

**Antimicrobial activity and fumonisins associated with  
cowpea (*Vigna unguiculata*)**

**BY**

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## **DECLARATION**

I, the undersigned, declare that these studies, except where acknowledged in the text, is my own work and has not been previously submitted in any other form to this or any other tertiary institution.

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**ABSTRACT**

A survey involving 71 farmers from rural communities in Mpumalanga, South Africa was conducted to gather information regarding the importance and utilisation of cowpea. Cowpea was rated third most important in contributing to household security, preceded by maize and vegetable production. Cowpea was mainly produced for own consumption, as a source of income and as fodder for livestock to a lesser extent. The crop was used by 8.5% of the farmers for medicinal purposes. Results indicated that 20% of the farmers encountered problems with mouldiness during storage, with insect infestation to a lesser degree.

Cowpea seed samples from South Africa and Benin, West Africa were analysed for seed mycoflora and various fungal genera, particularly *Aspergillus*, *Phoma* and *Lasiodiplodia* were recorded. The results indicated an array of *Fusarium* spp. including *F. equiseti*, *F. chlamydosporum*, *F. graminearum*, *F. proliferatum*, *F. sambucinum*, *F. scirpi*, *F. semitectum* and *F. subglutinans*. The seed samples and the *F. proliferatum* isolates, cultured on maize patty media, were analysed for fumonisin production. Samples were extracted with methanol/water (70:30) and cleaned-up on strong anion exchange solid phase extraction cartridges. High-performance liquid chromatography with pre-column derivatisation using *o*-phthaldialdehyde was used for the detection and quantification of FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub>. The cowpea cultivars from South Africa had levels of FB<sub>1</sub> ranging between 0.12 – 0.61 µg/g. All the *F. proliferatum* isolates produced FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> with total fumonisin concentration levels between 0.80 - 25.30 µg/g. The highest level of FB<sub>1</sub> detected was 16.86 µg/g.

Surface-disinfected seeds were imbibed in sterile distilled water amended with FB<sub>1</sub> to yield final concentrations of 10, 25, 50 and 100 ppm. Percentage germination was determined in paper towels according to the International Seed Testing Association (ISTA) rules. Root and shoot length was measured after 9 days. All the toxin concentrations significantly decreased seed germination whilst root

and shoot elongation was inhibited by the 50 and 100 ppm concentrations. Embryonic seed tissue treated with FB<sub>1</sub> indicated compaction of the protoplasm and separation of the plasma membrane from the cell wall. Lipid bodies accumulated and seemed to line the cell wall.

Acetone and ethanol extracts of the leaves of two cowpea cultivars exhibited significant inhibition of the growth of fungal plant pathogens at 5.0 mg/ml, with the exception of *Fusarium equiseti*. The growth of some fungi, in particular *Alternaria alternata*, was also reduced by lower concentrations of certain extracts. Acetone extracts of the Bechwana White cultivar inhibited growth of *Staphylococcus aureus* and *Enterococcus faecalis* at 2.5 mg/ml and *Bacillus cereus*, *B. subtilis* and *Enterobacter cloacae* at 5.0 mg/ml. Ethanol extracts of the same cultivar showed antibacterial activity against *E. faecalis* and *E. cloacae* at 5.0 mg/ml.

This study represents the first report on the natural occurrence of fumonisins on cowpea seed and the potential of *F. proliferatum* isolates from cowpea seed to produce fumonisins. The phytotoxic effects of FB<sub>1</sub> on cowpea seeds as well as the antimicrobial potential of cowpea leaf extracts were demonstrated for the first time.

**Keywords:** antimicrobial, cowpea, FB<sub>1</sub>, fumonisins, *Fusarium proliferatum*, germination, medicinal, mycoflora, phytotoxic, storage, ultrastructure, *Vigna unguiculata*

