

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1. AN INTRODUCTION TO COWPEA: *VIGNA UNGUICULATA* (L.) WALP

Cowpea is an indigenous African legume crop belonging to the Fabaceae/Leguminosae family (Wiersema & León 1999). This widely cultivated and adapted crop is of great importance in the tropical and subtropical countries of Asia, Oceania, the Middle East, southern Europe, Africa, southern United States of America and Central and South America (Brader 2002). Cowpea is commonly known as black-eyed pea and southern pea. Other names for the crop include dinawa (Sotho, Tswana), munawa (Venda), akkerboon (Afrikaans), niébé (French), Frijol de costa (Spanish) and augenbohne (German) (Wiersema & León 1999).

##### 2.1.1. Uses of cowpea

The crop is known to be economically important due to its soil improvement abilities by increasing soil nitrogen levels (Quin 1997; Wiersema & León 1999). Cowpea also suppresses weed growth and prevents soil erosion through excellent ground cover. Furthermore, a source of cash for rural communities is provided through the trade of seed (Quin 1997). Many subsistence farmers and rural communities living in less developed countries rely largely on the vegetable crop as a good source of nutritious food. Farmers also obtain fodder and forage for their animals (Wiersema & León 1999). It has been reported that all the parts of the cowpea plant (i.e. roots, leaves and seeds) are used medicinally (Nyazema 1987; van Wyk & Gericke 2000).

##### 2.1.1.1. Importance as a food crop

The leaves, pods and seeds provide a good source of protein, vitamins and carbohydrates. The seed particularly contains on average 23-25% protein and 50-67% starch (Quin 1997). The seed provides an important source of nourishment, especially protein, for relatively poor people who cannot afford milk and meat products (Brader 2002). In Africa, the seeds are consumed either fresh or rehydrated, as an ingredient in soups or as a paste in steamed ('moin-moin') and fried dishes ('akara') (Ogunsanwo *et al.* 1989; Hung *et al.* 1990; van Wyk & Gericke 2000). In India, it is mainly eaten as cooked whole seeds or immature seeds (Kachare *et al.* 1988). In Nigeria, the seeds are eaten after boiling to softness and mixed with pepper, salt and palm oil to form a porridge (Ogunsanwo *et al.* 1989; Maduekwe &

Umechuruba 1992). Seeds can also be eaten with yams (*Dioscorea* sp.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Maduekwe & Umechuruba 1992). The young pods and leaves are eaten as green vegetables (van Wyk & Gericke 2000).

#### 2.1.1.2. Traditional medicinal uses

Cowpea has been identified by traditional healers in Zimbabwe to treat urinary schistosomiasis (bilharzia) (Nyazema 1987). The decoction made from the seeds of cowpea and the roots of *Euclea divinorum* Hiern or *Terminalia sericea* Burch ex DC. is taken orally to treat this illness (Nyazema 1987). Similarly, cowpea seeds and the roots of *Lannea edulis* (Sond.) Engl. can be used to treat blood in the urine and bilharzia (van Wyk & Gericke 2000). In East Africa, roots are used to expel the afterbirth (placenta, umbilical cord and ruptured membranes associated with the foetus) after childbirth (Kokwaro 1976, as cited by Hutchings *et al.* 1996). The leaves can be chewed and applied on burns and used as a snuff to treat headaches (Hutchings *et al.* 1996) whilst the Zulu's make emetics from the plant and are then taken to relieve fever (Gerstner 1939, as cited by Hutchings *et al.* 1996). The seeds also hold diuretic and anthelmintic (destruction of parasitic worms, e.g. tape worms) properties, and are used to treat liver complaints associated with jaundice (Noorwala *et al.* 1995). Further medicinal uses include: a decoction of the seed taken orally to treat the abnormal absence of menstruation (amenorrhoea); powdered roots eaten with porridge to treat painful menstruation, epilepsy and chest pain; a root paste applied to the bitten area caused by a snake bite as an antidote and a root infusion given to infants for constipation (van Wyk & Gericke 2000).

#### 2.1.1.3. Useful compounds isolated from cowpea

In a study carried out by Ng *et al.* (2002) various antifungal proteins were isolated from legume seeds, including cowpea, and were assayed for ability to inhibit human immunodeficiency virus type 1 (HIV-1) reverse transcriptase, protease and integrase enzymes. These enzymes are essential to the life cycle of HIV-1. The results concluded that cowpea P-antifungal protein had a high potency in inhibiting HIV-1 protease and HIV-1 integrase enzymes. Furthermore, cowpea a-antifungal protein was potent in inhibiting HIV-1 reverse transcriptase and HIV-1 integrase (Ng *et al.* 2002). Carvalho *et al.* (2001) reported that two cysteine-rich peptides isolated from cowpea seeds showed antimicrobial activity against the phytopathogenic fungi *Fusarium oxysporum* Schlecht.: Fr. and *F. solani* (Mart.) Appel and Wollenw. emend. Snyd. and Hans and the yeast *Saccharomyces cerevisiae* Hansen in an *in vitro* assay. The proteins, defensin and lipid transfer proteins (LTP) inhibited early growth and caused

many hyphal morphological alterations of the fungi. The LTP's were immunolocalised in the cell walls and in intracellular compartments of the cotyledons and embryonic axes (Carvalho *et al.* 2001).

## 2.2. FUNGI AND MYCOTOXINS ASSOCIATED WITH COWPEA SEED

As in the case of many edible leguminous crops, the optimal utilisation of cowpea as a food crop is hampered by numerous constraints. Many losses of cowpea seed particularly are due to the inadequate post-harvest storage of the seeds (Uzogara & Ofuya 1992). Fungal infestation of stored seeds is highly dependent on seed moisture content and the storage temperature (Neergaard 1977). Seeds become susceptible to fungal infestation under conditions of relative high humidities and temperatures (Esuruoso 1975; Hitokoko *et al.* 1981; Seenappa *et al.* 1983). Some of these fungi are known to produce toxic secondary metabolites, namely mycotoxins, that can lead to severe health implications in both humans and animals when contaminated seed is ingested (Barrett 2000).

### 2.2.1. Storage fungi

There are numerous reports referring to storage fungi and seed-borne fungi associated with cowpea seed. A detailed list of the mycoflora associated with cowpea seed, which has been formulated from the available literature, is presented in Table 2.1. One of the earliest reports concerning seed-borne fungi associated with cowpea seed is from Singh & Chohan (1974). The authors analysed seed collected from local markets in Ludhiana, India for fungi using the agar plate and blotter method. Fungi including *Aspergillus niger* van Tieghem, *A. terreus* Thom, *Fusarium concolor* Reinking, *F. verticillioides* (Sacc.) Nirenberg (previously known as *F. moniliforme* Sheldon), *Penicillium crustosum* Thom and *Rhizopus arrhizus* Fischer were noted as new records of cowpea seed-borne fungi (Singh & Chohan 1974). In 1975, Esuruoso observed *Aspergillus flavus* Link ex. Fries, *A. niger*, *A. ochraceus* Wilhelm, *Penicillium digitatum* Sacc. and *R. arrhizus* to be associated with 81 samples of seed in western Nigeria. Other fungi isolated, including *Botryodiplodia theobromae* Pat, *Chaetomium globosum* Kunze ex. Fr., *Cladosporium cladosporioides* (Fresen.) de Vries., *C. herbarum* (Pers.) Link ex. S.F. Gray, *Colletotrichum lindemuthianum* (Sacc. & Magh.) Bri. & Cav. *Curvularia lunata* (Wakker) Boedijn, *C. pallescens* Boedijn, *F. semitectum* Berk. & Rav., *F. solani* and *Phoma* sp. were new records for seed-borne fungi on cowpea (Esuruoso 1975). In 1981, Kumari & Karan detected *Trichothecium roseum* Link ex. Fries, *Verticillium* sp., *Circinella* sp., *Cladosporium* sp. and *Alternaria tenuis* Nees for the first time on cowpea seeds, collected from local markets of Hyderabad, India

