



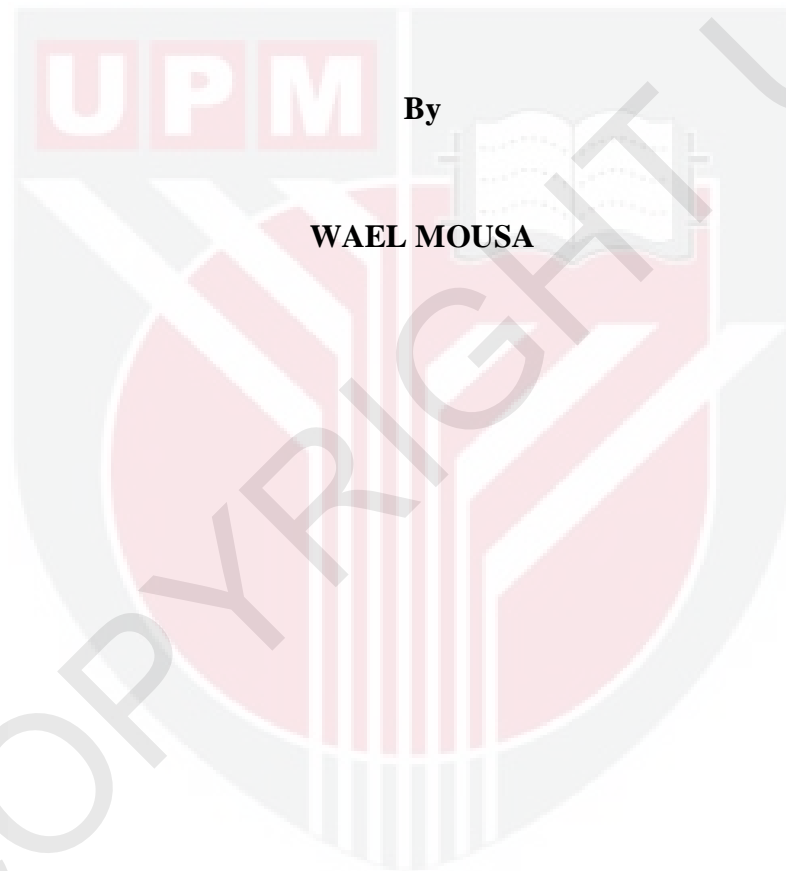
UNIVERSITI PUTRA MALAYSIA

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

Wael Mousa

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By

WAEEL 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

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: Farinazleen Mohammed Ghazali, PhD

Faculty: Food Science and Technology

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% CO₂ (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% CO₂) was examined using the two above-mentioned fungal isolates. Except at 0.92 a_w , as much as 80% CO₂ 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 CO₂ where growth rate and aflatoxin production were negatively correlated with CO₂ while the lag phase correlated positively with CO₂. At 0.98 a_w , atmosphere enriched with 20% and 80% CO₂ 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 CO₂ levels in the headspace were between 20-80% compared to the control. Finally, the effectiveness of three essential oils (cinnamon, clove 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_w (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_w of the paddy, the application of essential oils at $500 \mu\text{g g}^{-1}$ 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_{50}) 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.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENDEKATAN MODEL BERSEPADU KAWALAN LEPAS TUAI PADI
DAN BERAS DARI PERTUMBUHAN *Aspergillus flavus* DAN
PENGHASILAN AFLATOKSIN**

Oleh

Wael Mousa

Mei 2012

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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 mempamerkan 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 dan 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 pengesanan 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_w . Kedua-dua strain gagal untuk tumbuh pada suhu marginal (10 dan 43 °C) tanpa mengira a_w yang dikaji, dan pada tahap a_w 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_w di antara 0.86-0.99 dengan a_w optimal pada 0.98 dan suhu optimal dalam lingkungan 25-30 °C. Kemudian, kesan a_w (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_w dan suhu terhadap kadar pertumbuhan. Kesemua model telah disahihkan menggunakan data eksperimentasi bebas. Berpandukan kepada indeks penilaian, pelaksanaan model 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_w 0.82 pada beras putih manakala pertumbuhan dan aflatoxin telah dikesan pada a_w di antara 25-35 °C pada beras perang. Jumlah aflatoxin yang tertinggi telah dibentuk pada nilai a_w (0.90-0.92) yang lebih tinggi dan suhu 20 °C selepas 21 hari inkubasi untuk kedua-dua jenis beras di mana

