MYCOTOXIGENIC FUNGI AND MYCOTOXINS CONTAMINATING RED RICE AT CONSUMER LEVEL IN SELANGOR, MALAYSIA

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Field of Study : **Food Mycology**

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ABSTRACT

Red rice is a product of rice fermentation by fungi from the genus *Monascus* notably the *M. purpureus*, *M. ruber*, and *M. pilosus*. Although the traditional method of its preparation does not necessarily adhere to safety and health regulation, it is still widely consumed by Chinese as traditional herbal remedy believed to possess outstanding and diverse industrial, clinical, and food applications. The present study was undertaken to determine the occurrence of mycotoxigenic fungi and mycotoxins present on red rice at consumer level in Selangor, Malaysia. In this study, 50 samples of red rice were obtained from nine administrative districts of the state Selangor. Twenty nine were stored at cold temperature (refrigeration) and 21 at room temperature. For fungal isolation and enumeration, samples were plated on Dichloran Rose Bengal Chloramphenicol (DRBC) and Czapek Dox (CD) agars by direct- and dilution-plating techniques. Identification of fungi was done morphologically (micro and macro) according to published manuals. There was no significant difference ($p \geq 0.05$) in Colony Forming Unit (CFU) readings between cold and room temperatures with the highest being 2.1 x 10^6 CFU/g and the lowest 1.4 x 10^4 CFU/g. The readings were above the permissible limit set by Malaysian Ministry of Health of not more than 10^2 CFU/g, and International Commission for Microbiological Specification of Foods of 10^2 to 10^5 CFU/g. Besides the starter fungi *Monascus* spp. which were present in 50 samples (100%), three other species were also found to be predominant namely, *Penicillium chrysogenum* (62%), *Aspergillus niger* (54%), and *Aspergillus flavus* (44%) which were classified as potent mycotoxigenic fungi in foods and feedstuffs. Mycotoxins were determined by enzyme-linked immunosorbent assay (ELISA). Citrinin, aflatoxin, and ochratoxin-A were evaluated based on the presence of producer fungi in the samples. Citrinin was present in 50 samples (100%) at 229.50 to 20,648.75

ppb. Aflatoxin was present in 46 samples (92%) at 0.61 to 77.33 ppb. Ochratoxin-A was present in 50 samples (100%) at 0.23 to 2.48 ppb. A hundred per cent citrinin (50/50), 76.09% aflatoxin (35/46), and 0% ochratoxin-A (0/50) were present critically above the permissible limit set by Malaysian Ministry of Health of not more than 5 ppb of all mycotoxins in food, and European Union at 4 ppb for aflatoxin, and 3 ppb for ochratoxin in food. There is a slight but insignificant ($p \geq 0.05$) correlation between presence of mycotoxigenic fungi and mycotoxins as the presence of fungal contaminants cannot ultimately ascertain the presence of mycotoxins. Presence of fungal contaminants only served as an initial indicator of possibility of mycotoxins contamination. In conclusion, all red rice samples were contaminated with mycotoxigenic fungi and mycotoxins above the permissible limits for human consumption. This in turn might indicate improper production, handling, transportation, and storage practice of the commodity. It is henceforth suggested that traditional production of red rice should adhere to scientific inspection and clinical regulation, and toxicity studies on the nephrotoxic effect of citrinin produced by the starter fungi in red rice should be carried out to confirm the safety and health status of red rice.

ABSTRAK

Beras merah ialah satu produk penapaian beras oleh kulat dari genus Monascus antaranya M. purpureus, M. ruber, dan M. pilosus. Meskipun kaedah tradisional penyediaan produk ini tidak selalunya menepati panduan kesihatan dan keselamatan, ia masih lagi diambil secara meluas oleh masyrakat Cina sebagai ramuan herba yang dipercayai mempunyai khasiat tinggi dalam bidang industri, klinikal, dan permakanan. Kajian ini dilakukan untuk mengenalpasti kehadiran kulat bermikotoksin dan menentu-ukur mikotoksin dalam beras merah di peringkat pengguna di Selangor, Malaysia. Dalam kajian ini, 50 sampel beras merah diperolehi dari sembilan daerah di Selangor. Di peringkat pengguna, 29 sampel disimpan di suhu sejuk (peti sejuk), dan 21 sampel disimpan di suhu bilik. Bagi pemencilan dan pengiraan kulat, sampel dikultur pada agar Dichloran Rose Bengal Chloramphenicol (DRBC) dan Czapek Dox (CD) menggunakan teknik pemplatan terus dan pencairan. Pengenalpastian kulat dilakukan berdasarkan makro- dan mikromorfologi mengikut manual pengenalpastian. Tiada perbezaan ketara ($p \geq 0.05$) dalam bacaan Unit Pembentukan Koloni (CFU) antara sampel suhu sejuk dan suhu bilik dengan bacaan tertinggi berjumlah 2.1 x 10^6 CFU/g dan terendah berjumlah 1.4 x 10^4 CFU/g. Bacaan ini melebihi had yang dibenarkan oleh Kementerian Kesihatan Malaysia iaitu tidak melebihi 10^2 CFU/g, dan International Commission for Microbiological Specification of Foods pada julat 10^2 hingga 10^5 CFU/g. Selain ibu kulat iaitu Monascus spp. yang hadir pada 50 sampel (100%), terdapat tiga kulat lain yang juga hadir pada jumlah tinggi iaitu Penicillium chrysogenum (62%), Aspergillus niger (54%), dan Aspergillus flavus (44%) yang semuanya tergolong dalam kumpulan kulat bermikotoksin yang penting dalam makanan. Mikotoksin ditentu-ukur mengikut kaedah pencerakinan imunojerapan untaian enzim (ELISA). Citrinin, aflatoxin, dan ochratoxin-A dikenalpasti berdasarkan

