

**MANAGEMENT OF FUNGAL DISEASES OF PEPPER (*Capsicum* spp.) WITH  
HOST PLANT RESISTANCE AND SELECTED BIOPESTICIDES IN  
SOUTHWESTERN NIGERIA**

**BY**

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## ABSTRACT

Pepper is an important vegetable crop used as a food condiment worldwide. However, Fungal Diseases (FD) reduce yield and limit pepper production. Synthetic pesticides are effective in managing FD but could be detrimental to the environment. Resistant crop varieties and biopesticides have been employed to manage FD on some crops, but there is limited information on their use in controlling pepper FD. Therefore, FD of pepper, and their management with host plant resistance and biopesticides were investigated.

Infected pepper leaves (n=450) were randomly collected from farmers' fields in Oyo, Ogun, Osun, Ekiti and Ondo States where pepper were predominantly cultivated, and symptoms of FD were assessed. Fungi were isolated, identified and Frequency of Occurrence (FO) and their pathogenicity determined following standard procedures. On the field, ten pepper cultivars comprising three *Capsicum annuum*-CAN, three *Capsicum frutescens*-CFR and four *Capsicum chinense*-CCH were evaluated for resistance to *Colletotrichum coccodes*-CC, *Colletotrichum capsici*-CCA and *Pyrenochaeta lycopersici*-PL on a plot of 6 x 15m (40,000 plants/ha) area in a split-plot design replicated four times. Disease Severity (DS) was assessed using a rating scale of 1.0-2.0 (resistant), 2.1-2.5 (moderately resistant), 2.6-3.0 (susceptible), 3.0-5.0 (highly susceptible). On the field, eight biopesticides [dried powder of *Thevetia neriifolia* Leaf-TNL, *Azadirachta indica* Leaf-AIL and Seed-AIS, *Tagetes erecta* Shoot-TES and Root-TER each at 2.5g/100L of water and biocontrol agents; *Trichoderma harzianum*-TH, *Trichoderma pseudokonnigii*-TP each at  $2.06 \times 10^6$  spores/mL, and *Bacillus subtilis*-BS at  $2 \times 10^8$  CFU/mL] were evaluated separately on CAN-Tatashe (40,000 plants/ha) inoculated with the pathogens. Untreated and mancozeb treated plots served as controls. The experimental design was split-plot fitted into RCBD and replicated four times. Disease severity was determined at 12 weeks after sowing. Data were analysed using descriptive statistics and ANOVA at  $\alpha_{0.05}$ .

The FD symptoms identified were leaf spots, chlorosis, necrosis, blight and fruit rot. Six fungi (CC, TH, CCA, PL, TP and *Penicillium* sp) were isolated and identified. *Colletotrichum coccodes* had the highest FO of 81, 64, 60, 54% in Oyo, Ogun, Osun, and Ekiti States, respectively, while TP was highest in Ondo State (55.5%). Three isolates, CC, CCA and PL were pathogenic with 84.0, 72.0 and 50.0% levels of infection, respectively. *Penicillium* sp, TH and TP were not pathogenic (0.0%) on healthy pepper plants. *Capsicum chinense* cv. Batassa was resistant ( $2.0 \pm 0.1$ ) to CCA and PL and moderately resistant ( $2.4 \pm 0.2$ ) to CC. Other pepper cultivars ranged from susceptible ( $3.0 \pm 0.3$ ) to highly susceptible ( $4.7 \pm 1.0$ ) to the three pathogens, while uninoculated plants had the least DS of  $1.0 \pm 0.0$ . Plants treated with TP had the least DS ( $1.5 \pm 0.0$ ) which was significantly different from AIS ( $2.0 \pm 0.1$ ), AIL ( $2.5 \pm 0.2$ ) and TNL ( $2.5 \pm 0.2$ ) powders. The DS of plants sprayed with TH ( $2.7 \pm 0.3$ ) and BS ( $2.7 \pm 0.3$ ) were significantly lower than TES ( $3.7 \pm 0.7$ ), TER ( $4.0 \pm 0.9$ ), and mancozeb ( $4.1 \pm 1.0$ ).

Fungal diseases of pepper are prevalent in Southwestern Nigeria. *Colletotrichum coccodes*, *Colletotrichum capsici* and *Pyrenochaeta lycopersici* were pathogenic on all pepper cultivars. *Capsicum chinense* cv. Batassawas resistant to fungal diseases of pepper. *Trichoderma pseudokonnigii* at  $2.06 \times 10^6$  spore/mL reduced fungal infection on pepper.

**Keywords:** Pepper fungal diseases, *Trichoderma pseudokonnigii*, Biopesticides, *Capsicum* Spp., Pathogens

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**CERTIFICATION**

This is to certify that this study was carried out by Miss Felicia Omoyeme Aigbokhan in the Department of Crop Protection and Environmental Biology, Faculty of Agriculture, University of Ibadan, Oyo state, Nigeria.

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## **DEDICATION**

This research work is dedicated to God Almighty, the source of my strength and wisdom and also for His infinite mercies, and to Mr. and Mrs. M. O. Aigbokhan and those who answered anytime I called. I appreciate your love, support and encouragement.

## Table of Contents

| PAGE   |          |
|--|----------|
| Fly leaf   | i        |
| Title Page                                       | ii       |
| Abstract   | iii      |
| Acknowledgements                                 | iv       |
| Certification                                    | vi       |
| Dedication                                       | vii      |
| Table of content                                 | viii     |
| List of Tables                                   | xxi      |
| List of Plates                                   | xxiv     |
| List of Figures                                  | xxvii    |
| <b>CHAPTER ONE</b>                               | <b>1</b> |
| <b>INTRODUCTION</b>                              | <b>1</b> |
| <b>CHAPTER TWO</b>                               | <b>5</b> |
| <b>LITERATURE REVIEW</b>                         | <b>5</b> |
| Description of Pepper                            | 5        |
| Origin and Species of Pepper                     | 8        |
| Environmental Requirements for Pepper Production | 8        |
| Nursery Preparation                              | 10       |
| Cultivation of Pepper                            | 11       |
| Growth of Pepper Plants                          | 11       |
| Uses of pepper                                   | 12       |
| Insect Pests of Pepper Plants                    | 12       |



|   |    |
|---|----|
| Diseases of Pepper  | 13 |
| Cercospora Leaf Spot  | 14 |
| Anthracnose   | 14 |
| Damping-off   | 14 |
| White Mould   | 14 |
| Powdery mildew caused by <i>Leveillula taurica</i>  | 15 |
| Fusarium wilt caused by <i>Fusarium oxysporum</i>   | 15 |
| Phytophthora blight caused by <i>Phytophthora capsici</i>   | 15 |
| Southern blight caused by <i>Sclerotium rolfsii</i>   | 15 |
| Bacterial Spot Disease  | 16 |
| Bacterial wilt disease  | 16 |
| Virus Diseases of Pepper  | 16 |
| Management of Fungal Diseases   | 17 |
| Crop Rotation   | 18 |
| Use of Resistant Varieties  | 18 |
| Use of Chemical Control   | 19 |
| Biological Control Methods  | 20 |
| Use of Botanicals   | 20 |
| Management of Pathogens with Biopesticides  | 21 |
| Microbial Biopesticides   | 21 |
| <i>Trichoderma</i> species  | 21 |
| Botanical Biopesticides   | 21 |
| Use of Biochemical Pesticide (PlantExtracts) for the<br>Control of Fungal Diseases                      | 22 |
| <b>CHAPTER THREE</b>  | 23 |
| <b>MATERIALS AND METHODS</b>  | 23 |
| Characteristics and Perception of Pepper Growers in Three Selected<br>Locations in Southwestern Nigeria | 23 |

|   |    |
|---|----|
| Study Areas   | 23 |
| Population, Sampling Procedure and Sample Size  | 23 |
| Methods and Sources of Data   | 25 |
| Measurement of Variables  | 25 |
| Personal characteristics of the Respondents   | 25 |
| Production Resources of Pepper Farmers  | 25 |
| Constraints in Pepper Production  | 25 |
| Disease Management Practices of Pepper Farmers  | 26 |
| Use of Pesticides   | 26 |
| Sampling, Screening and Management of Fungal Diseases   | 26 |
| Experimental Sites  | 26 |
| Collection of Infected Pepper Samples, Disease Incidence and Severity                           | 26 |
| Source of Planting Seeds  | 27 |
| Sterilization of Materials  | 27 |
| Media Preparation   | 29 |
| Isolation and Identification of Fungi   | 29 |
| Isolation and Identification of Biocontrol Agents from Soil                                     | 30 |
| Fungal and Bacterial Spore Estimation   | 30 |
| Pathogenicity of Fungal Isolates on Three Local Cultivars of Pepper                             | 31 |
| Screening of Ten Pepper Cultivars for Resistance to the<br>Identified Fungal Pathogens in Pots  | 32 |
| Preparation and Inoculation of Fungal Pathogens   | 32 |
| Screening of Ten Pepper Cultivars for Resistance to Identified<br>Fungal Pathogens on the Field | 36 |
| Management of Pepper Diseases using Biopesticides   | 38 |
| <i>In vitro</i> activity of Bio-control agents Using Slide Culture Technique                    | 38 |
| Collection and Preparation of Botanicals  | 38 |
| <i>In vitro</i> Antifungal Activity of the Selected Plant Extracts on the                       |    |

|   |    |
|---|----|
| Isolated Pathogenic Fungi   | 40 |
| Antifungal Activity of the Biopesticides on Isolated Pathogenic Fungi                           | 40 |
| Data Collection   | 42 |
| Phytochemical Analyses of Neem, Marigold and Milk bush  | 43 |
| Determination of Tannin Total Content   | 43 |
| Determination of Total Saponin  | 43 |
| Determination of Total Alkaloid   | 43 |
| Determination of Total Flavonoid Content (TFC)  | 44 |
| Determination of Total Phenolic Content (TPC)   | 44 |
| Data Analyses   | 45 |
| <b>CHAPTER FOUR</b>   | 46 |
| <b>RESULTS</b>  | 46 |
| Characteristics and Perception of Pepper Growers in the<br>Three Selected Southwestern States   | 46 |
| Personal Characteristics of Respondents   | 46 |
| Crops Grown and Farmers' Perception of Factors affecting<br>Productivity of their Pepper Farms  | 46 |
| Disease Description Symptoms associated with Pepper<br>Plants in Farmers Fields                 | 49 |
| Disease Management Practices of Pepper Farmers  | 51 |
| Percentage of Respondents Using Pesticides in Pepper<br>Farms across Selected States            | 51 |
| Relationship between personal characteristics of respondents' and<br>the level of pesticide use | 51 |
| Symptoms Expression and Distribution of Fungal Diseases   | 56 |
| Symptom Observation from Pepper Farms   | 56 |
| Diseases Severity and Incidence in Pepper Farms in<br>Southwestern Nigeria                      | 56 |
| Fungi Isolated from Diseased Pepper Plants  | 56 |

|  |    |
|--|----|
| Percentage Frequency of Occurrence of Fungi Isolated from<br>Three Pepper Cultivars Sampled in Southwestern States                         | 68 |
| Pathogenicity Test of Isolated Fungi on Three Cultivars of Pepper  | 74 |
| Screening of Ten Pepper Cultivars for Resistance to<br>Pathogenic Fungi in Pot Experiment  | 80 |
| Effect of <i>Colletotrichum capsici</i> on Plant Height and<br>Number of Leaves of Pepper Plants in Pot Experiment                         | 80 |
| Effect of <i>Colletotrichum capsici</i> on Yield and Fungal<br>Disease Severity of Pepper in Pot Experiment                                | 80 |
| Effect of <i>Pyrenochaeta lycopersici</i> on Plant Height and<br>Number of Leaves of Ten Pepper Cultivars in Pot Experiment                | 84 |
| Effect of <i>Pyrenochaeta lycopersici</i> on Yield and Disease Severity of<br>Ten Pepper Cultivars in Pot Experiment                       | 84 |
| Effect of <i>Colletotrichum coccodes</i> on Plant Height and<br>Number of Leaves of Ten Pepper Cultivars in Pot Experiment                 | 84 |
| Effect of <i>Colletotrichum coccodes</i> on Yield and Fungal<br>Disease Severity of Ten Pepper Cultivars in Pot Experiment                 | 89 |
| Evaluation of Ten Pepper Cultivars for Resistance to Pathogenic<br>Fungi in First and Second Field Trials in Year 2016 and 2017            | 89 |
| Effect of <i>Colletotrichum capsici</i> on Plant Height and Number of<br>Leaves of Ten Pepper Cultivars in First and Second Field Trials   | 89 |
| Effect of <i>Colletotrichum capsici</i> on Yield and Fungal Disease Severity of<br>Ten Pepper Cultivars in First and Second Field Trials   | 94 |
| Effect of <i>Pyrenochaeta lycopersici</i> on Plant Height and Number of<br>Leaves of Ten Pepper Cultivars in First and Second Field Trials | 94 |
| Effect of <i>Pyrenochaeta lycopersici</i> on Yield and Fungal Disease<br>Severity of Ten Pepper Cultivars in First and Second Field Trials | 98 |
| Effect of <i>Colletotrichum coccodes</i> on Plant Height and Number of   |    |