penghasilan aflatoksin secara konsisten di dalam julat 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 aflatoksin yang lebih tinggi berbanding beras putih. Model logistik yang menghuraikan sempadan pertumbuhan dan penghasilan aflatoksin *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 pertumbuhan dan penghasilan aflatoksin, masing-masing. Kebarangkalian pertumbuhan dan penghasilan aflatoksin 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 berjaya mampu meramal kebarangkalian dengan kadar kesejajaran 85.2% dan 88.9% untuk pertumbuhan dan penghasilan aflatoksin, masing-masing. Manakala kesemua kes salah pengesanan telah didapati sebagai positif palsu. Kemudian, potensi pembungkusan atmosfera terubah suai dengan 20-80% CO₂ (diseimbangkan dengan nitrogen) dalam mengawal pertumbuhan dan penghasilan aflatoksin terhadap padi pada a_w (0.92-0.98) yang berbeza relatif kepada kawalan (0% CO₂) telah diselidik menggunakan dua isolat kulat yang dinyatakan di atas. Kecuali pada a_w 0.92, CO₂ 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 aflatoksin telah dipengaruhi dengan peningkatan dalam CO₂ di mana kadar pertumbuhan dan penghasilan aflatoksin adalah berhubung kait secara negatif

dengan CO₂ manakala fasa lag berhubung kait secara positif dengan CO₂. Pada a_w 0.98, atmosfera yang diperkaya dengan 20% dan 80% CO₂ membawa kepada sekurang-kurangnya 59% dan 88% pengurangan dalam pertumbuhan dan 47% dan 97% dalam penghasilan aflatoksin, masing-masing. Pada a_w 0.95, 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 aflatoksin oleh *A. flavus* yang dibiakkan pada agar tepung beras dan bijirin padi pada a_w (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 aflatoksin pada in vitro dan pada bijirin padi. Tanpa mengira a_w pada padi, penggunaan minyak-minyak esen pada 500 µg g⁻¹ mengurangkan pertumbuhan dan pembentukan aflatoksin dengan > 55% dan > 80%, berbanding dengan > 75% dan > 82% dengan penggunaan antioksidan (BHA, PP), masing-masing. Anggaran 50% dos-dos berkesan (ED₅₀) yang diperlukan untuk mengurangkan pertumbuhan dan pembentukan aflatoksin pada agar tepung beras adalah lebih rendah berbanding yang digunakan pada padi dan yang diperlukan untuk merencatkan pertumbuhan aflatoksin adalah lebih rendah berbanding yang diperlukan untuk pertumbuhan.

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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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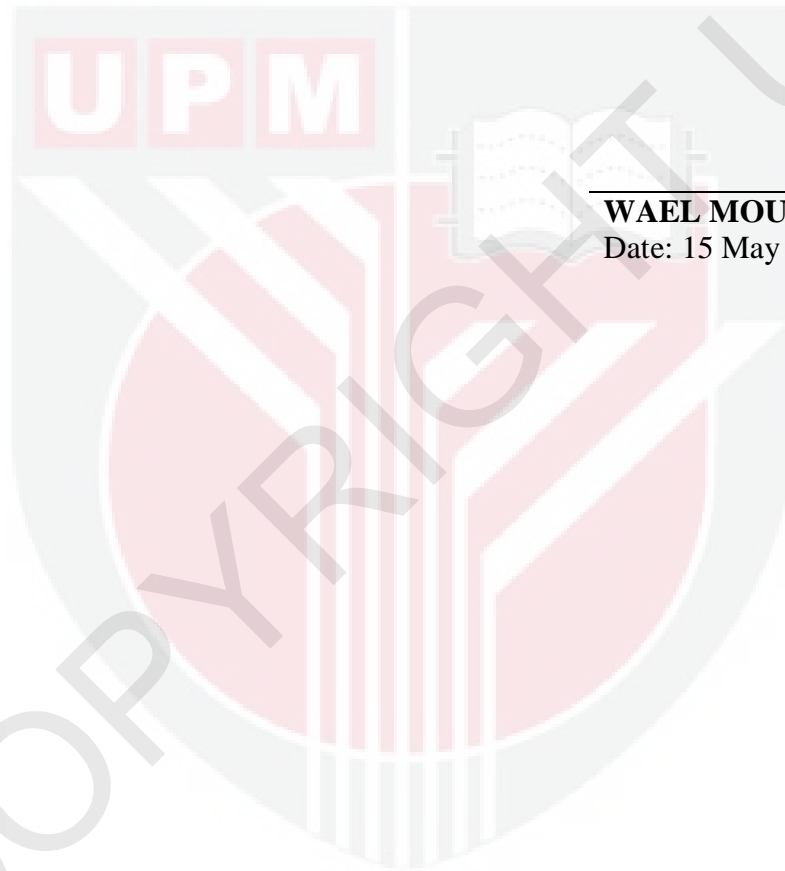
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DECLARATION

