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Rapid immunological assays for detection of aflatoxin B_1 in animal feed and its products

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(وَأَنْزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)

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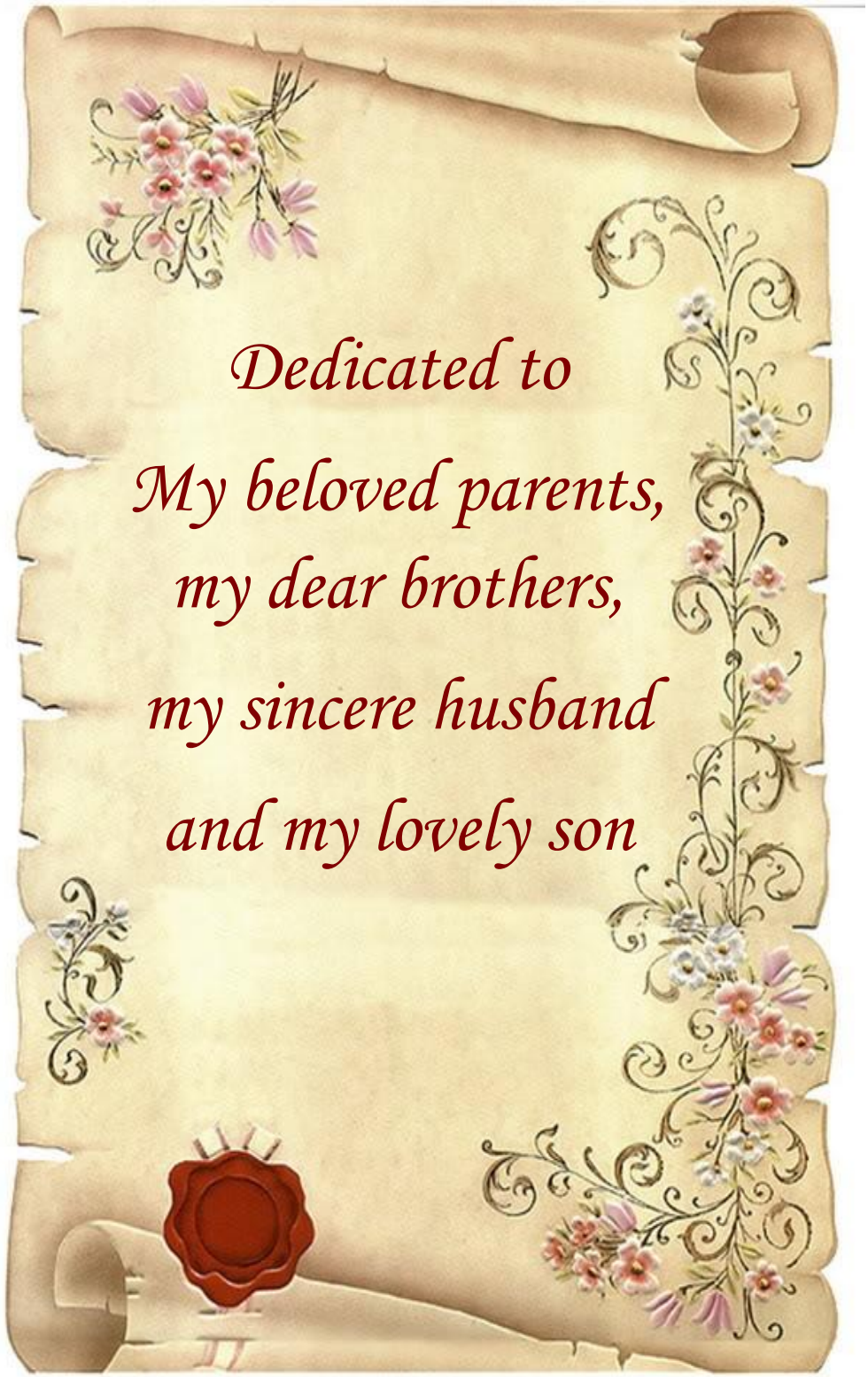
ABSTRACT