| a  | b |     |
|--|---|-----|
| Leaves of Ten Pepper Cultivars in First and Second Field Trials  |   | 103 |
| Effect of <i>Colletotrichum coccodes</i> on Yield and Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trials     |   | 103 |
| The Effect of Different Plant Crude Extracts on Three Fungal Isolates of Pepper <i>in vitro</i>  |   | 109 |
| The Effect of Plant Crude Extract on <i>Colletotrichum capsici in vitro</i>  |   | 109 |
| The Effect of Plant Crude Extracts on <i>Pyrenochaeta lycopersici in vitro</i>   |   | 113 |
| The Effect of Plant Crude Extracts on <i>Colletotrichum coccodes in vitro</i>  |   | 119 |
| The Effect of Bio-control Agents on Fungal Pathogens of Pepper <i>in vitro</i>   |   | 119 |
| Growth Parameters, Fungal Disease Severity and Yield of Three Fungal Isolates on Pepper Plants Treated with Biopesticides in the Field     |   | 122 |
| Effect of Biopesticides on Plant Height and Number of Leaves of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> in Field       |   | 122 |
| Effect of Biopesticides on Number of Fruits and Fruit Weight of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> in Field       |   | 122 |
| Effect of Biopesticides on Disease Severity of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> in Field                        |   | 122 |
| Effect of Biopesticides on Plant Height and Number of Leaves of Pepper Plants Inoculated with <i>Pyrenochaeta lycopersici</i> in Field     |   | 126 |
| Effect of Biopesticides on Number of Fruits and Weight of Fruits of Pepper Plants Inoculated with <i>Pyrenochaeta lycopersici</i> in Field |   | 126 |
| Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with <i>Pyrenochaeta lycopersici</i> in Field               |   | 130 |
| Effect of Biopesticides on Plant Height and Number of Leaves of Pepper Plants Inoculated with <i>Colletotrichum coccodes</i> in Field      |   | 130 |
| Effect of Biopesticides on Number of Fruits and Weight of Fruits of Pepper Plants Inoculated with <i>Colletotrichum coccodes</i> in Field  |   | 130 |
| Effect of Biopesticides on Fungal Disease Severity of Pepper Plants  |   |     |

|  |     |
|--|-----|
| Inoculated with <i>Colletotrichum coccodes</i> in Field  | 134 |
| Quantitative composition of Phytochemicals in <i>Azadirachta indica</i> seed, <i>Tagetes erecta</i> shoot, <i>Azadirachta indica</i> leaf, <i>Tagetes erecta</i> root and <i>Thevetia nerifolia</i> leaf | 134 |
| <b>CHAPTER FIVE</b>  | 138 |
| <b>DISCUSSION</b>  | 138 |
| <b>CHAPTER SIX</b>   | 146 |
| <b>CONCLUSION AND RECOMMENDATIONS</b>  | 146 |
| <b>REFERENCES</b>  | 148 |
| <b>APPENDIX</b>  | 162 |

## LIST OF FIGURES

|  |     |
|--|-----|
| Major pepper producing areas in Nigeria  | 9   |
| Pepper Diseases Assessment areas in Southwestern Nigeria.  | 28  |
| Layout of field experiment for the screening of pepper cultivars to fungal pathogens   | 37  |
| Layout of field experiment for the management of pepper diseases with biopesticides  | 41  |
| Percentage Respondents using Crop Rotation as a Management practice for Reducing Pepper Fungal Diseases in the Three Selected Southwestern, Nigeria, 2014          | 52  |
| Farmer's Sources of Planting Materials in Selected Southwestern States   | 53  |
| Percentage of Respondents Using Pesticides for Management of Disease in Selected Southwestern Nigeria, 2014  | 54  |
| Disease Severity of Pepper Plants in the Southwestern States   | 60  |
| Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> on the Field                   | 124 |
| Effect of Biopesticides on Number of Fruits (a) and Weight of fruits (b) of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> on the Field               | 125 |
| Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with <i>Colletotrichum capsici</i> in the Field                                     | 127 |
| Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants Inoculated with <i>P. lycopersici</i> in the Field                           | 128 |
| Effect of Biopesticides on Number of Fruits (a) and Fruit Weight (b) of Pepper Plants Inoculated with <i>P. lycopersici</i> in the Field                           | 129 |
| Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with <i>P. lycopersici</i> in the Field   | 131 |
| Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants of Pepper Plants Inoculated with <i>Colletotrichum coccodes</i> on the Field | 132 |
| Effect of Biopesticides on Number of Fruits (a) and Fruit Weight (b) of Pepper Plants Inoculated with <i>Colletotrichum coccodes</i> on the Field                  | 133 |

|  |     |
|--|-----|
| Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with<br><i>Colletotrichum coccodes</i> in the Field | 135 |
| Fruit shape of pepper cultivars  | 34  |



## LIST OF PLATES

|  |    |
|--|----|
| A plant of <i>Capsicum frutescens</i> flowering and fruiting   | 6  |
| Pepper fruits (a) <i>Capsicum frutescens</i> , (b) <i>Capsicum chinense</i> (c) <i>Capsicum annuum</i>   | 7  |
| Pictures of pepper cultivars-Cultivar  | 32 |
| Part of different botanicals used  | 39 |
| Symptoms observed on infected pepper plant from the survey   | 57 |
| Viral diseases of pepper plants observed on infected pepper plants the survey  | 58 |
| 4. 2: Seven day old culture of Isolated from infected pepper plants collected during the survey conducting in Southwestern Nigeria. MAG × 400  | 63 |
| 4.3: Seven day old culture of <i>Penicillium</i> species species isolated from infected pepper plants collected during the survey conducting in Southwestern Nigeria   | 64 |
| 4.4:Seven day old culture of <i>Trichoderma pseudokoningii</i> Isolated from infected pepper plants collected during the survey conducting in Southwestern Nigeria.  | 65 |
| 4.5: Seven day old culture of <i>Trichoderma harzianum</i> isolated from infected pepper plants during survey in Southwestern Nigeria.   | 66 |
| 4.6: Seven day old culture of <i>Colletotrichum capsici</i> isolated from infected pepper plants collected during the survey conducting in Southwestern Nigeria.   | 67 |
| 4. 7: (a) Seven day old culture of <i>Pyrenochaeta lycopersici</i> isolated from infected pepper plants collected during the survey conducting in Southwestern Nigeria.  | 69 |
| 4. 8:Chlorotic foliage and wilting (a) Acervuli masses on the fruits of bell pepper ( <i>Capsicum annuum</i> ) caused by <i>Colletotrichum coccodes</i> from inoculated pepper plant (b)   | 76 |
| 4.9: Small, brownish lesions surrounded by chlorotic halo on the leaves of Inoculated pepper plant(a). Fruit lesions on bell pepper ( <i>Capsicum annuum</i> ) concentrically arranged on fruit caused by <i>Colletotrichum capsici</i> from inoculated pepper plant (b) | 77 |
| 4.10: Leaf spot which include yellowing of leaves (a) Rot symptoms on hot pepper fruits caused by <i>Colletotrichum capsici</i> from inoculated pepper plant.  | 78 |
| 4.11: Leaves of pepper plants Inoculated with (a) <i>Penicillium</i> species (b) <i>T. harzianum</i>   |    |

|   |     |
|---|-----|
| (c) <i>T. pseudokoningii</i> from inoculated pepper plant (d) uninoculated plants   | 79  |
| 4. 12: Growth inhibition of <i>Colletotrichum capsici</i> on PDA media incorporated with <i>A. indica</i> seed extract at 5,000 mg/kg concentration (a), <i>Colletotrichum capsici</i> on PDA media seven days after Inoculation (b). | 111 |
| 4.:13 : Growth inhibition by <i>Colletotrichum capsici</i> on PDA media incorporated with <i>T. neriifolia</i> leaf extract at concentrations of 12,500 mg/kg   | 114 |
| 4.14: Growth inhibition of <i>Pyrenochaeta lycopersici</i> on PDA media incorporated with <i>A. indica</i> leaf plant extracts at 7,500 mg/kg concentration at seven days after Inoculation   | 115 |
| 4:15 : Growth inhibition of <i>Pyrenochaeta lycopersici</i> on PDA media incorporated with <i>Tagetes erecta</i> shoot extract at concentrations of 7,500 mg/kg   | 117 |
| 4.16: Growth inhibition of <i>Colletotrichum coccodes</i> on PDA media incorporated with <i>A. indica</i> seed extracts at 2,500 mg/kg concentration (b) seven days after Inoculation   | 118 |
| 4.17: Growth inhibitions between (a) <i>C.coccodes</i> (b) <i>P. lycopersici</i> and (c) <i>C. capsici</i> and <i>Trichoderma pseudokoningii</i> in a dual culture 7 days after incubation  | 123 |

## LIST OF TABLES

|   |    |
|---|----|
| 3.1: Agricultural Development Programmes and Number of Respondents from each States   | 24 |
| 3.2: List of Pepper Cultivars used in this Study  | 33 |
| 4. 1: Personal Characteristics of Pepper Farmers in Southwestern States of Nigeria, 2014  | 47 |
| 4.2: Perception of Pepper Growers in the Three Selected States  | 48 |
| 4.3: Percentage of Diseases Indicated and Constraints Observed by Pepper Farmers in the Three Selected States                                 | 50 |
| 4.4: Chi-square Analysis Showing the Relationship between Respondents' Personal Characteristics and their Level of Pesticides Usage           | 55 |
| 4.5: Percentage Disease Incidence from Infected Pepper Fields in Southwestern Nigeria   | 59 |
| 4.6. Isolated Fungi Categorized into Three Classes on the Basis of Diametric Mycelial Growth  | 62 |
| 4. 7: Frequency of Occurrence (%) of Isolated Fungi from Three Pepper Cultivars Sampled in Southwestern States, Nigeria, 2014                 | 70 |
| 4.8: Frequency of Occurrence (%) of Isolated Fungi From Hot Pepper ( <i>Capsicum chinense</i> ) in Southwestern States Nigeria, 2015          | 71 |
| 4.9: Frequency of Occurrence (%) of Fungi Isolated from Chilli Pepper ( <i>Capsicum frutescens</i> ) in Southwestern States, Nigeria, 2015    | 72 |
| 4. 10: Percentage Frequency of Occurrence of Fungi Isolated from Bell Pepper ( <i>Capsicum annuum</i> ) in Southwestern States, Nigeria, 2015 | 73 |
| 4.11: Number of Diseased Leaves (%) Inoculated with Six Isolated Fungi on Healthy pepper plants   | 75 |
| 4.12: Effect of <i>Colletotrichum capsici</i> on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment                  | 81 |
| 4.13: Effect of <i>Colletotrichum capsici</i> on Number of Fruits and Weight of Fruits of   |    |

|  |    |
|--|----|
| Ten Pepper Cultivars in Pot Experiment   | 82 |
| 4.14: Effect of <i>Colletotrichum capsici</i> on Disease Severity of Ten Pepper Cultivars in Pot Experiment                                  | 83 |
| 4.15: Effect of <i>Pyrenochaeta lycopersici</i> on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment               | 85 |
| 4.16: Effect of <i>Pyrenochaeta lycopersici</i> on Number of Fruits and Weight of Fruits of Ten Pepper Cultivars in Pot Experiment           | 86 |
| 4.17: Effect of <i>Pyrenochaeta lycopersici</i> on Disease Severity of Ten Pepper Cultivars in Pot Experiment                                | 87 |
| 4.18: Effect of <i>Colletotrichum coccodes</i> on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment                | 88 |
| 4.19: Effect of <i>Colletotrichum coccodes</i> on Number of Fruits and Weight of Fruits of Ten Pepper Cultivars in Pot Experiment            | 90 |
| 4.20: Effect of <i>Colletotrichum coccodes</i> on Disease Severity of Ten Pepper Cultivars in Pot Experiment                                 | 91 |
| 4.21: Effect of <i>Colletotrichum capsici</i> on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials                  | 92 |
| 4.22: Effect of <i>Colletotrichum capsici</i> on Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials                   | 93 |
| 4.23: Effect of <i>Colletotrichum capsici</i> on Number of Fruits of Ten Pepper Cultivars in First and Second Field Trials                   | 95 |
| 4.24: Effect of <i>Colletotrichum capsici</i> on Weight of Fruits (g) of Ten Pepper Cultivars in First and Second Field Trials               | 96 |
| 4.25: Effect of <i>Colletotrichum capsici</i> on Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trial Experiments | 97 |
| 4.26: Effect of <i>Pyrenochaeta lycopersici</i> on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials                | 99 |
| 4.27: Effect of <i>Pyrenochaeta lycopersici</i> on Number of Leaves of Ten Pepper Cultivars  |    |