Hedge & Hiremath (1987) noted that the frequency of cowpea seed mycoflora was less after storage than that of freshly harvested seed. Fungal genera including *Colletotrichum*, *Phoma*, *Curvularia*, *Trichothecium* and *Macrophomina* were not recorded in the seeds after storage, whereas *Aspergillus* dominated (Hedge & Hiremath 1987). Furthermore, the authors reported that more species of fungi were isolated from the seed coat and the cotyledons than the embryo. *Fusarium verticillioides* was isolated only from the embryo whereas *Aspergillus*, *Alternaria* and *Rhizopus* were mainly found in the seed coat (Hedge & Hiremath 1987).

Cowpea seed samples from India assayed for seed-borne fungi revealed that *F. verticillioides*, *F. oxysporum*, *Colletotrichum gleosporioides* (Penzig) Penzig and Saccardo, *A. niger* and *Penicillium* sp. were the most dominant fungi (Shama *et al.* 1988). Similarly, Ushamalini *et al.* (1998) reported that *M. phaseolina*, *F. oxysporum*, *Alternaria alternata* (Fr.:Fr.) Keissler, *A. flavus*, *A. niger* and *Penicillium* sp. were isolated from seeds collected from different districts in Tamil Nadu, India. Previous studies concerning storage fungi associated with cowpea seeds carried out by Kritzinger (2000) showed that the genera *Aspergillus*, *Penicillium* and *Alternaria* were the most common fungi found from seed of nine cultivars. Three species of *Aspergillus* were isolated from the cultivars, namely, *A. flavus*, *A. niger* and *A. ochraceus* with *A. niger* being the most common species (Kritzinger 2000).

A study done by Bulgarelli *et al.* (1988) showed that cowpea paste (prepared from dried seeds to make "akara") also supported an array of micro-organisms including bacteria, yeast and fungi. The paste, immediately collected after preparation from markets in Nigeria, showed the presence of *A. niger*, *F. sporotrichioides* Sherb., *F. verticillioides*, *Acremonium* sp., *Moniella* sp. and *Geotrichum candidum* Link & Fries (Bulgarelli *et al.* 1988). From the above reports and results of previous studies concerning storage and seed-borne fungi associated with cowpea seed, it is evident that the seed supports a wide range of fungi. These fungi can play an important role in the quality and longevity of the seeds. Furthermore, important seed transmitted diseases are caused by some fungi, eg. *Colletotrichum* spp. (Shama *et al.* 1988). As stated earlier, several of these fungi are capable of producing mycotoxins, thus producing a possible potential health threat to the consumers.

**Table 2.1.** Mycoflora associated with cowpea seed

	<b>Species</b>	<b>Reference(s)</b>
	en	
	us	
<i>Absidia</i>	spp.	Gowda & Sullia 1987
<i>Acremonium</i>	<i>strictum</i> Gams	Jindal & Thind 1990
	spp.	Kritzinger 2000
<i>Actinomucor</i>	<i>repens</i> Schostak	Gowda & Sullia 1987
<i>Alternaria</i>	<i>alternata</i> (Fr:Fr.) Keissler	Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Jindal & Thind 1990; Zohri <i>et al.</i> 1992; Ushamalini <i>et al.</i> 1998
	<i>cassiae</i> Juriar & Khan	Van den Berg <i>et al.</i> 2002
	<i>tenuis</i> Nees	Kumari & Karan 1981
	<i>tenuissima</i> (Kunze: Fries) Wiltshire	Gowda & Sullia 1987
	spp.	Gowda & Sullia 1987; Kritzinger 2000
<i>Ascochyta</i>	spp.	Emechebe & McDonald 1979
<i>Aspergillus</i>	<i>awamori</i> Nakazawa	Zohri <i>et al.</i> 1992
	<i>candidus</i> Link ex. Fries	Kumari & Karan 1981
	<i>carbonarius</i> (Bainier) Thom	Zohri <i>et al.</i> 1992
	<i>clavatus</i> Desmazières	Gowda & Sullia 1987
	<i>flavipes</i> (Bainier & A. Sartory) Thom & Church	Zohri <i>et al.</i> 1992

<i>flavus</i> Link ex. Fries	Esuruoso 1975; Sinha & Khare 1977, 1978; Kumari & Karan 1981; Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Jindal & Thind 1990; Cabrales 1992; Maduekwe & Umechuruba 1992; Zohri <i>et al.</i> 1992; Ushamalini <i>et al.</i> 1998; Kritzinger 2000
<i>fumigatus</i> Fresenius	Kumari & Karan 1981; Zohri <i>et al.</i> 1992
<i>glaucus</i> Link ex. Gray	Gowda & Sullia 1987
<i>janus</i> Raper & Thom	Zohri <i>et al.</i> 1992
<i>nidulans</i> (Eidam) Wingate	Kumari & Karan 1981; Jindal & Thind 1990
<i>niger</i> van Tieghem	Singh & Chohan 1974; Esuruoso 1975; Sinha & Khare 1977, 1978; Kumari & Karan 1981; Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Jindal & Thind 1990; Maduekwe & Umechuruba 1992; Zohri <i>et al.</i> 1992; Ushamalini <i>et al.</i> 1998; Kritzinger 2000
<i>ochraceus</i> Wilhelm	Esuruoso 1975; Zohri <i>et al.</i> 1992; Kritzinger 2000
<i>oryzae</i> (Ahlburg) Cohn	Zohri <i>et al.</i> 1992
<i>sulphureus</i> Thom chrucl	Kumari & Karan 1981
<i>sydowii</i> (Bainier & A. Sartory) Thom & Church	Zohri <i>et al.</i> 1992
<i>tamarii</i> Kita	Esuruoso 1975; Zohri <i>et al.</i> 1992
<i>terreus</i> Thom	Singh & Chohan 1974; Gowda & Sullia 1987; Shama <i>et al.</i> 1988; Maduekwe & Umechuruba 1992; Zohri <i>et al.</i> 1992
<i>ustus</i> (Bainier) Thom & Church	Zohri <i>et al.</i> 1992
spp.	Sinha & Khare 1977, 1978; Gowda & Sullia 1987; Cabrales 1992

