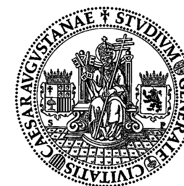
escuela
politécnica
superior
de huesca



UNIVERSIDAD DE ZARAGOZA

PROYECTO FIN DE MÁSTER

EVALUATION OF FOUR BOTANICAL FUNGICIDES AND SITUATIONAL REVIEW ON *VIGNA UNGUICULATA* (L. WALP.) ANTHRACNOSE PATHOGEN, *COLLETOTRICHUM* SPP. SACC & MANG.

AUTOR: VITUS IKECHUKWU OBI

ENSEÑANZA: Masters Degree On “Introduction To Research In Agricultural Science And Natural Environment

DIRECTOR/ES: Juan José Barriuso Vargas

PONENTE: Joaquín Aibar Lete

FECHA: 29/09/2011

DEDICATION

This work is dedicated to my lovely spouse, Theresa and children, Chike and Chinenye, for their wonderful companion and co-operation in the course of the work.

ACKNOWLEDGEMENT

To God almighty be the glory for making this work the success it is today. To my project supervisor Professor (Dr.) Juan Jose Barriuso I thank so much for accepting to act in this capacity. The great role played in both the course of the project and the masters' programme as whole by Dr. Jesus Carlos Romea of the *MECOHISA* cannot go undocumented. He was like an engineer as far as the programme was concerned and was always available to offer his wealth of experience in the successful completion of the project. Dr. Jesus Carlos accept my wholesome gratitude and may God almighty continue to grant you with all your heart desires according to His will, Amen.

To my family members I owe a lot as regards this work. I recognize the special effort of my spouse, Theresa Chinwe Obi, for her spirited care and encouragement in the course of the study. Chike and Chinenye, my kids also aided in their own way during the study. I appreciate all their understanding during the period.

ABSTRACT

The completion of this research involved the revision of about seventy one referred subject related journals and academic materials. The parasitic organism *Colletotrichum destructivum* O`Gara was associated as the actual causal pathogen of cowpea anthracnose. Of all the twenty different pathogens linked with the various cowpea fungal diseases, in this work, only *Colletotrichum* (*C. destructivum*) was found to have the virulence and propensity of afflicting a 100% infection on a single susceptible cowpea crop each at a given pathogenic situation. Twenty *Colletotrichum* species along with their specific primary hosts were identified in this work. The study also provided eighteen plant families under the pathogenic affliction of *Colletotrichum* identified alongside eighteen different plant families representing the entire plants and plant materials screened for biofungicidal properties within a span of eleven years. The screened botanicals of four plants of *Azadiractha indica*, *Cymbopogon citratus*, *Ocimum gratissimum*, and *Xylopia aethiopica* were effective in reducing the spore germination and radial growth of *Colletotrichum destructivum* O`Gara *in vitro* and the growth of the pathogen *in vivo*. Cold water botanicals of *C. citratus* were the best in reducing the growth of the pathogen *in vitro* and in checking the spread of anthracnose disease of cowpea *in vivo*. An evaluation with cowpea (*Vigna unguiculata*) indicated the extracts applied before or after pathogen inoculation to be significantly effective in reducing the size of pathogen induced lesion.

TABLE OF CONTENTS

Page

Dedication. -- **i**

Acknowledgement. -- **ii**

Abstract. -- **iii**

Table of content. --- **iv**

List of table. -- **vii**

List of figures. --- ix

CHAPTER ONE. -- 1

1.0 Introduction. -- 1

CHAPTER TWO. -- 2

2.0: Literature review. -- 2

2.1: Cowpea crop. -- 2

2.2: *Colletotrichum* and other phytopathogens of cowpea. ------ 7

2.3: *Colletotrichum lindemuthianum*. ------------------------------------ 9

2.4: *Colletotrichum destructivum*. -- 11

2.5: One disease two pathogens. --------------------------------------- 12

2.6: Anthracnose and the acervuli. -------------------------------------- 17

2.7: The genus *Colletotrichum*. -- 21

2.8: Systematic on *Colletotrichum species*. -------------------------- 23
2.9: Management of anthracnose problems of *Vigna unguiculata* 26
2.10: Chemical plant disease management. ------------------------- 28
2.11: The botanicals in plant disease management. --------------- 30

CHAPTER THREE. -- 37
3.0: Materials and methods. -- 37
3.1: Consulted material. -- 37
3.2: Botanical materials. --- 37
3.3: Botanical extractions. -- 43
3.4: Isolation and identification of pathogen. ------------------------- 43
3.5: Effect of extracts on spore germination. ------------------------- 44
3.6: Effect of extracts on colony growth of pathogen. ------------- 45
3.7: Field evaluation of extracts and Benomyl. ----------------------- 45
3.8: Experimental design. -- 46
3.9.1: *In vitro* experiment. -- 47
3.9.1.1: Botanical evaluation on pathogen spore germination. ---- 47
3.9.1.2: Botanical evaluation on pathogen colony growth. ---------- 47
3.9.2: *In vivo* experiment for botanical effectivity on disease development. --- 49

CHAPTER FOUR. -- 50
4.0: Results. -- 50
4.1: Effect of *Azadiractha* Treatment on Spore Germination, Colony Growth and Sporulation Density. -- 51
4.2: Effect of *Xylopia* Treatment on Spore Germination, Colony Growth and Sporulation Density. -- 52
4.3: Effect of *Cymbopogon* Treatment on Spore Germination, Colony Growth and Sporulation Density. -- 54
4.4: Effect of *Ocimum* Treatment on Spore Germination, Colony Growth and Sporulation Density. -- 55
4.5: Effect of Plant Extracts and Benomyl Treatments on *Vigna unguiculata* Disease Development by *Colletotrichum* spp. ------------- 55
4.6: Effect of plant Extracts and Benomyl on *in vivo* Sporulation Density of *Colletotrichum* Spp. -- 56
4.7: Effect of treatments on pathological activities of *Colletotrichum* spp. -- 59
4.7.1: Treatments effect *in vitro* on three pathological activities of *Colletotrichum spp*. -- 60
4.7.2: Effect of four botanical extracts and Benomyl fungicide treatment on *Vigna unguiculata* diseases development. ---------------- 61

CHAPTER FIVE. --- 70
5.1: Discussion. -- 70
5.2: Conclusion. -- 76

BIBLIOGRAPHY. -- 78

LIST OF TABLES

Table 1: Relative contributions by geographical area to scientific publications on cowpea diseases. -- 4
Table 2: Publications on cowpea disease according to pathogen groups. -- 5
Table 3: Publication on cowpea disease control according to types- 6
Table 4: *Colletotrichum* and other fungal pathogen of cowpea. ------ 8
Table 5: Morphological and growth characteristics of *Colletotrichum destructivum* and *Colletotrichum lindemuthianum.* ---------------------- 14
Table 6: Morphological and growth characteristics of LAR 056, three isolates of *Colletotrichum destructivum* and an isolate of *lindemuthianum.* --- 16
Table 7: Some species of *Colletotrichum* and their specific hosts.--- 20
Table 8: Plant families under the affliction of *Colletotrichum.* --------- 22
Table 9: Plant families screened for biofungicidal properties. -------- 33
Table10: Biological classification on Neem plant. ----------------------- 39
Table 11: Biological classification on Bush pepper plant.---------------- 40
Table 12: Biological classification on Lemon grass plant.--------------- 41
Table 13: Biological classification on Scent leaf plant. ------------------ 42
Table 14: *In vitro* experimental design. ------------------------------------ 48
Table 15: *In vivo* experimental design. ------------------------------------ 48
Table 16: Effects of extracts (HWE) *in vitro* on *C. lindemuthianum.* - 51
Table 17: Effect of extracts (HWE&OE) *in vitro* on *C. lindemuthianum.* --- 52

Table 18: Effect of extracts on cowpea anthracnose disease. ------ 53
Table 19: Effect of *Ocimum* Treatment on Spore Germination, Colony Growth and Sporulation Density. ------------------------------- 54
Table 20: Effect of Plant Extracts and Benomyl Treatments on *Vigna ungiculata* Disease Development by *Colletotrichum* spp.---- 58
Table 21: Sporulation Density at Indicated Treatment Time.--------- 59
Table 22: Treatment effect *in vitro* on the three pathological activities of *Colletotrichum* spp. --- 61
Table 23: Effect of four botanical extracts and Benomyl treatments on *Vigna unguiculata* anthracnose disease development. ----------- 64

LIST OF FIGURES

Figure 1a: Flowering and fruiting cowpea crop. ---------------------- 2
Figure 1b: Cowpea crop with matured green and *Ecru* colour pods. -- 2
Figure 1c: Healthy harvested cowpea grains. ------------------------- 2
Figure 2: Various organs of *Colletotrichum* infected cowpea crop - 7
Figure 3: *Colletotrichum* species. --- 18
Figure 4: Acervulus of an anthracnose fungus under compound microscope. -- 18
Figure 5: Anthracnose (*Colletotrichum*) infected cowpea leaves 19
Figure 6: Anthracnose (*Colletotrichum*) infected grains. ---------- 19
Figure 7: Classification on *Colletotrichum*. ---------------------------- 25
Figure 8: Botanicals in biopesticides. ---------------------------------- 31
Figure 9a: Matured fruit of *Azadiractha indica*. ---------------------- 38
Figure 9b: Dehisced seeds of *Azadiractha indica*. ----------------- 38
Figure 9c: *Azadiractha indica* young tree. ----------------------------- 38
Figure 9d: *Azadiractha indica* tree with immature fruits. ----------- 38
Figure 10a: *Xylopia aethiopica* tree with unripe fruits. -------------- 40
Figure 10b: *Xylopia aethiopica* harvested ripped fruits. ----------- 40
Figure 11a: *Cymbopogon citratus* (young lemon grass). ---------- 41
Figure 10b: *Cymbopogon citratus* (matured lemon grass. -------- 41
Figure 12a: *Ocimum gratissimum* (young scent plant). ------------ 42
Figure 12b: *Ocimum gratissimum* plant at fruiting stage. ---------- 42
Figure 13: *Azadiractha indica* oil. -- 43
Figure 14: *Xylopia aethiopica* oil. --- 43
Figure 15: Processed leaves of *Cymbopogon citratus*. ------------ 43
Figure 16: Koch pasture’s postulates. ---------------------------------- 44

Figure 17: Potted *Vigna unguiculata* crops.treated at three different levels including the control. -- 57
Figure 18: Infected cowpea crop (control) with stem anthracnose (K) and leaf excised lesion (J). -- 58
Figure19: Comparative effect of *Azadiractha, Cymbopogon, Ocimum,* and *Xylopia* extract base on *Colletotrichum* spore germination. --- 62
Figure 20: Comparative effect of *Azadiractha, Cymbopogon Ocimum* and *Xylopia* extract base on *Colletotrichum colony growth*. -- 63
Figure21: Comparative effect of*Azadiractha,Cymbopogon,Ocimum* and *Xylopia* extract base on *Colletotrichum sporulation density.*- 65
Figure 22: Comparative effect of treatment on disease spread based on period of inoculation on *Vigna unguiculata*. --------------- 66
Figure 23: Location of line of tendency on treatment and inoculation periods on *Vigna unguiculata*. ------------------------------ 67
Figure 24: Percentage regional pathogen infection on *Vigna unguiculata* crop. -- 68
Figure 25: Information on *Vigna unguiculata* disease control techniques utilized for a media decade. -------------------------------- 69

CHAPTER ONE

1.0 INTRODUCTION

Cowpea crop is an important source of protein and other essential nutrients, for both human and livestock particularly among the low income segment of the populace in both the semi-arid regions of the tropics and subtropics of the world (Adebanjo and Bankole, 2004). Cowpea crop has some old and new fungal pathogens that influence its existence, reproduction and survival. One important fungus induced pathogenic problems of this crop is the anthracnose.

Anthracnose disease of cowpea has long been associated with the species of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav**.** (Adebitan and Ikotun, 1996; Amadioha and Obi, 1998; Amadioha, 2003), and relatively of recent with the *Colletotrichum destructivum* O'Gara (Latunde-Dada *et al.*, 1996; Allen *et al.*, 1998; Akinbode and Ikotun, 2008). For the economic importance of this disease, anthracnose on cowpea, several control methods have been adopted including the application of chemical (fungicides) and integrated pest and disease management (Amadioha and Obi, 1998; Amadioha, 2003; Adebanjo and Bankole, 2004).

But for the conscious environmental sustenance and ecological compatibility, there is the need and desirability to search for the alternative which employs natural agro biological balance to address this all important cowpea disease. Two ways to look at the available natural agro biological balance in the control of plant disease are: (a) biological control of plant disease through the use of antagonistic micro organism (bioagents), (Amusa and Ikotun, 1995; Bankole and Adebitan, 1996; Adekunle *et al.,*1997; Akinbode and Ikotun ,2008), (b) botanical control of plant disease through the use of plant extracts (Amadioha and Obi, 1998; Amadioha, 2003; Adebanjo and Bankole, 2004; Akinbode and Ikotun, 2008). In addition therefore, to the fungicidal evaluation of four plant extracts, this study aims to ascertain the state of scientific investigation on botanicals, with cowpea anthracnose disease as a case study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 COWPEA

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous grain crop cultivated mostly in the humid tropics of the globe (Latunde-Dada *et al.,* 1996)**.**It is a primary and commonly cultivated legume crop by many farmers in Africa, where it is grown for its seeds, as a vegetable crop, for green manure, fodder, as a cash crop and or cover crop(Latunde –Dada *et al*.,1996; Allen *et al*., 1998; Akinbode and Ikotun, 2008)**.**Cowpea haulms are also valuable source of livestock protein(Owolade *et al*.,2006). Basically it is an annual crop with the growth habit of climbing, spreading or erect in the bean family (*Leguminosae)* also *Papilionaceae*. Cowpeas are native to Africa where they were domesticated over 4000 years ago. The crop exhibits much variation in growth habit, leaf shape, flower colour and seed size and colour (Emechebe and Lagoke 2002), (fig 1).

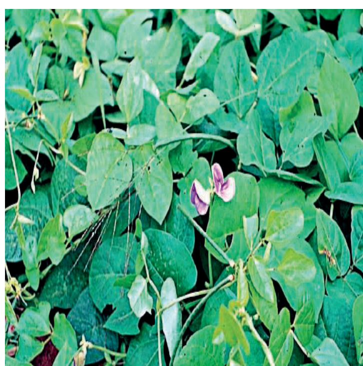


Fig 1a: Flowering and fruiting cowpea crop. **Source**: Http://www.bhg.com/gardening/plantdictionary/vegetable/cowpea/.

Fig 1b: Cowpea crop with matured green and Ecru colour pods. **Source**: Http://www.bhg.com/gardening/plantdictionary/vegetable/cowpea/.

Fig.1c: Healthy harvested Cowpea grains.

This crop is an important source of protein and other essential nutrients to a significant segment of the populace in both the semi-arid regions of the tropics

and subtropics of the world (Adebanjo and Bankole, 2004). The crop constitutes the cheapest source of dietary proteins for the low income sector of the populace of Africa especially west and Central sectors of the region according to Akinbode and Ikotun (2008), where they are rarely planted as a monocrop, but mostly intercropped with maize (*Zea mays* L.), Sorghum (*Sorghum bicolor* (L.) Moench), Pearl millet (*Pennisetum americanum* (L.) Leeke) and Cassava (*Manihot esculenta* Crantz) (Adebanjo and Bankole, 2004; Adebitan and Ikotun, 1996).

Many agro-ecological zones in Nigeria of West Africa tolerate the cultivation and growth of cowpea (Akinbode and Ikotun, 2008). In a relative geographical comparism of scientific publications on cowpea for five years time range according to the work of Emechebe and Lagoke (2002), the Asian region was associated with the most research interest of 35.9% and Australia with the least of 1.4% paper contribution (table 1). This according to Emechebe and Lagoke (2002) leaves the rest 62.7% of the scientific contributions to Africa (24.20%), North America (21.80%), Europe (8.20%), and 7.9% paper contribution from South America and the Caribbean in the “world cowpea conference” of the year 2000.

This all important global crop, however, encounters a number of operational constraints, including pests and diseases that limits its production and yield potentials from seedling to harvest (Emechebe and Lagoke, 2002; Adebanjo and Bankole, 2004; Akinbode and Ikotun, 2008).Cowpea diseases have been associated with species of phytopathogens belonging to various pathogenic groups, (Emechebe and Lagoke, 2000) including fungi, bacteria, viruses, nematodes and the parasitic flowering plants.

Table 1: Relative contributions by geographical area to scientific publication on cowpea diseases (1995-2000)

Geographical area	Number of papers	Percentage of papers contributed (%)
Africa	85	24.80
Asia	123	35.90
Australia	5	1.40
Europe	28	8.20
North America	75	21.80
S. America &the Caribbean	27	7.90
Total	343	100.00

Source: Emechebe and Lagoke (2002).

In their evaluation of scientific documents contributed on cowpea on cowpea diseases according to pathogen groups, between 1995 and 2000, a total of 35.6% was on fungi alone (table 2). This relatively high level percentage of scientific papers speaks volume on the major pathogenic constrains and its economic importance in the smooth cultivation of cowpea crops.

In an effort to checkmate the pathological problems standing on the smooth cowpea production, several control techniques are employed (Colpas *et al.*, 2009). On the evaluation of scientific papers on cowpea diseases control for a span of five years, only a meager value of 7.48% was associated with presentations on “botanicals” (table 3), an indication that more scientific research works and coordinated attention are needed in these areas considering, in addition, the ecological compatibility and environmental importance of botanicals in the control of plant pathogenic problems (Amadioha and Obi, 1999; Ogwulumba *et al.*, 2008; Colpas *et al.*, 2009).

Table 2: Publications on cowpea diseases according to pathogen groups (1995-2000).

Group of pathogen	Number of papers	Percentage of papers (%)
Bacteria	21	6.10
Fungi	122	35.6
Nematodes	69	20.10
Parasitic plants	19	5.50
Viruses	112	32.7

Source: Emechebe & Lagoke (2002)

One important fungus induced pathogenic problems of cowpea is the anthracnose. Anthracnose disease of cowpea has long been associated with the species of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cav. (Adebitan and Ikotun, 1996; Amadioha and Obi, 1998; Amadioha, 2003), and relatively of recent with the *Colletotrichum destructivum* O'Gara (Latunde-Dada *et al.*, 1996; Allen *et al.*, 1998; Akinbode and Ikotun, 2008).

Whichever way the anthracnose of *Colletotrichum* species affects the above ground parts with the products of water soaked lesion in all tissues of the cowpea crop (Akinbode and Ikotun, 2008). According to Adebitan and Ikotun (1996) cropping pattern affects significantly the incidence of anthracnose on cowpea at various stages of the crop growth in a season. Cowpea anthracnose pathogen is a seed borne fungus, usually found on soil surface or plant debris (Amusa *et al.*, 1994; Fokung *et al.*, 1997; Akinbode and Ikotun, 2008). It thrives for at least 2 years on diseased stem tissue either on the soil surface or beneath the soil (Akinbode and Ikotun, 2008).

Lush cowpea growth resulting from closer spacing, as indicated by Adebitan and Ikotun (1996), favored reduced air circulation, promoted higher humidity, prolonged dew periods and allowed cooler soil surface temperatures. All these

microclimatic changes as influenced by the exuberant cowpea vegetation they inferred are considered favorable for the development and progress of anthracnose disease on cowpea.

Cowpea exhibits anthracnose systems within 35 to 40 days after planting susceptible varieties in the form of lesions as small angular brown spots on the leaf petiole, the lower surface of leaves and leaf veins (Adebitan and Ikotun (1996). These spots according to these scientists later coalesced to produce a brick-red to brown discoloration of the leaf.

Table 3: Publication on Cowpea Diseases Control According to Types (1995-2000)

Control type	Number of papers	Percentage of papers (%)
Biocontrol.	17	11.49
Botanicals.	11	7.43
Cultural	22	14.87
Host Pest Resistance (HPR).	72	48.65
Pesticidal.	26	17.57

Source modified from: Emechebe & Lagoke (2002).

2.2 *COLLETOTRICHUM* AND OTHER PHYTOPATHOGENS OF COWPEA

Cowpea crop has some other old and new fungal pathogens that influence its existence, reproduction and survival. Each of these pathogens has regions of interest on a whole cowpea plant (table 4.). Some fungal pathogens attack and infect the roots of the crop (Fernando and Linderman, 1997), stem (Bankole and Adebanjo, 1998, Smith *et al.,* 1999a) leaves (Amadi, 1995, Santos *et al.*, 1997 and Emechebe and Lagoke, 2002), pods and fruits (Munoz and Tamayo, 1994, Roy and Ratnayake, 1997), seeds/seedlings (Smith *et al.*, 1999b, Aveling and Adandonon, 2002), whole plant parts (Akinbode and Ikotun, 2008).

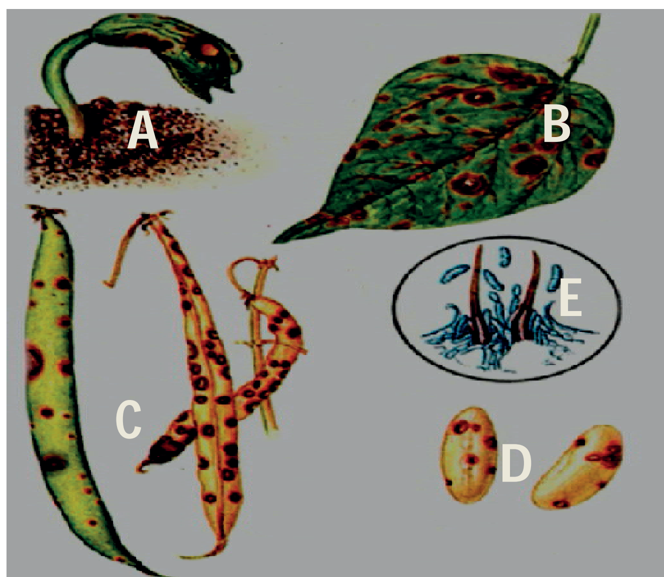


Fig 2: Various organs of *Colletotrichum* infected cowpea crop: A = cowpea seedling, B =cowpea leaf, C =cowpea pods, D= cowpea grains; E = *Colletotrichum* conidiophores and conidia. Adapted Source: Http://www.infonet biovision.org/default/ct/120/crops.

Interestingly of about twenty different pathogens associated with various cowpea crop fungal diseases, as indicated in this work, it is worthy to note that the *Colletotrichum* is the only one that has the potential to affect every part of its host (fig 2). From table 4, it is also obvious that about 30% of the fungal pathogens attack the foliar part of the crop, 25% on the stems, 15% on the roots, 10% on the pods /fruits, 25% on the seeds/seedlings and mere 10% on the whole parts of the plant. Incidentally, while the other fungal pathogens have the possibility each of attacking only about 20% of a stand, the *Colletotrichum spp.* (*C.destructivum* and *C.truncatum*) have the propensity of affecting 100% of a single crop each at a given situation.