|   |     |
|---|-----|
| in First and Second Field Trials  | 100 |
| 4.28: Effect of <i>Pyrenochaeta lycopersici</i> on Number of Fruits of Ten Pepper Cultivars in First and Second Field Trials  | 101 |
| 4.29: Effect of <i>Pyrenochaeta lycopersici</i> on Weight of Fruits (g) of Ten Pepper Cultivars in First and Second Field Trials  | 102 |
| 4.30: Effect of <i>Pyrenochaeta lycopersici</i> on Disease Severity of Ten Pepper Cultivars in First and Second Field Trials  | 104 |
| 4.31: Effect of <i>Colletotrichum coccodes</i> on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials  | 105 |
| 4.32: Effect of <i>Colletotrichum coccodes</i> on Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials   | 106 |
| 4.33: Effect of <i>Colletotrichum coccodes</i> on Number of fruits (g) of Ten Pepper Cultivars in First and Second Field Trials   | 107 |
| 4.34: Effect of <i>Colletotrichum coccodes</i> on Weight (g) of Fruits of Ten Pepper Cultivars in First and Second Field Trials   | 108 |
| 4.35: Effect of <i>Colletotrichum coccodes</i> on Disease Severity of Ten Pepper Cultivars in First and Second Field Trials   | 110 |
| 4.36: <i>In vitro</i> Growth Inhibition of <i>Colletotrichum capsici</i> by Plant Crude Extracts  | 112 |
| 4.37: <i>In vitro</i> Growth Inhibition of <i>Pyrenochaeta lycopersici</i> by Plant Crude Extracts  | 116 |
| 4.38: <i>In vitro</i> Growth Inhibition of <i>Colletotrichum coccodes</i> by Plant Crude Extracts   | 120 |
| 4.39: Growth Inhibition of Bio-control Agents on Pepper Fungal Pathogens <i>in vitro</i> using Slide Technique  | 121 |
| 4.40: Quantitative Composition of Phytochemicals present in <i>Azadirachta indica</i> seed, <i>Tagetes erecta</i> shoot, <i>Azadirachta indica</i> leaf, <i>Tagetes erecta</i> root and <i>Thevetia neriifolia</i> leaf | 136 |

## CHAPTER ONE

### 1.0 INTRODUCTION

Vegetables (pepper, tomatoes, onions, okra, lettuce and cabbages, etc) are the main constituents of human diet (Fayemi, 1999; Sikora and Fernandez, 2005). They are valuable cash crops for farmers (Sikora and Fernandez, 2005). On the African continent, the top three vegetable crops produced are tomatoes, onions and pepper (Sikora and Fernandez, 2005). Nigeria ranked tenth among the producers of vegetables in the world in 2016 with 13,000 million tonnes (FAO, 2017). Pepper is a major vegetable crop cultivated globally (Fayemi, 1999; Sikora and Fernandez, 2005)

*Capsicum* species is a plant of the family Solanaceae (Wells, 2008). It is indigenous to Mexico and has been predominantly planted over a period of years. Pepper has become a major spice in many food preparations. Pepper species have different names which depend on location as well as the type of variety. The most common variety is the chilli pepper usually called the spicy pepper. The big, moderate form is called the bell pepper in many countries (Latham, 2013).

Globally, pepper production exceeded 472,500 million metric tons (MT) in 2016 (FAO, 2017). Vietnam was the highest supplier of pepper worldwide with 140,000 MT in 2016 followed by Indonesia with 70,000 MT, India (48,500 MT) and Brazil the 4<sup>th</sup> producer of pepper in the world with about 45,000 MT (FAO, 2017). Malaysia is the 5<sup>th</sup> producer of pepper with 28, 300 MT as against Nigeria producing 13,000 MT in 2016 (FAO, 2017). Pepper consumption is about 40% of the total amount of vegetables

intake per day. Pepper can contribute between ₦138 million annually as profit after tax to the manufacturing industries in Nigeria (Aja, 2012).

Pepper has been established to be useful in medicines (Wells, 2008). Pepper contains capsaicin which is used in medicine for treating circulatory ailments. It is a stimulant and a painkiller. The extract of capsaicin from pepper has been made into aerosol form used by force agents to weaken people in a gathering during riots (Quattrocchi, 2000). Pepper extract has been used as a natural insecticide in gardens (Mason *et al.*, 1999).

Pepper fruits are used as food condiments or eaten raw. They are used as sauce together with meat or cheese for bread fillings (Eshbaugh, 1975). They are sold as jam, or dried and stored as dry pepper. Pepper can also be made into powder forms which are regularly added to vegetables, stews and soups. They are fundamental ingredients for some special delicacies, such as *nduja* (Wells, 2008). Different pepper types are often used in sauces to eat starch staples. Pepper is a major ingredient in making sauce such as hot suya powder (Fayemi, 1999). In Nigeria, pepper is readily dried, ground and packaged for export (Idowu-Agida *et al.*, 2010).

Globally, the total amount of fresh vegetables produced for the market as a percentage of total production has decreased slightly since 1990 (Sikora and Fernandez, 2005). Pepper production is constrained by biotic factors which include insects, weeds, birds, fungi, bacteria, viruses and nematodes throughout the world. *Capsicum* spp. are considered to be a major crop in the tropics. It is a primary profitable produce grown globally (Poulos, 1992) and severely affected by diseases causing 50% to 84% yield losses (Pakdeevaram *et al.*, 2005). Insect pests associated with pepper include; Beet armyworm (*Spodoptera exigua*), Flea beetles (*Chaetocnema pulicaria*), Leafminers (*Lyriomyza* spp.), Aphids (*Myzus persicae*), Leafroller (*Platynota stultana*), Pepper weevil (*Anthonomus eugeni*), Thrips (*Thrips tabaci*) and Spider mites (*Tetranychus urticae*) (Burt, 2005). Fungal diseases that are important limiting factors in pepper production include, Powdery mildew (*Leveillula taurica*), Fusarium wilt (*Fusarium oxysporum*), Phytophthora blight (*Phytophthora capsici*) and Damping-off (*Pythium* spp) (Pernezny *et al.*, 2003; Valenzuela, 2011). Generally, bacterial diseases include bacterial spot disease caused by *Xanthomonas vesicatoria* and bacteria wilt (*Ralstonia*

*solanacearum* = *Pseudomonas solanacearum*). Viruses are also major constraint to pepper production and they include *Cucumber mosaic virus* (CMV) and *Potato vein mottle virus* (PVMV) (Valenzuela, 2011).

Different methods have been used in managing diseases as well as pests associated with pepper. These methods include the use of botanicals, biological agents and soil amendments and they are known as biopesticides (Agbenin and Marley, 2006). Botanicals, such as Siam weed, have been used in the management of plant pathogens (Agbenin and Marley, 2006). *Azadirachta indica* has caused 100% reduction in the incidence of some plant pathogenic fungi (Amadioha, 2000). Biological agents such as *Bacillus subtilis*, *Trichoderma* and *Penicillium* species have been reported to possess fungicidal properties. *Bacillus* and *Trichoderma* species were indicated as effective antifungal organisms in the management of plant pathogens (Muthukumar, 2009).

Disease management option is an important aspect in agriculture and in order to reduce the use of pollutant chemicals such as pesticides that are released to the environment. Therefore, the need for alternative method which is the use of biopesticides that are environment friendly and do not require specialized application, as opposed to most synthetic chemicals. Knowledge of the adverse effects of synthetic pesticides has led to exploration of natural pesticides in disease management. Botanicals and biological control agents (biopesticides) have been employed in disease control of many economically important crops. An example is the *Trichoderma harzianum* which has been used extensively and successfully in the management of many soil borne diseases such as Phytophthora blight (*Phytophthora capsici*) (Pernezny *et al.*, 2003).

Pepper an important economic crop worldwide; its production and yield is low owing to effects of pathogens on the field. Losses due to these pathogens range from 50% to 84% (Pakdeevaporn *et al.*, 2005). Literature is scanty in the development of resistant pepper cultivars in the management of fungal diseases of pepper. Hence, the need to determine the resistance of different pepper varieties to fungal diseases. Food and environmental safety is important for sustainable development. The adverse effect of synthetic pesticides on human health and the natural ecosystem necessitate the need to explore natural mechanisms of disease control in plants. Botanicals and biological control agents (biopesticides) have been employed in disease control of many economically



important crops. However, biopesticides have not been extensively used in the diseases of pepper, especially, as a management for fungal diseases. Therefore, this research work was conducted to investigate resistant pepper cultivars and biopesticides in managing fungal diseases of pepper.

In view of the importance of fungal diseases as a serious biotic factor in pepper production, the objectives of these studies were to:

1. determine selected personal characteristics of pepper growers in Southwestern Nigeria
2. determine production resources and constraints encountered in the production process
3. investigate, isolate and identify fungal pathogens associated with pepper in Southwestern Nigeria
4. evaluate and select pepper varieties for resistance to fungal pathogens
5. evaluate biological agents and plant botanicals for pepper disease management

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1. Description of Pepper

Pepper (*Capsicum* spp.) belongs to the Solanaceae family. *Capsicum* originated from Mexico and America (Terry-Kelley and Boyhan, 2009). Pepper (Plate 2.1) is a warm climate, self-pollinating plant even though out-crossing can occur. It is grown as an annual or perennial (Motes *et al.*, 2009). The plant is sometimes woody at the base and may grow to 1-2 m tall. Leaves vary in size and shape, but are mainly oval with pointed tips. The flowers may be solitary or in clusters of two or three in the leaf axils. The fruit is a berry and varies in size, shape, colour and pungency depending on the variety (Plate 2.2) (Agbato, 1999). The fruit contains capsaicin, a volatile chemical which is an indication of the pungency (hotness) (Agbato, 1999). The fruits of *Capsicum annuum* are milder in pungency; others like the sweet pepper are not sharp in flavour (Terry-Kelley and Boyhan, 2009). Fruits of *Capsicum annuum* can be green, white or yellow in colour when immature and red, or brown when ripe and mature depending on the variety (Agbato, 1999; Soto-Ortiz and Silvertooth, 2008; Terry-Kelley and Boyhan, 2009).

The main varieties in Nigeria are *Capsicum annuum*, *C. frutescens* and *C. chinense* Jacq. (Lakshmi *et al.*, 1993). *Capsicum annuum* is a large fruit-bearing pepper variety (Odugbemi and Akinsulire, 2006) such as the bell or sweet (tatashe) pepper (Sahin and Miller, 1996). *Capsicum annuum* is an annual erect herb or sub-herb with many branches. Adetiloye (2005) reported that bell pepper (*Capsicum annuum*) is the oldest and common of all spices and is widely used as condiment, which blends well with most savoury dishes and has extensive culinary uses. Most of the bell pepper consumed in Nigeria are produced from the northern parts of the country (Figure 2.1) (Erinle, 1989).