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

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	v
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xviii
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxv
 CHAPTER	
1	
INTRODUCTION	1
1.1 Specific objectives	4
2	
LITERATURE REVIEW	6
2.1 Introduction	6
2.2 Water availability	7
2.3 Moisture sorption isotherm	9
2.4 Factor affecting fungal growth in the grains ecosystem	9
2.4.1 Effect of water activity on fungal growth	10
2.4.2 Effect of temperature on fungal growth	11
2.4.3 Fungal interaction in grain ecosystem	12
2.4.4 Effect of inter-gaseous composition on fungal growth	13
2.4.5 Relationship between insects manifestation and fungi in grain ecosystem	14
2.5 Possible effect of climate change on change on the growth of mycotoxingenic fungi	15
2.6 Mycotoxins	16
2.6.1 Aflatoxins	17
2.6.2 Aflatoxin analysis	24
2.7 Control of fungal growth and aflatoxins	29
2.7.1 Control of aflatoxigenic fungi using modified atmosphere storage	29
2.7.2 Control of aflatoxigenic fungi using antioxidants	30
2.7.3 Control of aflatoxigenic fungi using essential oils	31
2.7.4 Biological control of aflatoxigenic fungi	34
2.8 Effect of milling on aflatoxins level in rice	36
2.9 Predictive mycology	38
2.9.1 Primary models	39
2.9.2 Secondary models	42
2.9.3 Tertiary models	46
2.9.4 Probability models	46

3	SORPTION ISOTHERMS AND ISOSTERIC HEATS OF SORPTION OF MALAYSIAN PADDY	48
3.1	Introduction	48
3.2	Materials and methods	50
3.2.1	Materials	50
3.2.2	Experimental procedure	50
3.2.3	Fitting sorption data to various isotherm equations	51
3.2.4	Determination of the net isosteric heat of sorption	52
3.3	Results	53
3.3.1	Moisture sorption isotherm and sorption hysteresis	53
3.3.2	Modelling the sorption isotherm	56
3.3.3	Isosteric heat of sorption	62
3.4	Discussion	63
3.4.1	Moisture sorption isotherm and sorption hysteresis	63
3.4.2	Modelling sorption isotherm	65
3.4.3	Isosteric heats of sorption	67
3.5	Conclusion	68
4	MODELLING THE EFFECT OF WATER ACTIVITY AND TEMPERATURE ON THE GROWTH RATE OF AND AFLATOXIN PRODUCTION BY <i>Aspergillus flavus</i> IN PADDY	69
4.1	Introduction	69
4.2	Materials and methods	72
4.2.1	Fungal isolates	72
4.2.2	Experimental design	72
4.2.3	Preparation and inoculation of paddy grains	73
4.2.4	Assessment of fungal growth	74
4.2.5	Modelling fungal growth as a function of a_w and temperature	74
4.2.6	Modelling aflatoxin production as a function of a_w and temperature	76
4.2.7	Model validation	77
4.2.8	Determination of aflatoxin	77
4.3	Results	79
4.3.1	Effect of a_w and temperature on the growth rate of <i>A. flavus</i> on paddy	79
4.3.2	Model validation	87
4.3.3	Effect of a_w and temperature on Aflatoxin production by <i>A. flavus</i> on paddy	91
4.4	Discussion	94
4.5	Conclusion	98

