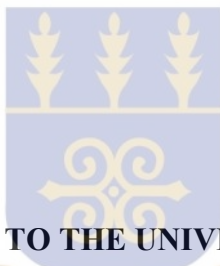


**AETIOLOGY, IMPORTANCE AND CONTROL OF A VINE-BROWNING AND DIE-BACK DISEASE OF WATER YAM (*DIOSCOREA ALATA* L.) IN THE VOLTA REGION OF GHANA**

**BY**

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**(10363967)**



**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF M. PHIL CROP SCIENCE (PLANT PATHOLOGY) DEGREE**

**JULY, 2013**

**DECLARATION**

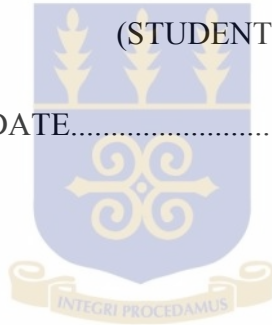
I do hereby solemnly declare that, this thesis is the original research of Shadrack Asiedu Coffie towards the award of the Master of Philosophy Crop Science (Plant Pathology) in the Department of Crop Science, College of Agriculture and Consumer Sciences (CACCS), University of Ghana, Legon except for other people's work which have been aptly cited and duly acknowledged and thus, neither part nor whole of this thesis has been presented elsewhere for another degree.

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## TABLE OF CONTENTS

|                                                                                                                      |      |
|----------------------------------------------------------------------------------------------------------------------|------|
| DECLARATION .....                                                                                                    | i    |
| ACKNOWLEDGEMENT .....                                                                                                | ii   |
| DEDICATION .....                                                                                                     | iv   |
| TABLE OF CONTENTS.....                                                                                               | v    |
| LIST OF TABLES.....                                                                                                  | ix   |
| LIST OF FIGURES .....                                                                                                | x    |
| LIST OF PLATES .....                                                                                                 | xi   |
| LIST OF ABBREVIATIONS .....                                                                                          | xiii |
| ABSTRACT .....                                                                                                       | xv   |
| <br>                                                                                                                 |      |
| CHAPTER ONE .....                                                                                                    | 1    |
| 1.0 INTRODUCTION .....                                                                                               | 1    |
| <br>                                                                                                                 |      |
| CHAPTER TWO .....                                                                                                    | 6    |
| 2.0 LITERATURE REVIEW .....                                                                                          | 6    |
| 2.1 Yam: Origin and distribution.....                                                                                | 6    |
| 2.2 Botany of yam.....                                                                                               | 7    |
| 2.3 Distinguishing characteristics of <i>D. alata</i> and <i>D. rotundata</i> .....                                  | 9    |
| 2.4 Economic importance and uses of yam .....                                                                        | 10   |
| 2.5 World production and trade of yam.....                                                                           | 13   |
| 2.6 Yam production in Ghana.....                                                                                     | 13   |
| 2.7 Climatic, water and soil requirements of yam .....                                                               | 15   |
| 2.8 Husbandry practices/staking .....                                                                                | 15   |
| 2.9 Harvesting.....                                                                                                  | 16   |
| 2.10 Field diseases of yam.....                                                                                      | 16   |
| 2.10.1 Foliar fungal diseases.....                                                                                   | 17   |
| 2.11 Economic importance of anthracnose disease of <i>D. alata</i> .....                                             | 18   |
| 2.12 Prevalence, incidence and severity of yam anthracnose disease.....                                              | 20   |
| 2.13 Symptoms of anthracnose disease of <i>D. alata</i> .....                                                        | 21   |
| 2.14 Etiology of anthracnose disease of <i>D. alata</i> .....                                                        | 22   |
| 2.15 <i>Colletotrichum</i> species.....                                                                              | 23   |
| 2.15.1 Existence of <i>Colletotrichum</i> species.....                                                               | 24   |
| 2.16 Host relations and specificity in <i>Colletotrichum gloeosporioides</i> and <i>C. truncatum</i> .....           | 27   |
| 2.17 Characterization of the strains of <i>Colletotrichum gloeosporioides</i> and <i>C. truncatum</i> isolated ..... | 28   |

|                                                                                                                                                                                  |        |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| 2.18 Screening of <i>D. alata</i> cultivars for resistance to anthracnose disease using tissue culture-derived whole-plant assay.....                                            | 30     |
| 2.18.1 Methods of screening of <i>D. alata</i> cultivars.....                                                                                                                    | 30     |
| 2.18.2 Methods of <i>in-vitro</i> propagation of <i>D. alata</i> .....                                                                                                           | 32     |
| 2.19 Development of IPM strategy for management of yam anthracnose disease.....                                                                                                  | 33     |
| 2.19.1 Stages of yam anthracnose disease development and its cycle.....                                                                                                          | 33     |
| 2.19.2 Epidemiology of yam anthracnose.....                                                                                                                                      | 34     |
| 2.20 Management of anthracnose disease of <i>D. alata</i> in the field.....                                                                                                      | 35     |
| <br>CHAPTER THREE.....                                                                                                                                                           | <br>37 |
| 3.0 MATERIALS AND METHODS.....                                                                                                                                                   | 37     |
| 3.1 Socio-economic background of the two important <i>D. alata</i> growing districts in the Volta region of Ghana.....                                                           | 37     |
| 3.1.1 Nkwanta-North District.....                                                                                                                                                | 37     |
| 3.1.2 Krachi-West District.....                                                                                                                                                  | 38     |
| 3.2 Assessment of farmers' perception on importance and control of a vine-browning and die-back disease of <i>D. alata</i> in the Volta region.....                              | 39     |
| 3.2.1 Selection of experimental sites within the two districts for the disease survey.....                                                                                       | 39     |
| 3.2.2 Survey to assess farmers' knowledge, perception, experiences and economic importance of a vine-browning and die-back disease of <i>D. alata</i> in the Volta Region.....   | 40     |
| 3.2.3 Symptoms description of vine-browning and die-back disease of water yam and samples collection.....                                                                        | 41     |
| 3.2.4 Determination of incidence and severity of a vine-browning and die-back disease of <i>D. alata</i> in the Nkwanta-North and Krachi-West districts of the Volta Region..... | 42     |
| 3.3 Isolation and identification of the causal agent(s) of a vine-browning and die-back disease of <i>D. alata</i> in the Volta region.....                                      | 45     |
| 3.3.1 Pathogen Isolation.....                                                                                                                                                    | 45     |
| 3.3.2 Identification of micro-organisms associated with anthracnose disease of <i>D. alata</i> .....                                                                             | 46     |
| 3.4 Characterization of the strains of <i>C. gloeosporioides</i> and <i>C. truncatum</i> .....                                                                                   | 50     |
| 3.4.1 Diversity study in <i>C. gloeosporioides</i> and <i>C. truncatum</i> infecting <i>D. alata</i> in Ghana.....                                                               | 50     |
| 3.4.2 Genome analysis of <i>C. gloeosporioides</i> and <i>C. truncatum</i> strains.....                                                                                          | 51     |
| 3.4.2.1 Rapid amplified polymorphic DNA (RAPD) analysis.....                                                                                                                     | 51     |
| 3.4.2.2 Restriction Enzyme Digestion of rDNA ITS regions.....                                                                                                                    | 53     |
| 3.5 Screening of <i>D. alata</i> cultivars for resistance to anthracnose disease using tissue culture-derived whole-plants assay.....                                            | 54     |
| 3.5.1 Determination of appropriate hormones concentration for the tissue culture test.....                                                                                       | 54     |
| 3.5.2 Raising of <i>D. alata</i> explants for the screening trial.....                                                                                                           | 55     |

|                                                                                                                                                                                             |     |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 3.5.3 Screening of 12 <i>D. alata</i> cultivars for resistance to anthracnose disease .....                                                                                                 | 57  |
| 3.6 Development of IPM strategies for management of anthracnose disease of <i>D. alata</i> .....                                                                                            | 59  |
| 3.6.2 Field management of anthracnose disease of <i>D. alata</i> in Ghana .....                                                                                                             | 61  |
| CHAPTER FOUR .....                                                                                                                                                                          | 64  |
| 4.0 RESULTS .....                                                                                                                                                                           | 64  |
| 4.1 Farmers' perception and practices on the cause, spread, control and importance of a vine-browning and die-back disease of <i>D. alata</i> in the Volta region.....                      | 64  |
| 4.1.1 General background of <i>D. alata</i> farmers .....                                                                                                                                   | 64  |
| 4.1.2 Farmers' knowledge and perception on disease symptoms, susceptibility of <i>D. alata</i> cultivars and prevalence of anthracnose disease of <i>D. alata</i> in the Volta Region ..... | 65  |
| 4.1.4 Knowledge and experience of farmers on, yield and economic impact of a vine-browning and die-back disease of <i>D. alata</i> on livelihoods of farmers.....                           | 70  |
| 4.1.5 Symptoms of a vine-browning and die-back disease of <i>D. alata</i> .....                                                                                                             | 73  |
| 4.1.6 Incidence and severity of a vine-browning and die-back disease of <i>D. alata</i> in the Nkwanta-North and Krachi-West districts.....                                                 | 74  |
| 4.2 Micro-organisms associated with a vine-browning and die-back disease of <i>D. alata</i> in the Volta Region .....                                                                       | 75  |
| 4.2.1 Identification using morpho-cultural characteristics of isolates .....                                                                                                                | 75  |
| 4.2.2 Molecular Identification of isolates .....                                                                                                                                            | 77  |
| 4.2.3 Pathogenicity test of isolates from the Volta Region .....                                                                                                                            | 78  |
| 4.3 Characterization of the strains of <i>C. gloeosporioides</i> and <i>C. truncatum</i> causing anthracnose disease of <i>D. alata</i> in Ghana.....                                       | 82  |
| 4.3.1 Diversity in <i>C. gloeosporioides</i> and <i>C. truncatum</i> causing anthracnose disease of <i>D. alata</i> in Ghana.....                                                           | 82  |
| 4.4.2 Incidence of anthracnose disease on two-month old potted explants of 12 <i>D. alata</i> cultivars using six <i>Colletotrichum</i> isolates.....                                       | 107 |
| 4.5 Integrated management of anthracnose disease of <i>D. alata</i> .....                                                                                                                   | 108 |
| CHAPTER FIVE .....                                                                                                                                                                          | 113 |
| 5.0 DISCUSSION.....                                                                                                                                                                         | 113 |
| 5.1 Assessment of farmers' knowledge and perception of a vine-browning and die-back disease of <i>D. alata</i> in the Volta Region.....                                                     | 113 |
| 5.4 Characterization of the strains of <i>C. gloeosporioides</i> and <i>C. truncatum</i> associated with anthracnose disease of <i>D. alata</i> in Ghana.....                               | 120 |
| 5.5 Screening of <i>D. alata</i> cultivars for resistance to anthracnose disease .....                                                                                                      | 123 |
| 5.6 Development of IMP strategy to manage anthracnose disease of <i>D. alata</i> .....                                                                                                      | 126 |

|                                                                                                                                                                                   |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| CHAPTER SIX.....                                                                                                                                                                  | 128 |
| 6.0 CONCLUSION AND RECOMMENDATION.....                                                                                                                                            | 128 |
| 6.1 Conclusion.....                                                                                                                                                               | 128 |
| 6.2 Recommendation.....                                                                                                                                                           | 129 |
| REFERENCES.....                                                                                                                                                                   | 130 |
| APPENDICES.....                                                                                                                                                                   | 151 |
| APPENDIX I: Questionnaire on etiology, importance and control of a vine-browning and die-back disease of water yam ( <i>Dioscorea alata</i> L.) in the Volta Region of Ghana..... | 151 |
| APPENDIX II: Surveyed Areas for the Diversity Study.....                                                                                                                          | 155 |
| APPENDIX IV: ANOVA for pathogenecity test for the Volta region strains.....                                                                                                       | 157 |
| APPENDIX V: ANOVA for the performance of <i>D. alata</i> explants on different hormones combinations in the main experiment.....                                                  | 157 |
| APPENDIX VI: ANOVA for Mean severity (AUDPCs) for a vine-browning and die-back disease on 12 tissue-cultured <i>D. alata</i> cultivars raised in a screen house.....              | 158 |
| APPENDIX VII: ANOVA for the mean disease incidence of a vine-browning and die-back disease of 12 tissue-cultured <i>D. alata</i> cultivars raised in a screen house.....          | 158 |
| APPENDIX VIII: ANOVA for fungicides inhibitory effect on radial mycelial growth and sporulation of three <i>Colletotrichum</i> spp.....                                           | 159 |

## LIST OF TABLES

|                                                                                                                                                                                                                                                     |     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Table 3.1: Experimental sites and number of farmers interviewed per site.....                                                                                                                                                                       | 40  |
| Table 3.3: Disease assessment key for severity of a vine-browning and die-back disease of <i>D. alata</i> .....                                                                                                                                     | 44  |
| Table 4.1: Educational background of respondents.....                                                                                                                                                                                               | 64  |
| Table 4.2: Symptoms used by farmers to identify a vine-browning and die-back disease of <i>D. alata</i> in the field.....                                                                                                                           | 66  |
| Table 4.5: Reasons for planting <i>D. alata</i> by farmers in the Nkwanta-North and Krachi-West districts. ....                                                                                                                                     | 70  |
| Table 4.6: Expected and actual yield of <i>D. alata</i> harvested by farmers in the Nkwanta-North and Krachi-West districts .....                                                                                                                   | 71  |
| Table 4.7: Farmers' reasons for not achieving expected yield of <i>D. alata</i> in the Nkwanta-North and Krachi-West districts .....                                                                                                                | 72  |
| Table 4.8: Socio-economic impacts of a vine-browning and die-back disease of <i>D. alata</i> on the livelihoods of farmers in the Nkwanta-North and Krachi-West districts. Number of farmers = 120. ....                                            | 73  |
| Table 4.9: Disease incidence and severity of anthracnose disease of <i>D. alata</i> in the selected communities of the Nkwanta-North and Krachi-West districts of the Volta Region. ....                                                            | 75  |
| Table 4.11: Detection of DNA fragments of <i>C. gloeosporioides</i> and <i>C. truncatum</i> strains obtained from diseased <i>D. alata</i> plants in the Volta Region using ITS1/ITS4 primer pair and CgInt/ITS4 species-specific primer pair. .... | 78  |
| Table 4.12: Disease infection (%) by <i>Colletotrichum</i> isolates on two-month old potted tissue-cultured <i>D. alata</i> cultivars (TDa008 and TDa010).....                                                                                      | 81  |
| Tabel 4.14 (86-96 pp): Distinguishable morphological and cultural characteristics of the 22 <i>Colletotrichum</i> spp. isolated from anthracnose disease of <i>D. alata</i> in Ghana. ....                                                          | 86  |
| Table 4.15: Detection of DNA fragments of <i>C. gloeosporioides</i> and <i>C. truncatum</i> strains obtained from diseased <i>D. alata</i> plants in Ghana using ITS1/ITS4 primer pair and CgInt/ITS4 species-specific primer pair. ....            | 98  |
| Table 4.21: Severity (mean AUDPCs) scores and ranks (parenthesis) of anthracnose disease on two-month old potted explants of 12 <i>D. alata</i> cultivars using six <i>Colletotrichum</i> isolates. ....                                            | 107 |
| Table 4.16: Incidence (%) of anthracnose disease on two-month old potted explants of 12 <i>D. alata</i> cultivars using six <i>Colletotrichum</i> isolates.....                                                                                     | 108 |

## LIST OF FIGURES

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Fig. 2.1: Origin and distribution of <i>Dioscorea</i> species worldwide. Source: Coursey (1975) and Degras (1993).....                                                                                                                                                                                                                                                                                                                                                                                                                                   | 6   |
| Fig. 3.2: Inoculated (INO) and non-inoculated (C) two-month old potted tissue culture-derived whole-plants of <i>D. alata</i> cultivars enclosed in transparent latex bags to maintain high RH% for disease infection. Pathogen used = <i>C. gloeosporioides</i> and <i>C. truncatum</i> with spore suspensions of $1.6 \times 10^6$ conidial/ml.....                                                                                                                                                                                                    | 50  |
| Fig. 4.2: Perception of <i>D. alata</i> farmers on the cause of a vine-browning and die-back disease of <i>D. alata</i> .<br>.....                                                                                                                                                                                                                                                                                                                                                                                                                       | 68  |
| Fig. 4.3: Perception of <i>D. alata</i> farmers on the spread of a vine-browning and die-back disease of <i>D. alata</i> within farms. ....                                                                                                                                                                                                                                                                                                                                                                                                              | 69  |
| Fig. 4.4: Perception of <i>D. alata</i> farmers on the spread of a vine-browning and die-back disease of <i>D. alata</i> among farms. ....                                                                                                                                                                                                                                                                                                                                                                                                               | 69  |
| Fig 4.5: Perception of <i>D. alata</i> farmers on measures needed for successful of control of a vine-browning and die-back disease of <i>D. alata</i> in the Volta Region.....                                                                                                                                                                                                                                                                                                                                                                          | 70  |
| Fig. 4.7: UPGMA dendrogram based on Jaccard's coefficient, indicating relationships among the 16 strains of <i>C. gloeosporioides</i> obtained from six Regions of Ghana. Genetic distances were based on the combined data from the random amplified polymorphic DNA (RAPD) and the internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) analyses. RAPD primers: OPA-11, OPA-18, OPA-05, OPC-07 and OPC-16 and Restriction enzymes used were: <i>HaeIII</i> , <i>HinfI</i> , <i>RsaI</i> , <i>MspI</i> and <i>HhaI</i> ..... | 103 |
| Fig. 4.8: UPGMA dendrogram based on Jaccard's coefficient, indicating relationships among the four strains of <i>C. truncatum</i> obtained from three Regions of Ghana. Genetic distances were based on the combined data from the random amplified polymorphic DNA (RAPD) and the internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) analyses. RAPD primers: OPA-11, OPA-18, OPA-05, OPC-07 and OPC-16 and Restriction enzymes used were: <i>HaeIII</i> , <i>HinfI</i> , <i>RsaI</i> , <i>MspI</i> and <i>HhaI</i> .....   | 104 |
| Fig. 4.9: Fungistatic effects of four fungicides (Carbendazim, Mancozeb, Nordox and Shatiz F1) on radial mycelial growth and sporulation of two <i>Colletotrichum</i> isolates – <i>C. truncatum</i> (A) and <i>C. gloeosporioides</i> Cg111 (B). PDA amended cultures and controls (*NF) were incubated at $26 \pm 2$ °C and 65-72% RH in the laboratory. Vertical bars indicate $LSD_{5\%}$ . Percentage reduction in radial mycelial growth of test fungi (I) was calculated based on the formula of Kiran <i>et al.</i> (2010). ....                 | 109 |

## LIST OF PLATES

- Plate 4.1: Stages of development of symptoms of a vine-browning and die-back disease of *D. alata*. Healthy plant (I); pin-point spots on lamina (II); pin-points spots surrounded by yellow halo on lamina (III); small irregular brown spots with yellow halo (IV); brown regular spots with gray centre bordered by dark brown ring (V); large brown spots bordered by yellow patches on leaves and die-back of vines (VI); and browning and/or blackening of vines and defoliation of leaves (VII). ..... 74
- Plate 4.6: Amplified DNA fragments of *C. gloeosporioides* strains (lanes – 2, 3, 4 and 6) and *C. truncatum* strains (lanes – 1 and 5) obtained from diseased *D. alata* in the Volta region using ITS1/ITS4 primer pair (A) and ITS4/CgInt species specific primer pair (B). M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); C = Negative controls (sterile distilled water). ..... 78
- Plate 4.7: Two-month old potted tissue culture-derived whole-*D. alata* plants showing symptoms of anthracnose disease. Die-back (blue arrows) (A-C) and necrotic lesions on leaves (brown rings) (D-G) 13 DAI with *C. gloeosporioides* isolate (Cg111) using spore suspension of  $1.6 \times 10^6$  conidia/ml. .... 79
- Plate 4.8: Two-month old potted tissue culture-derived whole-*D. alata* plants without symptoms of anthracnose disease. Plants were sprayed with sterile distilled water amended with two drops of Tween 80 (2% v/v) – as controls. .... 80
- Plate 4.10: Different growth patterns of *C. truncatum* strains on PDA at  $25 \pm 2$  °C, 7 days. Volta strains (I and II); Ashanti strains (E-G); Central strain (III); Eastern strain (IV). .... 83
- Plate 4.11: Different formation of setae and acervuli of *C. gloeosporioides* strains obtained from diseased *D. alata* plants – setae (A) and acervuli (B). Black bars in panels = 60  $\mu$ m. .... 83
- Plate 4.12: Different formation of setae and acervuli of *C. truncatum* strains obtained from diseased *D. alata* plants – setae (A-C) and acervuli (D and E). Black bars in panels = 60  $\mu$ m. .... 84
- Plate 4.13: Amplified DNA fragments of *C. gloeosporioides* strains (lanes - 1, 2, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19 and 20) and *C. truncatum* strains (lanes - 3, 6, 10 and 16) obtained from diseased *D. alata* in Ghana using ITS1/ITS4 primer pair (A) and ITS4/CgInt species specific primer pair (B). M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); C = Negative controls (sterile distilled water); Pc = Positive controls (*C. gloeosporioides* from mango). Lanes: 1 = Cg111, 2 = Cg114, **3 = Ct130**, 4 = Cg117, 5 = Cg125, **6 = Ct132**, 7 = Cg141, 8 = Cg147, 9 = Cg148, **10 = Ct142**, 11 = Cg149, 12 = Cg150, 13 = 151, 14 = Cg158 162, 15 = Cg162, **16 = Ct168**, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169. .... 97
- Plate 4.14: RAPD fingerprints obtained with three RAPD Primers OPA-18 (A), OPC-07 (B) and OPC-16 (C) for 20 *Colletotrichum* isolates (1-20) obtained from diseased *D. alata* in Ghana. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); Pc = Positive controls (*C. gloeosporioides* from Mango); Lanes: 1 = Cg111, 2 = Cg114, **3 = Ct130**, 4 = Cg117, 5 = Cg125, **6 = Ct132**, 7 = Cg141, 8 = Cg147, 9 = Cg148, **10 = Ct142**, 11 = Cg149, 12 = Cg150, 13 = 151, 14 = Cg158 162, 15 = Cg162, **16 = Ct168**, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169. .... 100
- Plate 4.16: Three-week old *in-vitro* explants of *D. alata* incubated at 16-hour light/8-hour dark photoperiod (cool, white fluorescent light –  $30 \mu\text{mol m}^{-2} \text{S}^{-1}$ ) and a temperature of 25 °C, 60-70% RH. .... 105
- Plate 4.17: Three-week old hardened explants of *D. alata* cultivars TDa019 (A), TDa007 (B) and TDa013 (C) without symptoms of anthracnose disease. Medium used – 1:1 vermiculite: compost (peat: sand 9:1) (IITA, Nigeria) maintained under bioclimatic chamber. .... 105

Plate 4. 18: Six-week old explants of *D. alata* cultivars TDa010 (A); TDa019 (B); TDa018 (C) and TDa007 (D) with varying number of roots and shoots. Explants were incubated at 16-hour light/8-hour dark photoperiod (cool, white fluorescent light –  $30 \mu\text{mol m}^{-2} \text{S}^{-1}$ ) and a temperature of 25 °C, 60-70% RH... 105

## LIST OF ABBREVIATIONS

|          |                                                                                    |
|----------|------------------------------------------------------------------------------------|
| AEA      | Agricultural Extension Agent                                                       |
| ANOVA    | Analysis of Variance                                                               |
| AR       | Ashanti Region                                                                     |
| BNARI    | Biotechnology of Nuclear Agriculture Research Institute, Accra                     |
| B/A-R    | Brong-Ahafo Region                                                                 |
| C        | Control                                                                            |
| CR       | Central Region                                                                     |
| CRD      | Completely Randomized Design                                                       |
| CPDA     | Carbendazim Potato Dextrose Agar                                                   |
| CSIR-CRI | Council for Scientific and Industrial Research-Crops Research Institute,<br>Kumasi |
| Ct       | <i>Colletotrichum truncatum</i>                                                    |
| Cg       | <i>Colletotrichum gloeosporioides</i>                                              |
| CTAB     | Cetyltrimethylammonium Bromide                                                     |
| DAI      | Days after Inoculation                                                             |
| DSDW     | Double Sterile Distilled Water                                                     |
| DNA      | Deoxyribonucleic Acid                                                              |
| EDTA     | Ethylene Diamino Tetra-acetic Acid                                                 |
| e.g.     | Example                                                                            |
| ELISA    | Enzyme Linked Immunosorbent Assay                                                  |
| ER       | Eastern Region                                                                     |
| F        | Fisher's Test                                                                      |
| FAO      | Food and Agricultural Organization of the United Nations                           |
| FAOStat  | Food and Agricultural Organization of the United Nations' Statistics               |
| g        | Gramme                                                                             |
| GAEC     | Ghana Atomic Energy Commission, Accra                                              |
| GEPC     | Ghana Exports Promotion Council                                                    |
| i.e.     | That is                                                                            |
| ISSER    | Institute of Statistical, Social and Economic Research                             |
| IITA     | International Institute of Tropical Agriculture                                    |
| IPM      | Integrated Pest Management                                                         |
| JHS      | Junior High School                                                                 |

|           |                                                                                                                |
|-----------|----------------------------------------------------------------------------------------------------------------|
| L         | Litre                                                                                                          |
| LSD       | Least Significant Difference                                                                                   |
| MAS       | Months after sprouting                                                                                         |
| MoFA      | Ministry of Food and Agriculture, Ghana                                                                        |
| MoFA-SRID | Ministry of Food and Agriculture – Statistics, Research and Information Directorate                            |
| MoFA-ESD  | Ministry of Food and Agriculture – Extension Service Directorate                                               |
| MPDA      | Mancozeb Potato Dextrose Agar                                                                                  |
| NPDA      | Nordox Potato Dextrose Agar                                                                                    |
| NAPDA     | Non-amended Potato Dextrose Agar                                                                               |
| NR        | Northern Region                                                                                                |
| P         | Probability                                                                                                    |
| RCBD      | Randomized Complete Block Design                                                                               |
| PCR       | Polymerase Chain Reaction                                                                                      |
| PDA       | Potato Dextrose Agar                                                                                           |
| PGRRI     | Plant Genetic Resource Research Institute, Bunso                                                               |
| RAPD      | Randomized Amplified Polymorphic DNA                                                                           |
| RFLP      | Restriction Fragment Length Polymorphism                                                                       |
| RH        | Relative Humidity                                                                                              |
| RNA       | Ribonucleic Acid                                                                                               |
| RT-PCR    | Reverse Transcription Polymerase Chain Reaction                                                                |
| RPM       | Revolution per Minute                                                                                          |
| SPDA      | Shafiz Potato Dextrose Agar                                                                                    |
| SHS       | Senior High School                                                                                             |
| SPSS      | Statistical Package for Social Sciences                                                                        |
| ssDNA     | Single Stranded Deoxyribonucleic Acid                                                                          |
| SDW       | Sterilized Distilled Water                                                                                     |
| TAE       | Tris-Acetic-Ethylene Diamino Tetra-acetic Acid                                                                 |
| TBC-BNARI | Tissue-Culture and Biotechnology Centre of the Biotechnology and Nuclear Agriculture Research Institute, Accra |
| TRIS      | Trizma Base                                                                                                    |
| TRIS-EDTA | Trizma Base-Ethylene Diamino Tetra-acetic Acid                                                                 |
| UG        | University of Ghana                                                                                            |
| UV        | Ultra Violet                                                                                                   |

## ABSTRACT

Vine-browning and die-back disease (anthracnose disease) is a major constraint and a predominant disease in all yam producing areas especially, in Africa. The disease reduces photosynthetic area of infected yam plants resulting in severe yield loss. Farmers and Agricultural Extension Agents in the Nkwanta-North and Krachi-West districts of the Volta Region have recently reported high incidence of the disease on their farms. A study was, therefore, conducted from January, 2012 to June, 2013 to assess farmers' knowledge and perception of the disease and its economic impacts on their livelihoods, determine the incidence and severity of the disease, identify the causal organisms of the disease, characterize the strains of the pathogens inducing the disease, use tissue culture-derived whole-plant assay to screen water yam cultivars for anthracnose resistance and develop effective integrated pest management strategy for the disease in the field. Assessment of farmers' knowledge and perception was ascertained through questionnaires and interviews of 120 *D. alata* farmers in the Nkwanta-North and Krachi-West districts of the Volta Region. The findings indicated that, most *D. alata* farmers in both districts were aware of the presence of a vine-browning and die-back disease in their fields and could identify it by its symptoms but were ignorant of its causal organisms, spread and control. The incidence and severity of the disease in the Nkwanta-North district (75.30% and 1.80) were higher compared to Krachi-West district (55.70% and 1.60). The pathogens responsible for the disease in the Volta region and other yam growing regions (Ashanti, Brong-Ahafo, Central, Eastern and Northern regions) in Ghana were confirmed as *Colletotrichum gloeosporioides* Penz. and Sacc. and *C. truncatum* Andrus and Moore using symptoms of the disease, morpho-cultural characteristics and genome analysis of isolates, and pathogenicity test. The vine-browning and die-back disease was identified as anthracnose disease of *D. alata*. Morpho-cultural characters and Polymerase Chain Reaction (PCR) with five Random Amplified Poly-Dimorphism (RAPD) primers (OPA-11, OPA-18, OPC-5, OPC-7 and OPC-16) and five Inter-Spacers Restriction Fragment Length Polymorphism (ITS-RFLP) enzymes (*Hha*I, *Hae*III, *Hinf*I, *Msp*I and *Rsa*I) were used to characterize 16 strains of *C. gloeosporioides* and four strains of *C. truncatum* obtained from diseased *D. alata* samples in the Volta Region and other five yam growing regions in Ghana (Ashanti, Brong-Ahafo, Central, Eastern and Northern). The 16 *C. gloeosporioides* strains clustered into two major groups (with similarity of 52%) and six sub-groups while the *C. truncatum* strains were put into two groups (with similarity of 30%) using unweighted pair-group method with arithmetic average (UPGMA). Screening of 12 *D. alata* cultivars for resistance to the disease was performed using tissue culture-derived whole-plant assay. Of the 12 cultivars, six (TDa008, TDa010, TDa014, TDa023, TDa025 and TDa027) were

resistant to the disease but at varying levels while the six other cultivars (TDa007, TDa013, TDa015, TDa018, TDa019 and TDa021) were susceptible at varying levels. Cultivar TDa023 was highly resistant while TDa019 was highly susceptible. The use of integrated pest management strategy consisting of hot-water treated seed yam ( $45\pm 1^{\circ}\text{C}$  for 20 minutes and drying for 30 minutes) of the highly resistant cultivar (TDa023) along with monthly weeding and Mancozeb sprays significantly ( $P < 0.05$ ) reduced anthracnose severity (1.2) compared to the control (3.0) in the field. Although, the technology developed could effectively reduce the disease, it requires additional research at two or more locations to be sure of its sustainability before recommending to farmers. Moreover, benefit-cost analysis ought to be undertaken on the developed IPM strategy.

## CHAPTER ONE

### 1.0 INTRODUCTION

Yam (*Dioscorea* spp.) constitutes an economically important staple food for millions of people in the tropics and subtropics. West Africa accounts for about 95% of the total yam production area (FAO, 2002). The crop is one of Ghana's major staples, being ranked second in importance (in tonnage terms) after cassava in staple food production but its relatively high unit price makes it the most important food crop in terms of value of production (Lebot, 2009). Along with cassava, it provides 31% of 'national food security' and supplies in excess of 50% of the calorie needs of the average Ghanaian (Dadzie, 2004). Yam is grown mainly by smallholders and serves as valuable income source for producers and retailers. In addition, it generates foreign exchange earnings for the Country and plays a significant role in the socio-cultural lives (such as celebration of New Yam Festivals) of some producing regions such as the Ashanti, Volta and Brong-Ahafo Regions (Narula *et al.*, 2007). The two most important cultivated edible yams in Ghana are white Guinea yam (*D. rotundata* Poir) and water yam (*D. alata* L.) (Ofori and Hahn, 1991).

*D. alata* is composed of food nutrients such as carbohydrates (starch, sugars, and fibers), proteins, minerals, a negligible amount of lipids and accounts for over 20% of the total dietary calorie intake in Africa and South Pacific (Opara, 2003; Bhandari and Kawabata 2004; Lebot, 2009). It is traditionally consumed as boiled, mashed, fried and roasted. However, some cultivars are well suited for pounding (Lebot *et al.*, 1998). Industrially, certain *D. alata* cultivars are processed into flour, crisps, chips or cubes, flakes and ice-cream (Degras, 1993).

In Ghana, *D. alata* is commercially produced in six major regions with Volta being the leading producer followed by the Eastern, Brong-Ahafo, Northern, Ashanti and Central regions (MoFA,

2010). In comparison with *D. rotundata*, *D. alata* has superior characteristics for sustainable production, including high yield potential, ease of propagation, early vigour for weed suppression and storability of tubers (Asiedu *et al.*, 2003). The limitation of *D. alata* production in most areas in Ghana is the susceptibility of most cultivars to a leaf necrosis, vine-browning and die-back disease. Recently, prevalence of this disease has been reported by farmers and agricultural extension agents (AEAs) in the Volta region but it appears to be less significant in the other *D. alata* growing regions (MoFA-ESD, Personal Communication, 2012). The disease has, therefore, become a major constraint to *D. alata* production in the Volta region. Infections that commenced prior to or during tuber formation resulted in excessive yield loss of about 90% (Atsu and Akator, Personal Communication, 2012). A similar disease was reported to limit the production of the crop in the Caribbean (Degras *et al.* 1984; Green and Simons, 1994). Abang *et al.* (2001a) reported 85% yield loss of *D. alata* due to the disease in Nigeria while 75-95% yield loss have been reported in the Caribbean (Mignucci *et al.*, 1988; Green and Simons, 1994).

Several important *D. alata* cultivars were lost in the Caribbean due to high incidence (85-100%) of a vine-browning and die-back disease (Degras *et al.*, 1984; Mignucci *et al.*, 1988; Green, 1994). Recently, the Nkwanta-North and Krachi-West districts of the Volta Region has been encountering high incidence of the disease which has led to abandoning of *D. alata* production by many farmers with a negative impact on their livelihoods (Atsu and Akator, Personal Communication, 2012). It was, therefore, imperative to assess the economic importance of the vine-browning and die-back disease affecting *D. alata* in the Volta Region of Ghana.

The vine-browning and die-back disease symptoms described by the farmers and AEAs in the Volta Region were typical of *Colletotrichum* spp. infection as similar symptoms were reported on *D. alata* in Nigeria (Abang *et al.*, 2001a) and the Caribbean (Green and Simons, 1994) were

associated with *Colletotrichum gloeosporioides* and *C. truncatum*. However, whether the same causal organisms were responsible for the disease in the Volta Region of Ghana was unknown. It was, therefore, necessary to identify the causal agents of the vine-browning and die-back disease of *D. alata* in the Volta region.

Although, *Colletotrichum* spp. are widely accepted as the causal agents of a vine-browning and die-back disease of *D. alata* (Singh *et al.*, 1966; Winch *et al.*, 1984), identification and differentiation of species based on morphological and cultural characteristics have often been inadequate (Alonso-Gonzalez *et al.*, 2006). *C. gloeosporioides* isolates from a vine-browning and die-back disease of *D. alata*, often appear similar in cultural and morphological characteristics but are highly variable in terms of virulence – from location to location (Abang *et al.*, 2001). Diversity studies among *Colletotrichum* spp. using molecular techniques such as polymerase chain reaction (PCR) with species-specific primers (Adaskaveg and Hartin, 1997), inter-spacers restriction fragment length polymorphism (ITS RFLP), random amplified polymorphic DNA (RAPD) markers (Thottappilly *et al.* 1999; Alleyne, 2001) and inter-spacers (ITS) sequence analysis (Sreenivasaprasad *et al.*, 1994; Sreenivasaprasad *et al.*, 1996; Freire *et al.*, 2001; Martín and García-Figueres, 1999) are more efficient in resolving the systematic issues in *Colletotrichum* spp. Thus, the combined use of molecular diagnostic tools and traditional morpho-cultural techniques would present an appropriate and good approach for studying *Colletotrichum* spp. complexes existing in the Volta region and/or Ghana at large.

The deployment of durable host plant resistance in *D. alata* would contribute significantly to a high level of field performance. The highly spatial and temporal variations among *Colletotrichum* spp. coupled with long growth cycle of *D. alata* and the high cost of conducting multiple-location trials more often than not, limit field evaluation and generally makes it unproductive (Green *et al.*,

2000). Previously, resistant cultivars in the Caribbean were reported to be susceptible as a result of new or previously unknown virulent strains of *C. gloeosporioides* (Ano *et al.*, 2005) and selection of *D. alata* cultivars under sub-optimal conditions for disease development under natural field infection.

Lack of standard screening procedures for yam anthracnose resistance delayed progress in resistance breeding until Onyeka *et al.* (2006) optimized tissue culture-derived whole-plant assay to evaluate *D. alata* cultivars for varying levels of resistance. This assay provides a probable rapid technique for evaluating breeding populations and studying the relationship between *D. alata* cultivars and pathogen isolates. Augmenting this method by taken into accounts, the disease development, rate of the disease progression and the final level of diseased area under the progress curve (AUDPC) (Walther *et al.* 1999; Jeger, 2004) would, therefore, not only provide a strong tool for identification of levels of resistance to the vine-browning and die-back disease but also the analysis of genetic basis for resistance in *D. alata* cultivars in the Volta region and/or Ghana at large. The study, then, proposed the use of tissue culture-derived whole-plant assay to screen *D. alata* cultivars in the Volta region and/or Ghana – taking into consideration, the AUDPC.

Currently, there are no available measures that ensure sustainable control of a vine-browning and die-back disease of *D. alata* in Ghana. Resistant *D. alata* cultivars seem to be the best means of controlling the disease in the Caribbean (Green and Simons, 1994) but cultivars which initially proved resistant to the disease later succumbed to it partly due to the high variability of the pathogen (Green *et al.*, 2000). Furthermore, since resistance is relative, not all the *D. alata* cultivars in the Volta region or collections in Ghana would prove resistant to the disease. Hence, integrated pest management (IPM) measures which involve the use of healthy planting materials,

good agronomic practices and judicious use of effective fungicides would, therefore, form the basis of sustainable management strategies for the vine-browning and die-back disease in Ghana.

The study, therefore, employed IPM strategy to manage the disease.

The objectives of the study were to:

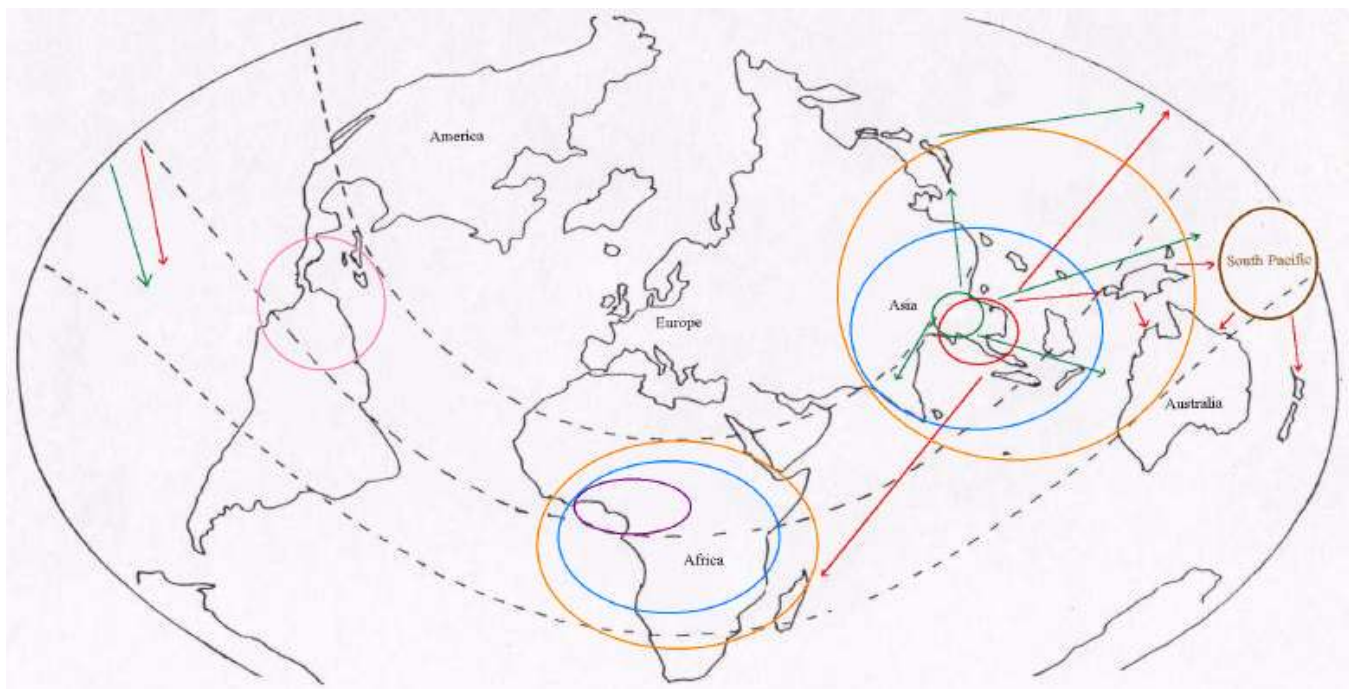
- Assess the farmers' knowledge, perception and practices on the cause, spread, control and importance of a vine-browning and die-back disease of *D. alata* in the Volta Region
- Determine the incidence and severity of the disease in the Volta Region
- Confirm the identity of the causal organisms as *Colletotrichum* spp.
- Characterize the strains of the pathogen isolated
- Utilize tissue culture-derived whole-plant assay to screen 12 *D. alata* cultivars for resistance to the disease.
- Develop an integrated pest management (IPM) strategy to manage the disease.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Yam: Origin and distribution

Yam (also called Ñamé -Spanish and Igname - French) belongs to the genus, *Dioscorea* and a family, *Dioscoreaceae*. Of the estimated 300-600 species available (Govaerts *et al.*, 2007), only over half a dozen principal species are grown for consumption (Ofori and Hahn, 1991) while others are grown for medicinal purposes (Wu *et al.*, 2005; Lebot, 2009). Yams originated in the Far East and spread westwards (Coursey, 1975) (Fig. 2.1). They have since evolved independently in the Eastern and Western Hemispheres and today yams are grown widely throughout the tropics (Asiedu *et al.*, 2003). In the West African yam zone, which is the principal producer on a global basis, *D. rotundata*, *D. alata* and *D. esculenta* are the most common species (Degras, 1993).