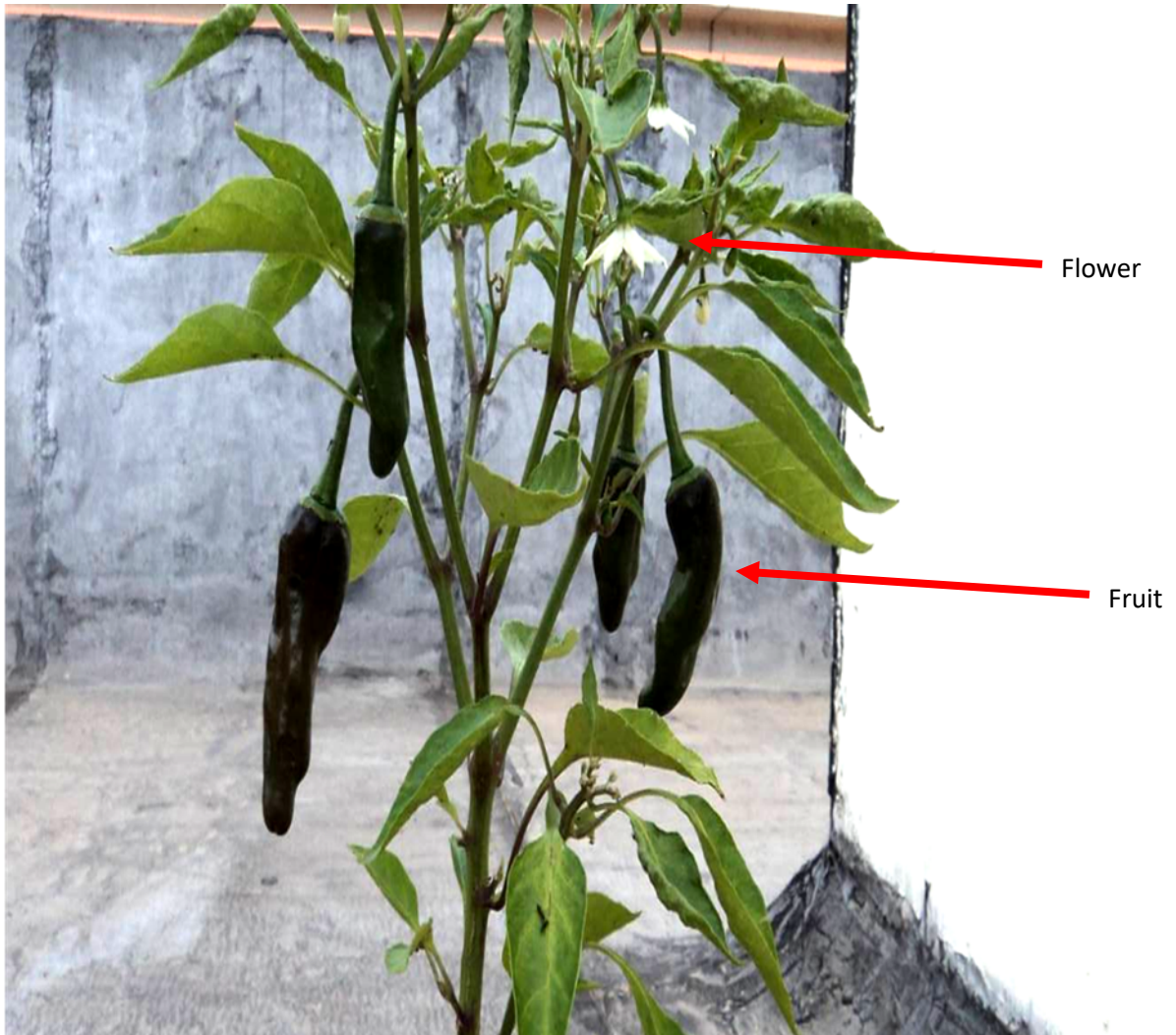


Plate 2.1: A plant of *Capsicum frutescens* flowering and fruiting



Plate 2.2: Pepper fruits (a) *Capsicum frutescens*, (b) *Capsicum chinense* (c) *Capsicum annuum*

Poor seed germination, poor seedling establishment and premature fruit drop due to pests and diseases reduce pepper production in southern parts of the country (Adetiloye, 2005). *Capsicum frutescens* Mill (bird pepper) is a perennial, sub-herb which thrives for three to four years. The fruits are narrower than those of *Capsicum annuum*, highly pungent, spindle-shaped and green when immature but red or yellow/orange while some remain green at maturity (Lakshmi *et al.*, 1993). *Capsicum chinense*, hot pepper (atarodo) is a perennial herb or sub-herb with fruits which are small, spindle-shaped, and green when immature but red or yellow/orange at maturity and highly pungent (Lakshmi *et al.*, 1993).

## **2.2 Origin and Species of Pepper**

Pepper originated from the Central and South Americas. Hot chilli and sweet pepper (*Capsicum annum*) originated from Mexico. The hot pepper (aromatic) type of pepper (*Capsicum chinense*) is indigenous to Amazonian zone and *Capsicum frutescens* (bird pepper) is from coastal zones of America. It is thought that pepper was brought to West African region through the Portuguese about five centuries ago. *Capsicum* consists of 27 species, five of which are cultivated and they include *C. baccatum*, *C. annum*, *C. pubescens*, *C. frutescens*, and *C. chinense* (Norman *et al.*, 1992).

*Capsicum annum*, *C. frutescens* and *C. chinense* are classified as single species in tropical Africa (Calpas, 2002). Calpas (2002) divided *C. annum* into four cultivar units: aromatic pepper, bird pepper, chilli pepper, and sweet pepper. Pepper has been naturalised as a native crop of the sub-region (Calpas, 2002).

## **2.3 Environmental Requirements for Pepper Production**

Planting of pepper is more suitable in sandy soil. Peppers are best planted in soil with pH of 6 and 7. Peppers can tolerate a well-drained, sandy loam, for optimum production as it grows best on deep, medium textured sandy soil or loamy, fertile soil or raised bed (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008; Valenzueka, 2011).

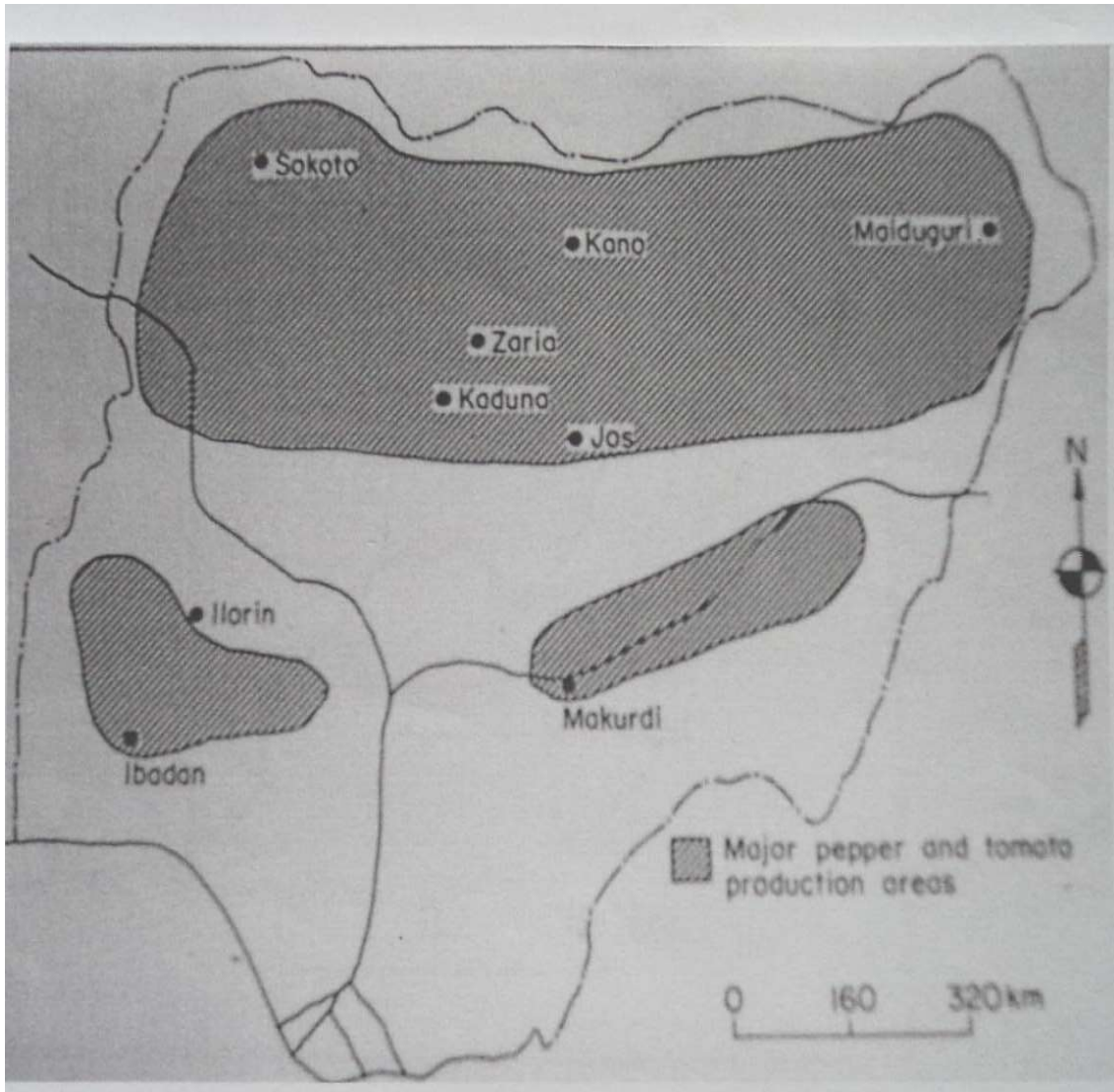


Figure 2.1: Major pepper producing areas in Nigeria (Erinle, 1989)

Pepper plants require a medium rainfall level of 600-750 mm. Excessive rainfall beyond 750 mm causes flowers to drop, poor fruit setting and fruit rot. Pepper requires warm growing season with a temperature of 18-30°C. Temperatures above 32°C with fairly low relative humidity can increase transpiration leading to bud, flower and fruit to drop (Agbato, 1999; Terry-Kelley and Boyhan, 2009).

Pepper seedlings are nursed and transplanted when they are about 8-10 cm in height or having four to six true leaves. Pepper requires a planting space of 60 cm x 75 cm apart with 35 cm x 45 cm between plants (Agbato, 1999). Pepper can survive in a warm climate condition with a temperature of between 18-30°C (Aram and Rangarajan, 2005). Peppers flourish in a series of soil types, although well drained soil is necessary for optimal growth. Soil with large lumps, stones or iron should be removed to allow good drainage (Agbato, 1999). Pepper responds to the addition of fertilizers (Agbato, 1999; Aram and Rangaran, 2005). Weeding is necessary as pepper is a poor competitor with weeds (Terry-Kelley and Boyhan, 2009).

### **2.3.1 Nursery Preparation**

Pepper requires a rich soil to raise seedlings (CABI, 2008). Soils are firmly pressed to the brim of the trays before watering. Nursery beds are prepared in the shaded areas of field into 1 meter wide with 1 meter path in between beds. The bed surfaces are leveled before seeds are planted on the beds (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008; Valenzueka, 2011). Sodium methyldithiocarbamate is applied at the rate of 1 liter to 20 liters in each bed to manage nursery diseases (Valenzueka, 2011). Beds or seedling trays containing soil can be watered a day before sowing. Drills can be made into about 5–10 cm apart across the beds and 100 seeds per drill are sown (CABI, 2008) then covered lightly. Seedlings are thinned to 1 stand per 2.5 cm of drill 15–20 days after sowing.

Watering of the seedlings can be done every morning. Watering can be reduced in quantity during hardening of seedlings. Shade can be provided in the nursery to protect seedlings from hot weather and heavy rains. A framework of palm-frond shed can be used to provide adequate shading. Palm fronds can be reduced to half at 30 days after

sowing while the other fronds can be removed on 40 days after sowing. This should be done to harden the seedlings before transplanting (CABI, 2008).

### **2.3.2 Cultivation of Pepper**

Land preparation is carried out seven days before transplanting of seedlings. These include ploughing, harrowing and making of beds. Beds of about 1.0 meter wide should be made (CABI, 2008).

Generally, moderate watering is encouraged. Over-watering may predispose plants to diseases such as damping off (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008). Circles can be drawn round the base of the plant and fertiliser carefully spread in the groove (Burt, 2005). The grooves are then covered lightly with soil. Circles are made around each plant at a distance of about 4 –7 cm from the stem of the plant. (Pernezyny *et al.*, 2003; CABI, 2008). Fruiting starts 2-3 months after transplanting, and, in favourable conditions this will continue for months (Agbato, 1999). Pepper is harvested with the hands and done before full maturity (Motes *et al.*, 2009) before the red colour develops.

### **2.4 Growth of Pepper Plants**

The growth stages of pepper (*Capsicum annuum*) are pre-bloom (46.5-48 days), early bloom (66-68 days), peak bloom-early fruit development (89-92 days), physiological maturity-green (117-122 days) and red harvest stage (154-160 days) (Soto-Ortiz and Silvertooth, 2008). The pre-bloom stage covers the nursery period to transplanting (Calpas, 2002; Terry-Kelley and Boyhan, 2009). The early bloom stage covers the period of foliage production to first flowering (Calpas, 2002; Terry-Kelley and Boyhan, 2009). The peak bloom extends to the first harvest (Calpas, 2002; Soto-Ortiz and Silvertooth, 2008). The physiological maturity stage is the maximum growth rate phase and the red harvest stage is senescence or decreasing phase (Soto-Ortiz and Silvertooth, 2008). The growth of *Capsicum* species is dependent on variety and environmental factors which influence the supply of water, nutrients and cultural practices for optimum production (Soto-Ortiz and Silvertooth, 2008).



## 2.5 Uses of pepper

Peppers are made into stews, sauces and soups for human consumption (Gill, 1988; Fayemi, 1999). They are also used to flavour beverages because they contains high amounts of Vitamin A (9.5 mg), B6 (0.2 mg), C (121.0 mg), thianine (0.5 mg), ash (0.9 mg), fat (2.3 g), protein (4.1 g), phosphorus (1.2 mg), iron (2.9 mg), potassium (3.2 mg), carbohydrates (2.9 g) and food energy (94 calories) per 100 g of raw edible red hot chilli (Fayemi, 1999; Odugbemi and Akinsulire, 2006; Terry-Kelley and Boyhan, 2009). Capsaicin, the fiery alkaloid found in the placenta of chilli fruits has anti-inflammatory and pain-killing effects, and can be used for the treatment of different neurophysiologic disorders. It is used for lowering blood cholesterol and it is used as an anti-obesity agent (Fayemi, 1999; Celocia *et al.*, 2006; Odugbemi and Akinsulire, 2006). Dried fruits of *Capsicum annuum* can be used for treatment of fowl cholera and dried, ground fruits of *C. frutescens* for the treatments of diarrhea, cold, Newcastle and coccidiosis of poultry (Egbunike and Nworgu, 2005). Dried pepper is mixed with grains/seeds in air-tight hermetic containers and stored for months without damage from insect pests. Dried pepper is insecticidal, an antifeedant and discourages oviposition (Nworgu, 2006). In Nigeria, cowpea seeds are protected well with powder from hot pepper at rate of 1% mixed with the grains over a period of eight weeks (Onu and Aliyu, 1995).