5	EFFECT OF WATER ACTIVITY AND TEMPERATURE ON THE GROWTH AND AFLATOXIN PRODUCTION BY <i>Aspergillus flavus</i> ON POLISHED AND BROWN RICE: DEVELOPMENT AND ASSESSMENT OF DIFFERENT GROWTH MODELS	100
	5.1 Introduction	100
	5.2 Materials and methods	101
	5.2.1 Fungal isolates	101
	5.2.2 Experimental design	101
	5.2.3 Preparation of rice	102
	5.2.4 Inoculation, incubation and growth assessment	102
	5.2.5 Model development	103
	5.3 Results	107
	5.3.1 Modelling the growth rate as a function of temperature and a_w	107
	5.3.2 Validation and evaluation of performance of the models	113
	5.3.3 Effect of temperature, a_w , and incubation time on the formation of aflatoxins	113
	5.4 Discussion	116
	5.5 Conclusion	119
6	MODELLING GROWTH/NO GROWTH AND AFLATOXIN PRODUCTION BOUNDARY OF <i>Aspergillus flavus</i> ON RICE	125
	6.1 Introduction	125
	6.2 Materials and methods	126
	6.2.1 Experimental design	126
	6.2.2 Fungal isolates	127
	6.2.3 Preparation of the medium	127
	6.2.4 Inoculum preparation, inoculation, incubation, and growth monitoring	127
	6.2.5 Determination of aflatoxins	128
	6.2.6 Modelling growth no /growth and aflatoxins production boundaries	129
	6.2.7 Model validation	130
	6.3 Results	131
	6.3.1 Modelling growth/no growth interface	131
	6.3.2 Modelling aflatoxins production/no production interface	138
	6.3.3 Model validation	144
	6.4 Discussion	145
	6.5 Conclusion	151

7	TEMPERATURE, WATER ACTIVITY AND GAS COMPOSITION EFFECTS ON THE GROWTH AND AFLATOXIN PRODUCTION BY <i>Aspergillus flavus</i> ON PADDY	152
	7.1 Introduction	152
	7.2 Materials and methods	153
	7.2.1 Experimental design	153
	7.2.2 Fungal isolates	153
	7.2.3 Influence of temperature and water activity on the relation between colony diameters and aflatoxins production on paddy	154
	7.2.4 Influence of modified atmosphere packaging (MAP) on the growth and aflatoxin production on paddy with different water activity	154
	7.2.5 Determination of aflatoxins	155
	7.2.6 Mathematical and statistical analysis	155
	7.3 Results	157
	7.3.1 Effect of temperature and a_w on the relation between colony diameter and aflatoxins production by <i>A. flavus</i> on paddy	157
	7.3.2 Effects of a_w and initial headspace CO_2 on the growth parameters of <i>A. flavus</i> on paddy	160
	7.3.3 Effects of a_w and initial headspace CO_2 on aflatoxins production in paddy	164
	7.4 Discussion	165
	7.5 Conclusion	170
8	EFFICACY OF ESSENTIAL OILS AND ANTIOXIDANTS IN CONTROLLING OF GROWTH AND AFLATOXIN PRODUCTION BY <i>Aspergillus flavus</i> ON PADDY	171
	8.1 Introduction	171
	8.2 Materials and methods	172
	8.1.2 Screening study for the effect of essential oil growth of <i>A. flavus</i>	172
	8.2.2 <i>In vitro</i> effect of essential oils and antioxidant on the growth and aflatoxin production by <i>A. flavus</i>	173
	8.2.3 Effect of essential oils and antioxidant on the growth and aflatoxin production by <i>A. flavus</i> on paddy	174
	8.2.3 Aflatoxin analysis	175
	8.2.3 Mathematical and statistical analysis	175
	8.3 Results	176
	8.3.1 Screening study for the effect of essential oil growth of <i>A. flavus</i>	176
	8.3.2 <i>In vitro</i> effect of essential oil and antioxidants on the growth of and aflatoxin production by <i>A. flavus</i>	176

	8.3.3	Effect of essential oil on the growth of and aflatoxin production by <i>A. flavus</i> on paddy grains	184
	8.4	Discussion	191
	8.5	Conclusion	194
9		SUMMARY, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	195
	9.1	Summary and conclusion	195
	9.2	Recommendation for future research	202
		REFERENCES	203
		BIODATA OF STUDENT	229
		LIST OF PUBLICATIONS	230