<i>Botryodiplodia</i>	<i>theobromae</i> Pat.	Esuruoso 1975; De Barros <i>et al.</i> 1985
<i>Botrytis</i>	<i>cinerea</i> Persoon: Fries	Sinha & Khare 1977
	spp.	Sinha & Khare 1978; Gowda & Sullia 1987
<i>Cacumisporium</i>	spp.	Sinha & Khare 1977
<i>Cephalophora</i>	<i>tropica</i> Thaxter	Zohri <i>et al.</i> 1992
<i>Cephalosporium</i>	spp.	Sinha & Khare 1977; Kumari & Karan 1981; Gowda & Sullia 1987; Shama <i>et al.</i> 1988
<i>Cercospora</i>	<i>canescens</i> Ellis & G. Martin	Emechebe & McDonald 1979
<i>Chaetomium</i>	<i>globosum</i> Kunze: Fries	Esuruoso 1975; Gowda & Sullia 1987; Shama <i>et al.</i> 1988; Zohri <i>et al.</i> 1992
	<i>indicum</i> Corda	Gowda & Sullia 1987
	spp.	Sinha & Khare 1977, 1978; Maduekwe & Umechuruba 1992; Kritzinger 2000
<i>Circinella</i>	spp.	Kumari & Karan 1981; Gowda & Sullia 1987
<i>Cladosporium</i>	<i>cladosporioides</i> (Fresenius) de Vries	Esuruoso 1975; Jindal & Thind 1990
	<i>herbarum</i> (Persoon: Fries) Link	Esuruoso 1975; Shama <i>et al.</i> 1988
	<i>sphaerospermum</i> Penzig	Zohri <i>et al.</i> 1992
	<i>vignae</i> Gardner	Hedge & Hiremath 1987
	spp.	Kumari & Karan 1981; Gowda & Sullia 1987; Kritzinger 2000
<i>Cochliobolus</i>	<i>lunatus</i> R.R. Nelson & Haasis	Singh & Chohan 1974
	<i>spiciferus</i> R.R. Nelson	Sinha & Khare 1977
<i>Colletotrichum</i>	<i>capsici</i> (Syd.) Butler & Bisby	Emechebe & McDonald 1979
	<i>dematium</i> (Persoon: Fries) Grove	Shama <i>et al.</i> 1988; Smith <i>et al.</i> 1999

	<i>gleosporioides</i> (Penzig) Penzig and Saccardo	Shama <i>et al.</i> 1988
	<i>lindemuthianum</i> (Saccardo & Magnus) Briosi & Cavara	Esuruoso 1975; Emechebe & McDonald 1979; Hedge & Hiremath 1987
	<i>truncatum</i> (Schw.) Andrus & Moore spp.	Emechebe & McDonald 1979 Gowda & Sullia 1987
<i>Corticium</i>	<i>rolfsii</i> Curzi	Emechebe & McDonald 1979
<i>Corynespora</i>	<i>cassiicola</i> (Berk. & Curt.) Wei	Esuruoso 1975
<i>Curvularia</i>	<i>lunata</i> (Wakker) Boedijn	Esuruoso 1975; Kumari & Karan 1981; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Maduekwe & Umechuruba 1992; Ushamalini <i>et al.</i> 1998
	<i>pallescens</i> Boedijn	Esuruoso 1975; Shama <i>et al.</i> 1988
	<i>tuberculata</i> Jain	Jindal & Thind 1990
	<i>verruculosa</i> Tandon & Bilgrami spp.	Singh & Chohan 1974; Sinha & Khare 1977, 1978 Kritzinger 2000
<i>Diaporthe</i>	<i>phaseolorum</i> (Lehman) Wehmeyer	Sinha & Khare 1977, 1978
<i>Diplodia</i>	spp.	De Barros <i>et al.</i> 1985
<i>Drechslera</i>	<i>hawaiiensis</i> (Bugnicourt) Subramanian & Jain spp.	Singh & Chohan 1974; Sinha & Khare 1977 Shama <i>et al.</i> 1988
<i>Emericella</i>	<i>nidulans</i> (Eidam) Vuillemin	Zohri <i>et al.</i> 1992
	<i>quadrilineata</i> (Thom & Raper) C.R. Benjamin	Zohri <i>et al.</i> 1992

<i>Epicoccum</i>	<i>nigrum</i> Link	Jindal & Thind 1990
<i>Eurotium</i>	<i>chevalieri</i> Mang	Zohri <i>et al.</i> 1992
	<i>heterocaryoticum</i> Chris., Lop., and Benj.	Jindal & Thind 1990
<i>Fusarium</i>	<i>concolor</i> Reinking	Singh & Chohan 1974
	<i>equiseti</i> (Corda) Sacc.	Sinha & Khare 1977, 1978; De Barros <i>et al.</i> 1985; Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Jindal & Thind 1990
	<i>fusarioides</i> (Fragoso & Ciferri) C. Booth	Sinha & Khare 1977
	<i>oxysporum</i> Schlecht.: Fr.	Esuruoso 1975; Emechebe & McDonald 1979; De Barros <i>et al.</i> 1985; Gowda & Sullia 1987; Shama <i>et al.</i> 1988; Zohri <i>et al.</i> 1992; Varma <i>et al.</i> 1995
	<i>oxysporum</i> f.sp. <i>tracheiphilum</i> (E.F. Smith) Snyder & Hansen	Ushamalini <i>et al.</i> 1998
	<i>semitectum</i> Berk. & Rav.	Esuruoso 1975; De Barros <i>et al.</i> 1985; Gowda & Sullia 1987; Shama <i>et al.</i> 1988; Jindal & Thind 1990
	<i>solani</i> (Mart.) Appel and Wollenw. emend. Snyd. and Hans	Esuruoso 1975; Emechebe & McDonald 1979; Shama <i>et al.</i> 1988
	<i>verticillioides</i> (Sacc.) Nirenberg	Singh & Chohan 1974; Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Maduekwe & Umechuruba 1992
	spp.	Kumari & Karan 1981; Gowda & Sullia 1987; Cabrales 1992; Kritzinger 2000
<i>Gibberella</i>	<i>fujikuroi</i> (Sawada) Wollenweber	Zohri <i>et al.</i> 1992
<i>Gilmaniella</i>	spp.	Kritzinger 2000

<i>Macrophomina</i>	<i>phaseolina</i> (Tassi.) Goid.	Esuruoso 1975; Sinha & Khare 1977, 1978; Emechebe & McDonald 1979; De Barros <i>et al.</i> 1985; Gowda & Sullia 1987; Hedge & Hiremath 1987; Maduekwe & Umechuruba 1992; Ushamalini <i>et al.</i> 1998
<i>Memnomiella</i>	spp.	Sinha & Khare 1977; Kumari & Karan 1981
<i>Mortierella</i>	spp.	Gowda & Sullia 1987
<i>Mucor</i>	<i>hiemalis</i> Wehmer	Gowda & Sullia 1987
	spp.	Gowda & Sullia 1987; Hedge & Hiremath 1987
<i>Nigrospora</i>	spp.	Sinha & Khare 1977; Shama <i>et al.</i> 1988; Kritzinger 2000
<i>Paecilomyces</i>	spp.	Gowda & Sullia 1987
<i>Penicillium</i>	<i>aurantiogriseum</i> Dierckx	Zohri <i>et al.</i> 1992
	<i>chrysogenum</i> Thom	Jindal & Thind 1990; Zohri <i>et al.</i> 1992
	<i>citrinum</i> Thom	Jindal & Thind 1990; Zohri <i>et al.</i> 1992
	<i>crustosum</i> Thom	Singh & Chohan 1974
	<i>digitatum</i> Sacc.	Esuruoso 1975
	<i>funiculosum</i> Thom	Esuruoso 1975; Zohri <i>et al.</i> 1992
	<i>oxalicum</i> Currie & Thom	Jindal & Thind 1990; Zohri <i>et al.</i> 1992
	<i>purpurogenum</i> Stoll	Zohri <i>et al.</i> 1992
	spp.	Sinha & Khare 1977, 1978; Gowda & Sullia 1987; Hedge & Hiremath 1987; Shama <i>et al.</i> 1988; Cabrales 1992; Maduekwe & Umechuruba 1992; Ushamalini <i>et al.</i> 1998; Kritzinger 2000