— *D. alata*; — *D. dumetorum* and *D. hipsida*; — *D. trifida*; — *D. esculenta*; — *D. cayenensis* and *D. rotundata* and — *D. bulbifera*

Fig. 2.1: Origin and distribution of *Dioscorea* species worldwide. Source: Coursey (1975) and Degras (1993).

## 2.2 Botany of yam

Yam (*Dioscorea* spp. L) is a polyploid and a vegetatively propagated tuber crop that is cultivated in the yam belt of West and Central Africa (IITA, 2009). It belongs to a group of crops labelled “orphaned crops”, which have not received research attention for a long period of time and, therefore, has attained very little improvement (Otoo, 2001b). The major edible species of yam of African origin are white Guinea yam (*D. rotundata* Poir.), yellow Guinea yam (*D. cayenensis* Lam.), and trifoliolate or bitter yam (*D. dumetorum* Kunth). Edible species from Asia include water or greater yam (*D. alata* L.), and lesser yam (*D. esculenta*). Cush-cush yam (*D. trifida* L.) originated from the Americas (IITA, 2009). White Guinea yam and water yam are the most important in terms of cultivation and use in West Africa. Yam is grown in about 51 countries in the tropics and subtropics with yields averaging about 11 million metric tons/hectare in the major producing countries of West Africa (Nigeria, Cote d’Ivoire, Ghana, and Benin) (Okwu and Ndu, 2006).

*Dioscorea* is a genus of over 600 species with subterranean tubers or rhizomes (Govaerts *et al.*, 2007). The tubers are storage organs and often grown to a substantial size; they produce short, fibrous roots and annual shoots, which are winding (except in dwarf species), the direction of winding being specific. The genus *Dioscorea* is divided into a number of taxonomic sections; the important food yams are grouped in the following: Enantiophyllum- *D. alata*, *D. rotundata*/*D. cayenensis* complex, *D. opposita* and *D. japonica*; Lasiophyton- *D. dumetorum* and *D. hispida*; Combilium - *D. esculenta* and Macrogynodium- *D. trifida*; Opsophyton- *D. bulbifera* (Lebot, 2009).

Yam has an annual vegetative system composed of a root apparatus (some extend throughout the upper layers of the soil, others consist of root hairs), a stem apparatus, and a foliar apparatus. The adventitious roots arising from the base of the stem absorb mineral nutrients, and water (Orkwor

*et al.*, 1998). The yam stem is usually a thin twining vine allowing the plants to climb. It is frequently winged and commonly spiny. Several species have deep striations in their stem, some contain anthocyanins while others have large thorns and generally, the direction of the twining is used as a taxonomic feature (Bourret, 1973). The leaves are petiolate (except for *D. dumetorum*, *D. hispida* and *D. pentaphylla*, which have trifoliate leaves and hairs on their stems), often cordate with strongly marked reticulate veining (unusual for a mono-cotyledon), sometimes lobed, occasionally palmately compound (Okwu and Ndu, 2006) and have an arrangement either opposite or alternate with axillary buds (Degras, 1993a).

It has small flowers borne in elongated racemes, with male and female separate and usually borne on different plants. The female flowers form dehiscent casing, usually trilocular, with 6 seeds, generally winged and flat for wind dispersal, though many of the cultivated forms have become partially or highly sterile (Akoroda and Hahn, 1995). The seed usually goes through a dormancy period of three to four months before germination can occur. The genus *Dioscorea* is dioecious with an extremely irregular production of male and female flowers pollinated by insects (Bourret, 1973). As flowering is rare, yams are vegetatively propagated using the basal nodal region, the tuber and the bulbils (Degras, 1993a). Plants usually produce a single annual tuber, which is usually 20 to 40 cm long and weighs from two to several dozen kilograms, depending on cultivar and growing conditions (Dadzie, 2004). The body can be elongated or spherical with a white, yellow or purple flesh. Bulbils, which are characteristics of *D. alata*, are produced in the axils of the leaves which have the morphology and appearances of condensed stems and in a few instances are relatively large and tuberous weighing about 20 to 100 g (Degras, 1993, 1993b). By contrast, *D. bulbifera* produces only aerial tubers, which can weigh up to 1 kg (Dadzie, 2004).

Yam tuber does not sprout during the early part of storage, even under suitable growth conditions (Alexander and Coursey, 1969). The length of tuber dormancy is endogenously controlled and

conditions such as availability of soil moisture or cool temperature are ineffective triggers of sprouting (Passam and Noon, 1977). The length of dormancy is also influenced by the yam species (Otoo, 2001b). The environmental conditions affecting yam tuber dormancy are photoperiod, white and coloured lights, temperature, relative humidity and partial oxygen pressure (Akem and Asiedu, 1994). Physiological age of tubers affects their readiness to sprout, but by approximately 6 months after harvesting, dormancy disappears completely and bud-less setts planted after that period will require nearly the same time to sprout (Onwueme and Charles, 1994).

### **2.3 Distinguishing characteristics of *D. alata* and *D. rotundata***

*D. alata* (water yam) and *D. rotundata* (white yam or white guinea yam) are the two most important yam species cultivated in Ghana and/or West Africa (Dadzie, 2004; IITA, 2009; Cornelius, 2011) with distinct characteristics. *D. alata* is a large climbing plant, which usually reaches 15 m in height, with quadrangular winged stems, twining anticlockwise (to the right) (Putnam and Gaskalla, 2010). Leaves are opposite, variable in size and shape, but usually ovoid to cordate with a deep basal sinus, acuminate. The male flowers are borne on panicles, up to 30 cm long whilst the female flowers are on small axillary spikes. Few cultivars produce fertile seed and most are completely sterile (Akoroda and Hahn, 1995). Bulbils are liberally formed in leaf axils of some cultivars (Degras, 1993b).

The tubers are usually single except few cultivars and exhibit wide variation in shape, colour and size: they are generally cylindrical but may be long and bending to almost globular and are often pronged or lobed, or even flattened and fan-shaped (Alexander and Coursey, 1969). Their weight is usually 5-10 kg though special cultivation can yield giant tubers of 15 kg or more (Putnam and Gaskalla, 2010). The flesh of some cultivars can be deep reddish-purple or pink and these forms have been classified as *D. purpurea* Roxb. and *D. afropurpurea* Roxb. (Lebot, 2009). *Dioscorea*

*rotundata* on the other hand, is a deciduous, herbaceous thorny vine, dying to the ground after physiological maturity (Bourett, 1973). The stems are twining, growing to 20 m or more, with four often purplish wings (cross-shaped or square in cross-section) (Alexander and Coursey, 1969). Tubers formed in some cultivars are elongated, hairy with rough-textured surface which grow to as much as 15 cm long (Amusa *et al.*, 2003). The leaves are opposite, but often alternate at the base of the vine. The blades are heart-shaped, with rounded basal lobes, or arrowhead-shaped, with angular basal lobes; the five to seven arching, longitudinal veins are conspicuous (IITA, 2009). Leaf blades are as much as eight inches long and six inches broad; the petioles are often as long as the blades. Flowering is frequent and conspicuous and fruits usually produced are 3-winged capsules borne in pendent racemes (Putnam and Gaskalla, 2010).

#### **2.4 Economic importance and uses of yam**

Yams are second to cassava as the most important tropical root crop (in terms of production or consumption) (Dadzie, 2004). Yams are a staple crop in many parts of Africa and Southeast Asia (Amusa *et al.*, 2003). In the South Pacific, the yam is a significant food crop, accounting for over 20%, 8.1%, and 4.6% of the total dietary calorie intake in the Kingdom of Tonga, Solomon Islands, and Papua New Guinea, respectively (Simons, 1993). Besides their importance as food source, yams also play a significant role in the socio-cultural lives of some producing regions like the celebrated New Yam Festival in West Africa, a practice that has also extended to overseas where significant population of tribes from yam growing areas observe it (Narula *et al.*, 2007). In some parts of Southeastern Nigeria, the meals offered to gods and ancestors consist principally of mashed yam. Yams store relatively longer in comparison with other tropical fresh produce, and therefore stored yam represents stored wealth which can be sold all-year-round by the farmer or marketer.

In parts of Igbo land in Southeastern Nigeria, it is customary for the parents of a bride to offer her yams for planting as a resource to assist them in raising a family (Egesi *et al.*, 2007). The *Dioscorea* species have long been cultivated for their medicinal properties (sapogenin steroids, used in production of cortisone and synthetic hormones) in the following order of importance: *D. bulbifera*, *D. cayenensis*, *D. dumetorum*, *D. alata*, *D. trifida*, *D. laxiflora*, and *D. microbotrya* (Karnick, 1969; Pedralli *et al.*, 2002). The wide array of saponins and steroidal sapogenins from various cultivars provides the pharmaceutical industry with compounds for oral contraceptives and prevention of diseases such as malaria, yellow fever, skin rashes and dengue (Bhandari and Kawabata, 2004; Lebot, 2009). Wu *et al.* (2005) analyzed the consumption of *D. alata* by post-menopausal women and concluded that although the mechanisms are not yet fully understood, its consumption reduces the risk of breast cancer and cardiovascular disease. Nutritionists stress the importance of yams in treating anaemic patients given their wealth of nutrients, vitamins, and minerals (Okwu and Ndu, 2006). In Africa, it was found that the tuber is responsible for increased fertility in women who habitually consume it (Balbach and Boarim, 1993). Diosgenin can be extracted from *D. bulbifera* (Narula *et al.*, 2007) and can also be used as an anti-tumor agent (Gao *et al.*, 2007).

According to FAOSTAT (2005), an estimated 48.7 million tons of yam were produced worldwide in 2005, with Sub-Saharan Africa accounting for 97 per cent of this figure. In Nigeria alone, export earnings from yams hit N56 bn in 2008, up from N37 bn realized in 2007 and about N70 bn was realized from yam export in 2010. In 2008, the World production of yam was 51.4 million tons out of which Nigeria accounted for an average of 36.7 million tons, Ghana for 3.6 million tons and Cote d'Ivoire for 4.8 million tons (FAOSTAT, 2010). Farmers have constantly affirmed that yam cultivation is a veritable source of income. According to statistics, average daily

consumption per capita of yams is highest in Benin (364 kcal), Côte d'Ivoire (342 kcal), Ghana (296 kcal), and Nigeria (258 kcal) (IITA, 2009).

A typical analysis of the edible portion of the tubers is: water 65-73 per cent; protein 1.12-2.78 per cent; fat 0.03-0.27 per cent; carbohydrate 22-29 per cent; fiber 0.65-1.40 per cent; ash 0.67-2.06 per cent (IITA, 2000). The starch contains a high proportion of fairly large granules: sizes ranging from 5 to 50 microns have been reported. The gelatinization temperature ranges from 69Â° to 88Â° and the viscosity from 100 to 200 Brabender units. Unlike most other yam species, starch from *D. alata* has high gel strength. Starch from white-fleshed and purple-fleshed cultivars have similar typical composition averaging: moisture 13.6 per cent; protein 0.14 per cent; ash 0.22 per cent; amylose 21.1 per cent; reducing sugars 0.18 per cent; pH 7.1 (Tetteh and Saakwa, 1991; Bailey and Jeger, 1992). Ascorbic acid contents ranging from 4.9 to 8.2 mg/100 g of edible portion have been reported, while certain cultivars in the South Pacific have been found to contain 6 mg/100 g of carotene. Three anthocyanins have been isolated from *D. alata* var. *atropurpurea* and *rubella* and found to be cyanidin glycosides (Degras, 1993a).

Yams are a source of food, a source of income to access food, leads to reduction in imported food and thus adds to local food self-sufficiency (FAO, 2000). This is even more significant when the untapped potential of yams is considered. Jamaica grows and exports several varieties of yams but the most popular and lucrative is yellow yams (*D. cayenesis*) which displaced Negro yam (*D. rotundata*) as the main yam variety by the mid-1970s. Yellow yams currently account for close to 60 percent of total yam production and perhaps as much as 90 percent of all Jamaican yam exports. Jamaica is the world's leading yam exporter accounting for 16 percent of the global yam trade but has a monopoly of the yellow yam market (FAOSTAT, 2010). It serves a critical employment role with thousands of rural families making a living from yam cultivation.

## 2.5 World production and trade of yam

Most of the world production of yam is from Africa (about 96%) with Nigeria alone accounting for nearly 75% of the total world production; World annual production was estimated to be 25 million metric tons in 1974, and 24 million metric tons in 1992 (Moura-Costa *et al.*, 1993). In 1995, total world production increased from 32.7 million metric tons to 37.5 million metric tons in 2000 (IITA, 2009). Also, in the period, export quantity declined slightly while export income remained fairly steady (FAOSTAT, 2010). During the period 1975-90, total yam cultivated area increased by about 38.8% globally, while the total production increased by 45.8%. However, the importance of yam in the economy of the main producing areas appears to be declining due partly to competition with other crops like cassava in Nigeria and taro in the South Pacific (Opara, 1999). The major producing areas have also continued to experience high population growth rates. During the last four decades, the annual growth rate (%) of per capita production in the major yam zones in Africa has declined (Egesi *et al.*, 2007).

## 2.6 Yam production in Ghana

In Ghana, yam is grown on free draining, sandy and fertile soil after clearing the first fallow. Land is prepared in the form of mound or ridge or heap of 1 meter (3 ft. 3 inch) height or on the flat. The yams recommended for such soil conditions are the white yam or white guinea yam (*D. rotundata*) and water yam or yellow yam (*D. alata*). Planting is done by seed yam or cut setts from ware tubers (CCPGS, 2011). The tubers are subjected to treatment with wood ash or a fungicide (thiabendazole) 24 hours before planting to prevent damage to the tubers. The setts are planted at an interval of 15–20 cm (5.9–7.9 inch) with the cut surface facing up. Mulching is essential during October–November with dry grass or plant debris weighed down with balls of mud. Dosage of fertilizer application, as essential, is decided after chemical analysis of the soil samples. Manual weeding by hoeing is done three or four times depending on rate of weed growth (Dadzie, 2004). Yam is currently the fourth most important tuber-root crop in the world, after

potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz), and sweet potato (*Ipomoea batatas* L.). In 2008, the estimated world production of yams was 51.7 million tons, with Africa leading the production. West Africa is the most important area for yam production in the world.

Ghana is the second largest producer of yams in the world next to Nigeria (FAOSTAT, 2010). In Ghana, during 1997 and 1998, the crop was ranked second in importance (in terms of tonnage) after cassava and was the most important crop in terms of value (Dadzie, 2004). A survey by GTZ in the Northern Region of Ghana identified yams as the most important cash and food crop in that region, followed by groundnuts, cassava and maize. Also, the National Agricultural Research Strategy Plan (NARSP) identified yam, cassava and cocoyam as first priority commodities for research, with yam receiving the highest priority rating of all crops. However, there are a number of serious constraints to yam production. These include: pests and diseases, the low multiplication rate (and hence low availability of planting material), declining soil fertility and the high and expensive labour inputs required (Degras, 1993). Yams are almost entirely vegetatively propagated by planting pieces of tubers, or setts or whole small tubers. Each seed piece usually weighs 115 to 140 g. They can be planted 7 to 9 cm deep in 1.2 m rows with plants spaced 60 cm apart, or in hill plantings 1 m apart.

Traditionally in Ghana, farmers obtain their planting materials either from their own farms, or by buying the surplus from neighboring farmers. This means that the planting material is often of low quality, being infected with fungal pathogens, virus and/or nematodes and may be relatively expensive (Tetteh and Saakwa, 1991; Latunde-Dada et al., 2001). In order to ensure the survival and growth of the planting materials, fairly large pieces of seed yam (220 g) are used which adds to the cost and results in a very low multiplication rate. Inter-cropping is the most common practice, with the distance between yam plants determined by the number and types of the other

crops in the field. However, where sole cropping is done, rows are 1 m apart, with intra-row spacing of 50-100 cm (Dadzie, 2004).

## **2.7 Climatic, water and soil requirements of yam**

Yam is a plant of the sub-humid to humid tropics but also does well in semi-arid regions. Reported temperature range for growth is 14 - 40 °C with the optimum between 20-32 °C (Abang *et al.*, 2003). Since it takes 7-10 months to mature in the field, a well-distributed annual rainfall (or water supply) ranging between 700-8000 mm with the optimum between 1200-4000 mm is required for yam production (Akem and Asiedu, 1994; Egesi *et al.*, 2007). However, dry spells of up to 1 month during the growing season do not severely reduce yields. Yam is sensitive to aluminium toxicity in the soil. It thrives in deep, fertile, well drained, loose, loams and sandy-loam soils with low levels of salinity and responds well to manuring. In comparison with other tropical tuber crops, yam requires soil of high fertility (Dadzie, 2004). Heavy clays tend to be waterlogged and result in tuber rots and difficult harvesting. Gravel or rocky soils tend to hinder tuber penetration. Required soil pH range for growth is 4.8 - 8.5 with the optimum between 5.5 - 6.5 (IITA, 2009).

## **2.8 Husbandry practices/staking**

Plants are usually staked soon after emergence. However, unstaked cultivation which suppresses weeds better but gives lower yields is also practiced (CCPGS, 2011). Weeding is done 3-4 times during the season, using hand tools. In general, yam is the first crop in the rotation after fallow. Some kind of support is usually required for good production of yams. *D. rotundata* and *D. cayenensis* are sometimes grown without support, but the sacrifice in yield is so great that only dire shortages of labour or staking material should prevent staking. The staking systems are usually built of local materials such as sticks or branches, slim bamboos, or bamboo with side branches. Sometimes dead materials from previous crops such as corn, sorghum, or tobacco are

used, but these usually do not last long enough to give support through the growing season. The use of living plants is also practiced but not recommended because they compete with the yams and severely reduce yields (Otoo, 2001b). However, because the staking of yams is so costly, further attempts to develop a living-plant system especially, with legumes such as *Cajanus* and *Sesbaniay* is being advocated (Dadzie, 2004). One of the best staking system currently designed in Nigeria by the IITA (2009) and being replicated in Ghana involves twelve-foot teak poles strung with 12-gage galvanized wire 6 to 8 feet off the ground and tied to stakes driven into the ground. Two rows of yams are then planted, one on each side and sufficiently developed vines are passed onto the wire with support of strings.

## **2.9 Harvesting**

In many parts of West African yam zone, mature yams are harvested at the end of the rainy season or early part of the dry season, which coincides with the end of vegetative growth (Dadzie, 2004). Yams for long-term storage (for marketing or seed) are usually harvested during the harmattan period (Dec-Jan) in many parts of southeastern Nigeria when the crops have attained maximum growth and maturity. Harvesting occurs 7-10 months after planting. Hand tools are used to dig up the tubers. Although the tubers can be kept quite well, they are easily damaged during and after harvest, and therefore need careful handling to avoid bruises.

## **2.10 Field diseases of yam**

Yam diseases do not only cause severe yield losses but also lead to genetic erosion as well as restricting international germplasm movement and exchange (IITA, 2000). However, it has been estimated that an average of over 25% of the yield is lost annually to diseases and pests (Arene, 1987; Ezeh, 1998; FAO, 1998). Anthracnose disease of yam is often referred to by a number of names including yam necrosis, yam scorch, yam chlorosis and vine-browning and die-back. Yam anthracnose disease caused by *C. gloeosporioides* has a wide distribution and is becoming a

major worldwide biotic constraint to yam (*Dioscorea* species) production especially, in the humid and sub-humid tropics (Emehute *et al.*, 1998). Fournet *et al.* (1974) and Nwankiti and Arene (1976) also reported the destruction of the disease to *D. alata* species especially, in the Caribbeans and the yam zones of tropical Africa. On the same account, Nwankiti and Ene (1984) and Winch *et al.*, (1984) reported that *D. alata*, the most widely distributed species are particularly susceptible to the disease whiles other yam species are affected to a much lesser extent (Mignucci *et al.*, 1988).

Despite the minor effects of pests on yam cultivars in comparison to other crops, phytosanitary problems represent the main difficulties for producers. The virus [*Yam mosaic virus* (YMV)], anthracnose disease of yam caused by the fungus (*Glomerella cingulata*), nematodes (*Meloidogyne* spp., *Pratylenchus* spp. and *Scutellonema bradys*) are the main diseases that account for the highest field losses in susceptible cultivars (Onayemi, 1983; Abang *et al.*, 2003; Amusa *et al.*, 2003). A vine-browning and die-back disease stands out as the principal phytosanitary problem in several areas of yam cultivation and production (IPGRI/IITA, 1997). Nematodes, which interact with fungi and bacteria, attack the tubers in the field and continue to cause damage in post-harvest. Yam meloidogynoses are diseases caused by nematodes of the genus *Meloidogyne*, which show high incidence and severity in production, causing heavy losses in yam production and marketing (Abang *et al.*, 2002; Mignouna *et al.*, 2003b). Yam mosaic virus caused by an aphid-transmitted potyvirus is another important field disease affecting yam (Egesi *et al.*, 2007).

### **2.10.1 Foliar fungal diseases**

Anthracnose disease is one of the major foliar diseases affecting yam and has had a considerable impact on water yam production world-wide (Nwankiti and Arene, 1976; Simons, 1993). It is caused mostly by the fungus *C. gloeosporioides* (Nwankiti *et al.*, 1987). The disease attacks

several species of *Dioscorea*, particularly *D. alata*, *D. cayenensis*, *D. rotundata* and *D. trifida*. The symptoms observed in each host can be vein banding, curling, mottling, green-spotting and flecking (Mantell, 1980; IITA, 1993; Asiedu *et al.*, 2003). Concentric leaf spot disease of yam caused by *Sclerotium rolfsii* has also been reported in south western Nigeria (Amusa, 2000). Symptoms of the disease include circular leaf spots of varying sizes that form concentric rings. At maturity, the center of the leaf spots contains sclerotia of the fungus. The lesion may merge, together with the center eventually falling out due to necrosis (Mantell, 1980; IITA, 1993). At nurseries, the pathogen causes complete blight of sprouting yam with sclerotia being produced at the base of the infected yam leaves. Soil, plant debris and several weeds such as *Aclipha ciliata*, *Chromolaena odoranta*, *Euphobia heterophilla*, *Ipomoea triloba* and *Commelina erecta* can harbour the pathogen (Amusa, 2000). The effect of the disease on yield has not been investigated, but it is anticipated that under severe attack yield loss of more than 50% could be obtained (IITA, 2009).

Zonate leaf spot caused by *Curvulariae ragroistides* and *Pestalotia macro trichia* is occasionally encountered on *D. alata* fields (PANS, 1984; Amusa *et al.*, 1993). Also, foliar symptoms of nematode infections on yams are sporadically observed on the field. Early yellowing of leaf and termination of vine growths have been observed on *D. rotundata* infected with *M. incognita*, but infections rarely reduces total tuber yield (Adesiyun and Odihirin, 1978). Cercospora leaf spot caused by *Cercospora* spp. also affect yam species resulting in appreciable yield reduction (IITA, 2000).

### **2.11 Economic importance of anthracnose disease of *D. alata***

Anthracnose disease has had a dramatic impact on *D. alata* cultivation in regions such as the Caribbean where mono-cultures with a single popular susceptible variety are common (Orkwor and Asiedu, 1995). In this region, susceptibility to anthracnose disease has made it virtually

impossible to grow the popular *D. alata* cultivars Pacala and White Lisbon. The disease is considered the single most important factor responsible for the decline of water yam production in the Caribbean (Ano *et al.*, 2002; McDonald *et al.*, 1998). Nwankiti and Ene (1984) reported *D. alata* yield loss of about 80% in Nigeria as a result of attack by anthracnose disease. On-farm yield-loss studies in three agro-ecologies within the water yam production zone of West Africa showed that the impact of the disease on yield is highly dependent on time of disease onset and the prevalent environmental conditions (Green, 1994). *C. gloeosporioides* was recently found to be associated with deep-seated (systemic) infection of botanic seeds of water yam (Abang, 1997).

Yam breeders do report instances where it has proved impossible to obtain viable seeds from parental genotypes because of the high flower abortion and low fruit set associated with the disease (Orkwor and Asiedu, 1995). Although the exact role of infected seed in the epidemiology of water yam vine-browning and die-back is yet to be ascertained, it poses a serious problem in the international distribution and exchange of water yam (seed) germplasm since materials have to be certified disease-free. Furthermore, the disease is implicated in the erosion of genetic diversity in large-scale field collections of water yam germplasm (Orkwor and Asadu, 1997). *C. gloeosporioides* is also known to cause 'dead skin' disease in the Caribbean, in which severely affected tubers fail to sprout and become rapidly infected with other pests and pathogens (Green and Simons, 1994). The pathogen is believed to play a role in tuber bio-deterioration in Nigeria; however, 'dead skin' symptoms have not been reported.

Vine-browning and die-back disease occurs wherever water yam is grown but extreme temperatures in parts of northern Nigeria appear unfavorable for disease development (Green, 1994; Nwankiti and Ene, 1984). In areas where vine-browning and die-back is not currently considered a constraint, there is a risk of higher yield losses in the future, as the trend towards

shorter fallows increases and as new hybrids replace a wide spectrum of local landraces. The disease causes leaf necrosis and die-back of *D. alata* vines, leading to a reduction in the effective photosynthetic surface area of the crop with a concomitant reduction in ability of the yam tuber to store food reserves (Abang *et al.*, 2002). Green (1994) recorded losses in excess of 85% while Mignucci *et al.* (1988) reported losses exceeding 90%. Farmers in the Caribbean can no longer rely on the hitherto effective benzimidazole fungicides due to the development of fungicide-resistant *C. gloeosporioides* strains (Bayart and Pallas, 1994). The presence of several pathogenic fungi on the yam phylloplane led Amusa *et al.* (2003) to suggest that anthracnose disease is a 'disease complex', requiring the concerted action of a number of fungal pathogens for significant symptom development. In virulence studies under controlled environment conditions, however, Abang (1997) showed that a single slow-growing grey isolate of *C. gloeosporioides* is capable of causing 100% leaf abscission and premature death of up to 76% of inoculated plants. Earlier reports showed that the disease was severe only on *D. alata* but more recent investigations have revealed that the disease is prevalent and severe on most of the widely cultivated species of yam (Akem and Asiedu, 1994).

## **2.12 Prevalence, incidence and severity of yam anthracnose disease**

Although, yam anthracnose prevalence is usually confused with and at times used interchangeably with the disease incidence, prevalence refers to the number of geographical units (field, farms, districts, regions or other defined populations) where the disease has been visually observed or detected, divided by the total of the geographical units sampled and evaluated (Nutter *et al.*, 1991; Cooke *et al.*, 2006; Nutter *et al.*, 2006). The disease prevalence data is usually multiplied by 100 to obtain a percentage of the geographical units diseased. Hu *et al.* (1995) also stated that disease (yam anthracnose) prevalence can be obtained by sampling whole plant samples or plant parts from a population and using appropriate indexing methods to detect the presence of the pathogen. According to Nutter (1997), pathogen prevalence may provide quantitative information on the

presence of the pathogen (*Colletotrichum* spp.) in symptomatic plants. Generally, samples collected from a single geographical location are bulked and indexed to detect the presence of the pathogen. Disease incidence on the other hand, is the number or proportion of plant units that are visibly diseased as a result of a pathogen (*Colletotrichum* spp.) in relation to the total number of plant units sampled and assessed (Madden and Huges, 1995; González-Pérez *et al.*, 2011).

### **2.13 Symptoms of anthracnose disease of *D. alata***

Anthracnose disease attack all parts of *D. alata* plant at all stages of development (Egesi *et al.*, 2009). Infections concentrated or expressed on the shoot tips generally result in vine and/or tip die-back (Abang *et al.*, 2006). Other symptoms expressed generally, depend on the age of the yam plant, the environmental conditions and the species and/or cultivar grown (Ayodele *et al.*, n.d). Typically, when infection is initiated on the leaves, symptoms first appear on lower older leaves close to the soil surface as pinpoint lesions that are dark brown, with or without pale brown or yellow chlorotic halos (Winch *et al.*, 1984; Akem and Asiedu, 1994). The lesions then expand rapidly and may coalesce, encompassing the entire leaf and resulting in premature leaf abscission. Severe infection results in defoliation leaving naked, black and drying vines (Arene, 1987; Amusa, 2000; Prasath and Ponnuswami, 2007). The enlarged spots form yellow margins and the centre may fall out giving a ‘shot-hole’ effect.

Infected leaves sometimes fall off reducing the effective photosynthetic surface of the plant and early infection may result in premature death of the plant. Shoot tip and stem die-back frequently occur under such severe infections (Abang, 2003). In some cases, there is a superficial bronzing and blackening of mature leaves on surfaces exposed to the sun, a condition often referred to as scorching. According to Green and Simons (1994), the disease affects the leaves, petioles, stems and veins of the plant, causing leaf spots, leaf blotches, petiole blights, pre-mature abscission, dieback and eventual death of the entire plant. The disease usually has a dramatic effect on

infected plants, converting a field of initially healthy yam plants from ‘green’ to ‘black’ within a few weeks (Green and Simons, 1994).

#### **2.14 Etiology of anthracnose disease of *D. alata***

The pathogen causing anthracnose disease of *D. alata* was first described as *Gloeosporium pestis* Masee from yam in Fiji (Winch *et al.*, 1984). It was later reported from *D. alata* in India (Singh *et al.*, 1966) and subsequently classified as *C. gloeosporioides*. There have been conflicting reports on the actual cause of anthracnose disease of *D. alata*. Previous work by Amusa (1997) indicates that yam anthracnose is a disease complex and has been associated with the activities of *C. gloeosporioides*, *Curvularia pallescens*, *C. eragrostides*, *Pestalotia* spp. and *Rhizoctonia solani*. Many researchers have referred to the disease by the common names of: yam chlorosis or yam scorch in reference to the symptoms observed on leaves (Ayodele *et al.*, n.d). The confusion associated with naming of the disease is due to uncertainties of its actual cause and has necessitated studies to determine the pathogen of the disease.

Although other pathogens are involved in the anthracnose disease complex, several researchers have confirmed that *colletotrichum* spp. is the causal organism of the disease (Winch *et al.*, 1984; Singh *et al.*, 1966). Extensive studies by Nwankiti and Ene (1984), Abang *et al.* (2002) and Aduramigba-Modupe *et al.* (2010) have confirmed *C. gloeosporioides* as the primary cause of anthracnose disease of *D. alata*. Akem (1999) argued that, due to the predominance of *C. gloeosporioides* from sample isolations and the reproduction of typical symptoms of anthracnose disease with the fungus, *C. gloeosporioides* is the causal agent of the disease on *D. alata*. Studies by McDonald *et al.* (1998) and Ano *et al.* (2002) revealed that *C. gloeosporioides* is the main cause of anthracnose disease of *D. alata* in the Carribean. Earlier reports in Ghana revealed that *Colletotrichum* spp. were the causal agents of anthracnose of *D. alata* (Oduro, 2000; Offei *et al.*,

2008). In Nigeria, both *C. gloeosporioides* and *C. truncatum* have been reported as the cause of anthracnose disease of *D. alata* (Ayodele *et al.*, n.d).

### **2.15 *Colletotrichum* species**

The genus *Colletotrichum* includes a number of plant pathogens of major importance, causing diseases of a wide variety of woody and herbaceous plants (Abang *et al.*, 2006). It has a primarily tropical and subtropical distribution, although there are some high-profile species affecting temperate crops. Fruit production is especially affected; both high value crops in temperate markets such as strawberry, mango, citrus and avocado, and staple crops such as banana (Waller *et al.*, 2002). *Colletotrichum* species cause devastating disease of coffee berries in Africa, and seriously affect cereals including maize, sugar cane and sorghum. The genus was recently voted the eighth most important group of plant pathogenic fungi in the world, based on perceived scientific and economic importance (Yuan *et al.*, 2011). As plant pathogens, *Colletotrichum* species are primarily described as causing anthracnose disease although other maladies are also reported such as red rot of sugar cane, coffee berry disease, crown rot of strawberry and banana, and brown blotch of cowpea (Rojas *et al.*, 2010). Many species may be seed-borne and can survive well in soil by growing saprobially on dead plant fragments and may be spread through water-splash dispersal of conidia and air transmission of ascospores from the sexual morph (Nicholson and Moraes, 1980).

Infection occurs through an appressorium that develops from the germinating spore on the plant surface, followed by turgor driven penetration of the cuticle and in some cases, of epidermal cells by infective hyphae (Bailey and Jeger., 1992). Establishment within plant tissues is aided through the production of host-induced virulence effectors by the fungus (Kleeman *et al.*, 2012; O'Connell *et al.*, 2012). Nascent colonies in most cases then enter a biotrophic phase with

infected tissues remaining externally symptomless which may be short (O'Connell and Reed, 2012) or extended and presumably involve dormancy (Prusky and Plumbley, 1992). Then, the fungus enters a necrotrophic phase that results in significant death of plant cells and the emergence of pathogenic lesions. This delayed onset of disease symptoms may lead to significant post-harvest losses, with apparently healthy crops degenerating in storage (Prusky and Plumbley, 1992). The biotrophic life strategies adapted by *Colletotrichum* species may also contribute to their prominence as symptomless endophytes of living plant tissues (Lu *et al.*, 2004; Joshee *et al.*, 2009; Rojas *et al.*, 2010; Yuan *et al.*, 2011).

### **2.15.1 Existence of *Colletotrichum* spp.**

*Colletotrichum* species have been reported to exist as endophytes, epiphytes, saprobes, plant pathogens and even human pathogens (Sutton, 1992; TeBeest *et al.*, 1997; Cano *et al.*, 2004; Kumar and Hyde, 2004; Photita *et al.*, 2004, 2005; Promputtha *et al.*, 2007). *Colletotrichum* species that cause serious plant disease are also commonly isolated as endophytes from healthy plants and have been identified as saprobes on dead plant material (Photita *et al.*, 2001a; Kumar and Hyde, 2004; Liu *et al.*, 2007; Promputtha *et al.*, 2007; Damm *et al.*, 2009; Prihastuti *et al.*, 2009). In many cases, the same species have been recorded with several lifestyles although with the ambiguity of species identification, it is not always clear whether they are definitely the same species. Prihastuti *et al.* (2009) described *C. fructicola* and *C. siamense* from coffee berries, isolated as epiphytes, endophytes and pathogens and these species have since been shown to be widespread on several hosts (Yang *et al.*, 2009). *C. dematium* also occurs as an endophyte, pathogen and saprobe (Latunde-Dada *et al.*, 2001; Damm *et al.*, 2009).

*Colletotrichum* is one of the most economically important genera of fungi, causing anthracnose disease, affecting a wide host range, especially on tropical and subtropical crops as well as fruit trees (Sutton, 1992). Above ground plant parts can be affected by anthracnose diseases at all

stages on stems, leaves, flowers and fruits. An example of anthracnose disease caused by *Colletotrichum* spp. familiar to many is the blackening of tropical fruits (Tang *et al.*, 2005; Crouch and Beirn, 2009), especially bananas and mangoes in fruit bowls. The disease often takes two forms, resulting in spots on leaves or the blackening of fruits, usually post-harvest. In the case of persimmon, *Colletotrichum horii* can infect fruits, twigs, cause dieback and even tree death (Zhang, 2008; Hyde *et al.*, 2009). *Colletotrichum acutatum*, *C. capsici* and *C. gloeosporioides* have been reported causing anthracnose disease on chili fruits in Thailand (Than *et al.*, 2008b).

### **2.15.2 *Colletotrichum* as endophytes**

*Colletotrichum* species have been found as symptomless inhabitants (endophytes) in plant tissues (Liu *et al.*, 2007; Damm *et al.*, 2009; Prihastuti *et al.*, 2009). For example, putative *C. gloeosporioides* and *C. acutatum* strains were isolated from healthy leaves and pseudo stems of banana (*Musa acuminata*), ginger (*Alpinia malaccensis*), *Eupatorium thymifolia* and wild ginger (*Amomum siamense*) in Thailand and in low frequencies from rhizomes of wild ginger (Bussaban *et al.*, 2001; Photita *et al.*, 2005). Lu *et al.* (2004) isolated *C. gloeosporioides* and *C. boninense* as endophytes from leaves of 12 different tree species in the Iwokrama Forest Reserve, Guyana. Hyde and Soyong (2008) reported that *Colletotrichum* spp. that exist as endophytes can become primary saprobic decomposers or as latent infections of pathogens that cause disease under specific conditions. Simmonds (1941) showed in field experiments that *Gloeosporium musarum* (= *C. musae*) can remain latent within the skin of green banana fruits for almost five months, and develop anthracnose disease of fruit rot as the fruit ripens. The development of the fungus in the latent phase is restricted due to a poor capacity for secreting macerating enzymes and pectin esterase (Simmonds, 1963). Quiescent infections of strawberry transplants by *C. gloeosporioides* are common and play an important role as inoculum sources of anthracnose disease of crown rot (Raman and Louws, 2008).

### **2.15.3 *Colletotrichum gloeosporioides* and *C. truncatum***

#### **2.15.4.1 *Colletotrichum gloeosporioides***

*Colletotrichum gloeosporioides* (Penzig) Penzig and Sacc. is an asexual filamentous fungal pathogen belonging to the Phylum: Ascomycota and genus: *Colletotrichum* that causes anthracnose disease on various tropical, sub-tropical and temperate fruits across the world and also in Ghana (Akem and Asiedu, 1994). Mycelial colour ranges from gray to white, dark-brown to orange and rosy with colony elevation ranging from thick floccose aerial mycelia to flat-mat mycelia (IMI, 1995). *C. gloeosporioides* easily sporulate producing abundant, visible fruiting bodies with submerged or superficial acervuli (Ayodele *et al.*, n.d). Some strains sporulate superficially with scattered acervuli while others produce both superficial and submerged sporulation. Conidia produced are usually cylindrical with obtuse ends, hyaline, aseptate, uninucleate formed in setose, globose and/or saucer-shaped acervuli. Setae produced by *C. gloeosporioides* ranges from dark brown to black, highly profused to sparse, slightly curved to straight, 1-4 septate, swollen at the base and tapering towards the apex measuring 50-240 µm long (IMI, 1995; Onyeka *et al.*, 2005). Acervuli produced by *C. gloeosporioides* vary from round to irregular and globose to saucer-shaped. The *C. gloeosporioides* species complex has been studied by Weir *et al.* (2012) and it is a well-supported clade on a very long branch and shows few differences in the gene loci. However it is a diverse clade in terms of morphology and includes a number of important plant pathogens. Weir *et al.* (2012) recognized two sub-clades within the species complex based on an eight-locus analysis, both of which were supported by Bayesian posterior probability values of 1.

#### **2.15.3.2 *Colletotrichum truncatum***

*Colletotrichum truncatum* Andrus and Moore is a filamentous fungal pathogen belonging to the Phylum: Ascomycota and genus: *Colletotrichum* that causes anthracnose disease of on few selected crops such as yam (Ayodele *et al.*, n.d; IMI, 1995). It has array of mycelial colour from

dark brown to salmon. Conidia produced are strongly curved, fusiform with acute apex and obtuse base, hyaline, aseptate and uninucleate usually produced in acervuli ranging from sparse to saucer-shaped. Setae are frequently produced ranging from sparse to profuse, black to dark brown, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex measuring 40-190  $\mu\text{m}$  long (IMI, 1995). Acervuli formed ranges from setose to saucer-shaped.

### **2.16 Host relations and specificity in *Colletotrichum gloeosporioides* and *C. truncatum***

*Colletotrichum gloeosporioides* and *C. truncatum* are cosmopolitan with either multiple species occurring on a single host or a single species occurring on multiple hosts (Sander and Korsten, 2003). Fungus-host relationships are broad, imprecise and often overlapping (Freeman and Shabi, 2000). *Colletotrichum* species can infect many hosts and may adapt to new environments (Sanders and Korsten, 2003; Photita *et al.*, 2004), leading to serious cross infection problems in plant production. Earlier reports showed that the anthracnose disease caused by *C. gloeosporioides* and *C. truncatum* was severe only on *D. alata* but more recent investigations have revealed that the disease is prevalent and severe on most of the widely cultivated yam species (Akem and Asiedu, 1994). *C. gloeosporioides* has been reported as a pathogen on a wide range of host species throughout the world (Cannon *et al.*, 2008). Weeds such as *Spigella anthelmia*, *Calapogonium mucunoides* and *Commelina* sp. and several other non-yam hosts harbour populations of *C. gloeosporioides* that can be highly virulent on yam (Abang *et al.*, 2006). Citrus spp. harboured populations of *C. gloeosporioides* that were highly virulent on yam (Green, 1994) and isolates from lime (*Citrus aurantiifolia*) were highly virulent on *D. alata* (Simons, 1993).

## **2.17 Characterization of the strains of *Colletotrichum gloeosporioides* and *C. truncatum* isolated**

### **2.17.1 Polymerase chain reaction (PCR)**

PCR amplification is a rapid and sensitive method for detecting and characterizing members of pathogen groups. It can be used to confirm the results obtained from ELISA and electron microscopy. PCR was first used for the amplification of  $\beta$ -globin genomic sequences for diagnosis of sickle cell anemia (Saiki *et al.*, 1985). Since then, it has been employed extensively for the identification and differentiation of distinct strains of pathogens by amplification of sequences (Ward *et al.*, 1992; Ward and Shukla, 1991; Shukla and Ward, 1989).

PCR amplification requires Deoxynucleotide triphosphates (dNTPs) for the DNA extension, an optimized buffer containing magnesium, reverse and forward primers, which are complementary to the opposite strands of DNA and a thermostable DNA polymerase enzyme isolated from e.g. the thermophilic bacterial species, *Thermus aquaticus* (*Taq*) (Gelfand and White, 1990). Specific or degenerate primers are designed according to the available sequence data of viral genomic nucleic acids of the same group of viruses. Degenerate primers are a mixture of oligonucleotides varying in base sequence but with the same number of bases. Isolated DNA goes through temperature cycles. First DNA strands are disassociated at high temperature (94-96°C). The temperature is then lowered (50-55°C) to allow primers to anneal to the DNA strands. Finally, the DNA is subjected to the optimum extension temperature for the polymerase (usually 72°C). The three steps are repeated 30-45 times, allowing an exponential accumulation of DNA fragments. Amplified products are analyzed by agarose gel electrophoresis and DNA markers are included to allow the size of the PCR products to be estimated (Saiki *et al.*, 1985). PCR works directly for the amplification of DNA templates and badnaviruses (Ward *et al.*, 1992; Nie and Singh, 2000; Sharman *et al.*, 2000).

