potential than *A. niger* as bioremediation agent to be applicable to remove dimethoate or pyrethroid from soil. This finding is important as it suggests the possible use of the rhizosphere fungi in metabolizing pyrethroid and dimethoate and is in agreement with the findings of Sasek[31], who reported the ability of a white rot fungus *Phanerochaete chrysosporium* to metabolize a number of various important environmental pollutants. The need to remediate contaminated sites has led to the development of new technologies which emphasize the destruction of the contaminants rather than the conventional approach of disposal[32], hence possible use of fungi isolated from the rhizosphere of *Juglans regia L.* may be an important factor in this solution.

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