<i>Pestalotiopsis</i>	<i>mangiferae</i> (Henn.) Steyaert	Sinha & Khare 1977
<i>Phoma</i>	<i>bakeriana</i> Henn.	Sinha & Khare 1977; Sinha & Khare 1978
	<i>exigua</i> Desmazières	Shama <i>et al.</i> 1988
	<i>glomerata</i> (Corda) Wollenw. and Hochapf	Jindal & Thind 1990
	<i>vignae</i> P. Henn.	Hedge & Hiremath 1987
	spp.	Esuruoso 1975; De Barros <i>et al.</i> 1985; Shama <i>et al.</i> 1988; Kritzinger 2000
<i>Phomopsis</i>	spp.	De Barros <i>et al.</i> 1985
<i>Pithomyces</i>	spp.	Sinha & Khare 1977; Kritzinger 2000
<i>Pleospora</i>	<i>infectoria</i> Fuckel	Singh & Chohan 1974; Sinha & Khare 1977, 1978
<i>Pyrenochaeta</i>	<i>decipiens</i> Marchal	Gowda & Sullia 1987
<i>Rhizoctonia</i>	<i>bataticola</i> (Taubenhaus) E. J. Butler	Singh & Chohan 1974
	<i>solani</i> Kühn	Emechebe & McDonald 1979; Gowda & Sullia 1987; Shama <i>et al.</i> 1988
	spp.	Kritzinger 2000
<i>Rhizopus</i>	<i>arrhizus</i> Fischer	Singh & Chohan 1974, Esuruoso 1975; Hedge & Hiremath 1987
	<i>nigricans</i> Ehrenberg	Gowda & Sullia 1987
	<i>nodosus</i> Namysl.	Gowda & Sullia 1987
	<i>oryzae</i> Went and Prinsen	Jindal & Thind 1990
	<i>stolonifer</i> (Ehrenberg: Fries) Vuillemin	Esuruoso 1975; Gowda & Sullia 1987; Zohri <i>et al.</i> 1992; Ushamalini <i>et al.</i> 1998
<i>Scopulariopsis</i>	spp.	Kumari & Karan 1981; Gowda & Sullia 1987; Kritzinger 2000
	<i>brumptii</i> Salvanet-Duval	Zohri <i>et al.</i> 1992
	<i>halophilica</i> Tubaki	Zohri <i>et al.</i> 1992

<i>Septoria</i>	<i>vignae</i> Henn.	Emechebe & McDonald 1979
<i>Sphaceloma</i>	spp.	Shama <i>et al.</i> 1988
<i>Stachybotrys</i>	spp.	Sinha & Khare 1977, 1978
<i>Syncephalastrum</i>	<i>racemosum</i> Cohn ex J. Schroeter	Sinha & Khare 1977; Gowda & Sullia 1987; Shama <i>et al.</i> 1988; Zohri <i>et al.</i> 1992
<i>Syncephalis</i>	spp.	Gowda & Sullia 1987
<i>Thamnidium</i>	<i>elegans</i> Link: Fries	Gowda & Sullia 1987
<i>Torula</i>	<i>viride</i> Persoon: Fries	Shama <i>et al.</i> 1988
	spp.	Gowda & Sullia 1987; Kritzinger 2000
<i>Trichothecium</i>	<i>roseum</i> (Persoon: Fries) Link	Kumari & Karan 1981; Gowda & Sullia 1987; Hedge & Hiremath 1987; Jindal & Thind 1990
<i>Tripospermum</i>	spp.	Kritzinger 2000
<i>Ulocladium</i>	<i>chartarum</i> (Preuss) E. Simmons	Sinha & Khare 1977
<i>Verticillium</i>	spp.	Kritzinger 2000
<i>Zygorhynchus</i>	spp.	Gowda & Sullia 1987

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#### 2.2.1.1. Effects of storage fungi on cowpea seed germination and seedling development

It is well known that storage fungi have a negative impact on the germination of various seeds and grains. Maheshwari *et al.* (1984) reported that *F. verticillioides* and various *Aspergillus* spp. adversely affected the seed germination, root length and shoot length of cowpeas to various degrees. *Aspergillus nidulans* (Eidam) Winter was most effective in inhibiting seed germination (38.8%) and root length (82.8%) whilst *F. verticillioides* was the least effective. The inhibitory activity was due to the presence of amino acids, organic acids and phenols (Maheshwari *et al.* 1984). On the other hand, Rheeder *et al.* (2002) found that *F. verticillioides* infection alone of maize seeds did not affect seed germination. Furthermore, Jindal & Thind (1990) reported that *A. flavus*, *F. equiseti* (Corda) Sacc., *F. semitectum*, *Rhizopus oryzae* Went and Prinsen and *T. roseum* also significantly reduced germination of cowpea seeds.

#### 2.2.2. Mycotoxins and their effect on cowpea seed

These secondary metabolites cause mycotoxicosis when ingested by higher vertebrates and other animals. Liver and kidney functioning can deteriorate when these metabolites are ingested through contaminated plant-based foods and animal-derived foods. Mycotoxins can also be neurotoxic, interfere with protein synthesis and can produce skin sensitivity or necrosis and extreme immunodeficiency (Sweeney & Dobson 1998).

Although legumes do not generally support the growth of toxigenic fungi and the production of mycotoxins (Webley *et al.* 1997), there are numerous reports regarding mycotoxins and legume seeds (Ahmad & Singh 1991; El-Kady *et al.* 1991; Saber 1992; Pitt *et al.* 1994; Tseng *et al.* 1995, Tseng and Tu 1997; Saber *et al.* 1998). However, the information pertaining to the production of various mycotoxins on cowpea seed is scant. Most of the literature regarding this aspect focuses on *Aspergillus* infection and aflatoxin production (El-Hag & Morse 1976; Seenappa *et al.* 1983; Zohri *et al.* 1992; El-Kady *et al.* 1996). Seenappa *et al.* (1983) reported that all cowpea seed samples collected in Tanzania were susceptible to *Aspergillus parasiticus* Speare infection and subsequent aflatoxin production. El-Hag & Morse (1976) investigated the production of aflatoxins by *Aspergillus oryzae* (Ahlburg) Cohn when grown on cowpeas or rice. It was found that this variant strain was capable to produce significant quantities of aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>.