kehadiran kulat pengeluar dalam sampel. Citrinin terdapat pada 50 sampel (100%) dengan bacaan 229.50 hingga 20,648.75 ppb. Aflatoxin terdapat pada 46 sampel (92%) dengan bacaan 0.61 hingga 77.33 ppb. Ochratoxin-A terdapat pada 50 sampel (100%) dengan bacaan 0.23 hingga 2.48 ppb. Seratus peratus citrinin (50/50), 76.09% aflatoxin (35/46), dan 0% ochratoxin-A (0/50) melebihi had yang dibenarkan oleh Kementerian Kesihatan Malaysia iaitu tidak melebihi 5 ppb bagi semua jenis mikotoksin dalam makanan, dan Kesatuan Eropah pada 4 ppb untuk aflatoxin, dan 3 ppb untuk ochratoxin dalam makanan. Terdapat perkaitan tipis namun tidak ketara ($p \geq 0.05$) antara kehadiran kulat bermikotoksin dan kewujudan mikotoksin kerana kehadiran kulat tidak semestinya mengesahkan kewujudan mikotoksin. Kehadiran kulat hanyalah berfungsi sebagai penunjuk awal terhadap kemungkinan wujudnya sesuatu mikotoksin tersebut. Sebagai rumusan, kesemua sampel beras merah mempunyai kulat bermikotoksin dan mikotoksin yang melebihi had yang dibenarkan dalam permakanan manusia. Ini secara tidak langsung menunjukkan bahawa proses penghasilan, pembungkusan, pengangkutan, dan penyimpanan yang dilakukan adalah tidak sempurna. Adalah dicadangkan bahawa kaedah tradisional penyediaan beras merah ini perlu mematuhi kawalan saintifik dan panduan klinikal, dan kajian mengenai kesan barah buah pinggang oleh citrinin yang dihasilkan oleh ibu kulat beras merah iaitu Monascus spp. perlu dijalankan untuk mengesahkan status kesihatan dan keselamatan Beras Merah.

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LIST OF ABBREVIATIONS

A.D	*Anno Domini* (Latin), translated as "*in the year of Our Lord"*
ca.	*circa* (Latin), translated as *"around"*
CABI	Centre for Agricultural Biosciences International
CAST	Council of Agricultural Science and Technology
CBS	Centraalbureau voor Schimmelcultures (Netherlands)
CD	Czapek Dox agar
CFU	Colony-forming unit
DNA	Deoxyribo-nucleic acid
DRBC	Dichloran Rose Bengal Chloramphenicol agar
E	E numbers, codes for food additives used in European Union
ELISA	Enzyme-linked Immunosorbent Assay
et al.	*et alia* (Latin), translated as *"and others"*
HEK293	Human Embryonic Kidney cell line
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IAC	immuno-affinity column
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
MARDI	Malaysian Agricultural Research and Development Institute
ppb	part per billion, (µg/kg)
ppm	part per million, (mg/kg)
ppt	part per trillion, (ng/kg)
RNA	Ribo-nucleic acid
TCM	Traditional Chinese Medicine
UPM	Universiti Putra Malaysia

LIST OF SYMBOLS

°C	*degree Celsius*
%	*percent*
g	*gram*, derived into ng, µg, mg, kg
k	*kilo*, prefix of a measurement unit (10^3)
µ	*micro*, prefix of a measurement unit (10^{-6})
L	*Liter*, derived into µL, mL
m	*milli*, prefix of a measurement unit (10^{-3})
n	*nano*, prefix of a measurement unit (10^{-9})
pH	*potential Hydrogen*, scale for acidity and alkalinity
psi	*per square inch*, unit for pressure

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