UNIVERSITI PUTRA MALAYSIA

AN INTEGRATED MODELLING APPROACH TO POSTHARVEST CONTROL OF Aspergillus flavus GROWTH AND AFLATOXIN PRODUCTION IN PADDY GRAINS AND RICE

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FSTM 2012 15
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CONTROL OF Aspergillus flavus GROWTH AND AFLATOXIN
PRODUCTION IN PADDY GRAINS AND RICE

By

WAEL MOUSA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

May 2012
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Chairman: Farinazleen Mohammed Ghazali, PhD

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The main aim of the present study was to develop an integrated postharvest strategy for controlling the growth of and aflatoxin production by Aspergillus flavus on paddy and rice. Initially, equilibrium moisture content of paddy was studied at 20, 30, 40 and 50°C at relative humidity (RH) between 0.113 and 0.976 using gravimetric technique. The isotherms displayed the general sigmoidal curve Type II and exhibited hysteresis where it was more pronounced at lower temperatures. At fixed RH, the sorption characteristics were temperature-dependent where the sorption capacity of the paddy increased as the temperature was decreased. Among the models assessed for their ability to fit the sorption data, the Oswin equation was the best followed by the third order polynomial, GAB, Smith, Chung-Pfost, and Henderson models. Therefore, the Oswin model was chosen to estimate the amount of water required to rehydrate dried paddy to the desired water activity ($a_w$) in subsequent studies. Thereafter, the growth of two aflatoxigenic A. flavus on paddy and aflatoxin production were studied following a full factorial design with seven $a_w$ levels between 0.82 and 0.99 and seven temperatures between 10 and 43°C. The
growth of the fungi, expressed as colony diameter (mm), was measured daily and aflatoxin production was analyzed using isocratic HPLC with a fluorescence detector. The maximum colony growth rates of both isolates were estimated by fitting the primary model of Baranyi to growth data. Three potentially suitable secondary models; Rosso, polynomial, and Davey, were assessed for their ability to describe the radial growth rate as a function of temperature and $a_w$. Both strains failed to grow at the marginal temperatures (10 and 43°C) regardless of the $a_w$ studied, and at the $a_w$ level of 0.82, regardless of the temperature. Despite that the predictions of all studied models showed good agreement with the observed growth rates, the Davey model proved to be the best predictor of the experimental data. Aflatoxins were detected at $a_w$ between 0.86-0.99 with an optimal $a_w$ of 0.98 and the optimal temperature was in the range of 25-30°C. Then, the effect of $a_w$ (0.82-0.92) and temperature (15-42°C) on the growth and aflatoxin production by A. flavus on polished and brown rice was also studied. Four secondary models were used to implicit the combined effects of $a_w$ and temperature on the growth rates. All models were validated using independent experimental data. According to the assessment indices, the performance of the Davey model in describing the experimental data was the highest, followed in decreasing order by the polynomial, Gaussian and Rosso models. The estimated optimal growth temperature was between 30-34°C. Neither growth nor aflatoxins were detected at $a_w$ 0.82 on polished rice while growth and aflatoxins were detected at this $a_w$ between 25-35°C on brown rice. The highest amounts of aflatoxins were formed at the higher $a_w$ values (0.90-0.92) and a temperature of 20°C after 21 days of incubation for both types of rice where the consistency of aflatoxin production within a wider range of $a_w$ values occurred between 25-30°C. The results also showed that brown rice tended to support higher
A. flavus growth and aflatoxin production than the polished rice. Logistic models describing the growth and aflatoxin production boundaries of A. flavus were also developed. Experiments were conducted at a$_w$ between 0.80 and 0.99 and temperature between 10 °C and 45°C on rice meal agar (RA) for duration of four weeks. The degree of agreement between the predicted and observed data in terms of concordance was > 97% and > 98% for growth and aflatoxin production, respectively. Probabilities of growth and aflatoxin production at 21 days were almost equal to those at 28 days. The polynomial logistic models that were developed were validated with data obtained from repeated experiments on paddy. The models were successfully able to predict the probabilities with concordance rates of 85.2% and 88.9% for growth and aflatoxin production, respectively, whereas all the misidentified cases were found to be false positive. Then, the potential of modified atmosphere packaging with 20-80% CO$_2$ (balanced with nitrogen) in controlling the growth and aflatoxin production on paddy at different a$_w$ (0.92-0.98) relative to the control (0% CO$_2$) was examined using the two above-mentioned fungal isolates. Except at 0.92 a$_w$, as much as 80% CO$_2$ failed to inhibit the growth of the fungi completely. However, at all a$_w$ levels studied, the growth parameters as estimated by Baranyi function and aflatoxin production were affected by the increment in CO$_2$ where growth rate and aflatoxin production were negatively correlated with CO$_2$ while the lag phase correlated positively with CO$_2$. At 0.98 a$_w$, atmosphere enriched with 20% and 80% CO$_2$ led to at least 59% and 88% reduction in growth and 47% and 97% in aflatoxin production, respectively. At 0.95 a$_w$, the lag phases of both isolates on average increased by a factor of 1.7-12.0 when the CO$_2$ levels in the headspace were between 20-80% compared to the control. Finally, the effectiveness of three essential oils (cinnamon, glove and thyme) and three antioxidants [butylated
hydroxyanisole (BHA), propyl paraben (PP) and butylated hydroxytoluene (BHT)] on controlling the growth of and aflatoxin formation by the *A. flavus* grown on rice meal agar and paddy grains at different *a*<sub>w</sub> (0.92, 0.95, and 0.98) were evaluated. Two of the antioxidants (BHA and PP) and the three essential oils displayed significant inhibitory effect on the growth and aflatoxin formation on *in vitro* and on the paddy grains. Regardless of *a*<sub>w</sub> of the paddy, the application of essential oils at 500 µg g<sup>-1</sup> reduced the growth and aflatoxin formation by > 55% and >80%, compared with >75% and > 82% with usage of antioxidants (BHA, PP), respectively. The estimated effective doses 50% (ED<sub>50</sub>) required to reduce growth and aflatoxin formation on rice meal agar were lower than their counterpart on paddy and those required to inhibit aflatoxin formation were lower those of growth.
PENDEKATAN MODEL BERSEPADU KAWALAN LEPAS TUAI PADI DAN BERAS DARI PERTUMBUHAN *Aspergillus flavus* DAN PENGHASILAN AFLATOKSIN