Table: 4.Colletotrichum and Other Fungal Pathogens of Cowpea (*Vigna unguiculata).*

Pathogen	Disease	Organs affected	Cited Ref.
Alteneria cassiae Juria &Khan	*Alternaria* leaf spot	Foliar	Grange and Aveling(1998)
Cercospora canescens Ellis & Martin	*Cercospora* leaf spots	Foliar	Amadi (1995)
Choanephora cucurbitarum (Berk & Rav.)	*Choanephora* pod rot	Pods /fruits	Munoz and Tamayo(1994)
Colletotrichum dematium (Pers. ex Fr.)	*Colletotrichum* stem disease	Stem	Smith *et al.,* (1999a)
Colletotrichum destructivum O`Gara	Anthracnose	Every part	Akinbode &Ikotun(2008)
Colletotrichum capsici (Syd.)Butl. & Bisb. *(=Colletotrichum truncatum* (Schw.)	Brown blotch	All parts	Latunde-Dada *et al* ., (1999); Latunde-Dada and Lucas (2007)
Fusarium oxysporum f.sp *tracheiphilum*	*Fusarium* wilt	Seedlings	Smith *et al.,* (1999b)
Fusarium oxysporum f.sp *vasinfectum* (E.F.Smith)Synd & Hans	*Fusarium* wilt	Seedlings	Ushamlini *et al.,*(1997a)
Macrophomina phaseolina	*Macrophomina* blight	Seedlings(severe mortality)	Ratnoo e*t al.,* (1997)
Mycospharella cruenta Latham.(Anormoph of *Pseudocercospora*)	*Pseudocercospra* leaf spots	foliar	Emechebe &Lagoke(2002)
Phomopsis longicola	*Phomopsis* pod spot	Pods/fruits	Roy and Ratnayake(1997)
Protomycopsis phaseoli Ramak&Subram.(=*Entyl oma vinae* Batista)	Leaf smut	Foliar	Santos *et al.,* (1997)
Pythium aphanidermatum (Edison) Fitz	*Pythium* soft rot	Stem	Bankole and Adebanjo(1998)
Pythium ultimum	Damping of(pre/post)	Seed/seedling	Aveling and Adandonon(2000)
Phytophthora cactorum (Leb. &Chon.) Schroet.	Red stem canker	Stem/root	Fernando and Linderman(1997)
Phytophthora vignae Puress.	*Phytophtora stem* rot	Stem/root	Fernando and Linderman(1997)
Sphaceloma sp.(Anamorph of *Elsinoe phaseoli* Jenkin)	*Sphaceloma* scab.	Hypocotyls & epicotyls	Emechebe &Lagoke(2002)
Sclerotium rolfsii Sacc. (Teliomorph: *Corticum rolfsii* Curzi).	Basal stem rot/wilt	Stem	Muqit *et al.,* (1996)
Uromyces appendiculatus (pers.) Unger (=*U.vinae* Barclay).	Brown rust	Foliar	Heath (1997)
Thanatephorus cumeris (Frank)Donk(=*Rhizocto nia solani* Kuhn)	Web blight Root (root rot /seedling disease complex	Root/seedling	Emechebe &Lagoke(2002)

2.3 <u>*COLLETOTRICHUM LINDEMUTHIANUM*</u>

Colletotrichum lindemuthianum (Sacc. and Magn.) Bri. And Cav. is one of the species of Colletotrichum being linked with the anthracnose pathogenic problem of cowpea. Anthracnose of cowpea has been described as the major disease of cowpea (*Vigna unguiculata* (L.)Walp), causing severe damage and lose under low temperature, high humidity and free moisture (Adebitan and Ikotun, 1996; Emechebe and Lagoke, 2002).

In an infected cowpea crop the anthracnose symptoms first appears at thirty five to forty days after planting in the form of lesions as small angular brown spots on the leaf petiole, the lower surface of leaves and leaf veins of cowpea grown under different cropping patterns (Adebitan and Ikotun, 1996). These various spots created later coalesced to produce a brick red to brown discoloration of the entire leaf. Symptoms are usually delayed until production of flowering buds.

Cropping pattern affects significantly, according to Adebitan and Ikotun, (1996) the incidence of anthracnose on cowpea at various stages of the growth. For instance anthracnose incidence of severity have been reported to be lower in the intercrop relative to the sole crop while reduction in both inter and intra row spacing resulted in an increase in the incidence and severity of anthracnose(O`Connell *et al.*, 1993; Adebitan and Ikotun, 1996; Emechebe and Lagoke, 2002).

Some scientists attributed the higher diseases incidence and severity values obtained on monocropped cowpea farming to lack of physical barriers provided in the intercrop. Adebitan and Ikotun, (1996) reported that up to forty to forty nine percent reduction in disease reduction can be obtained in cowpea intercropped system.

Crop treatment with some phosphorus (P_2O_5) fertilizer has also been reported to reduce the severity of anthracnose problem when compared with crops without any fertilizer application (O`Connell *et al.*, 1993; Emechebe and Lagoke, 2002). These same researchers reported that weed free plots of cowpea crops

can decrease incidence of anthracnose by about 56% and severity reduction by about 43% when compared to weed infested cowpea plots.

The reduction in the possibility of infection has been reported in an intercropped cowpea by Adebitan and Ikotun (1996) who attributed the no host crop as being responsible due to their ability to slow down the dispersion and movement of spores and pathogen propagules within the cropping system. Adebitan and Ikotun, (1996) were of the view that the conidia which fall on a no host are eventually lost, resulting in a slower increase of disease in an intercropping system.

Other observed mechanisms for disease reduction on intercropped stands as deduced by Adebitan and Ikotun, (1996) are: (a) that in a pure stand of cowpea crops with uniform susceptibility to species of *Colletotrichum*, for example, the replacement of a portion of these crops by resistant ones, like maize for example, reduces the amount of inoculums available for subsequent dispersal within the stands. (b) That the resistant crop may interfere with the passage of inoculums between crops. This is so, especially in the case of air borne diseases where the foliage of the resistant crop could act as a trap for the spore or potential propagules. This would subsequently reduce the number of propagules available for infecting the susceptible crop, and (c) that the resistant host provides an unsuitable environment for the development of the disease within the immediate or extended cropping system.

As part of the observation on the *Colletotrichum* species effect on cowpea within a cropping systems, Adebitan and Ikotun (1996) reported that the closer the rows were together the greater the possibility of infection by the pathogen and that the disease was more severe on cowpea crops grown in rows which were more closely spaced than those which were grown in wider rows. Narrower plant spacing between and within rows led to increased incidence and disease severity. This according to Adebitan and Ikotun (1996) indicates that as a cowpea crops become more crowded together per given area, especially in the monocropped, the tendency for crop to contact increase. As a result the micro environment within the crop becomes more moist and thus more

conducive for disease development (Adebitan and Ikotun, 1996).Grain yield loss by 35-50% could be observed in *Colletotrichum lindemuthianum* susceptible cowpea cultivars (Amadioha and Obi, 1998; Amadioha, 2003).

2.4 *COLLETOTRICHUM DESTRUCTIVUM*

There has been scientific suggestions that the cowpea anthracnose pathogen be regarded as species that is distinct from *Colletotrichum lindemuthianum* (Emechebe and Lagoke, 2002), a phytopathogens also associated with the *Phaseolus* bean anthracnose (Amadioha and Obi, 1998).

Colletotrichum destructivum is the other pathogen of the species being associated with the anthracnose disease of cowpea. According to Akinbode and Ikotun, 2008; Allen *et al.,* 1998, anthracnose disease of cowpea (*Vigna unguiculata* (L.) Walp) is initiated by *Colletotrichum destructivum* O`Gara. The pathogen is described as seed borne fungus which can be found on soil surface or plant debris (Amusa *et al.*, 1994; Fokung *et al.*, 1997), and can survive for at least two years on diseased stem tissues either on the soil surface or beneath (Akinbode and Ikotun, 2008).

Colletotrichum destructivum is hemibiotrophic pathogen which is associated with a whitish fluffy mycelia colour (Akinbode and Ikotun, 2008). *Ricinus communis* plant extracts has been found to encourage the growth and survival of this pathogen instead of its inhibition for which it was bio-assayed. This pathogen *C. destructivum* sporulates readily on infected cowpea at localized infection foci and produce symptom within 96hrs of inoculation (Latunde-Dada *et al.*, 1999).

In the note of Latunde-Dada *et al.*, (1999) the anthracnose disease of cowpea induced by *Colletotrichum destructivum* O'Gara was erroneously, in the past, ascribed to *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav. The pathogen gains ingress into the host by elaborating from the melanized

aspersorium, an infection peg, which penetrates the cuticle directly to initiate infection (Latunde-Dada *et al.*, 1999).

According to Akinbode and Ikotun (2008) a weekly or biweekly application of Benomyl are effective against *C. destructivum* pathogen. They also observed in their investigation that an application of phosphorus fertilizer gave lowest severity of *C. destructivum*. Fungicide application and an integrated control system are some of the efforts geared towards the management of problems from *C. destructivum* in cowpea crop reported Akinbode and Ikotun (2008).

Only of recent *Colletotrichum destructivum* has also been linked to *Colletotrichum higginsianum* as synonyms. This, according to Sun and Zhang (2009) has been supported in scientific investigations where the sequences of the rDNA ITSxs region of *C. destructivum* isolates from cowpea were identical with 100% similarity to that of isolates of *C. higginsianum* originating from cruciferous plants.

2.5 ONE DISEASE TWO PATHOGENS

Two phytopathogens, *Colletotrichum lindemuthianum* (Saccardo & Mangnus) Briosi & Cavara and *Colletotrichum destructivum* (O`Gara) have at different times and space been allegedly linked with the anthracnose of cowpea (*Vigna unguiculata* (L.) Walp). But recently, based on many scientific investigations and findings on detailed, physiological and morphological characteristics of the different organisms and in comparism to the characteristics of the diseases inflicted, mycologist of the phytopathology are already on to the distinction and proper classification of these agents.

In the works of Sheriff *et al.*, 1994; Adebitan and Ikotun, 1996; Amadioha and Obi, 1998; Amadioha, 2003; Adebanjo and Bankole, 2004, the anthracnose of cowpea was linked with the *Colletotrichum lindemuthianum* as the sole causal organism and no mention was made of *Colletotrichum destructivum*. But anthracnose disease of cowpea according to Latunde-Dada *et al.,* (1999) was erroneously ascribed to the *Colletotrichum lindemuthianum* pathogen.

There have been numerous scientific calls that the cowpea anthracnose pathogen be regarded as a species that is completely distinct from the *Colletotrichum lindemuthianum*, which instead should be restricted to as a causal agent responsible for the anthracnose of kudzu bean (*Phaseolus vulgaris* L.) (Emechebe and Lagoke, 2002). Incidentally researchers in the field of plant pathology have before now also associated the anthracnose of Kudzu bean with the same *Colletotrichum lindemuthianum* pathogen. In their work Amadioha and Obi (1998) documented *Colletotrichum lindemuthianum* as the causal organism responsible for the anthracnose disease in both cowpea (*Vigna unguiculata* (L.) Walp) and Kudzu bean (*Phaseolus vulgaris* L.).

The anthracnose disease of cowpea according to Latunde-Dada *et al.,* (1996) is caused by a species of *Colletotrichum* which produces ovoid shaped conidia that are not of *Colletotrichum lindemuthianum* but of the *Colletotrichum destructivum*. They indicated that the initial accreditation of the cowpea anthracnose to the *Colletotrichum lindemuthianum* was faulty since the pathogen is more characterized with the anthracnose of Kudzu bean (*Phaseolus vulgaris* L.). And considering that the data arising from scientific studies of *Colletotrichum destructivum* pathogen morphology, mode of infection and rDNA sequences are distinct from that of *C. lindemuthianum* (O'Connell *et al.*, 1993; Sheriff *et al.*, 1994; Latunde-Dada *et al.*, 1996), an increasing body of evidence has questioned the designation of *C. lindemuthianum* as the ovoid spored cowpea anthracnose pathogen (Latunde-Dada *et al.*, 1996).

In the investigation of Latunde-Dada *et al.,* (1996) though, both the cowpea anthracnose fungus and the bean anthracnose fungus infect their respective hosts through an initial intracellular biotrophic phase, it was however, observed that the infection structures produced by the cowpea anthracnose fungus differ markedly from those produced by *C. lindemuthianum* (table 4).

Within a comparative evaluation of the hyphae lacerating structure, it was observed that during the biotrophic phase, *C. lindemuthianum* produces spherical vesicles and swollen primary hyphae which pass from initially-infected

epidermal cell to several other epidermal and cortical cells (Latunde-Dada *et al.*, 1996), whilst the biotrophic phase of the hemibiotrophic cowpea anthracnose fungus was restricted to single epidermal cells (Table 5).

According to Latunde-Dada *et al.*, (1996 &1999) the causal organism of cowpea anthracnose produces large, multilobed infection vesicles during the biotrophic infection of the host. The pathogen gains ingress into the host by elaborating from melanized appressoria, an infection peg, which penetrates the cuticle directly to initiate infection and never through the stomata (Latunde-Dada *et al.,* 1999). In addition Latunde-Dada *et al.*,(1996 &1999) inferred that anthracnose of cowpea has a straight conidia (14-18 µm) and penetrate host cells directly to establish transient intracellular biotrophic infections which are restricted to the initially infected epidermal cells (Table 5).

Table 5: Morphological and growth characteristics of *C. destructivum* and *C. lindemuthianum.*

Structures and Activities	*C. destructivum*	*C. lindemuthianum*
Conidia shape	Ovoid	Ovoid
Conidia size (µm)	16.3±2.0x4.0	12.1±1.0x4.0
Conidial Septation (upon germination)	Yes	No
Appressorial shape	Subglobose (with variable margins)	Globose
Stomatal penetration	No	No
Intracellular biography	Large,multilobed vesicles	Small spherical vesicles+primary hyphae
Intracellular	One cell	Many cells
Ability to infect cowpea	Yes	No
Prolonged symptomless infection	No	No
Acervulus	One seta	Many setae

Source: modified from Latunde-Dada *et al.,* (1999).

Latunde-Dada *et al.,* (1999) have provided strong evidence in favour of considering the cowpea anthracnose pathogen as a form of C. destructivum (O`Gara). Recognizing *C. destructivum* pathogen as a real causal organism of anthracnose in cowpea crop has been accepted by some authors on recent review of cowpea diseases (Emechebe & Lagoke, 2002; Allen *et al.*, 1998).

In a further investigation on the controversial anthracnose pathogen of cowpea, an electron microscopy analysis of the nucleotide sequences of the amplified D2 and ITS-2 regions of rDNA revealed very close similarities (97-99%) between the cowpea isolate and three isolates of *C. destructivum* obtained from Lucerne (*Medicago sativa*) (table 6).

It was indicated that the isolate LARS 056 is most closely related to the *C. destructivum* isolates, LARS 202, 319 and 709, with which it shares 97.7% to 98.3% homology. In contrast it was observed that the homology with *C. lindemuthianum* is about 87.1%.Latunde-Dada *et al.*, (1996) associated *C. lindemuthianum* pathogen with the intracellular biotrophic tissue colonization activity while the intracellular hemibiotrophic strategy of host colonization was said be unique with cowpea isolates LARS 056 in their work.

Although conidial size differed slightly, all isolates of *C. destructivum* showed significant morphological similarities to LARS 056, most notably by having conidia which become septate upon germination and subglobose appressoria with variable, irregular outlines (table 6).

In view of the several body of evidence as articulated by researchers such as Latunde-Dada *et al.*, (1996 &1999) this author is of the conclusive opinion that the organism***,*** *Colletotrichum destructivum* O`Gara be accepted the true pathogen of the anthracnose of Cowpea (*Vigna unguiculata* (L) Walp.). Already Latunde-Dada *et al.*, (1996) concluded in their work that for the fact that the actual taxonomic status of this pathogen remains to be defined, it is in appropriate to ascribe the pathogen to *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav.

In plant pathology the two species of *Colletotrichum,* have interestingly been found to posses some Biopesticides activities, so that their economic value should not be viewed only from the negative qualities. The metabolites from both *Colletotrichum lindemuthianum* and *Colletotrichum destructivum* have

been effectively employed in the control of several plant diseases (Amusa and Ikotun, 1995).

Table 6: Morphological and growth characteristics of LARS 056, three isolates of *Colletotrichum destructivum* and an isolate of *Colletotrichum lindemuthianum.*

Structures and Activities	*Colletotrichum destructivum*				*Colletotrichum lindemuthianum*
	LARS 056	LARS 202	LARS319	LARS 709	LARS 009
Conidial shape	Ovoid, predominantly straight, but slightly curved with a truncate.				Ovoid, straight rounded apex & base
Conidial size (µm)*	16.3±1.97x 4	17.8±1.64x 4	17.2±1.86x4. 4	16.4±2.09x 4	12.1±1.0x4
Septation of conidium	Yes	Yes	Yes	Yes	No
Appressorial shape	Subglobose with irregular and variable margins				Globose
Intracellular hyphae	Large multilobed vesicles and variable margins.				Small spheric vesicles+primary hyphae
Intracellular biotrophy	One cell	One cell	One cell	One cell	Many cells
Ability to infect Cowpea	Yes	Yes	Yes	Yes	No
Acervulus	One seta	One seta	One seta	One seta	Many setae

*Mean of 30 measurements ±S.D

Source: modified from Latunde- Dada *et al.*, (1996).

Results of the bioassay by Amusa and Ikotun (1995) showed that metabolites produced by *C. lindemuthianum and C. destructivum* pathogens were toxic to all the non host evaluated plants in their investigation. Both organisms have been recommended for inclusion as mycoherbicides for their effective pathotoxin activities on the bio assayed non-host weed plants (Amusa and Ikotun, 1995). *Colletotrichum destructivum* is also reported sensitive to the activities of *Pseudomonas fluerescens* and *Bacillus subtilis*, phytobacterial organisms, as bioagents in the work of Akinbode and Ikotun (2008).

2.6 ANTHRACNOSE AND ACERVULI

Anthracnose is a common name of plant diseases characterized by black lesions, usually sunken, caused by certain imperfect fungi that produce spores, e.g. *Colletotrichum*, *Gloeosporium* and some closely-related *Sphaceloma* species.

The disease is caused by certain Ascomycetes that produce asexual fruiting bodies called conidiomata in generic terms. These groups of pathogens are also referred to as polycyclic pathogens, (Schumann and D`Arcy, 2006), hence they complete several too many generations during the growing season, meaning that primary and secondary inocula are produced during a particular growing season. It refers to a group of diseases with variable symptoms but the common sign is acervuli filled with sticky conidia. In their work, Schumann and D`Arcy (2006) grouped anthracnose and vascular wilt as some of the common plant disease caused by necrtrophs. Necrotrophs usually produces destructive toxins and enzymes that destroy plant tissues. Other characteristics of Necrotrophs as stated by Schumann and D`Arcy (2006) are: their ability to attack young, weak, or senescent tissues; kill host cells rapidly by producing toxins or destructive enzymes and penetrate host through wounds or natural openings. They have wide host ranges and can grow as saprophytes, these scientists observed in addition.

The lifecycle of anthracnose diseases involves essentially production of spores on susceptible hosts, dispersal of spores, penetration of host tissue, initiation of an infection process within the cells, development of lesions, formation of bristly spores and dispersal usually by water-splash, air currents, insects or other forms of contact (Schumann and D'Arcy, 2006).

The name anthracnose itself is derived from the Greek word for coal which describes the black asexual fruiting bodies, (acervuli) of the casual fungi reported Schumann and D'Arcy (2006). The acervuli, they said are sometimes produced in concentric rings that may be observed on infected fruits and vegetables (Figs: 5 & 6).

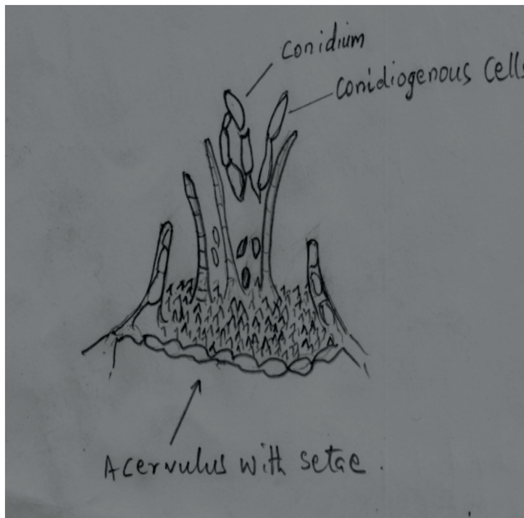


Fig. 3: *Colletotrichum* spp.Top: conidia, Conidiogenous cells. Bottom: Acervulus with setae (manually drawn).

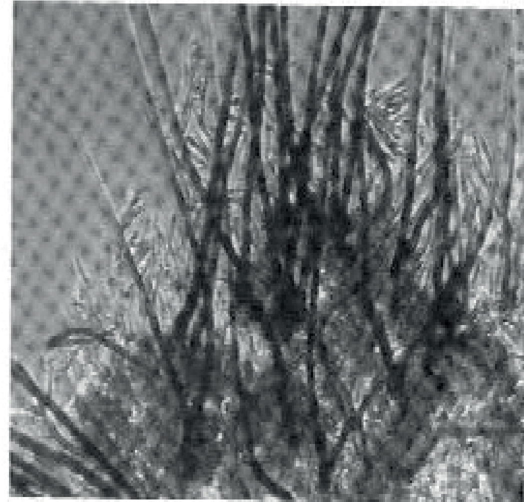


Fig. 4: Acervulus of an anthracnose fungus under compound microscope. Note the black setae (hair like projections) (Schumann and D`Arcy, 2006).

An Acervulus is composed of conidiophores arranged in a thin layer below the host epidermis, becoming exposed by erupting through the plant epidermis, sometimes containing whisker like hyphae (setae), characteristics of anthracnose diseases produced at asexual fruiting stage of the organism (Schumann and D`Arcy, 2006).

Pathogenic producers of conidia in partially enclosed structure are in the two forms of asexual stages of fungi reproduction. The other group is the Hyphomycetes. The Coelomycetes have their conidia formed from conidiophores within different types of fruiting bodies enclosed by a layer of fungal and /or host tissue. It is from here that we have the acervuli.

