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Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides

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Abstract

Twenty three soil samples were characterized for the incidence of fungal strains from pesticides contaminated agricultural soils. A total of 59 fungal strains were isolated and 33 fungi were characterized using various isolation and identification methods. Soil samples were also characterized for physiochemical properties. The isolated fungal strains were successfully identified belonging to the phylum ascomycota (7 genera), deuteromycota (2) and zygomycota (1). Alternaria, Aspergillus, Drechslera and Fusarium were predominant genera. Curvularia, Exserohilum, Humicola, Rhizopus and Torula were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used incubation conditions. Such strains were designated as Mycelia sterilia. Further, these species will be used in biodegradation of commonly used pesticides.

Key words: Agricultural soil, ascomycota, deuteromycota, zygomycota, mycelia sterilia, physiochemical, pesticides.

Introduction

Increasing environmental awareness has resulted in regulatory measures that aim to remedy past mistakes and protect the environment from future contamination and exploitation. These measures intend to preserve the environment and protect human health. Some of the pollutants of concern are chemical from pesticide, were banned when it was discovered that they are hazardous to human health. India is the largest consumer of pesticides among South Asian countries where maximum (44.5%) consumption of the total pesticides is used for cotton crop[1]. Pesticides get sorbed to soil and sediments owing to its hydrophobic nature. This makes it persistent in soil and sediments[2,3] and water[4,5]. This results in rapid increase in aquatic and soil environments that lead to accumulation in crop wastes[6], macrophytes[7], phyto- plankton[8], fishes[9], vegetables, milk, and milk products[10]. Pesticides and their degradation products generally get accumulated in the top soil and influence not only the population of various groups of soil microbes but also their biochemical activities like nitrification, ammonification, decomposition of organic matter and nitrogen fixation[11].

In our country, about 99 per cent of the pesticides are imported in bulk and in concentrated form (based on 1996 statistic). They are diluted and/or mixed with other chemicals by local manufacturers to obtain the formulation desired for local conditions. Unfortunately, in many cases, these compounds are also persistent in nature. Long after their use has been discontinued, these chemicals remain in soils and sediments where they can enter the food chain directly or percolate down to the water table. Once accumulated in the groundwater, these pollutants can enter drinking water wells and cause health problems.

Indirect accumulation in higher tropic level organisms, such as mammals, may cause health problems over time because of the increasing levels of toxic compounds within the body. There are two main reasons that these compounds persist in nature. First, the conditions necessary for their biodegradation are not present. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site[12]. If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavorable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation[13].

However, there are some microorganisms that can survive in pesticide contaminated site. Pesticide may affect soil microbial populations, stimulating growth of certain microorganisms and exerting toxic effects and inhibiting growth of others. So, identification and characterization of these microbial species is important to study about its potential candidate used in bioremediation. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source[14, 15], rendering the contaminants harmless or less toxic products in most cases. Therefore, in present investigation an attempt has made to isolate and characterize fungal strains from pesticides polluted sites.

Material and Methods

Collection of soil samples: Twenty three soil samples collected from different sites having history of repeated pesticides application were used for the isolation of fungal organisms. Samples were collected in sterile zipper polyethylene bags and stored at 4°C before processing.

Reagent and chemicals: All media components and chemicals used in the studies were of analytical grade and purchased from Hi-media laboratory Pvt. Ltd, Mumbai and Sigma–Aldrich, USA.