In 1992, Zohri *et al.* investigated the natural occurrence of citrinin, ochratoxin A, patulin, sterigmatocystin, T-2 toxin, diacetoxyscirpenol, zearalenone and aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> in 20 cowpea cultivars. Thin layer chromatographic (TLC) analyses of the chloroform extracts showed that only four seed samples were naturally infected with aflatoxins B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>. None of the other

toxins tested for were detected in these cultivars (Zohri *et al.* 1992). Hitokoko *et al.* (1981) found aflatoxin B<sub>1</sub> (4 µg/g), sterigmatocystin (1 µg/g), ochratoxin A (50 µg/g) and T-2 toxin (3.8 µg/g) in cowpea seed samples. Zohri (1993) inoculated 16 mycotoxin-free cowpea seed samples with *A. flavus* to determine the varietal differences of aflatoxin production in the cultivars. Three cultivars showed high resistance whilst eight revealed partial resistance and the remaining cultivars were highly susceptible to toxin accumulation (Zohri 1993). It was reported that there was no relationship between morphological characters (seed colour, shape and size) or testa thickness and the amount of toxin produced by the different cultivars. The author concluded that the susceptibility or resistance of cowpea cultivars to *A. flavus* colonisation and aflatoxin production was influenced by an interaction of several factors. Zinc and sodium (essential trace elements for aflatoxin synthesis) levels were increased in susceptible cultivars when compared to the resistant cultivars (Zohri 1993).

Similarly, El-Kady *et al.* (1996) reported cowpea seed to be susceptible to *A. flavus* infection and aflatoxins were produced on two of the three cultivars analysed. Morphological and histological characters of the different cultivars tested did not show any relation to the amount of aflatoxin produced. The one cultivar Balady, however, was found to be very resistant to toxin production and this seed contained low levels of sodium and high levels of phosphate and potassium (El-Kady *et al.* 1996). Reddy *et al.* (1992) suggested that the higher the lipid content of seeds or seed components, the higher was the growth of *A. parasticus* and aflatoxin B<sub>1</sub> biosynthesis. This was demonstrated using seeds of crops with different lipid contents, including cowpea.

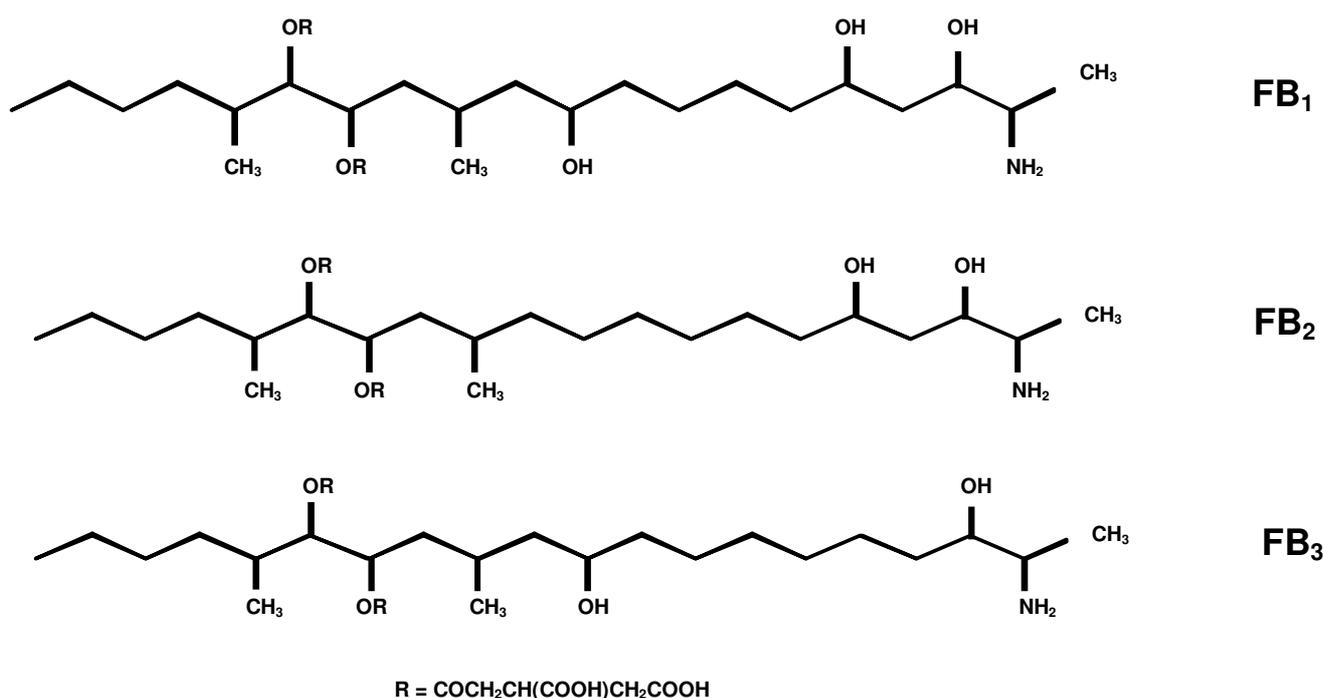
It has been reported by Adekunle & Bassir (1973) that aflatoxin B<sub>1</sub> and crude aflatoxins inhibited chlorophyll formation and seed germination of cowpea. Koehler & Woodworth (1938) induced chlorophyll deficiency in seedlings of citrus and maize. The authors suggested that the crude aflatoxins present in the walls of the fungal spores of *A. flavus* were responsible for this observation. The same trend was noted in mung seeds (*Vigna radiata* (L.) R. Wilcz), where seed germination, seedling growth, chlorophyll, protein and nucleic acid formation was inhibited by different concentrations of aflatoxin B<sub>1</sub> (Sinha & Kumari 1990). Maximum seed germination inhibition was caused by a 1000 µg/l concentration of aflatoxin B<sub>1</sub>.

## **2.3. THE FUMONISIN MYCOTOXINS**

### **2.3.1. Characterisation and toxicity**

The fumonisins are recently characterised mycotoxins with significant toxicological consequences. It has been reported that 15 *Fusarium* species (Rheeder *et al.* 2002) are capable of producing

fumonisin with the most important producers being *F. verticillioides* and *F. proliferatum* (Matsushima) Nirenberg (Rheeder *et al.* 2002). Other producers include *F. globosum* Rheeder, Marasas et Nelson (Sydenham *et al.* 1992) and *F. nygamai* Burgess and Trimboli (Thiel *et al.* 1991). *Alternaria alternata* f. sp. *lycopersici* is the only fungus that does not belong to the genus *Fusarium* that produces fumonisin B<sub>1</sub> (FB<sub>1</sub>), fumonisin B<sub>2</sub> (FB<sub>2</sub>) and fumonisin B<sub>3</sub> (FB<sub>3</sub>) in culture (Chen *et al.* 1992; Abbas & Riley 1996). The fumonisins are a structurally related group of diesters of propane-1, 2, 3-tricarboxylic acid and various 2-amino-12, 16-dimethylpolyhydroxyeicosanes in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of tricarboxylic acid (Bezuidenhout *et al.* 1988). Twenty-eight fumonisin analogues have been characterised and have been placed into series A, B, F and P based on their chemical structure (Rheeder *et al.* 2002). FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> (Figure 2.1.) are regarded to be the most abundant and most toxic of the naturally occurring analogues (Sydenham *et al.* 1992; Rheeder *et al.* 2002).