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Tujuan utama kajian semasa adalah untuk membina strategi lepas tuai bersepadu untuk mengawal pertumbuhan dan penghasilan aflatoksin oleh *Aspergillus flavus* pada padi dan beras. Permulaannya, kandungan kelembapan keseimbangan padi dikaji pada 20, 30, 40 dan 50 °C pada kelembapan relatif (RH) di antara 0.113 dan 0.976 menggunakan teknik gravimetrik. Isoterma menunjukkan lengkungan sigmoidal Jenis II am dan memperlihatkan histerisis di mana ia adalah lebih ketara pada suhu lebih rendah. Pada RH tetap, ciri penyerapan adalah suhu-kebergantungan di mana kapasiti penyerapan padi meningkat apabila suhu menurun. Antara model-model yang dinilai untuk keupayaan mereka yang sesuai dengan data penyerapan, persamaan Oswin adalah yang terbaik diikuti dengan model-model polinomial tertib ketiga, GAB, Smith, Chung-Pfost dan Henderson. Oleh itu, model Oswin telah dipilih untuk menganggar kandungan air diperlukan untuk menghidrat semula padi kering kepada aktiviti air (\(a_w\)) diingini dalam kajian selanjutnya. Berikutnya, pertumbuhan dua aflatoksigenik *A. flavus* pada padi dan penghasilan aflatoksin dikaji mengikut reka bentuk faktorial penuh dengan tujuh tahap \(a_w\) di antara 0.82 dan
0.99 and tujuh suhu di antara 10 dan 43 °C. Pertumbuhan kulat, dinyatakan sebagai diameter koloni (mm), diukur setiap hari dan penghasilan aflatoxin telah dianalisis menggunakan HPLC isokratik dengan pengesan pendafluoran. Kadar pertumbuhan koloni yang maksimum daripada kedua-dua isolat telah dianggarkan dengan menyesuaikan model utama Baranyi kepada data pertumbuhan. Tiga model sekunder berpotensi yang sesuai; Rosso, polinomial dan Davey, telah dinilai keupayaan mereka untuk menerangkan kadar pertumbuhan radial sebagai fungsi suhu dan a<sub>w</sub>. Kedua-dua strain gagal untuk tumbuh pada suhu marginal (10 dan 43 °C) tanpa mengira a<sub>w</sub> yang dikaji, dan pada tahap a<sub>w</sub> 0.82, tanpa mengira suhu. Meskipun ramalan kesemua model yang dikaji menunjukkan persetujuan yang baik dengan kadar pertumbuhan yang diperhatikan, model Davey terbukti sebagai peramal terbaik untuk data eksperimentasi. Aflatoxin telah dikesan pada a<sub>w</sub> di antara 0.86-0.99 dengan a<sub>w</sub> optimal pada 0.98 dan suhu optimal dalam lingkungan 25-30 °C. Kemudian, kesan a<sub>w</sub> (0.82-0.92) dan suhu (15-42 °C) pertumbuhan dan penghasilan aflatoxin oleh A. flavus pada beras putih dan beras perang juga dikaji. Empat model sekunder telah digunakan secara mutlak untuk kesan gabungan a<sub>w</sub> dan suhu terhadap kadar pertumbuhan. Kesemua model telah disahkan menggunakan data eksperimentasi bebas. Berpandukan kepada indeks penilaian, perlaksanaan mmodel Davey dalam menghuraikan data eksperimentasi adalah yang tertinggi, diikuti dalam susunan menurun oleh model-model polinomial, Gaussian dan Rosso. Anggaran suhu pertumbuhan optima adalah di antara 30-34 °C. Tiada pertumbuhan mahupun aflatoxin telah dikesan pada a<sub>w</sub> 0.82 pada beras putih manakala pertumbuhan dan aflatoxin telah dikesan pada a<sub>w</sub> di antara 25-35 °C pada beras perang. Jumlah aflatoxin yang tertinggi telah dibentuk pada nilai a<sub>w</sub> (0.90-0.92) yang lebih tinggi dan suhu 20 °C selepas 21 hari inkubasi untuk kedua-dua jenis beras di mana