Physiochemical characterization of soil samples: Physiochemical parameters such as organic carbon / nitrogen, pH, water content and temperature affect microbial population density which generally decreases with depth as a function of the availability of organic carbon and molecular oxygen, parameters which typically decrease with depth. Temperature and color of the soil samples was recorded on the spot. Moisture content, pH and % organic carbon and % organic nitrogen was measured according to standard procedure as given below:

Moisture content: Soil samples (10 g) collected from pesticides contaminated fields were dried at 60 ^{0}C for 72 hrs. in an oven and then the moisture content was calculated[16]. Dry weight of the sample was taken till it showed its constant weight. The percent moisture was expressed as follows:

$$\text{Moisture \%} = \frac{W_1 - W_2}{100} \times 100$$

Where W_1 = Weight of soil before oven drying
W_2 = Weight of soil after oven drying

pH of soil sample: Soil samples were dried at 60 ^{0}C for 72 hrs. grinded in pestle and mortar and filtered through 2 mm sieve and the sieved soil was dissolved in distilled water (2.5w/v) and vortexed for 5 minutes at 120 rpm and then pH was measured by digital pH meter[16].

Percent organic Carbon / Nitrogen: One gram soil sample was mixed in 10 ml potassium dichromate (1 N) and 20 ml concentrated H_2SO_4. Then 150 ml distilled water and 25 ml $FeSO_4$ (0.5 M) were added and the solution was titrated against potassium permanganate (0.1 N) solution to pink end point[17, 18].

$$\text{\% Organic carbon} = \frac{A - B}{C} \times 0.3 \times 1.33$$

Where: A- Volume of $K_2Cr_2O_7$ X Normality of $K_2Cr_2O_7$, B - Volume of $KMnO_4$ X Normality of $KMnO_4$, C - Weight of sample.

Soil organic nitrogen was calculated using following equation:
Organic nitrogen (%) = 0.862 × % organic carbon

Isolation and characterization of fungi: Serial dilution agar plating[19], Warcups soil plate and Waksman Direct inoculation methods were employed for the isolation of soil microbes; suspension was diluted up to 10^{-5}. The aliquots were cultured for isolation of fungal strains on Czapek Dox Agar ($NaNO_3$ 2.0 g/L, KCl 0.5 g/L, K_2HPO_4 1.0 g/L, $MgSO_4$. $7H_2O$ 0.5 g/L, $FeSO_4$. $7H_2O$ 0.01 g/L); and Potato Dextrose Agar (Peeled potato200.0 g/L, Dextrose 20.0 g/L) media. For primary isolation, Rose Bengal (30mg/L) was also added to the medium[20]. Three plates from each soil samples were incubated for 24-96 hrs at 25$\pm$2^{o}C, and each morphologically unique fungal colony was sub-cultured and purified using standard techniques.

The fungal species were identified and characterized based on their morphological characters and microscopic analysis by using taxonomic guides and standard procedures[21, 22, 23, 24]. The morphological characteristics evaluated were: colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production etc. The procedure of isolation and characterization of fungal species is shown in flow chart.

Growth rate: Growth rate (kd) was determined with the following equation: kd= D/T, where D is the experimentally determined average diameter of the fungal colony in mm exclusive of the diameter of the inoculum (8 mm) and T= time period.

Results and Discussion

Characterization of physiochemical parameters of soil samples: The physiochemical properties of soil samples used for isolation of microbial species were analyzed in the present study. The color of soil samples was brown to black, with variation in pH (7.82 - 8.65). The temperature of the soil was high (30.2 – 33.2^{o}C) with great variation in percent moisture content (0.35 – 0.95), percent organic carbon (0.2568 – 0.4125) and percent organic nitrogen (0.2213 – 0.3555). Table-1 results revealed that the soil samples from various sites shown heterogeneity in physicochemical parameters.

Soil properties like organic matter, pH and moisture content affect the density and diversity of microbes in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. The moisture content in soil acts as solvent and is essential for microbial functioning. A certain minimum level of organic matter and moisture content is essential to ensure the presence of an active microbial population in the soil[25]. Long term storage and usage of pesticides can increases organic carbon content, pH and heavy metals accumulation in soils[26-28]. In the present study, the important physicochemical properties of the soils used for the evaluation of natural fungal density, were determined.