**Figure 2.1.** General chemical structure of fumonisins

Studies have shown that fumonisins have been known to cause various toxicological problems in animals. These include leukoencephalomacia (LEM), a fatal brain disease in horses, and pulmonary edema syndrome (PES) in pigs (Norred & Voss 1994; Marasas 1996). Recent studies have suggested

that fumonisin consumption is a risk factor for neural tube defects (NTD) and other birth defects in humans. Fumonisins interfere with the utilization of folic acid, which is used to reduce the incidence of NTD (Marasas *et al.* 2004). Further toxicological effects include their hepatotoxicity and hepatocarcinogenicity to rats, and cytotoxicity to mammalian cell cultures (Marasas 1996). Fumonisin B<sub>1</sub> is statistically linked to the incidence of oesophageal cancer in humans in Transkei, South Africa and China (Marasas 1996) and evidence does suggest that it may play a role in the etiology of this disease in humans (Norred & Voss 1994; de Nijs *et al.* 1998). The International Agency for Research on Cancer (IARC) classed FB<sub>1</sub> in group 2B, which implies that it could possibly be carcinogenic to humans (IARC 2002). The joint FAO/WHO Expert Committee on Food Additives (JECFA) allocated a group provisional maximum tolerable daily intake (PMTDI) for fumonisins B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, alone or in combination, of 0.002 mg/kg body weight (JECFA 2001).

### 2.3.2. Occurrence on legume crops

Tseng *et al.* (1995) analysed three types of mouldy navy bean (*Phaseolus vulgaris* L.) samples for the *Fusarium* mycotoxins (diacetoxyscripenol, deoxynivalenol, T-2 toxin and FB<sub>1</sub>), namely, healthy beans without discolouration, beans with pink discolouration and a mixture of beans with whitish grey and pink discolouration. FB<sub>1</sub> was found to be present in the two latter samples at 0.5 µg/g and 1.1 µg/g, respectively. *Fusarium* species isolated from the mouldy beans included *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. graminearum* Schwabe, *F. verticillioides*, *F. oxysporum* and *F. solani*. The *Fusarium* species responsible for the production of the toxin was not determined during this study. In a later study conducted by Tseng & Tu (1997), FB<sub>1</sub> was detected by TLC analysis in *Fusarium*-infected adzuki beans (*Phaseolus angularis* (Willd.) W.F. Wight) and mung beans (*Phaseolus aureus* Roxb.). *Fusarium avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. verticillioides*, *F. oxysporum*, *F. solani* and *F. sporotrichioides* were isolated from the mouldy and discoloured seeds. The quantification of FB<sub>1</sub> by high performance liquid chromatography (HPLC) revealed that the mouldy and discoloured adzuki and mung bean samples contained 261±43.8 and 230±21.6 µg/g of FB<sub>1</sub>, respectively (Tseng & Tu 1997).

### 2.3.3. Phytotoxic effects of fumonisins

Not only do fumonisins play a negative role in the health of animals and possibly humans, they are known to show toxic effects towards plant species. Doehlert *et al.* (1994) conducted a study to assess the phytotoxic effects of FB<sub>1</sub> on maize seedlings. The germination of the seeds treated with zero to 1000 ppm FB<sub>1</sub> was unaffected. However, the toxin inhibited radical elongation by up to 75% after 48 h

imbibition. Amylase production in the endosperm was also inhibited, and which could suggest that FB<sub>1</sub> interfered metabolically with germination (Doehlert *et al.* 1994). Danielsen and Jensen (1998) found a significant negative correlation ( $r = -0.52$ ) between fumonisin content and maize seed germination. However, it was not established whether the fumonisins had a direct effect on germination. It was suggested that further research should study the effect of applying the purified toxin directly to the seeds (Danielsen & Jensen 1998). Van Asch *et al.* (1992) reported that maize callus growth was reduced as the concentration of FB<sub>1</sub> in the culture medium increased. The toxin significantly inhibited the growth of the calli at a concentration of 1.0 mg/l and at higher levels (van Asch *et al.* 1992).

Furthermore, FB<sub>1</sub> caused changes in the ultrastructure of treated maize callus cells, which included cell wall thickening, accumulation of phenolics in the vacuoles and accumulation of large starch grains in swollen plastids (van Asch 1990).

Abbas *et al.* (1991) reported that FB<sub>1</sub> could be exploited as a bioherbicide to control jimsonweed (*Datura stramonium* L.). Spores and mycelia of *F. verticillioides* isolated from jimsonweed incorporated into potted soil in which jimson weed plants were planted, caused local lesions and inhibited growth. The toxin caused the same symptoms on excised leaves (Abbas *et al.* 1991). These findings were supported by research carried out by Abbas & Boyette (1992). FB<sub>1</sub> sprayed onto jimsonweed plants at 10 to 200 µg/ml caused chlorosis and necrosis and reduced the height and biomass. Various other plants including sunflowers (*Helianthus annuus* L.), soybeans (*Glycine max* (L.) Merr.) and hemp (*Cannabis sativa* L.) showed varied degrees of symptoms (chlorosis, necrosis, black leaf lesions, tissue curl, stunting, defoliation, death) caused by the toxicity of FB<sub>1</sub>. However, barley (*Hordeum vulgare* L.), maize, rice, sorghum (*Sorghum bicolor* (L.) Moench) and wheat (*Triticum aestivum* L.) were not visibly affected by the toxin (Abbas & Boyette 1992). Lamprecht *et al.* (1994) showed that FB<sub>1</sub>, FB<sub>2</sub> and FB<sub>3</sub> caused leaf necrosis on detached tomato (*Lycopersicon esculentum* Mill.) leaves at the lowest concentration of 0.1 µM of each toxin and necrosis increased at higher concentrations. The fumonisins also caused reductions in shoot and root length and dry mass of maize and tomato seedlings at the varied concentrations (Lamprecht *et al.* 1994). The results from this study also showed that FB<sub>1</sub> was more phytotoxic to the seedlings than FB<sub>2</sub> and FB<sub>3</sub>.

#### **2.3.4. Mode of action of toxicity of fumonisins**

The mechanisms responsible for the diseases caused by fumonisins in animals have been widely studied (Riley *et al.* 1994). *In vitro* studies have shown that fumonisins are potent inhibitors of the enzyme sphinganine (sphingosine) N-acyl transferase (ceramide synthase) (Riley *et al.* 1994). Animals or cultured cells exposed to fumonisins show a dramatic increase in the free sphingoid base,

sphinganine, in tissues, serum and urine. Free sphingosine concentration increases, complex sphingolipid concentration decreases and sphingoid base degradation products and other lipid products also increase. Riley *et al.* (1994) hypothesised that this disruption in sphingolipid metabolism is an early molecular event in the onset and progression of cell injury and the diseases associated with the consumption of fumonisins e.g. LEM and PES. The exact mechanisms are not understood since the role of sphingolipids in cells is very complex.

Sphingolipids have various important functions in cell membranes including stabilisation of the membrane, sorting of lipids and proteins, binding to cytoskeletal elements and cell-cell recognition (Riley *et al.* 1994). A depletion of sphingolipids in membranes will lead to the disruption of the normal function of the membrane. In plants, however, there is little information regarding the function of sphingolipids. They do however play a role in cell signalling, membrane stability, stress response, pathogenesis and apoptosis (Sperling & Heinz 2003).