For the fact that the conidia component of an acervulus are sticky, anthracnose pathogens like the *Colletotrichum lindemuthianum* and *C. destructivum* are easily dispersed by water, insects, human activities and machinery reported Schumann and D`Arcy (2006). *Colletotrichum lindemuthianum and C. destructivum* are found within the plant pathogenic order of *Melanconiales* and family *Melanconiaceae.*

According to Dugan (2006) the *Melanconiaceae* are the acervuli fungi with aseptate and hyaline referred to as hyalosporae (Figs.3 & 4). They are often associated with setae and phialidic conidiogenesis. The term *Melanconiales* though appears obsolete just as Sphaeropsidales, for their lack of phylogenetical information, plant pathologists continue to note the importance of the acervuli as a useful information structure on *Colletotrichum* species induced anthracnose problem (Dugan, 2006).

Fig.5: Anthracnose (*Colletotrichum)* infected cowpea leaves .Source: Http://infonet.biovision.org

Anthracnose diseases attack all plant parts at any growth stage (Schumann and D`Arcy, 2006). The symptoms are most visible on leaves (fig 5) and ripe fruits and seeds (fig 6). At first, anthracnose generally appears on leaves as small and irregular yellow, brown, dark-brown or black spots. The spots can expand and merge to cover the whole affected area. The colour of the infected part darkens as it ages. The disease can also produce cankers on stems, mostly in woody plants. Infected fruit has small, water-soaked, sunken, circular spots that may increase in size up to 1 cm in diameter. As it ages, the center of an older spot becomes blackish and emits gelatinous pink spore masses. Wet conditions favour the activities of anthracnose disease. Anthracnose disease, however, are commonly managed by minimizing leaf wetness and removing infected plants and infected plant debris when possible, proffered Schumann and D`Arcy (2006). Reduction of primary inoculums and reduction in the rate of infection have also been recommended for the management of this problem.

Fig.6: Anthracnose (*Colletotrichum)* infectedcowpeagrains.Source: Http://www.infonet-biovision.org/default/ct/120/crops

Table 7: Some Species of *Colletotrichum* and Their Specific Hosts

Species	Associated Host	Common Name	Family	Cited Ref.
Colletotrichum acutatum	*Olea europeae*	Olive	*Oleaceae*	Salazar *et a.,l*(2007) Gomes *et a.,l*(2009)
C. acutatum	*Malus domestica* (=*M. pumila*)	Apple	*Roasaceae*	Liu *et al.*,(2007)
C. acutatum	*Fragaria ananassa*	Strawberry	*Roasaceae*	Salazar *et al.,*(2007) Garrido *et al.,*(2008)
C. capsici (=*C.truncatum*)	*Vigna unguiculata*	Cowpea	*Leguminosae(=Papilionoideae)*	Latunde-Dada *et al.,*(1999),Emechebe &Lagoke(2002)
C. boninense	*Coffea* spp.	Cooffee	*Rubiaceae*	Moriwaki *et al.*,(2003),& Nguyen *et al.,* (2010)
C. dematium	*Spinacia oleracea*	Spinach	*Amaranthaceae*	Liu *et al.,* (2007)
C .dematium	*Vigna unguiculata*	Cowpea	*Leguminosae*	Emechebe &Lagoke (2002)
C. destructivum	*Vigna unguiculata*	Cowpea	*Leguminosae*	Latunde-Dada(1999), Emechebe &Lagoke (2002)
C. destructivum	*Cuscuta epthymum* *Cuscuta campestris*	Dodder Field dodder	*Convovulaceae. Cuscutaceae*	Amusa & Ikotun(1995)
C. fragariae	*Fragaria ananassa*	Strawberry	*Roasaceae*	Salazar *et al.,*(2007) Garrido *et al.,*(2008)
C. gloeosporioides	*Persea americana* *Mangifera indica*	Avocado Mango	*Lauraceae* *Anacardiaceae*	Sanders *et al.,* (2000)
C. gloeosporioides	*Aeschynomene virginica* *Stylosanthes scabra*	Sensitve jointvetch Shrubby stylo.	*Fabaceae* *Fabaceae (=Leguminosae)*	Latunde-Dada *et al.,*(1999)
C. gloeosporioides	*Fragaria ananassa*	Strawberry	*Roasaceae*	Salazar *et al.,*(2007) Garrido *et al.,*(2008
C.gloeosporioides	*Carica papaya*	Pawpaw	*Caricaceae*	Palhano *et al.,*(2004)
C .graminicola	*Zea mays* *Oryza sativa*	Maize Rice	*Poaceae*	Amusa & Ikotun(1995)
C. higginsianum	*Arabidopsis thaliana*	Wall cress	*Brassicaceae*	Sun & Zhang(2009)
C. lindemuthianum	*Vigna unguiculata*	Cowpea	*Leguminosae*	Amadioha(2003) Adebitan& Ikotun(1996)
C. lindemuthianum	*Phaseolus vulgaris*	Green(kudzu) bean	*Fabaceae (=Leguminosae)*	Amadioha & Obi(1998); Liu *et al.*,(2007)
C. linicola	*Linum usitatissimum*	flax	*Linaceae*	Latunde-Dada &Lucas(2007)
C. magna	*Cucurbita pepo*	Pumpkin	*Cucurbitaceae*	Liu *et al.*,(2007)
C. malvarum	*Sida spinosa*	Prickly sida	*Malvaceae*	Liu *et al.*,(2007)
C. musae	*Banana*	Banana	*Musaceae*	Liu *et al.,*(2007)
C. orbiculare	*Cucumis lanatus* *Xanthium spinosum*	Water melón Cocklebur	*Cucurbitaceae* *Asteraceae*	Liu *et al.,*(2007)
C. sulineolum	*Sorghum bicolor*	Sorghum	*Poaceae*	Moore *et al.,* (2008)
C. trifolii	*Medicago sativa*	Alfalfa	*Fabaceae*	Mould *et al.*,(1992) Liu *et al.*,(2007)
C .truncatum	*Pisum sativum*	Snow peas	*Fabaceae*	Latunde-Dada *et al.,*(1999)
C. xanthi	*Xanthium spinosum*	Burr weed	*Asteraceae*	Amusa & Ikotun(1995)

2.7 <u>THE *GENUS: COLLETOTRICHUM*</u> Corda

The plant pathogenic genus, *Colletotrichum*, is an important genus within the parasitic microorganisms of crop production. Its damaging activities have been reported in the temperate, subtropical and tropical agricultural crops (Gomes *et al.*, 2008). It is a genus associated with setose acervuli, relatively large cylindrical or falcate phialoconidia, and appressoria, (Thaung, 2008), attacking a very broad range of host plants (table 6). The genus embraces some forty species of plant parasites and provides anamorphs of *Glomerella* with a large reservoir of synonymy too cumbersome for accurate and conclusive systematic study stated Thaung, (2008). The present work has been able to associate about twenty seven *Colletotrichum* species to their specific primary plant hosts (table 7). Scientists such as Gomes *et al.*, (2008) reported the genus to include many of the most damaging plant pathogens in the field of agriculture, whose anthracnose effects on a wide range of plants are well documented (Martin & Garcia- Figures, 1999).

Colletotrichum species have also been reported as the casual agents of anthracnose and blight on cereal crops (Amusa & Ikotun, 1995) grasses (Gomes *et al.*, 2009), grain crops (Adebitan & Ikotun, 1996, Amadioha & Obi, 1998, Latunde-Dada, 1999, and Amadioha, 2003), vegetable plants (Liu *et al.*, 2007), perennial tree crops (Latunde-Dada *et al.*,1999 and Gomes *et al.*, 2009) and even feeds (Mould *et al.*, 1992 and Liu *et al.*, 2007). Some postharvest disease problems have also been attributed to some species of the *Colletotrichum* genus of the plant pathogen (Sanders *et al.*, 2000). The genus with its devastating prowess can infect all plant surfaces, but favours young leaves, branches and fruits of herbaceous species growing in humid climates reported Gomes, *et al.,* (2008). Incidentally, this work has counted about eighteen total plant families under the pathogenic attack and disease affliction of *Colletotrichum* (table 8).

It has been pretty documented that the ability of *Colletotrichum* to develop a series of specialized infection structure, including germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae including the evolving of a hemibiotrophic strategies singled out the wide host range linked with the

species of this pathogenic genus (Sun and Zhang, 2008). Studies have also shown that *Colletotrichum* conidia will adhere rapidly to a wide range of plant and artificial surfaces, including cellophane, polystyrene, polycarbonate and glass.

These strong and relatively unique pathogenic characteristics must have led to the acceptance of the genus as an excellent model for studying the molecular and cellular bases of fungal pathogenicity as indicated by Sun and Zhang (2009).

Table 8: Plant Families Under The Affliction Of *Colletotrichum* Corda (1995-2009)

Family	Cited Authors
Amaranthaceae Juss.	Liu *et al.,(*2007)
Anacardiaceae Lindl.	Sanders *et al.,*(2000)
Asteracea Bercht. & J. Presl.	Amusa & Ikotun(1995), Liu *et al*.,(2007)
Brassicaceae Juss.	Sun & Zhang(2009)
Carcaceae Dumort.	Palhano *et al.,*(2004)
Convovulaceae Juss.	Amusa & Ikotun (1995)
Cucurbitaceae Juss.	Liu *et al.,*(2007)
Cuscutaceae Dum. (=Convovulaceae)	Amusa & Ikotun (1995)
Fabaceae Lindl.	Mould*etal.,* (1992), Amadioha & Obi (1998), Latunde-Dada *et al.,* (1999), Emechebe & Lagoke (2002), Liu *et al*., (2007).
Lauraceae Juss	Sanders *et al* (2000)
Leguminosae Juss., Non. con (=*Fabaceae* Lindl.)	Adebitan & Ikotun (1996), Latunde-Dada *et al.,* (1996),Emechebe & Lagoke (2002),Amadioha (2003)
*Linaceae.*L	Latunde-Dada and Lucas(2007)
Malvaceae Juss	Liu *et al*.,(2007)
Musaceae Juss	Liu *et al.,* (2007)
Oleaceae Hoffmgg.& Link	Salazar *et al.,* (2007) Gomes *et al.,* (2009)
Poaceae Barnhart (=Gramineae Juss, Non. Con.)	Amusa & Ikotun (1995), Moore *et al*., (2008)
Roasaceae Adans	Liu *et al*., (2007) Salazar *et al*., (2007) Garrido *et al.,* (2008)
Rubiaceae Linn	Moriwaki *et al*., (2003), & Nguyen *et al*., (2010)

Colletotrichum species are haploid organisms which can be cultured axenically and transformed. This in extension, according to Sun and Zhang (2009) greatly facilitates mutational analysis and the critical assessment of gene function by targeted gene disruption. For example, isolates of *Colletotrichum* from cruciferous plants has recently been reported to lead to the production of a new *Arabidopsis* pathosystem for infecting *Arabidopsis thaliana* plant (Sun and Zhang, 2009).

2.8 SYSTEMATICS ON *COLLETOTRICHUM*

The genus *Colletotrichum* Corda was first described by Tode in 1790 under the name *Vermicularia,* but was later established as *Colletotrichum* by Corda in 1837. According to Thaung, 2008, and Gomes *et al.*, 2008, *Colletotrichum* parasite is the known single genus among the Coelomycetes that has garnered most attention probably because of the diversity, distribution and devastating activities of most of its members. Coelomycetes are Deuteromycetes referred to as fungi imperfecti associated with structural acervuli, pycnidia, or stromata as conidiomata (Dugan, 2006; Schumann & D`Arcy, 2006 and Thaung, 2008). And within the Deuteromycetes, fungi Coelomycetes are Mitosporic and microscopic (Thaung, 2008), ubiquititious (Gomes *et al.*, 2008), parasitic, saprobic or facultative phytopathogens (Schumann & D`Arcy, 2006).

The Coelomycetes generally exist as conidial, spermatial / microconidial states or anamorphs of ascomycetes (Thaung, 2008). This group might as well be called fungi mitospori or fungi Anamorphic. According to Thaung, (2008) some authors have referred to Coelomycetes as polyphyletic, artificial (non phyletic) (Schumann & D`Arcy, 2006) and untenable or redundant form of fungi (Fig. 7).

The characterization and identification for species classification within the genus of the form Coelomycetes traditionally has been based on conidial shape & size, presence of sclerotia, appressoria production, and often on host range and pathogenicity (Liu *et al.*, 2007). Though molecular technologies based on the analysis of DNA have come on stream for the examination in details of the relationship that exist within species of *Colletotrichum* (Liu *et al.*, 2007), the

traditional methods based on both morphological characteristics and host specificity are still employed in the *genus* studies (Garrido & Carbu, 2008).

Analysis of DNA sequences continues to be a valuable tool to help resolve relationships among and within species and species complexes of *Colletotrichum*. According to Liu *et al.*,(2007),DNA sequence along with morphology and host range were used to delineate species within the broader *Colletotrichum graminicola* complex on sorghum (*Sorghum bicolor*) , maize (*Zea mays)*, wheat (*Triticum aestivum*),oat *(Avena sativa)*, feeds, and amenity grasses.

For lack of sexual (perfect meiospore) stages in their life cycles, Coelomycetes are usually consigned to a form – class/division of Deteromycota (also Dikaryyomycota) or fungi imperfecti (Thaung, 2008). This author (Thaung, 2008) in his studies pointed out that the form – taxa classificatory system in the Coelomycetes are exclusive, restrictive and of limited use and not indicative of clear relationships among taxa, because in his words “they serve nomenclature and identification purpose only”.

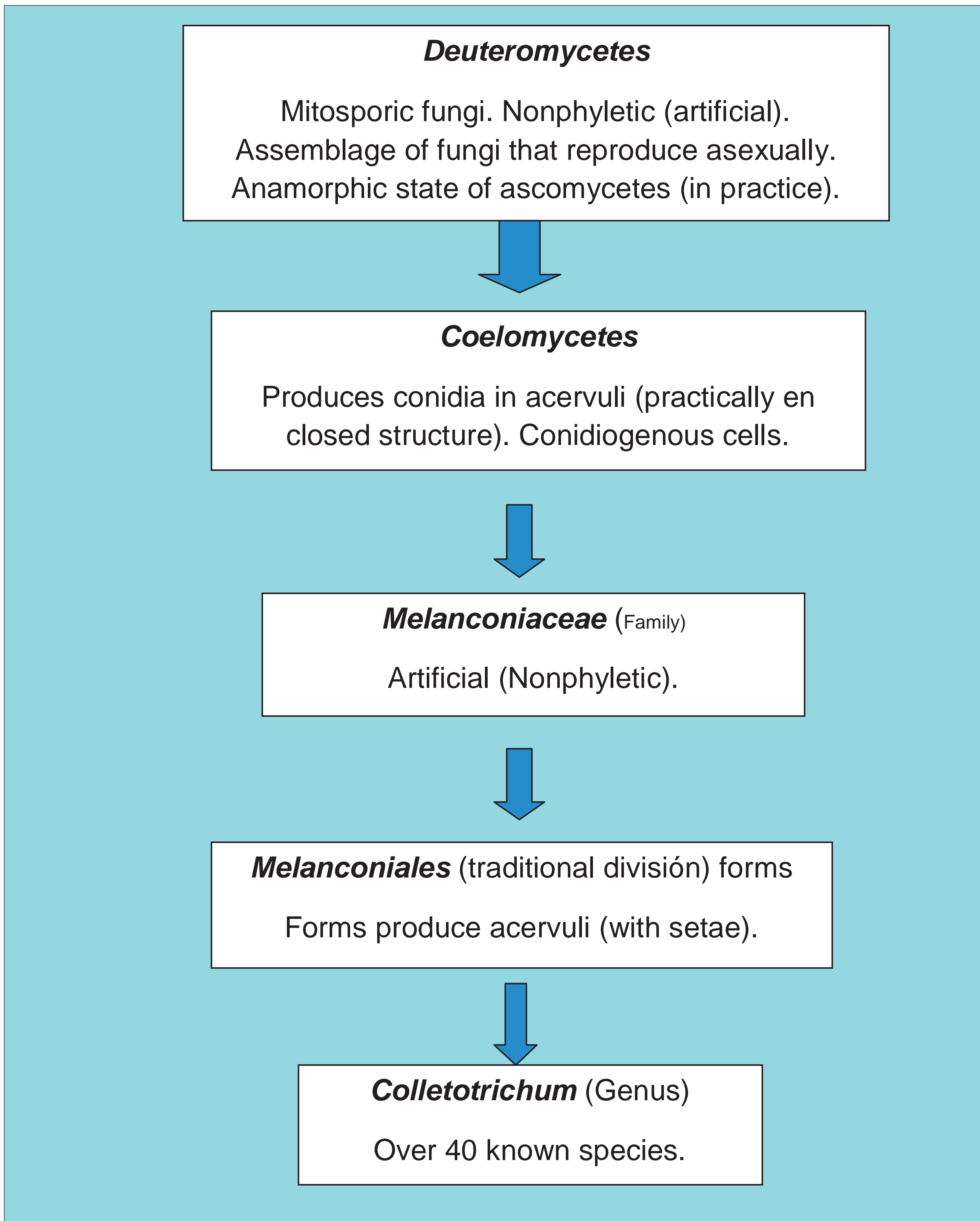


Fig. 7: Classification on *Colletotrichum*
Source: Schumann and D`Arcy (2006).

2.9 MANAGEMENT OF ANTHRACNOSE PROBLEM

The great economic importance of the *Colletotrichum* phytopathogens in agriculture cannot be over emphasized (Thanug *et al.,* 2008). On animal feeds, anthracnose by *Colletotrichum trifolii* of alfalfa (*Medicago sativa* L.), has been reported to lead to reduced forage yields, losses in plant vigour and stand depletion (Mould *et al.*, 1992). Species of *Colletotrichum* parasites like *C. acutatum* have been creating some known food health problems in the European regions over some time. According to Garrido *et al.,* (2008), this dreaded phytopathogens, *Colletotrichum acutatum*, thought to have been introduced in to European territory from California (USA) was first reported on strawberry plants in France in the year 1981.

For the impact of this organism, it is considered a quarantine pathogen in the European union and, as revealed by Garrido *et al.,* (2008), has subsequently been included in the list of regulated A2 pests in the European and Mediterranean Plant Protection Organization (EPPO) region since 1997 (EPPO/CABI,1997).

An understanding of the mode of infection of individual *Colletotrichum* species is a prerequisite for developing effective control strategies, particularly those based on host plant resistance. Knowledge of the factors influencing infection processes also provides epidemiologists with information which can be developed into forecasting models, and aids agronomists developing appropriate agricultural practices, based on crop sanitation and removal of volunteer, reservoir or collateral hosts.

Nevertheless, control of anthracnose problem of *Colletotrichum* species tends to focus mainly on inoculum reduction and prevention of latent infection (Sanders *et al.*, 2000). Adequate preharvest spray programme, therefore, have been recommended to check the post harvest anthracnose problems of agricultural products such as Avocado and Mango fruits.

In some countries previously registered fungicides programmes included monthly pre-harvest application of Benomyl, followed by cupric hydroxide or copper oxychloride for fruits such as avocado *(Persea americana)* and cupric hydroxide alone or with alternate mancozeb or Benomyl sprays for fruits such as mango (*Mangifera indica*). According sanders *et al*., (2000) with the exception of Benomyl, the aforementioned compounds are all contact fungicides whose timing of application should, therefore, coincide with periods of high rainfall when inoculum is dispersed.

The early 1960 introduction of benzimidazole agrochemicals such as Benomyl, carbendazin and thiophanates revolutionized fungicides disease control in crop production (Sanders *et al.,* 2000). However, the extended use of such agrochemicals as revolutionized by the introduction of benzimidazole fungicides in early 1960s resulted in selection for resistant pathogen genotypes which has remained predominant for several years after discontinued use indicated Sanders *et al.,* (2000). Some *Colletotrichum* species resistant to Benomyl, thiabendazole and prochloraz fungicides are documented (Sanders *et al.,* 2000).

The use of resistant cultivars implying recurrent phenotypic selection has been reported to increase anthracnose resistance from 1-20% to 59- 88% (Mould *et al.,* 1992). In the study of the infection process of *Colletotrichum destructivum* ,a hemibiotrophic fungus, using a light microscope in two cowpea cultivars, (TVX3236 and IT82E-60) resistant and susceptible respectively, Latunde-Dada *et al.,* (1999), observed that the production of appressoria and their melanisation were impaired in the resistant cultivar, resulting in reduced organ penetration. The scientists further observed that where penetration occurred in the course of their research, within the resistant varities, the initially infected epidermal cells underwent a hypersensitive response, restricting the destructive necrotrophic phase of the disease development. This action they attributed to the activities of the phytoalexins, Kieviton and Phaseollindin substances which they observed accumulated earlier and more rapidly in the stem tissues of the resistant cultivars, associated with the appearance of delimited necrotic spots on inoculated surfaces.

It is the presence and accumulation of the same substance, Kieviton, in times of problems, in the resistant cultivars that give *Phaseolus vulgarus (Fabaceae)* protection against ravaging pathogens such as *Colletotrichum lindemuthianum* (Diabate *et al.,* 2010).

In the susceptible cowpea cultivar IT82E-60, as against the resistant cultivars, Latunde-Dada (1999) revealed that there was delayed and slower accumulation of phytoalexins, Kieviton and Phaseollindin in the compatible interaction, together with the development of typical spreading water –soaked, anthracnose lesion.

2.10 CHEMICAL PLANT DISEASES MANAGEMENT

The agricultural crop and forestry production is presently largely dependent on the use of chemicals to control various pests and diseases or to retard unwanted soil microbial processes. Though some of these agrochemicals are designed to affect only specific target organisms or processes, most of them, however, have general toxic effects and hence often cause strenuous interactions with the biological soil ecosystem. In general species compositions of the soil microflora and fauna is reconstituted by pesticide substances. The inhibitory effect of copper sulphate on microbial glucose degradation in red latosol soil is reported by Airoldi and Critter, 1996. They discovered that increasing masses of copper sulphate caused a decrease of the original thermal effect to reach a null value at 6.19mg of inhibitor.

Soil life and fertility for crops, according to Pell *et al.,* (1998) depends partly on the delicate “balance” that exists between the various types of microorganisms that determine the turnover of carbon, nitrogen, and other valuable plant nutrients. Thus, it is clear that the addition of any potentially toxic compound is a serious threat to this equilibrium and hence to the sustainable fertility of the soil.

The use of fungicides in agriculture to protect plants from both soils and non soil born pathogen are a common practice. According to a review of the ecological effects of the accumulation of copper in soil by Jansch *et al.,* (2009) copper and copper based fungicides were introduce into European agriculture since 1885 and for its non degradable quality, and extended toxicity to the soil and its inhabitants, is under review whether they can be included in the recent positive list of active substance authorized for use in plant protection products in Europe (Annex 1) of the EU council Directive 91/4141. However, there exists a dearth of information on the side effects of fungicides (conventional) on key soil ecological processes (Chen *et al.,* 2001; Sahin and Ugur, 2003; and Jansch *et al.,* 2009).