Isolation and characterization of fungal strains: A total of 59 fungal isolates were obtained from the analysis of 23 soil samples taken from pesticide contaminated soils by soil dilution agar plating, Warcups soil plate and Waksman Direct inoculation methods. All fungal isolates were obtained in pure cultures by using standard techniques. The photomicrographs of all the fungal isolates were taken that helped in identification of the fungal isolates. The cultural characteristics and the sporulating structures of these isolates are shown in figure-1.

Thirty three fungal isolates were identified as, *Aspergillus allhabadii, A. aultaceus, A. candidus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. oryzae, A. sclerotiorum, Aspergillus* sp, *A. sulphureus, A. sydowii, A. terrus, Alternaria brassicola, A. citri, Alternaria* sp., *Alternaria* sp., *Alternaria* sp., *A. vitis, Curvularia brachyspora, Exserohilum turcicum, Drechslera australiensis, D. halodes, D. hawaiiensis, Drechslera* sp., *Humicola insolens, H. brevis, Fusarium acuminatum, Fusarium* sp, *Rhizopus* sp, *Torula herbarum* and *Ulocladium* sp.

The isolates from agricultural soils were identified as filamentous fungi belonging to the phyla Ascomycota (7 genera), Deuteromycota (2) and Zygomycota (1). Most of the fungal isolates were identified to the species level. *Alternaria, Aspergillus, Drechslera* and *Fusarium* were predominant genera. *Curvularia, Exserohilum, Humicola, Rhizopus* and *Torula* were the most frequently isolated genera. Rests of the strains were not identified owing to the lack of sporulating structures under presently used culture conditions. Such strains were designated as Mycelia sterilia. In such soil fungi may occur either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions[29]. Some of the species have been reported as common isolates from pesticides polluted soil and used for biodegradation of xenobiotics[30-33]. The fast growing and concurrent appearance of these species revealed that these fungal strains are much adapted to the pesticide contaminated soil sites and utilize the pesticides/xenobiotic compounds as novel growth and energy substrate. The fungal species which concurrently appeared will be used further for the degradation of the commonly used pesticides.

Conclusion

From the present investigation it is concluded that a total of fifty nine fungal strains were recovered from pesticide contaminated agricultural soil. Thirty three fungal species of ten genera belonging to the phyla ascomycota, deuteromycota and zygomycota were successfully identified after staining with lactophenol cotton blue based on their morphological characters and microscopic analysis. Most of the fungal species were able to grow efficiently and appear concurrently which means these indigenous fungi have the capacity to adapt to xenobiotic compounds as novel growth and energy substrate. Therefore, these fungi have a potential to degrade xenobiotic compounds which will be tested in further study for degradation of commonly used pesticides.

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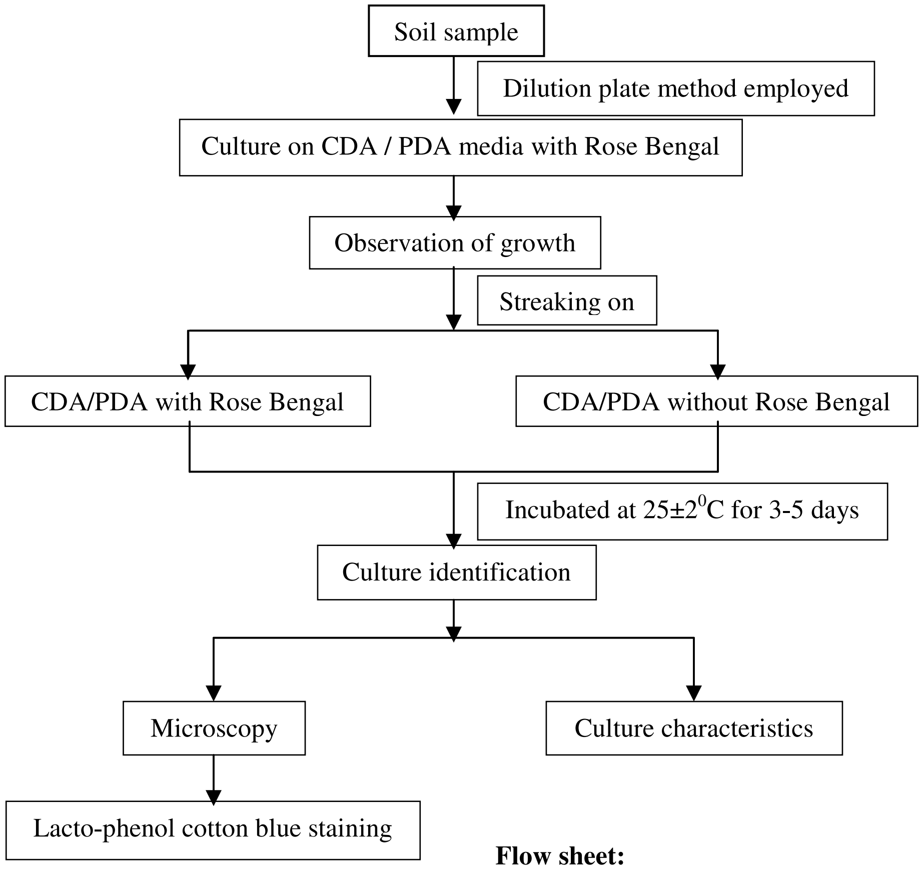