### 2.17.2 Diversity study in *Colletotrichum gloeosporioides* and *C. truncatum*

Traditionally, *Colletotrichum gloeosporioides* and *C. truncatum* have been identified and delimited on morphological characters; several features have been utilized by taxonomists including size and shape of conidia and appressoria; presence or absence of setae, sclerotia, acervuli and teleomorph state and cultural characters such as colony colour, growth rate and texture (Simmonds, 1965; Smith and Black, 1990; Sutton, 1992; Photita *et al.*, 2005; TeBeest *et al.*, 1997; Than *et al.*, 2008a-c; Thaug, 2008). These criteria alone are not always adequate for reliable differentiation among these two species due to variation in morphology and phenotype among species under environmental influences (Abang *et al.*, 2002).

To overcome the inadequacies of these traditional schemes, several kinds of molecular techniques have been used to study genetic diversity, for example, labelled probes to detect restriction fragment length polymorphism (RFLP) (Hegedeus and Khachatourians, 1993; Sreenivasaprasad *et al.*, 1996; Maurer *et al.*, 1997; Abang *et al.*, 2002; Peres *et al.*, 2002; Moriwaki *et al.*, 2002; Guerber *et al.*, 2003; Du *et al.*, 2005; Photita *et al.*, 2005; Whitelaw-Weckert *et al.*, 2007; Shenoy *et al.*, 2007b; Peres *et al.*, 2008; Than *et al.*, 2008a-c) and methods based on the polymerase chain reaction (PCR) such as the use of random amplified polymorphic DNA (RAPD) (Fegan *et al.*, 1993; Bidochka *et al.*, 1994; Piatti *et al.*, 1998; Freire *et al.*, 2001; Jensen *et al.*, 2001; Gaitan *et al.*, 2002), simple sequences repeat (SSR or microsatellites) analysis (Kretzner *et al.*, 2000; Enkerli *et al.*, 2001, 2005) and internal transcribed spacer (ITS)-rDNA sequence RFLP analysis (Rakotonirainy *et al.*, 1994; Buscot *et al.*, 1996; Coates *et al.*, 2002a).

Even though, they have different and complementary discriminatory power, RAPD and RFLP analyses have been very effective in detecting genetic diversity in several species (Couteaudier *et al.*, 1998; Glare and Inwood, 1998; Coates *et al.*, 2002b; Enkerli *et al.*, 2005). PCR technique combined with RAPD and RFLP analyses have been extensively used to determine the genetic

diversity of various entomopathogenic fungi (Welsh and McClelland, 1990; Williams *et al.*, 1990; Cobb and Clarkson, 1993; Bidochka *et al.*, 1994; Neuvéglise and Brygoo, 1994; Neuvéglise *et al.*, 1994; Bidochka *et al.*, 1995; Hodge *et al.*, 1995; Fungaro *et al.*, 1996; Maurer *et al.*, 1997) including *Metarhizium* (Fegan *et al.*, 1993; Leal *et al.*, 1994) and to relate genetic diversity to pathogenicity index (Bridge *et al.*, 1997) and to identify strains (Milner *et al.*, 2002).

The combined use of molecular diagnostic tools such as rapid amplified poly-dimorphism (RAPD) banding pattern (Thottappilly *et al.*, 1999), inter-spacers restriction fragment length polymorphism (ITS RFLP), polymerase chain reaction (PCR) using species-specific primers (Adaskaveg and Hartin, 1997) alongside the traditional morpho-cultural techniques is at present an appropriate and good approach for studying *Colletotrichum* species complexes (Cannon *et al.*, 2000; Cai *et al.*, 2009). This approach was employed in the characterization of the strains of *Colletotrichum gloeosporioides* and *C. truncatum* that existed in the Volta Region and the five other yam growing regions in Ghana – for accurate identification. Once a species is accurately named, it unlocks data that can be used for developing and implementing effective disease management strategies (Freeman *et al.*, 1993).

## **2.18 Screening of *D. alata* cultivars for resistance to anthracnose disease using tissue culture-derived whole-plant assay**

### **2.18.1 Methods of screening of *D. alata* cultivars**

*D. alata*, like any other crop, is often evaluated for disease incidence and severity in green house and the field using artificial or natural inoculation of the pathogen. Various *in-vitro* methods have been described for assessment of yam response to anthracnose disease based on the inoculation of *in-vitro* plantlets in glass tubes and purified mesophyll protoplasts (Moura-Costa *et al.*, 1993) and inoculation of detached leaves (Green *et al.*, 2000; Jacqua *et al.*, 2005). These methods are useful for pathogenicity tests and characterization of *C. gloeosporioides* isolates and could be used for

pre-screening of varietal resistance (Abang *et al.*, 2001); they are, nonetheless, unsuitable for analysis of the relationship between yam genotypes and pathogen isolates (Onyeka *et al.*, 2006) because they involve inoculation of plant parts in the artificial states. Abang *et al.* (2001) proposed a tissue culture-derived whole plant technique for assessing resistance to yam anthracnose disease. This method involves spore inoculation of potted tissue culture-derived whole-plants and disease evaluation and has the advantage of using whole-plants in a controlled environment. It therefore, provides a probable rapid technique for evaluating breeding populations and studying the relationship between yam genotypes and pathogen isolates. This technique was adapted in screening of popularly grown *D. alata* cultivars in Ghana in the present study.

Tissue culture-derived whole-plant method (Abang *et al.*, 2001) provides a rapid technique for assessment of yam accessions and segregating populations for resistance to anthracnose disease. Due to the long time needed for field evaluation and the need sometimes, to screen genotypes for resistance to distinct pathogen isolates (Onyeka *et al.*, 2006), breeding programs continue to rely on controlled environmental studies to achieve rapid progress towards development of resistant varieties. Besides, the high cost of conducting multiple-location trials coupled with the long growth cycle of water yam (Otoo, 2001b) and the highly spatial and temporal variations among *C. gloeosporioides* isolates limits field evaluation and often makes it unproductive (Green *et al.*, 2000).

### **2.18.2 Disease assessment**

Disease assessment based on percentage whole plant area (WPA) scoring method proposed for field assessment of yam anthracnose disease (Simons and Green, 1994) and spray inoculation method (Onyeka *et al.*, 2006) enhance the efficiency of the tissue culture-derived whole-plant technique. The critical stage for optimal single disease assessment of tissue culture-derived whole-plant is seven days after inoculation (7 DAI) (Onyeka *et al.*, 2005b). Nevertheless, the use

of more than one assessment is a requirement to satisfactorily compare different yam genotypes. Thus, the use of AUDPC derived from assessment before and after the critical stage is the most suitable for quantitative resistance assessment (Onyeka *et al.*, 2005a). This study then encompassed the assessment of area under the disease progress curve (AUDPC) for a defined period of crop growth, rather than the critical point model approach (Jeger and Viljanen-Rollinson, 2001).

### **2.18.3 Methods of *in-vitro* propagation of *D. alata***

There are several methods of *in-vitro* propagation of *D. alata* (Moura-Costa *et al.*, 1993; Abang *et al.*, 2001). However, the most widely used methods are the plant tissue and meristem cultures.

#### **2.18.3.1 Plant tissue culture**

The most important economic use of plant tissue culture to date is probably for the rapid propagation of plants, which facilitates the interchange of plant breeding material. *In-vitro* culture in combination with other techniques allows the elimination of viruses and other diseases from valuable clones (Mantell *et al.*, 1978). Germplasm conservation *in-vitro* is also important for the preservation of the genetic resources. Micro-propagation is the *in-vitro* asexual reproduction of plant materials from very small pieces of plants in an artificial medium under aseptic conditions (Hartmann and Kester, 1975c). Tissue culture allows the propagation of a wide variety of plant materials such as foliage plants, woody ornamentals, vegetables, fruits and horticulture. Axillary shoot production is the most common form of propagation used by commercial propagators. The indirect formation of shoots can also be made from callus. However, callus is genetically unstable and new plants may not be true to type (Mantell *et al.*, 1978). The production of *in-vitro* culture does not necessarily free plants from viruses. For safe germplasm movement, the plant materials need to be tested and only that material found virus-free used (Walkey, 1991).

### **2.18.3.2 Meristem cultures**

Morel and Martin (1952) were the first to demonstrate that virus-free plants can be recovered from infected plants through meristem culture. Plant virus particles are generally found in the conducting systems of plants and are rarely present in actively dividing meristematic cells (Sahin *et al.*, 2003). Evans *et al.* (1983) demonstrated that the shoot and root apices of virus-infected plants are frequently devoid of viral particles or contain very low virus concentrations. Meristem-tip culture is the most important and effective method in comparison with plant tissue culture since meristem-tips grow more quickly into plantlets than cultured tissues from other sources. In addition to that, the regenerated plantlets usually retain the genetic characteristics of the parent plants (Mantell *et al.*, 1978). Meristem-tip culture was found to be efficient for the eradication of viruses and fungi in garlic (Verbeek *et al.*, 1995), in sweet potato (Frison and Ng, 1981) and also in *D. trifida* (Saleil *et al.*, 1990). This method was used to raise the *D. alata* cultivars used in the screening trial of the current study.

## **2.19 Development of IPM strategy for management of yam anthracnose disease**

Fair knowledge of the stages of yam anthracnose disease development and its spread (i.e. epidemiology) aid in the disease management (Onyeka *et al.*, 2006). Hence, extensive reviews of these areas were done in order to effectively devise appropriate strategy to manage the disease.

### **2.19.1 Stages of yam anthracnose disease development and its cycle**

*C. gloeosporioides* was previously thought to survive in soil but Ekefan *et al.* (2000) showed that survival in soil is unlikely. Microbial antagonism appears to play a significant role in the apparent non-survival of *C. gloeosporioides* in soil. The pathogen over-seasons in diseased stems, leaves and tubers as spores. The ascospores produced by surviving mycelium produce the primary inoculum. Conidia can spread over relatively short distances by rain splash or overhead irrigation. Ascospores are airborne and important in long distance dispersal (Galvan *et al.* 1997; Ebenebe,

1980). Important source of *C. gloeosporioides* inoculum is infested crop debris (Green, 1994) hence older leaves closer to the soil are first infested by the pathogen or young leaves and vines that have reached the tops of supporting poles and drops near to the soil. When the spores come in contact with the leaves and vines, it penetrates through natural openings such as stomata (Nwankiti and Okpala, 1981) and wounds, however, wounding do not increase symptom severity on *D. alata* (Kolattukudy *et al.*, 2000; Abang *et al.*, 2001).

Mature leaves with well-developed cuticle are known to be considerably more resistant to attack by *C. gloeosporioides* than are younger leaves (Sweetmore *et al.*, 1994; Abang, 1997). The stages of anthracnose disease development are enhanced by optimum temperature for growth and sporulation of the pathogen which is known to vary between 26 °C and 32 °C. Spore viability and germination are extremely sensitive to atmospheric humidity; such that, at 99% relative humidity (RH), germination was half that at 100% RH and was found to be negligible below 97% RH (Wharton, 1994). Lesion formation is, therefore, dependent on periods of 100% RH or leaf wetness of sufficient duration to allow for spore germination and host penetration.

### **2.19.2 Epidemiology of yam anthracnose**

Studies investigating the epidemiology of yam anthracnose highlighted three major sources of infection: infected planting material (tubers) (Simons and Green, 1994); alternative hosts (Simons, 1993; Green, 1994); plant debris (Green, 1994). Infected soil was however, discounted as a source of inoculums (Ekefan *et al.*, 2000; Arene, 1980). However, the relative importance of these sources of inoculum varies depending on geographical location. Survival of the pathogen from one season to the next may occur, but is unlikely to be important where growers practice crop rotation and plough-in crop debris. The fungus, *C. gloeosporioides*, infects many crops and weeds and is likely that spores from these plants also affect the yam crop (Morse *et al.*, 2000). If tuber rots occur in the Pacific as they do in the Caribbean, then infected planting material is likely

to be the most important way that new crops become diseased (Osai, 1993). Small immature tubers, derived from early shoot death, may be a major source of infection of the shoots as they develop (Amusa and Ayinla, 1997). Once infection is established in the crop, development of management measures against the disease depends on rainfall and host variety.

Severe outbreaks develop on susceptible varieties following rainstorms or cyclones (Amusa and Ayinla, 1997). Spores are formed in large numbers on the leaf spots and are splashed in rain and or carried by dripping dew to adjacent and lower leaves and stems (Akinnusi *et al.*, 1987; Ogali *et al.*, 1991). Abang (2003) obtained different strains of *C. gloeosporioides* (FGS and SGG) from dissected botanic seeds that were surface sterilized and plated on culture media but the incidence of infection in seed lots and the efficiency of *C. gloeosporioides* transmission through seed remains to be established. The importance of infected tubers as a source of inoculum was established in epidemiological studies, which found a correlation between infected tubers sown and the amount of foliar disease subsequently observed. However, long periods of rain favour epidemics of the disease because conidia are spread by rain splash (Galvan *et al.* 1997; Ebenebe, 1980; Schwartz and Mohan, 1995).

## **2.20 Management of anthracnose disease of *D. alata* in the field**

Management of anthracnose disease of *D. alata* in the field has always been challenging although management measures which include chemical and cultural methods give appreciable results (Akem and Asiedu, 1994). Measures for the management of yam anthracnose disease in the field include cultural practices, use of chemicals, and resistant varieties as planting materials that provide partial control by reducing the rate of disease development (Nwakiti and Arene, 1976; Ogundana, 1981; Nwakiti, 1982; Walther *et al.*, 1999). Chemical sprays (Benlate) reduced anthracnose disease of *D. alata* to some extent in the Caribbean however, regular application of chemicals led to the development of fungicide resistance besides being inappropriate for resource-

poor farmers (Green and Simons, 1994; Bayart and Pallas, 1994). Use of host plant resistance is one of the sustainable management strategies for controlling yam anthracnose.

The development of yam populations with multiple resistances to the disease presents a cheaper and more effective approach to its control, necessitating the screening of local and introduced germplasm for reaction to anthracnose disease in different agro-ecological zones (Nwakiti and Ene, 1984; Nwakiti *et al.*, 1987). The use of anthracnose-resistant cultivars bred and released by the International Institute of Tropical Agriculture (IITA) has been advocated (IITA, 1993). However, it is reported that *D. alata* cultivars which were initially resistant to anthracnose disease later succumbed to the disease due to the emergence of different strains of *C. gloeosporioides* in the Caribbean (Green and Simons, 1994; Ano *et al.*, 2005). It is, therefore, argued that, integrated disease management strategy which combines cultural methods (the use of crop rotation, fallowing, planting of healthy materials, staking, good farm sanitation and the destruction of infected crop cultivars), use of host resistant cultivars and chemical sprays (effective fungicides) proves most appropriate in managing anthracnose disease on the field (IITA, 2000; Onyeka *et al.*, 2005a; Offei *et al.*, 2008). The study, then, used the integrated disease management approach in managing anthracnose disease of *D. alata* established on the field.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Socio-economic background of the two important *D. alata* growing districts in the Volta region of Ghana

Nkwanta-North and Krachi-West are the two major districts in the Volta Region of Ghana where *D. alata* is commercially produced (Dadzie, 2004). These two districts were the areas where the study was specifically conducted.

##### 3.1.1 Nkwanta-North District

The Nkwanta-North (red arrowed) (Fig. 3.1) district is one of the 18 districts in the Volta region and located between Latitude 7° 30' North and 8° 45' North and Longitude 0° 10' West and 0° 45' East. The district capital, Kpassa is located 270 km to the north of Ho (the Regional Capital) (Anonymous, 2006). It has a surface area of approximately 11,510 km<sup>2</sup> thus making it one of the smallest districts in the region. The area experiences wet and dry seasons with the dry season occurring between November and March. Mean annual rainfall ranges from 922 mm to 1,874 mm and temperatures between 11 °C and 39 °C (Anonymous, 2006). The district lies in the transitional vegetation zone and is covered by Sanannah woodland and grassland with occasional pockets of semi-deciduous forest. The soil in the district is mainly of the ferric acrisols and dystic fluvisols type. The ferric acrisols covers about 70% of the entire area and supports the cultivation of a wide variety of crops including root crops (especially, yam), cereals, legumes, and oil palm. The structure of the local economy is skewed towards agriculture, which employs about 70% of the working population (MoFA-SRID, 2010). Unlike in other parts of the country, where farming is left in the hands of the elderly, people of all ages in the district are involved in farming due to the high returns derived from yam production. The district is one of the leading producers of yam in the country. About 80% of the working population is involved in its production as either farm owners, tenants or farm labourers.

### 3.1.2 Krachi-West District

Krachi-West district (black arrowed) (Fig. 3.1) lies in between the northern part of the central uplands of the Volta Region dominated by highlands ranging from 850 m to 1000 m above sea level (Anonymous, 2006). However, the northern part is flat with slopes rising from 85 m to 300 m above sea level. The entire district forms part of the Voltaian Basin and the district capital is Kete-Krachi. The population distribution in the district shows that 51.7 percent are males while 48.3 percent are a female which is different from the national distribution where there are more females (50.68) than males (49.32 percent) (Ghana Statistical Service, 2010). The district covers an area of 4169 sq. km out of which 37% is covered by water. The northern part of the district is part of the northwest savannah zone in the North and the forest zone in the south. About 80% of the district is covered with savannah grassland. However, forests and woodlands can be found along the lake, streams, rivers, foothills and the southern portion of the Oti zones. The major soils in the District include the Techiman Association (mainly sandy soils) in the north, the Kpelesawgu association (sandy clay soils) in the mid-portions and southern-most tip, the Dormabin-Dentesso association (silty sand) in the south and the Ejura-Amantim association (sandy loam) in the west. The district has about 70% of the population actively engaged in agricultural related ventures (MoFA-SRID, 2010). The crops cultivated include yam, cassava, cowpea, maize and rice. The district is one of the leading producers of yam in the country and, therefore, faces the problem of glut in good harvests (Dadzie, 2004). Some farmers rear animals such as goats, sheep, cattle and poultry.

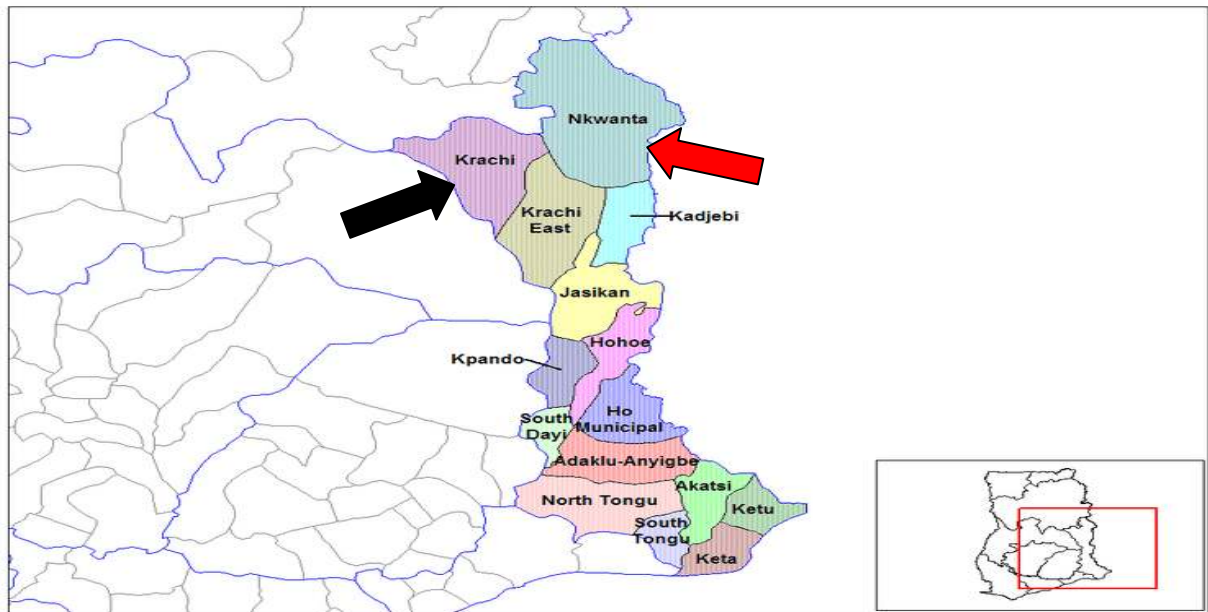


Fig. 3.1: Map of Volta Region indicating the study area. Nkwanta-North (red arrowed) and Krachi-West (black arrowed). Source: Anonymous (2006).

## 3.2 Assessment of farmers' perception on importance and control of a vine-browning and die-back disease of *D. alata* in the Volta region

### 3.2.1 Selection of experimental sites within the two districts for the disease survey

Two districts (Nkwanta-North and Krachi-West) where anthracnose disease of *D. alata* had been reported (Atsu and Akator, Personal Communication, 2012) were selected and three communities were randomly selected in each district for the study. The selection was done stepwisely by first obtaining a list of all *D. alata* producers from the Agricultural Extension Agents (AEAs) working in the two districts. Initial focus group discussions were then held with the AEAs of each district to determine the communities in their respective districts where the disease was most predominant. Fifteen *D. alata* farmers per community with disease hot-spots were randomly selected from the lists of *D. alata* farmers provided by the AEAs. Experimental sites and number of farmers selected for the survey are presented in Table 3.1.

Table 3.1: Experimental sites and number of farmers interviewed per site.

| <b>Districts</b> | <b>Communities</b> | <b>Number of farmers interviewed</b> |
|------------------|--------------------|--------------------------------------|
| Nkwanta-North    | Kpasa-Tindani      | 23                                   |
|                  | Kpasa-Abunyanya    | 18                                   |
|                  | Bajimpoa-Akura     | 19                                   |
| Krachi-West      | Gyidiasu           | 17                                   |
|                  | Osramae            | 23                                   |
|                  | Ohiamankyene       | 20                                   |

### **3.2.2 Survey to assess farmers' knowledge, perception, experiences and economic importance of a vine-browning and die-back disease of *D. alata* in the Volta Region**

A field survey was conducted between February and June, 2012 to obtain baseline data on farmers' knowledge, perception, experiences and economic importance of anthracnose disease of *D. alata* in the Nkwanta-North and Krachi-West districts of Volta Region of Ghana. One hundred and twenty *D. alata* farmers (from both smallholders with  $\leq 1$  ha of *D. alata* and plantation growers with  $\geq 10$  ha of *D. alata*) were randomly chosen from the experimental sites in the two districts. Pre-tested semi-structured questionnaires designed were read and interpreted to the farmers and their responses documented. The survey was aimed at establishing farmers' knowledge, perception and experiences concerning prevalence, spread, control and economic importance of the disease especially its impact on the livelihood of the farmers. The data collected using the questionnaire included the demographical data of the farmers; knowledge, perception and experience of farmers concerning cause of the disease, spread and control and the socio-economic impact of the disease including the livelihood of farmers (Appendix 1). All data collected through the questionnaires were analyzed using Statistical Package for Social Sciences (SPSS) version 18 (SPSS Inc., 2009).

### 3.2.3 Symptoms description of vine-browning and die-back disease of water yam and samples collection

Symptoms of a vine-browning and die-back disease consistently observed on *D. alata* plants in all the farms visited in the Nkwanta-North and Krachi-West districts of the Volta Region were described by the stages of the diseases. This was done by examining diseased leaves and vines of selected *D. alata* cultivars [using systematic sampling technique with ‘W-shaped’ sampling path (Lin *et al.*, 1979)] and symptoms compared with the description and/or pictures provided by Egesi *et al.* (2007); Abang *et al.* (2006); Akem and Asiedu (1994); Ayodele *et al.* (n.d.). During the field survey, other *D. alata* diseases such as leaf curl, witches broom and leaf spots were observed but not considered important in the study. Photographic records were taken on diseased *D. alata* cultivars, agronomic practices and mode of storage of planting materials with DSC–S750 Sony Digital Camera.

Samples (leaves and vines) of *D. alata* cultivars exhibiting a vine-browning and die-back disease symptom together with healthy samples were collected, sealed in sterile transparent latex bags – each of size 15 cm x 20 cm, well-labelled, packaged in an ice chest container and transported to the Plant Pathology laboratory of the Department of Crop Science, University of Ghana – Legon. The samples were kept in a Lec laboratory refrigerator model BK 700 at approximately 4 °C and used for the isolation and identification of the causal organism. Besides, healthy matured tubers of nine *D. alata* cultivars grown in the Volta region (Table 3.2) were collected and transported along with the diseased samples. In addition, three *D. alata* cultivars were collected from the Plant Genetic Resources Research Institute (PGGRI) – Bunso, Eastern region (Table 3.2). These cultivars were planted in both the Experimental Garden of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission (GAEC), Accra and Sinna’s Garden of the Department of Crop Science, University of Ghana – for nodal

cuttings to be used in the tissue culture experiment, personal observation of their growth and yield characters (Table 3.2), germplasm maintenance and for subsequent studies.

Table 3.2: General characteristics of the 12 *D. alata* cultivars used in the study.

| <b>Cultivar code</b> | <b>Local name/ source no.</b> | <b>Characteristics</b>                                                            | <b>Original Source</b> |
|----------------------|-------------------------------|-----------------------------------------------------------------------------------|------------------------|
| TDa007               | Niboltutu                     | Multiple tuberling, low yielding and oval-cylindrical dotted tubers               | Volta region           |
| TDa008               | TDa008-12                     | Oval-cylindrical tuber shape, high yielding                                       | PGRRI-Bunso            |
| TDa010               | Sum ne hyen                   | Irregular tuber shape, pinkish-white tubers, high yielding, purple vines          | Volta region           |
| TDa013               | TDa013-12                     | Irregular tuber shape, high yielding                                              | PGRRI-Bunso            |
| TDa014               | Chachribon                    | Cylindrical tuber shape, high yielding, light-purple vines with less-thick leaves | Volta region           |
| TDa015               | TDa015-12                     | Irregular tuber shape, high yielding                                              | PGRRI-Bunso            |
| TDa018               | Akomea                        | Cylindrical tuber shape, moderate yielding                                        | Volta region           |
| TDa019               | Matches (short tubered)       | Multiple tuberling, oval tuber shape, thin-leaves                                 | Volta region           |
| TDa021               | Matches (long tubered)        | Cylindrical tuber shape, multiple tuberling                                       | Volta region           |
| TDa023               | Akaba (thorn-less)            | Oval-cylindrical tuber shape, high yielding, thick-leaves                         | Volta region           |
| TDa025               | Poka                          | Cylindrical tuber shape, low yielding                                             | Volta region           |
| TDa027               | Akaba (thorned vine)          | Oval-cylindrical tuber shape, high yielding, thick-leaves.                        | Volta region           |

#### **3.2.4 Determination of incidence and severity of a vine-browning and die-back disease of *D. alata* in the Nkwanta-North and Krachi-West districts of the Volta Region**

The incidence and severity of a vine-browning and die-back disease of *D. alata* in the two districts were determined to assess the extent of spread in farms and to effect appropriate management of the disease. In each district, a total of three communities and fifteen *D. alata* farms per community were randomly selected and in every farm, a minimum of 20 and maximum of 50 plants (depending on farm size) were selected (using systematic sampling technique with ‘W-shaped’ sampling path (Lin *et al.*, 1979) and assessed for disease incidence and severity. In order to get rid of selection bias, every eighth *D. alata* plant was selected, assessed and scored for

both incidence and severity (IMI, 1995). Severity of a vine-browning and die-back disease of *D. alata* in the six communities of the two districts were determined using a disease assessment key with a subjective score-scale of 0-5 (modified after Akem and Asiedu, 1994; Prasath and Ponnuswami, 2007) (Table 3.3). Percentage whole-plant area scoring method (Simons and Green, 1994; Abang *et al.*, 2006) was employed in the assessment of the selected plants. The proportion of diseased tissues' (leaves and vines) area of each selected plant (i.e. percent whole-plant lesion area) against non-diseased tissues' area was scored on a scale of 0-5. The scores were then converted to disease severity index (Kim *et al.*, 2000) as:

$$\text{Disease severity index (I)} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Total number of observations} \times \text{maximum disease score}}$$

Disease incidence was determined using +/- scoring method (Abang *et al.*, 2006) whereby (+) represented presence of the disease whereas (-) represented absence of the disease. The number of both diseased and healthy inspected plants in each farm was counted respectively and the incidence value calculated by the formulae:







$$\text{Disease incidence (Farm)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected per farm}} \times 100\%$$

$$\text{Disease incidence (Community)} = \frac{\sum \text{Disease incidence in each farm} \times \text{Total number of farms inspected}}{\text{Total number of farms inspected}} \times 100\%$$

$$\text{Disease incidence (District)} = \frac{\sum \text{Disease incidence in each Community} \times \text{Total number of Communities inspected}}{\text{Total number of Communities inspected}} \times 100\%$$

The data obtained from the two districts were then compared using student *t-test* and GenStat Statistical Software version 9.0 was used to perform the analysis. Due to the importance and greater economic impacts of the anthracnose disease on the livelihoods of farmers in the Nkwanta-North and Krachi-West in the Volta Region, there was the need therefore, to confirm the identity of the pathogens causing the disease in order to devise appropriate IPM strategy to manage it.

Table 3.3: Disease assessment key for severity of a vine-browning and die-back disease of *D. alata*

| Index | Qualitative Rating                                                                               | Pictorial Rating                                                                      |
|-------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| 0.0   | No symptoms on leaves and vines                                                                  |    |
| 1.0   | 1 – 10 per cent of leaves and vines showing disease symptoms                                     |    |
| 2.0   | 11 – 25 per cent of leaves showing disease symptoms with browning of vines                       |   |
| 3.0   | 26 – 40 per cent of leaves showing disease symptoms with browning of vines and some tip die-back |  |
| 4.0   | 41 -70 per cent of leaves showing disease symptoms with heavy browning of vines and tip die-back |  |
| 5.0   | 71 – 100 per cent of leaves defoliated and whole-plant dying with naked black drying             |  |

**Source:** Modified after Akem and Asiedu (1994) and Prasath and Ponnuswami (2007).

### **3.3 Isolation and identification of the causal agent(s) of a vine-browning and die-back disease of *D. alata* in the Volta region**

#### **3.3.1 Pathogen Isolation**

Isolation of pathogens associated with the disease was first done on water agar (WA) from Agar agar (OXOID) and then sub-cultured onto potato dextrose agar (PDA). The WA was prepared by dissolving 15 g of Agar powder in 250 ml of sterilized distilled water (SDW) in a 1 L conical flask. The flask was shaken vigorously to get a uniform mixture and then topped to the 500 ml mark. The flask was plugged with cotton wool and covered with Aluminium foil and autoclaved at 121 °C for 15 minutes at a pressure of 15 psi. On the other hand, to prepare 500 ml PDA, 19.5 g of the PDA powder (OXOID) was dissolved in 250 ml of SDW in a 1 L conical flask. The flask was shaken vigorously to get a uniform mixture and then topped to the 500 ml mark. The flask was plugged with cotton wool and covered with Aluminium foil and autoclaved at 121 °C for 15 minutes at a pressure of 15 psi.

The infected tissues (leaves and vines) of *D. alata* showing typical symptoms of anthracnose disease were selected and washed thoroughly in several changes of SDW and blotted with sterile cotton wool. Portions of advancing necrotic margins on both leaves and vines were then cut out into small bits (measuring about 2 mm) and surface sterilized in 1% sodium hypochlorite solution for one minute. Sterilized tissues were severally rinsed in double sterile distilled water to remove the traces of sodium hypochlorite and blotted with sterile cotton wool. Blotted tissues were transferred into Petri plates containing WA under aseptic conditions of standard tissue isolation (Rangaswami, 1958) and incubated at 26±2 °C in the laboratory for seven days under 12-h artificial light/darkness cycle. Healthy *D. alata* tissues (leaves and vines) were used as controls. Incubated plates were examined macroscopically after 72 hours for pathogen growth (Thangamani *et al.*, 2011). Where there signs of pathogen growth on five-day old WA plates,

were sub-culturing was done by transferring 2 mm diameter mycelial plugs (taken from the advancing disease margins) onto freshly prepared PDA plates and incubated at  $26\pm 2$  °C for 7-10 days. Mono-conidial cultures were then prepared for each pure isolate after identification and maintained on both PDA plates and slants at 4°C in a refrigerator for later use.

### **3.3.2 Identification of micro-organisms associated with anthracnose disease of *D. alata***

Isolated pathogens from the diseased *D. alata* samples were identified using morphological, cultural and molecular methods and confirmed with pathogenecity or infectivity test.

#### **3.3.2.1 Identification of isolates using culture characteristics**

Cultural characteristics such as conidial masses, colony growth and colour were observed on PDA at  $26\pm 2$  °C; 10 replicates were prepared for each isolate. The mean colony growth for each isolate from 48 to 168 hours (i.e. 2 – 7 days) was calculated. The colour of each colony and conidial masses were also recorded on the seventh day. The description and naming of the *Colletotrichum* species was done according to Chaube and Pundhir (2005); IMI (1995); Barnett and Hunter (1998); Mordue (1971); Sutton and Waterston (1970); Thom and Raper (1945).

#### **3.3.2.2 Identification of isolates using morphological characteristics**

In the morphological identification, the shapes and sizes of conidia, setae and acervuli of each isolate were examined under a light microscope after incubation at  $26\pm 2$  °C for seven days on PDA. The mean size (length and width) of conidia per isolate was measured with the aid of electron scanning microscope.

#### **3.3.2.3 Genome analysis of isolates**

Molecular techniques such as polymerase chain reaction (PCR) with Oligonucleotide and species-specific primers (Adaskaveg and Hartin, 1997) were used to confirm the identity of isolates and discriminate pathogens (*C. gloeosporioides* from *C. truncatum*) involved in the disease.

### **3.3.2.3.1 Culturing of isolates for DNA extraction**

Fungal isolates obtained from *D. alata* cultivars in the different geographic districts and maintained in the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana – Legon were used for the analysis. Mono-spore cultures of each fungal isolate were obtained by plating multi-spore cultures stored in the refrigerator onto Petri plates containing freshly prepared PDA and incubating the plates at  $26 \pm 2$  °C for 7 days and then cultivating single spore colonies (from the 7-day old culture) on Sabouraud media: containing ( $\text{g L}^{-1}$ ) Peptone, 10; Dextrose, 40; agar, 20; supplemented with 1% (w/v) of yeast extract (Difco, France) to produce mycelium for DNA extraction.

### **3.3.2.3.2 DNA extraction**

A modified cetyltrimethylammonium bromide (CTAB) method (McGarvey and Kaper, 1991) was used for DNA extraction. Aerial mycelia-conidia were scraped off 7-day old cultures (about 50 mg) from each isolate and ground in liquid nitrogen to a fine powder. Samples were thawed in 2 mL homogenization buffer (0.1 M Tris-HCl, 1.4 M NaCl, 20 mM EDTA pH 8.0) and incubated at 37 °C for 15 min. Extraction buffer (1 mL) (7% CTAB, 1% polyvinylpyrrolidone, 0.1 M Tris-HCl, 1.4 M NaCl, 20 mM EDTA pH 8.0), 300  $\mu\text{L}$  20% sodium dodecyl sulphate and 0.5% mercaptoethanol (v/v) were added and mixed by vortexing. After incubation at 65 °C for 1 hour, the homogenate was extracted twice with an equal volume of phenol/chloroform/ isoamylalcohol (25:24:1, v/v/v). The aqueous phase was then separated by centrifugation and incubated with RNase A (100 mg  $\text{mL}^{-1}$ ) and proteinase K (10 mg  $\text{mL}^{-1}$ ) for 15 min at 37 °C. After a final chloroform/isoamylalcohol (24: 1, v/v) extraction, DNA was precipitated by adding 0.6 vol isopropanol and incubating on ice for 30 min. After centrifugation (30 min at 10 000 revs/min) to collect DNA, nucleic acid was further purified and re-precipitated by adding 2.5 vol ice-cold 70% ethanol and 0.5 vol 7.5 M ammonium acetate pH 7.7, standing at 23 °C for 30 min and then centrifuging for 20 min at 10 000 revs/min. The final pellet was washed twice in 70% ethanol, air-

dried and re-suspended in 100  $\mu$ L Tris-EDTA (TE) (10 mM Tris-Cl/1 mM EDTA) buffer pH 7.4 (Sahin *et al.*, 2003).

#### **3.3.2.3.3 PCR amplification using Oligonucleotide and species-specific primers**

Oligonucleotide primer pair, ITS1 (5'-TCCGTAGGTGAACCTCCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with rDNA genes flanking the entire ITS1-5.8S-ITS4 region (about 500-600 bp) was used for ITS PCR-amplification (White *et al.*, 1990). A species specific primer pair, ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and CgInt (5'-GGCCTCCCGCCTCCGGGCGG-3') (Mills *et al.*, 1992) was used in the species-specific PCR amplification. For each of the twenty isolates, PCR of the extracted DNA was performed in a BIO-RAD PTC-220 thermocycler (Dyad MJ Research) for 30 cycles of 35 s at 94 °C, 35 s at 55 °C and 1 min at 72 °C and a final additional 10 mins cycle at 72 °C. Reactions were carried out in a total volume of 25  $\mu$ L containing 0.2  $\mu$ M of dNTPs, 0.5  $\mu$ M of the primer, 2.5  $\mu$ M of 1x PCR amplification buffer, 0.5 mM of MgCl<sub>2</sub>, 0.4 U *Taq* DNA polymerase (Invitrogen) and 0.4  $\mu$ M of DNA template and 50 ng ultrapure water. Reactions were done twice to evaluate the consistency of the banding patterns for all isolates studied. Products were separated on a 2% (w/v) agarose gel in 1x TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 125 V for 3 hours. The gels were stained with ethidium bromide and visualized with UV light using a Cole Palmer FLUO-LINK FLX apparatus and photographed for later assessment.

#### **3.3.2.4 Pathogenicity test of *Colletotrichum* isolates from the Volta region**

Pathogenicity test was done to confirm the identity of the pathogen isolated from *D. alata* on a randomly selected tissue culture-derived whole-*D. alata* cultivar TDa 008.

##### **3.3.2.4.1 Inoculum preparation**

To prepare inoculum for spray-inoculations, 2 mm mycelia plugs of three *Colletotrichum* isolates (Cg111, Cg125 and Ct132) – which represented genetic variations in the characterization study of

the Volta Region isolates, were taken from the sub-cultures established from single spores and stored in the fridge and transferred onto freshly prepared PDA plates; and left to sporulate under continuous light for 7-10 days at  $26\pm 2$  °C. Inoculum suspensions were then prepared by flooding each culture plate with 50 ml double sterile distilled water (DSDW) with two drops of Tween 80 (2% v/v). The surface of each culture plate was gently brushed with a fine paintbrush (Egesi *et al.*, 2009) to dislodge spores. The mycelia-spore suspensions were then filtered through double-layered cheesecloth to remove mycelia. The resulting spore suspension per isolate was adjusted to  $1.6 \times 10^6$  conidia/ml using Eubauer haemocytometer and a tally counter.

#### **3.3.2.4.2 Inoculation of *D. alata* cultivars**

A day to the experimental inoculation, two-month old potted tissue culture-derived whole-*D. alata* cultivars (TDa 008 and TDa 013) supported with 1.5 m wooden stakes were sprayed with carborandum using a fine De-vilbis atomizer. Before inoculation was done, all *D. alata* plants were enclosed in transparent latex bags, each of size 0.5 m x 1.5 m to prevent cross-inoculation by the different isolates. Plants were then either spray-inoculated with the prepared inoculum of each isolate or sterile distilled water amended with two drops of Tween 80 (2% v/v) (as controls) with a 1 litre hand-held sprayer until run-off. Inoculums were allowed to adhere onto the leaves and vines for about 30 minutes (Abang *et al.*, 2006), after which each plant was covered again with the transparent latex bag misted with sterile distilled water to maintain a moist environment (conditions: natural 12-h daylight/night cycle,  $26\pm 2$  °C and 98–100% RH) for 48 hours – for infection to occur (Fig. 3.2). The experiment was replicated 15 times, using 6 x 12 factorial treatments in completely randomized design (CRD).

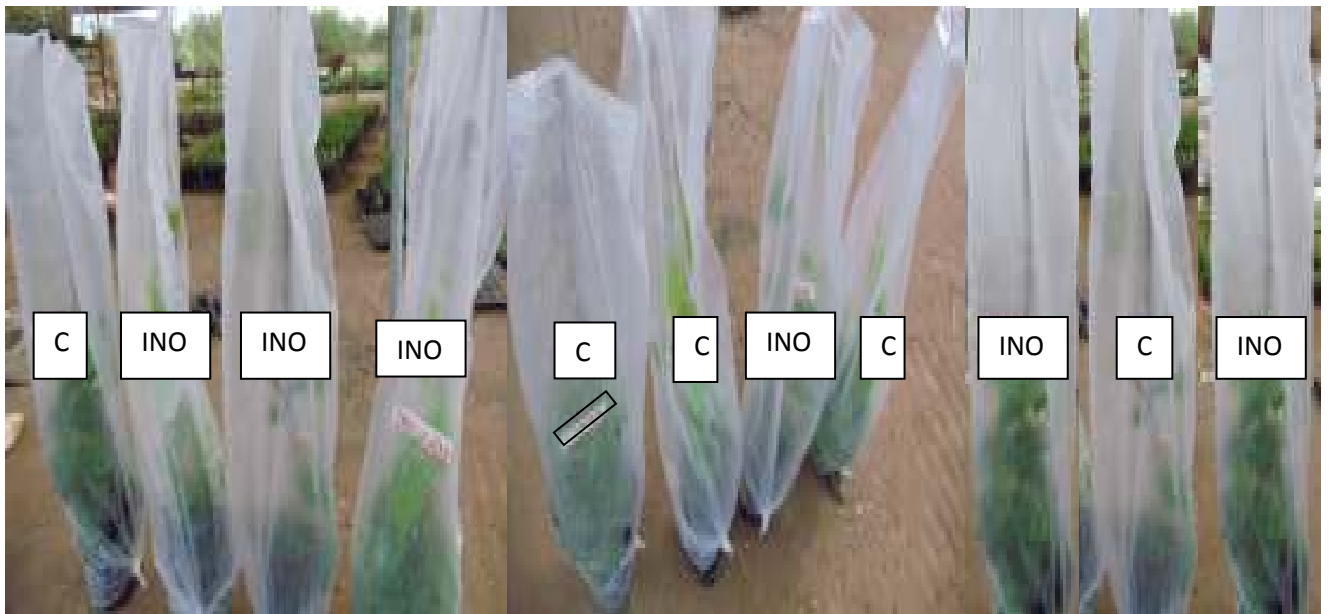


Fig. 3.2: Inoculated (INO) and non-inoculated (C) two-month old potted tissue culture-derived whole-*D. alata* cultivars enclosed in transparent latex bags to maintain high RH% for disease infection. Pathogen used = *C. gloeosporioides* and *C. truncatum* with spore suspensions of  $1.6 \times 10^6$  conidial/ml.