In their investigation, Perrin and Plenchette (1993**)** observed that "because of its harmful effect, Benomyl must be avoided in any management strategy that aims to preserve arbuscular mycorrhizal fungi". According to Chen *et al.,* (2001), Benomyl, Captan and Chlorothalonyl exhibited adverse effect on Soil microbial activity (substrate induced respiration and dehydrogenase activity) and nitrogen dynamics (NH4-N and NO3-N) in their laboratory batch incubation. All the three fungicides suppressed the peak soil respiration in unamended soil by 30-50%. However the researchers observed that captan appeared to have more pronounced overall effects on soil microbial activity and nitrogen dynamics than either Benomyl or chlorothalomyl. Due to high side effect of copper based fungicides to soil microbial activities, Sonmiez *et al.,* (2006) observed in their study that fruit number, total yield, dry root weight and plant height decreased with increasing copper application to soil. Combined applications of copper to soil and leaves could be more deleterious to the plant, soil and the entire ecology than when the product is applied only to soil or leaves, they inferred. They reported that copper toxicity, as expressed by reduced root length, appeared to be a direct result of the accumulation of excess copper in the soil. Important natural antimicrobials such as streptomycin and actinomycetes are soil inhabitants and should be protected and not dislodged using fungicides or other copper based pesticides, these researches further argued. In their investigation of the antimicrobial activity of some Streptomyces isolates, Sahin

and Ugur (2003) recovered from 46 soil samples, a total of 74 different Streptomyces isolates. The researchers listed rhizosphere of plants, agricultural soil, reserved areas and forest soils as the potential habitats of these microbial elements. It is worth the interest to note, however, that these aspects of the result would not have been possible if the soil regions were under the bombardment of synthetic fungicides.

In recent time the active search for bioactive molecules in insect, micro organisms and plants for disease control in Agriculture has been on the increase (Amadioha and Obi, 1999; Enikuomehin and kehinde, 2007; Colpas *et al.,* 2009). This is to give a commensurate check and balance to the rapid devastation our soils and ecology are being subjected to from conventional pesticide.

2.11 THE BOTANICALS IN PLANT DISEASES MANAGEMENT

For the economic importance of this disease ,anthracnose on cowpea , several control methods have been adopted including the application of chemical (fungicides) and integrated pest and disease management (Amadioha and Obi, 1998;Amadioha,2003;Adebanjo and Bankole, 2004).But for the conscious environmental sustenance and ecological compatibility , there is the need and desirability to search for the alternative which employs natural agro biological(Biopesticides) balance to address this all important cowpea disease.

The Environmental protection Agency (EPA-USA) defines a Biopesticides as a pesticide derived from natural materials such as animals, plants, bacteria and certain minerals (http://www.polyversumla.com). Biopesticides of plant origin are the botanicals (Fig: 8).

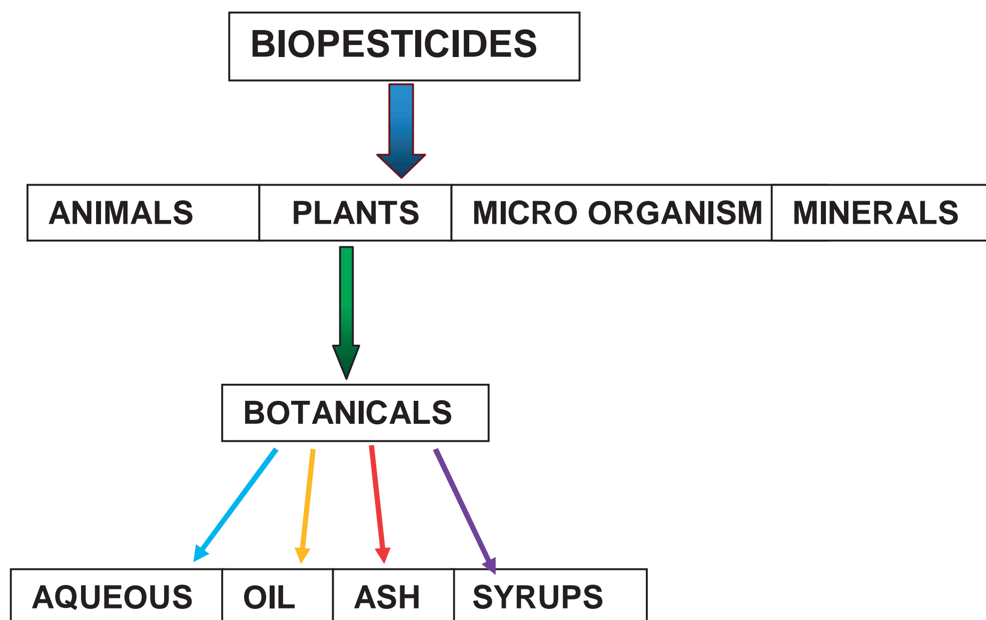


Fig. 8: Botanicals in Biopesticides.

Two ways to look at the available natural agro biological balance in the control of plant disease are: (a) biological control of plant disease through the use of antagonistic micro organism (bioagents), (Amusa and Ikotun, 1995; Bankole and Adebitan, 1996; Adekunle *et al.,* 1997; Akinbode and Ikotun, 2008), (b) botanical control of plant disease through the use of plant extracts (Amadioha and Obi, 1998; Amadioha, 2003; Adebanjo and Bankole, 2004; Akinbode and Ikotun, 2008).

The search for bioactive molecules in plants as alternative to chemicals in the control of plant health issues is no doubt on the increase. These are remotely aimed at protecting the soil that supports the life of these crops among other horticultural plants and in extension safeguard the environment for every other *vivo* organism. Control of *Aspergillus flavus* in Maize with Plant Essential oils and their compounds is documented. Montes-Belmont and Carvajal (1998) who used the essential oils from eleven different plants for maize kernel protection pointed out that a residual effect of *Cinnamomum zeylanicum*, though was detected 4 weeks of kernel treatment, no phytotoxic effect on germination and

corn growth was detected with any of these oil; hence annexing them a bundle of fungicide alternatives for soil protection and sustainability.

In the evaluation of some botanicals in *in vitro* control of *Colletotrichum destructivum*, pathogen of the dreaded anthracnose of cowpea, Akinbode and Ikotun (2008) inhibited the growth of the pathogen using *Nicotiana tabacum* plant extract. Ogwulumba *et al.,* (2008) in their research revealed that extracts from paw-paw (*Carica papaya)* leaf and bitter leaf (*Ocimum gratissimum*) can be used to protect groundnuts from the menace of foliar fungal pathogens such as *Phoma arachidicola* and *Botrytis cinerea.*The induced production of phytoalexins in soybean cotyledons and sorghum mesocotyls in addition to the induced systemic resistance in cucumber to *Colletotrichum lagenarium* by the leaf extracts of *Ocimum gratissimum* has been documented by Colpas *et al.,* (2009).

Table.9:Plant families screened for biofungicidal properties(1998-2009)

Family	Samples	Bioassay	Botanical Form	Reference
Alliaceae	*Garlic* spp	*In vitro*	Syrup	Win *et al.,*(2007)
Annonaceae	*Xylopia aethiopica;Annona reticulata*	*Invitro/In vivo*	Oil extract; Aqueous	Amadioha & Obi (1998); Bautista-Baño (2003)
Arecaceae	*Elaeis guineensis; Cocos nucifera*	*In vitro; In vitro*	Ashes; Ashes	Enikumehin & Kehinde(2007); Obi&Ugwunze(2009)
Asteraceas	*Chromoleana odorata; Vernonia amygdalina*	*In vitro; In vivo*	Aqueous; Aqueous	Nduagu *et al.,* (2008);Ogwulumba *et al.*, (2008)
Caricaceae	*Carica papaya*	*In vivo*	Aqueous	Ogwulumba *et al.*, (2008)
Cochlospermaceae	*Cochlospermum planchonii*	*In vitro*	Aqueous	Nduagu *et al.,* (2008)
Euphorbiaceae	*Ricinus comunis; Hymenocardia acida; Euphorbia prostrata*	*In vitro; In vitro; In vitro*	Aqueous; Aqueous; Ashes	Akinbode & Ikotun (2008);Nduagu *et al.,*(2008);Obi &Ugwunze (2009)
Fabaceae	*Tephrosia vogelii; Senna alata*	*In vitro; In vitro*	Aqueous; Ashes	Nduagu *et al.,* (2008);Obi &Ugwunze (2009)
Lamiaceae	*Thymus vulgaris; Ocimum gratissimum; O. sanctum*	*In vivo; In vivo; In vitro*	Oil extract; Aqueous; Aqueous/Syrup	Montes-Belmont& Carvajal (1998); Amadioha & Obi (1999); Nduagu *et al.,* (2008); Colpas *et al.* (2009)
Lauraceae	*Cinnamon zeylanicum*	*In vivo; In vitro*	Oil extract ;Syrup	Montes-Belmont, & Carvajal (1998) Win *et al.,* (2007)
Meliciaceae	*Azadiractha indica*	*In vitro/ In vivo.*	Oil extract; Aqueous	Amadioha & Obi (1998); Nduagu *et al.,* (2008)
Myrtaceae	*Psidium guajava.*	*In vitro;*	Aqueous.	Nduagu *et al.,*(2008)
Piperaceae	*Piperaceae*	*In vitro/ In vivo*	Aqueous/syrup; Syrup	Amadioha (2003); Win *et al.,* (2007)
Plumbaginaceae	*Plumbago zeylanica*	*In vitro*	Ashes	Obi & Ugwunze(2009)
Poaceae	*Cymbopogon citratus*	*In vitro/ Invivo;In vitro*	Aqueous; Oil extract	Amadioha & Obi (1999); Palhan (2004)
Potederiaceae	*Eichhomia crassipes*	*In vitro*	Ashes	Enikumehin & Kehinde (2007)
Rutaceae	*Citrus limon*	*In vitro*	Aqueous/Syrup; Aqueous	Amadioha (2003); Nduagu *et al.,* (2008)
Solanaceae	*Nicotiana tabacum*	*In vitro*	Aqueous	Akinbode & Ikotun (2008)

Their findings were in reflection to the reduction in disease incidence and an increase in Chitinase production of the test crops. Crude botanical extracts from stem back and root bark of *Azadiractha indica, Vernonia amygdalina* and *Cochlospermum planchonii* exhibited strong fungi toxicity against *Colletotrichum capsici* as reported by Nduagu *et al.,* (2008) who similarly proffered that they be formulated into products for the potential control of anthracnose health problem of sweet pepper (*Capsicum annuum.*).These evaluated items are favored over conventional pesticides for their non-toxic effect on the test crops and easy biodegradability for the stable health and safety of the soil (Nduagu *et al.*, 2008).

In continuation of the search for an environmentally non toxic and soil/crop friendly products plant health protectants, Palhano *et al.,*(2004) inactivated spores of *Colletotrichum gloeosporiodes* using high hydrostatic pressure separate and combined with Citral or lemongrass (*Cymbopogon citrates*) essential oil. Their work suggested the use of high hydrostatic pressure and plant essential oils as an alternative for fruit health problems for the safety and stability of the soil and its environment. A combination of *Chitosan* and botanical extracts from custard apple leaves, papaya leaves and seeds were reported to posses antifungal effects (Bautista-baños *et al.*, 2003).The combination of 2.5% *Chitosan* with all the tested extracts had fungistatic rather than fungicidal effect on the test pathogens reported Bautista-baños *et al.* (2003).

Chitosan, according to these scientists is a given name to a deacetylated form of chitin from crustacean elements. As non toxic biodegradable material, as well as an elicitor, *Chitosan* as inferred by Bautista-baños (2006) has the potential to become a new class of plant protectants on direct soil friendly interaction, assisting towards the goal of sustainable agriculture.

The search for bioactive substances from the plant world has led researches to distinctive regions of separate plants. Hence, Obi and Onuoha (2000) observed that, plant flowers, leaves, barks, seeds, fruits, roots and at times whole plant could be employed in the search for botanicals for use towards an effective management of phytopathological problems. The seeds of neem, Azadiractha

indica A.Juss and fruits of bush pepper, *Xylopia aethiopica* (Dunal) A. Rich were used in the work of Amadioha and Obi (1998), while in another similar study the same authors employed the leaves of scent plant, *Ocimum gratissimum* (L.) and lemon grass, *Cymbopogon citratus* D.C.Stapf. for anthracnose disease of cowpea (Amadioha and Obi, 1999). Ogwulumba *et al.,* (2008) used the leaves of paw-paw, *Carica papaya* and bitter leaf, *Vernonia amygdalina* during their research. This is not too different from the work of Amadioha (2003) where the leaves of *Piper nigrum*, *Ocimum sanctum* and *Citrus limon* were of use in their antifungal evaluation against *Colletotrichum lindemuthianum* pathogen.

In the examination of induction of plant disease responses to botanical extracts, Colpas *et al.,* (2009) utilized the leaves of *Ocimum gratissimum* for the screening. The leaves of tobacco, *Nicotiana tabacum* and *Ricinus communis* were of most interest in the scientific investigation of some bioagents and botanicals in *in vitro* control of *Colletotrichum destructivum* Akinbode and Ikotun (2008).

The screening of a total of eleven plants on growth of *Colletotrichum capsici* (Synd) Butler & Bisby, causal agent of pepper anthracnose, concentrated choice on leaves, stem bark and root barks of the concerned plants (Nduagu *et al*., 2008). In a relatively recent study by Obi and Ugwunze (2009), the twigs of *Senna alata* (L.) and *Asimina triloba* ; the endocarp (shell) of *Cocos nucifera*; whole plants of *Plumbago zeylanica* and *Eurphobia prostrata* were screened against the tuber rots of *Colocasia esculenta* L., *Fusarium solani* and *Botrydiplodia theobromae*.

It is also of note that scientists often adopt different methods and techniques in the extraction and characterization of products from plants probably due to the type of plant used and the sole aim of the experiment (Obi and Onuoha, 2000). Plant material could thus be used in its fresh form or dried, air/sun or oven dried. Researchers have carried out their extraction exercises within different methods (fig.8) such as using hot or cold water (Amadioha and Obi,1999; Akinbode and Ikotun, 2008; Colpas *et al*., 2009; Ogwulumba *et al.,* 2008; Nduagu *et al.* ,2008),organic solvents for steam(oil)distillate (Amadioha, and

Obi, 1998) ,crude ashes (Enikumehin & Kehinde, 2007; Obi &Ugwunze,2009), in place of aqueous extracts (Obi and Onuoha ,2000). Propagators of this scientific initiative and their collaborators are of the strong argument among others that: (1) Biopesticides tend to pose fewer risks than conventional pesticides. (2) Biopesticides are usually inherently less toxic than conventional pesticides. (3) When used as a component of integrated pest management (IPM) programmes, Biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. (4) Requires much less data and time frame to register a Biopesticides than to register a conventional pesticide (Http://www.epa.gov/PR_Notices/pr97-3.html). (5) They are non residue producing control agent. And (6) Are eco-friendly and easy to use. In support of all these the European Community, like in other developed parts of the world ,has since established a European Commission Working Document which specifies data requirements for active substances of plants protection products made from plants or plant extracts (SANCO/10472rev.5).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 CONSULTED MATERIALS: Materials on literatures bordering principally on cowpea anthracnose by *Colletotrichum lindemuthianum* and *Colletotrichum destructivum*, and botanicals spanning through 1992 to 2010 were sourced and reviewed accordingly. A total of seventy one referred literatures and four *on line* materials provided direct information for the study. Crop families under the afflicting influence of *Colletotrichum* were grouped, and species of *Colletotrichum* with their specific hosts identified. *Colletotrichum* with other related pathogens of cowpea crop were assembled and percentage virulence comparism evaluated among them. The plant families so far screened (between 1998 and 2009) for potential source of biofungicidal substances, together with their botanical forms were also included in the work.

3.2 BOTANICAL MATERIALS: Seeds from mature dehisced fruits of *Azadiractha indica* A.Juss (neem) (fig 9; table 10) were oven dried for two days at 60^oC and the seed coats then split to remove the cotyledons which were subsequently washed in sterile, distilled water and oven dried together with the sterile water washed fruits of *Xylopia aethiopica* (fig10; table 11) at 60^oC for 24h. After drying, the fruits and the seeds were separately ground in a mortar to obtain 1000g of dry powder from each material. Harvested fresh leaves of *Cymbopogon citratus* (fig 11; table 12) and *Ocimum gratissimum* (fig12; table 13) were washed thoroughly in tap water and sterile distilled water, air- dried at 27^oC, weighed (100g) and ground separately in sterile mortar.

Fig 9a: Matured and ripped fruit of *Azadiractha indica.*
*Source:*UPTH, Porthacourt

Fig 9b**:** Dehisced seeds of *Azadiractha indica***.**
Source: UPTH, Porthacourt

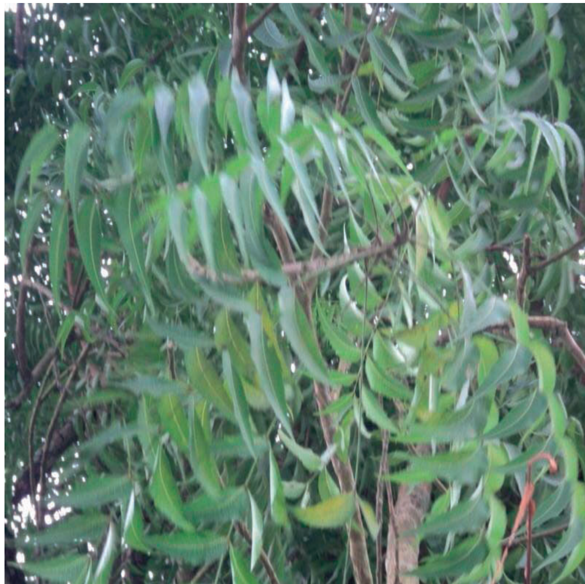


Fig 9c**:** *Azadiractha indica*, young tree.
Source: UPTH, Porthacourt

Fig 9d: *Azadiractha indica* tree with immaturefruits.Source:Http://www.bhg.com/ gardening/plant dictionary/vegetable/cowpea/.

Table10 :Biological Classification on Neem plant

Kingdom	*Plantae*	Plants
Subkingdom	*Tracheobionta*	Vascular Plants
División	*Magnoliophyta*	Flowering Plants
Superdivision	*Spermatophyta*	Seed Plants
Clase	*Magnoliopsida*	Dicotyledons
Subclase	*Rosidae*	N A
Order:	*Sapindales(Rutales)*	NA
Family:	*Meliaceae*	Mahogany family
Subfamily:	*Melioideae*	NA
Tribe:	*Melieae*	NA
Genus	*Azadirachta A.Juss*	azadirachta
Species:	*Azadirachta indica* A.Juss	neem

NA: Not available.

Fig10a: *Xylopia aethiopica tree with unripe fruits. Source:* Spiced Africa – Grains of Paradise and Grains of Selim **(http://www.justfoodnow.com)**

Fig10b: *Xylopia aethiopica harvested ripe fruits.source: ChAI EXOTICOS ZGZ.*

Table 11: Biological Classification on bush pepper plant

Kingdom	*Plantae*	Plants
Subkingdom	*Tracheobionta*	NA
División	*Magnoliophyta(*Angiospermae**)**	Seed producing
Superdivision	NA	NA
Clase	*Magnoliopsida*	Dicotyledoneae
Subclase	*Magnoliidae*	NA
Order:	*Magnoliales*	woody Plants
Family:	*Annonaceae Juss*	Woody Plants
Subfamily:	NA	NA
Tribe:	NA	NA
Genus	*Xylopia*	Pepper
Species:	*Xylopia aethiopica* (Dunal) A. Rich**.**	Guinea pepper

NA: Not available.

Fig11a: *Cymbopogon citratus (Young Lemon grass*
Source:UPTH, Porthacourt

Fig11b: *Cymbopogon citratus plant (Matured).*
Source:UPTH, Porthacourt

Table12: Biological Classification on Lemon grass plant

Kingdom	*Plantae*	Plant
División	*Magnoliophyta*	Angiospermas (flower plant)
Clase	*Liliopsida*	Monocots
Subclase	*Commelinidae*	Commelinids
Order	*Poales*	Monocots flowering Plants
Family	*Poaceae*	Herbácous plants,rarely woody
Subfamily	*Panicoideae*	Racemos inflorescence
Tribe	*Andropogoneae*	NA
Genus	*Cymbopogon*	Tea grass
Species	*Cymbopogon citratus*	Lemon grass

NA: Not Available.

Fig12a. *Ocimum gratissimum* (Young scent plant).
Source: UPTH, Porthacourt

Fig12b.*Ocimum gratissimum* plants at fruiting stage.
Source: UPTH, Porthacourt.

Table13: Biological Classification on scent leaf plant

Kingdom	*Plantae*	Plants
Subkingdom	*Tracheobionta*	Vascular Plants
División:	*Angiosperms*	Flowering Plants
Superdivision	*Spermatophyta*	Seed Plants
Clase:	*Magnoliopsida*	Dicotyledons
Subclase:	*Asteridae*	NA
Order:	*Lamiales*	NA
Family:	*Lamiaceae*	Mint family
Subfamily:	*NA*	NA
Tribe:	*NA*	NA
Genus	*Ocimum L.*	basil
Species	*Ocimum. gratissimum L.*	African basil/Scent leaf

NA: Not available.

3.3 BOTANICAL EXTRACTION: An oil soluble extract was made of each sample of *A. indica* (fig 9) and *X. aethiopica* (fig 10) by placing 80g of the dry powder in thimble and extracting with 500 ml diethyl ether for 6h using *Soxhlet* extractor (Amadioha & Obi 1998 and 1999). The ether was evaporated initially using water and then left overnight at a laboratory temperature for evaporation of the remaining ether. Hot water extracts (HWE) were obtained by infusing the four ground test materials separately with 100ml sterile distilled water using 250ml Erlenmeyer flasks in water bath at 80°C for 1.5h. Each paste from *Cymbopogon citratus* (fig 11) *and Ocimum gratissimum* was added to 100ml beaker, stirred vigorously and allowed to stand for 1h and then filtered to obtain cold water extracts (CWE). The crude hot and cold extracts were both obtained by several filtrations through 4 folds of sterile cheese cloth.

Fig13. *Azadiractha indica* oil.

Fig.14. *Xylopia aethiopica* oil.

Fig15.Processed *Cymbopogon citratus leaves.*

3.4 ISOLATION AND IDENTIFICATION OF PATHOGEN. The test pathogen, *Colletotrichum destructivum* was isolated from an infected cowpea plant. An infected cowpea plant with anthracnose symptoms was collected from the farm site of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Lesions were observed under a stereobinocular microscope(X 12-60) for the presence of acervuli .Mounts of the fruiting bodies were examined under the microscope to ascertain the identity of the isolates with reference to

illustrated genera of imperfect fungi (Dugan, 2006). Pure cultures of the pathogen were prepared through aseptic transfer of acervuli to PDA in Petri dishes and Koch Pasteur `s postulates (fig 16) observed for further authenticity.