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**TABLE OF CONTENTS**

<b>LIST OF TABLES AND FIGURES</b>		<b>x</b>
<b>LIST OF ABBREVIATIONS</b>		<b>xiii</b>
<b>CHAPTER 1</b>	<b>GENERAL INTRODUCTION</b>	<b>1</b>
<b>1.1.</b>	<b>BACKGROUND AND MOTIVATION OF THE STUDY</b>	<b>1</b>
<b>1.2.</b>	<b>OBJECTIVES OF THE STUDY</b>	<b>3</b>
<b>1.3.</b>	<b>STRUCTURE OF THESIS</b>	<b>3</b>
<b>1.4.</b>	<b>LITERATURE CITED</b>	<b>5</b>
<b>CHAPTER 2</b>	<b>LITERATURE REVIEW</b>	<b>8</b>
<b>2.1.</b>	<b>AN INTRODUCTION TO COWPEA: <i>VIGNA UNGUICULATA</i> (L.) WALP</b>	<b>8</b>
<b>2.1.1.</b>	<b>Uses of cowpea</b>	<b>8</b>
<i>2.1.1.1.</i>	<i>Importance as a food crop</i>	<i>8</i>
<i>2.1.1.2.</i>	<i>Traditional medicinal uses</i>	<i>9</i>
<i>2.1.1.3.</i>	<i>Useful compounds isolated from cowpea</i>	<i>9</i>
<b>2.2.</b>	<b>FUNGI AND MYCOTOXINS ASSOCIATED WITH COWPEA SEED</b>	<b>10</b>
<b>2.2.1.</b>	<b>Storage fungi</b>	<b>10</b>
<i>2.2.1.1.</i>	<i>Effects of storage fungi on cowpea seed germination and seedling development</i>	<i>20</i>
<b>2.2.2.</b>	<b>Mycotoxins and their effect on cowpea seed</b>	<b>20</b>
<b>2.3.</b>	<b>THE FUMONISIN MYCOTOXINS</b>	<b>21</b>
<b>2.3.1.</b>	<b>Characterisation and toxicity</b>	<b>21</b>
<b>2.3.2.</b>	<b>Occurrence on legume crops</b>	<b>23</b>
<b>2.3.3.</b>	<b>Phytotoxic effects of fumonisins</b>	<b>23</b>
<b>2.3.4.</b>	<b>Mode of action of toxicity of fumonisins</b>	<b>24</b>
<b>2.4.</b>	<b>ANTIMICROBIAL EFFECTS OF PLANT EXTRACTS ON BACTERIAL AND FUNGAL PATHOGENS</b>	<b>25</b>
<b>2.5.</b>	<b>SECONDARY METABOLITES ASSOCIATED WITH COWPEA</b>	<b>26</b>
<b>2.5.1.</b>	<b>Secondary metabolites</b>	<b>26</b>

2.5.2.	Antimicrobial and medicinal activity of secondary metabolites	28
2.6.	THE INFLUENCE OF PHYSICAL FACTORS ON THE ULTRASTRUCTURE OF COWPEA SEEDS	28
2.7.	LITERATURE CITED	29
<b>CHAPTER 3</b>	<b>SURVEY ON THE IMPORTANCE AND UTILISATION OF COWPEA BY RURAL COMMUNITIES IN THE MPUMALANGA PROVINCE OF SOUTH AFRICA</b>	<b>41</b>
3.1.	INTRODUCTION	41
3.2.	METHODOLOGY	42
3.2.1.	Survey area	42
3.2.2.	Survey questionnaire	42
3.2.3.	Analysis of results	44
3.3.	RESULTS AND DISCUSSION	44
3.3.1.	Biographical profile	44
3.3.2.	Agricultural profile	45
3.3.3.	Importance and role of cowpea	46
3.3.3.1.	<i>Use as a food crop</i>	46
3.3.3.2.	<i>Source of income</i>	47
3.3.3.3.	<i>Feed for livestock</i>	48
3.3.3.4.	<i>Medicinal uses</i>	49
3.3.4.	Cultivation practices	49
3.3.5.	Storage practices	50
3.3.6.	Health aspects	51
3.3.7.	Major constraints associated with cowpea production	52
3.4.	CONCLUSION	53
3.5.	ACKNOWLEDGEMENTS	54
3.6.	LITERATURE CITED	54
<b>CHAPTER 4</b>	<b>Mycoflora and fumonisins associated with cowpea (<i>Vigna unguiculata</i> (L.) Walp) seeds</b>	<b>57</b>
	Abstract	58