#### 3.3.2.4.3 Re-isolation of pathogen

Infected leaves and vines of *D. alata* cultivars were collected, labelled and packaged in an ice chest and sent to the Plant Pathology laboratory, Department of Crop Science, University of Ghana, Legon for re-isolation of the suspected causal agents. The isolation was done first on water agar (WA) and incubated at  $26 \pm 2$  °C, about 70-80% RH for five days and sub-cultured onto PDA and maintained at the same incubation conditions for seven days. The pathogen was then observed and identified as described in sections under 3.3.2.

### 3.4 Characterization of the strains of *C. gloeosporioides* and *C. truncatum*

#### 3.4.1 Diversity study in *C. gloeosporioides* and *C. truncatum* infecting *D. alata* in Ghana

A survey was carried out between July and December, 2012 to obtain baseline knowledge on the diversity of the pathogen associated with vine-browning and die-back disease of *D. alata* in Ghana. Prior to the survey, a list of all the major *D. alata* growing districts in each of the five regions (Ashanti, Brong-Ahafo, Central, Eastern and Northern) was obtained from the Extension Service Department of the Ministry of Food and Agriculture, Ghana. Two major *D. alata* growing

districts per region were then randomly selected for the study. Initial focus group discussions were held with the AEAs of each of the selected districts to determine the communities within their respective districts where the disease was most predominant. A maximum of three communities per district were randomly selected and fifteen *D. alata* farms per community were further selected randomly from the list of *D. alata* farms provided by the respective AEAs.

A total of 12 districts, 27 communities and 405 farms (Appendix II) were surveyed for the study. During the survey, several samples of *D. alata* leaves and vines exhibiting varying symptoms of a vine-browning and die-back disease were collected using systematic sampling technique with ‘W-shaped’ sampling path (Lin *et al.*, 1979) from different locations and cultivars. The samples were then sealed in sterile transparent latex bags of size 0.5 m x 1.5 m, well-labelled, packaged in ice chest container and transported to the Plant Pathology Laboratory for isolation (as discussed in section 3.3.1), identification (as discussed in section 3.3.2) and characterization (as discussed in sections 3.4.2 to 3.4.3) of the micro-organisms associated with the diseased *D. alata* tissues. Photographic records were taken on the diseased *D. alata* plants and agronomic practices undertaken by farmers with DSC–S750 Sony Digital Camera. Pathogenicity test was carried out as described in section 3.3.2.4 using six selected *Colletotrichum* isolates (Cg146, Cg147, Cg149, Cg151, Cg165 and Ct168) which were diverse from the other isolates in the genome diversity study conducted using five Random Amplified Poly-Dimorphism (RAPD) primers (OPA-11, OPA-18, OPC-5, OPC-7 and OPC-16) and five Inter-Spacers Restriction Fragment Length Polymorphism (ITS-RFLP) enzymes (*Hha*I, *Hae*III, *Hinf*I, *Msp*I and *Rsa*I).

### **3.4.2 Genome analysis of *C. gloeosporioides* and *C. truncatum* strains**

#### **3.4.2.1 Rapid amplified polymorphic DNA (RAPD) analysis**

In the RAPD analysis of isolates, PCR-RAPD of the extracted genomic DNA was performed in a BIO-RAD PTC-220 thermocycler (Dyad MJ Research) for 45 cycles of 35 s at 94 °C, 35 s at 55

°C and 1 min at 72 °C and a final additional 10 mins cycle at 72 °C. Reactions were carried out in a total volume of 25 µL containing 0.2 µM of dNTPs, 0.5 µM of the primer, 2.5 µM of 1x PCR amplification buffer, 0.5 mM of MgCl<sub>2</sub>, 0.4 U *Taq* DNA polymerase (Invitrogen) and 0.4 µM of DNA template and 20.5 µM ultrapure water. Reactions were done twice to evaluate the consistency of the banding patterns for all isolates studied. Products were separated on a 2% (w/v) agarose gel in 1x TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 125 V for 3 hours. The gels were stained with ethidium bromide and visualized with UV light using a Cole Palmer FLUO-LINK FLX apparatus and photographed for later evaluation.

A pre-screening of twenty *Colletotrichum* isolates with ten primers from the OPA, OPC and OPD series (Operon Technologies, California, USA) was performed to select the most informative primers. For the final evaluation of isolates, five primers: OPA-11 (5'-GGGTAACGCC-3'); OPA-18 (5'-AGCCAGCGAA-3'); OPC-05 (5'-GATGACCGCC-3'); OPC-07 (5'-GTCCCGACGA-3') and OPC-16 (5'-TTCCGAACCC-3') were used. Computer analysis of RAPD patterns were performed as described by Halmschlager *et al.* (1994), in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively). For each enzyme, polymorphic bands were considered as binary characters and scored as present (1) or absent (0). Genetic similarity between pairs was estimated using Jaccard's similarity coefficient with the SIMQUAL option (Jaccard, 1908). The similarity matrix was run on sequential, agglomerative, hierarchical and nested clustering (SAHN) (Sneath and Sokal, 1973) using the unweighted pair-group method with arithmetic average (UPGMA) clustering algorithm to generate a dendrogram. All computations were undertaken using the NTSYSpc 2.1 program (Rohlf, 2000).

#### 3.4.2.2 Restriction Enzyme Digestion of rDNA ITS regions

All fungal isolates obtained from the six regions were used for PCR restriction fragment analysis. Oligonucleotide primers, ITS1 (5'-TCCGTAGGTGAACCTCCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with rDNA genes flanking the entire ITS1-5.8S-ITS4 region (about 500-600 bp) were used for ITS PCR-amplification (White *et al.*, 1990). The PCR reactions were carried out in a BIO-RAD PTC-220 thermocycler using a total volume of 25  $\mu$ L containing 0.2  $\mu$ M of dNTPs, 0.2  $\mu$ M of the ITS forward and reverse primers, 1 x PCR amplification buffer, 0.25 mM of MgCl<sub>2</sub>, 1 U *Taq* DNA Polymerase (Invitrogen) and 25 mg of DNA template and an initial amplification step of 2 min at 94 °C, followed by 45 cycles of 35 s at 94 °C, 35 s at 55 °C and 10 min at 72 °C. Restriction of the PCR products was carried out according to the manufacturer's instructions using five endonucleases: *Hha*I, *Hae*III, *Rsa*I, *Hinf*I and *Msp*I to study polymorphisms in the ITS regions of the isolates. Enzyme treatments were made by taken a 3  $\mu$ L aliquot of the PCR reaction and incubating it with 1 unit of the respective enzyme for 1–2 hours at 37 °C using the digestion buffer specified by the manufacturer.

Restriction fragments generated were analyzed by electrophoresis on 2% polyacrylamide gel (20 x 20) in 1 x Tris- Borate-EDTA (TBE) pH 8.0 buffer, at 125 V for 3 hours. Gels were stained with ethidium bromide and thereafter, DNA fragments were visualized under UV light using a Cole Palmer FLUO-LINK FLX apparatus and the molecular weights of the fragments determined by comparison with a 100 bp DNA ladder.

For each marker, polymorphic bands were considered as binary characters and scored as present (1) or absent (0). Genetic similarity between pairs was estimated using Jaccard's similarity coefficient with the SIMQUAL option (Jaccard, 1908). The similarity matrix was run on sequential, agglomerative, hierarchical and nested clustering (SAHN) (Sneath and Sokal, 1973)

using the unweighted pair-group method with arithmetic average (UPGMA) clustering algorithm to generate a dendrogram. All computations were undertaken using the NTSYSpc 2.1 program (Rohlf, 2000).

### **3.5 Screening of *D. alata* cultivars for resistance to anthracnose disease using tissue culture-derived whole-plants assay**

Nine *D. alata* cultivars grown in the Volta Region and three cultivars collected from PGGRI, Bunso – Eastern Region of Ghana were screened for their resistance to the *Colletotrichum* spp. isolated from the diseased *D. alata* obtained in the various regions. The methods (adapted to accomplish this objective are elaborated in sections 3.5.1 to 3.5.3 below.

#### **3.5.1 Determination of appropriate hormones concentration for the tissue culture test**

In order to maximize reagents and/or hormones use (Svabova and Lebeda, 2009; Sebastiani *et al.*, 1994), preliminary experiment was conducted at the Tissue Culture and Biotechnology Centre (TCBC) of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) in Ghana Atomic Energy Commission (GAEC) in April, 2012. The sole aim of this trial was to arrive at the choice of appropriate treatment combination (i.e. hormones combination) that could give best performance of explants to be used in the tissue culture experiment. Six randomly selected *D. alata* cultivars: TDa008, TDa013, TDa018, TDa019, TDa025 and TDa027 and six-combination of hormones (BAP + NAA + Kinetin: T<sub>1</sub> = [0.25 + 0.25 + 0.25 mg/l], T<sub>2</sub> = [0.25 + 0.5 + 0.5 mg/l], T<sub>3</sub> = [0.25 + 1.0 + 1.0 mg/l], T<sub>4</sub> = [0.25 + 1.5 + 1.5mg/l], T<sub>5</sub> = [0.25 + 2.0 + 2.0 mg/l] and T<sub>6</sub> = [0.25 + 2.5 + 2.5 mg/l]) were used in the trial. A 6 x 7 factorial arrangement in CRD with 25 replications (i.e. culture tubes) per cultivar per hormones combination was used and each replication consisted of a single tube. Auxin-free basal medium (BAP + NAA + Kinetin: 0.0 + 0.0 + 0.0 mg/l) was used as the control. All activities that were carried out were done as described in the sections of the main experiment (section 3.5.3) with the exception of the design used together with the number of culture tubes and/or replications.

Data on root-shoot responses (i.e. days required to root and shoot initiation, mean number of roots and shoot per cultivar and mean shoot growth over a period of six weeks) of each cultivar were recorded. The main tissue culture experiment was then performed after the appropriate hormones combination and/or concentration had been selected from the preceding trial. Photographic records were taken on every stage of the experiment with Sony Digital Camera model DSC-S750.

### **3.5.2 Raising of *D. alata* explants for the screening trial**

Explants of the 12 *D. alata* cultivars were raised using tissue culture-derived whole-plant assay with the detailed methods described in sections 3.5.2.1 to 3.5.2.3 below.

#### **3.5.2.1 Plantlets source and preparation**

Nodal cuttings (about 2.5-3.0 cm long) of healthy vines with active buds (i.e. meristematic buds) were made from the 12 *D. alata* cultivars maintained in the Experimental Garden, BNARI – GAEC and Sinna’s Garden, Department of Crop Science, University of Ghana – Legon. Samples collected were well packaged, properly labelled and transported to the Tissue Culture and Biotechnology Centre, BNARI during August and September, 2012. The vines were then cut into 1.5 cm to 2.0 cm length with single node intact. These nodal vine cuttings were washed with 5% (v/v) detergent solution (Teepol, Qualigen, Mumbai, India) for 10 minutes and rinsed several times with running tap water. These nodal cuttings were surface sterilized with bavistin 0.3 % (w/v) and streptomycin 0.2% (w/v) for 10 minutes each and then washed with sterile distilled water and transferred to laminar air flow cabinet. In the laminar chamber, nodal segments were again treated with 70% ethyl alcohol for 30 seconds to one minute followed by another treatment in 0.1% (w/v) mercuric chloride (HgCl<sub>2</sub>) for another 5 minutes. Finally, the nodal cut vines were washed thoroughly in 3 to 4 changes of double sterile distilled water and soaked on sterile blotting paper. Sterilized blotted explants were then implanted on a fortified solid MS medium (Murashige and Skoog, 1962) prepared according to Maura-Costa and Mantell (1993).

All cultures were incubated at 16-hour light/8-hour dark photoperiod (cool, white fluorescent light –  $30 \mu\text{mol m}^{-2} \text{S}^{-1}$ ) and a temperature of 25/29 °C (night/day) growth room, in 2.5 cm diameter x 10 cm long tubes under 60-70% relative humidity (RH). Fifty replicate tubes per cultivar were used in the experiment with each replication comprising of a single culture tube.

#### **3.5.2.2 Plantlets multiplication**

Plantlets cultures were maintained by regular sub-culturing at 2-week intervals on freshly prepared MS medium (Flow Laboratories), 2% sucrose, 0.8% agar, pH 5.8, amended with root-shoot hormones (Mantell *et al.* 1978). *In-vitro* raised shoots measuring about 4-5 cm length grown in multiplication medium were excised from the shoot clumps and transferred onto a half-strength MS basal medium supplemented with 2.0 mg/l  $\alpha$ -Naphthalene acetic acid (NAA), 2.0 mg/l Kinetin and 0.25 mg/l BAP (which proved best amongst the six hormones-combinations in the preliminary experiment) for root and shoot formation. In addition to the aforementioned root-shoot hormone supplements, the medium was amended with ascorbic acid 100 mg/l (as an antioxidant) and artificial charcoal (making the medium assume the natural darkness condition in the root-region of the soil). The root-shoot responses of each cultivar *in-vitro* which included: rooting and shooting percentage (i.e. % explants response), days required to root and shoot initiation, mean number of roots and shoot per cultivar and mean shoot growth over a period of six weeks were recorded. Auxin-free basal medium was used as the control.

#### **3.5.2.3 Acclimatization and establishment of plantlets**

Six-week old rooted micro-propagules were removed from culture tubes and the roots were washed under running tap water to remove the agar. Plantlets were then transferred into sterile poly pots (small plastic cups) of size 22 x 35 x 18 cm containing pre-soaked sterile 1:1 vermiculite: compost (peat: sand, 9:1) (IITA, Nigeria) and maintained under moist condition by covering plantlets with sterile poly pots misted with double sterile distilled water for three days at

28/24 °C day/night temperatures, 70-80% RH and 16-h photoperiod. Mean weaning survival per cultivar was tabulated. After the three days, the coverings were removed and the plantlets were kept under bioclimatic chamber for further three weeks for advanced hardening. Following that, plantlets were transplanted into black plastic pots measuring 40 cm wide x 50 cm long containing a mixture of soil + sand + compost in 1:1:1 ratio and were still kept under the bioclimatic chamber for a period of one week after which they were transferred to 70% luminous screen house and remained there for the pathogen-screening experiment. Each plant was supported with a wooden stake of height, 1.5 m. No pesticide was applied during the course of the trial. Rather, hand-pickings of weeds were done when necessary and potted plants were irrigated thrice a week.

### **3.5.3 Screening of 12 *D. alata* cultivars for resistance to anthracnose disease**

Six *Colletotrichum* isolates obtained from the Volta region and the other five regions (Ashanti, Brong-Ahafo, Central, Eastern and Northern regions) were used in the screening tests. Inoculums were prepared as described in section 3.3.2.4.1. A day to the experimental inoculation, two-month old potted tissue culture- derived whole-*D. alata* cultivars (TDa007, TDa008, TDa010, TDa013, TDa014, TDa015, TDa018, TDa019, TDa021, TDa023, TDa025 and TDa027) supported with 1.5 m wooden stakes were sprayed with carborandum using a fine De-vilbis atomizer. Before inoculation was done, each *D. alata* plant was covered with a transparent latex bag of size 0.5 m x 1.5 m to prevent cross-inoculation by the different isolates. Fifteen potted *D. alata* plants per cultivar received a spray-inoculum treatment of an isolate at a concentration of  $1.6 \times 10^6$  conidia/ml amended with two drops of Tween 80 (2% v/v) with a 1 litre hand-held sprayer until run-off. The control plants (15 per cultivar), however, were sprayed with sterile distilled water amended with two drops of Tween 80 (2% v/v).

Inoculums were allowed to adhere onto the leaves and vines for about 30 minutes, after which each plant was covered again with the transparent latex bag misted with sterile distilled water to maintain a moist environment (conditions: natural 12-h daylight/night cycle,  $26\pm 2$  °C and 98–100% RH) for 48 hours – for infection to occur (Fig. 3.3). The layout of this experiment was a 6 x 12 factorial arranged in CRD with 20 replicates and each replication consisting of a single plant. The height and number of leaves per plant at the time of inoculation were recorded. Disease severity for each cultivar was evaluated using the percentage whole plant area scoring method (Egesi *et al.*, 2007), in which percentage of whole plant area affected by a vine-browning and die-back disease was scored based on a scale of 0-5 modified after Akem and Asiedu (1994) and Prasath and Ponnuswami (2007). 0 = no symptoms (highly resistant); 1 = 1-10 per cent of plant area showing disease symptoms (resistant); 2 = 11-25 per cent of plant area showing disease symptoms (moderately resistance); 3 = 26-40 per cent of plant area showing disease symptoms (susceptible); 4 = 41-70 and 5 = 71-100 per cent of plant area showing disease symptoms were ranked highly susceptible.

The disease severity was scored at 5, 9, 12 and 14 days after inoculation (DAI) (modified after Onyeka *et al.*, 2006) whilst disease incidence (presence or absence of the disease) was recorded on 14 DAI – for all strains. Variations in resistance of *D. alata* cultivars were evaluated based on the ideal method for assessment of partial or rate reducing resistance (Onyeka *et al.*, 2006) by taking into account, the beginning of the disease development, rate of the disease progression and final level of the diseased cumulative area under the progress curve (AUDPC) before and after the critical stage after inoculation and calculated using Shaner and Finney (1977) formula as indicated below. The disease progress rate ( $R_d$ ) was then calculated as the regression slope coefficient of disease severity over time (Onyeka *et al.*, 2006). Data were pooled and subjected to

analysis of variance (ANOVA) and the differences between individual means were tested using Fisher's Least Significant Difference Test (LSD Test) at 5% (Steel *et al.*, 1997).

$$\text{AUDPC} = \sum_{i=1}^n [(X_{i+1} + X_i)/2] [t_{i+1} - t_i]$$

Where,  
 $X_i$  = the proportion of the host tissue damaged at  $i^{\text{th}}$  day  
 $t_i$  = the time in days after appearance of the disease at  $i^{\text{th}}$  day  
 $n$  = the total number of observations

The highly resistant (TDa023) and highly susceptible (TDa019) (but farmers' preference) *D. alata* cultivars obtained from the screening test were purposively selected and used in the integrated management strategies for the disease.

### **3.6 Development of IPM strategies for management of anthracnose disease of *D. alata***

Integrated method of disease management which involved selection of healthy planting materials, hot-water treatment ( $45 \pm 1$  °C for 20 minutes and drying for 30 minutes), frequent weeding (monthly) and chemical (fungicides sprays) was employed in the study to manage anthracnose disease of *D. alata*.

#### **3.6.1. *in-vitro* evaluation of fungistatic effect of four fungicides on radial mycelial growth and sporulation of *C. gloeosporioides* and *C. truncatum***

The efficacies of four fungicides readily available on the local market were assessed for the management of anthracnose disease of *D. alata*. *In-vitro* fungicides screening experiment was conducted in the Plant Pathology Laboratory of the Department of Crop Science, University of Ghana, Legon to assess the efficacies of four fungicides on the radial mycelial growth and sporulation of the selected *C. gloeosporioides* (Cg111) and *C. truncatum* (Ct132) strains – which proved highly pathogenic in the screening trial and showed distinct diversities in the genome

analyses). Poisoned food technique (Kiran *et al.*, 2010) was employed in the study for the measurement of the radial mycelial growth and sporulation of the isolates on both amended and non-amended potato dextrose agar (PDA). Efficacies of systemic (Carbendazim) and three protectant (Mancozeb, Shafiz F1 and Nordox) fungicides' efficacies were assessed in the Plant Pathology Laboratory by plating each isolate on fungicide-amended PDA (modified after Maldonado *et al.*, 2005); Carbendazim potato dextrose agar (CZPDA), Mancozeb potato dextrose agar (MPDA), Shafiz potato dextrose agar (SPDA) and Nordox potato dextrose agar (NPDA) with treatment levels shown in Table 3.4.

Tabel 3.4: Types of fungicides and application rates used in the *in-vitro* screening of fungicides

| Fungicide                                          | Recommended concentration |
|----------------------------------------------------|---------------------------|
| Carbendazim (Bendazim® 50WP)                       | 1.3 g/litre               |
| Shafiz F1 ® 71.5 WP                                | 3.1 g/litre               |
| Mancozeb (Agrithane® 80 WP)                        | 2.7 g/litre               |
| Nordox (Cuprous oxide (Cu <sub>2</sub> O) ® 86 WP) | 5.0 g/litre               |

The control used in the experiment was non-amended potato dextrose agar (NAPDA). Each of the media was centrally inoculated with a 2 mm diameter fungus plug taken from the advancing margin of a five-day old single colony culture of each isolate and incubated at 26±2 °C and 65-72% RH in the laboratory. The design used in this experiment was 4 x 4 factorial experiments in completely randomized design (CRD) layout with ten replications per treatment. Daily observation of plates was made up to the seventh day and colony diameter measured by taking the average of the two perpendicular distances across the centre of the colony using a meter rule. Kiran *et al.* (2010) formula for calculating percentage reduction in radial growth (I) of test fungus over control (C) was used for the percentage radial growth reduction.

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent reduction in growth of test fungi.

C = Radial growth (mm) in control.

T = Radial growth (mm) in treatment.

A sterile cork borer of diameter 2 mm was used to take mycelial plugs from relative positions equidistant to the center of Petri dish with mycelial growth after the eighth day of incubation. Each detached plug was put into 10 ml sterile distilled water with two drops of Tween 80 solution in a McCartney bottle and capped (Maldonado *et al.*, 2005). The spores of each plug were then detached by electronic shaking for about 20 seconds and counted under a compound microscope with the aid of eubauer haemocytometer and a tally counter. Kiran *et al.* (2010) formula for determining the percentage reduction in sporulation (I) over control (C) was employed in calculating the percentage reduction in sporulation of the *C. gloeosporioides* isolates.

$$I = \frac{C - T}{C} \times 100$$

Where, I = Percent reduction in sporulation of test fungi.

C = Number of spores in control.

T = Number of spores in treatment.

### **3.6.2 Field management of anthracnose disease of *D. alata* in Ghana**

The experiment was conducted at the University of Ghana Farm, Legon (5 ° 58' N, 0 ° 8' W; 153 m asl) in the Greater Accra Region of Ghana which is located within the Coastal Savannah Zone, with annual mean rainfall of 750 mm and average temperature of 26 °C. The soil belongs to the Adenta series, ferric Acrisol which is light in texture, well drained and easy to work with. The topsoil profile is sandy loam and consists of approximately 20 cm of pale-brown to brown sand with loose, friable consistence and a weak, fine granular structure (FAO/UNESCO, 1990).

Clean seed-yam (i.e. setts) of the two purposively selected *D. alata* cultivars TDa023 (highly resistant) and TDa019 (highly susceptible but farmers' preference) were established for integrated

management of the disease from November, 2012 to May, 2013. Prior to planting, setts of both cultivars (each of weight, 200 g) were dipped into hot-water (45 °C) for 20 minutes and allowed to dry for 30 minutes (Small, 1988). The non-treated setts (controls) were, however, not treated with the hot-water. The total land area was 45 x 30 m<sup>2</sup> prepared by ploughing and harrowing with a tractor after which ridges of height 50 cm and length 12 m separated at 1 m were raised manually with hoe. The whole plot was divided into three main blocks and each block was further divided into two sub-plots (i.e. plots A and B) separated at 2 m apart where the treated and control setts were randomly assigned (to either of the plots). Allocation of seed-pieces per sub-plot was done randomly. The design used was 2 x 4 factorial in randomized complete block design (RCBD) with three replications. Setts were placed 8 cm deep and 1m apart within the 50 cm high ridges giving a population of 10,000 plants per hectare. Each plant was supported with a wooden stake of height 2.5 m. Each block contained 20 ridges with each treatment appearing 25 times in a block. The sub-plots assigned with treated setts were weeded once every month whilst the control sub-plots were left unweeded.

Two months after sprouting (MAS) of setts, all *D. alata* plants on the treated sub-plots (except the bordered ones) were covered with transparent latex bags each of size 0.5 m x 2.5 m – a day to the fungicides treatment in order to prevent fungicides-cross effect. Each of the three fungicides: Carbendazim, Mancozeb and Nordox (Cu<sub>2</sub>O) (which significantly inhibited the radial mycelial growth and sporulation of the *Colletotrichum* isolates Cg111 and Ct132 in *in-vitro* assay) with preparations of 2.7, 5.0 and 1.3 g/l of water respectively was sprayed onto seven randomly selected *D. alata* plants per cultivar per sub-plot using a 15 l Knapsack sprayer at 30 psi after carefully removing the latex bags (one at a time). The control plants were, however, sprayed with SDW. Fungicides sprays were either repeated on every 14 days (Carbendazim and Mancozeb) or 28 days (Nordox) for two months.

A day after the first fungicides application, all *D. alata* plants (i.e. both treated and controls) were sprayed with carborandum using a fine De-vilbis atomizer. Next day after the carborandum application, all the *D. alata* plants (except the bordered plants) were spray-inoculated with mixed spore-suspension of *Colletotrichum* isolates (Cg111 + Ct132) with concentration,  $1.6 \times 10^6$  conidia/ml. The inoculum concentration was amended with two drops of Tween 80 (2% v/v) before spraying until run-off with a 1 litre hand-held sprayer. Inoculum was allowed to adhere onto the leaves and vines of *D. alata* for about 30 minutes, after which each plant was covered with new latex bag misted with SDW – to maintain high humidity for infection to occur. The experiment was conducted under natural irrigation condition, however, when necessary, artificial irrigation was done. No fertilizer was applied in the course of the trial.

#### **3.6.2.1 Disease assessment**

Disease ratings per cultivar per plot were undertaken a week after inoculation (WAI) for severity of anthracnose disease using a subjective score-scale of 0-5 (modified after Prasath and Ponnuswami, (2007); Akem and Asiedu, 1994) (Table 3.3). The scoring was repeated weekly for two months (Mignucci *et al.*, 1988) to estimate foliage and vine necrosis caused by *Colletotrichum* spp. Variation of treatments in managing and/or controlling the disease were evaluated based on ideal method for assessment of disease *D. alata* plant (Onyeka *et al.*, 2006) by taking into consideration, the beginning of the disease development, rate of the disease progression and final levels of the diseased cumulative area under the progress curve (AUDPC) before and after the critical stage of the disease. The disease progress rate was then taken as the slope of regression line for disease severity against time. Data collected were statistically analyzed with GenStats software version 9.0 and the differences between individual means were separated using Fisher's Least Significant Difference (LSD) test at 5% (Steel *et al.*, 1997).

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Farmers' perception and practices on the cause, spread, control and importance of a vine-browning and die-back disease of *D. alata* in the Volta region

##### 4.1.1 General background of *D. alata* farmers

The survey revealed that majority (93.3%) of *D. alata* farmers in the Nkwanta-North and Krachie-West districts of the Volta region were males and 6.7% were females. Majority of the respondents were aged 41-50 years (56.7%). The rest were aged 21-30 years (4.2 %), 31-40 years (33.3%) or 51 years and above (5.8%).

Majority (31.0%) of the *D. alata* farmers in the two districts had no formal education (Table 4.1). The rest had formal education up to the Junior High School (JHS) level (27.0%), Senior High School (SHS) (22%) and Primary (15%). However, a small population of the farmers (5%) had received Tertiary education.

Table 4.1: Educational background of respondents

| Education Level     | Frequency  | Percent      | Cumulative Percent |
|---------------------|------------|--------------|--------------------|
| No formal education | 37         | 31.0         | 31.0               |
| Primary             | 18         | 27.0         | 58.0               |
| JHS                 | 32         | 22.0         | 80.0               |
| SHS                 | 27         | 15.0         | 95.0               |
| Tertiary            | 6          | 5.0          | 100.0              |
| <b>Total</b>        | <b>120</b> | <b>100.0</b> |                    |

Majority (62.0%) of the farmers had between 1-5 years farming experiences in *D. alata* production. The rest had 6-10 years (30.0%) or below 1 year (8.0%) farming experience.

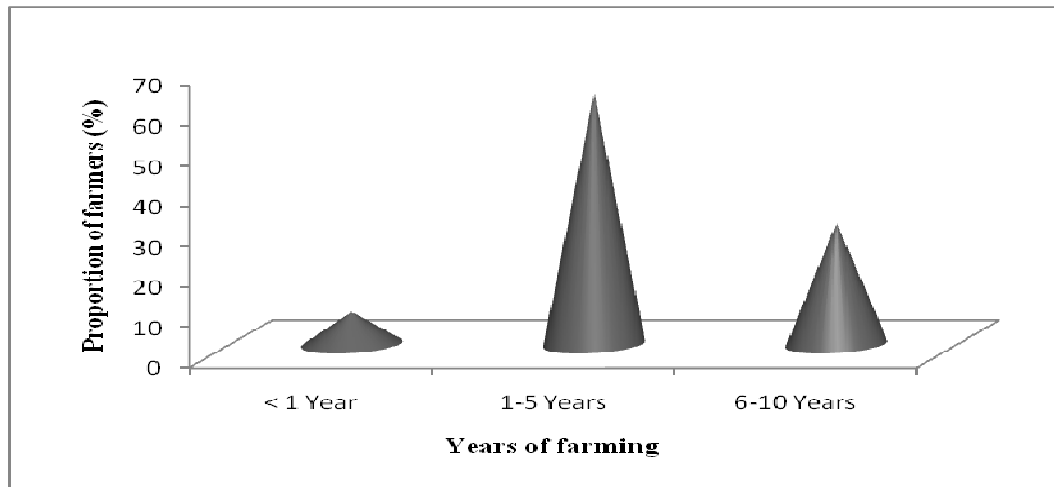


Fig. 4.1: Years of farming of *D. alata* by farmers in the Nkwanta-North and Krachi-West districts of the Volta Region

#### 4.1.2 Farmers' knowledge and perception on disease symptoms, susceptibility of *D. alata* cultivars and prevalence of anthracnose disease of *D. alata* in the Volta Region

A greater proportion (58%) of the farmers in the two districts indicated that in the presence of vine-browning and die-back disease, *D. alata* plants exhibit black spots with dark brown borders merging to form lesions and defoliation while 37% described large brown spots on the leaves bordered by yellow patches and die-back (Table 4.2). Some farmers (18%), however, described brown regular spots on leaves surrounded by dark brown rings with gray centres whilst a few (7%) indicated that small regular brown spots with yellow halos were seen on leaves. A handful of the farmers (2%) indicated that the disease appears as pin-point spots scattered on the lamina surrounded by yellow halos whilst 1% described the disease as pin-point spots scattered on the lamina without yellow halos (Table 4.2).

Table 4.2: Symptoms used by farmers to identify a vine-browning and die-back disease of *D. alata* in the field.

| Disease symptoms                                                                   | Frequency  | Percent of respondents | Cumulative percent |
|------------------------------------------------------------------------------------|------------|------------------------|--------------------|
| Pin-point spots scattered on the lamina without yellow halos                       | 1          | 0.8                    | 0.8                |
| Pin-point spots scattered on the lamina surrounded by yellow halo                  | 2          | 2.5                    | 3.3                |
| Small irregular brown spot with yellow halo                                        | 7          | 5.8                    | 9.1                |
| Brown regular spots surrounded by a dark brown ring and having a gray center       | 18         | 15.1                   | 24.2               |
| Large brown spots bordered with yellow patches and die-back                        | 37         | 30.8                   | 55.0               |
| Black spots with dark brown border merging to form lesions and causing defoliation | 55         | 45.0                   | 100.0              |
| <b>Total</b>                                                                       | <b>120</b> | <b>100.0</b>           |                    |

*D. alata* cultivars, ‘Matches’ (long and short tubered), ‘Akaba’ (thorned and thorn-less), ‘Akomea’, ‘Nibultutu’, ‘Chachribon’, ‘Poka’ and ‘Sum ne hyen’ were the popularly grown cultivars in both districts. Farmers (100%) indicated that all the cultivars grown were susceptible to the vine-browning and die-back disease but to different levels. Majority (40.8%) of the farmers perceived Matches ‘short tubered’ as the highly susceptible cultivar, followed by Matches ‘long tubered’ (30.0%) and ‘Chachribon’ (15.1%). However, ‘Akomea’ (5.8%) and ‘Poka’ (4.2%) were perceived to be moderately susceptible whiles ‘Sum ne hyen’ (2.5%), ‘Nibultutu’ (1.6) and ‘Akaba’(thorned and thorn-less) (0.8%) were the least susceptible (Table 4.3).

Table 4.3 Farmers knowledge on susceptibility of *D. alata* cultivars to a vine-browning and die-back disease of *D. alata* in the Volta Region.

| <b>Cultivar</b>         | <b>Frequency</b> | <b>Percent of respondents</b> | <b>Cumulative Percent</b> | <b>Host Responses*</b> |
|-------------------------|------------------|-------------------------------|---------------------------|------------------------|
| Matches (short tubered) | 48               | 40.0                          | 40.0                      | <b>HS</b>              |
| Matches (long tubered)  | 36               | 30.0                          | 70.0                      | <b>S</b>               |
| Akaba (thorned)         | 1                | 0.8                           | 70.8                      | <b>LS</b>              |
| Akaba (thorn-less)      | 1                | 0.8                           | 71.6                      | <b>LS</b>              |
| Akomea                  | 6                | 5.0                           | 76.6                      | <b>MS</b>              |
| Poka                    | 5                | 4.2                           | 80.8                      | <b>MS</b>              |
| Sum ne hyen             | 3                | 2.5                           | 83.3                      | <b>LS</b>              |
| Nibultutu               | 2                | 1.6                           | 84.9                      | <b>LS</b>              |
| Chachribon              | 18               | 15.1                          | <b>100.0</b>              | <b>S</b>               |
| <b>Total</b>            | <b>120</b>       | <b>100.0</b>                  |                           |                        |

\*Host responses: HS = Highly susceptible; S = Susceptible; LS = Less susceptible; MS = Moderately susceptible.

Majority (55.8%) of the farmers perceived to have encountered disease incidence of 50-70% while 27.5% of the farmers recorded 25-50% disease incidence. Seventeen percent of the farmers perceived an incidence of less than 25% whereas 3.0% had between 75 and 100% of their farms affected by the disease (Table 4.4).

Table 4.4: Percentage of *D. alata* plants in farms affected by a vine-browning and die-back disease of *D. alata* in Nkwanta-North and Krachi-West districts.

| <b>Incidence (%) of disease on farms</b> | <b>Frequency</b> | <b>Percent of respondents</b> | <b>Cumulative Percent</b> |
|------------------------------------------|------------------|-------------------------------|---------------------------|
| Less than 25                             | 17               | 14.2                          | 14.2                      |
| 25 - 50                                  | 33               | 27.5                          | 41.7                      |
| 50 - 75                                  | 67               | 55.8                          | 97.5                      |
| 75 - 100                                 | 3                | 2.5                           | <b>100.0</b>              |
| <b>Total</b>                             | <b>120</b>       | <b>100.0</b>                  |                           |

#### 4.1.3 Farmers' perception, knowledge and experience on the cause, spread and control of a vine-browning and die-back disease of *D. alata*

A wide range of causes of a vine-browning and die-back disease of *D. alata* were perceived by the farmers in the two districts (Fig. 4.2). Most of the farmers (73.3%) had no idea of the cause of

the disease while 12.5% it to excessive rainfall. Poor management practices by farmers (6.7%), excessive sunlight (5.0%), superstition and belief (2%) and fungus (0.8%) were also perceived as causes of the disease.

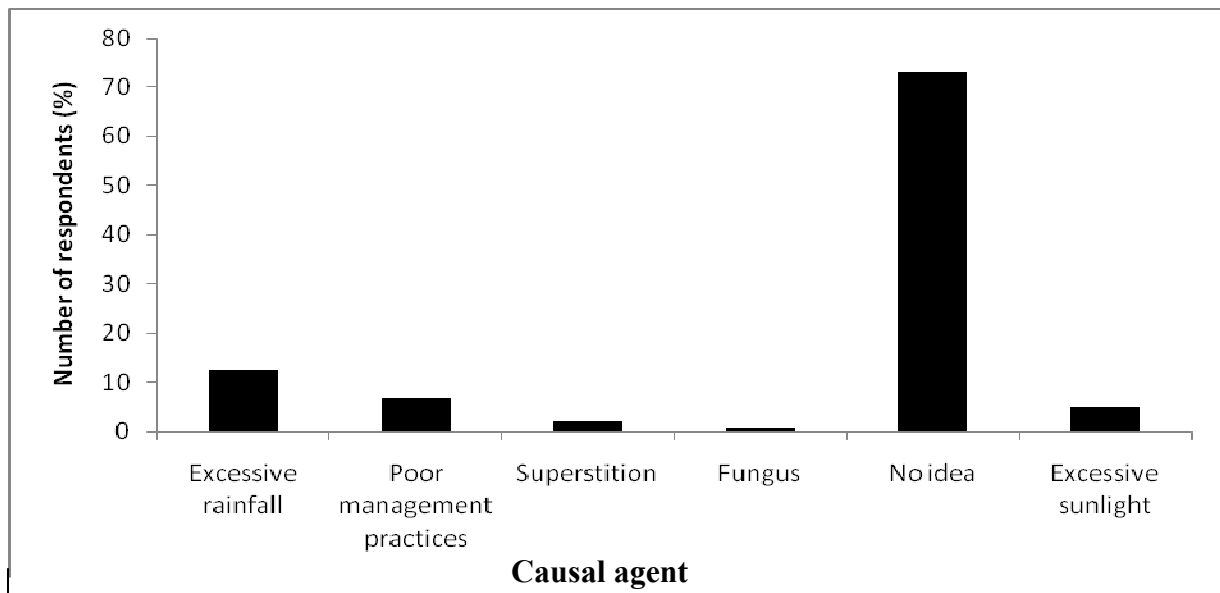


Fig. 4.2: Perception of farmers on the cause of a vine-browning and die-back disease of *D. alata*.

*D. alata* farmers in both districts had different perceptions on the mode of spread of a vine-browning and die-back disease of *D. alata* within and among farms (Fig. 4.3). A high proportion of the farmers (63.3%) had no idea as to how the disease spread within a given farm, while 20.0% attributed the spread to planting of infected materials (i.e. seed-yam). On the other hand, 7.5% perceived the disease spread to be by contact between healthy and diseased plants while 5.0% and 4.2% ascribed it to rainfall and wind respectively

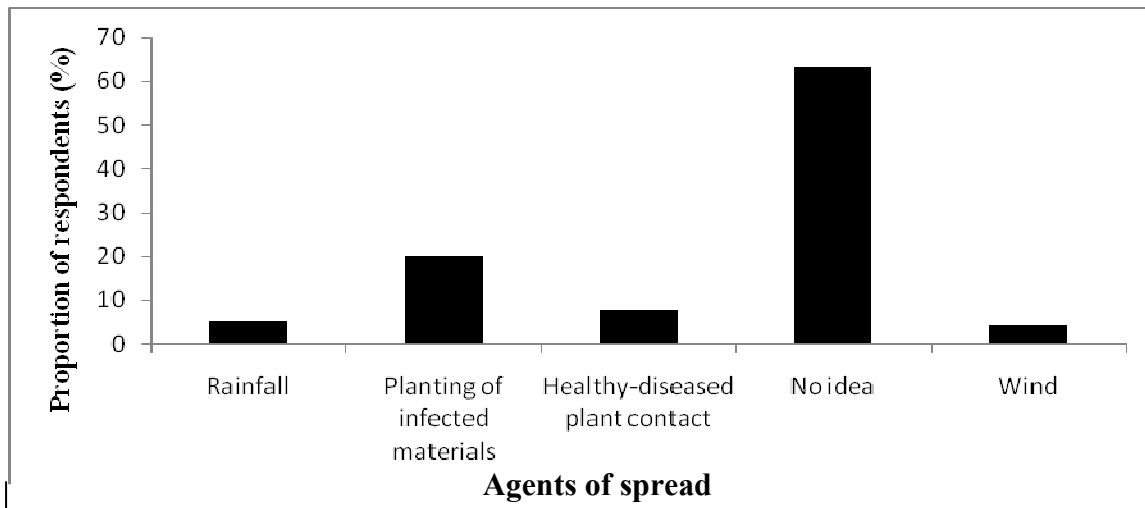


Fig. 4.3: Perception of farmers on the spread of a vine-browning and die-back disease of *D. alata* within farms.

Farmers' perception on the spread of the disease between and within farms was the same (Fig. 4.4). Majority (69.2%) of the farmers did not have any idea as to how the disease is spread from one farm to another, however, some (15.8%) believed the disease spread was due to exchange of infected planting materials among farmers while others (9.2%) attributed the spread to wind. The rest credited the spread of the disease among farms to rainfall (6.0%) and contaminated tools (0.8%) respectively.

