Molecular and Biochemical Studies on Some Aflatoxin-Producing Fungi in Egypt

A Thesis Submitted by

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(M. Sc. in Biochemistry, 2006)

For the Awerd of the Doctor of Philosophy Degree in

Biochemistry

Under Supervision of

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2014

Approval Sheet

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بِسْمِ اللّهِ الرَّحْمـَنِ الرَّحِيمِ

(رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِكَ فِي عِبَادِكَ الصَّالِحِينَ)

صدق الله العظيم

﴿ سورة النمل– آية ١٩﴾

I declare that this dissertation has been composed by myself and the work herein has not been submitted for a degree at this or any other university.

Heba Yousef Rizk Mohamed

I dedicate this work to my mother's soul, my father and my family. I have to thank them for supporting me with kindness and patience.

Heba Yousef Rizk Mohamed

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ABSTRACT

Aflatoxin producing fungi are serious plant pathogens attacking several economical host plants resulting in remarkable defect in economic quality. *Aspergillus flavus* is imperfect filamentous fungus that has a worldwide distribution in the environment. The isolation of *A. flavus* from corn and peanut samples from several Egyptian governorates is an indication for the wide spreading of this fungus in Egypt.

Molecular and biochemical techniques were applied for the detection of those isolates. The biochemical studies include quantification of aflatoxin levels in *A. flavus* isolates using HPLC. Various approaches were evaluated for the control of *A. flavus* growth and aflatoxins production using hydrogen peroxide and gallic acid. The molecular techniques include random amplified polymorphic DNA (RAPD) and quantitative real-time PCR amplification (qPCR).

The obtained results showed that all screened isolates of *A. flavus* have aflatoxins producing ability. Quantification of aflatoxins levels in *A.flavus* isolates using HPLC showed that isolates of *A. flavus*, which isolated from peanut has ability to aflatoxin production more than those isolated from corn, and aflatoxin B_1 was produced in all isolates. Analysis of DNA fingerprinting indicated that there was a considerable genetic variation within the *A. flavus* isolated from corn and peanut depending on the primer used. Amplification of ITS region using ITS4-ITS5 primers produced a unique band of 600bp. Specific PCR used for the detection of *Aspergillus flavus* using specific primer pair (PEPO1-PEPO2) that produces characteristic band size 200 bp. Detection of nor-1 gene in *A. flavus* isolated from corn and peanut using specific primer (NOR1-NOR2) produces single band at 400bp. Expression of the Nor-1 gene is the main factor responsible for AFs production. For this reason analysis of Nor-1 gene using real time polymerase chain reaction (qPCR), in nine samples from peanut, corn and soil, was applied. The results showed that DNA concentrations ranged from 15×10^{-6} to 8×10^{-7}ng by using Nor-1and Nor-2 primers. Aflatoxins are highly resistant to various physical and chemical treatments, so, addition of H_2O_2 and gallic acid to the growth medium was found to be more effective in inhibiting aflatoxin production. In conclusion, the current study provides guidelines for monitoring the quality control of agricultural commodities and management strategy for controlling growth rate of *A. flavus* and aflatoxin production.

***Keywords*:** Aflatoxin, *Aspergillus flavus*, real-time PCR, HPLC, DNA fingerprinting, ITS.

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LIST OF ABBREVIATIONS

AF	Aflatoxin
AFLP	Amplified fragment length polymorphism
AOAC	Association of official analytical chemists
APA	Aflatoxin producing ability
BP	Base pair
CAM	Coconut agar medium
CAT	Catalase activity
DNA	Deoxyribonucleic acid
dNTP	2'-deoxynucleoside 5'-triphosphate
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immunoabsorbent assay
EtBr	Ethidium bromide
FDA	Food and drug administration
GC-MS	Gas chromatography-mass spectrometry
HPLC	High performance liquid chromatography
ITS	Internal transcribe spacer
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PKA	Palm kernel agar
PPb	Part per billion
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
Rt-PCR	Real time polymerase chain reaction
SDS	Sodium dodecyl sulfate
TAE	Tris-Acetate –EDTA
TE	Tris-EDTA
TLC	Thin layer chromatography
Tm	Melting temperature
UPGMA	Un-weighed pair group method using average linkages
UV	Ultraviolet