Peppers are also used for anti-microbial activity on meat. The phenol derivate (coumaric acid) extracted from *Capsicum annuum* is responsible for the anti-microbial action (Kim *et al.*, 1995). Pepper can be ground into powder to extend the durability of meat sausages in a modified environment. Pepper can also be used to put off odour produced by meaty foods (Martinez *et al.*, 2006).

## 2. 6 Insect Pests of Pepper Plants

Pests of pepper plants include beet armyworm (*Spodoptera exigua*), flea beetles (*Chaetocnema pulicaria*), leafminers (*Lyriomyza* spp.), aphids (*Myzus persicae*), tomato fruit worm (*Helicoverpa zea*), leafroller (*Platynota stultana*), pepper weevil (*Anthonomus eugenii*), thrips (*Thrips occidentallis*) and spider mites (*Tetranychus urticae*) (Vos *et al.*, 1993).

Pests of pepper can be managed by early planting which allows establishment of seedlings before infestation and have proven to be the best method of control. Oils such as the neem oil are incorporated into the affected plants which serve as a non-chemical method of control. Carbaryl, spinosad, bifenthrin and permethrin are insecticides that provide satisfactory measures for the management of flea beetles for weeks although re-application will be needed (Valenzuela, 2011).

## **2.7 Diseases of Pepper**

Diseases affecting plants are major factors limiting pepper production in the world (Terry-Kelley and Boyhan, 2009). These diseases are caused by environmental factors, fungi, bacteria, viruses and nematodes. Diseases affect leaves, roots, stem, flowers and fruits. There are also postharvest infections that affect peppers in storage. A diseased crop is considered a key limitation in production of pepper (Vos *et al.*, 1993).

Among the main diseases of pepper are fungal diseases such as *Cercospora* leaf spot caused by *Cercospora capsici* (Terry-Kelley and Boyhan, 2009). Anthracnose infection by *Colletotrichum nigrum* and *C. gloeosporioides* occurs on fruits of affected plants (Terry-Kelley and Boyhan, 2009). Phytophthora fruit and crown rot caused by *Phytophthora capsici* (Terry-Kelley and Boyhan, 2009) is a main problem in pepper cultivation. Phytophthora fruit and crown rot are destructive diseases of pepper surpassed only by bacterial spot and *Tomato spotted wilt virus* (TSWV) diseases in order of importance and yield losses. Symptoms include wilting of plant and complete death of plants. The crown region of the plant base is often darkened, hollow and somewhat necrotic (Terry-Kelley and Boyhan, 2009). Most virus diseases affecting pepper cause symptoms such as stunting, leaf distortion, mosaic leaf discolouration, and spots or discolourations on the fruit (Terry-Kelley and Boyhan, 2009).

Pepper virus diseases are the key limiting factors in pepper cultivation throughout the world. Some of these viruses are *Pepper veinal mottle virus* (PVMY), *Tomato spotted wilt virus* (TSWV) *Cucumber mosaic virus* (CMV) and *Pepper mild mottle virus* (PMMV)

### **2. 7. 1 Cercospora Leaf Spot**

Cercospora leaf spot is caused by *Cercospora capsici*. Symptoms of cercospora leaf spot include small, round to oblong lesions which are surrounded by light gray masses at the centers of the leaves. The stalks and stem of the plants are heavily affected with these symptoms as well. Infected leaves are generally shed prematurely (Terry-Kelley and Boyhan, 2009).

### **2.7.2 Anthracnose**

Anthracnose is found to be associated with fruit diseases of plant especially ripe fruits. *Colletotrichum capsici* and *C. gloeosporioides* are fungal pathogens associated with this disease (Boucher and Richard, 2001). Symptoms on hot and sweet peppers under appropriate conditions such as low temperature, low humidity includes immature fruit, lesions on stems, and leaf spots. Infections may appear as hollow lesions on the pepper fruit. Sometimes the lesions change to black with a pattern of setae and sclerotia surrounding the lesion which may develop into acervuli containing grey coloured masses of spores. *Colletotrichum* usually produced micro-sclerotia which allow the fungus to take over the soil where they can continue to exist for years. A three-year crop rotation from the susceptible crops such as the solanaceous crops can considerably lower the inoculum level in the soil (Boucher and Richard, 2001).

### **2.7.3 Damping-off**

Damping-off in pepper is caused by *Pythium* species and *Rhizoctonia solani*. Damping-off causes poor seed germination, and death of collapsed seedlings. Discoloured roots can also be seen. Appearance of disease is caused by overcrowded population of plants and too much fertilizer application of nitrogen (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008).

### **2.7.4 White Mould**

White mould is a soil-borne fungus caused by *Sclerotinia sclerotiorum*. It is also known as watery soft rot, stem rot and blossom blight. The disease leads to about 5% yield loss of pepper plants during the cool and wet growing season (Boucher and

Richard, 2001). Symptoms include water soaked lesions on stems of infected plants. Portions of infected stems develop a wilting appearance consisting of white fungal growth especially when environmental conditions are favourable (Pernezyny *et al.*, 2003).

#### **2.7.5 Powdery mildew caused by *Leveillula taurica***

*Leveillula taurica* causes white powdery growth on the underside of leaves which starts in patches but spreads all over the leaves, including the top surface of the leaves. There are also yellow-brown discolourations on the underside of the affected leaves (Boucher and Richard, 2001). Diseases commonly occur in older leaves both in humid and dry conditions (Boucher and Richard, 2001).

#### **2.7.6 Fusarium wilt caused by *Fusarium oxysporum***

Symptoms of fusarium wilt of pepper range from yellowing of foliage and wilting of upper leaves which spread to all parts of plant and leaves that remain attached to plant are dark green in colour. Red-brown discolouration of vascular tissue and, eventually death of plant. Disease emergence is favoured by high soil moisture content (Pernezyny *et al.*, 2003, Burt, 2005; CABI, 2008; Valenzuela, 2011).

#### **2.7.7 Phytophthora blight caused by *Phytophthora capsici***

This disease is caused by the pathogen *Phytophthora capsici*. It causes blight of leaves, crown, root, and fruit rot in infected plants. It is one of the most devastating diseases of pepper. The symptoms include black lesions and wilting of leaves, fruits, infected plants (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008, Valenzuela, 2011). Roots and stems closer to the soil becomes water-soaked, dark brown in colour and eventually the whole plants collapse (Gevens *et al.*, 2008)

#### **2.7.8 Southern blight caused by *Sclerotium rolfsii***

Infected plants show rapid leaf wilting, chlorosis of the foliage and are dark grey around the soil. The branches becomes dark grey in colour with wave-like mat of

mycelia covering the stem may also be present (Pernezyny *et al.*, 2003, Burt, 2005; CABI, 2008).

### **2.7.9 Bacterial Spot Disease**

*Xanthomonas vesicatoria* is the pathogen causing the bacterial spot disease. (Pernezyny and Collins, 1997; Terry-Kelley and Boyhan, 2009). This disease is the most frequent disease affecting pepper. Bacterial spot symptom is often found around stems, leaves and fruits of plants. The disease takes place at all the stages of growing period of the plant (Terry-Kelley and Boyhan, 2009). The disease symptoms include water-soaked lesions and cracks on stem of plants (Burt, 2005; CABI, 2008).

### **2.7.10 Bacterial wilt disease**

*Ralstonia solanacearum* is the causal organism of the bacterial wilt disease. The disease symptom occurs as a spotted form on the plants or in collections on the infected plants. Wilting in infected plants begins with the immature leaves during the day and the plants recover briefly in the evening in a cooler environment. After some days, wilting will take place. The wilted leaves retain their leafy-green colour without falling as the disease increases. The roots and lower part of the stem turn dark grey with a water soaked portion (vascular system) of the plant (AVRDC, 2004).

### **2.7.11 Virus Diseases of Pepper**

*Cucumber mosaic virus* (CMV) disease is a very common disease of pepper and is transmitted by aphids. *Cucumber mosaic virus* causes stunting, mottling and necrosis of foliage. Fruits produced will be distorted and begin to break down at the blossom end, particularly in the seams that separate the capsules (Terry-Kelley and Boyhan, 2009). (Pernezyny *et al.*, 2003; Burt, 2005; CABI, 2008; Valenzuela, 2011).

Virus infection causes a great yield loss on pepper in Africa, Asia and the Middle East (Mbaye and Zitter, 1997). The diseased leaf becomes distorted and curled with stunted growth of infected plant. The disease is transmitted through infected weeds that harbour the virus and insects. It is one of the most common viruses affecting pepper.

Transmission is by thrips and can affect pepper at any stage of development (Terry-Kelley and Boyhan, 2009).

## **2.8 Management of Fungal Diseases**

Fungal disease management poses a major problem because these pathogens have extensive host range for most species. The control of fungal diseases can be through any of the following measures such as chemical, cultural and biological control (Martinelli *et al.* (2014). Fungal diseases can be managed with the use of disease-free planting materials, proper sanitation practices before planting and after harvesting, appropriate planting space which has been used in the management of Damping-off disease (Burt, 2005; CABI, 2008, Pernezyny *et al.*, 2003). Damping-off diseases are controlled by removing infected plants with appropriate plant spacing to avoid overcrowding of plants which promote air circulation as well as crop rotation.

The use of mycoparasites organism has been employed to eradicate the growth of sclerotia on infected plants and commercially made products which contain *Coniothyrium minutans* that are found to be of great measure in reducing the sclerotial level to the minimum. These products can be applied to the soil prior to planting. This is done by incorporating it into hole of 4 cm deep (Boucher and Richard, 2001).

Cercospora leaf spot is a also a major disease affecting pepper production. They occur from seedling stage to vegetative stage of plant and are being managed by the use of clean seeds and seedlings which are free from disease. This disease are reduced to minimal with good water management techniques, crop rotation and application of appropriate fungicides (Pernezyny *et al.*, 2003, Burt, 2005; CABI, 2008; Valenzueka, 2011). Diseases such as powdery mildew and white mould are being managed by providing the plants with a barrier to reduce infection this is done by covering the lower part of the stems with aluminum foil to wrap the lower part of the stem (Pernezyny *et al.*, 2003, Burt, 2005; CABI, 2008) and with the use of fungicides as well as planting on well drained soil (Boucher and Richard, 2001).

Blight diseases such as phytophthora blight can be managed by avoiding the use of polluted water for irrigation and soil treatment with fungicides (AVRDC, 2004). Wilting diseases in pepper are being handled by integrated method as the pathogen has

numerous strains/races and a broad host range which include tomato, tobacco, banana, eggplant, potato, plantain, sweet potato, peanut, and several weeds (Terry-Kelley and Boyhan, 2009).

### **2.8.1 Crop Rotation**

This method is one of the oldest, thriving and less expensive methods for managing the spread of fungi (Martinelli *et al.*, 2014; Nicole, 2009). It is done by substituting susceptible crops with resistant as completely immune crops, leading to decrease of fungal spores (Nicole, 2009). Disease problems and control become difficult when susceptible crops are grown without rotation. Crop rotation reduces the spread of fungal spores that remain in the soil for at least one-year after harvesting of the previous crops. The significant reduction of fungal spores lessens yield loss from the field during another planting season to a susceptible crop (Nicole, 2009).

The highly resistant pepper (Carolina Cayenne) was used in the United States of America as a rotational crop which reduced infection that allowed subsequent production of susceptible vegetable crops (Nicole, 2009).

### **2.8.2 Use of Resistant Varieties**

This method is an effective and inexpensive method to the farmer (Nicole, 2009). Fungi-resistant varieties have been stated to be the most workable and feasible method in fungal disease management (Nicole, 2009). The use of resistant varieties will reduce the reproduction of fungal spores and thereby reducing the level of inoculum considerably (Martinelli *et al.*, 2014).

Plants with inherited resistance traits to diseases are one of the most viable, cost-effective methods of management of disease (Chung and Black, 1997). When available, the resistant variety is the most excellent measure for Africa. However, crop varieties that are resistant to fungi are few and most African fungal pests are polyphagous (Martinelli *et al.*, 2014). In some instances, resistance may be related to the existence of fungicidal materials present in the plant (Martinelli *et al.*, 2014).