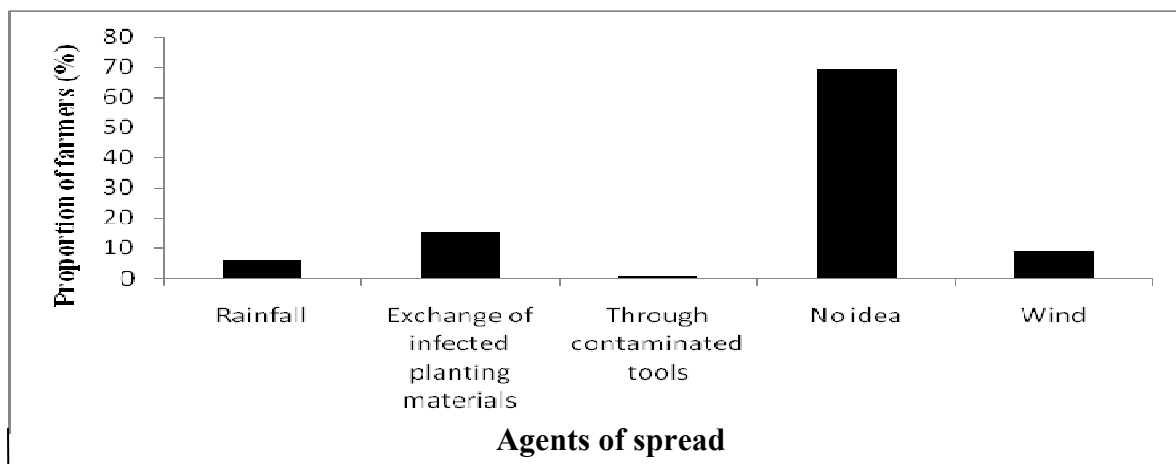


Fig. 4.4: Perception of farmers on the spread of a vine-browning and die-back disease of *D. alata* among farms.

Majority (63.30%) of farmers in both districts were of the view that the disease could be controlled. Nonetheless, 36.67% of them believed the disease was uncontrollable. Out of the

63.3% farmers who perceived that the disease could be controlled, 50.0% indicated that application of chemicals was likely to control the disease while 27.6% considered regular weeding to be the best control measure. About 11.8% of the respondents had no idea of any control measure, though they believed the disease could be controlled whereas 10.5% perceived use of healthy planting materials as the most effective disease control measure (Fig. 4.5).

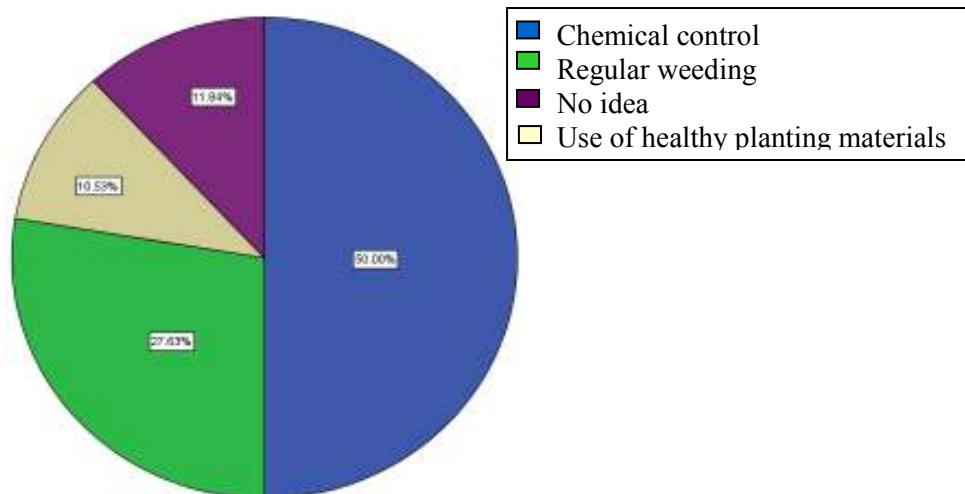


Fig 4.5: Perception of farmers on measures needed for successful of control of a vine-browning and die-back disease of *D. alata* in the Volta Region.

#### 4.1.4 Knowledge and experience of farmers on, yield and economic impact of a vine-browning and die-back disease of *D. alata* on livelihoods of farmers

Majority (55.8%) of the farmers in both districts produced *D. alata* production mainly for food security. Some (40.8%) produced it exclusively for income generation while the rest (3.4%) produced the crop in order to buy assets such as houses, lands and bullock (Table 4.5).

Table 4.5: Reasons for planting *D. alata* by farmers in the Nkwanta-North and Krachi-West districts.

| Reasons for planting <i>D. alata</i> by farmers | Frequency  | Percent of respondents | Cumulative Percent |
|-------------------------------------------------|------------|------------------------|--------------------|
| For food security                               | 67         | 55.8                   | 55.5               |
| For income generation                           | 49         | 40.8                   | 96.6               |
| Purchase of assets                              | 4          | 3.4                    | 100.0              |
| <b>Total</b>                                    | <b>120</b> | <b>100.0</b>           |                    |

The yields expected by farmers in both districts were between 4.0 and 21.0 tons per 2 ha and 15 ha farm size respectively but the actual harvest of most farmers (51) was between 4 and 6 tons (Table 4.6). Out of 27.5% who cultivated 2 ha farmlands, only 5.8% realized their expectations in terms of yield (i.e. between 4-6 tons/2 ha farm size) while the majority (26) did not reach their harvest target and had a yield between 1-3 tons/2 ha. None of the farmers who cultivated farmlands ranging from 5 to 15 ha achieved his/her expected yield; implying that, 72.5% of the farmers did not reach their anticipated yields (Table 4.6).

Table 4.6: Expected and actual yield of *D. alata* harvested by farmers in the Nkwanta-North and Krachi-West districts.

| Farm size/ha | Expected yield (tons) of <i>D. alata</i> per annum | Actual yield (tons) per annum |           |           |           |           |          |          | Freq.      | %    |
|--------------|----------------------------------------------------|-------------------------------|-----------|-----------|-----------|-----------|----------|----------|------------|------|
|              |                                                    | 1-3                           | 4-6       | 7-9       | 10-12     | 13-15     | 16-18    | 19-21    |            |      |
| 2            | 4.0 – 6.0                                          | 26                            | 7         | 0         | 0         | 0         | 0        | 0        | 33         | 27.5 |
| 5            | 7.0 – 9.0                                          | 4                             | 19        | 0         | 0         | 0         | 0        | 0        | 23         | 19.2 |
| 7            | 10.0 – 12.0                                        | 2                             | 11        | 5         | 0         | 0         | 0        | 0        | 18         | 15.0 |
| 10           | 13.0 – 15.0                                        | 0                             | 14        | 6         | 9         | 0         | 0        | 0        | 29         | 24.2 |
| 13           | 16.0 – 18.0                                        | 0                             | 0         | 0         | 5         | 7         | 0        | 0        | 12         | 10.0 |
| 15           | 19.0 – 21.0                                        | 0                             | 0         | 0         | 1         | 3         | 1        | 0        | 5          | 100  |
|              | <b>Total</b>                                       | <b>32</b>                     | <b>51</b> | <b>11</b> | <b>15</b> | <b>10</b> | <b>1</b> | <b>0</b> | <b>120</b> |      |

Majority (63.7%) of the respondents attributed their yield losses to *D. alata* diseases. The rest ascribed their losses to excessive drought (15.8%), inadequate labour (8.0%) and excessive rainfall (6.2%) (Table 4.7). Some farmers attributed low yield to non-staking (2.7%) of plants, flooding (2.7%) during heavy down-pour of rain and assigned their yield losses to poor land preparation (0.9%).

Table 4.7: Farmers' reasons for not achieving expected yield of *D. alata* in the Nkwanta-North and Krachi-West districts.

| Reasons for not achieving expected yield | Frequency  | Percent of respondents | Cumulative percent |
|------------------------------------------|------------|------------------------|--------------------|
| Diseases of <i>D. alata</i>              | 72         | 63.7                   | 63.7               |
| Excess rainfall                          | 7          | 6.2                    | 69.9               |
| Extensive drought                        | 18         | 15.8                   | 85.7               |
| Poor land preparation                    | 1          | 0.9                    | 86.6               |
| Non-staking                              | 3          | 2.7                    | 89.3               |
| Flooding                                 | 3          | 2.7                    | 92.0               |
| Inadequate labour                        | 9          | 8.0                    | <b>100.0</b>       |
| <b>Total</b>                             | <b>113</b> | <b>100.0</b>           |                    |

Responses of *D. alata* farmers in Nkwanta-North and Krachi-West districts concerning the socio-economic impacts of a vine-browning and die-back disease on their livelihoods are presented in Table 4.8. The diseases impacted negatively on the farmers' livelihoods as majority were unable to pay: their school fees (80.8%), service loans (90.8%) and medical bills (69.2%) and purchase of assets (88.4%). Nearly all the farmers (95.8%) reported that poor yield resulting from the disease really impacted negatively on their social relations with their spouses and in some cases, even extends to the extended family body. Most of them (76.7%) were shy to contact extension agents in times of poor yield. However, few respondents (7.6%) stated that poor yields due to the disease did not negatively influence their relationships with extension agents.

Table 4.8: Socio-economic impacts of a vine-browning and die-back disease of *D. alata* on the livelihoods of farmers in the Nkwanta-North and Krachi-West districts. N = 120.

| Economic and social responsibilities of farmers | Level of Influence* |      |      |     |     |
|-------------------------------------------------|---------------------|------|------|-----|-----|
|                                                 | 1                   | 2    | 3    | 4   | 5   |
| Payment of school fees                          | 80.8**              | 10.0 | 7.5  | 1.7 | 0.0 |
| Payment of service loans                        | 90.8                | 9.2  | 0.0  | 0.0 | 0.0 |
| Payment of medical bills                        | 69.2                | 18.3 | 12.5 | 0.0 | 0.0 |
| Purchase of assets                              | 88.4                | 5.8  | 5.8  | 0.0 | 0.0 |
| Relationship with spouse and neighbours         | 95.8                | 3.4  | 0.8  | 0.0 | 0.0 |
| Relationship with Extension Officers            | 76.7                | 13.3 | 3.4  | 5.0 | 1.6 |

\*1 = Highly negatively influenced; 2 = Negatively influenced; 3 = Neutral; 4= Positively influenced; 5 = Highly positively influenced; \*\* Values represent proportion of farmers.

#### 4.1.5 Symptoms of a vine-browning and die-back disease of *D. alata*

When *D. alata* cultivars infected by a vine-browning and die-back disease were examined at the various farms in the Nkwanta-North and Krachi-West districts during the field survey, different symptoms of the disease were observed. The most common field symptoms were:

- Pin-point spots scattered on the lamina (stage 1);
- Pin-point spots scattered on the lamina and surrounded by yellow halo (stage 2);
- Small brown spot with yellow halo (stage 3);
- Brown regular spots surrounded by a dark brown ring and having a gray center (stage 4);
- Large brown spots bordered by yellow patches and die-back of vines (stage 5); and
- Black spots with dark brown border merging to form lesions and causing defoliation as well as browning of vines (stage 6).

In the advanced stage of the disease, some plants die before maturity leaving the tuber mummified and/or shriveled; harvested tubers decay shortly after storage. In some cases, mycelial mat and acervuli of *Colletotrichum* sp. were found on well defined lesions on leaves, petioles and vines. It was also noted that, some local *D. alata* cultivars, especially ‘Nibultutu’ exhibited black coatings on upper leaf surfaces exposed to the sun (named of ‘yam scorch syndrome’) (Plate 4.1).

Generally, most cultivars in the surveyed areas exhibited the typical characteristic symptoms that start with circular black spots randomly distributed on leaf surfaces, expanding to leaf edge necrosis and then progressing to vine blackening and tip die-back.



Plate 4.1: Stages of development of symptoms of a vine-browning and die-back disease of *D. alata*. Healthy plant (I); pin-point spots on lamina (II); pin-points spots surrounded by yellow halo on lamina (III); small irregular brown spots with yellow halo (IV); brown regular spots with gray centre bordered by dark brown ring (V); large brown spots bordered by yellow patches on leaves and die-back of vines (VI); and browning and/or blackening of vines and defoliation of leaves (VII).

#### 4.1.6 Incidence and severity of a vine-browning and die-back disease of *D. alata* in the Nkwanta-North and Krachi-West districts

The mean incidence of a vine-browning and die-back disease of *D. alata* recorded in the Nkwanta-North (75.3%) was significantly higher ( $P < 0.05$ ) than that of the Krachi-West (55.7%)

(Appendix III) (Table 4.9). However, the mean severity of the disease in the two districts were not significantly different ( $P > 0.05$ ) (Table 4.9).

Table 4.9: Disease incidence and severity of anthracnose disease of *D. alata* in the selected communities of the Nkwanta-North and Krachi-West districts of the Volta Region.

| District      | Community                             | Disease Incidence (%) | Disease Severity |
|---------------|---------------------------------------|-----------------------|------------------|
| Nkwanta-North | Kpasa-Tindani                         | 73.01                 | 1.81             |
|               | Kpasa-Abunyanga                       | 88.17                 | 2.01             |
|               | Bajimpoa-Akura                        | 65.12                 | 1.57             |
|               | <b>Mean</b>                           | <b>75.30</b>          | <b>1.80</b>      |
| Krachi-West   | Osramae                               | 67.14                 | 1.65             |
|               | Gyidaesu                              | 49.11                 | 1.52             |
|               | Ohiamankyene                          | 51.13                 | 1.63             |
|               | <b>Mean</b>                           | <b>55.75</b>          | <b>1.60</b>      |
|               | <b>LSD (<math>P &lt; 0.05</math>)</b> | <b>6.52</b>           | <b>1.46</b>      |

## 4.2 Micro-organisms associated with a vine-browning and die-back disease of *D. alata* in the Volta Region

### 4.2.1 Identification using morpho-cultural characteristics of isoaltes

When *D. alata* leaves and vines exhibiting typical symptoms of yam anthracnose disease collected from the Volta Region of Ghana were cultured on PDA and incubated at  $26 \pm 2$  °C, 70-85% RH for seven days, *C. gloeosporioides* Penz. and Sacc. and *C. truncatum* Andrus and Moore were consistently isolated.

#### 4.2.1.1 *Colletotrichum gloeosporioides*

*C. gloeosporioides* was the micro-organism consistently isolated from lesions of the *D. alata* samples collected from the Volta Region. The morpho-cultural studies revealed different growth patterns, mycelial colours and other distinguishable morphological characters in PDA culture (Plate 4.2). Colony growths of isolates were visible 48 hours after sub-culturing onto freshly prepared PDA. A total of four different growth patterns ranging from concentric with distinct rings to growth with less pronounced rings were observed among isolates (Plate 4.2). Mycelial

colour of isolates ranged from gray to white to dark brown and orange. Colony elevation also ranged from thick floccose aerial mycelia (Cg111) to flat-mat mycelia (Cg114) (Plate 4.2). The isolates sporulated producing abundant and visible fruiting bodies with submerged acervuli. Some isolates sporulated superficially with scattered acervuli while others produced both superficial and submerged sporulation. Mean radial growth of colony on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days was 4.2 cm. Conidia of isolates were cylindrical with obtuse ends, hyaline, aseptate, uninucleate (Plate 4. 3) formed in globose to sparse acervuli.

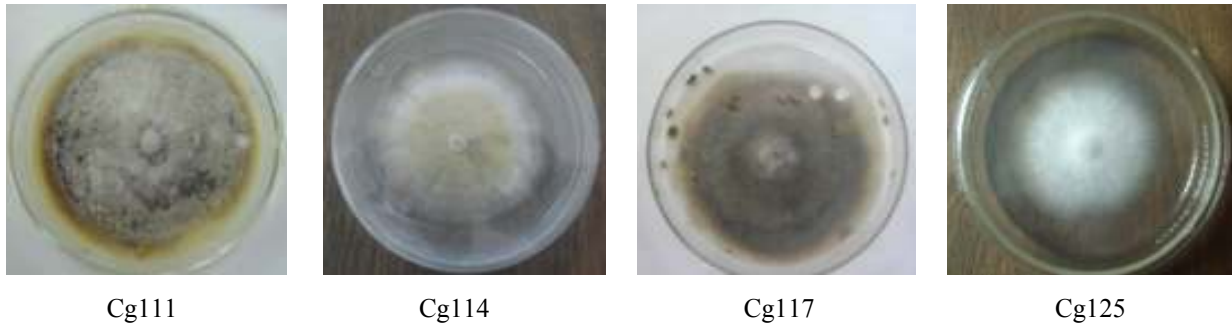


Plate 4.2: Culture characteristic of *C. gloeosporioides* isolates (obtained from diseased *D. alata* in the Volta Region) on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days. Whitish thick floccose aerial mycelial growth with light yellow ring at the border (Cg111); Light-yellowish flat-mat mycelial growth (Cg114); Dark-brown mycelial growth with white pustule at the centre (Cg117); and whitish fluffy mycelia growth with less pronounced rings (Cg125).



Plate 4.3: Morphological characteristics of *C. gloeosporioides* on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days. Conidia – cylindrical with obtuse ends, hyaline, aseptate and uninucleate; Black bar in each panel = 20 µm long.

#### 4.2.1.2 *Colletotrichum truncatum*

*C. truncatum* isolates produced different growth pattern and distinct morphological characters in PDA culture. Colony growths were visible 48 hours after sub-culturing onto PDA. Isolates produced dark/blackish mycelial growth on culture surface with colony margin ranging from uniform

(Ct131) to distinct profuse branching of mycelia (Ct130) (Plate 4.4). The isolates produced abundant superficial sporulation with peculiar black acervuli. Two distinct colony growth patterns ranging from concentric with distinct rings to growth with profused branching were observed among isolates (Plate 4.4). The mean radial mycelial growth of colony on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days was 3.5 cm. Conidia of isotaes were strongly curved, fusiform with acute apex and obtuse base, hyaline, aseptate and uninucleate (Plate 4.5) formed in acervuli ranging from saucer-shaped to sparse type.

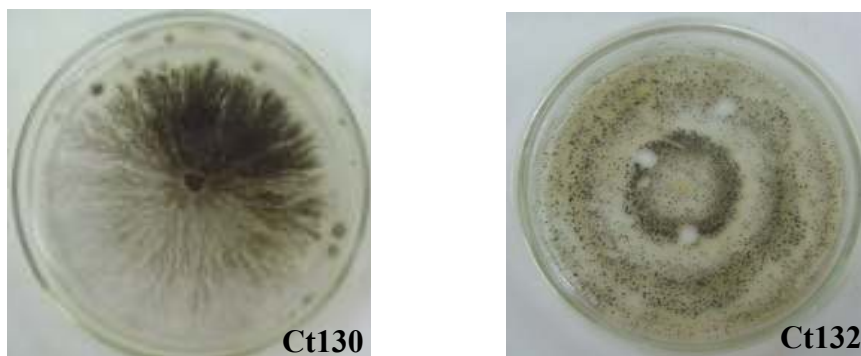


Plate 4.4: Morphological characteristics of *C. truncatum* isolates (obtained from diseased *D. alata* in the Volta Region) on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days. Dark mycelial growth with profuse branching of mycelia (Ct130) and concentric growth with distinct rings and uniform margin (Ct132).

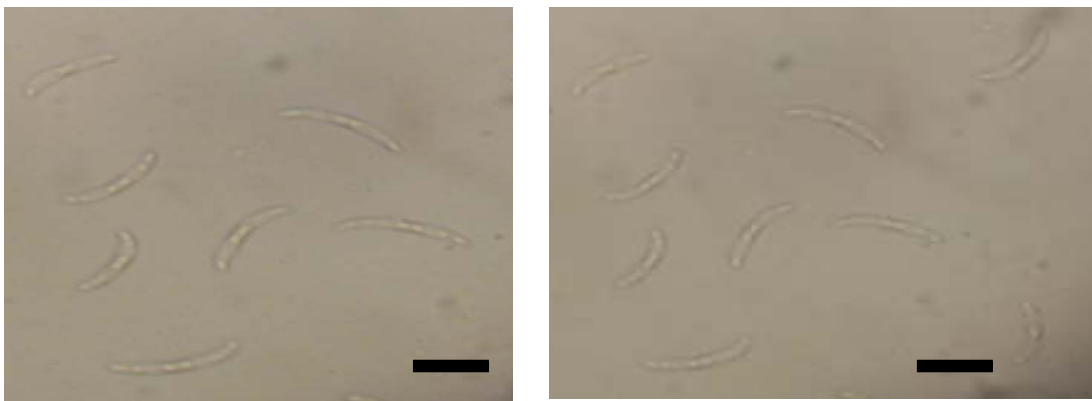


Plate 4.5: Cultural characteristics of *C. truncatum* on PDA incubated at  $26\pm 2$  °C and 70-85% RH for seven days. Conidia – strongly curved, fusiform with acute apex and obtuse base, hyaline, aseptate and uninucleate; Black bar in each panel = 20 µm long.

#### 4.2.2 Molecular identification of isolates

Amplification of the ITS1-5.8s-ITS4 locus with the Oligonucleotide primer pair (ITS1/ITS4) generated PCR products of approximately 600-bp for both the *C. gloeosporioides* (2, 3, 4 and 6)

and *C. truncatum* strains (1 and 5) (Plate 4.6A). Using the ITS4/CgInt species-specific primer pair, PCR products of about 530-bp was generated for all the *C. gloeosporioides* strains (2, 3, 4 and 6) but no amplicon was obtained in *C. truncatum* strains (1 and 5) (Plate 4.6B). The negative controls (C) did not produce any PCR product (Table 4.11).

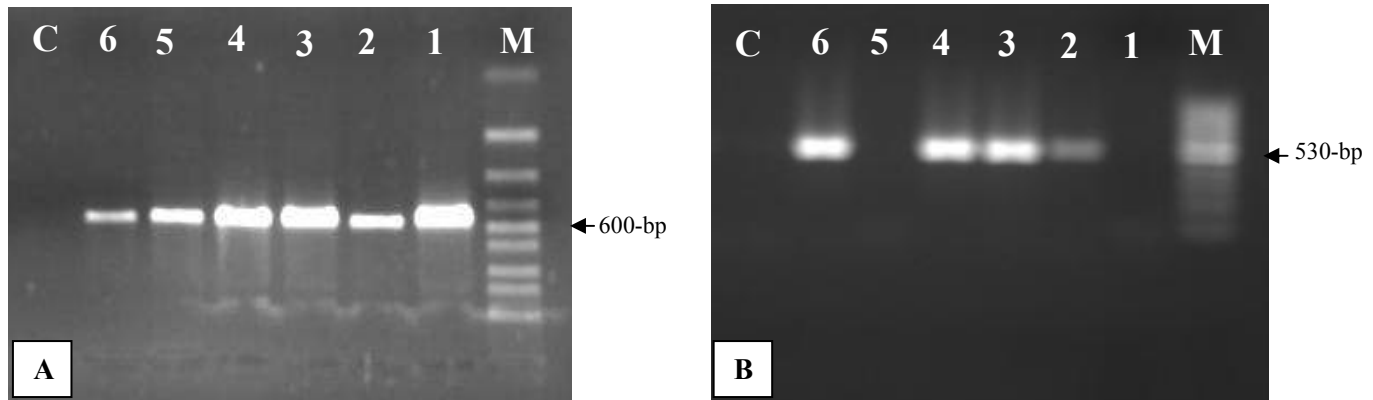


Plate 4.6: Amplified DNA fragments of *C. gloeosporioides* strains (lanes – 2, 3, 4 and 6) and *C. truncatum* strains (lanes – 1 and 5) obtained from diseased *D. alata* in the Volta region using ITS1/ITS4 primer pair (A) and ITS4/CgInt species specific primer pair (B). M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); C = Negative controls (sterile distilled water).

Table 4.11: Detection of DNA fragments of *C. gloeosporioides* and *C. truncatum* strains obtained from diseased *D. alata* plants in the Volta Region using ITS1/ITS4 primer pair and CgInt/ITS4 species-specific primer pair.

| Isolate Code       | Lab ID | ITS1/ITS4 | ITS4/CgInt |
|--------------------|--------|-----------|------------|
| *Ct130             | 1      | +         | -          |
| <sup>a</sup> Cg111 | 2      | +         | +          |
| <sup>a</sup> Cg114 | 3      | +         | +          |
| <sup>a</sup> Cg117 | 4      | +         | +          |
| *Ct132             | 5      | +         | -          |
| <sup>a</sup> Cg125 | 6      | +         | +          |

<sup>a</sup>Cg = *C. gloeosporioides*; \*Ct = *C. truncatum*; (+) = presence of amplified DNA fragment and (-) = Absence of DNA fragment.

#### 4.2.3 Pathogenicity test of isolates from the Volta Region

Pathogenicity test of *Colletotrichum* isolates on two-month old potted tissue culture-derived *D. alata* plants with inoculum suspension of  $1.6 \times 10^6$  conidia/ml carried out in the greenhouse

revealed all isolates to be pathogenic and thus, incited disease lesions true to type (i.e. anthracnose disease symptoms) on the *D. alata* plants (Plate 4.7). The control plants did not show any symptoms of the disease (Plate 4.8). Infection caused by the various isolates appeared similar but re-isolation yielded only the isolate with which the particular *D. alata* cultivar was inoculated. Small, irregular, brown spots with yellow halo symptoms were more often observed on younger than older leaves. Brown, regular spots bordered by dark brown ring with gray center, however, dominated the older leaves with sporadic die-back symptoms.

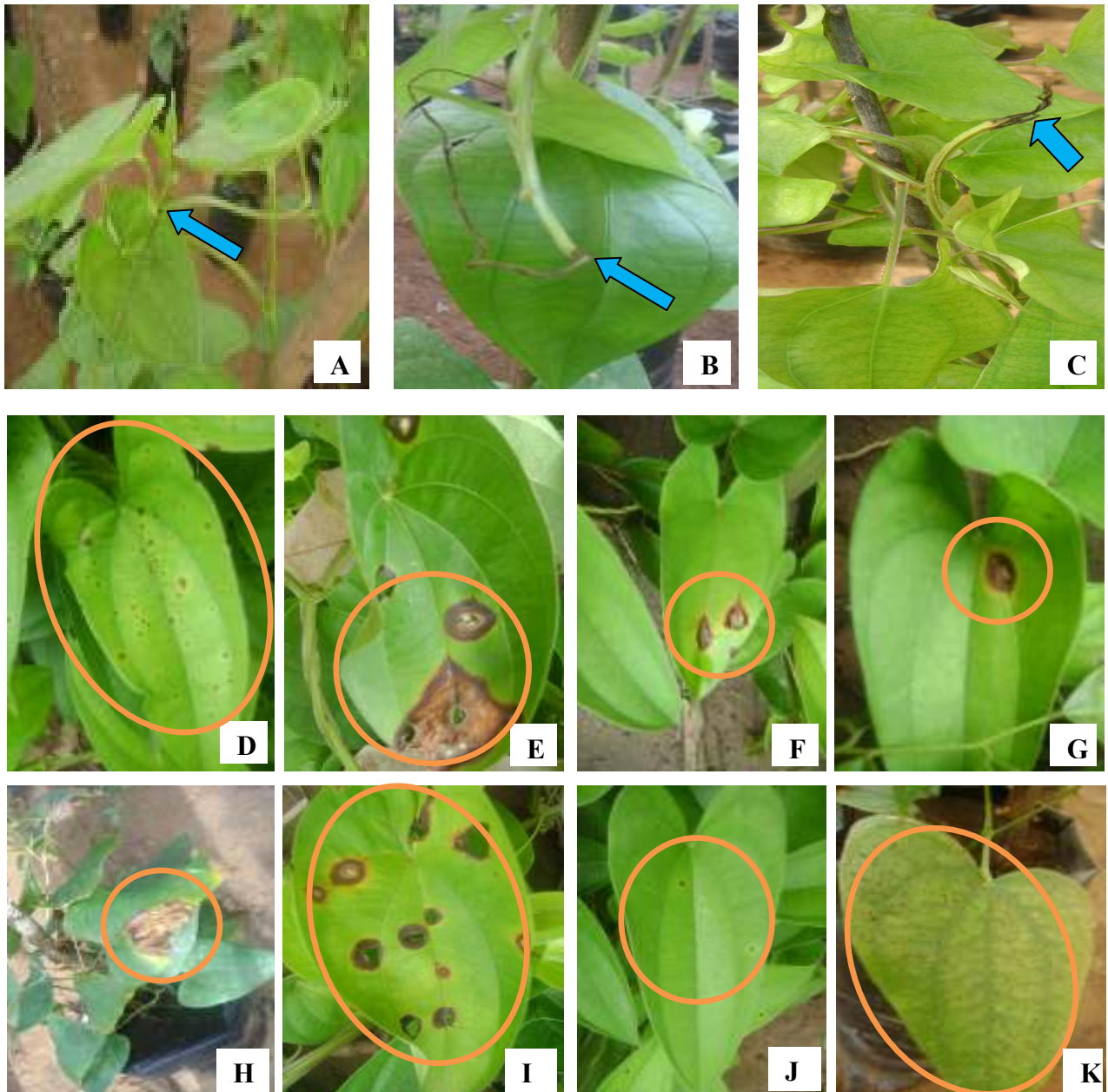


Plate 4.7: Two-month old potted tissue culture-derived whole-*D. alata* plants showing symptoms of anthracnose disease. Die-back (blue arrows) (A-C) and necrotic lesions on leaves (brown rings) (D-G) 13 DAI with *C. gloeosporioides* isolate (Cg111) using spore suspension of  $1.6 \times 10^6$  conidia/ml.



Plate 4.8: Two-month old potted tissue culture-derived whole-*D. alata* plants without symptoms of anthracnose disease. Plants were sprayed with sterile distilled water amended with two drops of Tween 80 (2% v/v) – as controls.

The first evidence of infection was noted on the 3<sup>rd</sup> day after incubation (DAI) in cultivar TDa 008 whilst cultivar TDa 010 exhibited disease symptoms on the 4<sup>th</sup> DAI. The symptoms caused by the two strains of *C. gloeosporioides* and the *C. truncatum* isolate were undistinguishable at the initial stage of infection. The lesion first appeared as pin-point spots scattered on the lamina and later marked as brown, regular spots bordered by dark brown rings and gray center. However, as the infection advanced, three clearly discernible foliage symptoms were detected: (1) brown regular spots surrounded by dark brown ring with gray center; (2) large brown spots bordered by yellow patches; and (3) die-back. In *D. alata* cultivar TDa008, Ct132 isolate caused significantly higher ( $P < 0.005$ ) disease with mean whole-plant area infection of 17.3% than isolate Cg125 with a mean whole-plant area infection of 10.8% (Table 4.12). However, between isolates Cg111 and Cg125, there was no difference. In cultivar TDa010, Ct132 caused significantly higher ( $P < 0.005$ ) disease with mean whole-plant area infection of 11.3% than isolate Cg125 with a mean whole-plant area infection of 8.9%. Isolate Cg111 recorded significantly higher ( $P < 0.005$ ) mean whole-plant area infection of 13.0% in TDa 008 than in TDa010 (9.1%). Nonetheless, Cg111 was not significantly ( $P > 0.005$ ) virulent from Cg125 in both cultivars (TDa 008 than in TDa010) (Appendix IV).

Table 4.12: Disease infection (%) by *Colletotrichum* isolates on two-month old potted tissue-cultured *D. alata* cultivars (TDa008 and TDa010).

| <i>D. alata</i> Cultivar               | <i>Colletotrichum</i> Isolate |             |             | Control (SDW*) | LSD ( $P < 0.05$ ) |
|----------------------------------------|-------------------------------|-------------|-------------|----------------|--------------------|
|                                        | Cg111                         | Cg125       | Ct132       |                |                    |
| TDa008                                 | 13.0                          | 10.80       | 17.30       | 0.00           | <b>2.90</b>        |
| TDa010                                 | 9.1                           | 8.91        | 11.32       | 0.00           | <b>1.30</b>        |
| <b>LSD (<math>P &lt; 0.005</math>)</b> | <b>1.80</b>                   | <b>1.41</b> | <b>2.62</b> | <b>0.00</b>    |                    |

\*SDW = Sterile distilled water.

The varied strains of *C. gloeosporioides* and *C. truncatum* revealed in the culture studies necessitated the need to genetically characterize them in order to resolve the systematic issues that existed among them.

### 4.3 Characterization of the strains of *C. gloeosporioides* and *C. truncatum* causing anthracnose disease of *D. alata* in Ghana

#### 4.3.1 Diversity in *C. gloeosporioides* and *C. truncatum* causing anthracnose disease of *D. alata* in Ghana

*C. gloeosporioides* and *C. truncatum* were consistently isolated from the *D. alata* samples which exhibited typical symptoms of anthracnose disease collected from the six regions of Ghana (Volta, Ashanti, Brong-Ahafo, Central, Eastern and Northern Regions). A total of 18 different strains of *C. gloeosporioides* and four of *C. truncatum* were obtained which exhibited varied growth patterns in PDA culture. Out of the 18 strains of *C. gloeosporioides* found, a total of four were obtained from the Volta, three from Ashanti, three from Brong-Ahafo, two from Central two from Eastern and four from Northern Region. In the case of *C. truncatum*, two strains were isolated from the Volta, one from Central and one from the Eastern Region.

##### 4.3.1.1 Diversity in growth patterns of *C. gloeosporioides* and *C. truncatum* strains

###### 4.3.1.1.1 *Colletrichum gloeosporioides*

A total of 18 different growth patterns of *C. gloeosporioides* ranging from concentric growth with distinct rings to growth with less pronounced rings were observed on PDA (Plate 4.9). Mycelial colour ranged from white to gray to dark brown and orange. Colony elevation also ranged from thick floccose aerial mycelia to fluffy and flat. All isolates sporulated within 3 to 10 days with either copious or scarce conidia. Some isolates sporulated superficially with scattered acervuli while some too exhibited both superficial and submerged sporulations.

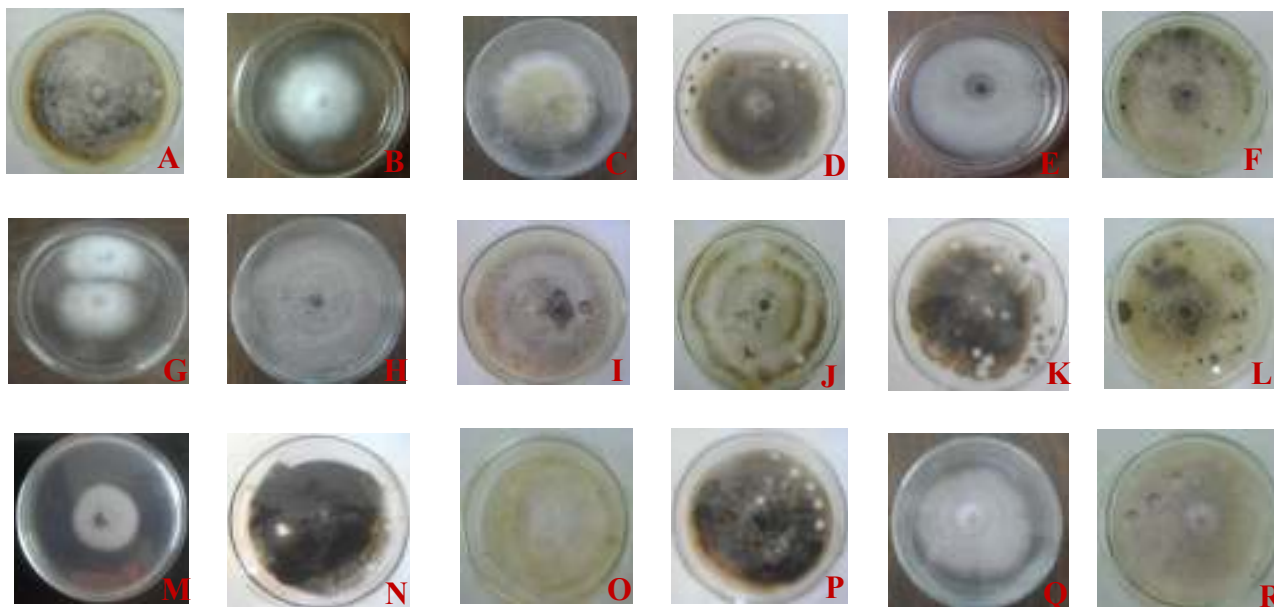


Plate 4.9: Different growth patterns of *C. gloeosporioides* strains on PDA at  $25\pm 2$  °C, 7 days. Volta strains (A-D); Ashanti strains (E-G); Central strains (H and I); Eastern strains (J and L); Brong-Ahafo strains (M-O); and Northern strains (P-R).

#### 4.3.1.1.2 *C. truncatum*

Four different colony growth patterns of *C. truncatum* ranging from concentric growth with distinct rings to growth with profused branching were observed (Plate 4.10). The strains produced either dark/blackish or salmon mycelial growth on culture surface with colony margin ranging from uniform to distinct profuse branching of mycelia. There was production of abundant superficial sporulation with peculiar black acervuli (Plate 4.10).

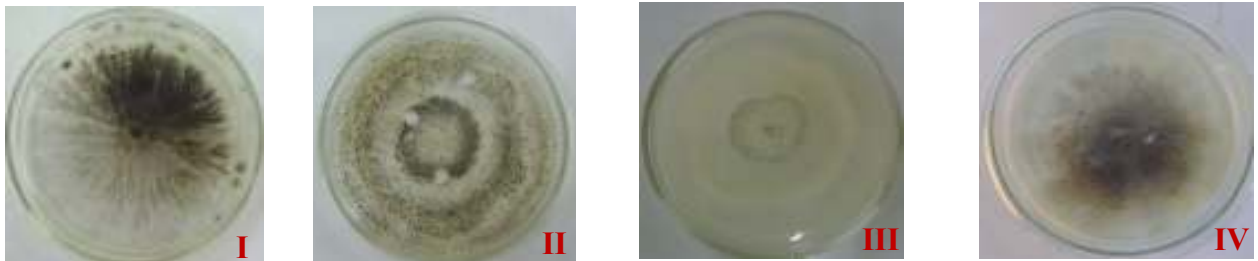


Plate 4.10: Different growth patterns of *C. truncatum* strains on PDA at 25±2 °C, 7 days. Volta strains (I and II); Ashanti strains (E-G); Central strain (III); Eastern strain (IV).

#### 4.3.1.2 Diveristy in setae and acervuli formation of *C. gloeosporioides* and *C. truncatum* strains

##### 4.3.1.2.1 *C. gloeosporioides*

Setae formed in some selected strains of *C. gloeosporioides* ranged from highly profused to sparse, dark brown to black, slightly curved to straight, 1-4 septate, swollen at the base and tapering towards the apex measuring 55-174 µm long (Plate 4.11A). However, in some strains there was no setae formation. Acervuli formed also ranged from round to irregular, globose to saucer-shaped measuring 50-260 µm in diameter (Plate 4.11B).

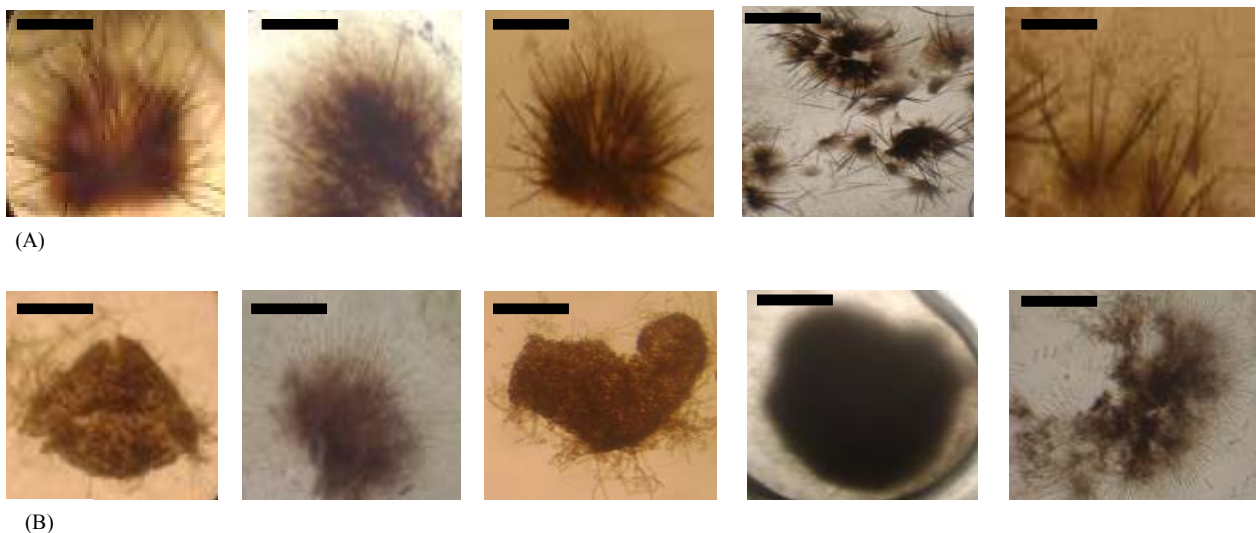


Plate 4.11: Different formation of setae and acervuli of *C. gloeosporioides* strains obtained from diseased *D. alata* plants – setae (A) and acervuli (B). Black bars in panels = 60 µm.

#### 4.3.1.2.2 *C. truncatum*

Setae formed in *C. truncatum* strains ranged from sparse to profuse, black to dark brown, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex measuring 50-190  $\mu\text{m}$  long (Plate 4.12a-c). Acervuli formed ranged from setose to saucer-shaped measuring 70-190  $\mu\text{m}$  in diameter (Plate 4.12d-e).

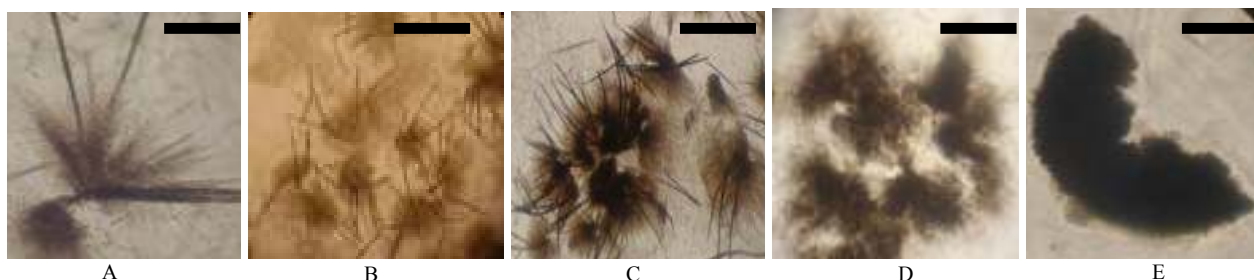


Plate 4.12: Different formation of setae and acervuli of *C. truncatum* strains obtained from diseased *D. alata* plants – setae (A-C) and acervuli (D and E). Black bars in panels = 60  $\mu\text{m}$ .