<b>INTRODUCTION</b>	<b>59</b>	
<b>MATERIALS AND METHODS</b>	<b>60</b>	
<b>Seed samples</b>	<b>60</b>	
<b>Isolation and identification of seed-borne fungi</b>	<b>60</b>	
<b>Maize patty media</b>	<b>60</b>	
<b>Sample extraction and clean-up</b>	<b>60</b>	
<b>Fumonisin analyses</b>	<b>61</b>	
<b>RESULTS AND DISCUSSION</b>	<b>61</b>	
<b>ACKNOWLEDGEMENTS</b>	<b>64</b>	
<b>LITERATURE CITED</b>	<b>64</b>	
<b>CHAPTER 5</b>	<b>Phytotoxic effects of fumonisin B<sub>1</sub> on cowpea seed</b>	<b>72</b>
	<b>ABSTRACT</b>	<b>72</b>
	<b>MATERIALS AND METHODS</b>	<b>74</b>
	<b>Seed material</b>	<b>74</b>
	<b>Toxin</b>	<b>74</b>
	<b>Seed treatments</b>	<b>74</b>
	<b>Seed germination</b>	<b>74</b>
	<b>Transmission electron microscopy</b>	<b>74</b>
	<b>Statistical analysis</b>	<b>75</b>
	<b>RESULTS AND DISCUSSION</b>	<b>75</b>
	<b>Effect on seed germination and root and shoot elongation</b>	<b>75</b>
	<b>Effect on ultrastructure</b>	<b>75</b>
	<b>LITERATURE CITED</b>	<b>77</b>
<b>CHAPTER 6</b>	<b>Antimicrobial activity of cowpea (<i>Vigna unguiculata</i> (L.) Walp) leaf</b>	
	<b>extracts</b>	<b>87</b>
	<b>Abstract</b>	<b>88</b>
	<b>Introduction</b>	<b>89</b>
	<b>Materials and methods</b>	<b>90</b>
	<i>Plant material</i>	<b>90</b>
	<i>Preparation of extracts</i>	<b>90</b>



	<i>Micro-organisms</i>	90
	<i>Antimicrobial tests</i>	91
	<b>Results and discussion</b>	91
	<b>References</b>	93
<b>CHAPTER 7</b>	<b>GENERAL DISCUSSION</b>	<b>102</b>
<b>7.1.</b>	<b>LITERATURE CITED</b>	<b>105</b>
	<b>SUMMARY</b>	<b>107</b>
<b>APPENDIX A</b>	<b>SURVEY OF COWPEAS</b>	<b>109</b>
<b>APPENDIX B</b>	<b>LIST OF PUBLICATIONS AND PRESENTATIONS</b>	<b>117</b>

## LIST OF TABLES AND FIGURES

### TABLES

#### CHAPTER 1

<b>Table 1.1.</b>	Production figures of different grain, pulse and oilseed crops in South Africa for 2003 (FAOSTAT 2004)	1
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#### CHAPTER 2

<b>Table 2.1.</b>	Mycoflora associated with cowpea seed	12
-------------------	---------------------------------------	----

#### CHAPTER 3

<b>Table 3.1.</b>	Size of land used for agricultural activities by farmers interviewed in Mpumalanga	45
-------------------	--	----

#### CHAPTER 4

<b>Table 1.</b>	Fungi isolated from four cultivars of cowpea seeds obtained in South Africa	68
<b>Table 2.</b>	Fungi isolated from cowpea seeds obtained from three localities in Benin	69
<b>Table 3.</b>	Fumonisin production by <i>Fusarium proliferatum</i> isolates grown on maize patty medium	70

#### CHAPTER 6

<b>Table 1.</b>	Antibacterial activity of acetone and ethanol leaf extracts of two cowpea cultivars	101
-----------------	---	-----

## FIGURES

### CHAPTER 2

- Figure 2.1.** General chemical structure of fumonisins 22
- Figure 2.2.** Chemical structures of selected flavonoids associated with cowpea 27

### CHAPTER 3

- Figure 3.1.** Survey area, indicated by the red ring, in the Mpumalanga Province, South Africa 43
- Figure 3.2.** Farmer and his family together with interviewers during an interview in Mpumalanga 43
- Figure 3.3.** Age demographics of people interviewed in Mpumalanga 44
- Figure 3.4.** Source of income of the people interviewed in Mpumalanga 45
- Figure 3.5.** Frequency of cowpea consumption by people in Mpumalanga 46
- Figure 3.6.** Percentage contribution of cowpea to household income in Mpumalanga 48
- Figure 3.7.** Frequency of cowpea consumption by animals in Mpumalanga 48
- Figure 3.8.** Number of years that the respondents have been producing cowpea in Mpumalanga 49
- Figure 3.9.** Various storage containers observed during the survey in Mpumalanga; a. plastic bucket, b. cement room, c. wooden room and d. metal box 51
- Figure 3.10.** Major constraints associated with cowpea cultivation in Mpumalanga 53