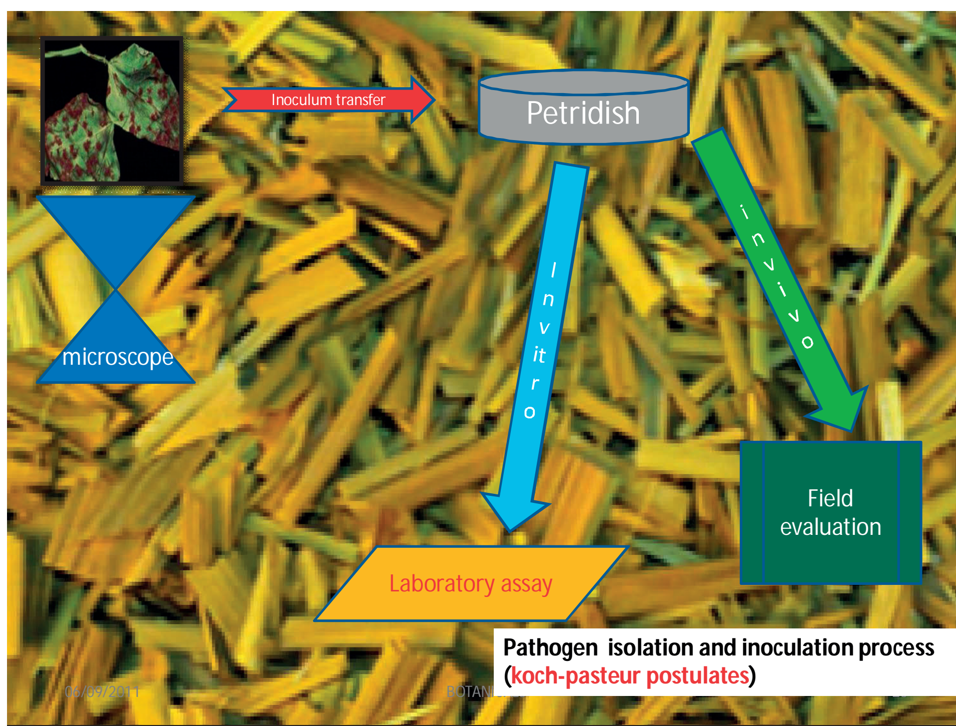


Fig 16: Pathogen isolation and inoculation process according to Koch-Pasteur postulates

3.5 EFFECT OF EXTRACTS ON SPORE GERMINATION.

Suspension of 10-day –old cultures of the pathogen were prepared using a disc (3mm diameter) in 1ml each of the undiluted (100% concentration) and diluted (50% concentration) extracts in test tubes. Similar spore suspensions were prepared in sterile distilled water as control. The contents of the tubes were subsequently centrifuged at 100 revolution/min for 10 minutes and then filtered through four folds of cheese cloth. With a Pasteur pipette , a drop (0.05ml) of each spore suspension ($10x10^4$ spores /ml) was placed on triplicate sterile slides inside Petri dish-moisture chambers and incubated at 27±1°C for 24h.Further spore germination was then stopped by adding a drop of lactophenol cotton blue (a biological stain) to each spore suspension on the

slide and 100spores were observed at random with microscope (X 10) and recording the number generated for each replicate treatment to determine the number that germinated which was used to determine the percentage inhibition of spore germination.

3.6 EFFECT OF EXTRACTS ON COLONY GROWTH.

The effect of botanicals on fungal growth was determined by growing *Colletotrichum lindemuthianum* on a PDA (potato dextrose agar) media containing extract in a Petri plate. A 50 percent concentration of crude extract in PDA was prepared by adding 50ml of the oil or hot water extract to 50 ml molten PDA (prepared by dissolving 3.7g PDA in 50 ml sterile distilled water). One hundred percent botanicals in PDA was prepared by spreading 1ml of full strength of each botanical on the surface of the solidified PDA-botanical medium contained in Petri dish previously marked at the bottom with two perpendicular lines indicating the center of the Petri plates. The control contained PDA without added botanicals.

A disc (3 mm in diameter) of 10-day old *Colletotrichum lindemuthianum* culture was aseptically transferred to the center of the solidified PDA-extract medium in the Petri plates (above the marked perpendicular lines). The Petri plates were subsequently incubated for 7 days at 27ºC. Radial growth of the *Colletotrichum lindemuthianum* was measured on each plant and compared with the growth of the organism in plates without botanicals. The experiment was replicated five times per treatment.

3.7 FIELD EVALUATION OF EXTRACTS AND BENOMYL

The effects of botanicals on disease development *in vivo* were determined using cowpea crops. From the international Institute of Tropical Agriculture (IITA), seeds of cowpea, cv.ITE 2246-4, susceptible to *Colletotrichum lindemuthianum* were surface sterilized in 0.5 percent sodium hypochlorite solution for 1 min and rinsed in sterile distilled water. The sterile seeds, at three seeds per pot (fig18) were sown in 4 kg of top -soil contained in 22.5cm diameter earthen pots, previously sterilized with metham sodium or Vapam (a soil fumigant).

Potted plants were randomly arranged in three groups (fig 17) in a glass-house and watered twice daily through a tap water source. Crops in the first group were spray-inoculated with a spore suspension ($10x10^5$ spores /ml distilled water) of the pathogen two days before spraying with either botanicals (undiluted) or Benomyl (3.0 g/l). Crops in the second were inoculated two days after application of botanicals or Benomyl .Plants in the third group were sprayed with the botanicals or Benomyl after symptoms of anthracnose were evident (21 days after inoculation).Control crops within each group were similarly inoculated with spore suspension ,but sprayed with sterile water instead of with neither botanicals nor Benomyl. The size of individual leaf lesions (fig 18) were measured with a metric ruler 25 days after spraying the leaves with botanicals or Benomyl. Each test consisted of five replicates pots per treatment in a completely randomized block design.
Twenty

3.8: Effect of tissue extracts of *Azadiractha*, Cymbopogon *Ocimum, a*nd *Xylopia* on sporulation density of C*olletotrichum* spp

The culture plate used for the study on colony growth were used In the study to study the effect of the tissue extracts on sporulation density of *Colletotrichum* spp. Five mllimometres of sterile distilled water was added to each of four replicate plates per treatment .Spores from each plate were washed into suspension with the aid of a flame–sterile scalpel and filtered through 3 layers of muslin cloth into a test tube. Spores count in each of 5 small squares per replicate spore suspension were made using hemacytometer slide for each treatment. Number of spores per treatment was calculated using the formular:
(A+B+C+D+E) x50 equal numbers per cubic millimeter ,multiplied by 1000 to get value in milimetre.,where letters A,B,C,D and E represent spore counts in 5 one square millimeter rulings of the hemacytometer. The ruled surface is 0.100mm below the cover glass on the hemacytometer grid and volume of liquid over a square millimeter is $0.1mm^{3.}$ To compensate for differences in colony

growth, sporulation densities were divided by related colony areas using the formular: πr^2 where r =radial growth and π =3.142.

3.9 Experimental design.

In this investigation two major experiments were conducted: *in vitro* and *in vivo*.

3.9.1 *In vitro* experiment.

The study on *in vitro* includes the extracts evaluation on fungal spore germination and colony growth rate of test organism.

3.9.1.1 Botanical evaluation on pathogen spore germination

Four plant extracts of *Azadiractha indica*, *Xylopia aethiopica*, *Cymbopogon citratus* and *Ocimum gratissimum* were used at two different experimental levels of 100 and 50 % concentration. A 3mm (diameter) disc of 10-day -old culture of test pathogen provided the source of spore suspension .Spore suspension was carried out on a test tube with the aid of centrifugation .A drop of spore suspension (0.05ml) was placed on triplicate sterile slides inside Petri-dish – moisture chambers and incubated at 27°c. Pathogen spores at rate of 100 per slide were observed at the random after 24hrs of incubation for extract effectivity measure on spore germination. Therefore, five treatments including control (water) at two grade levels of 3 replicates were evaluated.

3.9.1.2 Botanical evaluation on pathogen colony growth.

Four plant extracts of *A. indica, X. aethiopica, C. citratus and O. gratissimum* were employed in the evaluation of pathogen colony growth rate .Extracts were used at two different levels of 100 and 50% concentration .Potato Dextrose Agar (PDA) was used as a growth medium for the organism .Therefore, five treatments, including control (blank PDA) at two levels of five replications were evaluated at 26 ±1°c. Plates were arranged in a completely randomized block design (CRBD), and obtained data subjected to a statiscal analysis of the Duncan multiple range test.(DMRT).

Table 14: *In vitro* experimental design.

	Spore germination	Colony growth
Materials evaluated/Treatment	*Azadiractha indica, Xylopia aethiopica, Cymbopogon citrates ,Ocimum gratissimum* and water	*Azadiractha indica, Xylopia aethiopica, Cymbopogon citratus ,Ocimum gratissimum* and water
Substrate	Suspension	PDA
Pathogen	*Colletotrichum* spp.	*Colletotrichum* spp.
Factor of study	Spore germination	Colony growth
Evaluation	Number of spore germination (7 days.)	Extent of colony spread
Replications	Three	Five
Levels of Treatment	Two (100% and 50%)	Two
Data presentation	Percentage reduction on spore germination	Percentage reduction on colony growth

Table 15: *In vivo* experimental design.

Materials evaluated/ Treatment	*Azadiractha indica, Xylopia aethiopica, Cymbopogon citrates ,Ocimum gratissimum* and Benomyl
Test crop	Test pathogen susceptible *Vigna unguiculata.*
Pathogen of interest	*Colletotrichum* spp.
Factor of study	Lesion and lesion spread.
Evaluation	Disease development.
Replications	Five (3seeds/pot).
Levels of Treatment	Three (2days before inoculation; 2days after inoculation; and 21days after inoculation).
Data presentation	Percentage reduction in lesion spread.

3.9.2 *In vivo* experiment for botanical effectivity on disease development.

The *in vivo* experiment was for the evaluation of *A. indica*, *X. aethiopica*, *C.citratus* and *O. gratissimum* botanical effectivity on disease development in comparism with the standard Benomyl fungicide. Three seeds of sterile *Colletotrichum lindemuthianum* susceptible cowpea seeds were cultivated per pot. In total five treatments at 3 levels of each with five replicates were conducted in the *in vivo* experiment. Disease development was evaluated based on lesions and lesion spread.cum sporulation density

Twenty five days after spraying the leaves with the plant extracts and Benomyl, four lesions per pot were selected at random and cut out, using a surgical blade, into test tubes containing 5ml distilled water. The tubes were then taken to the laboratory and subjected to centrifugation at 100 rpm to release conidia from lesions into suspension. Three replicate drops of each spore suspension were placed on a hemacytometer slide and sporulation density was determined as described in section 3.8 above. The mean sporulation density of three replicate tubes was then divided by four to obtain the mean sporulation density per lesion for each extract treatment .The size of individual leaf lesion used for sporulation density were later measured with a metric ruler and mean values per treatment calculated. Potted plants were arranged in a completely randomized block design (CRBD), and obtained data subjected to a statistical analysis applying the Duncan multiple range test. (DMRT). Option.

CHAPTER FOUR

4.0 RESULTS

Contributions based on scientific research at a continental level showed Asia to have over 35% information treating cowpea and its associated disease and pathogenic problems between 1995 and 2000 (table 1). This was closely followed by Africa with slightly over 24% and the least being Australia with paper contribution of less than 2%. Fungi pathogen were found to be highest on cowpea disease groupings according to pathogen for the same period of time (table 2).The least group of pathogens associated with cowpea infection was parasitic plants (5.50%) as indicated in table 2.

Based on cowpea disease control methods, scientific contribution on HPR scaled highest (48.65%) as against the lowest attention from botanicals (7.43%) for five years of evaluation (table 3). Interestingly of about twenty different pathogens associated with various cowpeas fungal diseases, as indicated in table 4, *Colletotrichum* is the only one with the virulence to affect almost every part of its host and in extension the propensity of afflicting almost a 100% infection on a single susceptible cowpea crop each at a given pathogenic situation (fig 2). On table 7 were indicated a total of twenty *Colletotrichum* species including their specific primary hosts.

Plant families under the pathogenic affliction of *Colletotrichum* were provided as shown on table 8.
The different forms of botanicals in use were also identified and grouped in fig: 8. A total of eighteen plant families were found to have represented the entire plants and plant materials screened for biofungicidal characteristics between 1998 and 2009 (table 9). As can also be seen in table 9, of this work a total of eighteen plant families have been scientifically screened, between 1998 and 2009, and found to habour a large spectrum of species containing substances of different degree of biofungicidal properties.

4.1 Effect of *Azadiractha* Treatment on Spore Germination, Colony Growth and Sporulation Density.

At the four different levels of treatment all extracts of *Azadiractha* had a significant inhibitory effect on spore germination 24 hours after incubation. Spore germination of 100% was recorded in sterile distilled water as against the 44% and 54.3% germination recorded at 100% and 50% hot water seed extracts respectively. Thirty one percent (31%) spore germination was associated with 50% oil while at 100% concentration *Azadiractha* oil did not allow for any spore germination (Table16.). The extracts also had a significant inhibitory effect on colony growth of the *Colletotrichum*

Table 16. : Effect of *Azadiractha,* treatment on spore germination, colony growth and sporulation density.

Treatment	Mean percentage spore germination ^{X}	Mean colony growth ^{Y}	Mean sporulation density ^{Z} ($x10^3$)
100% Hot water-Extract (Ahwe)	44.0c * 56.0) **	26.0b * (51.5)**	6.3c* (-26.0)**
50% Ahwe	54.3d (45.7)	26.4b (50.8)	12.0d (-140.0)
100% Oil (**Aoe**)	0.0a (100.0)	23.5a (56.2	1.0a (80.0)
50% Oil	31.0b (69.0)	23.6a (56.0)	14.2e (-184.0)
Water	100.0e	53.6c	5.0b

X =Mean of 3replicates each of 100spores /extract, 24hrs after incubation.
Y =means of 5 replicates 7 days after incubation.
Z =Means of 4 replicates 7 days after incubation.
* =Means in the same column followed by the same letter are not significantly different at 0.05 level of significance (Duncan's Multiple Range Test, DMRT).
** =Numbers in bracket are percentage reduction or increase in parameter indicated.

Azadiractha oil treatment caused a significantly higher percentage growth reduction than water extracts. In either case however, inhibitory effect on growth was not concentration dependent. Hence there was no significant difference in both levels of hot water and oil treatments within colony growth control. (table16).

The oil and water extracts had a differential significant effect on sporulation density of *Colletotrichum* organism. While the 100% concentration *Azadiractha* oil significantly inhibited sporulation density by 80%, its 50% concentration caused as much as 184% increase in sporulation density (Table 16) One hundred per cent and fifty per cent hot water extracts increased sporulation density by 26% and 140% respectively.

Table 17: Effect of *Xylopia* treatment on spore germination, colony growth, and sporulation density.

Treatment.	Mean percentage spore germination.^{X}	Mean colony growth(mm).^{Y}	Mean sporulation density ^{Z}(x10^3).
100% Hot water-Extract (**Xhwe**)	21.3b* (78.7)*	24.7b* (53.9)**	0.7a* (86.0)**
50% Xhwe	41.0d (59.0)	25.4b (53.4)	2.4b (52.0)
100% Oil(**Xoe)**	0.0a (100.0)	22.9a (57.3)	4.1c (18.0)
50% Oil(Xoe)	28.0c (72.0)	25.6b (52.2)	2.1b (58.0)
Water(**C)**	100.0e	53.6c	5.0c

X=Mean of 3 replicates each of 100spores /extract, 24hrs after incubation.

Y=means of 5 replicates 7 days after incubation.

Z=Means of 4 replicates 7 days after incubation.

*=Means in the same column followed by the same letter are not significantly different at 0.05 level of significance (DMRT).

**=Numbers in bracket are percentage reduction or increase in parameter indicated.

4.2 Effect of *Xylopia* Treatment on Spore Germination, Colony Growth and Sporulation Density

Table 17 shows the effect of *X. aethiopica* on *in vitro* spore germination, colony growth and sporulation density of *Colletotrichum* spp. All the four levels of treatment had a significant inhibitory effect on spore germination 24 hours after incubation.

Pathogen spore germination of 100% was recorded in sterile distilled water treatment as against low 21.3% and 41% spore germination recorded in 100% and 50% hot water extract concentration respectively. Twenty eight percent spore germination were associated with 50% oil as against zero spore germination with 100% oil concentration. (Table 17)

Table 18: Effect of *Cymbopogon* Treatment on spore germination ,colony growth and sporulation density

Treatment	Mean percentage spore germination X	Mean colony growth (mm) Y	Mean sporulation density Z ($x10^3$).
100% Hot water-Extract (Chwe)	79.7b * (20.3)**	24.7a* (53.9)**	9.3b* (-86.0)**
50% Chwe	90.7d (9.3)	25.0a (53.4)	16.6c (-232.0)
100% Oil (Coe)	68.7a (31.3)	24.9a (55.2)	8.6b (-72.0)
50% Oil (Coe)	85.0c (15.0)	24.4a (54.5)	16.1c (-222.0)
Water (C)	100.0c (0)	53.6b	5.0a

X = Mean of 3replicates each of 100spores /extract, 24hrs after incubation.
Y = means of 5 replicates 7 days after incubation.
Z = Means of 4 replicates 7 days after incubation.
* = Means in the same column followed by the same letter are not significantly different at 0.05 level of significance (Duncan's Multiple Range Test, DMRT).
** = Numbers in bracket are percentage reduction or increase in parameter indicated.

The *Xylopia* extracts also had some significant inhibitory effect on colony growth of *Colletotrichum.* There was no concentration dependence in hot water case, hence there was no significant difference within the treatment unlike with the oil treatment where there was significant difference within mean colony values. One hundred per cent and fifty per cent water extracts caused 86% and 52% reductions in sporulation density, respectively, whereas 100% and 50% oil extracts were associated with 18% and 58% reduction in sporulation density. There was no significant difference between oil extract at full strength and the control mean sporulation density.

4.3: Effect of *Cymbopogon* Treatment on Spore Germination, Colony Growth and Sporulation Density.

The expression of *Cymbopogon* on *in vitro* spore germination, colony growth and sporulation density of *Colletotrichum* spp. is presented in table18. All extract treatment had a significant inhibitory effect on spore germination 24 hours after incubation. The highest percentage reduction of 31.3% in spore germination was recorded in 100% cold water extract.

Table19 :Effect of *Ocimum* Treatment on spore germination ,colony growth and sporulation density

Treatment.	Mean percentage spore germination X	Mean colony growth (mm).Y	Mean sporulation density Z($x10^3$).
100% Hot water-Extract (Ohwe)	60.7c* (39.7)**	25.9a* (51.7)**	1.8b* (64.0)**
50% Ohwe	80.3d (19.7)	27.1a (49.4)	4.4c (12.0)
100% Oil (Ooe)	51.3a (48.7)	25.5a (52.4)	1.2a (76.0)
50% Oil(Ooe)	59.0b (41.0)	26.8a (50.0)	4.1c (18.0)
Water (c)	100c	53.6b	5.0d

X = Mean of 3replicates each of 100spores /extract, 24hrs after incubation.
Y = means of 5 replicates 7 days after incubation.
Z = Means of 4 replicates 7 days after incubation.
* = Means in the same column followed by the same letter are not significantly different at 0.05 level of significance (Duncan's Multiple Range Test, DMRT).
** = Numbers in bracket are percentage reduction or increase in parameter indicated.

This was followed by 100% hot water extract (20.3%), 50% cold water extract (15%), and 50% hot water extract (9.3%). Though the extracts showed over 50% inhibitory effect on colony growth of the pathogen at all levels, the leaf extract was not concentration dependent and statistically, therefore, were no significant difference within the treatment values under mean colony growth. The leaf extracts instead significantly stimulated sporulation of *Colletotrichum* spp. One hundred per cent and fifty percent hot water leaf extracts impacted 86% and 232% increase in sporulation density respectively, while 100% and 50% cold water extracts were associated with 72% and 222% increase in sporulation density respectively.

4.4. Effect of *Ocimum* Treatment on Spore Germination, Colony Growth and Sporulation Density

The record on the effect of *Ocimum* is shown on Table19 Also all extract treatments (four levels) had a significant inhibitory effect on spore germination 24 hours after incubation. The inhibitory effect was all concentration dependent .As against the 100% germination recorded in sterile distilled water , only 80.3 and 60.7 germination per cent were recorded in 50% and 100% hot water leaf extract respectively. The extracts showed significant reduction in sporulation density with all treatments. One hundred per cent cold water extract was the most potent causing 76%reduction in sporulation density .This was followed by 100% hot water extract associated with 64% reduction in sporulation density. At 50% concentration, the potency of both hot and cold water extracts caused 12% and 18% reduction in sporulation density respectively. Again mean colony growth, though, were reduced close to, or above 50 %,(table 19) the values on analysis did not show the existence of any significant difference within values at all treatment levels.

4.5 Effect of Plant Extracts and Benomyl Treatments on *Vigna ungiculata* Disease Development by *Colletotrichum* spp

The effect of botanical extracts and Benomyl on *Vigna ungiculata* is presented in table 20. Oil plant extracts of *Azadiractha* and *Xylopia* reduced lesion spread by the same margin of 37.8% on plants sprayed 2 days before inoculation. This value was significantly different from the 25.7% reduction recorded for Benomyl treatment two days before pathogen inoculation (2dbi). Lesion sizes in leaves sprayed with *Ocimum* and *Cymbopogon* cold water extracts were similar to those on Benomyl sprayed leaves but significantly smaller than those on water-sprayed control leaves. Similar, but significantly smaller lesion spreads were observed on leaves sprayed with *Azadiractha* oil, *Xylopia* oil and *Xylopia* hot water extract than those observed on control leaves. Least control of lesion spread was associated with *Ocimum* and *Cymbopogon* hot water extracts with only 8.9% and 10.4% reduction in lesion spread respectively.

Lesion development was significantly inhibited in all leaves sprayed with either plant extract or Benomyl two days after artificial inoculation with *Colletotrichum* spp. spore suspension. Treatments with *Ocimum,* hot and cold, and *Cymbopogon* hot and cold water extracts were as effective as foliar spray with Benomyl .Foliar spray with *Azadiractha* and *Xylopia* oil and hot water extract were more effective than foliar spray with Benomyl.

Lesion spread was also significantly reduced in leaves sprayed with all plant extracts (except *Cymbopogon* cold water extracts) and Benomyl twenty one days after inoculation with test pathogen: Foliar spray with Benomyl, *Cymbopogon* hot water extract and *Ocimum* cold and hot water extracts resulted in similar but significantly lower percentage reduction in lesion spread than percentage lesion spread reduction associated with *Azadiractha*-oil, *Xylopia*-oil and *Xylopia*-hot-water extracts.