#### 4.3.1.3 Diveristy in radial mycelial growth and pathogenicity test of some selected strains of *C. gloeosporioides*

Radial mycelial growth of the 10 selected strains of *C. gloeosporioides* on PDA incubated for seven days at  $26 \pm 2^\circ\text{C}$  and 70-85% RH in 12/12 h light/dark cycle increased progressively with time (i.e. duration of incubation) (Fig. 4.6). The growth ranged from 2.3 cm (Cg169) to 4.4 cm (Cg141 and Cg162) on day 7. At day 2, there were no significant differences ( $P > 0.05$ ) in mycelial growths among the 10 strains. However, at day 3, 4, 5, 6 and 7, strain Cg141 produced significantly higher ( $P < 0.05$ ) mycelial growth of 2.5, 3.4, 4.0, 4.2 and 4.4 cm respectively compared with strains Cg169 (0.9, 1.1, 1.4, 1.6 and 1.8 cm) and Cg151 (1.0, 1.2, 1.5, 1.7 and 1.9 cm) (Fig 4.6). Strain Cg162 initially grew slowly but at day 7, it produced the highest radial mycelial growth (4.4 cm).

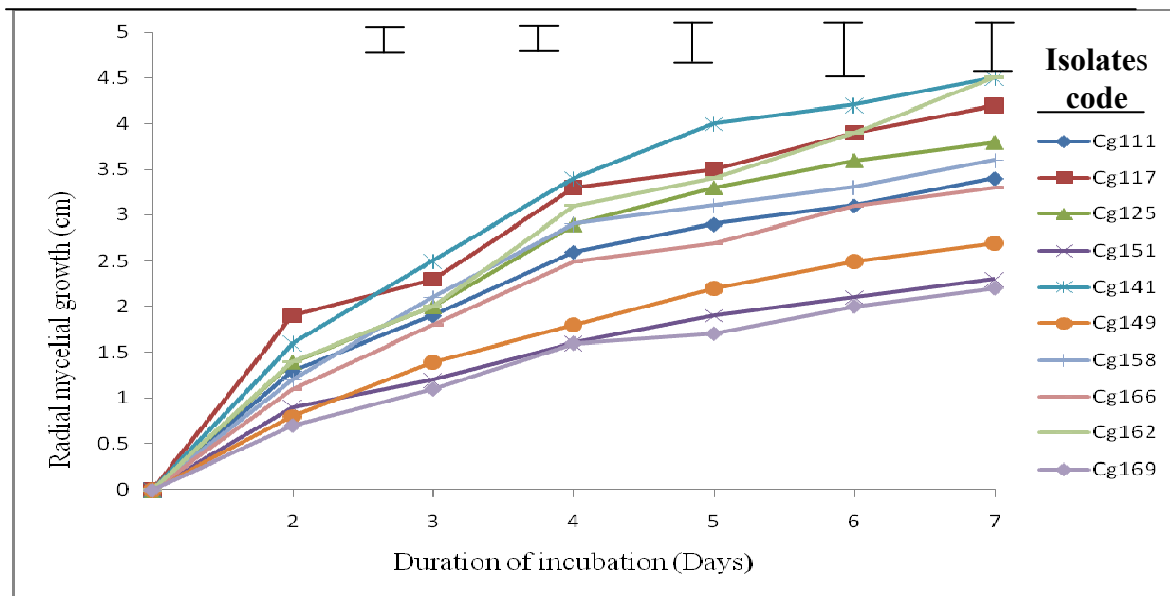




Fig. 4.6: Radial mycelial growth of 10 selected *C. gloeosporioides* strains on PDA incubated at  $26 \pm 2$  °C, 70-85% RH for seven days in 12/12 h light/dark cycle. Vertical bars indicate LSD at 5%.


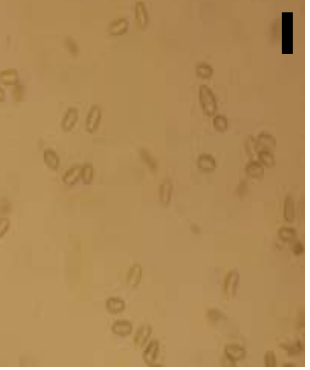

All the *Colletotrichum* isolates (Cg146, Cg147, Cg149, Cg151, Cg165 and Ct168) used in the pathogenicity test proved pathogenic but to varying levels. Isolate Ct168 produced significant ( $P < 0.05$ ) higher disease severity (2.1) compared with Cg146 (1.3) and Cg147 (1.4). However, there were no significant ( $P > 0.05$ ) differences among the isolates Cg149, Cg151, Cg165 and Ct168.



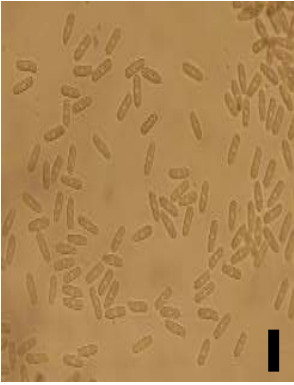



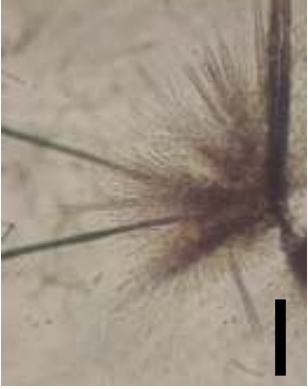

#### 4.3.1.4 Distinguishable morphological and cultural characteristics of the 22 *Colletotrichum* isolates from anthracnose disease of *D. alata* in Ghana



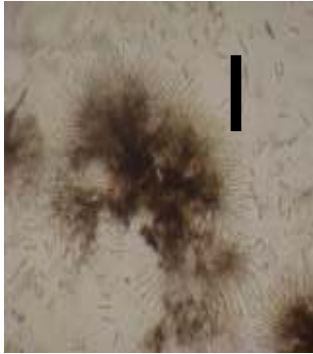
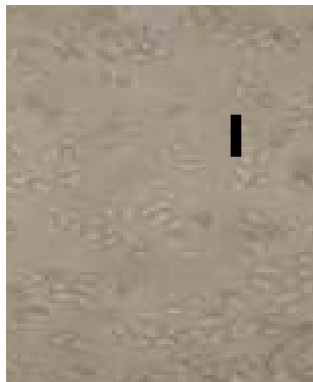

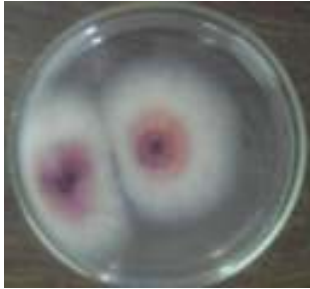


The macro and microscopic studies of each of the 22 *Colletotrichum* isolates revealed varied growth patterns, mycelial colours and other distinguishable morphological and cultural characters in PDA culture (Table 4.14; 92-101 pp). Conidial measurements ranged from  $2.0\text{-}13\ \mu\text{m} \times 1\text{-}4\ \mu\text{m}$  in isolate Cg111 to  $15.5\text{-}24\ \mu\text{m} \times 3.5\text{-}4\ \mu\text{m}$  (Ct130). Average radial growth of 7-day old PDA cultures of isolates incubated at  $26 \pm 2$ °C and 70-85% RH ranged between 2.2 to 4.5 cm. Setae and acervuli were present in some isolates with varying characters.









Tabel 4.14 (86-96 p): Distinguishable morphological and cultural characteristics of the 22 *Colletotrichum* spp. isolated from anthracnose disease of *D. alata* in Ghana.



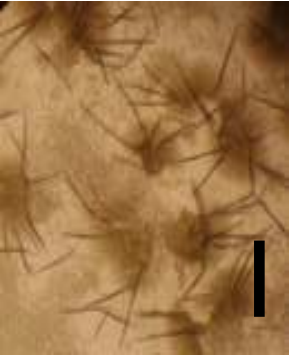



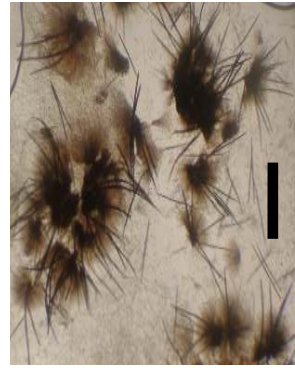
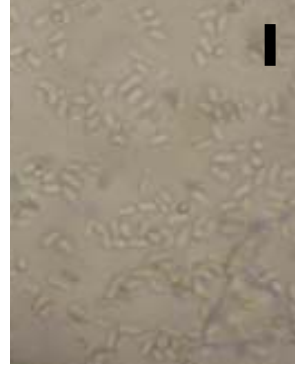
| Source                  | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                         | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Kpasa-Tindani,<br>V/R*  | Cg 111       | <p><b>Top</b><br/>Fluffy white growth with an outer light yellow growth ring on surface and submerged sporulation. Dark brown growth surrounded by light brown ring on underside of colony. <b>Radial growth:</b> 4.2 cm.</p> <p><b>Underside</b></p>                                           |  <p>Conidia: x 400</p> <p><b>Setae:</b> Present – sparse to profuse, dark brown to black, straight to slightly curved, 1–4 septate swollen at the base and tapering towards the apex, 55–168 µm long. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 6–20 µm x 2–6 µm, formed in globose acervuli. Acervulus round to elongated 62–230 µm in diameter.</p> |
| Kpasa-Abunyanga,<br>V/R | Cg 117       | <p><b>Top</b><br/>Dark gray mycelia with less pronounced rings on the surface with white pustule at the centre and submerged sporulation. Underside of colony with pronounced dark brown ring growths and white growth at the centre. <b>Radial growth:</b> 3.9 cm.</p> <p><b>Underside</b></p> |  <p>Conidia: x 400</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 12–23 µm x 1–4 µm, formed in setose acervuli. Acervuli setose measuring 50–210 µm in diameter.</p>                                                                                                                                                         |



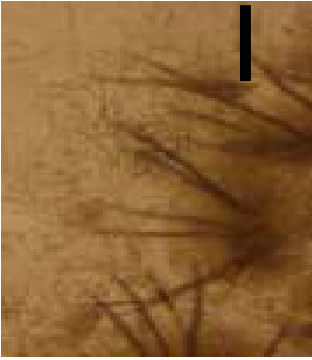



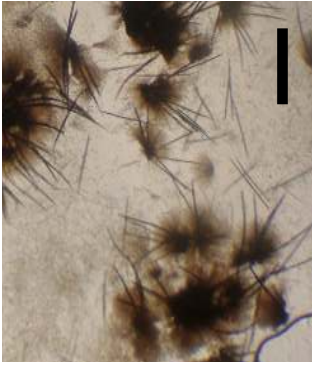

| Source                 | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|------------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bajimpoa-Akura,<br>V/R | Cg 125       |  <p>Top</p>  <p>Underside</p> <p>Whitish fluffy mycelia growth with less pronounced growth rings on the surface and submerged sporulation. Underside exhibits whitish growth with light brown centre. <b>Radial growth:</b> 2.6 cm</p>                                                                                 |  <p>Conidia: x 400</p>  <p>Round acervulus: x 400</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 2-13 µm x 1-4 µm, formed in round acervuli with diameter ranging from 80-260 µm.</p>                                                                                                                                                       |
| Osramae,<br>V/R        | Ct 132       |  <p>Top</p>  <p>Underside</p> <p>Salmon-grey, fast concentric growths with more pronounced superficial sporulation on surface but less pronounced mycelia producing numerous sclerocia in culture. Underside of colony with dark ring growth with pronounced submerged sporulation. <b>Radial growth:</b> 4.4 cm</p> |  <p>Setae: x 400</p>  <p>Conidia: x 400</p> <p><b>Setae:</b> Present – profused, black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex measuring 50-120 µm long. <b>Conidia:</b> Strongly curved, fusiform with acute apex and obtuse base, hyaline, aseptate, uninucleate, 15-24 µm x 3.5-4 µm, formed in setose acervuli of 65-170 µm in diameter.</p> |



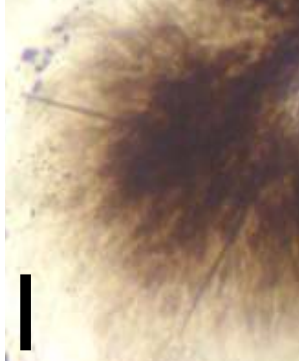
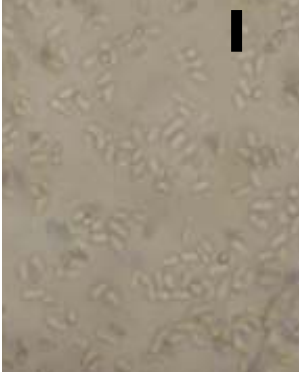




| Source               | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|----------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gyidaesu,<br>V/R     | Cg 114       |  <p>Top</p>  <p>Underside</p> <p>Light yellowish growth at the centre surrounded with whitish ring of mycelia, slow growth, less distinct concentric rings on surface whilst underside with yellowish-green colour bordered with white ring and production of submerged distinct dark-brown conidial pustules at the central region. <b>Radial growth:</b> 2.5 cm.</p> |  <p>Conidia: x 400</p>  <p>Acervulus: x 400</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> Cylindrical with rounded ends, hyaline, aseptate, uninucleate, 4-20 <math>\mu\text{m}</math> x 2-5 <math>\mu\text{m}</math>, formed in brownish saucer-shaped acervuli of diameter, 60-235 <math>\mu\text{m}</math>.</p>                                                                                                                                                                                                                  |
| Ohiamankyene,<br>V/R | Ct 130       |  <p>Top</p>  <p>Underside</p> <p>Dark/blackish mycelial growth on both culture surface and underside, slow growth, distinct profuse branching of mycelia, abundant superficial sporulation of peculiar black acervuli. <b>Radial growth:</b> 3.5 cm.</p>                                                                                                             |  <p>Setae: x 400</p>  <p>Conidia: x 400</p> <p><b>Setae:</b> Present – profuse, dark brown, straight, 1-4 septate, swollen at the base and tapering towards the apex measuring 65-190 <math>\mu\text{m}</math> long. <b>Conidia:</b> Strongly curved, fusiform (tapering towards both ends) with acute apex and obtuse base, hyaline, aseptate, uninucleate, 15.5-24 <math>\mu\text{m}</math> x 3.5-4 <math>\mu\text{m}</math>, formed in dark saucer-shaped acervuli measuring 70-190 <math>\mu\text{m}</math> in diameter.</p> |

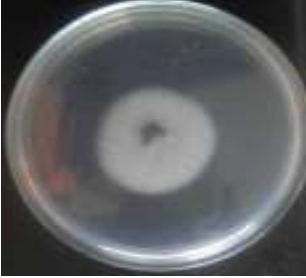





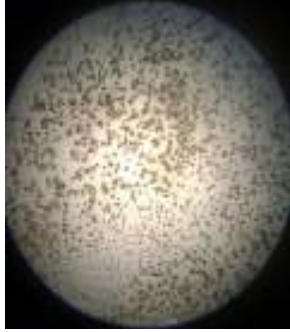
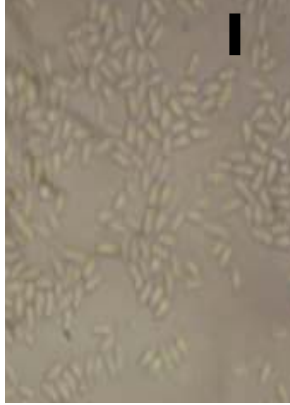
| Source                 | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                       |                                                                                     | Morphology of conidia, mycelia and fruiting bodies                                 |                                                                                    |
|------------------------|--------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| New Edubiase, A/R*     | Cg 151       |                                                                                                                                                                                                                                            |   |   |   |
|                        |              | Top                                                                                                                                                                                                                                                                                                                           | Underside                                                                           | Irregular Acervulus: x 400                                                         | Conidia: x 400                                                                     |
|                        |              | <p>Grayish yellow growth with thick floccose aerial mycelia and dark conidial pustules at the centre and superficial sporulation. Underside of colony with less pronounced light brown rings interspersed with dark conidial pustules and pronounced dark centre and submerged sporulation. <b>Radial growth:</b> 4.5 cm.</p> |                                                                                     |                                                                                    |                                                                                    |
| CSIR-CRI, Fumesua, A/R | Cg 158       |                                                                                                                                                                                                                                           |  |  |  |
|                        |              | Top                                                                                                                                                                                                                                                                                                                           | Underside                                                                           | Conidia: x 400                                                                     | Aerial mycelia with Sporulation: (arrow) x 400                                     |
|                        |              | <p>Whitish cottony growth with thick floccose aerial mycelia, less pronounced growth rings on surface and aerial sporulation within cottony mycelial growth. Underside of colony with distinct central rosy buff zonations surrounded with whitish ring of mycelia. <b>Radial growth:</b> 2.6 cm.</p>                         |                                                                                     |                                                                                    |                                                                                    |




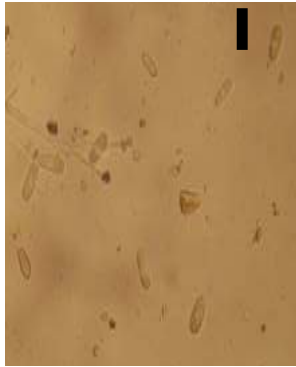


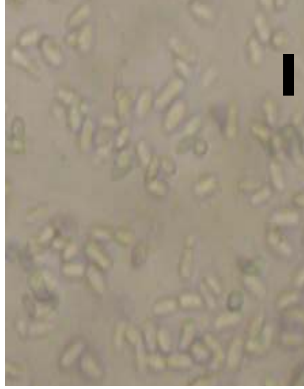
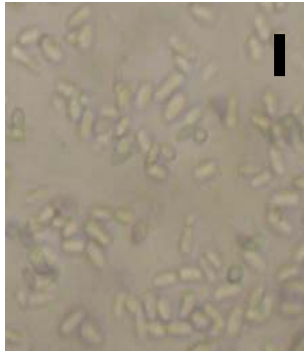
| Source             | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------|--------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Bronikurom,<br>A/R | Cg 147       |  <p>Top</p>  <p>Underside</p> <p>Whitish-gray cottony fast growth with less pronounced rings on surface having dark-gray pustule at the centre. Underside of colony with light gray to brown pronounced growth rings bordered with whitish region. <b>Radial growth:</b> 4.5 cm.</p>                                           |  <p>Conidia: x 400</p>  <p>Acervulus: x 400</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> Cylindrical with round ends, hyaline, aseptate, binucleate, 4-23 µm x 2-5 µm, formed in dark saucer-shaped acervuli. Acervulus elongated measuring 60-180 µm in diameter.</p>           |
| Bawjiase,<br>C/R*  | Cg 141       |  <p>Top</p>  <p>Underside</p> <p>Grayish fluffy growth with less pronounced concentric rings on the surface, submerged sporulation, black mass at the centre. Underside with light gray to white pronounced growth with less distinct dark rings and black conidial pustule at the centre. <b>Radial growth:</b> 4.4 cm.</p> |  <p>Acervulus: x 400</p>  <p>Conidia: x 400</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4.5-24 µm x 3.5-4 µm, formed in brown to dark setose acervuli. Acervulus irregular measuring 55-170 µm in diameter.</p> |









| Source                 | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                  | Morphology of conidia, mycelia and fruiting bodies                                                 |                                                                                                      |
|------------------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Odumasi,<br>C/R        | Ct 142       | <br>Top                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <br>Underside  | <br>Setae: x 400  | <br>Conidia: x 400  |
|                        |              | <p>Salmon growth with scanty mycelia, pronounced ring growths on both surface and underside of colony. Abundant superficial sporulation appearing as dark ring at the centre for both the surface and the reverse of colony. <b>Radial growth:</b> 2.7 cm.</p> <p><b>Setae:</b> Present – sparse to profuse, dark brown, straight to slightly curved, 1-3 septate, swollen at the base and tapering towards the apex, 40-70 µm long. <b>Conidia:</b> Weakly curved, fusiform (tapering towards both ends) with acute apex and obtuse base, hyaline, aseptate, uninucleate, 16-25 µm x 3-4 µm, formed in brown setose acervuli measuring 70-190 µm in diameter.</p> |                                                                                                  |                                                                                                    |                                                                                                      |
| Assin-Edubiase,<br>C/R | Cg 143       | <br>Top                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <br>Underside | <br>Setae: x 400 | <br>Conidia: x 400 |
|                        |              | <p>Smoky gray colony with thick floccose aerial mycelia and dark conidial pustules at the centre. Underside of colony with distinct olivaceous gray zonation alternated with dark buff zonation and pronounced superficial and submerged sporulation. <b>Radial growth:</b> 4.4 cm.</p> <p><b>Setae:</b> Present – profuse, black, slightly curved, 1-4 septate, swollen at base and tapering towards the tip, 50-150 µm long. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4-20 µm x 2.5-4 µm, formed in dark setose acervuli. Acervulus irregular measuring 65-170 µm in diameter.</p>                                          |                                                                                                  |                                                                                                    |                                                                                                      |

| Source               | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                    |                                                                                                  | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                                                    |                                                                                                      |
|----------------------|--------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| PGRRI-Bunso,<br>E/R* | Cg 167       | <br>Top                                                                                                                                                                                 | <br>Underside  | <br>Setae: x 400                                                                                                                                                                                                                                     | <br>Conidia: x 400  |
|                      |              | <p>Dark gray mycelial growth with serrated-end growth and interspersed with white pustules on the surface and submerged sporulation. Underside of colony with ring of dark brown growth separating rings of two light brown growths.<br/><b>Radial growth:</b> 3.7 cm.</p> |                                                                                                  | <p><b>Setae:</b> Present – sparse, dark brown, straight to slightly curved, 1–4 septate, swollen at base and tapering towards the apex, 65–175 µm long. <b>Conidia:</b> Cylindrical with obtuse ends, hyaline, aseptate, ununucleate, 15–22 µm x 3.5–5 µm, formed in brown irregular acervuli measuring 60–180 µm in diameter.</p>    |                                                                                                      |
| Asesewa,<br>E/R      | Ct 168       | <br>Top                                                                                                                                                                                | <br>Underside | <br>Setae: x 400                                                                                                                                                                                                                                    | <br>Conidia: x 400 |
|                      |              | <p>Light yellowish brown mycelia, fast growth, distinct concentric rings, abundant sporulation, submerged and superficial production of distinct brown acervuli on both surface and underside.<br/><b>Radial growth:</b> 4.3 cm.</p>                                       |                                                                                                  | <p><b>Setae:</b> Present – profuse, black, straight, 1–4 septate, swollen at base and tapering towards the end, 50–145 µm long. <b>Conidia:</b> Strongly curved, fusiform with acute apex and obtuse base, hyaline, aseptate, ununucleate, 13.5–23 µm x 2.5–4 µm, formed in dark setose acervuli measuring 60–170 µm in diameter.</p> |                                                                                                      |

| Source         | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                        |                                                                                     | Morphology of conidia, mycelia and fruiting bodies                                 |                                                                                    |
|----------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|
| Sekesoa, E/R   | Cg 169       |                                                                                                                                                                                                                                                             |   |   |   |
|                |              | Top                                                                                                                                                                                                                                                                                                                                            | Underside                                                                           | Setae: x 400                                                                       | Conidia: x 400                                                                     |
|                |              | Dark brown mycelia, slow growth with abundant superficial sporulation on the surface. Underside of colony with dark brown with submerged sporulation. <b>Radial growth:</b> 2.2 cm.                                                                                                                                                            |                                                                                     |                                                                                    |                                                                                    |
| Tanoso, B-A/R* | Cg 162       |                                                                                                                                                                                                                                                            |  |  |  |
|                |              | Top                                                                                                                                                                                                                                                                                                                                            | Underside                                                                           | Conidial pustules of colony: x 400                                                 | Conidia: x 400                                                                     |
|                |              | Light yellowish gray growth with light floccose aerial mycelia interspersed with dark conidial pustules and a light brown ring at the centre with superficial sporulation. Underside of colony with light yellow with grayish brown centre interspersed with dark pustules of conidia and submerged sporulation. <b>Radial growth:</b> 4.5 cm. |                                                                                     |                                                                                    |                                                                                    |

| Source              | Isolate Code | Culture characteristics                                                                                                                                                                           |                                                                                                  | Morphology of conidia, mycelia and fruiting bodies                                                                                                                                                                                                                                                  |                                                                                                      |
|---------------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Twumia,<br>B-A/R    | Cg 165       | <br>Top                                                                                                        | <br>Underside  | <br>Acervulus: x400                                                                                                                                                                                                | <br>Conidia: x 400  |
|                     |              | White gray, slow growth with submerged dark round to irregular acervuli and no distinct ring growth on both surface and underside. <b>Radial growth:</b> 2.2 cm.                                  |                                                                                                  | <b>Setae:</b> Absent. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4-20 $\mu\text{m}$ x 3-4 $\mu\text{m}$ , formed in dark globose acervuli measuring, 70-230 $\mu\text{m}$ in diameter.                                                                           |                                                                                                      |
| Sunkundei,<br>B-A/R | Cg 166       | <br>Top                                                                                                       | <br>Underside | <br>Conidial pustules: x 400                                                                                                                                                                                      | <br>Conidia: x 400 |
|                     |              | Dark gray mycelial growth, less pronounced growth rings with submerged sporulation. Dark brown growth on underside interspersed with small-sized conidial pustules. <b>Radial growth:</b> 3.3 cm. |                                                                                                  | <b>Setae:</b> Absent. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 2-19 $\mu\text{m}$ x 2-4 $\mu\text{m}$ , formed in setose or globose acervuli or on solitary phialides on mycelium. Acervulus round to elongated to irregular 65-190 $\mu\text{m}$ in diameter. |                                                                                                      |

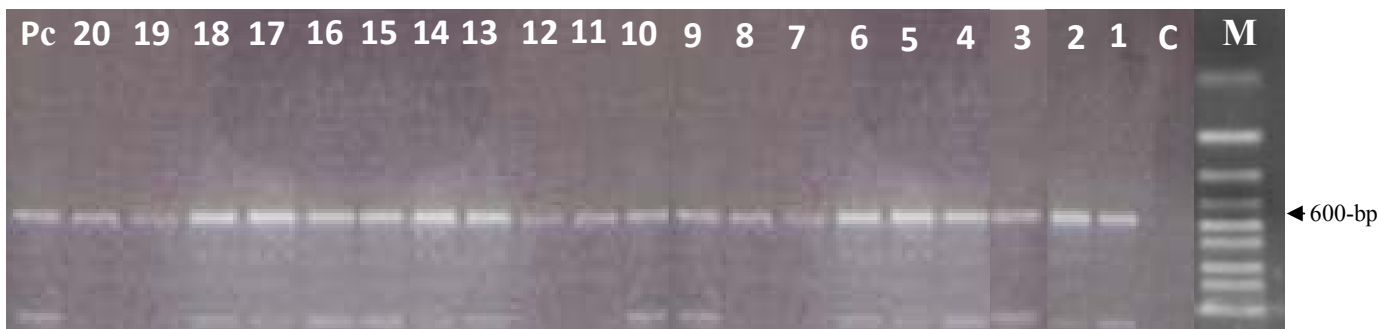
| Source          | Isolate Code | Culture characteristics                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                  | Morphology of conidia, mycelia and fruiting bodies                                                   |                                                                                                      |
|-----------------|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| SARI,<br>N/R*   | Cg 146       | <br>Top                                                                                                                                                                                                                                                                                                                                                                                                                                     | <br>Underside  | <br>Setae: x 400    | <br>Conidia: x 400  |
|                 |              | <p>Smoky gray colony with light floccose aerial mycelia and white pustule at the centre. Underside of colony with distinct rosy-coloured mycelia.<br/> <b>Radial growth:</b> 4.2 cm.</p> <p><b>Setae:</b> Present – highly profused, dark brown, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, 60-180 µm long. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4-21 µm x 3-4.5 µm, formed in setose acervuli measuring 60-190 µm in diameter.</p> |                                                                                                  |                                                                                                      |                                                                                                      |
| Nyariga,<br>N/R | Cg 148       | <br>Top                                                                                                                                                                                                                                                                                                                                                                                                                                    | <br>Underside | <br>Conidia: x 400 | <br>Conidia: x 400 |
|                 |              | <p>Whitish gray fluffy mycelia growth with less pronounced rings growth on the surface. Underside with white gray growth with light rosy centre. <b>Radial growth:</b> 3.7 cm.</p> <p><b>Setae:</b> Absent. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 2.5-18 µm x 3-5 µm, formed in setose acervuli measuring 60-170 µm in diameter.</p>                                                                                                                                                   |                                                                                                  |                                                                                                      |                                                                                                      |

| Source        | Isolate Code | Culture characteristics                                                                                                                                                                                                                           |                                                                                     | Morphology of conidia, mycelia and fruiting bodies                                 |                                                                                    |                                                                                                                                                                                                                                                                                                                                  |
|---------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tolon,<br>N/R | Cg 149       |                                                                                                                                                                |   |   |   |                                                                                                                                                                                                                                                                                                                                  |
|               |              | Top                                                                                                                                                                                                                                               | Underside                                                                           | Setose acervulus: x 400                                                            | Conidia: x 400                                                                     |                                                                                                                                                                                                                                                                                                                                  |
|               |              | Dark gray colony with black pustule at the centre and superficial sporulation at the surface. Underside of colony with distinct brownish colour and submerged sporulation with dark centre. <b>Radial growth:</b> 3.8 cm.                         |                                                                                     |                                                                                    |                                                                                    | <b>Setae:</b> Absent. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 6-23 µm x 3.5-5 µm, formed in setose acervuli measuring 70-230 µm in diameter.                                                                                                                                               |
| Bole, N/R     | Cg 150       |                                                                                                                                                               |  |  |  |                                                                                                                                                                                                                                                                                                                                  |
|               |              | Top                                                                                                                                                                                                                                               | Underside                                                                           | Setae: x 400                                                                       | Conidia: x 400                                                                     |                                                                                                                                                                                                                                                                                                                                  |
|               |              | Whitish light mass mycelia, bordered with light yellow to gray ring towards the end on the surface with submerged sporulation. Underside of colony with light rosy interspersed with light dark pustules of conidia. <b>Radial growth:</b> 3.6 cm |                                                                                     |                                                                                    |                                                                                    | <b>Setae:</b> Present – highly profused, dark brown, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, 50-170 µm long. <b>Conidia:</b> cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 13-23 µm x 3-5 µm, formed in irregular acervuli measuring 60-180 µm in diameter. |

\* V/R = Volta region, A/R = Ashanti region, C/R = Central region, E/R = Eastern region, B-A/R = Brong-Ahafo region and N/R = Northern region. Black bar in each panel of conidia measures 20 µm whilst that of setae and acervuli measures 60 µm. Radial growth of isolates was recorded on the 7<sup>th</sup> day of incubation at 26±2°C, 70-85% RH.

#### 4.3.1.5 Molecular characterization of *Colletotrichum* spp. obtained from anthracnose disease of *D. alata* in the six regions of Ghana

Of the 22 isolates obtained from the six regions (Ashanti, Brong-Ahafo, Central, Eastern, Northern and Volta Regions), 20 were used in the genome analysis since two of the *C. gloeosporioides* strains exhibited similar morpho-cultural characters. In the ITS1/ITS4 primer pair analysis, amplification of the ITS1-5.8s-ITS4 locus of all the 20 *Colletotrichum* isolates generated PCR products of approximately 600-bp on agarose gel (Plate 4.13a) as was indicated in section 4.2.2. However, ITS4/CgInt species specific primer pair produced PCR products of about 530-bp for all strains of *C. gloeosporioides* (lanes: 1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18 and 19) (Plate 4.13b). No amplified products were recorded in *C. truncatum* strains (lanes: 3, 10, 16 and 20) (Plate 4.13b). This indicates that the *C. truncatum* strains identified among the isolates in the culture studies were actually confirmed. The negative controls (C) in both analyses did not produce any PCR product. All of the profiles generated were examined visually and bands scored (as present or absent) (Table 4.15).



(A)



(B)

Plate 4.13: Amplified DNA fragments of *C. gloeosporioides* strains (lanes - 1, 2, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 17, 18, 19 and 20) and *C. truncatum* strains (lanes - 3, 6, 10 and 16) obtained from diseased *D. alata* in Ghana using ITS1/ITS4 primer pair (A) and ITS4/CgInt species specific primer pair (B). M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); C = Negative controls (sterile distilled water); Pc = Positive controls (*C. gloeosporioides* from mango). Lanes: 1 = Cg111, 2 = Cg114, 3 = Ct130, 4 = Cg117, 5 = Cg125, 6 = Ct132, 7 = Cg141, 8 = Cg147, 9 = Cg148, 10 = Ct142, 11 = Cg149, 12 = Cg150, 13 = 151, 14 = Cg158 162, 15 = Cg162, 16 = Ct168, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169.

Table 4.15: Detection of DNA fragments of *C. gloeosporioides* and *C. truncatum* strains obtained from diseased *D. alata* plants in Ghana using ITS1/ITS4 primer pair and CgInt/ITS4 species-specific primer pair.

| Source of Isolate<br>(Region) | Isolate Code | Lab ID | ITS1/ITS4 | ITS4/CgInt |
|-------------------------------|--------------|--------|-----------|------------|
| Volta                         | #Cg111       | 1      | +         | +          |
| Volta                         | Cg114        | 2      | +         | +          |
| Volta                         | *Ct130       | 3      | +         | -          |
| Volta                         | Cg117        | 4      | +         | +          |
| Volta                         | Cg125        | 5      | +         | +          |
| Volta                         | *Ct132       | 6      | +         | -          |
| Central                       | Cg141        | 7      | +         | +          |
| Central                       | *Ct142       | 10     | +         | -          |
| Northern                      | Cg148        | 9      | +         | +          |
| Northern                      | Cg149        | 11     | +         | +          |
| Northern                      | Cg150        | 12     | +         | +          |
| Ashanti                       | Cg151        | 13     | +         | +          |
| Ashanti                       | Cg158        | 14     | +         | +          |
| Ashanti                       | Cg147        | 8      | +         | +          |
| Brong-Ahafo                   | Cg162        | 15     | +         | +          |
| Brong-Ahafo                   | Cg165        | 17     | +         | +          |
| Brong-Ahafo                   | Cg166        | 18     | +         | +          |
| Eastern                       | *Ct168       | 16     | +         | -          |
| Eastern                       | Cg167        | 19     | +         | +          |
| Eastern                       | Cg169        | 20     | +         | +          |

#Cg = *C. gloeosporioides*; \*Ct = *C. truncatum*; (+) = presence of amplified DNA fragment; (-) = Absence of DNA fragment.

In the analyses of the RAPD primers (OPA-11, OPA-18, OPC-5, OPC-07 and OPC-16) tested on the genome of the 20 *Colletotrichum* isolates collected from diseased *D. alata* in the Volta Region and

the other five Regions (Ashanti, Brong-Ahafo, Central, Eastern and Northern), DNA fingerprints were generated and evaluated for overall clearness of banding patterns (Plate 4.14 a-c). All the primers used generated short amplicons located between: 100 and 400-bp (OPC-16) (Plate 4.14c), 100 and 700-bp (OPA-18) (Plate 4.14a), 100 and 750-bp (OPC-07) (Plate 4.14b), 150 and 650-bp (OPC-05) (data not shown) and 130 and 730 (OPA-11) (data not shown). Sets of major amplicons were generated in each primer and were shared by two or more isolates. In OPA-18, amplicon of approximately 100-bp was shared by almost all isolates while 250 and 530-bp were shared by three *C. gloeosporioides* strains (Cg125, Cg150 and Cg158: Lanes 5, 12 and 14) and a *C. truncatum* strain (Ct130: Lane – 3) (Plate 4.14a).

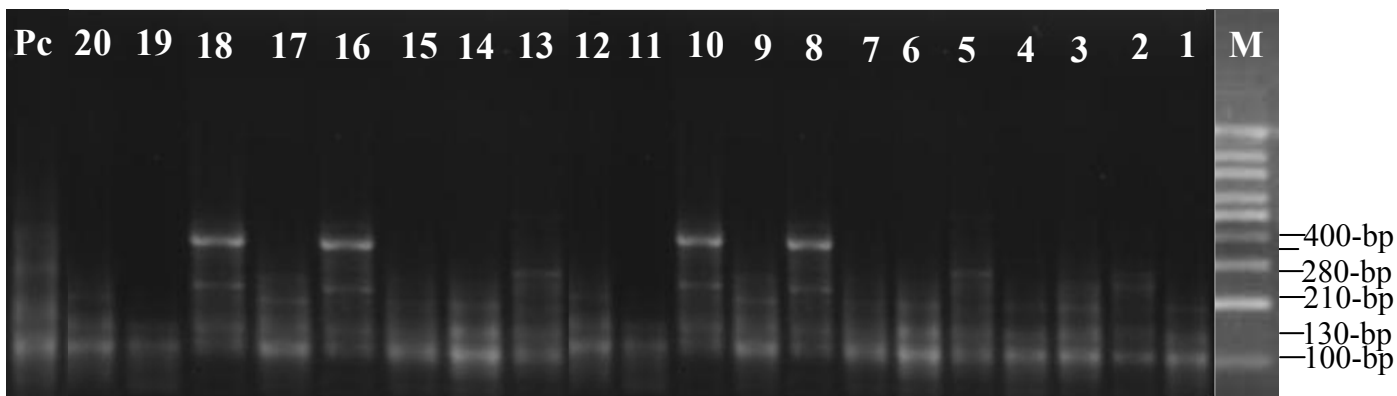
In using primer OPC-07, amplicon of approximately 120-bp was shared by almost all isolates (both *C. gloeosporioides* and *C. truncatum* isolates); 400 and 680-bp were shared by isolates (Ct130, Cg125 and Cg150: Lanes 3, 12 and 14 respectively); 750-bp amplicon was shared by seven *C. gloeosporioides* strains (Cg117, Cg148, Cg150, Cg158, Cg162, Cg166 and Cg169: Lanes 4, 9, 12, 14, 15, 18 and 20 respectively) (Plate 4.14b). Using OPC-16 primer, discernible amplicons of about 100 and 130-bp were shared by almost all the isolates (both *C. gloeosporioides* and *C. truncatum* isolates) whilst amplicons of approximately 280 and 400-bp were shared by two *C. gloeosporioides* strains (Cg147 and Cg166: Lanes – 8 and 18 respectively) and two *C. truncatum* strains (Ct142 and Ct168: Lanes – 10 and 16 respectively). Similar results were demonstrated with primers OPA-11 and OPC-05 (data not shown).



(A) RAPD Primer OPA-18



(B) RAPD Primer OPC-07



(C) RAPD Primer OPC-16

Plate 4.14: RAPD fingerprints obtained with three RAPD Primers OPA-18 (A), OPC-07 (B) and OPC-16 (C) for 20 *Colletotrichum* isolates (1-20) obtained from diseased *D. alata* in Ghana. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); Pc = Positive controls (*C. gloeosporioides* from Mango); Lanes: 1 = Cg111, 2 = Cg114, 3 = **Ct130**, 4 = Cg117, 5 = Cg125, 6 = **Ct132**, 7 = Cg141, 8 = Cg147, 9 = Cg148, 10 = **Ct142**, 11 = Cg149, 12 = Cg150, 13 = 151, 14 = Cg158 162, 15 = Cg162, 16 = **Ct168**, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169.

In the analyses of the restriction fragments of the amplification products with five endonucleases (*Hae*III, *Hin*fI, *Rsa*I, *Msp*I and *Hha*I), *Hae*III enzyme produced the most fingerprints ranging from 100 to 100

1500-bp (Plate 4.15) whilst enzyme *MspI* gave the simplest fingerprints ranging between 100 to 800-bp (data not shown). *HaeIII* enzyme produced discernible DNA fragment amplified at about 100-bp band was shared by almost all isolates whilst 250-bp band was shared by nine isolates (Cg117, Cg125, Cg149, Cg141, Cg148, Cg149, Cg162, Cg166 and Cg169: Lanes – 4, 5, 6, 7, 9, 11, 15, 18 and 20 respectively) (Plate 4.15). The positive control (Pc – reference isolate) also produced an amplicon at this band region (250-bp). However, none of the *C. truncatum* strains (Ct130, Ct132, Ct142 and Ct168: Lanes – 3, 6, 11 and 16 respectively) produced fingerprints that matched with that of the reference isolate (*C. gloeosporioides* from mango) (Plate 4.15).

Using *HhaI* digestion, *C. gloeosporioides* strains (Cg111 and Cg118: Lanes – 1 and 18) produced marked DNA fragment of about 150-bp; Cg162: Lane – 15 (200 and 300-bp); and Cg149: Lane – 10 (330 and 490-bp) (Plate 4.16). Among the *C. truncatum* strains, Ct130: Lane – 3 produced discernible fragment of approximately 200-bp; Ct132: Lane – 6 (200-bp) and Ct168: Lane – 16 (150, 200 and 250-bp) (Plate 4.16). *MspI* digestion yielded 400-bp fragments for seven *C. gloeosporioides* strains (Cg111, Cg141, Cg147, Cg148, Cg151, Cg165 and Cg169: Lanes – 1, 7, 8, 9, 13, 17 and 20 respectively); 350-bp fragments for four isolates (Cg125, Cg149, Cg158 and Cg166: Lanes – 5, 10, 14 and 18 respectively); 150 and 300-bp for isolates (Ct130 and Ct132: Lanes – 3 and 6 respectively); and 400-bp for isolates (Ct142 and Ct168: Lanes – 11 and 16 respectively) (data not shown). *HinfI* and *RsaI* produced major bands ranging between 100 – 450-bp and 120 – 750-bp respectively (data not shown).



Plate 4.15: RFLP fingerprints obtained with *HaeIII* primer for 16 *C. gloeosporioides* strains (Lanes – 1, 2, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19 and 20) and four *C. truncatum* strains (Lanes – 3, 6, 11 and 16) obtained from diseased *D. alata* in Ghana. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); Pc = Positive control (*C. gloeosporioides* from mango); Lanes – 1 = Cg111, 2 = Cg114, 3 = Ct130, 4 = Cg117, 5 = Cg125, 6 = Ct132, 7 = Cg141, 8 = Cg147, 9 = Cg148, 10 = Cg149, 11 = Ct142, 12 = Cg150, 13 = Cg151, 14 = Cg158, 15 = Cg162, 16 = Ct168, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169.