Flow sheet:
Protocol for isolation and characterization of fungal strains

Table-1
Physiochemical parameters of soil samples

Sample	Color	Temperature (^{o}C)	pH	% Moisture	% Carbon	% Organic Nitrogen
A1	Black	30.2	8.26	0.62	0.2568	0.2213
A2	Gray	30.5	8.56	0.37	0.2615	0.2254
A3	Gray	31.0	8.56	0.52	0.2687	0.2316
A4	Black	32.4	8.55	0.51	0.2589	0.2231
A5	Dark Gray	30.3	8.43	0.62	0.261	0.2249
B1	Brown	30.5	7.96	0.42	0.3125	0.2693
B2	Brown	32.6	8.57	0.7	0.3528	0.3041
B3	Gray	31.2	8.55	0.59	0.3687	0.3178
C1	Black	30.5	8.65	0.38	0.3921	0.3379
C2	Dark Gray	30.6	8.57	0.61	0.3872	0.3337
C3	Gray	32.3	8.63	0.51	0.3625	0.3124
C4	Black	30.7	8.44	0.36	0.3569	0.3076
D1	Brown	31.3	8.35	0.58	0.3458	0.298
D2	Brown	30.4	8.26	0.95	0.3631	0.3129
D3	Gray	33.2	8.4	0.9	0.4125	0.3555
D4	Gray	30.6	8.26	0.78	0.3267	0.2816
E1	Black	31.1	8.41	0.35	0.2891	0.2492
E2	Brown	30.5	7.82	0.69	0.3128	0.2696
E3	Gray	30.2	8.61	0.8	0.2938	0.2532
F1	Dark Gray	31.2	8.3	0.46	0.3426	0.2953
F2	Dark Gray	33.1	7.86	0.73	0.3524	0.3037
F3	Gray	30.7	8.18	0.43	0.3618	0.3118
F4	Gray	31.1	8.43	0.7	0.3705	0.3193