Fig. 17 Shows *Vigna* crops treated at three different levels including the control Fig 17A Shows crops treated two days before inoculation (**2dbi**) .Fig 17B were crops treated two days after inoculation (**2dai**) .Crops treated twenty one days after inoculation (**21dai**) are shown in fig 17C. Fig 17D indicates crop samples from the control lots .Health conditions of the test crops tend to decrease in the order of **D >C >B >A** as indicated by the general appearance .Fig 18 shows heavily infected control crops with stem anthracnose (K) and almost completely blighted leaf with excised lesion(J).

4.6 Effect of plant Extracts and Benomyl on *in vivo* Sporulation Density of *Colletotrichum* Spp.

The effect of plant extracts and Benomyl on *in vivo* sporulation density of *Colletotrichum* is shown on table21 .Generally, sporulation density control on *Vigna* crops treated two days before inoculation (**2dbi**) was higher than that on crops treated two days after pathogen inoculation (**2dai**) but lower than sporulation density on crops treated twenty ones days after inoculation (**21dai**).

In all cases, sporulation density control on lesions of non–treated leaves (**C**) was significantly lower than sporulation density control associated with lesions on leaves sprayed with plant extracts and Benomyl.

A highly significant reduction in sporulation density of 88.1% was associated with Benomyl on crops treated two days before inoculation.(**2dbi**) This was closely followed by *Azadiractha* oil and *Xylopia* oil which caused 78% and 69.6% reduction in sporulationdensity respectively .The least fungitoxic effect was expressed by *Ocimum* cold water extract (Ocwe) with only 22% reduction in sporulation density.

Fig 17: Potted *Vigna unguiculata* crop treated at three different levels ,including the control.

There was also no significant difference within the values of *Ocimum* and *Cymbopogon* extracts at the whole treatment levels ,two days before crop inoculation(2dbi) On *Vigna* crops treated two days after artificial inoculation (**2dai**), the highest fungitoxic effect was shown by *Azadiractha* oil with 63.2% reduction in sporulation density.

Benomyl was the second in effectiveness with 57.9% reduction in sporulation density two days after inoculation (2dai). This, however was not significantly different (P<0.05) from the 56.1% inhibition from *Xylopia* oil Extract from *Cymbopogon* (cwe) was the least effective with only 13.8% inhibition on sporulation density. two days after inoculation (2dai)

Table20 :Effect of Plant Extracts and Benomyl treatments on *Vigna unguiculata* disease development by *Colletotrichum* spp

Treatment.	Lesion diameter (mm)* at indicated treatment time.		
	2 days before inoculation (2dbi).	2days after inoculation (2dai).	21days after inoculation (21dai).
Azadiractha Hot Water (**Ahwe**).	9.5c** (29.6) ***	10.4d** (23.0)***	11.7dc** (13.3)***
Azadiractha Oil (**Aoe**).	8.4f (37.8)	9.4c (30.4)	10.8f (20.0)
Xylopia Hot Water (**Xhwe**).	9.1ef (32.6)	11.3c (16.3)	11.4c (15.6)
Xylopia Oil **(Xoe)**.	8.4f (37.8)	10.1de (25.2)	10.5f (22.2)
Ocimum Hot Water (**Ohwe**).	12.3b (8.9)	12.2b (70.4)	12.3bc (8.9)
Ocimum Cold Water (**Ocwe**).	10.6cd (21.5)	11.9bc (11.9)	11.9cd (11.9)
Cymbopogon Hot Water (**Chwe**).	12.1b (10.4)	12.1b (10.4)	12.3bc (8.9)
Cymbopogon Cold Water (**Ccwe**).	10.0de (25.9)	11.9bc (11.9)	12.7ab (8.0)

* Data are means of 5 replicates each of 4 lesions at random.

** Columns means followed by the same letters are not significantly different at 5% level (DMRT)

*** Numbers in brackets are percentage reduction in lesion spread

Fig 18: Infected *Vigna unguiculata* crop (control) with stem anthracnose (K), and leaf lesion ((J).

Table 21. Sporulation Density at Indicated Treatment Time

Treatment	Lesion Diameter (mm)* at indicated treatment time.		
	2daysbefore (2dbi) inoculation (x10^{6}).	2daysafter (2dai) inoculation (x10^{6}).	21daysafter (21dai) inoculation (x10^{6}).
Azadiractha Hot Water (Ahwe)	2.1d** (64.4)***	3.1c (45.6)***	5.1dc (21.5)***
Azadiractha Oil (Aoe).	1.3c (78.0)	2.1c (63.2)	2.8g (56.9)
Xylopia Hot Water (Xhwe).	2.7cd (54.2)	3.0 ef (47.4)	4.9e (24.6)
Xylopia Oil (Xoe)	1.8dc (69.5)	2.5 fg (56.1)	3.2fg (50.8)
Ocimum Hot Water (Ohwe)	4.5 b (23.7)	4.3cd (24.6)	6.1 b (6.2)
Ocimum Cold Water (Ocwe).	4.6b (22.0)	3.9d (31.6)	5.5c (15.2)
Cymbopogon Hot water (Chwe).	4.5b (23.7)	4.7bc (17.5)	6.0bc (7.7)
Cymbopogon Cold Water (Ccwe).	4.3 b (27.1)	4.8b (13.8)	5.3cde (18.5)
Benomyl (B).	0.7 f (88.1)	2.4fg (57.9)	3.3 f (49.2)
Water (C).	5.9a	5.7a	6.5a

*Data are means of 5 replication each of two hemacytometer readings

**Columns mean followed by the letters are not significantly different at 5% level (DMRT).

***Numbers in brackets are percentage reduction in sporulation density.

Observation on crops treated twenty one days after inoculation(**2dai**) showed *Xylopia* oil to be more fungitoxic with 56.9% inhibition on sporulation density compared to the significantly lower percentage inhibition of 49.2 associated with the Benomyl fungicide .*Ocimum* hot water extract (Ohwe) was the least effective causing only 6.2% reduction in sporulation density,. twenty one days after artificial crop inoculation (21dai

4.7 Effect of treatments on pathological activities of *Colletotrichum* spp.

This heading tends to present in total but concise the various treatments evaluated on the pathological activities of *Colletotrichum* spp in the course of this study.These include the major *in vitro* and *in vivo* fungicidal determination of four botanical extracts from *Azadiractha indica, Cymbopogon citratus, Ocimum gratissimum and Xylopia aethiopica* and in comparation with Benomyl, a conventional fungicide.

4.7.1 Treatments effect of extract *in vitro* on three pathological activities of *Colletotrichum* spp.

Table 22 displays the inhibitory potential s of the extracts *in vitro* of *Azadirachta indica, Cymbopogon citratus, Ocimum gratissimum* and *Xylopia aethiopica* on three pathological activities *(*spore germination. colony growth and sporulation density).

The comparative effect of treatment as indicated in fig 19 showed the oil extract of *Azadiractha* and *Xylopia* to inhibit spore germination of *Colletotrichum* up to 100% at full strength extract concentration. Least extracts effectiveness contra spore germination was obtained with *Cymbopogon* water extract at half dose concentration In all spore germination of *Colletotrichum* was controlled between 10 % and 100%., with the average from the activity of *Ocimum* at full strength water extract.

Fig 20 indicates a comparative effect of extract treatments on colony growth of test pathogen. Extract activities of test plants existed on 48 to 58%. Again the *Xylopia* treatment at full strength oil concentration showed the highest potential against colony growth while the least was obtained with *Ocimum* hot water at half dose concentration.

A rather fascinating result was obtained of extracts` activities on sporulation density. (fig 21).Though there was inhibitory extract effect of over 60%from both *Azadiractha* (100% oil), *Ocimum* (100%cold water) and *Xylopia* (100%hot water), *Azadiractha* and *Cymbopogon* extracts supported sporulation in the test pathogen While *Azadiractha* extracts (hot water and oil at half dose) aided pathogen sporulation with well over 180% intensity, *Cymbopogon* extracts at all levels of treatment concentration assisted heavily pathogen sporulation with more than 230% (fig 21)

Table22: Treatments effect (WE&OE)[1] *in vitro* on three pathological activities of *Colletotrichum* spp.

Treatment	Percentage inhibition[2]											
	Spore germination				Colony growth				Sporulation density			
	Water Extract		Oil Extract		Water Extract		Oil Extract		Water Extract		Oil Extract	
	A[3]	B[4]	C[3]	D[4]	E[3]	F[4]	G[3]	H[4]	I[3]	J[4]	K[3]	L[4]
Azadiractha indica	56,0	45,7	100	69	51,5	50,8	56,2	56,0	-26	-140	80	-184
Cymbopogon.citratus	20,3	9,3	31,3	15	53,9	53,4	55,2	54,5	-86	-232	-72	-222
Ocimum.gratissimum	39,3	19,7	48,7	41	51,7	49,4	52,4	50,0	64	12	76	18
Xylopia.aethiopica	78,7	59	100	72	53,9	53,4	57,3	52,2	86	52	18	58

1: Extracts were water (WE) or oil (OE) with data means of 5replicates
2: Inhibition measured as a reduction on number of spore germination, extent of colony spread and quantity of inocula available.
3: Treatment at full extract concentration.
4: Treatments at diluted (50%) extracts concentration.

4.7.2 Effect of four botanical extracts and Benomyl fungicide treatment on *Vigna unguiculata* diseases development

The information on table 23 is on the effect the four evaluated botanical extracts and Benomyl fungicidal expressed on the test pathogen, *Colletotrichum* spp In this *in vivo* treatment evaluation lesion development was inhibited at various degrees in all leaves sprayed with either botanicals or Benomyl through all periods tested. Comparatively the highest value of 70.4% lesion reduction (fig 22) was associated with hot water extract of *Ocimum* at 2days after crop inoculation (2dai)

The two subsequent values of relatively high percentages associated with reduction in lesion development after the 70.4% were 37.8% each from extracts of *Azadiractha* and *Xylopia* oil but from crop plants treated 2days before pathogen inoculation (2dbi).

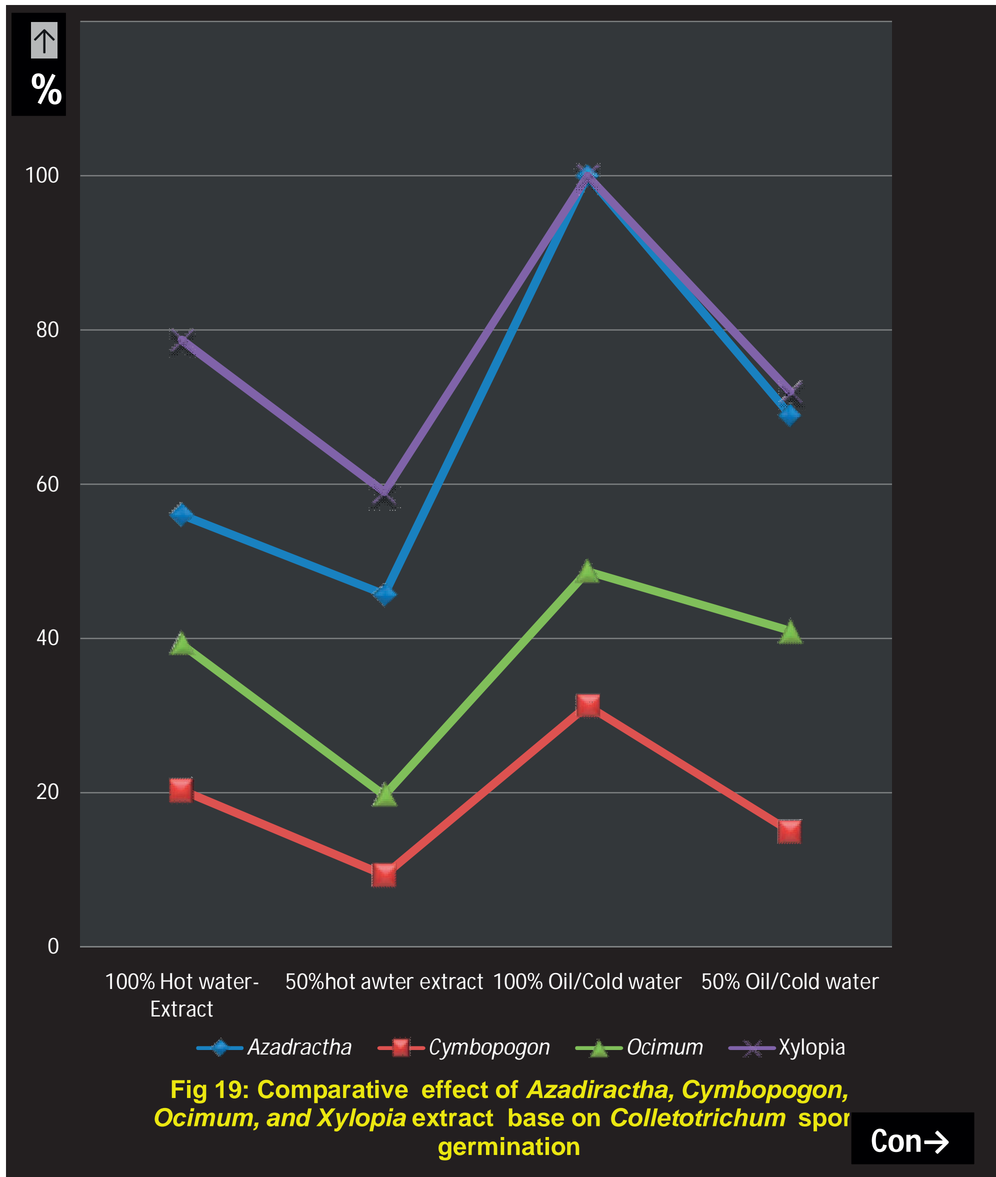


Fig 19: Comparative effect of *Azadiractha, Cymbopogon, Ocimum, and Xylopia* extract base on *Colletotrichum* spor germination

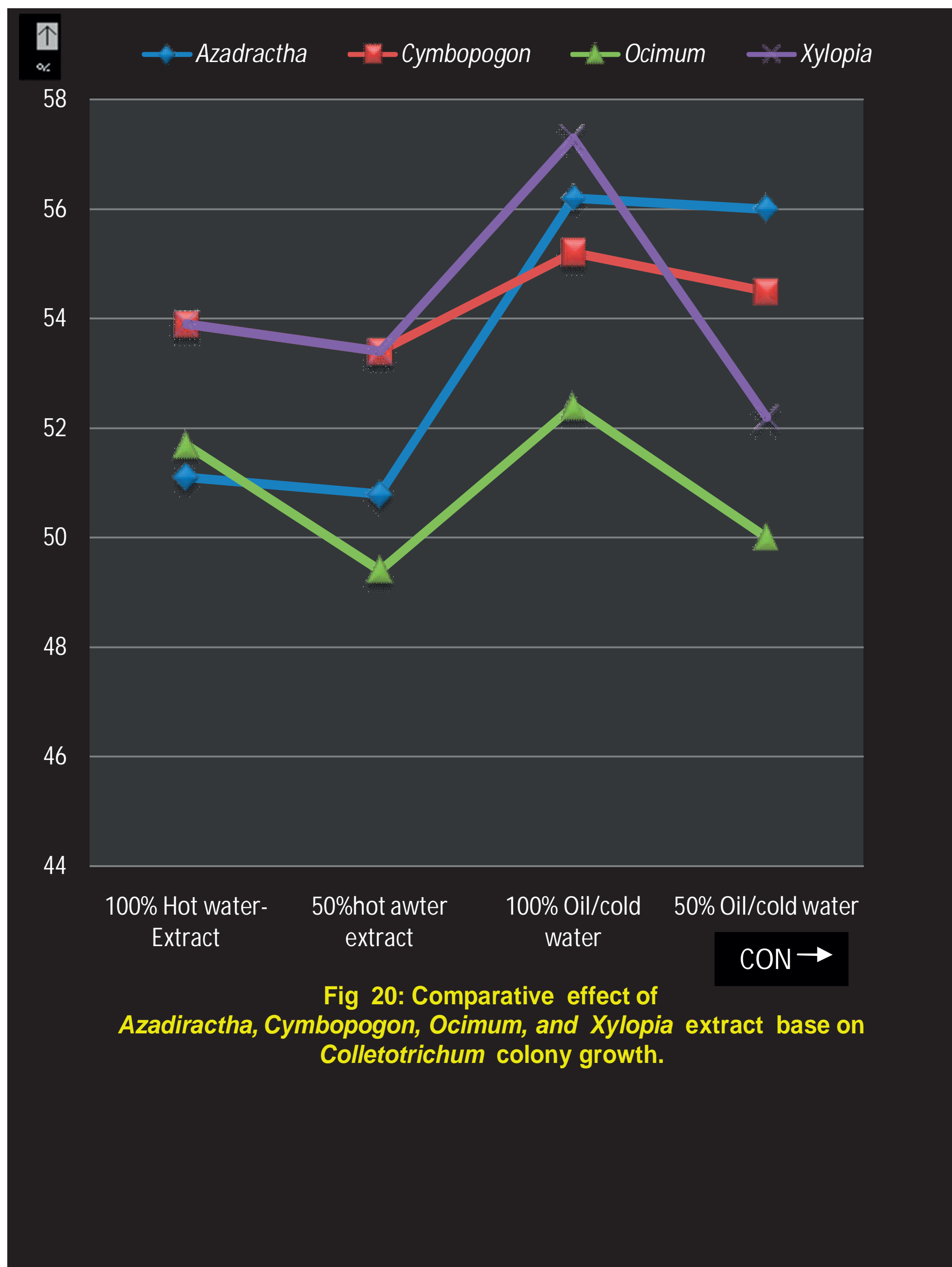


Fig 20: Comparative effect of *Azadiractha, Cymbopogon, Ocimum, and Xylopia* extract base on *Colletotrichum* colony growth.

The chemotherapeutic treatment at 21 days after pathogen inoculation (21dai) reduced lesion spread by its highest value of 22.2% from the extract of *Xylopia aethiopica*.The conventional fungicide of Benomyl impacted its highest effect on lesion spread protectively on the crop by 25.9%.

In all the treatments the poorest control measure was from the extract of *Cymbopogon citratus* with a meager 8% lesion spread inhibition. According to the graphical representation of the data on table 23, the trace of *line of tendency* (fig23) indicates that the lesion spread on crops treated were best reduced 2days period before pathogen inoculation (2dbi).

Table 23: Effect of four botanical extracts and Benomyl treatments on cowpea anthracnose disease development

Treatment	Treatment form[2]	Percentage reduction in lesion spread[1]		
		2 days before inoculation (2dbi)	2 days after inoculation (2dai)	21 days after inoculation (21dai)
Azadiractha indica	Ahwe	29,6	23	13,3
	Aoe	37,8	30,4	20
Xylopia aethiopica	Xhwe	32,6	16,3	15,6
	Xoe	37,8	25,2	22,2
Ocimum gratissimum	Ohwe	8,9	70,4	8,9
	Ocwe	21,5	11,9	11,9
Cymbopogon citratus	Chwe	10,4	10,4	8,9
	Ccwe	25,9	11,9	8
Benomyl	Bn	25,9	12,6	12,6

1: Inhibition measured as a reduction in lesion spread as compared with those on control crops, means of 10 lesions of 5 replicates.

2: Extracts in the form of *Azadiractha* hot water (**Ahwe**) ,*Azadiractha* oil (**Aoe**), *Xylopia* hot water **(Xhwe**), *Xylopia* oil (**Xoe**), *Ocimum* hot water (**Ohwe**) *Ocimum* cold water (**Ocwe**), *Cymbopogon* hot water (**Chwe**) and *Cymbopogon* cold water (**Ccwe**) treatments at full concentration.