#### **2.4. ANTIMICROBIAL EFFECTS OF PLANT EXTRACTS ON BACTERIAL AND FUNGAL PATHOGENS**

With the dramatic increase in opportunistic systemic mycoses associated primarily with AIDS and treatment with immunosuppressive agents, new antifungal compounds are urgently required. There are numerous reports regarding the use of plant extracts to control human pathogens, and these include bacterial and fungal pathogens (Lall & Meyer 2000; Wiart *et al.* 2004). However, there is limited information on the use of plant extracts as an alternative means in controlling plant pathogens. With the decrease in the use of chemical formulations to control bacterial and fungal plant pathogens, plant extracts have been exploited as a novel means of control. Poswal *et al.* (1993) investigated the fungicidal properties of various plant parts of ten plant species against the fungal pathogens *M. phaseolina*, *Alternaria zinnae* Pape ex M.B. Ellis and *Sclerotium rolfsii* Sacc. Eksteen *et al.* (2001) reported that methanolic crude extracts of *Eucomis autumnalis* (Miller) Chitt. showed significant antifungal activity against fungal plant pathogens including *F. oxysporum*, *S. rolfsii*, *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow. This extract also compared favourably to the inhibition of the mycelial growth by a broad spectrum synthetic fungicide (carbendazim/difenoconazole). Barreto *et al.* (1997) found that ethanolic extracts of selected seaweed extracts including *Caulerpa filiformis* (Suhr) Hering, *Zonaria tournefortii* (Lamour.) and *Hypnea spicifera* (Suhr) Harv. inhibited the fungal growth by more than 50% of the phytopathogens *Verticillium* sp. and *R. solani*. Pretorius *et al.* (2003) tested crude extracts from 26 South African plant species *in vitro* for their potential to inhibit the growth of

various plant pathogenic fungi and bacteria. None of the crude extracts showed growth inhibition of the fungi tested. The crude extracts of *Euclea crispa* (Thunb. Guerke) subsp. *crispa*, *Acacia erioloba* E. Mey, *Senna italica* Mill. subsp. *arachoides* and *Buddleja saligna* Willd. inhibited the growth of all five bacteria, namely, *Agrobacterium tumefaciens* Smith and Townsend, *Clavibacter michiganense* Spiekermann pv. *michiganense* Smith, *Erwinia carotovora* pv. *carotovora* Jones, *Pseudomonas solanacearum* Smith and *Xanthomonas campestris* Pammel pv. *phaseoli* Smith. The crude extract of *E. crispa* compared more favourably to that of dimethyl dodecyl ammonium chloride (DDAC), a commercial bactericide (Pretorius *et al.* 2003).

## 2.5. SECONDARY METABOLITES ASSOCIATED WITH COWPEA

Flavonoids are low molecular weight 15-carbon secondary metabolites that are widely distributed in the vegetable kingdom (Salisbury & Ross 1992). They play vital roles in defence against pathogens and predators. However, some do have a negative impact on the use of seeds and grains in animal feed and human food e.g. proanthocyanidins (Shirley 1998). Flavonoids can be subdivided into groups, which include the flavonols and flavones (Salisbury & Ross 1992). Legumes are a particularly rich source of flavonoid compounds and could be explored for their increased use in medicine and disease control (Dakora 1995).

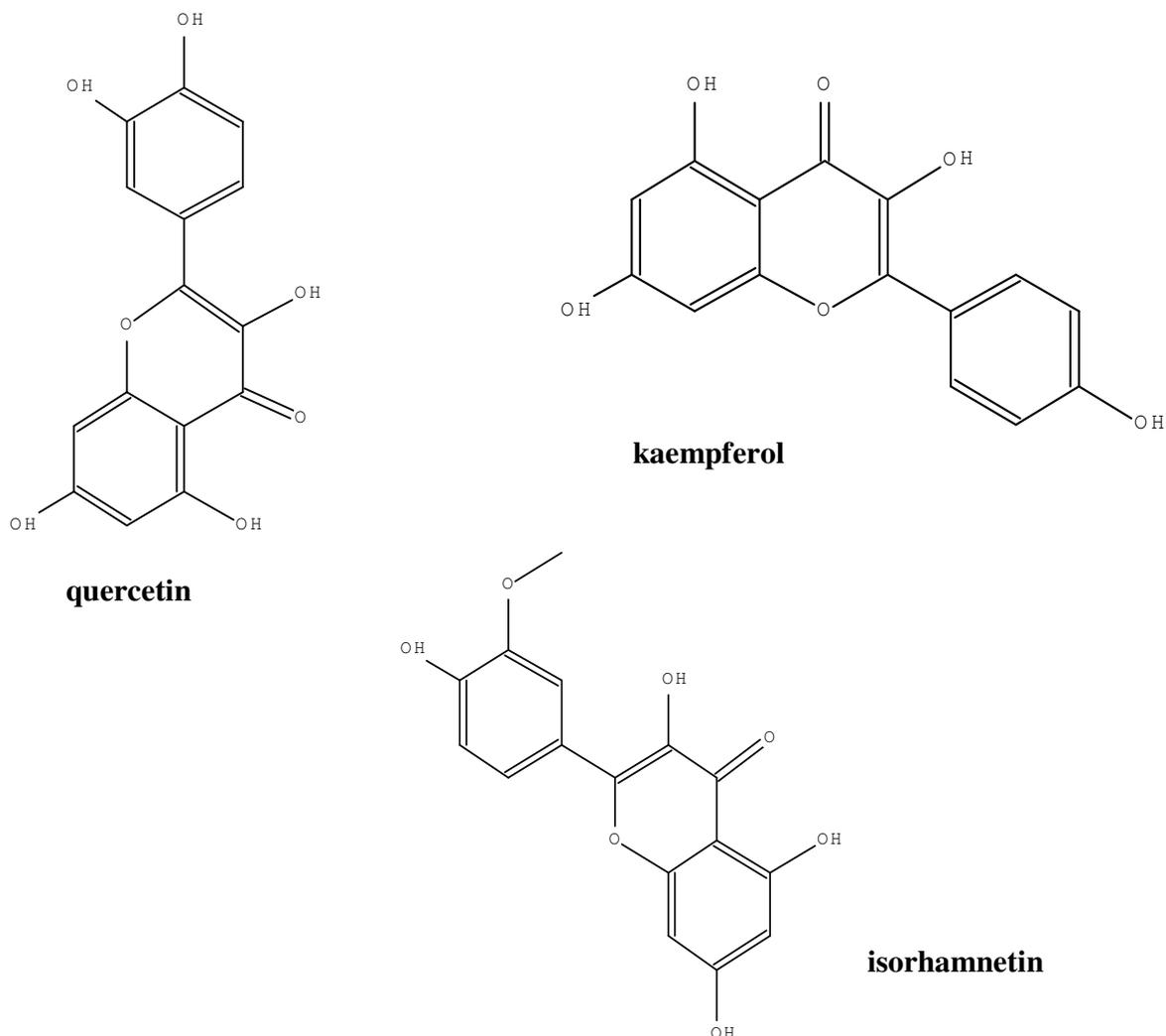
Isoflavonoids (which differ in chemical structure from flavonoids) (Salisbury & Ross 1992) formed in plants during attacks by plant pathogens, play an important role in host-plant resistance to diseases. Since these compounds are toxic to various microbes and the fact that their accumulation restricts microbial growth within plant tissue, they function as phytoalexins (Dakora 1995).

