بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

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ECOFRIENDLY BIODEGRADATION OF SYNTHETIC DYES

By

SAMAR AL REFAEY ABDELSALAM AHMED
B.Sc. Agric. Sc. (Agric. Microbiology), Fac. of Agric., Ain Shams Univ., 2010
M.Sc. Agric. Sc. (Agric. Microbiology), Fac. of Agric., Ain Shams Univ., 2017

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This thesis for Ph.D. degree has been approved by:

Dr. Wafaa Mohamed Abd El-Rahim ……
Researcher Prof. of Environmental Microbiology, Agricultural and Biological Research Institute, National Research Centre (NRC).

Dr. Rawia Fathy Gamal ……
Prof. Emeritus of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

Dr. Khadiga Ahmed Ahmed Abou-Taleb ……
Prof. of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

Dr. Abd El-Wahab Mohamed Abd El-Hafez ……
Prof. Emeritus of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

Date of Examination: / / 2022

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Under the supervision of:

Dr. Abd El-Wahab Mohamed Abd El-Hafez
Prof. Emeritus of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University (Principal Supervisor).

Dr. Khadiga Ahmed Ahmed Abou-Taleb
Prof. of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

Dr. Samah Hashem Abo-Hussein
Lecturer of Agricultural Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University.

ABSTRACT

Samar Al-Refaey Abdelsalam "Ecofriendly biodegradation of synthetic dyes" Unpublished Ph.D. Thesis, Department of Agric. Microbiology. Faculty of Agriculture, Ain shams University, 2022.

The present study aimed to apply the microbial technique to decolorize and detoxify dye. Decolorization of mono-azo dyes (reactive red 189 (RR189), reactive orange 13 (RO13), and reactive violet 5 (RV5)), di-azo dyes (reactive black 5 (RK5) and (reactive blue 172 (RB172)), and anthraquinone dye (reactive blue 19 (RB19)) by 239 microbial isolates (176 bacterial isolates and 63 fungal isolates) isolated from contaminated soil and effluent samples. Among 239 dyes decolorizing microbial isolates, ten isolates (35RB, 2GB, 15VB, 2GF, 1VF, 16KB, 7KF, 15NB, 7NF, and 5BF) were chosen as the most efficient dyes decolorizing isolates, with dyes removal concentrations ranging from 57.00 to 95.80 ppm. The germination of broccoli (*Brassica oleracea* var. *italic*) and lettuce (*Lactuca sativa* var. *capitata*) seeds were examined for the toxicity of tested dyes and 10 microbial dyes metabolites. According to the data, dye metabolites are not toxic to plants. Out of ten dye decolorizing isolates, two pioneer fungal isolates (5BF and 7KF) were chosen as having the highest percentage of RB172 or RK5 dyes decolorization and seed germination. These isolates were identified to be phenotypically and genotypically identical to *Aspergillus flavus* 5BF and *Aspergillus niger* 7KF, respectively, and were accredited in GenBank as *Aspergillus flavus* isolate MICSR2021 (accession no. OM681337) and *Aspergillus niger* strain MICR2021 (accession no. OM681337) (accession no. ON010743). Thin-layer chromatography revealed that these strains were aflatoxins-free. After 72 hours of incubation, these *A. flavus* isolate MICSR2021 and *A. niger* isolate MICR2021 strains degraded RB19 or RK5 dyes in a medium containing mannitol or glycerol as carbon sources and NH_4Cl or NH_4NO_3 as nitrogen sources. Laccase and manganese

peroxidase (MnP) activities were found to be active during dye decolorization by tested strains. Furthermore, metabolites produced by the treatment of RB172 or RK5 dyes with *A. flavus* and *A. niger* were analyzed using gas chromatography-mass spectrometry. Cyclohexene, 1,3,5-triazine-2,4-diamine-6chloro-N-ethyl, and 2,4-di-tert-butylphenol were the RB172 metabolites. RK5 metabolites included benzene, 1,3,5-trimethyl, 1,3,5-triazine-2,4-diamine, 6-chloro-N-ethyl, and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione. In a pot experiment, fungal metabolites were applied in the agricultural field to improve the vegetative growth of the faba bean *(Vicia faba*). The results revealed that the fungal metabolites were not toxic to the plant and increased plant growth parameters (root length, root dry weight, shoot length, shoot dry weight, and leaves number per plant) as water treatment (control).On the other hand, dyes were harmful to the growth of plants.

The *A. flavus* and *A. niger* strains were safe when removing dye from a synthetic medium with high efficiency, and their metabolites had no negative influence on the environment.

Keywords: *Aspergillus flavus*, *Aspergillus niger*, Biodegradation, Decolorization, Degrading enzymes, Phytotoxicity, Synthetic dyes and GC-MS.

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