Plate 4.16: RFLP fingerprints obtained with *HhaI* primer for 16 *C. gloeosporioides* strains (Lanes – 1, 2, 4, 5, 7, 8, 9, 10, 12, 13, 14, 15, 17, 18, 19 and 20) and four *C. truncatum* strains (Lanes – 3, 6, 11 and 16) obtained from diseased *D. alata* in Ghana. M = Molecular size marker GeneRuler™ DNA Ladder Mix (100-10,000 bp); Pc = Positive control (*C. gloeosporioides* from mango); Lanes – 1 = Cg111, 2 = Cg114, 3 = Ct130, 4 = Cg117, 5 = Cg125, 6 = Ct132, 7 = Cg141, 8 = Cg147, 9 = Cg148, 10 = Cg149, 11 = Ct142, 12 = Cg150, 13 = Cg151, 14 = Cg158, 15 = Cg162, 16 = Ct168, 17 = Cg165, 18 = Cg166, 19 = Cg167 and 20 = Cg169.

#### 4.3.1.5.1 Cluster analyses of the 20 *Colletotrichum* isolates

##### 4.3.1.5.1.1 *C. gloeosporioides* strains

The dendrogram of genetic relatedness generated by scoring of the presence (1) or absence (0) of reproducible bands of the five RAPD primers OPA-11, OPA-18, OPC-05, OPC-07 and OPC-16 (Operon Technologies, California, USA) and the five RFLP endonucleases *HaeIII*, *HinfI*, *RsaI*, *MspI* and *HhaI* clearly showed the genetic variability between the 16 *C. gloeosporioides* strains isolated from diseased *D. alata* in Ghana (Fig. 4.7). The 16 *C. gloeosporioides* strains identified in the culture study (i.e. traditional approach) were summarized by the cluster analysis into two main groups: Group A (Cg111 and Cg117 – both from Volta Region) and group B (Cg114, Cg125, Cg141, Cg146, Cg147, Cg149, Cg150, Cg152, Cg151, Cg158, Cg162, Cg165, Cg166 and Cg169 – from Volta and the other five Regions) (Fig. 4.7). However, there were five main sub-groupings (B<sub>1</sub>-B<sub>5</sub>) among the strains found in group B. Strains in group A shared 52% similarity with group B. The four strains from the Volta Region (Cg111, Cg114, Cg117 and Cg125) were basically grouped into two – Cg111 and Cg117 (group A) and Cg114 and Cg125 (group B) with Cg111 recording 100% similarity with Cg117 and Cg114 (78%) with Cg125 (Fig. 4.7).

Strains obtained from the Ashanti Region were grouped into three: Cg147 (group B<sub>2</sub>), Cg151 (group B<sub>1</sub>) and Cg158 (group B<sub>5</sub>); Brong-Ahafo Region into three: Cg162 (group B<sub>1</sub>), Cg165 (group B<sub>3</sub>) and Cg166 (group B<sub>5</sub>); Central Region into two: Cg141 (group B<sub>5</sub>) and Cg146 (group B<sub>2</sub>); Eastern into two: Cg167 (group B<sub>2</sub>) and Cg169 (group B<sub>5</sub>); and Northern Region into two: Cg149 (group B<sub>5</sub>) and Cg150 (group B<sub>2</sub>). Strains Cg114 and Cg117 from the Volta Region were similar whilst Cg111 and Cg125 were 80% similar. Again, Cg125 and Cg149 from Volta and Northern Regions respectively were 100% similar. Three isolates Cg158, Cg166 and Cg169 from Ashanti, Brong-Ahafo and Eastern Regions respectively also revealed 100% similarity (Fig. 4.7).

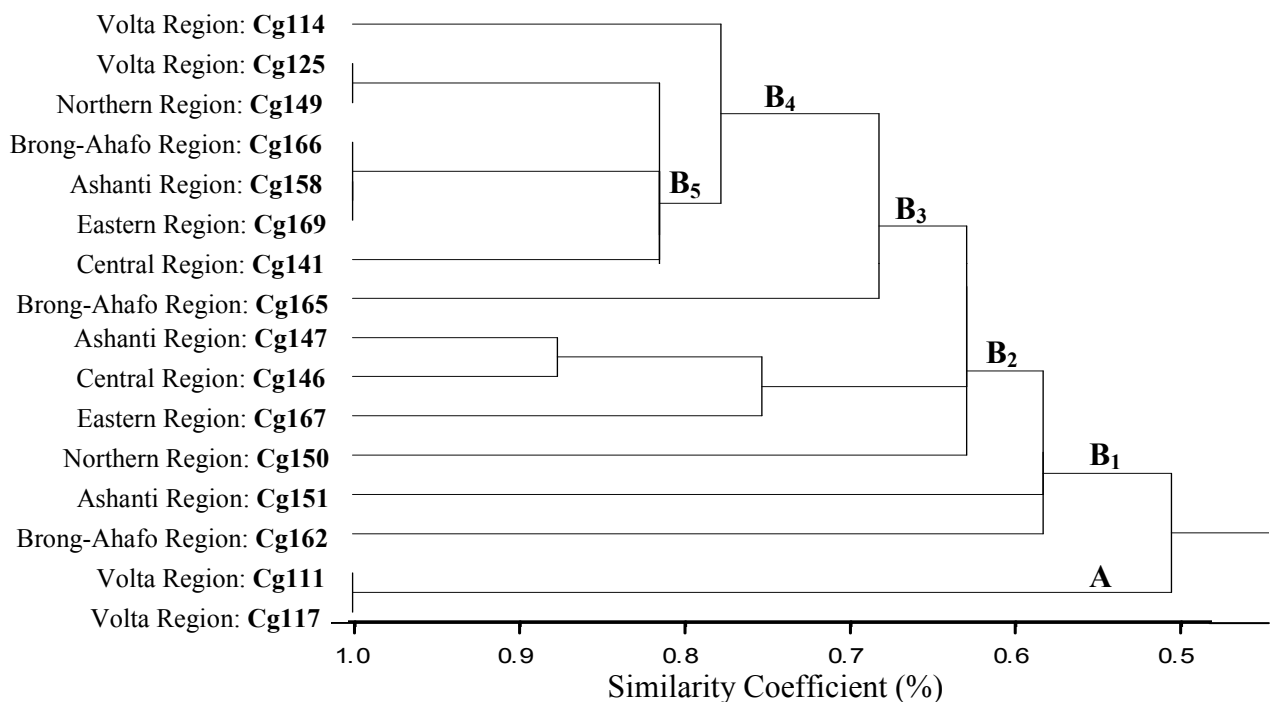


Fig. 4.7: UPGMA dendrogram based on Jaccard's coefficient, indicating relationships among the 16 strains of *C. gloeosporioides* obtained from six Regions of Ghana. Genetic distances were based on the combined data from the random amplified polymorphic DNA (RAPD) and the internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) analyses. RAPD primers: OPA-11, OPA-18, OPA-05, OPC-07 and OPC-16 and Restriction enzymes used were: *Hae*III, *Hinf*I, *Rsa*I, *Msp*I and *Hha*I.

#### 4.3.1.5 .1.2 *C. truncatum* strains

Cluster analysis of the restriction fragments from the amplification products of the rDNA genes flanking the entire ITS1-5.8S-ITS4 region of the isolates using the five RFLP and the five RAPD primers showed the existence of two distinct groups (group A and B) (Fig. 4.8). However, sub-groupings occurred in the group B strains (i.e. group B<sub>1</sub> and B<sub>2</sub>) which resulted in three groupings. The first group comprised

two identical strains Ct130 and Ct168 (group B<sub>2</sub>) from the Volta and Eastern Regions respectively and were 100% similar (Fig. 4.8). The second group (B<sub>1</sub>) was strain from the Central Region (Ct142) which was 65% similar to Ct130 and Ct168 obtained from the Volta and Eastern Regions respectively. The strain Ct132 which proved highly virulent in the pathogenicity test was grouped separately (i.e. put into group A) from the other strains and recorded 30% similarity to the first and second groups of isolates. The Volta Region strains (Ct130 and Ct132) were put into two different groups, B<sub>2</sub> and A respectively.

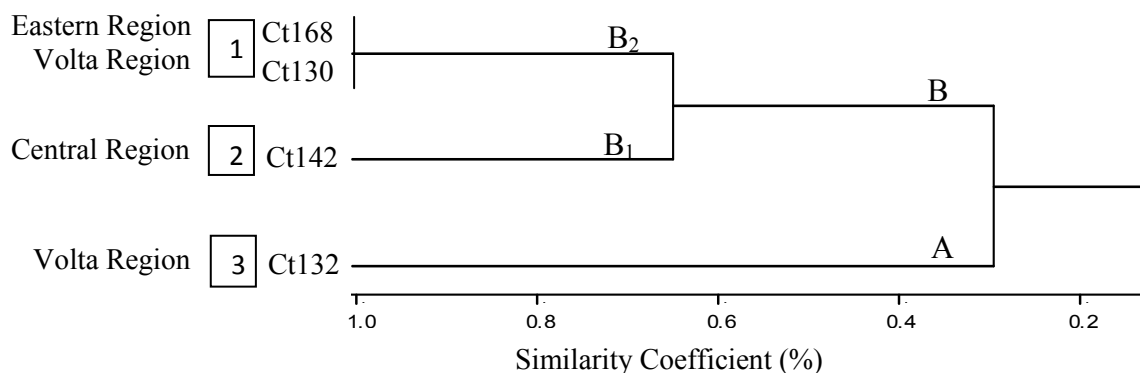


Fig. 4.8: UPGMA dendrogram based on Jaccard's coefficient, indicating relationships among the four strains of *C. truncatum* obtained from three Regions of Ghana. Genetic distances were based on the combined data from the random amplified polymorphic DNA (RAPD) and the internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP) analyses. RAPD primers: OPA-11, OPA-18, OPA-05, OPC-07 and OPC-16 and Restriction enzymes used were: *Hae*III, *Hinf*I, *Rsa*I, *Msp*I and *Hha*I.

#### 4.4 Screening of *D. alata* cultivars for resistance to anthracnose disease of *D. alata*

The 12 *D. alata* tissue culture-derived whole-plants used for the screening trial gave higher (70-90%) explants response (i.e. root-shoot). However, the rate of explants growth *in-vitro* (Plat 4.16) was slow in comparison with the hardened explants on 1:1 vermiculite: compost (peat: sand 9:1). No anthracnose disease symptoms were observed on hardened *D. alata* explants (Plate 4.17). Days to root initiation and bud break, number of root and shoot per explants (Plate 4.18), root and shoot length and nodes per shoot of explants were significantly different ( $P < 0.05$ ) among the cultivars (Appendix V).



Plate 4.16: Three-week old *in-vitro* explants of *D. alata* incubated at 16-hour light/8-hour dark photoperiod (cool, white fluorescent light –  $30 \mu\text{mol m}^{-2} \text{S}^{-1}$ ) and a temperature of  $25^\circ\text{C}$ , 60-70% RH.

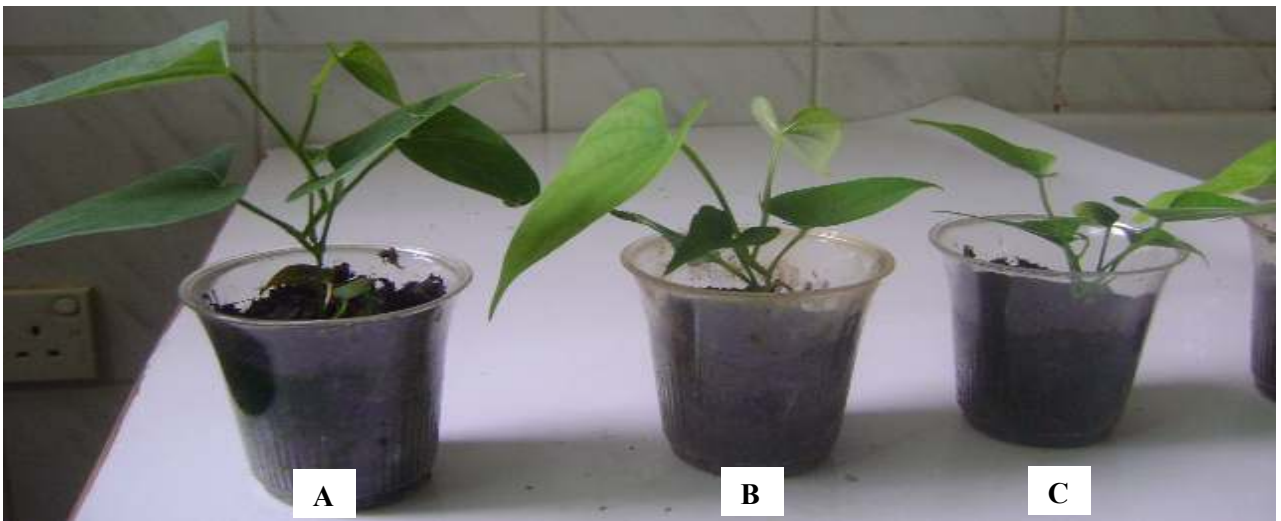


Plate 4.17: Three-week old hardened explants of *D. alata* cultivars TDa019 (A), TDa007 (B) and TDa013 (C) without symptoms of anthracnose disease. Medium used – 1:1 vermiculite: compost (peat: sand 9:1) (IITA, Nigeria) maintained under bioclimatic chamber.



Plate 4.18: Six-week old explants of *D. alata* cultivars TDa010 (A); TDa019 (B); TDa018 (C) and TDa007 (D) with varying number of roots and shoots. Explants were incubated at 16-hour light/8-hour dark photoperiod (cool, white fluorescent light –  $30 \mu\text{mol m}^{-2} \text{S}^{-1}$ ) and a temperature of  $25^\circ\text{C}$ , 60-70% RH.

#### 4.4.1 Mean severity (AUDPCs) of anthracnose disease of the 12 *D. alata* cultivars

The AUDPC scores of anthracnose disease in the two-month old potted tissue culture-derived whole-*D. alata* cultivars (12) revealed wide range of resistance among the cultivars. The cultivars were grouped into two main categories: resistant – TDa008, TDa010, TDa014, TDa023, TDa025 and TDa027 and susceptible – TDa007, TDa013, TDa015, TDa018, TDa019 and TDa021. However, the levels of resistance and susceptibility varied among cultivars (Table 4.16). *D. alata* cultivar TDa023 had AUDPC value of 29.67 which was significantly lower ( $P < 0.005$ ) than each of the remaining five resistant cultivars TDa027 (36.53), TDa025 (45.02), TDa008 (47.48), TDa014 (48.49) and TDa010 (49.40) (Table 4.16) but there were no significant ( $P > 0.005$ ) difference among the cultivars TDa008, TDa010, TDa014, TDa025 and TDa027.

Among the six susceptible cultivars, TDa018 had a significantly lower ( $P < 0.05$ ) AUDPC value (54.37) compared with TDa007 (66.61), TDa021 (74.31) and TDa019 (97.90). There was significant ( $P < 0.05$ ) interaction between the 12 *D. alata* cultivars and six *Colletotrichum* isolates (Table 4.16) (Appendix VI). The ranking of cultivars varied among isolates (Table 4.21). Nonetheless, cultivar TDa023 had the lowest rank (1) in almost all the isolates (Cg111, Cg151, Ct132 and Ct168) whilst cultivar TDa019 recorded the highest rank (12) in nearly all isolates (Cg111, Cg151, Ct132 and Ct168) used (Table 4.16)

Table 4.21: Severity (mean AUDPCs) scores and ranks (parenthesis) of anthracnose disease on two-month old potted explants of 12 *D. alata* cultivars using six *Colletotrichum* isolates.

| Cultivar    | Mean AUDPCs*                   |               |              |              |              |              | SDW** | Mean         | Host Response*** |
|-------------|--------------------------------|---------------|--------------|--------------|--------------|--------------|-------|--------------|------------------|
|             | <i>Colletotrichum</i> Isolates |               |              |              |              |              |       |              |                  |
|             | Cg111                          | Ct132         | Cg150        | Cg151        | Cg162        | Ct168        |       |              |                  |
| TDa007      | 55.81 (7)                      | 152.40(1)#    | 69.22(10)    | 26.61 (4)    | 36.22 (3)    | 59.30 (10)   | 0.00  | 66.61(10)    | LS               |
| TDa008      | 45.92 (5)                      | 110.71 (7)    | 21.91 (1)    | 24.92 (3)    | 49.71 (8)    | 31.72 (7)    | 0.00  | 47.48 (4)    | LR               |
| TDa010      | 34.82 (3)                      | 132.11(1)     | 41.91 (6)    | 28.33 (5)    | 23.02 (1)    | 36.21 (8)    | 0.00  | 49.40 (6)    | LR               |
| TDa013      | 60.30 (8)                      | 119.30 (8)    | 38.52 (5)    | 33.71 (9)    | 59.36 (9)    | 26.90 (5)    | 0.00  | 56.35 (8)    | LS               |
| TDa014      | 46.32 (6)                      | 78.42 (5)     | 44.90 (8)    | 29.85 (6)    | 34.53 (2)    | 49.36 (9)    | 0.00  | 48.97 (5)    | LR               |
| TDa015      | 66.53 (9)                      | 75.01 (3)     | 36.41 (4)    | 30.91 (7)    | 61.51 (10)   | 23.47 (2)    | 0.00  | 56.54 (9)    | LS               |
| TDa018      | 85.11(11)                      | 73.91 (2)     | 70.00(11)    | 38.64(10)    | 45.80 (7)    | 25.75 (4)    | 0.00  | 54.37 (7)    | LS               |
| TDa019      | 93.24(12)                      | 167.20 (12)   | 68.20 (8)    | 86.22(12)    | 89.92 (12)   | 82.61(12)    | 0.00  | 97.90 (12)   | HS               |
| TDa021      | 71.20(10)                      | 123.82 (9)    | 75.60(12)    | 46.71(11)    | 68.33 (11)   | 60.25(11)    | 0.00  | 74.31 (11)   | S                |
| TDa023      | 23.61 (1)                      | 49.70 (1)     | 23.60 (2)    | 22.90 (1)    | 38.27 (4)    | 19.91 (1)    | 0.00  | 29.67 (1)    | HR               |
| TDa025      | 40.91 (4)                      | 83.21 (6)     | 43.11 (7)    | 33.36 (8)    | 43.80 (6)    | 25.71 (3)    | 0.00  | 45.02 (3)    | R                |
| TDa027      | 28.70 (2)                      | 75.83 (4)     | 32.20 (3)    | 24.52 (2)    | 39.11 (5)    | 30.83 (6)    | 0.00  | 36.53 (2)    | R                |
| <b>Mean</b> | <b>54.37</b>                   | <b>103.47</b> | <b>47.13</b> | <b>35.56</b> | <b>49.13</b> | <b>39.34</b> |       | <b>54.83</b> |                  |

LSD ( $P < 0.001$ ): Isolate = 10.21, Cultivar = 6.38, and Isolate x cultivar = 22.07

\*AUDPC = Area under disease progress curves for anthracnose disease of *D. alata* using 0-5 scale and calculated using the formula of Shaner and Finney (1977); \*\*SDW = Sterile distilled water (negative control); \*\*\*Host responses: LS = Less susceptible; S = Susceptible; HS = Highly susceptible; LR = Less resistant; R = Resistant; HR = Highly resistant; and #Lower values in parenthesis indicate higher resistance and vice versa.

#### 4.4.2 Incidence of anthracnose disease on two-month old potted explants of 12 *D. alata* cultivars using six *Colletotrichum* isolates

The incidence of anthracnose disease generated by scoring the presence (1) or absence (0) of the disease on two-month old potted explants of the 12 *D. alata* cultivars using six *Colletotrichum* isolates was not significant ( $P > 0.005$ ) (Appendix VII). However, *D. alata* cultivar

TDa023 had a mean disease incidence value of 71.95% which was relatively lower than cultivar TDa019 (89.53%) (Table 4.16). Among the isolates, Ct132 recorded the highest mean disease incidence (86.76%) while Cg151 recorded the least (75.97%). Although, there were no significant ( $P > 0.005$ ) interactions among the isolates and cultivars, interaction between isolate Ct132 and cultivar TDa019 recorded the highest disease incidence (96.72%) whilst isolate Cg151 and cultivar TDa023 produced the lowest mean disease incidence (65.94%).

Table 4.16: Incidence (%) of anthracnose disease on two-month old potted explants of 12 *D. alata* cultivars using six *Colletotrichum* isolates.

| Cultivar    | Disease Incidence              |              |              |              |              |              | *SDW        | Mean         |
|-------------|--------------------------------|--------------|--------------|--------------|--------------|--------------|-------------|--------------|
|             | <i>Colletotrichum</i> Isolates |              |              |              |              |              |             |              |
|             | Cg111                          | Ct132        | Cg150        | Cg151        | Cg162        | Ct168        |             |              |
| TDa007      | 80.81                          | 90.3         | 78.40        | 81.87        | 82.73        | 79.30        | 0.00        | <b>82.24</b> |
| TDa008      | 85.21                          | 86.4         | 81.93        | 79.24        | 81.40        | 80.12        | 0.00        | <b>82.38</b> |
| TDa010      | 76.94                          | 79.93        | 80.01        | 75.01        | 79.21        | 76.35        | 0.00        | <b>77.92</b> |
| TDa013      | 88.12                          | 91.62        | 78.99        | 76.03        | 83.17        | 77.03        | 0.00        | <b>82.49</b> |
| TDa014      | 75.97                          | 80.53        | 74.75        | 69.56        | 77.92        | 70.17        | 0.00        | <b>74.83</b> |
| TDa015      | 80.68                          | 86.41        | 79.89        | 78.16        | 84.26        | 79.16        | 0.00        | <b>81.45</b> |
| TDa018      | 84.45                          | 88.87        | 81.12        | 79.92        | 83.19        | 80.24        | 0.00        | <b>82.97</b> |
| TDa019      | 93.69                          | 96.72        | 85.03        | 81.08        | 91.09        | 89.58        | 0.00        | <b>89.53</b> |
| TDa021      | 79.64                          | 91.44        | 83.56        | 76.82        | 81.21        | 78.98        | 0.00        | <b>81.94</b> |
| TDa023      | 73.33                          | 79.10        | 67.23        | 65.94        | 70.18        | 69.91        | 0.00        | <b>71.95</b> |
| TDa025      | 84.03                          | 86.09        | 74.92        | 70.25        | 75.24        | 73.18        | 0.00        | <b>77.29</b> |
| TDa027      | 79.12                          | 83.76        | 75.33        | 67.74        | 74.02        | 72.51        | 0.00        | <b>75.42</b> |
| <b>Mean</b> | <b>81.83</b>                   | <b>86.76</b> | <b>78.43</b> | <b>75.97</b> | <b>80.30</b> | <b>77.21</b> | <b>0.00</b> | <b>80.09</b> |

LSD ( $P > 0.001$ ): Isolate = ns, Cultivar = ns, Isolate x cultivar = ns

\*SDW = Sterile distilled water; Disease incidence was scored on the 14<sup>th</sup> DAI using a score of (+) for disease presence and (-) for disease absence and percent disease incidence calculated using the formula below:

$$\text{Disease incidence (I)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants inspected}} \times 100$$

#### 4.5 Integrated management of anthracnose disease of *D. alata*

When the strains of *C. gloeosporioides* (Cg111) and *C. truncatum* (Ct132) which proved highly virulent in the cultivar screening trial were selected and treated with four fungicides (Carbendazim, Mancozeb, Nordox and Shafiz F1), there was significant ( $P < 0.005$ ) fungistatic effect on radial

mycelial growth and sporulation of isolates *in-vitro* (Appendix VIII). Radial mycelial growth and sporulation inhibition ranged from 100% in Carbendazim, Mancozeb and Nordox to 0% in the control (PDA without fungicide) (Fig. 4.9). Shafiz F1 had significantly lower ( $P < 0.05$ ) fungistatic effect (80%) on radial mycelial growth and sporulation of isolate Ct132 compared with Carbendazim (100%), Nordox (100%) and Mancozeb (100%) (Fig. 4.9a). Similarly, in isolate Cg111, Shafiz F1 produced significantly lower ( $P < 0.05$ ) fungistatic effect (86%) on radial mycelial growth and sporulation of isolate Cg111 compared to Carbendazim (100%), Mancozeb (100%) and Nordox (100%) (Fig. 4.9b).

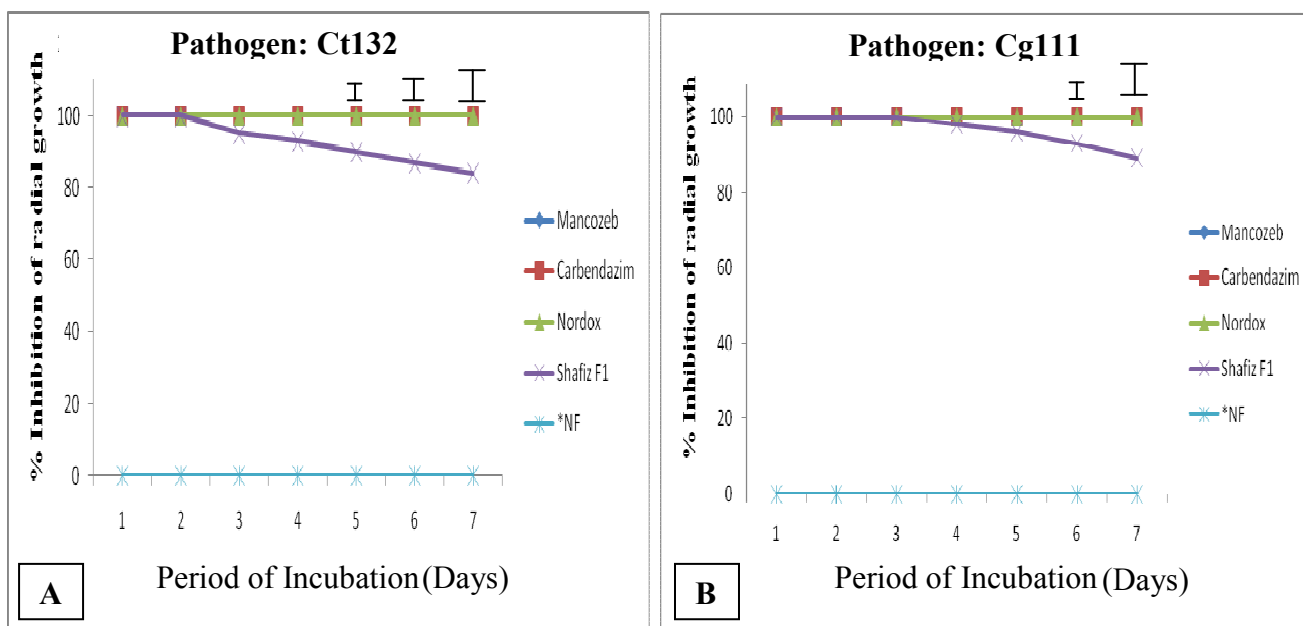


Fig. 4.9: Fungistatic effects of four fungicides (Carbendazim, Mancozeb, Nordox and Shatiz F1) on radial mycelial growth and sporulation of two *Colletotrichum* isolates – *C. truncatum* (A) and *C. gloeosporioides* Cg111 (B). PDA amended cultures and controls (\*NF) were incubated at  $26 \pm 2$  °C and 65-72% RH in the laboratory. Vertical bars indicate  $LSD_{5\%}$ . Percentage reduction in radial mycelial growth of test fungi (I) was calculated based on the formula of Kiran et al. (2010).

The information on mean rainfall, relative humidity and temperature of the study area obtained from the *Ghana Metrological Station, 'Mpehuasem'* during the period of study (November, 2012 to May, 2013) revealed that there was positive correlation between mean rainfall and mean relative humidity. However, there was no positive correlation between the mean temperatures recorded and mean rainfall or relative humidity (Table 4.17). The study area had a maximum mean rainfall in

May, 2013 (245.3 mm) with 33.5 mm as the minimum in December, 2012 (Table 4.17). The maximum and minimum mean temperatures were 34.3°C and 19.3°C respectively recorded in December, 2012. The mean relative humidity ranged from 64 % in December, 2012 to 79% in May, 2013.

Table 4.17: Mean rainfall, relative humidity and temperature distributions of the study area during the period of the experiment (November, 2012 to May, 2013).

| Month<br>(2012/13) | Mean<br>Rainfall<br>(mm) | Mean Relative<br>Humidity<br>(%) | Mean Temperature (°C) |      |
|--------------------|--------------------------|----------------------------------|-----------------------|------|
|                    |                          |                                  | Max.                  | Min. |
| November           | 36.1                     | 66                               | 32.1                  | 20.2 |
| December           | 33.5                     | 64                               | 34.3                  | 19.3 |
| January            | 42.1                     | 67                               | 29.1                  | 23.3 |
| February           | 70.8                     | 69                               | 29.6                  | 23.5 |
| March              | 96.9                     | 72                               | 31.4                  | 24.0 |
| April              | 98.3                     | 74                               | 29.4                  | 23.9 |
| May                | 245.3                    | 79                               | 28.7                  | 22.4 |

Source: Ghana metrological station (2013).

The integrated disease management trial using artificial inoculation of mixed *Colletotrichum* isolates (Cg111 and Ct132 – highly virulent strains) with spore suspension of  $1.6 \times 10^6$  conidial/ml revealed that, *D. alata* cultivars which received hot-water treatments, fungicides sprays and weeding in an integration (i.e. cultivar + hot-water treatment (H) + weeding (W) + fungicide sprays) gave significantly lower ( $P < 0.05$ ) disease severities compared to cultivars that did not receive hot-water treatment, fungicide sprays and weeding (controls) (i.e. cultivar, no hot-water treatment (NH), no weeding (NW) and no fungicide sprays) (Fig. 4.10). The disease severity of both the treated cultivars and the controls increased with time (weeks) such that mean disease severities of 0.1 and 1.3 in week 1 and 8 respectively were recorded in the treated cultivars whilst the controls recorded mean disease severities of 0.6 in week 1 and 3.4 in week 8. In cultivar

TDa019, treatment combination (TDa019 + H + W + Mancozeb sprays) had a significantly lower ( $P < 0.05$ ) disease severity (1.1) in week 8 compared with the treatment combination (TDa019 + H + W + Nordox sprays) (2.0). However, there was no significant difference ( $P > 0.05$ ) between the treatment combinations (TDa019 + H + W + Mancozeb sprays) and (TDa019 + H + W + Carbendazim sprays). In cultivar TDa023, there were no significant differences ( $P > 0.05$ ) among the treatment combinations (TDa023 + H + W + Mancozeb sprays), (TDa023 + H + W + Carbendazim sprays) and (TDa023 + H + W + Nordox sprays) (Fig. 4.10). However, TDa023 + H + W + Mancozeb sprays had the least disease severity value (1.2). Among the controls, cultivar TDa023 had significantly lower ( $P < 0.05$ ) disease severity (3.0) in week 8 in comparison with TDa019 (3.7).

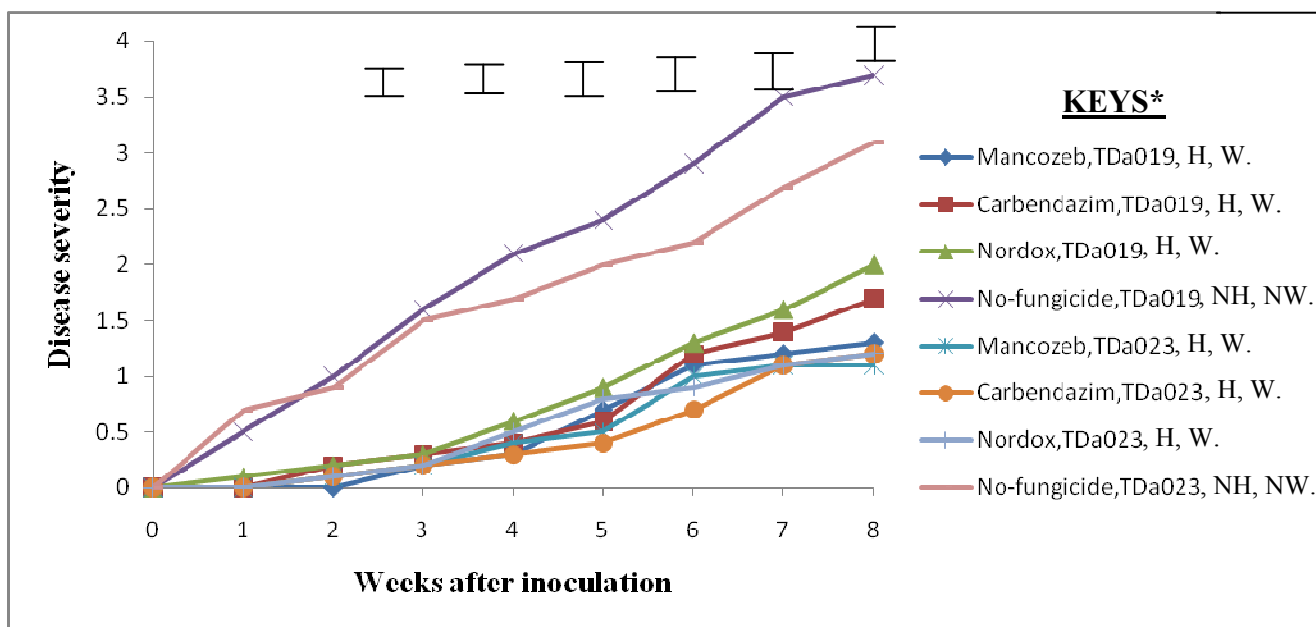


Fig. 4.10: Progress of anthracnose disease of *D. alata* cultivars (TDa019 and TDa023) on the field following artificial inoculation of mixed *Colletotrichum* isolates (Cg111 and Ct132) with spore suspension  $1.6 \times 10^6$  conidial/ml. \*Keys: TDa019 and TDa023 = *D. alata* cultivars; H = Hot-water treatment; W = Weeding; NH = No hot-water treatment; NW = No weeding. Vertical bars indicate LSD at 5%.

#### 4.5.1 Mean AUDPC scores associated with each IPM strategy for the management of anthracnose disease of *D. alata* on the field

The mean AUDPC of the mixed isolates on the two *D. alata* cultivars (TDa019 and TDa023) revealed significance ( $P < 0.05$ ) (Table 4.18). In cultivar TDa019, the mean AUDPC scores ranged

from 24.06 to 102.05. The treatment combination (TDa019 + H + W + Mancozeb sprays) had significantly lower ( $P < 0.05$ ) mean AUDPC score (24.06) compared with (TDa019 + H + W + Nordox sprays) (52.27) and the control (102.05). However, there was no significant difference ( $P > 0.05$ ) between treatment combinations (TDa019 + H + W + Mancozeb sprays) and (TDa019 + H + W + Carbendazim sprays). In cultivar TDa023, the mean AUDPC scores ranged from 16.54 to 91.76 (Table 4.18). Treatment combination (TDa023 + H + W + Mancozeb sprays) again recorded significantly lower ( $P < 0.05$ ) mean AUDPC score (17.01) compared with (TDa023 + H + W + Nordox sprays) (30.99). There was no significant difference ( $P > 0.05$ ) between the treatment combinations (TDa023 + H + W + Mancozeb sprays) and (TDa023 + H + W + Carbendazim sprays) (Table 4.18).

Table 4.18: Mean AUDPC scores associated with each IPM strategy for the management of anthracnose disease of *D. alata* on the field and ranks (parenthesis). Isolates used = (Cg111 + Ct132).

| <b>Treatment Combination*</b>         | <b>Mean AUDPC**</b> |
|---------------------------------------|---------------------|
| Mancozeb + TDa019 + H + W             | 24.06 (1)#          |
| Carbendazim + TDa019 + H + W          | 25.18 (2)           |
| Nordox + TDa019 + H + W               | 52.27 (3)           |
| No-fungicide + TDa019 + NH + NW       | 102.05 (4)          |
| Mancozeb + TDa023 + H + W             | 16.54 (1)#          |
| Carbendazim + TDa023 + H + W          | 17.01 (2)           |
| Nordox + TDa023 + H + W               | 40.99 (3)           |
| No-fungicide + TDa023 + NH + NW       | 91.76 (4)           |
| <b>LSD (<math>P &lt; 0.05</math>)</b> | <b>4.78</b>         |

\* TDa019 and TDa023 = *D. alata* cultivars; H = Hot-water treatment (45°C for 20 minutes); W = Weeding (monthly); NH = No hot-water treatment; NW = No weeding. Fungicides sprays were initiated 2 months after sprouting (MAS) and terminated at 4 MAS. Total Carbendazim application (fortnightly) = 4 at 500 g a.i./ha; Total Mancozeb application (fortnightly) = 4 at 800 g a.i./ha; and total Nordox application (tri-weekly) = 3 at 800 g a.i./ha.

\*\*AUDPC = Area under disease progress curves for anthracnose disease of *D. alata* using 0-5 scale and calculated using the formula of Shaner and Finney (1977).

#Lower scores in parenthesis indicate higher disease reduction (AUDPC).

## CHAPTER FIVE

### 5.0 DISCUSSION

#### **5.1 Assessment of farmers' knowledge and perception of a vine-browning and die-back disease of *D. alata* in the Volta Region**

Analyses of the data collected from the field survey to assess the knowledge and perception of *D. alata* farmers in the Nkwanta-North (60) and Krachi-West (60) districts of the Volta Region revealed that most of the farmers have little or no formal education. Probably, most people with secondary or tertiary education seek for jobs in the cities and thus, leave the farming activities in the hands of those who have had little or no formal education. This finding corroborates Sesay *et al.* (2013) report that farming in Sierra Leone is left in the hands of the little or no educated people while the highly educated ones seek for jobs in the cities. The involvement of people with little or no formal education in water yam production in both districts might have influenced the disease spread since they perceived diseased *D. alata* plants as early maturing cultivars. Strategic programs to educate farmers on novel advancements and improved technologies in farming for business could make farming lucrative to attract educated people in the districts into *D. alata* production.

Most farmers in the two districts could identify the vine-browning and die-back disease of *D. alata* by its symptoms and referred to it as “brown brown” which connotes browning of leaves and vines of the *D. alata* cultivars. Majority (88.0%) of the respondents were, however, ignorant of the cause of the disease and rather attributed it to heavy rainstorm after a prolonged drought and superstition. The causal organisms have been identified as the fungi *Colletotrichum gloeosporioides* and *Colletotrichum truncatum* by earlier workers (Ayodele, *et al.*, n.d; Singh *et al.*, 1966; Nwankiti and Ene, 1984; Akem, 1999; Abang *et al.*, 2002; Aduramigba-Modupe *et al.*, 2010). Since the real cause of the disease was unknown to most of the farmers, diseased *D. alata* plants were left intact on the field and farm sanitation and other suitable cultural practices to minimize the spread of the disease were not carried out. The association of heavy rainfall with the occurrence of the disease by

the farmers (15.0%) supported the report that fungal infection of plant hosts is influenced by adequate moisture (Schwartz and Mohan, 1995; Agrios, 2005). The ignorance of farmers in both districts about the spread of the disease within and among farms might have contributed to the high incidence of the disease as the sources of inoculum (diseased plant parts) were left on farms after harvest. Nevertheless, a few farmers (24.0%) ascribed the disease spread within farms to planting of infected seed yam and among farms to exchange of infected seed yam (19.0%). This agrees with the findings of Orkwor and Asadu (1997) and Kambaska *et al.* (2009) that 30% of previous harvests of farmers' seed yam reserved for the next cropping season in Nigeria served as primary sources of inoculum on farms especially, when exchanged among farmers. Moreover, infected planting materials have been identified as triggering epidemics of the disease as fungi survive beneath the periderm of setts (Simons and Green 1994).

The few farmers (9.0%) who attributed the spread of the disease within farms to contact between diseased and healthy plant were in agreement with Schwartz and Mohan (1995), Agrios (2005) and Chaube and Pundhir (2005) who related the spreading of the disease to plants contact, insects, irrigation water, infected tools and debris. Wind (11.0%) and rainfall (6.0%) were perceived by some farmers as modes of the disease spread among farms. This agrees with findings of Ebenebe (1980), Schwartz and Mohan (1995) and Galvan *et al.* (1997) who reported that *C. gloeosporioides* infection on leaves is mainly caused by air-borne conidia. Hence, the disease spread in the Volta Region might have been influenced by both wind and water.

*D. alata* cultivars commonly grown by farmers in the districts were 'Akaba' (thorned and thornless), 'Matches' (long and short tubers), 'Akomea', 'Poka', 'Nibultutu', 'Chachribon' and 'Sum nyen' in decreasing order of popularity. This may have been influenced by 'Akaba' cultivars being less prone to anthracnose and high yielding with better quality compared to the other cultivars. Cultivar resistance to diseases in yam greatly influences farmers' selection for cultivation (Sesay *et*

*al.*, 2013). High dry matter content and low percent peel loss (i.e. yield and food quality) are also important selection criteria for yam genotype especially, if it is to be processed into secondary products (Egesi *et al.*, 2007). Although, 'Matches' was highly susceptible to the vine-browning and die-back disease, it was the second prime cultivar to 'Akaba' commercially grown in both districts. This may partly be due to its ability to sprout from small seed or sett sizes (even, as small as matches-box size) and multiple tuber formation. The susceptibility of 'Matches' cultivar to the disease may probably be due to a lower concentration of phenolics (antifungal compounds) and other resistance factors to prevent or reduce disease infection. The strains of the pathogen in the region might have also produced excessive phenolases (tyrosinase) in the cultivars to breakdown their resistance (Plumbley and Sweetmore, 1994).

Furthermore, *D. alata* has a longer maturity period of about 9 to 12 months which increases its exposure to the strains of the pathogen in the field and continuous high production of a given *D. alata* cultivar (especially, 'Matches') on the same piece of land year after year, might have also contributed to the high losses (50 to 75%) encountered by the farmers. It was, however, observed during the field survey that 'Nibultutu', 'sum ne hyen' and 'Akomea' which were resistant to the anthracnose disease were cultivated on a small-scale; usually, for consumption (Coffie, Personal Observation, 2012). The pathogen strains might have developed resistant-breakdown gene(s) to match the resistant gene(s) in the cultivars as supported by the concept of 'gene for gene matching' (Maloy, 1993). McDonald *et al.* (1998) and Ano *et al.* (2002a) reported 90-100% loss of *D. alata* cultivars to *C. gloeosporioides* strains in the Caribbean where monocultures with a single popular susceptible cultivar were common.