### 2.5.1. Secondary metabolites

Lattanzio *et al.* (1997) showed through flavonoid HPLC analyses of cultivated cowpea lines, that three flavonoid aglycones, namely, quercetin, kaempferol and isorhamnetin (Figure 2.2.), were always present in the leaves. Quercetin was noted as being the most abundant. The flavonoid glycoside pattern showed 10 different glycosides, including 2 *p*-coumaroylglycosides of kaempferol and five of quercetin (Lattanzio *et al.* 1997). Other phenolic aglycons that were identified from leaf extracts of cowpea lines and some wild species of *Vigna* include vanillic acid, *p*-coumaric acid, caffeic acid, cinnamic acid, ferulic acid, protocatechuic acid, sinapic acid and apigenin (Lattanzio *et al.* 2000; Cai *et al.* 2003). Isobe *et al.* (2001) found the flavonoids coumestral, daidzein and genistein in cowpea root extracts. A new pentacyclic triterpenoid saponin as well as other known compounds including cycloartenol,

stigmasterol, 3-o-acetyl-oleanolic acid and 3-o-β-D-glucoside were also isolated from cowpea seeds (Noorwala *et al.* 1995).

Various phytoalexins have been isolated from cowpea after fungal or virus infection. These include the isoflavonoids, kievitone (Bailey 1973; Keen 1975), phaseollin (Bailey 1973), phaseollidin (Bailey 1973), 2-O methylphaseollidiniso flavan (Preston *et al.* 1975) and demethylhomopterocarpin (Lampard 1974). Vignafuran (Preston *et al.* 1975) is the first reported 2-aryl-benzofuran phytoalexin following inoculation with *C. lindemuthianum*. Vignafuran was active against two prevalent Nigerian races of *C. lindemuthianum* (Preston *et al.* 1975). Munn & Drysdale (1975) showed that kievitone can be induced in cowpea by abiotic treatments including topical application of copper chloride (CuCl<sub>2</sub>), actinomycin D or cycloheximide solutions.



**Figure 2.2.** Chemical structures of selected flavonoids associated with cowpea

### 2.5.2. Antimicrobial and medicinal activity of secondary metabolites

The isoflavonoids, pterocarpins, isoflavones and isoflavanones are very toxic to fungal pathogens. They can cause permanent damage to membrane systems, and therefore inhibit fungal spore germination, germ-tube elongation and hyphal growth (Dakora 1995). Quercetin is a naturally occurring bioflavonoid found in high concentrations in red wines, onions and green tea. Its properties include activity as an anti-oxidant and anti-inflammatory. Quercetin, extracted from the leaves of *Geranium dissectum* L. (cut-leaf geranium), showed a good inhibitory effect on the growth of fungi including *F. oxysporum*, *R. solani*, *M. phaseoli* and *Aspergillus carneus* Blochwitz (El-Gammal & Mansour 1986). This same trend could be seen regarding the bacteria *Staphylococcus aureus* Rosenbach, *Sarcina lutea* (Schroeter) Schroeter and various *Bacillus* species. Kaempferol, extracted from *Tribulus pentandrus* L. (devil's-thorn), showed similar results as with quercetin but did not inhibit the growth of *S. lutea* and *Bacillus mycoides* Flugge (El-Gammal & Mansour 1986). Aziz *et al.* (1998) reported that quercetin and *p*-coumaric acids inhibited the growth of *A. parasiticus* and *A. flavus* by 100% at 0.3 mg/ml while caffeic acid inhibited the fungal growth and aflatoxin production at 0.2 mg/ml. Furthermore, caffeic acid inhibited growth of the bacteria *Escherichia coli* (Migula) Castellani and Chalmers and *Klebsiella pneumoniae* (Schroeter) Trevisan at 0.3 mg/ml and *Bacillus cereus* Frankland and Frankland at 0.5 mg/ml. *P*-coumaric acid completely inhibited the growth of the three above-mentioned bacteria at 0.4 mg/ml (Aziz *et al.* 1998).

The increased inhibitory action of phenolic compounds is due to the presence of a phenolic OH group (Gourma *et al.* 1989). The OH group is much more reactive and can easily form hydrogen bonds with active sites of enzymes. Lueck (1980) reported that the antimicrobial action of these compounds was due to the inhibition of certain enzyme reactions or enzyme synthesis in the microbial cell by chemicals. This makes it possible to inhibit the enzyme involved in the basic metabolism of the cell or the synthesis of important cell constituents. El-Gammal & Mansour (1986) reported that quercetin was successful in inhibiting microbial growth of various pathogens used in medicinal and industrial fields. It has been reported that therapy with quercetin provides significant symptomatic improvement in most men with chronic pain syndrome (Shoskes *et al.* 1999).

## 2.6. THE INFLUENCE OF PHYSICAL FACTORS ON THE ULTRASTRUCTURE OF COWPEA SEEDS

The principle features of cowpea seed noted by transmission electron microscopy (TEM) examination include round, ellipsoidal or kidney shaped starch grains and thick cell walls with pit-pairs

(Saio & Monma 1993). Other cellular materials such as vacuoles, protein bodies and lipid bodies can also be observed. Lipid bodies are rarely found adjacent to the cell walls (Saio & Monma 1993).

Suspension culture cells of cowpea (unadapted and thermoadapted cells) underwent various structural changes when exposed to heat stress. These modifications included: almost complete loss of polyribosomes, rough endoplasmic reticulum and dictyosomes; migration of intracellular waste material into the vacuole; retraction of the tonoplast from the cytoplasm into vacuoles and the swelling of the nucleolus with assumed accumulation of preribosomal RNP granules (Dylewski *et al.* 1991). Hung *et al.* (1990) found that severe heat treatment damaged the middle lamella of cotyledon cells and changed the birefringence property of starch granules. Enwere *et al.* (1998) investigated the effect of a drying treatment on the microstructure of cowpea seed and found that cavities occurred in the cotyledons of the 80°C and 120°C dried seeds. The high temperatures weakened the binding forces between the starch granules and protein matrix and the force applied during sectioning was enough to dislodge the granules (Enwere *et al.* 1998). In certain cases the entire cell content was lost after sectioning. It also appeared that the cell content was shrinking away from the cell wall (Enwere *et al.* 1998).

There are various reports of the effects of imbibition on cowpea seed structure. Thomson & Platt-Aloia (1982) reported that the plasmalemma in cowpea seeds is quite permeable during the early stages of imbibition. This was noted by the leakage of electrolytes and the localisation of chloride within the cells of NaCl-imbibed seeds. Freeze-fracture electron microscopy of the radical after 24 h of imbibition revealed a major change in the subcellular organisation. The endoplasmic reticulum and dictyosome were easily visualised and the protein and lipid bodies were spherical. The plasmalemma was also more regular even when compared to the dry seeds (Thomson & Platt-Aloia 1982). The ultrastructure of the dry seeds, after a non-aqueous primary fixation, showed the cytoplasm containing numerous ribosomes. The organelles were ill defined and irregular in outline. Freeze-fracture of the dry embryos revealed that the lipid droplets were closely appressed to the plasmalemma (Thomson & Platt-Aloia 1982).

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