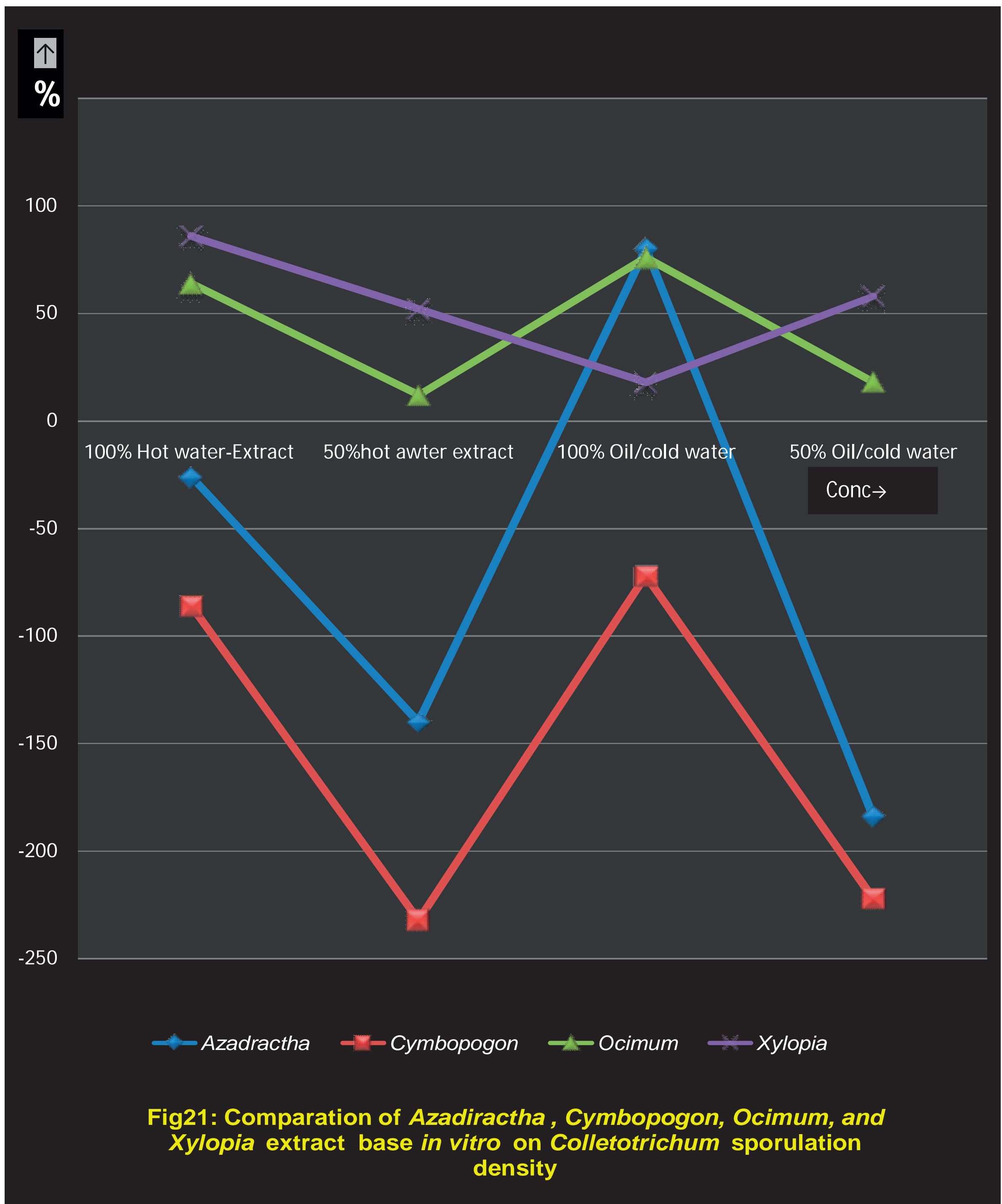


Fig21: Comparation of *Azadiractha* , *Cymbopogon, Ocimum, and Xylopia* extract base *in vitro* on *Colletotrichum* sporulation density

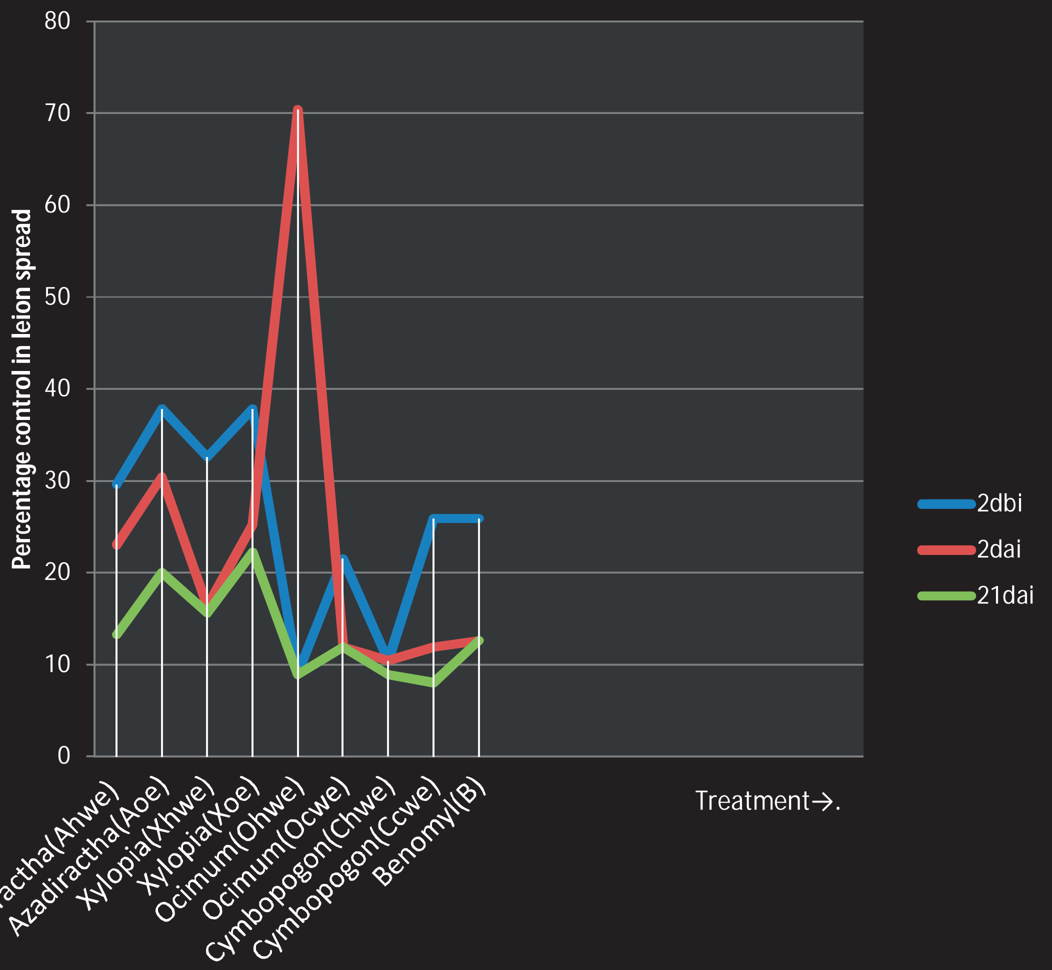


Fig22 :Comparative effect of treatmenst on disease spread based on period of inoculation on *Vigna unguiculata*

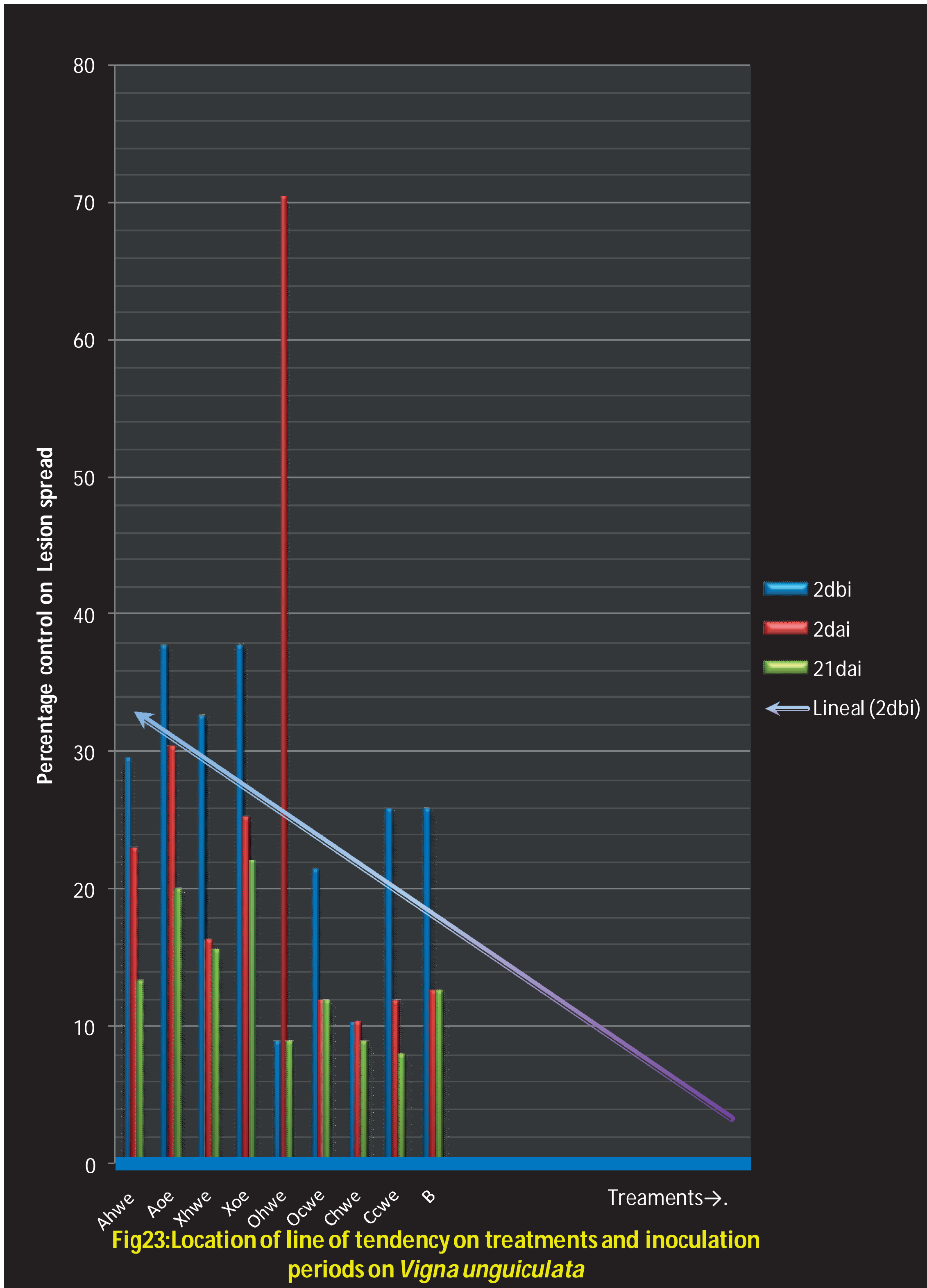


Fig23:Location of line of tendency on treatments and inoculation periods on *Vigna unguiculata*

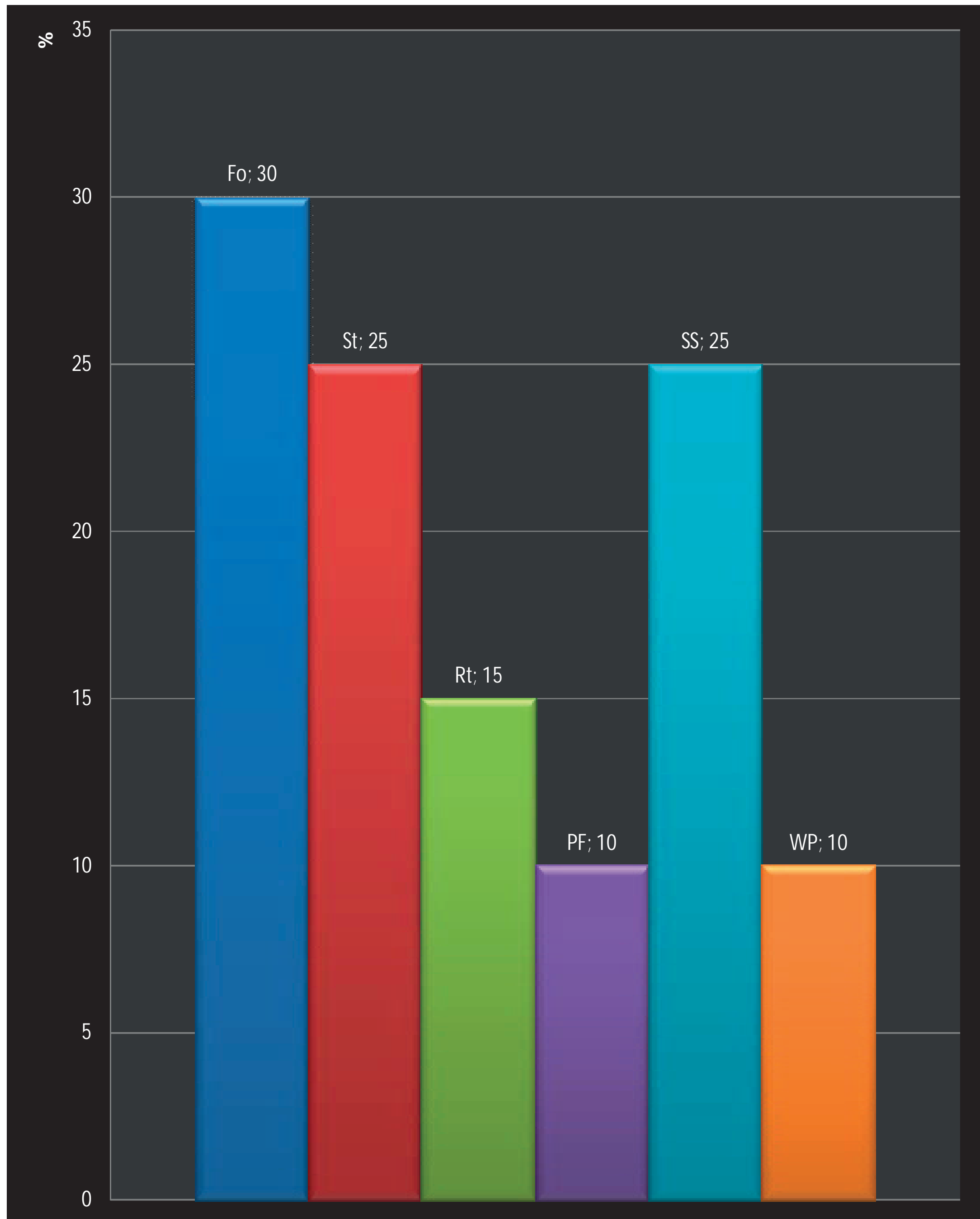


Fig 24: Percentage regional fungal attack on *Vigna unguiculata* crop.
(Fo = foliar; St = stem; Rt = root; PF = pod/fruit; SS = seed/seedlings; WP = whole plant).

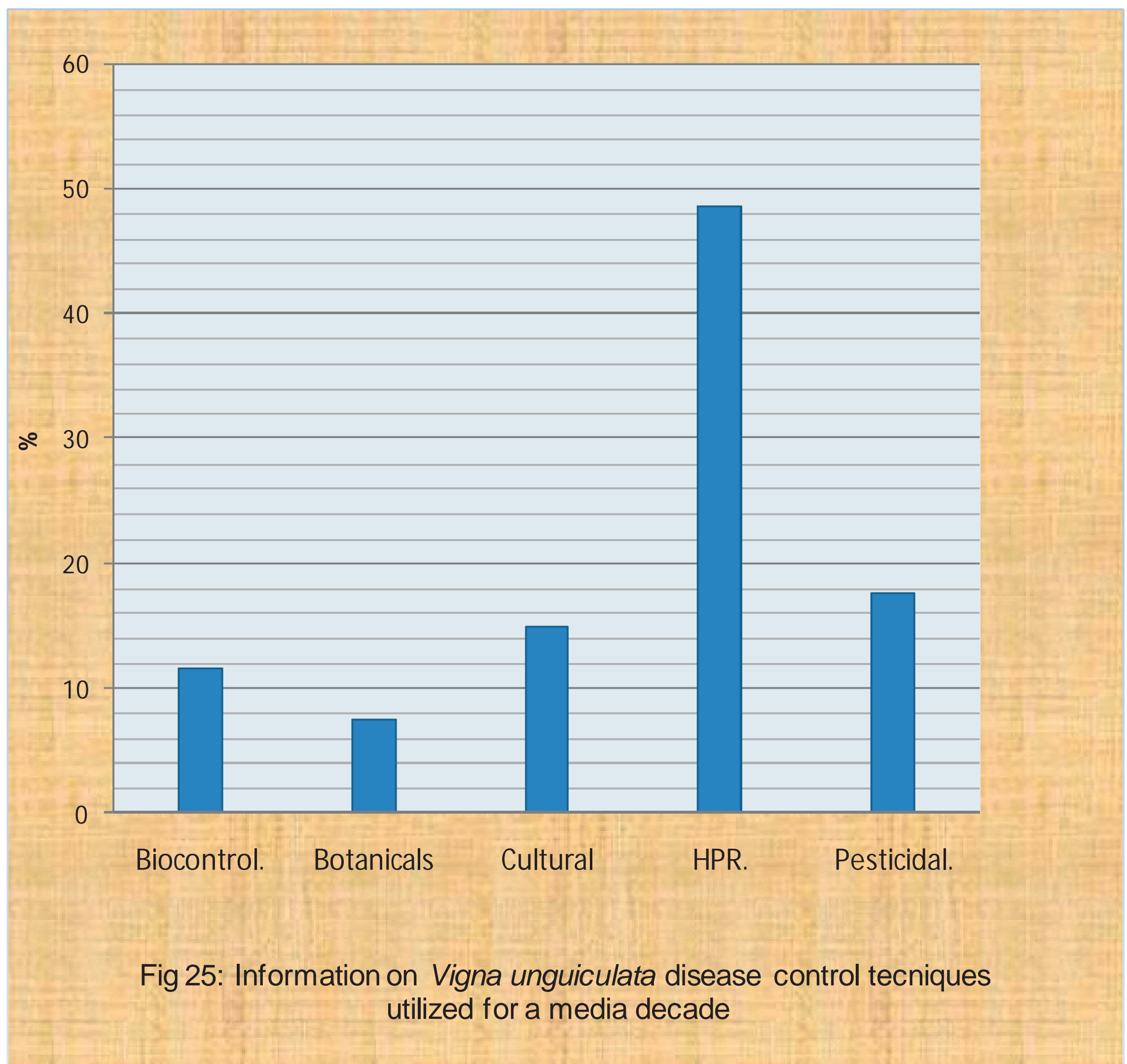


Fig 25: Information on *Vigna unguiculata* disease control tecniques utilized for a media decade

CHAPTER FIVE

5.1 DISCUSSION

Cowpea crop (*Vigna unguiculata* L) is an important and indispensable crop whose livestock and human nutritive values cannot be underestimated at least in the world regions with limited source of protein supply. This opinion has been corroborated by many scientific works in this field including Emechebe & Lagoke (2002), Adebanjo & Bankole (2004), Owolade *et al.,* (2006). This precious crop has always been exposed to the affliction of several pathogens ranging from Bacteria, to viruses, fungi to nematodes and of course recently the ravaging parasitic plants. However, the crops problem appears to originate most from the fungi group of pathogens. In addition to the information in support of this assertion (Emechebe & Lagoke, 2002), this study also identified about twenty different fungi pathogens associated with various cowpea diseases (table 4). In this work it was observed that in the consideration of single cowpea crop into six parts(fig 24), 30% of the fungal infections occur on the foliar part of the crop, 25% on the stems, 15% on the roots, 10% on the pods/fruits, 25% on the seeds/seedlings and 10% on the whole parts of the plant (table 4). Incidentally, while the other fungal pathogens posses the ability each of attacking only about a meager 20% of a stand, the *Colletotrichum* species (*C.destructivum* & *C. truncatum*) have in stock 100% virulence on a single crop each at a given pathogenic situation(table 4). This is in line with the findings of some scientists such as Latunde-Dada et *al.,* (1999), Latunde-Dada &Lucas (2007), and Akinbode & Ikotun (2008).

This degree of virulence on cowpea often leads to grain yield loss between 35 % and 50% as indicated by Amadioha & Obi (1998) and Amadioha (2003). After due scientific documents collation and an in-depth review of same, the author also affirm the pathogen *Colletotrichum destructivum* O`Gara the answerable causal organism of the anthracnose disease of cowpea (tables 5&6).This is also in support of the earlier assertion by Latunde-Dada *et al.,* (1999) and Emechebe & Lagoke (2002). In the work of Akinbode & Ikotun (2008), the causal organism

of cowpea anthracnose was also recognized as the *Colletotrichum destructivum* O`Gara.

Attempts have severally been made to adequately arrest the pathogenic problems of cowpea arising from *Colletotrichum* affliction among other pathogens.(table 4).These management efforts includes the use of Biocontrol systems (bioagents), Pesticides (conventional/synthetic chemicals), cultural observations (clean seeds/hygienic fields and practices), HPR (host plant resistance) and botanicals (Biopesticides/no synthetic chemicals) (table 3). Regrettably the Biopesticides (Botanicals) which tends to confirm to the global yearning for a natural agro biological balance in the fight against agricultural pests and disease is associated with about a meager 7% of the total cowpea disease management options (Fig 25). This was also the observation in the work of Emechebe & Lagoke (2002). These findings appear to confirm to the indication on table 9 of this work, where it was recorded that of all the entire plant families in existence only about eighteen (18) were screened for their biofungicides characteristics between 1998 and 2009.

The study also reported that the products screened during this eleven years span were of various forms or state, such as (i) Aqueous: botanicals extracted using water as the solvent. The water also forms the extract solution (Akinbode & Ikotun, 2008; Nduagu *et al.*, 2008); (ii) Syrup: botanicals of a higher measure of viscosity having been extracted with a solvent other than water , and also containing some of the extracting liquid in its solution (Win *et al.*,2007; Colpas *et al.*, 2009);(iii) Oil: botanicals in the form of essential oil of the test plants, usually extracted through a condensation system (Amadioha & Obi, 1998); and (iv) Ash: botanicals produced in the form of residues powder left after the combustion of a test plant material (Enikumehin & Kehhinde, 2007; Obi & Ugwunze, 2009).

Nevertheless, the botanical forms enumerated in this study could be extended to produce additional form by the application of further processing treatment on the original form. For example the Syrup produced in the work of Win *et al.*,

(2003) was utilized in its dry crude botanical extract state after subjecting the initial extract syrup to an evapoconcentration system. However, this study observed that most of the botanicals screened for this span of eleven years (1998 to 2009) were produced and also utilized in their aqueous form. And considering the total 33 frequency occurrence of botanical forms (table 9), the aqueous botanicals was 51.52%, followed by the syrup (15.15%), ash (18.18%) and the oil form of 15.15%.

The relative easy and economy of production could be responsible for the high percentage value obtained with aqueous botanical evaluation. Close to 28% family interaction existed between plant families under the affliction of *Colletotrichum* and the plant families screened for antifungal properties as expressed in tables 8 & 9. These occurrences were observed within the five plant families of *Asteraceas, Caricaceae, Fabaceae, Lauraceae and poaceae*. The rest of about 72% were unique in occurrence as there were no family interactions among them (tables 8 & 9). The reason for this pattern of existence leaves for further investigation.

Tissue extracts of *Azadiractha indica, Cymbopogon citratus, Ocimum gratissimum and Xylopia aethiopica* in the present study demonstrated, both *in vitro* and *in vivo*, to contain some degree of antimicrobial substances. These antimicrobial activities could be attributed to the presence of some toxic substances in these plants that were fungitoxic to *Colletotrichum destructivum* O`Gara, both *in vitro* and *in vivo.* (Tables 16 to 21)

It was observed that the tissue extracts of *Xylopia aethiopica* and *Azadiractha indica* were generally more effective than the *Ocimum gratissimum* and *Cymbopogon citratus* leaf extracts in the *in vitro* experiments. (Tables 16 to 19)

However, the same *Azadiractha* and *Cymbopogon* extracts (at different concentrations) were found to rather stimulate, greatly, sporulation in the *Colletotrichum*. Test pathogen.(Fig21) All levels of extract concentration evaluated in *Cymbopogon* positively influenced sporulation in *Colletotrichum destructivum* while *Azadiractha* tissue extract (water and oil) could archive the

same feet only at product dilution levels with 140% sporulation density in water and 184% in oil and a relatively small sporulation density of 26% from the full strength of water extract

The sporulation potentiality conversely exhibited by these two materials could suggest the suitability of extracts from *Azadiractha indica* and *Cymbopogon citratus* for substrate base/components in the culture of phytopathogens such as *Colletotrichum destructivum.*

The *Azadiractha* seed extracts is known to contain some phytoalkaloids such as azadirachtin as one of its active ingredients (Amadioha &Obi, 1998). The high antifungal activity exhibited by the oil extract in the present study may be attributed to such a substance (Nduagu *et al*, 2008).

In the *Cymbopogon* treatment one hundred per cent and 50% hot water leaf extracts caused 86% and 232% increase in sporulation density respectively, while 100% and 50% cold water leaf extracts were associated with 72% and 222% increase in sporulation density respectively

The inhibitory effect of the cold water leaf extract of *Cymbopogon* on colony growth of *Colletotrichum* was concentration dependent unlike with the hot water leaf extracts *Cymbopogon citratus* contains between 75-85% aldehydes consisting largely of the organic substance called citral, a preservative and food flavorings especially in India and Java.