Most farmers perceived that the disease could be controlled by the use of chemicals but chemical control was not practiced. The farmers rather resorted to cultural control practice such as regular weeding (27.63%) and use of healthy planting materials (10.53%). It is, however, suggested that

integrated disease management measures involving the use of resistant cultivars, judicious use of chemicals and good phytosanitary measures such as weeding, burning or burying of debris may give desirable results in controlling the disease (Ogundana *et al.*, 1970; Nwakiti and Arene, 1976; Nwakiti and Okpala, 1981; IITA, 1993). Anthracnose disease of *D. alata* was identified as the most important factor affecting farmers' income as they were unable to realize their expected yield. Many farmers have, therefore, abandoned the production of *D. alata* in both districts for other food crops. The disease had been estimated to cause excessive yield loss of about 85 to 100% in several fields in the Carribeans especially, when the disease epidemics commenced prior to or during tuber formation (Degras *et al.*, 1984; Mignucci *et al.*, 1988; Green, 1994). Some farmers, however, suggested extensive drought, excessive rainfall and poor land preparation as major causes of yield loss in *D. alata*. Germination of conidia of *Colletotrichum* spp. under high humidity and long period of drought significantly influence tuber formation of yam (Abang, 1997).

Farmers in the Nkwanta-North and Krachi-West districts produce *D. alata* principally for food consumption (food security) followed by income generation and then, for purchasing assets. Failure of farmers to realize their expected yield poses a threat not only to their economic responsibilities but also, their social relations as farmers in both districts were unable to settle school fees, medical bills, loans and acquire assets due to low or no returns from farms. The social relation of farmers with family members especially, spouse and Extension Officers were also negatively affected.

## **5.2 Incidence and severity of the disease in the Volta Region**

The incidence of vine-browning and die-back disease in Nkwanta-North (75.30%) and Krachi-West (55.80%) were very high. During the survey, it was observed that diseased *D. alata* debris from previous harvests were gathered and left on the field and on top of mounds which might have retained the inoculum (Akem and Asiedu, 1994; Abang *et al.*, 2006; Egesi *et al.*, 2007) and

contributed to the high disease incidence. *D. alata* plants were also left unstaked resulting in inter-twinning of vines (both healthy and diseased) which may have facilitated the spread of the disease. The practice of liberal exchange of germplasm (planting materials exchange) among farmers may account for the high disease incidence. The higher disease incidence at Nkwanta-North district may have resulted from the higher rainfall recorded during the production season (1700 mm) compared to Krachi-West district (900 mm) (Baddim and Aboni, Personal Communication, 2012).

Disease severity was similar in the two districts [Nkwanta-North (1.80) and Krachi-West (1.60)]. During the survey, it was observed that initiation of tubers in *D. alata* cultivars in the Nkwanta-North district had initiate tubers which coincided with the peak of the disease in the district around June/July when rainfall was very high (Atsu, Personal Communication, 2012). Translocation of resources for tuber formation at the expense of defensive mechanisms in leaves and vines might have contributed to the high disease severity (Onyeka *et al.*, 2005).

### **5.3 Identification of the causal agents of anthracnose disease of *D. alata* in the Volta Region**

The symptoms on diseased *D. alata* plants, morpho-cultural characteristics of the isolates, genome analysis, and pathogenicity test confirmed *Colletotrichum gloeosporioides* Penz. and Sacc. and *C. truncatum* Andrus and Moore as the causal agents of vine-browning and die-back disease of *D. alata* in the two districts of the Volta region. *Colletotrichum* spp. have been reported as the cause of anthracnose disease of *D. alata* in Ghana, but the actual species were not confirmed (Oduro, 2000; Offei *et al.*, 2008). This work, therefore, is the first confirmation of the actual species of *Colletotrichum* causing anthracnose disease of *D. alata* in Ghana and the foremost to report of a new species (*C. truncatum*). The same fungi were also implicated in the disease in the Central and Eastern regions. *Colletotrichum* spp. have been reported to be the pathogen causing anthracnose

disease of *D. alata* (Singh *et al.*, 1966; Winch *et al.*, 1984). *C. gloeosporioides* and *C. truncatum* have also been reported as the pathogens responsible for the disease in Nigeria (Ayodele, *et al.*, n.d). However, in the Ashanti, Brong-Ahafo and Northern Regions, only *C. gloeosporioides* was confirmed as the prime cause of the disease. This finding corroborates reports of Nwankiti and Ene (1984), McDonald *et al.* (1998), Akem (1999), Abang *et al.* (2002), Ano *et al.* (2002) and Aduramigba-Modupe *et al.* (2010) who found *C. gloeosporioides* as the causal agent of anthracnose disease of water yam.

The yam breeding program at IITA had lost valuable *D. alata* germplasm which could have been protected by fungicide sprays due to the erroneous concept that the disease was of complex etiology involving a virus until *Colletotrichum gloeosporioides* was confirmed as the principal cause of yam anthracnose (Akem, 1999). The presence of *C. truncatum* strains in the three regions of Ghana (Central, Eastern and Volta) could partly be due to the introduction of the strain from Nigeria (where it is well noted) through the importation of *D. alata* germplasm to the Southern-most part of Ghana (possibly, Plant Genetic Resource Research Institute (PGRRI), Bunso) and might have been spreading to the major *D. alata* growing areas. This then calls for internal quarantine to restrict the spread of this strain into other regions because, the strain is more virulent and thus, has the potential of degrading the *D. alata* industry in Ghana as was reported in the Caribbean (Degras *et al.*, 1984; Mignucci *et al.*, 1988; Green, 1994; Green and Simons, 1994).

The varying levels of pathogenicity in the *D. alata* cultivars could probably be due to different levels of phenolases (tyrosinase) produced by strains of *C. gloeosporioides* and *C. truncatum* (Plumbley and Sweetmore, 1994). Studies by Abang *et al.* (2001) confirms that variations exist among *C. gloeosporioides* responsible for anthracnose disease of *D. alata* and described four forms associated with anthracnose of yam in Nigeria: the slow-growing grey, the fast-growing salmon, the fast-growing grey and the fast-growing olive forms. These variations have wide-ranging effect

on disease severity as they possess different levels of virulence (Abang, 1997). The present study revealed that anthracnose disease attacks mainly leaves and vines of *D. alata* which confirms earlier works (Simons, 1993; Akem and Asiedu, 1994; Onyeka *et al.*, 2005; Abang *et al.*, 2006; Egesi *et al.*, 2009). In the initial stages of the disease development, small regular, brown necrotic spots with yellow halo symptoms were more frequently observed on the younger leaves than the older. However, as the disease progressed, brown regular spots surrounded by dark brown rings with gray centres or shot-holes dominated on the older leaves with occasional symptoms of die-back. This corroborates the findings of Offei *et al.* (2008). The rate of progression of the symptoms of anthracnose disease of *D. alata* was affected by the age of leaf and vine, stage of the epidemic, cultivar susceptibility, rainfall and agronomic practices such as fungicide application (Sweetmore *et al.*, 1994; Wharton, 1994).

The earlier expression of the disease symptoms on the younger leaves may probably be due to the tenderness of the cuticle membrane of the younger leaves than the older (Chaube and Pundhir, 2005). Again, the restriction of disease development on the younger leaves could partly be due to higher accumulation of phenols (antifungal compounds) (Plumbley and Sweetmore, 1994). Shot-holes observed later in the leaves may possibly be due to the activities of secondary microbes (Akem, 1999). *Fusarium sp.*, *Rhizopus stolonifer*, *Curvularia pallescens* and *C. eragrostides* were isolated from anthracnose diseased yam samples (Amusa, 1997).

Currently, no work has been done on the actual causes of the vines die-back and the browning of leaves. Perhaps, the plant might have been induced to release excessive phenols as counter attack to the pathogen phenolases (tyrosinase) (Green, 1994; Plumbley and Sweetmore, 1994; Agrios, 2005) which might have caused reduction in chlorophyll contents at the infected portions; and subsequently, led to browning of leaves and dying back of vines – a necrotic structural defense reaction (Winch *et al.*, 1993). Even though, *Fusarium sp.*, *Cercospora sp.* and *Colletotrichum spp.*

were isolated from diseased *D. alata* samples collected in the present study – making it a disease complex (Amusa, 1997), spray-inoculation of healthy potted tissue culture-derived whole-plants of *D. alata* cultivars with the *Colletotrichum* isolates alone proved pathogenicity with typical symptoms of a vine-browning and die-back disease on both leaves and vines similar to that ascribed by Winch *et al.* (1984). Akem (1999) also argued that, the predominance of *Colletotrichum* spp. from sample isolations and the reproduction of typical symptoms with *C. gloeosporioides* were proves that *C. gloeosporioides* is the main causal agent of anthracnose disease of *D. alata*. It is, therefore, suggested that *Colletotrichum* isolates which have been identified as the primary causal agent of the disease be used to screen *D. alata* cultivars for resistance to the disease.

#### **5.4 Characterization of the strains of *C. gloeosporioides* and *C. truncatum* associated with anthracnose disease of *D. alata* in Ghana**

*Colletotrichum* isolates from diseased *D. alata* in the Volta region together with the countrywide collections exhibited variable cultural and morphological characters such that, even, isolates with similar conidial measurements differed in some morphological characters, sporulation and growth patterns – from one location which is similar to observations made in Nigeria (Ayodele *et al.*, n.d). Traditionally, *Colletotrichum* spp. have been identified and classified based on morphological characters (Simmonds, 1965; Smith and Black, 1990; Sutton, 1992) and several features have been utilized by taxonomists including size and shape of conidia and appressoria; presence or absence of setae, sclerotia, acervuli and teleomorph state and cultural characters such as colony colour, growth rate and texture (Photita *et al.*, 2005; TeBeest *et al.*, 1997; Than *et al.*, 2008a-c; Thaung, 2008).

The high morpho-cultural variations that existed among the isolates could be due to collections of diseased samples from a wide area with varied environmental conditions as samples collected too closely did not show any or much variations. *Colletotrichum* spp. easily become varied, both in morphology and phenotype under environmental influences (Abang *et al.*, 2002; Moriwaki *et al.*,

2002; Whitelaw-Weckert *et al.*, 2007). Cannon *et al.* (2000) also suggested nucleic acid analyses should provide the most reliable framework for classifying *Colletotrichum* spp. as DNA characters are not directly influenced by environmental factors. Based on the morphological and cultural characters obtained in the present study, rapid identification and/or differentiation of *Colletotrichum* spp. from *D. alata* could be done.

The 16 *C. gloeosporioides* and four *C. truncatum* strains identified in the culture studies were clustered into two major groups (Groups: A and B) after the genome analyses with the RAPD primers and RFLP enzymes. This finding corroborates that of Xiao *et al.* (2004) who noted clustering of 15 isolates of *C. gloeosporioides* from strawberry into two separate groups with RAPD primers. The two major groupings observed could probably be due to long period of independent evolution from a single *Colletotrichum* strain that might have gained ability to infect a particular *D. alata* cultivar (Peres *et al.*, 2002). Asexual reproduction by conidia of isolates which results in occurrences of identical genotypes and linkages between independent sets of genetic markers might have also accounted for the observation made (Milgroom *et al.*, 1996).

The close relatedness of the isolates could lead to incorrect identification using morpho-cultural characteristics. Once the species are inaccurately named and/or identified, it is probable that vital information needed for effective management of the disease would not be provided (Freeman *et al.*, 1993). *Colletotrichum* isolates Cg111 and Ct132 (from the Volta Region) which were highly pathogenic to the potted tissue culture-derived whole-plant *D. alata* cultivars in the screening trial were grouped separately from the less pathogenic; indicating that pathogenicity was decisive in group formation. This finding agrees with that of Ratanacherdchai *et al.* (2010) who reported that RAPD markers and RFLP enzymes are quick and reliable alternatives for differentiating isolates of *Colletotrichum* spp. into their respective pathogenic group. It is possible that these two isolates possessed distinct genetic make-ups from the others. As both isolates were found in the Volta

Region, effective internal quarantine measures have to be implemented to prevent the spread of these isolates (especially, the most virulent (Ct132) from the Volta Region to other yam growing regions in Ghana. Moreover, sustainable management measures have to be deployed to manage the spread of the pathogens within the region.

The high genetic relatedness of the isolates affirms the argument that identification and differentiation of *Colletotrichum* spp. based on morphological and cultural characteristics have often been inadequate (Brown *et al.*, 1996). The combined usage of molecular diagnostic tools along with traditional morpho-cultural techniques in this study, therefore, present an appropriate approach for studying *Colletotrichum* spp. complexes (Cannon *et al.*, 2000). Accurate identification of *Colletotrichum* spp. can be achieved by combining multigene analysis and morpho-cultural characters (Cai *et al.*, 2009).

Although, the isolates were closely related, high genetic diversity existed among isolates within a specified region especially, Volta which may probably be due to the high evolutionary and genetic recombination rates of the isolates (Abang *et al.*, 2006). Analyzing *C. gloeosporioides* from diseased *D. alata* in the Caribbean using RAPD markers demonstrated a high level of genetic variability among isolates (Thottappilly *et al.*, 1999; Alleyne, 2001). Extensive studies have also confirmed high genetic diversity in *Colletotrichum* spp. (Freeman *et al.*, 1998; Sahin *et al.*, 2003). It is more probable that age (i.e. period of colonization), higher mutation rates and varied environmental conditions at sampling locations/sites have accounted for the wider genetic diversity found among the isolates (Hartl *et al.*, 1989; Hodson *et al.*, 1993). The wider variations among strains within regions especially, Volta affirms the high incidence of anthracnose disease in the region in comparison to the other five regions (Coffie, Personal Observation, 2012). There is, therefore, the need to develop sustainable management measures for managing the disease in the region, however, same could be replicated in the other regions of Ghana.

### 5.5 Screening of *D. alata* cultivars for resistance to anthracnose disease

The general performance of explants of the 12 *D. alata* cultivars which were further used for screening was very good. This may be due to the ratio of hormones (BAP + NAA + Kinetin: 0.25 + 2.0 + 2.0 mg/l respectively) used. The same ratio gave significantly higher ( $P < 0.05$ ) root-shoot number and nodes per explants of sweet potato (Maura-Costa and Mantell, 1993). The marked differences observed among explants of the cultivars in the present study regarding days to bud break, mean root, mean shoot and shoot length per explant as well as nodes per shoot indicated genetic variability in responding to growth regulators, traumatic or osmotic shock, mechanical perturbation and unusual culture and environmental conditions (Gaspar *et al.*, 2004).

The screening of explants of the 12 *D. alata* cultivars in Ghana for resistance to anthracnose disease revealed six cultivars (TDa008, TDa010, TDa014, TDa023, TDa025 and TDa027) resistant to the anthracnose disease but to varying degrees. TDa023 was the highly resistant cultivar while TDa025 and TDa027 were resistant with TDa008, TDa010 and TDa014 being the least resistant. The presence of varying levels of antifungal compounds (including phenols) and leaf thickness among others may have accounted for the relative disease resistance exhibited by the cultivars to the disease. The production of phenolases (tyrosinase) is a likely mechanism by which host resistance is overcome by some *C. gloeosporioides* strains (Plumbley and Sweetmore, 1994). Structural defensive mechanisms such as cell wall thickness, formation of cork layers and tyloses are also used by plants against pathogens (Agrios, 2005).

The least resistant cultivars TDa008, TDa010 and TDa014 are amongst the early-breeding lines in Ghana (Oppong, Personal Communication, 2012). Hence, they might have lost their resistance to the pathogens due to the development of severe strains over several years of exposure to varying environmental conditions. Field resistance of *D. alata* cultivars to yam anthracnose disease have

been observed to vary between locations resulting in differences in performance (Aduramigba-Modupe *et al.*, 2008). It is worth noting, that TDa027 which recorded lower disease severity (in comparison to the other resistant cultivar (TDa025) is a parent of TDa023 – which was highly resistant to the disease (Oppong, Personal Communication, 2012). Plant breeders should, therefore, breed for novel resistant *D. alata* cultivars periodically as strains of *Colletotrichum* spp. change their infective mechanisms ('pathogen race shift') (Maloy, 1993)

The results also revealed six *D. alata* cultivars (TDa007, TDa013, TDa015, TDa018, TDa019 and TDa021) susceptible to the disease but to varying levels such that TDa019 was highly susceptible whereas TDa021 was susceptible and TDa007, TDa13, TDa15 and TDa018 were the least susceptible. Although, cultivars TDa019 and TDa021 were bred and/or released not too long (Atsu, Personal Communication, 2012), their extensive cultivation (monocropping) might have enabled the pathogen adapt to them as substrates and developed resistant-breakdown gene(s) to overcome the resistant gene(s) in these cultivars. Several studies (Ayodele *et al.*, n.d; Akem and Asiedu, 1994; Onyeka *et al.*, 2006) have confirmed that pathogen and/or pests' damage is prevalent and severe on widely cultivated crop species – of which yam was emphatically stressed (Ayodele, *et al.*, n.d; Akem and Asiedu, 1994; Onyeka *et al.*, 2006).

The high susceptibility of TDa019 to the pathogen could also mean that phenols (antifungal compounds) in the cultivar are expressed in low quantity and thus, unable to overcome phenolases (tyrosinase) released by the pathogen (Plumbley and Sweetmore (1994). In addition, the thin-leaf of TDa019 in comparison with the highly resistant cultivar TDa023 (Coffie, Personal observation, 2013) might have augmented the pathogen infection since there is the likelihood of easy penetration of infection pegs (haustoria) of the pathogen. Cultivars TDa013 and TDa018 which were the best performers among the least susceptible in the screening trial, has not been extensively cultivated in comparison with the highly susceptible cultivar (TDa019) (Klomega and Aboni, Personal

Communication, 2012). The results corroborate finding of Svabova and Lebeda (2009) who inoculated tissue cultured plants with spore and mycelial filtrate as inocula. The pathogen might not have adapted to them as substrates. Differences in phenolic contents of the cultivars may also account for the difference in susceptibility observed. The biochemistry of the *D. alata* cultivars screened, therefore, needs to be studied so that the true nature of resistance of the test *D. alata* cultivars to anthracnose disease could be identified. The possible chemical resistant factors produced by the pathogen should be comprehensively researched and potential pathotoxins identified used for screening cell suspensions from which anthracnose disease tolerant cell lines could be obtained.

The *C. gloeosporioides* isolate (Cg111) and *C. truncatum* (Ct132) found in the Volta Region were more virulent compared to isolates from the other five regions. These isolates (Cg111 and Ct132) may possess complex disease-infection mechanisms (Abang *et al.*, 2006) than the others which might have contributed to the separate groupings in the cluster analyses. This affirms the high occurrence and severity of anthracnose disease of *D. alata* in the region compared to the other regions (Atsu and Akator, Personal Communication, 2012; Abdallah and Isaac, Personal Communication, 2012; Coffie, Personal Observation, 2012; Charles, Personal Communication, 2013). The resistant cultivars identified in this study have to be subjected to field-screening trials in two or more other regions of Ghana to ascertain their true resistance before recommending them to farmers. However, at present, efficient management of the disease in the Volta region would require the planting of the resistant cultivars (TDa008, TDa010, TDa014, TDa023, TDa025 and TDa027) alongside judicious use of fungicides and farm sanitation in an integrated disease management strategy.

## 5.6 Development of IMP strategy to manage anthracnose disease of *D. alata*

The *in-vitro* screening of fungicides for control of *C. gloeosporioides* (Cg111) and *C. truncatum* (Ct132) isolates revealed Carbendazim (systemic fungicide), Mancozeb (protectant fungicide) and Cuprous oxide (Nordox) as 100% effective in inhibiting radial mycelial growth and sporulation of the isolates while Shafiz F1 was the least effective. This confirms the findings of Naphtali (2012) that Carbendazim and Mancozeb are effective fungicides inhibiting radial mycelial growth and sporulation of *C. gloeosporioides* from diseased onion seedlings. The poor performance of Shafiz F1 could partly be owed to the inability of its active ingredient to degrade the cell-wall of the isolates (Gullino *et al.*, 2000).

The integrated disease management studies on the field revealed that integration of the highly resistant *D. alata* cultivar (TDa023), hot-water treatment of seed yam ( $45 \pm 1$  °C for 20 minutes), monthly weeding and Mancozeb sprays was an active strategy for reducing the disease severity followed by the selection of the highly resistant cultivar, hot-water treatment, weeding and Carbendazim sprays with the least being the integration of the highly resistant cultivar, hot-water treatment, weeding and Nordox sprays. Integrated pest management measure that combines selection of healthy planting materials, hot-water treatment, good sanitation, crop rotation, destruction of infected crop residues, chemical application and proper time of harvest has often been the best approach in controlling crop diseases and/or pests (Ogundana, 1971; Nwakiti and Arene, 1976; Nwakiti, 1982; Small, 1988; Egesi *et al.*, 2007; Obeng-Ofori, 2007). Selection of resistant cultivars, use of clean pathogen-free planting setts, crop rotation practices, spraying of recommended fungicides, removing weeds that may be alternative hosts, early staking and planting of barrier crops like maize in integration is effective in the management of anthracnose disease of yam (Offei *et al.*, 2008).

The better performance of the disease management package containing Mancozeb sprays partly agrees with the finding of Schwartz and Mohan (1995) who reported that Mancozeb spray is effective in controlling diseases in many crops. Mancozeb sprays significantly reduce anthracnose disease of yam Akem and Asiedu (1994) and onion Naphtali (2012). Mancozeb is multi-site in its mode of action and hence, could delay resistance of the pathogen (Gullino *et al.*, 2000). It is therefore, recommended that a disease management measure which integrates selection of healthy planting materials (setts) of the resistant cultivar (TDa023) alongside hot-water treatment, regular weeding and Mancozeb sprays be used to manage anthracnose disease of *D. alata* in the Volta Region and/or Ghana. However, Mancozeb sprays could be rotated with Carbendazim sprays to delay build-up of pathogen resistance.

The performance of the fungicides on the field contrasted the *in-vitro* findings (where all recorded 100% inhibition of radial mycelial growth and sporulation of the test fungi). This could partly be attributed to reaction of the respective active ingredients (a.i) to the varied environmental conditions (rainfall, temperature and relative humidity) that existed on the field at the period of the trial (Gullino *et al.*, 2000). Although, the cultivar TDa019 (farmers' preference) could not reduce the disease severity as did cultivar TDa023, it is recommended that, if possible, the two cultivars be planted as a 'mixed-crop' to reduce the disease build-up and/or spread (Maloy, 1993; Akem and Asiedu, 2004; Egesi *et al.*, 2007) alongside the use of Mancozeb-Carbendazim sprays in rotation and proper farm sanitation in an integrated disease management in the Volta Region. Currently, the development of *D. alata* populations with multiple resistances to anthracnose disease by the International Institute of Tropical Agriculture (IITA) has been advocated (IITA, 1993) in Nigeria which could be replicated in Ghana to manage the disease.

## CHAPTER SIX

### 6.0 CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

- The results of the study indicated that most *D. alata* farmers in the Nkwanta-North and Krachi-West districts of the Volta Region were aware of the presence of anthracnose disease of water yam on their fields and could identify it by its symptoms. They were, however, ignorant of the causal organisms and spread of the disease within and among farms. Although, the disease was not controlled, some farmers perceived use of chemicals as the right option for control of the disease. The disease impacted negatively on the livelihoods of the farmers.
- The Nkwanta-North district had higher disease incidence (75.30%) than Krachi-West district (55.70%) but disease severity was similar in both districts – Nkwanta-North (1.80) and Krachi-West (1.60).
- The vine-browning and die-back disease was identified as anthracnose disease of water yam. The pathogens responsible for the disease of *D. alata* in the Volta region and other yam growing regions (Ashanti, Brong-Ahafo, Central, Eastern and Northern regions) in Ghana are strains of *Colletotrichum gloeosporioides* and *C. truncatum*.
- A total of four strains of *Colletotrichum gloeosporioides* and two *C. truncatum* were found in the Volta region. However, in the six regions (Volta, Ashanti, Brong-Ahafo, Central, Eastern and Northern), a total of 18 strains of *C. gloeosporioides* and four of *C. truncatum* were found.
- *D. alata* cultivars TDa008, TDa010, TDa014, TDa023, TDa025 and TDa027 were resistant to anthracnose disease but at varying levels. Cultivar TDa023 was highly resistant while TDa025 and TDa027 were resistant and TDa008, TDa010 and TDa014 were the least resistant. Six other cultivars (TDa007, TDa013, TDa015, TDa018, TDa019 and TDa021) were susceptible to the disease but also, at varying levels. Cultivar TDa019 was highly susceptible whereas TDa021 was susceptible and TDa007, TDa013, TDa015 and TDa018 were the least susceptible.
- IPM strategy involving the use of highly resistant cultivar (TDa023) alongside hot-water treatment of seed yam (45°C for 20 minutes and drying for 30 minutes), monthly weeding and Mancozeb sprays was effective for managing anthracnose disease of *D. alata* in the field.

## 6.2 Recommendations

- Education of *D. alata* farmers in the Nkwanta-North and Krachi-West districts on the causes and mode of spread of the pathogens could change their perception and contribute to effective management of the disease.
- Effective internal quarantine measures should be implemented to prevent and/or reduce the spread of the *C. truncatum*, the most virulent strain from Volta Region to other yam growing regions in Ghana.
- Identification of disease resistance factors in *D. alata* should be done to aid Plant breeders in breeding for novel resistant cultivars to manage anthracnose disease.
- RAPD and ITS-RFLP techniques using different primers could be used to further characterize *Colletotrichum* strains in the country. SSR primers could be employed alongside the RAPD and ITS-RFLP primers for characterization of wide number of strains.
- Benefit-cost analysis of the IPM strategy developed for the management of anthracnose disease of water yam should be undertaken.
- Screening of the resistant *D. alata* cultivars identified should be repeated on the field at different locations to assess their performance and yield. Resistant *D. alata* cultivars bred in IITA, Nigeria could be sourced and screened for resistance to the *Colletotrichum* isolates found in Ghana.

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## APPENDICES

### APPENDIX I: Questionnaire on aetiology, importance and control of a vine-browning and die-back disease of water yam (*Dioscorea alata* L.) in the Volta Region of Ghana

*Documentation of farmers' knowledge, perception and experiences concerning prevalence, spread, control and economic importance of a vine-browning and die-back disease of D. alata.*

Questionnaire No.....

#### A. Background

1. Name of farmer.....Male/female.....
2. Level of education.....
3. Name of District.....
4. Name of village.....

#### 5. Size of yam farms, year of cultivation, sources of planting materials and expenditure

| Year | Size | Varieties | Source of planting materials | Expenditure |        |                    |        |
|------|------|-----------|------------------------------|-------------|--------|--------------------|--------|
|      |      |           |                              | land        | labour | Planting materials | others |
| 2008 |      |           |                              |             |        |                    |        |
| 2009 |      |           |                              |             |        |                    |        |
| 2010 |      |           |                              |             |        |                    |        |
| 2011 |      |           |                              |             |        |                    |        |

#### B. Farmers Knowledge, perception and experiences concerning disease prevalence

6. Please mention the diseases that affect the following parts of your yam
  - I. Leaves.....
  - II. Vines.....
  - III. Tubers.....
7. What is the size of the farm affected?.....
8. What percentage of your yam farm has been affected by anthracnose? A) Less than 25% B) Between 25% and 50% C) Between 50% and 75% , D) Between 75% and 100%, E) 100%

9. How do the yam tubers look like when they are affected by anthracnose disease?  
.....  
.....
10. How do the leaves and vines look like when the yam is affected by anthracnose disease?  
.....  
.....
11. Please describe the stages of the disease?  
.....  
.....
12. What periods of the year do your yams suffer from anthracnose disease?  
.....  
.....
13. At what age from date of planting do the yams suffer from anthracnose?  
.....  
.....
14. Which varieties of yams are usually affected by the disease?  
.....  
.....
15. What percentage of the yam farms in this town/village do you think are affected by yam anthracnose? A) less than 25%, B) between 25% and 50%, C) between 50% and 75% , D) between 75% and 100%, E) 100%

**C. Knowledge, perception and experiences concerning cause and spread of the disease**

16. What do you think are the causes of yam anthracnose disease?  
.....  
.....
17. Which year and month was the first time your yam was affected by yam anthracnose disease?  
Year..... Month.....
18. What condition was the farm before your yam first suffered the disease?  
A) Weedy [ ] B) Flooded [ ] C) Drought [ ] D) Normal condition [ ]
19. What did you do when you noticed your yams were affected with the disease?  
.....  
.....
20. Do your yams still suffer from yam anthracnose?  
.....  
.....
21. How does yam anthracnose disease spread on the same farm?  
.....  
.....
22. How does the yam anthracnose spread from one farm to another?  
.....  
.....  
.....

23. If your yam fails due to anthracnose disease what crop will you plant and why?

.....  
 .....

**D. Knowledge, perception and experiences concerning control**

24. Can yam anthracnose be controlled? Yes..... No.....

25. If yes, what do you think can be done to control the spread of yam anthracnose disease?

| What can be done | Who should do it | Why |
|------------------|------------------|-----|
|                  |                  |     |
|                  |                  |     |
|                  |                  |     |
|                  |                  |     |

26. What have you done to your yam to control anthracnose disease?

.....  
 .....

27. If no to question 26, give reasons

.....  
 .....

**E. Knowledge, perception and experiences concerning economic importance of yam anthracnose disease**

28. Expected yield, volume harvested and reasons for yield difference

| Year | Farm size | Expected yield | Volume harvested | Reasons for difference |
|------|-----------|----------------|------------------|------------------------|
| 2008 |           |                |                  |                        |
| 2009 |           |                |                  |                        |
| 2010 |           |                |                  |                        |
| 2011 |           |                |                  |                        |

29. Reasons for planting yam and what was done when yam was diseased

| Year | Reasons for planting yam | % of yam affected | What did you do when was diseased? |
|------|--------------------------|-------------------|------------------------------------|
| 2008 |                          |                   |                                    |
| 2009 |                          |                   |                                    |
| 2010 |                          |                   |                                    |
| 2011 |                          |                   |                                    |

30. Please explain the effect of yam anthracnose disease on the following:

- I. Money to pay your children's school fees  
.....  
.....
- II. Money to repay loans  
.....  
.....
- III. Money to pay medical bills  
.....  
.....
- IV. Money to buy assets  
.....  
.....
- V. Your relationship with your spouse and neighbours  
.....  
.....
- VI. Your relationship with extension officers  
.....  
.....

Farmer's contact number.....

**APPENDIX II: Surveyed Areas for the Diversity Study**

Table of Regions, Districts, Communities and number of farms selected for the Diversity Study.

| <b>Regions</b>     | <b>Districts</b> | <b>Communities</b>   | <b>Number of farms</b> |
|--------------------|------------------|----------------------|------------------------|
| <b>Ashanti</b>     | Ejisu-Juabeng    | Fumesua              | 15                     |
|                    |                  | Kentinkrono          | 15                     |
|                    |                  | Kwamo                | 15                     |
|                    | Adansi-South     | New Edubiase         | 15                     |
|                    |                  | Bronikurom           | 15                     |
|                    |                  | Odumasi              | 15                     |
| <b>Brong-Ahafo</b> | Tanoso-Central   | Twumia No. 1         | 15                     |
|                    |                  | Twumia No. 2         | 15                     |
|                    |                  | Sunkunde             | 15                     |
|                    | Tanoso-South     | Tanoso               | 15                     |
|                    | <b>Central</b>   | Awutu Senya District | Bawjiase Central       |
|                    |                  |                      |                        |
| Assin-Central      |                  | Odumase              | 15                     |
|                    |                  | Assin-Fosu           | 15                     |
|                    |                  | Assin-Edubiase       | 15                     |
| <b>Eastern</b>     | Nkurakan         | Sekesoa              | 15                     |
|                    |                  | Asesewa              | 15                     |
|                    | Akim-Tafo        | Bunso                | 15                     |
| <b>Northern</b>    | Tolon Kunbung    | SARI                 | 15                     |
|                    |                  | Nyankpala-Central    | 15                     |
|                    |                  | Nyanriga             | 15                     |
|                    | Bole             | Bole Central         | 15                     |
| <b>Volta</b>       | Nkwanta-North    | Kpasa-Tindani        | 15                     |
|                    |                  | Kpasa-Abunyanya      | 15                     |
|                    |                  | Bajimpoa-Akura       | 15                     |
|                    | Krachi-West      | Osramae              | 15                     |
|                    |                  | Ehiamankyene         | 15                     |
|                    |                  | Gyidiasu             | 15                     |
|                    |                  |                      | <b>405</b>             |

### APPENDIX III: ANOVA for the analyses of incidence and severity of a vine-browning and die-back disease in the Nkwanta-North and Krachi-West districts

Disease\_Incidence

Two-sample t-test

Variates: Nkwanta\_North, Krachi\_West

Summary

| Sample                                                                             | Size | Mean  | Variance | Standard deviation | Standard error of mean |
|------------------------------------------------------------------------------------|------|-------|----------|--------------------|------------------------|
| Nkwanta-North                                                                      | 15   | 75.30 | 984.37   | 32.90              | 9.55                   |
| Krachi-West                                                                        | 15   | 55.70 | 531.92   | 23.13              | 5.19                   |
| Difference of means:                                                               |      |       | 19.55    |                    |                        |
| Standard error of difference:                                                      |      |       | 4.81     |                    |                        |
| 95% confidence interval for difference in means: (3.09, 39.28)                     |      |       |          |                    |                        |
| Test of null hypothesis that mean of Nkwanta_North is equal to mean of Krachi_West |      |       |          |                    |                        |
| Test statistic t = 6.52 on 28 df; Probability < 0.001                              |      |       |          |                    |                        |

Disease\_Severity

Two-sample t-test

Variates: Nkwanta\_North, Krachi\_West

Summary

| Sample                                                                             | Size | Mean | Variance | Standard deviation | Standard error of mean |
|------------------------------------------------------------------------------------|------|------|----------|--------------------|------------------------|
| Nkwanta-North                                                                      | 15   | 1.80 | 1.251    | 1.013              | 0.291                  |
| Krachi-West                                                                        | 15   | 1.60 | 1.095    | 0.984              | 0.177                  |
| Difference of means:                                                               |      |      | 0.27     |                    |                        |
| Standard error of difference:                                                      |      |      | 0.16     |                    |                        |
| 95% confidence interval for difference in means: (-1.013, 1.286)                   |      |      |          |                    |                        |
| Test of null hypothesis that mean of Nkwanta_North is equal to mean of Krachi_West |      |      |          |                    |                        |
| Test statistic t = 1.46 on 28 df; Probability = 0.013                              |      |      |          |                    |                        |

#### APPENDIX IV: ANOVA for pathogenicity test for the Volta region strains

| Variate: Pathogenicity test |      |        |       |       |
|-----------------------------|------|--------|-------|-------|
| Source of variation         | d.f. | s.s.   | m.s.  | v.r.  |
| Pathogen                    | 2    | 38.61  | 19.31 | 16.09 |
| Cultivar                    | 1    | 23.10  | 23.10 | 19.25 |
| Pathogen.Cultivar           | 2    | 64.33  | 32.17 | 26.81 |
| Residual                    | 55   | 65.97  | 1.20  |       |
| Total                       | 60   | 192.01 |       |       |

F pr.  
<.001  
<.001  
<.001

#### APPENDIX V: ANOVA for the performance of *D. alata* explants on different hormones combinations in the main experiment

| Variate: Days to _root |      |          |         |      |
|------------------------|------|----------|---------|------|
| Source of variation    | d.f. | s.s.     | m.s.    | v.r. |
| Variety                | 11   | 24370.84 | 2215.53 | 4.07 |
| Residual               | 108  | 58723.00 | 543.73  |      |
| Total                  | 119  | 83093.84 |         |      |

F pr  
<.001

| Variate: %Explant_response |      |         |       |      |
|----------------------------|------|---------|-------|------|
| Source of variation        | d.f. | s.s.    | m.s.  | v.r. |
| Variety                    | 11   | 20.20   | 1.84  | 0.08 |
| Residual                   | 108  | 2504.29 | 23.19 |      |
| Total                      | 119  | 2524.49 |       |      |

F pr  
.063

| Variate: Days to _shoot |      |        |       |      |
|-------------------------|------|--------|-------|------|
| Source of variation     | d.f. | s.s.   | m.s.  | v.r. |
| Variety                 | 11   | 231.51 | 21.04 | 10.2 |
| Residual                | 108  | 300.38 | 2.78  |      |
| Total                   | 119  | 331.89 |       |      |

F pr  
<.001

#### Variate: Number of \_roots

| Source of variation | d.f. | s.s.    | m.s.   | v.r.  |
|---------------------|------|---------|--------|-------|
| Variety             | 11   | 1030.76 | 139.09 | 11.88 |
| Residual            | 108  | 1293.73 | 11.98  |       |
| Total               | 119  | 2388.00 |        |       |

F pr  
<.001

#### Variate: Shoot\_number

| Source of variation | d.f. | s.s.   | m.s. | v.r. |
|---------------------|------|--------|------|------|
| Variety             | 11   | 104.88 | 9.45 | 6.22 |
| Residual            | 108  | 164.09 | 1.52 |      |
| Total               | 119  | 268.09 |      |      |

F pr  
<.001

#### Variate: Shoot\_length

| Source of variation | d.f. | s.s.   | m.s.  | v.r. |
|---------------------|------|--------|-------|------|
| Variety             | 11   | 114.15 | 10.34 | 7.95 |
| Residual            | 108  | 140.66 | 1.30  |      |
| Total               | 119  | 254.81 |       |      |

F pr  
<.001

**APPENDIX VI: ANOVA for Mean severity (AUDPCs) for a vine-browning and die-back disease on 12 tissue-cultured *D. alata* cultivars raised in a screen house**

Variate: Screening sum\_AUDPC

| Source of variation | d.f. | s.s.     | m.s.    | v.r.  | F pr. |
|---------------------|------|----------|---------|-------|-------|
| Pathogen            | 5    | 8263.75  | 1652.55 | 20.98 | <.001 |
| Cultivar            | 11   | 22189.76 | 2017.25 | 25.61 | <.001 |
| Pathogen.Cultivar   | 55   | 45274.33 | 823.17  | 10.45 | <.001 |
| Residual            | 289  | 22764.23 | 78.77   |       |       |
| Total               | 360  | 98492.07 |         |       |       |

**APPENDIX VII: ANOVA for the mean disease incidence of a vine-browning and die-back disease of 12 tissue-cultured *D. alata* cultivars raised in a screen house**

Variate: Screening mean\_incidence

| Source of variation | d.f. | s.s.       | m.s.    | v.r. | F pr. |
|---------------------|------|------------|---------|------|-------|
| Pathogen            | 5    | 37983.65   | 7596.73 | 1.82 | .035  |
| Cultivar            | 11   | 52189.76   | 4744.52 | 1.14 | .107  |
| Pathogen.Cultivar   | 55   | 105274.33  | 1914.08 | 0.46 | .429  |
| Residual            | 289  | 1203044.37 | 4162.78 |      |       |
| Total               | 360  | 1398492.11 |         |      |       |

**APPENDIX VIII: ANOVA for fungicides inhibitory effect on radial mycelial growth and sporulation of three *Colletotrichum* spp.**

**Variate: Fungicides inhibitory\_effect, Day\_1**

| Source of variation | d.f | s.s.  | m.s. | v.r. | F pr  |
|---------------------|-----|-------|------|------|-------|
| Treatments          | 3   | 9.34  | 3.11 | 2.76 | <.001 |
| Residual            | 26  | 29.38 | 1.13 |      |       |
| Total               | 29  | 38.72 |      |      |       |

**Variate: Fungicides inhibitory\_effect, Day\_2**

| Source of variation | d.f | s.s.  | m.s. | v.r. | F pr  |
|---------------------|-----|-------|------|------|-------|
| Treatments          | 3   | 15.92 | 5.31 | 4.96 | <.001 |
| Residual            | 26  | 27.89 | 1.07 |      |       |
| Total               | 29  | 43.81 |      |      |       |

**Variate: Fungicides inhibitory\_effect, Day\_3**

| Source of variation | d.f | s.s.  | m.s. | v.r. | F pr  |
|---------------------|-----|-------|------|------|-------|
| Treatments          | 3   | 20.41 | 6.80 | 4.15 | <.001 |
| Residual            | 26  | 42.63 | 1.64 |      |       |
| Total               | 29  | 63.04 |      |      |       |

**Variate: Fungicides inhibitory\_effect, Day\_4**

| Source of variation | d.f | s.s.  | m.s.  | v.r. | F pr  |
|---------------------|-----|-------|-------|------|-------|
| Treatments          | 3   | 32.11 | 10.70 | 6.49 | <.001 |
| Residual            | 26  | 42.80 | 1.65  |      |       |
| Total               | 29  | 74.91 |       |      |       |

**Variate: Fungicides inhibitory\_effect, Day\_5**

| Source of variation | d.f | s.s.  | m.s.  | v.r. | F pr  |
|---------------------|-----|-------|-------|------|-------|
| Treatments          | 3   | 49.78 | 16.59 | 9.32 | <.001 |
| Residual            | 26  | 46.36 | 1.78  |      |       |
| Total               | 29  | 96.14 |       |      |       |

**Variate: Fungicides inhibitory\_effect, Day\_6**

| Source of variation | d.f | s.s.  | m.s.  | v.r.  | F pr  |
|---------------------|-----|-------|-------|-------|-------|
| Treatments          | 3   | 67.19 | 22.40 | 13.57 | <.001 |
| Residual            | 26  | 30.85 | 1.19  |       |       |
| Total               | 29  | 98.04 |       |       |       |

**Variate: Fungicides inhibitory\_effect, Day\_7**

| Source of variation | d.f | s.s.   | m.s.  | v.r.  | F pr  |
|---------------------|-----|--------|-------|-------|-------|
| Treatments          | 3   | 89.55  | 29.85 | 11.44 | <.001 |
| Residual            | 26  | 67.89  | 2.61  |       |       |
| Total               | 29  | 157.44 |       |       |       |