penghasilan aflatoxins secara konsisten di dalam jutai nilai $a_w$ yang lebih luas berlaku di antara 25-30 °C. Keputusan juga menunjukkan bahawa beras perang cenderung untuk menyokong pertumbuhan A. flavus dan penghasilan aflatoxins yang lebih tinggi berbanding beras putih. Model logistik yang menghuraikan sempadan pertumbuhan dan penghasilan aflatoxins A. flavus juga telah dibangunkan. Eksperimen telah dijalankan pada $a_w$ di antara 0.80 dan 0.99 dan suhu di antara 10 °C dan 45 °C pada agar tepung beras (RA) untuk jangka masa empat minggu. Darjah persetujuan di antara data ramalan dan pemerhatian dalam terma kesejajaran adalah > 97% dan > 98% untuk untuk pertumbuhan dan penghasilan aflatoxins, masing-masing. Kebarangkalian pertumbuhan dan penghasilan aflatoxins pada 21 hari adalah hampir sama dengan kebarangkalian pada 28 hari. Model-model logistik polinomial yang telah dibangunkan telah disahkan dengan data yang diperolehi daripada eksperimen ulangan terhadap padi. Model-model tersebut dengan berjayanya mampu meramal kebarangkalian dengan kadar kesejajaran 85.2% dan 88.9% untuk pertumbuhan dan penghasilan aflatoxins, masing-masing. Manakala kesemua kes salah pengesanan telah didapati sebagai positif palsu. Kemudian, potensi pembungkusan atmosfera terubah suai dengan 20-80% CO$_2$ (diseimbangkan dengan nitrogen) dalam mengawal pertumbuhan dan penghasilan aflatoxins terhadap padi pada $a_w$ (0.92-0.98) yang berbeza relatif kepada kawalan (0% CO$_2$) telah diselidik menggunakan dua isolat kulat yang dinyatakan di atas. Kecuali pada $a_w$ 0.92, CO$_2$ sebanyak 80% gagal untuk merencat pertumbuhan kulat sepenuhnya. Bagaimanapun, pada kesemua tahap $a_w$ yang dikaji, parameter-parameter pertumbuhan seperti yang dianggar dengan fungsi Baranyi dan penghasilan aflatoxins telah dipengaruhi dengan peningkatan dalam CO$_2$ di mana kadar pertumbuhan dan penghasilan aflatoxins adalah berhubung kait secara negatif
with CO₂ manakala fasa lag berhubung kait secara positif dengan CO₂. Pada aₜₗ₀.98, atmosfera yang diperkaya dengan 20% dan 80% CO₂ membawa kepada sekurang-kurangnya 59% dan 88% pengurangan dalam pertumbuhan dan 47% dan 97% dalam penghasilan aflatoxsin, masing-masing. Pada aₜₗ₀.95, fasa-fasa lag kedua-dua isolat secara purata meningkat dengan faktor 1.7-12.0 apabila tahap CO₂ di dalam ruang kepala adalah di antara 20-80% berbanding kawalan. Akhirnya, keberkesanan tiga minyak esen (kayu manis, bunga cengkih dan thyme) dan tiga antioksidan [butylated hidroksianisol (BHA), propyl paraben (PP) dan butylated hidroksitoluena (BHT)] dalam mengawal pertumbuhan dan pembentukan aflatoxsin oleh A. flavus yang dibakukan pada agar tepung beras dan bijirin padi pada aₜₗ₀ (0.92, 0.95 dan 0.98) yang berbeza telah dinilai. Dua daripada antioksidan (BHA dan PP) dan ketiga-tiga minyak esen mempamerkan kesan perencatan yang signifikan terhadap pertumbuhan dan pembentukan aflatoxsin pada in vitro dan pada bijirin padi. Tanpa mengira aₜₗ pada padi, penggunaan minyak-minyak esen pada 500 μg g⁻¹ mengurangkan pertumbuhan dan pembentukan aflatoxsin dengan > 55% dan > 80%, berbanding dengan > 75% dan > 82% dengan pengguna antioksidan (BHA, PP), masing-masing. Anggaran 50% dos-dos berkesan (ED₅₀) yang diperlukan untuk mengurangkan pertumbuhan dan pembentukan aflatoxsin pada agar tepung beras adalah lebih rendah berbanding yang digunakan pada padi dan yang diperlukan untuk merencatkan pertumbuhan aflatoxsin adalah lebih rendah berbanding yang diperlukan untuk pertumbuhan.
ACKNOWLEDGEMENTS