### CHAPTER 4

- Figure 1.** Structures of fumonisin B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>. 71

### CHAPTER 5

- Figure 1.** Chemical structure of fumonisin B<sub>1</sub> 81
- Figure 2.** Effect of FB<sub>1</sub> and *Fusarium* spp. on cowpea seed germination. Each bar is a mean of 3 replicates. Values of the bars not followed by the same letter are significantly different ( $P=0.05$ ) according to the student's *t* test. 82

- Figure 3.** Effect of FB<sub>1</sub> and *Fusarium* spp. on cowpea seeds and seedlings. Values of the lines with the same symbol not followed by the same letter are significantly different ( $P=0.05$ ) according to the student's *t* test. 83
- Figure 4.** Effect of FB<sub>1</sub> and *Fusarium* spp. on root and shoot elongation. Values of the lines with the same symbol not followed by the same letter are significantly different ( $P=0.05$ ) according to the student's *t* test. 84
- Figure 5.** TEM micrographs of the embryonic axes of cowpea seed, a) imbibed for 10 h in sterile distilled water, b) imbibed for 10 h in moist paper towels, c) imbibed for 10 h in sterile distilled water with the addition of FB<sub>1</sub> at 10 ppm, d) 25 ppm, e) 50 ppm, f, g and h) and 10 ppm. (CW = cell wall, L = lipid, N = nucleus, PM = plasma membrane, V = vacuole, arrows – plasma membrane separated from cell wall). Bar = 1  $\mu$ m. 85
- Figure 6.** TEM micrographs of cotyledon tissue of cowpea seed, a) slow imbibed for 10 h in moist paper towels, b) imbibed for 10 h in sterile distilled water, c) imbibed for 10 h in sterile distilled water with the addition of FB<sub>1</sub> at 10 ppm, d) 25 ppm, e) 50 ppm, f) and 100 ppm. (CW = cell wall, L = lipid, N = nucleus, V = vacuole, arrows – plasma membrane separated from cell wall). Bar = 1  $\mu$ m. 86

## CHAPTER 6

- Figure 1.** Antifungal activity of Bechwana White acetone leaf extracts (a), Bechwana White ethanol leaf extracts (b), Kpodjiguégué acetone leaf extracts (c) and Kpodjiguégué ethanol leaf extracts (d) on selected fungal pathogens. \* Each value of a bar is a mean of 3 replicates. Values of the bars within each fungal species not followed by the same letter are significantly different ( $P=0.05$ ) according to the student's *t* test.

96-99

## LIST OF ABBREVIATIONS

AIDS	-	Acquired immunodeficiency syndrome
ANOVA	-	analysis of variance
BW	-	Bechwana White
CuCl <sub>2</sub>	-	copper chloride
DDT	-	dichloro diphenyl trichloroethane
DDAC	-	-dimethyl dodecyl ammonium chloride
FAO	-	Food and Agriculture Organisation of the United Nations
FB <sub>1</sub>	-	fumonisin B <sub>1</sub>
FB <sub>2</sub>	-	fumonisin B <sub>2</sub>
FB <sub>3</sub>	-	fumonisin B <sub>3</sub>
HPLC	-	high performance liquid chromatography
HIV-1	-	human immunodeficiency virus type 1
IARC	-	International Agency for Research on Cancer
ISTA	-	International Seed Testing Association
JECFA	-	Joint FAO/WHO Expert Committee on Food Additives
Kpod	-	Kpodjiguégué
LEM	-	leukoencephalomalacia
LTP	-	lipid transfer proteins
MEA	-	malt extract agar
MRC	-	Medical Research Council
MIC	-	minimum inhibitory concentration
NTD	-	neural tube defects
NA	-	nutrient agar
OPA	-	<i>o</i> -phthaldialdehyde
PDA	-	potato dextrose agar
PES	-	pulmonary edema syndrome
PMTDI	-	provisional maximum tolerable daily intake
NaH <sub>2</sub> PO <sub>4</sub>	-	sodium dihydrogen phosphate
SPE	-	solid phase extraction
SAX	-	strong anion exchange
TEM	-	transmission electron microscopy

- TLC - thin layer chromatography
- WHO - World Health Organisation