The utilization of cultivars resistant to fungi has been identified as a possible means of replacing methyl bromide, when applied in integrated pest control approach

(Giannakou and Anastasiadis, 2005). The small-fruited hot pepper (*Capsicum frutescens* var *longum*) is resistant to some fungal pathogens. Resistant varieties are less expensive, simple, secure, and a successful means of managing diseases in many crops (Pernezyny *et al.*, 2003). Planting of resistant cultivars is not just a way to reduce losses from plant disease, but a way to reduce operating costs for spraying and other measures of disease control to avoid the accumulation of toxic compounds in the environment which are used to managing diseases in plants (Martinelli *et al.*, 2014).

Furthermore, several diseases such as vascular diseases (root rot) that are not frequently controlled by other measures are not economical to control for most countries but the use of resistant varieties is a successful means to manage the disease (Bafti *et al.*, 2005). The use of resistant varieties gives a means of supplying good yields with no pesticides residue (Martinelli *et al.*, 2014).

### **2.8.3 Use of Chemical Control**

Chemical control is an effective method for fungal disease management (CABI 2008). It is achieved by the use of chemicals known as fungicides (CABI 2008). Fungicides are pesticides used in managing fungal pathogens or prevent the growth of fungi. Some of these pesticides are detrimental to the environment and non-target organisms. The use of fungicides has been reported to control diseases such as powdery mildew (CABI 2008). Fungicides are commonly used as protectants on plants to eliminate pathogens (Drost, 2010). Fungicide treatments applied at post planting are meant to eliminate or significantly reduce the inoculum level on plants. This soil treatment includes fumigating the soil to control insect pests and pathogens on the field prior to planting (Drost, 2010).

Fungicides of various types have been successful in controlling most of the fungal diseases in growing crops. The commercially important diseases are leaf spot diseases, fruit rots, cereal stem diseases, smuts, rusts and seed-borne diseases which are controlled with fungicides (CABI 2008). Fungicides are also used to manage postharvest diseases that cause rapid and extensive breakdown of commodities therefore posing a serious problem in storage. An example of such a fungicide is 2-butylamin (CABI 2008).



#### **2.8.4 Biological Control Methods**

Biological control method is a way of using many diverse microorganisms in the management of diseases. They can be used as seed treatment, soil treatment and plant treatment. This can be done by coating the seeds, incorporating them into the soil or spraying them on infected plants. It has been known to be a way of controlling many plant diseases all over the world (Tsrer *et al.*, 2001; Henderson *et al.*, 2009). Biological control measures are direct methods with overall protection to plants from pathogens such as fungi. It involves the exploitation of antagonistic organisms to attack infection before and after infection has taken place (Noling and Becker, 1994; Radwan *et al.*, 2007; Khan *et al.*, 2008). Biological control mechanisms engaged the use of organisms in attacking the pathogens that are destroying the plants. Organisms such as *Bacillus* species, *Trichoderma* species and *Penicillium* species has been used in the management of many fungal diseases (Jurgen, 2017). *Trichoderma harzianum* has been used extensively and successfully in the management of many soil borne diseases such as damping-off disease caused by *Phytophthora* spp., root rot caused by *Pellicularia filamentosa*, seedlings blight caused by *Pythium*spp., black scurf caused by *Rhizoctonia solanii* and dry rot caused by *Macrophomina phaseoli* (Khan *et al.*, 2008)

#### **2.8.5 Use of Botanicals**

The use of botanicals has been effective in managing fungal diseases. They are readily available and are bio-degradable (Noling and Becker, 1994; Radwan *et al.*, 2007; Khan *et al.*, 2008). The use of plant materials such as Siam weed (*Chromolaena odorata*) containing fungicidal secondary metabolites has been used in the control of nematodes in pepper (Agaba *et al.*, 2016). Various plants and their parts have been used to suppress and repel pathogens by disrupting their life cycle or discouraging the pathogens from attacking the plant (Khan *et al.*, 2008). More than 2400 plant species are currently known to possess pest control properties (Stoll, 2000). Several plants have been identified with fungicidal properties either in their seeds, fruits, leaves, barks, roots or in their root exudates. Among many include the castor plant (*Ricinus communis*), and hemp (*Cannabis sativa*) used in the management of fungal diseases (Khanna and Sharma,

1998). Many plant extracts have been used to manage fungal infection such as the use of *Manilka zapota* in the control of chilli fruit rot disease caused by *Collectotrichum capsici* in bell pepper (Ajith *et al.*, 2012)

## **2.9 Management of Pathogens with Biopesticides**

### **2.9.1 Microbial Biopesticides**

Microbial pesticides consist of microorganisms (e.g., bacterium, fungus, virus or protozoan) as the active ingredient. Examples include *Bacillus thuringiensis*, *Pseudomonas* spp, *Streptomyces* spp, *Trichoderma* spp and *Coniothyrium minitans*, etc. (PMNI, 2013).

#### **2.9 1. 1 *Trichoderma* species**

*Trichoderma* is a filamentous (PMNI, 2013) fungus the species of which were previously considered to be culture contaminants. *Trichoderma* has been described as a resourceful fungus by suppressing the growth of pathogens in the rhizosphere (Gary, 2007). Although many people refer to it as a nuisance organism. It is a fungus that is useful for industry and biological control. *Trichoderma* spp. is prevalent in the soil, decayed materials and other environments. It is the most common and culturable fungus usually found in the soil (PMNI, 2013).

In recent years, considerable success has been achieved by the use of fungal bioagent. There is feasible and effective formulation of bioagents to exploit in the commercial industry (Bravo *et al.*, 2007). The possibility of making use of different formulation of *T. viride* has been an effective, practicable, and economically viable option for improving the disease control. Apart from biological control, in many cases *Trichoderma* also has the ability to increased plant growth response after application (Kumar *et al.*, 2017).

### **2.9.2 Botanical Biopesticides**

This entails the use of plant materials such as leaves, roots, stems and bark of trees for the management of diseases. They include plant extracts from Neem, Marigold plant, *Citrus* species, Seaweed/Kelp extracts and Giant knotweed (PMNI, 2013).

### 2.9.3 Use of Biochemical Pesticide (Plant Extracts) for the Control of Fungal Diseases

As the use of synthetic agrochemicals is becoming less favourable because of environmental pollution and detrimental effects on a variety of non-target organisms (Bonjar *et al.*, 2006), it is important that a solution is found to check the besetting problems of low production. This has resulted in worldwide interest in the use of bio-control methods including natural plant products because most of them are locally available, environment friendly, having no side effects while development of resistance to diseases is rare (Soytong *et al.*, 2001).

Several workers have reported the use of plant materials in managing plant diseases (Ekpo, 1991; Amadioha, 2000; Okigbo and Nneka, 2005; Kehinde, 2008). A list of 700 plant species was reported by Secoy and Smith (1983) that are used in controlling pests in different locations around the globe. For instance, *Cymbopogon citratus* (lemon grass) and Surinam cherry (*Eugenia uniflora*) plant oils were fungitoxic on fungi associated with melon seeds in storage (Kehinde, 2008). Extracts of *Vernonia amygdalina* Del. have been found to be effective on *Curvularia lunata* and *Fusarium semitectum* (Ekpo, 1991). Also, *Azadirachta indica* extracts were found to control the seed-borne fungi of cowpea (Ekpo, 1999). It was found that spraying crude neem oil on lilac bushes when done before any outbreak, prevented powdery mildew from breaking out for the rest of the season and it also gave hundred percent control in hydrangea as better than Benlate (benomyl). Bean rust was also controlled by 90% when neem extract was applied before the plants were exposed to the fungus (Bernd, 1999). In another study, extract of *Azadirachta indica*, *Carica papaya* L., *Costus afer* Ker-Gawl, *Mangifera indica* L. and *Ocimum gratissimum* were tested on different pathogens such as *Rhizopus stolonifer* and *Penicillium chrysogenum* with reports showing that *Costus afer* inhibited all the fungi isolated, *Azadirachta indica* inhibited all except *Rhizopus stolonifer*. Extracts of *Carica papaya* inhibited the growth of *Penicillium chrysogenum* while *Ocimum gratissimum* and *Azadirachta indica* reduced *Penicillium sclerotigenum* (Madunagu and Ebena, 1994).

## **CHAPTER THREE**

### **3.0**

## **MATERIALS AND METHODS**

### **3.1 Characteristics and Perception of Pepper Growers in Three Selected Locations in Southwestern Nigeria**

#### **3.1.1 Study Areas**

The study areas, Ogun, Oyo and Ekiti States of Southwestern Nigeria were purposively selected because they were predominantly cultivated areas. The study areas comprised eleven Agricultural Development Projects (ADP) zones. Most of the people living in these areas are farmers growing pepper, tomato, maize, cassava, yam and okra (ADP, 2015).

#### **3.1.2 Population, Sampling Procedure and Sample Size**

The target population for this study comprised pepper farmers in the three selected states among the five Southwestern States of Nigeria. A three-stage sampling technique was used to select respondents from the population of farmers in the study area. The study areas comprised of three ADP zones in Ekiti State and four each in Ogun and Oyo States.

In the first stage, three ADP zones were purposively selected from each of the States: making nine ADP zones all together (Table 3.1). In Ogun State zones one, two and four were selected while zones one, two and four were selected in Oyo State and one, two, and three in Ekiti State.

In the second stage, purposive sampling was used to select respondents representing 60% of the total registered farmers with the State Agricultural Development Project (ADP) (Table 3.1).

**Table 3.1: Agricultural Development Programmes and Number of Respondents from each States**

| <b>States</b> | <b>Selected ADP</b> | <b>Number of Registered Farmers</b> | <b>No of Respondents Selected</b> |
|---------------|---------------------|-------------------------------------|-----------------------------------|
| <b>Oyo</b>    | 1                   | 53                                  | 29                                |
|               | 2                   | 50                                  | 28                                |
|               | 4                   | 45                                  | 32                                |
| <b>Ogun</b>   | 2                   | 40                                  | 21                                |
|               | 3                   | 47                                  | 25                                |
|               | 4                   | 31                                  | 15                                |
| <b>Ekiti</b>  | 1                   | 41                                  | 21                                |
|               | 2                   | 30                                  | 15                                |
|               | 3                   | 49                                  | 24                                |
| <b>Total</b>  |                     | 382                                 | 210                               |

### **3.1.3 Methods and Sources of Data**

Primary and secondary sources were used for data collection. Primary data were collected using interview schedules. The interview schedule had three sections: farmer's personal information, challenges encountered by farmers and farmer's perception of the diseases affecting vegetable production. The interview schedule contained open and close – ended questions relating to knowledge of diseases and use of pesticides among farmers. Focus group discussion was conducted with farmers in the village while secondary data were sourced from literature.

### **3.1.4 Measurement of Variables**

#### **A. Personal characteristics of the Respondents**

- i. Gender: Respondents were required to indicate their gender whether they are male or female
- ii. Age: Respondents were required to indicate their age
- iii. Marital status: Respondents were asked to indicate their marital status whether they were married, single or divorced
- iv. Educational qualification: Respondents were asked to indicate their level of education such as primary, secondary, tertiary or other qualifications
- v. How many years of vegetable/ tomato/ pepper farming experience: Respondents were asked to indicate (in years) farming experience

#### **B. Production Resources of Pepper Farmers**

Respondents were requested to provide information on different types of crops grown by their household. These include the different crops that are grown in the dry and raining season, factors affecting production of pepper in their farm(s), list of vegetable crops that are more profitable and ranking of vegetables. Respondents were asked to indicate their source(s) of information.

#### **C. Constraints in Pepper Production**

Respondents were asked to provide information regarding the type of disease (s) observed on their pepper farm, the effect of diseases on farmers yield, the amount lost to

diseases in field per annum, changes in the environment, changes in diseases severity and incidence. Respondents were asked to indicate those challenges by ticking Yes/No.

#### **D. Disease Management Practices of Pepper Farmers**

Farmers were asked if they engaged in crop rotation, plants used in practicing crop rotation and sources of planting materials over the years. Respondents were asked to indicate with (Yes) or (No) for various practices.

#### **E. Use of Pesticides**

The respondents were asked to indicate if they apply pesticides and to list different types of pesticides applied on their fields.

### **3.2. Sampling, Screening and Management of Fungal Diseases**

#### **3.2.1 Experimental Sites**

The study was conducted in farmers' field, screen-houses and research laboratories in the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan Nigeria.

#### **3.3 Collection of Infected Pepper Samples, Disease Incidence and Severity**

*Capsicum* species that were infected with visible symptoms were taken following a Z-pattern from farmers' fields in Oyo, Osun, Ogun, Ekiti and Ondo States of Nigeria. The sample areas comprised all the ADP zones in all the States surveyed (Figure 3.1). In Ogun, Ondo and Oyo states, four zones were surveyed while three zones were sampled in Osun and Ekiti States. A minimum of five major pepper growers were visited per zone. A total of 90 farms were surveyed. Five infected leaves and plants were cut with scalpel per field visited, placed inside paper bags and labelled. Soil samples were taken to a depth of 10 cm and five samples per field were collected in planting bags and labelled. Coordinates of each farm were taken with the aid of Global Positioning System (GPS) device (Google Maps, 2015).