“In the Name of Allah, the Most Merciful and the Most Beneficent”

All praise do to allah, Lord of the universe. Only by his grace and mercy this thesis was completed. First and for most I would like to express my profound gratitude to my honourable supervisor Dr. Farinazleen Mohamad Ghazali for her invaluable advice, guidance, encouragement. I would like to wish her unending success in her family and professional lives. I am extremely grateful to my supervisory committee members, Prof. Dr. Jinap Selamat, Pof. Dr. Hasanah Mohd. Ghazali and Prof. Dr. Son Radu for their valuable contribution and suggestions.

I am grateful to my colleagues in the laboratory of food safety and quality; Kabir Umar, Elham Farahany, Sahar Arzandeh, Gisja Daniali, Wendy Lim, Diyana Syamim, Afsaneh Farhadian, Dr. Parvaneh Hajeb for being supportive friends and wish them all the very best for the future. I am indebted to the staff of the Faculty of Food Science and Technology, University Putra Malaysia who assisted me in one way or the other. Special thanks to Norliza Othman for her assistance through my study.

I would like to express my sincere gratitude to my parents, brothers and sisters for their prayers, unending encouragement and continuous moral support. Last but not least, my beloved wife, Myssa Mousa and my dear kids (Abdulrahman and Abdullah) deserve my deepest and heartfelt gratitude for their bearing with me for the many months I spent away from home.
I certify that a Thesis Examination Committee has met on 15 May 2012 to conduct the final examination of Wael A. S. Mousa on his thesis entitled “An Integrated Modelling Approach to Postharvest Control of *Aspergillus flavus* Growth and Aflatoxin Production in Paddy Grains and Rice” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

WAEL MOUSA
Date: 15 May 2012
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