Fungi and their toxins are extensively widespread as those fungi are ubiquitous in nature. Aflatoxin B_1, a fungal secondary metabolite excreted mainly by toxigenic *Aspergillus flavus*, considered as the most dangerous naturally occurring toxin, has carcinogenic effect on both human and animals. Aflatoxin B_1 was successfully produced from local toxigenic *Aspergillus flavus* strain on YES medium and its amount was determined using HPLC. Aflatoxin B_1 is a toxic fungal metabolite of low molecular weight, and hence is devoid of antigenicity. Those toxins also lack a reactive group for coupling with a macromolecule carrier for antibody production. Therefore, the main goal of this study was to produce polyclonal antibody (IgG) against aflatoxin B_1 through immunization of New Zealand white rabbits by the prepared antigen (AFB_1-BSA Conjugate). The produced polyclonal antibodies were used in the production of a diagnostic product that was tested against spiked and naturally contaminated samples. Spiked samples were tested using both sandwich and competitive ELISA. In case of sandwich ELISA, the recovery % obtained from finished cattle feed samples ranged from 80.3% and 96% while in meat samples AFB_1 ranged from 78.1% and 93%. For competitive ELISA, the mean recovery % of AFB_1 obtained from finished cattle feed samples ranged from 88.7% and 97% , while in meat samples it ranged from 89.6% and 103%. Using competitive ELISA, The produced product gave promising results and was efficient in detecting AFB_1 in the examined naturally contaminated samples. In conclusion, the diagnostic product produced in the present study is simple and economic. ELISA procedure that can be used to screen aflatoxin B_1 in variety of samples (animal feed samples, meat and meat products). Thus, it is possible to screen large number of samples simply and inexpensively for the presence of aflatoxin B_1.

Key words: Aflatoxin B_1, HPLC, Polyclonal antibody, ELISA, TLC, Animal feed, Meat, Meat products.


Dedicated to
My beloved parents,
my dear brothers,
my sincere husband
and my lovely son

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOS

LIST OF ABBREVIATIONS

A.	Aspergillus
Ab	Antibody
AFB_1	Aflatoxin B_1
AFLA	Aflatoxin
AFM_1	Aflatoxin M_1
Ag	Antigen
$\mathbf{a_w}$	Water activity
BSA	Bovine serum albumin
cfu/g	Colony forming unit per gram
conc.	Concentration
CV	Coefficient of variation
Dis.	Distilled
DON	DeOxyNivalenol
EDPC	1- Ethyl - 3 (3- dimethyl aminopropyl) carbodiimide
ELISA	Enzyme Linked Immunosorbent Assay
fn	False-negative values
fp	False-positive values
F.	Fusarium
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
fig.	Figure
$FUMB_1$	Fumonisin B_1
gm	Gram
HRP	Horseradish peroxidase
HPLC-FLD	High Performance Liquid Chromatography with Fluorescence detector
IC_{50}	Half maximal inhibitory concentration
IgG	Immunoglobulin G
IC - ELISA	Indirect competitive ELISA
Kg.	Kilogram
L	Liter
LOD	Limit of detection
LPCB	Lactophenol cotton blue

MeOH	Methanol water
Max.	Maximum
Min.	Minimum
mg.	Milligram
µg.	Microgram
ml	Milliliter
mM	Millimolar
MTLs	Maximum tolerated levels
NB	Nota bene
ND	Not detectable
nm	nanometer
NMR	Nuclear magnetic resonance
No.	Number
OPD	Ortho–phenylenediamidine
OD	Optical density
OTA	Ochractoxin A
P.	Penicillium
pAbs	Polyclonal antibodies
PBS	Phosphate buffer saline
PDA	Potato dextrose agar
pH	Hydrogen ion concentration
ppb	Parts-per billion
pps	Phosphate buffered saline
SDA	Sabouraud dextrose agar
SE	Standard error
spp.	Species
T-2	Trichothecene toxin
TLC	Thin layer chromatography
tn	True-negative values
tp	True-positive values
UV	Ultra violet
WHO	World Health Organization
YES	Yeast extract sucrose media
ZON	Zearalenone