Incidence of disease was assessed on the farms sampled and was calculated using the formula below;

% incidence =  $n \times 100/N$  ..... equation 1

Where n = number of plants showing symptoms on the field

N = Total number of plant on the field.

$$\text{Disease severity} = \frac{\text{Proportion of total damaged tissue}}{\text{Total surface area of the plant parts.}} \times \frac{100}{1} \quad \dots \text{equation 2}$$

This was converted to a modification of disease rating scale of Kehinde(2011). Low=1-30% damage portion; Medium=31-60% damage portion); High = > 60% damage portion).

### **3.3.1. Source of Planting Seeds**

Seeds of pepper were collected from the National Horticultural Research Institute (NIHORT), Ibadan, seed stores and farmers field in Southwestern States of Nigeria.

### **3.4 Sterilization of Materials**

All glass Petri dishes were washed with detergent and allowed to dry. Glass Petri dishes were then placed inside canisters and sterilized in a hot air oven at 160°C for 1 hour. Conical flasks and beakers were wrapped with aluminum foil and sterilized in a hot air oven at 160°C for 1 hour. Inoculating needles, scalpels and cork borers were sterilized by dipping in 70% ethanol and flaming to red-hot just before use. Laminar flow hood and work benches were surfaced-sterilized by swabbing with cotton wool soaked in 70% ethanol.

Top soil was collected from the Practical Year Training Programme (PYTP) farm of Faculty of Agriculture, University of Ibadan (UI), Ibadan and sterilized at 90°C for two hours following recommended procedure of the electrical soil sterilizer in the Department of Crop Protection and Environmental Biology (CPEB).



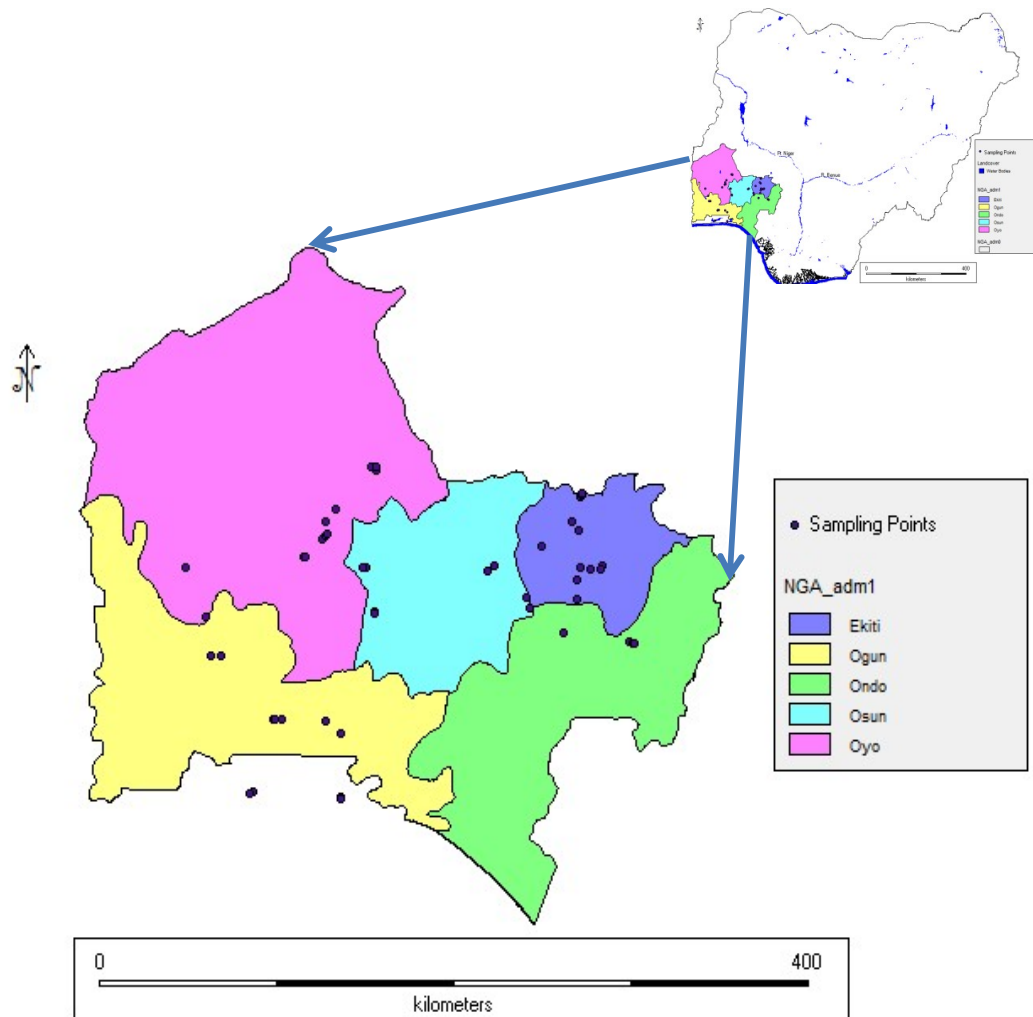


Figure 1: Pepper Diseases Assessment areas in Southwestern Nigeria. (Google Maps, 2015)

### 3.5. Media Preparation

Thirty-nine grams of commercial Potato Dextrose Agar (PDA) was weighed into 1000 ml distilled water in a 2000 ml Erlenmeyer flask. Twenty-eight grams of Nutrient Agar (NA) powder was weighed into 1000 ml distilled water in a 2000 ml Erlenmeyer flask. They were dissolved by heating on an electric cooker for 30 minutes and 100 ml were dispensed into 250 ml Erlenmeyer flasks capped with non-absorbent cotton wool and wrapped with aluminium foil. The suspension was autoclaved at  $1.05 \text{ kg cm}^{-2}$  for 15 minutes. Both media were then placed in a sterile laminar flow hood to cool to about  $45^{\circ}\text{C}$ . The Potato Dextrose Agar (PDA) was acidified with 10 drops of sterile lactic acid to suppress the growth of bacteria while lactic acid was not added to NA because it is used for bacteria culture. Both media were poured into separate Petri dishes at  $42\text{-}45^{\circ}\text{C}$ .

### 3.6. Isolation and Identification of Fungi

A section of leaf tissue was cut at the border of healthy and infected part using a sterile scalpel. Small pieces ( $1 \times 1 \text{ mm}$ ) of these samples were surface-sterilized for 1 min in 10 % sodium hypochlorite solution, rinsed in five changes of sterile distilled water then allowed to drain dry on two layers of sterilized filter paper. The sterilized leaf pieces were placed on acidified PDA under aseptic conditions. Isolation per samples was replicated four times and inoculated plates were incubated at  $27^{\circ}\text{C}$  for 7 days. Data on diametric mycelial growth was taken with ruler and categorized into  $> 80 \text{ mm}$ - fast growing pathogens,  $61\text{-}80 \text{ mm}$ - medium growing pathogens and  $< 61 \text{ mm}$ -slow growing pathogens (Gkoreziset *al.*, 2016). Percentage frequency of occurrence of isolated fungi was calculated as follows;

$$\% \text{ frequency of occurrence} = N \times 100 / T_n \dots\dots \text{equation 3}$$

Where, N = No of time the organism occurred.

$T_n$  = Total no of organism isolated

The pure cultures of each isolate were established for identification. The wet mounts of each isolate were prepared by taking small bits of each isolate with a sterile inoculating needle. The piece was then placed on the slide and stained with lactophenol cotton blue. The slide was then covered with a cover slip and observed under the microscope at  $\times 400$  magnification. A detailed structural feature of each isolate was

recorded by photography. The features of each organism were compared with those described by Barnett and Hunter (1998) for identification.

### **3.7. Isolation and Identification of Biocontrol Agents from Soil**

*Trichoderma pseudokoningii*, *Trichoderma harzianum* and *Bacillus subtilis* isolates that were selected for this study were obtained from soil samples collected during the field sampling in 2014-2015. The soil was serially diluted; 9 mL of water was dispensed inside vials and 1 g of soil samples were measured and poured inside the vials containing sterile distilled water subsequently serially diluted into tubes of 10 ( $10^1$  -  $10^{10}$ ). Thereafter, 0.1 mL of the solution was taken from each vial and dispensed separately on solidified PDA and NA.

Isolates from cultures above were sub-cultured on NA and PDA respectively to obtain pure cultures for identification of bacteria and fungi. At 24 hours, a single colony of bacteria was taken with a sterile inoculating loop and tested for biochemical reaction to KOH, catalase and oxidase. Gram staining, spore staining test and culture characteristics were also conducted on the bacterial isolates. Fungal isolates were identified as previously described (Section 3:6). The isolates were maintained on PDA and NA and stored at 4°C until required.

### **3.8. Fungal and Bacterial Spore Estimation**

The conidial suspension was prepared by adding 10 ml of distilled water unto the surface of each plate containing the 7-day old pure culture of each fungal isolate. Small sterile spatula was then used to dislodge the conidia and the solution was poured into a sterile beaker. The solution was thereafter filtered through four layers of sterile cheese cloth. The filtrate containing the spores was collected and made up to 50 ml by adding sterile distilled water. Using a sterile syringe, 0.1mL of homogenous conidial suspension was loaded into a haemocytometer through the V-shape groove. The suspension was allowed to settle for two minutes after which the spores were counted. Five small squares from each chamber of the haemocytometer with five large squares and each containing 25 small squares were counted from the two chambers of the haemocytometer and an

average count was obtained. The following formula was used to calculate spore per 0.1 mL

$$= \frac{X+Y}{2} \times 2000 (16 \times 25 \times 5 \text{ squares}) \dots \dots \text{equation 4 (Claudius-Cole, 2015)}$$

2

Where X= average spores on the first chamber, Y= average spores in the second chamber, spore concentration 0.1 mL in the haemocytometer. The amount of spores present in the suspension was calculated using  $C_1V_1 = C_2V_2$ . Where  $C_1$  = concentration of initial spores counted,  $V_1$  = volume of water used to wash the Petri dishes,  $C_2$  = concentration of spores needed,  $V_2$  = volume of water (suspension) needed to get the amount of spores to be used.

Estimation of *Bacillus subtilis* was prepared by serial dilution on the *Bacillus subtilis* to determine the population of bacteria cells that were used to inoculate each plant. The stock solution was prepared by washing plates with sterilised distilled water containing pure culture of *Bacillus subtilis* into conical flask. Ten test-tubes containing nine millilitres of sterile distilled water were used to dilute the stock solution with diluent factors of  $10^{-1}$  to  $10^{-10}$ . Then 0.1 mL were taken from each tube and dispensed on freshly prepared solidified nutrient agar plates using spread plate method and incubated for 24 hours. Countable plates were selected and calculated using the formula of Claudius-Cole, (2015)

$$\text{No of cells in stock culture} = \frac{\text{No of colonies on plate} \times \text{volume of solution in the tube} \times \text{diluent factor}}{\text{volume of stock culture}}$$

.....Equation 5

### 3.9. Pathogenicity of Fungal Isolates on Three Local Cultivars of Pepper

Pathogenicity test was carried out on four week old pepper seedlings of the local cultivars of *C. annuum* (Bell pepper-Tatashe), *C. frutescens* (Chilli pepper-Bawa) and *C. chinense* (Hot pepper-Rodo) using the six isolated fungi from infected samples collected during the survey according to Koch's postulate. Experimental design used was complete

randomized design with four replications. The suspension of fungal spores of  $2.06 \times 10^6$  was sprayed onto the leaves of pepper seedlings. Inoculated leaves were covered with transparent polyethylene bag for 24 hours to generate high relative humidity. Plants were observed for 7-days and the number of diseased leaves was counted by visual observation and recorded. Percentage number of diseased leaves was calculated using the formula of Akinbode (2012) below;

$$\% \text{ Number of diseased leaves} = N_d \times 100 / T_n \dots \dots \text{equation 6 (Akinbode, 2012)}$$

Where  $N_d$  = Number of diseased leaves

$T_n$  = Total Number of leaves

### **3.10. Screening of Ten Pepper Cultivars for Resistance to the Identified Fungal Pathogens in Pots**

Surface sterilised seeds of ten cultivars of pepper (Table 3.2) with their pictures (Plate 3.3) were planted in nursery trays containing sterilized soil and kept under shade for four weeks when the leaves were five before transplanting. Seedlings were transplanted into pots containing 5 kg (12 mm) sterilized soil. The pots were arranged in a Completely Randomized Design (CRD) with four replications. Cultural practices such as weeding was on weekly basis and watering was done at 2 day intervals.