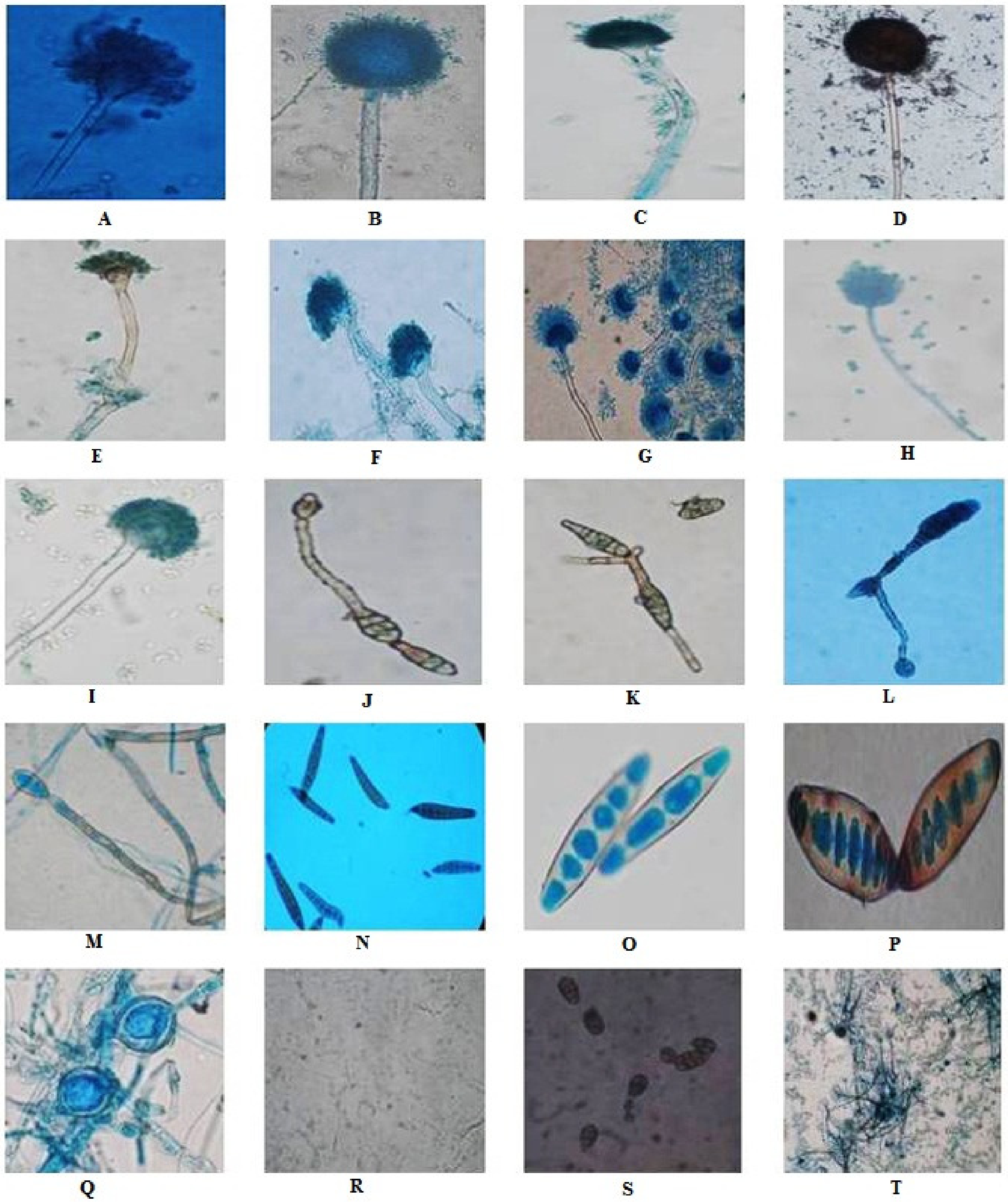

Figure-1

A: *Aspergillus allhabadii*; B: *A. alutaceus*; C: *A. flavus*; D: *A. niger*; E: *A. ochraceous*; F: *A. sclerotiorum*; G: *Aspergillus* sp.; H: *A. sydowii*; I: *A. terrus;*. J: *Alternaria* sp.; K: *Alternaria* sp.; L: *Alternaria* sp.; M: *Curvularia brachyspora*; N: *Drechslera halodes*; O: *Drechslera* sp.; *P*: *Excerohilum turcicum*; Q: *Fusarium acuminatum*; R: *Fusarium* sp.; S: *Ulocladium* sp.; T: *Rhizopus* sp.