It is possible that the antifungal property exhibited in the study was due to the citral content. This seems to support the work of Palhano *et al*, 2004, who successfully inactivated spores of *Colletotrichum gloeosporiodes* using high hydrostatic pressure separate and combined with citral essential oil. That also needs a further study to isolate the pure oil and determine the exact chemical content(s) that is (are) responsible for such antifungal activity. in conjunction with its converse sporulative potentials on *Colletotrichum destructivum.o*f *Vigna unguiculata*

The oil of *Xylopia aethiopica* was found to possess a chocking and irritating odour. The antimicrobial property of *X.aethiopica* exhibited on *Colletotrichum destructivum* test pathogen could perhaps be due to the *Xylopia* acid among other interesting diterpenes contained in the fruit extract (Amadioha and Obi 1998) Further work, however is needed to ascertain the specific chemical responsible for the fungitoxicity of this oil.

All treatment with *Ocimum* leaf extracts had a significant inhibitory effect on spore germination. The inhibitory was concentration dependent. There was also significant reduction in sporulation density with all treatments. In colony growth however, its effectiveness fell below that of *A. indica*, *C. citratus* and *X. aethiopica* at half dose concentration of both hot and cold water extract. (Fig 20)

In the *in vivo* experiment , the tissue extracts of *Xylopia* and *Azadiractha* also exhibited a more antifungal effect on *Colletotrichum destructivum* anthracnose than the leaf extracts of *Cymbopogon* and *Ocimum* (Tables 20 & 21) .Plant tissue extracts and the standard fungicide exhibited more fungitoxic effect as protective or prophylactic than as eradicative or chemotherapeutic fungicides against *Vigna unguiculata* disease *in vivo* This suggestion is based on the fact that the percentage reduction in lesion spread on plant treated 2 days before artificial inoculation(2dbi) was more than those treated after the expression of disease symptoms. This experimental assertion was further supported by the graphic run of *line of tendency* (Fig 23*)* where the lineal distribution located more points from treatments administered two days before the artificial crop inoculation. (2dbi).

The more prophylactic tendency of the test materials was further expressed in the physiological condition of the crop shown in fig 17, where plants treated 2 days before artificial inoculation (17A) looked healthier than the ones treated two days after pathogen inoculation (17B), which in turn were healthier than those treated after macroscopic symptom expression (17C)

The oil extract of *Azadiractha* seeds used in the present study caused some physiological wilting of the sprayed crops.

The plants, however recovered at a later stage. The synthetic fungicides could not, however reduce the spread of the disease as readily as the test plant extracts *in vivo* This relatively unimpressive control from the synthetic Benomyl fungicide (tables 20 & 21) could be due to the reported tolerance of the pathogen to the standard formulation (Amadioha, 2003 and Akinbode *et al*, 2008).

In general the fungitoxic activities of the plant tissue extracts, especially *in vitro*, tend to be adversely affected by heat, as the cold water tissue extracts showed more fungitoxic effect than the hot water tissue extracts on the test pathogen, colony growth and spore germination (Figs 19 &20).

.The considerable disease control potentials expressed in this study, however suggest that the extracts of the plants are suitable for exploitation as potent fungicides for enhanced crop production practices and safer and ecological compatibility.

The four test plants could be possible source of substitutes for synthetic chemicals in controlling anthracnose development in *Vigna unguiculata*, at least in the tropical regions of the globe. Interestingly the advantage and potential use of the higher plants in the controlling crop diseases have been emphasized (Amadioha, 2003; Hernandez-Albiter *et al,* 2007; Akinbode & Ikotun, 2008; Ogwulumba *et al* 2008; and Colpas *et al*, 2009)

5.2 CONCLUSION

1. Anthracnose disease remains a devastating health problem to cowpea crop and an equated hindrance to its economic cultivation. The afflicting pathogen *Colletotrichum destructivum* O`Gara has the virulence of 100% infection on a single crop stand (that is every part of the crop is subject to attack and infection by *Colletotrichum destructivum* O`Gara at a given pathogenic situation).The use of botanicals remain suitable contest to adequate disease management options, at least for its characteristics ease of production economy and ecological amiability.

2. Results from the present study, established the fact that the four test plants: *Azadiractha indica, Xylopia aethiopica, Cymbopogon citratus, and Ocimum gratissimum* possess antifungal substances significantly toxic to *Colletotrichum destructivum* O`Gara. It is however pertinent to state here that further studies are needed to isolate and characterize these antifungal substances from the four test plants.

3. The potential of these botanicals as source of fungicides is enormous including the following:

(i) The plants can be locally grown particularly in the global tropics.

(ii) The extracts can also be obtained with crude or relatively refined cheap methods.

(iii) The extracts have no mammalian toxicity, judged from the fact that they are already in use by man as food and flavorings or drugs and associated medicaments.

(iv) They have little or no phytotoxic effect on sprayed crops.

4. Finally, this study has availed the fact that with the high global yearning for the urgent replacement of conventional (chemical) fungicides in disease management with the ecologically compatible bio-fungicides the seemingly several works in this direction is merely about 7% of the different management systems as indicated on cowpea disease control options and therefore, is

advocating for more exploration in this important area of scientific evaluation which definitely would in addition provide an eco-friendly global environment.

BIBLIOGRAPHY

Adebanjo, A. & S.A.Bankole (2004). Evaluation of some fungi and bacteria for Biocontrol of anthracnose disease of cowpea. **J. Basic Microbial. 44(1) 3-9.**

Adebitan, S.A. (1996). Effect of phosphorus and weed interference on anthracnose of cowpea in Nigeria**. Forage Research, 22(1)11-19.**

Adebitan, S.A & T.Ikotun (1996). Effect of plant spacing and cropping pattern on anthracnose *Colletotrichum lindemuthianum* of cowpea. **Fitopatología Brasileira 21 (1) 5-12.**

Adejumo, T.O., T. Ikotun & D.A. Florini (1999). Biological control of *Protomycopsis phaseoli*, the causal agent of leaf smut of cowpea**. Journal of Phytopathology 147 (6):371-375.**

Adekunle, A.T, K.F. Cardwell, D.A. Florini & T.Ikotun (2001). Seed treatment with *Trichoderma* species for control of Damping-off of cowpea caused by *Macrophomina phaseolina*. **Biocontrol. Sci. Technol. 11 (2001) 449-457.**

Airold Claudio & Silvana, A .M. Critter (1996). The inhibitory effect of Copper sulphate on microbial glucose degradation in red latosol soil. **Thermochimica Acta: vol. 288 (1-2) 73-82.**

Akinbode, O.A & T. Ikotun (2008). Evaluation of some bioagents and botanicals in *in vitro* control of *Colletotrichum destructivum*. **African Journal of Biotechnology 7 (7) 868-872.**

Allen, D.J, G.Thottappilly, A.M. Emechebe & B.B Singh (1998**). Disease of cowpea,** in the **"Pathology of Food and Pasture Legumes"** by D.J Allen and J.M Lenne. CAB International, Wallingford, U.K. **267-324.**

Amadi, J.E (1995). Chemical control of Cercospra leaf spot disease of cowpea (*Vigna unguiculata* (L.)Walp.). **Agrosearch 1 (2):101-107.**

Amadioha, A.C & V.I.Obi (1998). Fungitoxic activities of extracts from *Azadirachta indica and Xylopia aethiopica* on *Colletotrichum lindemuthianum* in cowpea. **Journal of Herbs, Spices, and Medicinal Plants, 6 (2) 33-40.**

Amadioha, A.C (2003). Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. **Acta Phytopathologica ET. Entomological 38 (3-4) 259-265.**

Amadioha, A.C & V.I. Obi (1999). Control of Anthracnose disease of cowpea by *Cymbopogon citrates* and *Ocimum gratissimum*. **Acta Phytopathologica et. Entomologica Hungarica 34 (1-2) 85-89.**

Amusa, N.A, T. Ikotun, & Y.O.K. Osikanlu (1994). Screening cowpea and soybean cultivars for resistance to Anthracnose and Brown blotch diseases using phytotoxic metabolites. **Afr. Crop Sci. J. 2 (2) 221-224.**

Amusa, N.A.,& T. Ikotun (1995). Phytotoxicity of metabolites produced by some *Colletotrichum species* on weeds and crop plants in Nigeria. **Inter. J. Tropical plant diseases 13 (1995) 113-119.**

Aveling, T.A.S. & A.Adandonon (2000). First report of pre- and post emergence damping off of cowpea caused by *Pythium ultimum* in South Africa. **Plant Disease Reporter 84 (8):922.**

Bankole, S.A, & A. Adebanjo (1996). Biocontrol of Brown botch of cowpea caused by *Colletotrichum truncatum* with *Trichoderma viride*: **Crop Protection 15 (1996) 633-636.**

Bankole, S.A & A.Adebanjo (1998). Efficacy of some fungal and bacteria isolates in controlling wet rot disease of cowpea caused by *Pythium aphanidermatum.* **J. Plant Prot. 11, 37-43.**

Bautista –Baños, S., M.Hernandez-Lopez, E.Bosquez-Molina & C.L. Wilson (2003). Effects of chitosan and plant extracts on growth of Colletotrichum gloeospiodes, anthracnose levels and quality of papaya fruit. **Crop Protection 22 (9) 1087-1092.**

Bautista-Baños, S., A. N Hernandez-Lauzardo, M.G. Velázquez-Del Valle, M.Hernandez-Lopez, E.Ait Barka, E.Bosquez-Molna, & C.L. Wilson (2006). Chitosan as a potential Natural compound to control pre and post harvest diseases of horticultural commodities. **Crop protection 25 (2) 108-118.**

Colpas, F.T, K.R.F Schwan-Estrada, J.R. Stangarlin, M.L.Ferrarse, C.A Scapim, & S.M. Bonaldo (2009). Induction of plant disease responses by *Ocimum gratissimum* L., leaf extracts **.Summa Phytopathol.Botucatu 35 (3) 191-195.**

Diabaté Sékou, Aké Séverin, Kouamé Roger, Coulibaly Ouolo Arsène, & N'guessan Walet Pierre (2010). Phenolic diversity in the defence reaction of the oil palm against vascular wilt disease. **Agric. Biol. J. N. Am., 2010, 1 (3): 407-415.**

Dugan. M .Frank (2006). The identification of Fungi**. An illustrated introduction with keys, glossary, and guide to literature 176pp.**

El Ghaouth Ahmed, Joseph Arul, Alain Asselin, & Nicole Benhamou (1992). Antifungal activity of Chitosan on post harvest pathogens: Induction of morphological andcytologicalalterations in Rhizopus stolonfer**. Mycological Research 96(9)769-779.**

Emechebe, A.M., & S.T.O. Lagoke (2002). Recent advances in research on cowpea diseases in challenges and opportunities for the enhancing

sustainable cowpea production: proceeding of the world cowpea conference III, held at the International Institute of Tropical Agriculture **(IITA) Ibadan Nigeria 4-8 sept. 2000 by C.A. Fatokun, S.A. Tarawali, B.B Singh, P.M. Kormawa, and M.Tamo (2002) pp94-123.**

Enikumehin, A.O. & I.A. Kehinde (2007). *In vitro* screening of some tropical ash samples against seed borne pathogen of wheat (*Tritticum aestivum* L.) **Australasian Plant Pathology 36 (6) 587-590.**

Fernando, W.G.D & R.G. Linderman (1997). The effect of mycorrhizal (*Glomus intraradisces*) colonization on the development of root and stem rot (Phytophthora vignae) of cowpea**. Journal of the Natural Sci. Council of Sri Lanka 25 (1):39-47.**

Fokung, C.N., T. Ikotun, A.G.O. Dixon, & C.N. Akem (1997). First report of *Colletotrichum gloeosporioides f.sp. Manihot*, cause of cassava anthracnose disease being seed born and seed transmitted in cassava. **Plant Disease 81 (1997) 695.**

Garrido Carlos, Maria Carbu, Francisco Javierfernandez-Acero, Giles Budge, Inmaculada Vallego, Alison Colyer & Jesús M. Cantoral (2008). Isolation and pathogenicity of *Colletotrichum Spp*. Causing anthracnose of Strawberry in South West Spain. **Eur. J Plant Pathol. (2008) 120:409-415.**

Gomes Sonia, Pilar Prieto, Paula Martins-Lopes, Teresa Carvalho,Antonio Martin & Henrique Guedes-Pinto(2009). Development of *Colletotrichum acutatum* on Tolerant and Susceptible *Olea europaea* L. cultivars:A microscopic analysis**. Mycopathologia (2009) 168:203-211.**

Grange, N.L.A & T.A.S. Aveling (1998). First report of *Alternaria cassiae* on cowpea **.Plant Disease 882 (10):1171.**

Heath Michele, C. (1998). Involvement reactive oxygen species in the response of resistant (hypersensitive) or susceptible cowpea to the cowpea rust fungus. **New Phytol. (1998) 138,251-263.**

Howell, C.R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant disease: the history and evaluation of current concepts. **Plant Disease, 87 (1) 4-10.**

Jansch Stephan, Jorg Rombke & Tobias Frische (2009). Ecological effects of the accumulation of copper in soil: Results of a literature review. **Extended Abstract Zum gleichnamigen vortragbeider Vol 4 (1.3) 70pp.**

Latunde –Dada, A.O, R.J. O`Connell, C. Nasa, R.J.Pring, J.A. Lucas & J.A. Bailey (1996). Infection process and identity of the Hemibiotrophic anthracnose fungus *Colletotrichum destructivum* from cowpea (*Vigna unguiculata)*. **Mycological Res. 100 (9) 1133-1141.**

Latunde-Dada, A.O., R.J. O`Connell, C. Nash & J.A. Lucas (1999). Stomatal penetration of cowpea (*Vigna unguiculata*) leaves by a *Colletotrichum* species causing latent anthracnose**. Plant Pathology 48 (1999) 777-785.**

Latunde-Dada, A.O., R.J. O'Connell, P.Bowwyer & J.A.Lucas (1999). Cultivar resistance to anthracnose disease of cowpea (*Vigna unguiculata* (L.)Walp.) caused by *Colletotrichum destructivum* O`Gara**. European Journal of Plant Pathology 105:445-451.**

Latunde-Dada, A.O & J.A.Lucas (2007). Localized hemibiotrophy in *Colletotrichum: cytological* and molecular taxonomic similarities among *C. destructivum, C. linicola and C. truncatum***. Plant Pathology (2007) 56:437-447.**

Liu, B., L.A.Wasilwa, T.E.Morelock, N.R.O`Neil, &J.C.Correll (2000). Comparison of *Colletotrichum orbiculare* and Several Allied *Colletotrichum* spp. for mtDNA RFLPs ,Intron RFLP and Sequence Variation,Vegitative

Compatibility, and Host Specifity. **The American Phytopathological Society 97 (10) 1305-1314.**

Martin, M.P. & F. Garcia –Figueres (1999). *Colletotrichum acutatum* and *C. gloeosporiodes* cause anthracnose on Olives**. European Journal of Plant Pathology 105:733-741.**

Mohammed, S. & N.A.Amusa (2003). *In vitro* inhibition of growth of some seedling blight inducing pathogens by compost-inhibiting microbes. **African Journal of Biotechnology.Vol.2 (6), 161–164.**

Montes-Belmont, R. & Carvajal.M (1998). Control of *Aspergillus flavus* in maize with plant essential oils and their components. **Journal of Food Protection vol. 61 (5), 616-619.**

Moore, J.W., M.Ditmore, & D.O.Tebeest (2008). Pathotypes of *Colletotrichum sublineolum* in Arkansas. **Ame. Phyto.Soc. 92 (10) 1415-1420.**

Moriwaki Touji, Toyozo Sato, & Takao Tsukiboshi (2003). Morphological and molecular Characterization of *Colletotrichum boninense*. **Mycoscience (2003) 44:47-53.**

Mould M.J.R., J. Robb, & G.J. Boland (1992). Loss of resistance to *Colletotrichum trifolii* in *in vitro* regenerated alfalfa plant**. Cell Tissue and Organ Culture 28:207-213.**

Munoz, M.A. & M.P.J Tamayo (1994). The process of *Choanephora cucurbitarum* in cowpea (*Vigna unguiculata* (L.) Walp.) **ASCOLF Informa 20 (1) 4-5.**

Muqit, A., M.S.Haque, & M.M.Hossain (1996). Reaction of cowpea lines against *Sclerotium rolfii*. **Bangladish Journal of Plant Pathology 12 (1-2):63.**

Nduagu, C., E.J. Ekefan & A.O.Nwankiti (2008). Effect of some crude plant extracts on growth of *Colletotrichum capsici* (Synd) Butler and Bisby, causal agent of pepper anthracnose**. Journal of applied Biosciences 6 (2) 184-190.**

Nguyen, P., O.V.Petersson, P. Olsson, & E. Liljeroth (2010). Identification of *Colletotrichum* species associated with anthracnose diseases of coffee in Vietnam**. European Journal Plant Pathology 127 (1) 73-87.**

Obi, V.I &. Onuoha (2000). Extraction and characterization methods of plants and plant products, in **Biological and Agricultural Techniques edited by Ogbulie, J.N and O.A.Ojiako. Webs media Publishers, Owerri 351pp.**

Obi, V.I., & N.N.Ugwunze (2009). Control of tuber roots of *Colocasia esculenta* L. Using plant ash. **Recent Progress in Medicinal Plants 23 (5) 345-355.**

O`Connell, J.R., B.A. Uronu, G.Waksman, C. Nash, J. P. R. KEON & A.J. Bailey (1993). Hemibiotrophic infection of *Pisium sativum* by *Colletotrichum truncatum*. **Physiological Plant Pathology, 27, 75-98.**

Ogwulumba, S.I., K.I. Ugwuoke & C. Iloba (2008). Prophylactic effect of pawpaw leaf and bitter leaf extracts on the incidence of foliar mycopathogens of groundnut (*Arachis hypogaea* L.) in Ishiagu, Nigeria**. African Journal of Biotechnology 7 (16) 2878-2880.**

Owolade O.F., J.A. Adediran, M.A.Akande, & B.S.Alabi (2006). Effect of application of phosphorus fertilizer on brown blotch disease of cowpea. **African Journal of Biotechnology 5 (4) 343-347.**

Palhano Fernando L., Thabita, T. B.Vilches, Reginaldo, B. Santos, Marcos, T.D.Orllando, J. Aires Ventura, & Patricia M.B. Fernandos (2004). Inactivation of *Colletotrichum gloeosporiodes* spores by high hydrostatic pressure combined with Citral or Lemon grass essential oil**. International Journal of Food Microbiology 95 (1) 61-66.**

Pell Mikael, Bo Stenberg & Lennart Torstensson (1998). Potential denitrification and Nitrification Test for Evaluation of pesticide effects in soil. **Ambio, vol.27 (1) 24-28.**

Perrin, R. & C. Plenchette (1993). Effect of some fungicides applied as soil drenches on the mycorrhizal infectivity of two cultivated soils and their receptiveness Glomus intraradices. **Crop protection vol. 12 (2) 127-133.**

Ratnoo, R.S., K.L.Jain, & M.k Bhatnagar (1997). Effects of atmospheric temperature on the development of ashy stem blight of cowpea**. Journal of mycology and plant pathology 27 (1) 90-91.**

Roy, K.W. & S.Ratnayake (1997). First report of *Phomopsis lengicolla* infection of cowpea pods and seeds in Mississippi**. Plant Diseases 81 (6):693.**

Salazar, S.M., A.P. Castagnaro, M.E.Arias, N.Chalfoun, U.Tonello & J.C.Diaz Ricci (2007). Induction of a defense response in strawberry mediated by a virulent strain of *Colletotrichum*. **European Journal of Plant Pathology 117:109-122.**

Sahin Nurettin & Aysel Ugur (2008). Investigation of the antimicrobial activity of some *Streptomyces* isolates. **Turk J. Biol. 27 (2003) 79-84.**

Sanders Gina M., L. Korsten & F.C. Wehner (2000). Survey of fungicides sensitivity in *Colletotrichum gloeosporiodes* from different avocado and mango production areas in South Africa. **European Journal of Plant Pathology 106:745-752.**

Santos, A.A., M.A.W. Quindere, & M.B. Melo (1997). Evaluation of cowpea genetypes for resistance to cowpea smut (*Entyloma vignae*)**. Fitopatologia Brasileira 22 (1) 77-78.**

Schumann, L.G. & Cleora, J.D`Arcy (2006). Essential plant pathology, published by**: The American Phytological Society, U.S.A 338pp.**

Sherriff, C., J.M. Whelan, M.G.Arnold, M.J. Lafay, J Brygoo & A.J .Bailey (1994). Ribosomal DNA sequence analysis reveals new species groupings in the genus *Colletotrichum*. **Experimental Mycology 18:131-138.**

Smith, J.E., L.Christian, & T.A.S. Aveling (1999a). Infection process of *Colletotrichum dematium* on cowpea stems. **Mycological Research 103 (2) 230:234.**

Smith, S.N., D.M. Helms & S. R. Temple (1999b). The distribution of *Fusarium* wilts of blackeyed cowpea within California caused by *Fusarium oxysporum* f.sp *tracheiphilum* race 4**. Plant Diseases 83 (7) 694.**

Sonmiez Sahriye; Mustafa Kaplan; Namik Kemal Sonmez; Harun Kaya & Iker Uz (2006).High level of copper application to soil and leave reduce the growth and yield of tomato plant. **Sci. Agric. Vol.63 (3), 213-218.**

Sun Hu & Jing-Ze Zhang (2009). *Colletotrichum destructivum* from cowpea infecting *Arabidopsis thaliana* (wall cress) and its identity to *C. higginsianum*. **Eur. J. Plant Pathol. (2009) 125:459-469.**

Thaung Maung Mya (2008). *Coelomycete* systematics with special reference to *Colletotrichum*. **Mycoscience (2008) 49:345-350.**

Ushamalini, C., K. Rajappan, & K Gangadharan (1997a). Inhibition of *Macrophomina phaseolina* and *Fusarium oxysporum* f.sp *tracheiphilum* by antagonists under in vitro conditions**. Plant Disease Research 74 (4) 324-328.**

Win. N.K., P. Jitareerat, S. Kanlayanarat, & S. Sangchote (2007). Effect of Cinnamon extract, Chitosan coating, hot water treatment and their

combination on crown rot disease and quality of banana fruit. **Post harvest Biology and Technology 45, 333-340.**

Http://www.epa.gov/PR_Notices/pr97-3.html [On line, consulted: 31st August, 2011].

Http://www.bhg.com/gardening/plant-dictionary/vegetable/cowpea/ [On line, consulted: 26/02/2011].

Http://www.seedsplants.com [On line, consulted: 26/02/2011].

Http://www.infonet-biovision.org/default/ct/120/crops [On line, consulted: 17th May, 2011].

Http://www.justfoodnow.com/2009/10/01/spiced-africa [On line, consulted: 11th September 2011].

Http://www.polyversumla.com[On line, consulted: 27th September, 2011].