#### **3.10.1 Preparation and Inoculation of Fungal Pathogens**

Conidial suspension of the three pathogenic fungi was prepared as previously described (Section 3.8). The inoculation was conducted one week after transplanting on four-week old seedlings using a foliar spray method. The surface of the leaves was swabbed with 70% ethanol before spraying. A drop of Tween 20 was added to each 1 mL spore suspension containing  $2.06 \times 10^6$  spores/mL in a high-pressure sprayer and sprayed on the leaves of pepper plants. Uninoculated plants served as control. The inoculated and un-inoculated plants were covered for 24 hours with transparent bags to generate high humidity. Data were taken at inoculation and at 14 day intervals for 12 weeks. Plant height was taken with meter rule from above the soil line to the meristem, number of leaves and numbers of fruits were counted using visual observation. Weight of fruits per plant was determined by putting the fruits on a weighing balance.

**Table 3.2: List of Pepper Cultivars used in this Study**

| Cultivars | Pepper Genotype                                  | Source |
|-----------|--|--------|
| C1        | <i>Capsicum annuum</i> cv Tatashe-Bell pepper    | Local  |
| C2        | <i>Capsicum chinense</i> cv. Bawa-Chilli pepper  | Local  |
| C3        | <i>Capsicum frutescens</i> cv. Sombo-Bird pepper | Local  |
| C4        | <i>Capsicum chinense</i> cv. Rodo-Hot pepper     | Local  |
| C5        | <i>Capsicum chinense</i> cv. Avenir-Rodo         | Exotic |
| C6        | <i>Capsicum chinense</i> cv. Cameroon-Rodo       | Local  |
| C7        | <i>Capsicum frutescens</i> cv. Ijosi-Bird pepper | Local  |
| C8        | <i>Capsicum annuum</i> cv Nikitta -Bell          | Exotic |
| C9        | <i>Capsicum annuum</i> cv Pizzaro -Bell          | Exotic |
| C10       | <i>Capsicum chinense</i> cv. Batassa-Hot         | Exotic |

C-Cultivar



a



b



c



d



e



f

Plate 3.3: Fruit shape of pepper cultivars; Cultivar I- Bell pepper (a); Cultivar 2 - Chilli pepper (b); Cultivar 3- Bird pepper (c); Cultivar 4 –Bendel hot pepper (d); Cultivar 5- Avenir hot pepper (e) Cultivar 6- -Cameroon hot pepper (f)



g



h



i



j

Plate 3.3 contd: Pictures of pepper cultivars-Cultivar 7- Bird pepper (g); Cultivar 8- Bell pepper-Nikitta (h); Cultivar 9- Pizzaro Bell pepper (i) and Cultivar I0-Batassa hot pepper (j)



Disease severity rating was taken per plant using a scale of 1-5 as described by Akinbode (2012), where;

1 = no symptom, 2 = 1-25 % of plant showing symptoms, 3 = 26-50 % of plant showing symptoms, 4 = 51-75 % of plant showing symptoms, 5 > 75 % of plant showing symptoms or complete death of the plant.

Disease host assessment status was done using a scale of 1-3 (Roane *et al.*, 1974) where; 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 =Highly susceptible (HS).

### **3.11. Screening of Ten Pepper Cultivars for Resistance to Identified Fungal Pathogens on the Field**

The first and second field trials were carried out at the Crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan during the 2016 and 2017 growing seasons. The dry season was from August to December, 2016 and March to July, 2017. Surfaced sterilised seeds of ten cultivars of pepper (Table 3.2) were planted in nursery trays containing sterilised soil and kept under shade for four weeks when the leaves were five before transplanting. Seedlings were transplanted into each block containing the ten cultivars of pepper at the spacing of 30 cm by 30 cm as shown in Figure 3.2. The experiment was a split plot design with four pathogens (*C. coccodes*, *C. capsici*, *P. lycopersici* and control) as the main plots and cultivars (10) as sub-plots. The design was fitted into Randomized Complete Block Design with four replications. Five plants of each cultivar were transplanted into the sub-plot. The conidial suspension of *C. coccodes*, *C. capsici* and *P. lycopersici* were prepared and inoculation was conducted as previously described (Section 3.10.1). Data on plant height, number of leaves and number of fruits and weight of fruits per plant and disease severity were taken for the period of 12 weeks after transplanting as previously described (Section 3.10.1).

The second trial was conducted following the same procedure. Cultural practices such as weeding was done on a weekly basis and watering was done at 2-3 day intervals.

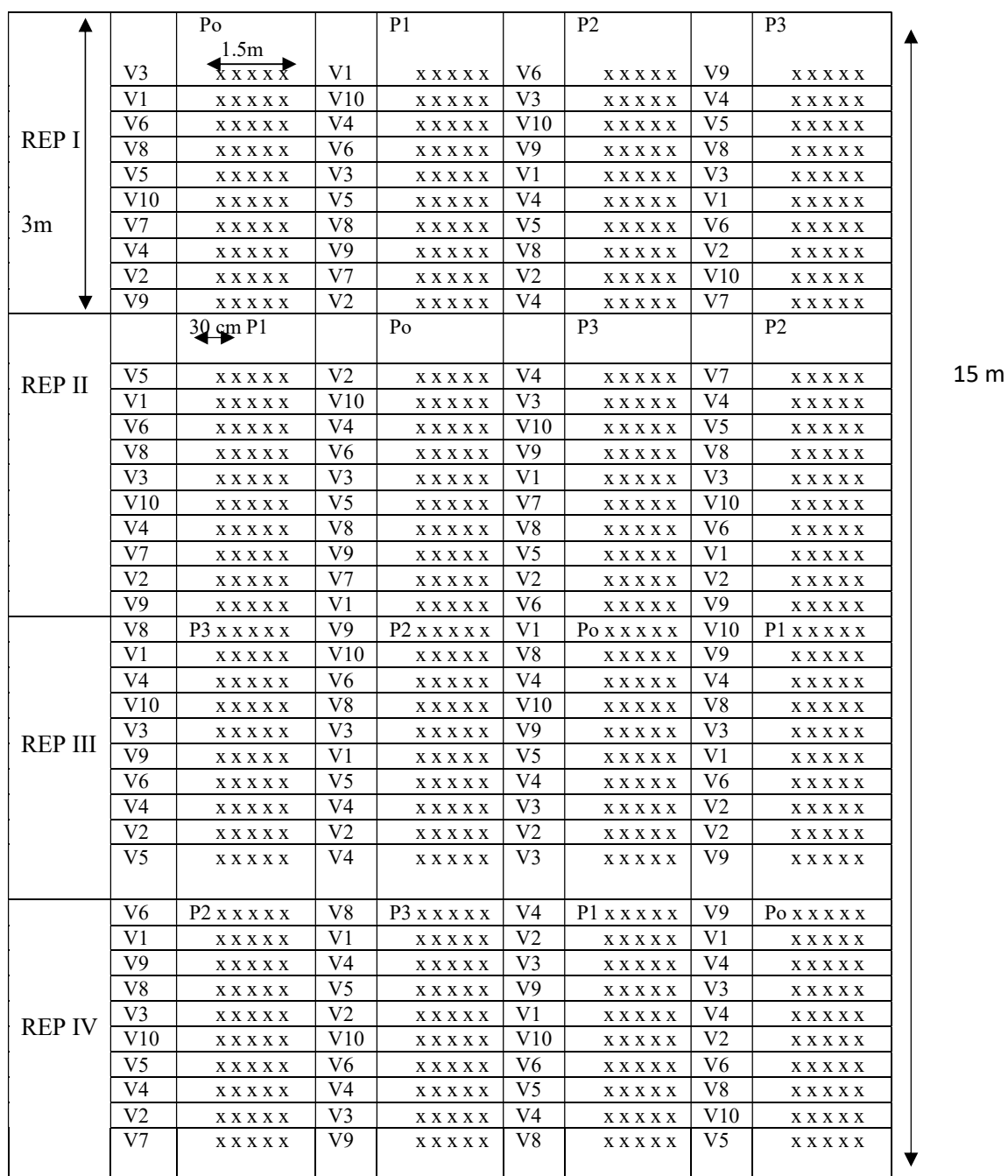


Figure 3.2: Layout of field experiment for the screening of pepper cultivars to fungal pathogens

P1- *Colletotrichum capsici*, P2-*Pyrenochaeta lycopersici*, P3- *Colletotrichumcoccodes*, P0- control (no pathogen), Cultivar 1- Bell pepper; Cultivar 2 – Chilli pepper; Cultivar 3- Bird pepper; Cultivar 4 - Hot pepper Cultivar 5-Hot pepper; Cultivar 6- Hot pepper; Cultivar 7- Chilli pepper; Cultivar 8- Bell pepper; Cultivar 9- Bell pepper; Cultivar 10-Hot pepper; x-plant stand

### 3.12 Management of Pepper Diseases using Biopesticides

#### 3.12.1. *In vitro* activity of Bio-control agents Using Slide Culture Technique

A slide was placed inside Petri dishes and then sterilised. Liquid Potato Dextrose Agar of 1mL was spread over the slide to make a thin film of PDA on the slide. Then discs of 5 mm of 7-day old culture were cut from the growing margin of *C. coccodes*, *C. capsici* and *P. lycopersici* cultures and similar discs from the plates of *T. pseudokoningii*, *T. harzianum* and *B. subtilis* were cut and placed at opposite sides of each slide 3 cm apart on the PDA film surface. Water soaked cotton wool was added to the plate to humidify the environment and they were incubated at  $25 \pm 2^{\circ}\text{C}$  for 7-days. At the end of the incubation period, the area between the bio-agents and pathogens was measured as the zone of inhibition. The percentage inhibition zone was determined by the measured inhibition zone divided by distance between the pathogen-bioagent disc then multiplied by 100 according to the modified method of Amadioha, (2000). The second trial was conducted following the same procedure.

$$\text{Mycelial inhibition (\%)} = \frac{\text{Inhibition Zone}}{\text{Distance between pathogen-bioagent}} \times 100 \dots\dots\dots\text{equation 7}$$

### 3.13. Collection and Preparation of Botanicals

Neem (*Azadirachta indica*) leaves, neem fruits, marigold (*Tagetes erecta*) plant and milk bush (*Thevetia neriifolia*) (Plate 3.4) were collected from National Horticultural Research Institute (NIHORT) Ibadan and University of Ibadan gardens. Mancozeb (fungicide) was purchased from a chemical store. The leaves, roots, shoots and seeds were air-dried on in the laboratory for three weeks. The air-dried parts were ground to powder form. Five concentrations of the different plant parts were prepared by weighing 2.5 g, 5.0 g, 7.5 g, 10.0 g and 12.5 g each of into 100 mL of sterile distilled water. The mixtures were left for 48 hrs to produce 2.5% (2,500 mg/kg), 5.0% (5000 mg/kg), 7.5% (7,500 mg/kg), 10.0% (10000 mg/kg) and 12.5% (12,500 mg/kg) extract concentrations. The preparations were filtered through two layers of muslin cloth (Amadioha, 2000). Mancozeb (fungicide) of 0.5 g was weighed into 100 mL of water according to the recommendation and water serve as control.



Plate 3.4: Part of different botanicals used- (a) *Azadirachta indica* (Neem) Fruits (b) *Azadirachta indica* (Neem) Leaves, (c) *Thevetia nerifolia* (milk bush) and (d) *Tagetes erecta* (Marigold) plant

### 3.13.1. *In vitro* Antifungal Activity of the Selected Plant Extracts on the Isolated Pathogenic Fungi

The prepared concentrations of 2.5%, 5.0%, 7.5%, 10.0% and 12.5% as well as control (0%) were incorporated into 3.9 g of PDA inside conical flask (250 ml) containing 100 mL of distilled water before autoclaving at  $1.05 \text{ kgcm}^{-2}$  for 15 minutes. Control treatment had no plant extract incorporated into the PDA. Experimental design used was Completely Randomized Design with four replications. Discs of 5 mm of *C. coccodes*, *C. capsici* and *P. lycopersici* culture, obtained from the 7 day old fungal culture were placed at the center of the plates containing different concentrations of plant extract-medium and incubated at  $25 \pm 2^{\circ} \text{C}$  for 7 days. Diametric growth (mm) of fungus was measured once at 7 days after inoculation. Percentage mycelial inhibition was calculated using the formula of Amadioha (2000).

$$\% \text{inhibition of mycelia growth} = \frac{D_c - D_t}{D_c} \times 100 \dots \dots \text{equation 8}$$

$D_c$

$D_c$  = diameter of control

$D_t$  = diameter of test pathogens

### 3. 14. Antifungal Activity of the Biopesticides on Isolated Pathogenic Fungi Trial

Surface sterilised seeds of the susceptible *Capsicum annuum* cv Tatase- bell pepper were planted in nursery trays containing sterilized soil and shaded for three weeks before transplanting. Seedlings of the sweet pepper cultivar were transplanted in each block at spacing of  $30 \times 30 \text{ cm}$  as shown in Figure 3.3. The experiment was a split plot design fitted into Randomized Complete Block Design with pathogens (3) as the main plots and biopesticides (10) as sub-plots and replicated four times. Conidial suspension of *C. coccodes*, *C. capsici* and *P. lycopersici* were prepared, estimated and inoculated as previously described (Section 3.10.1) at 7 days after transplanting.

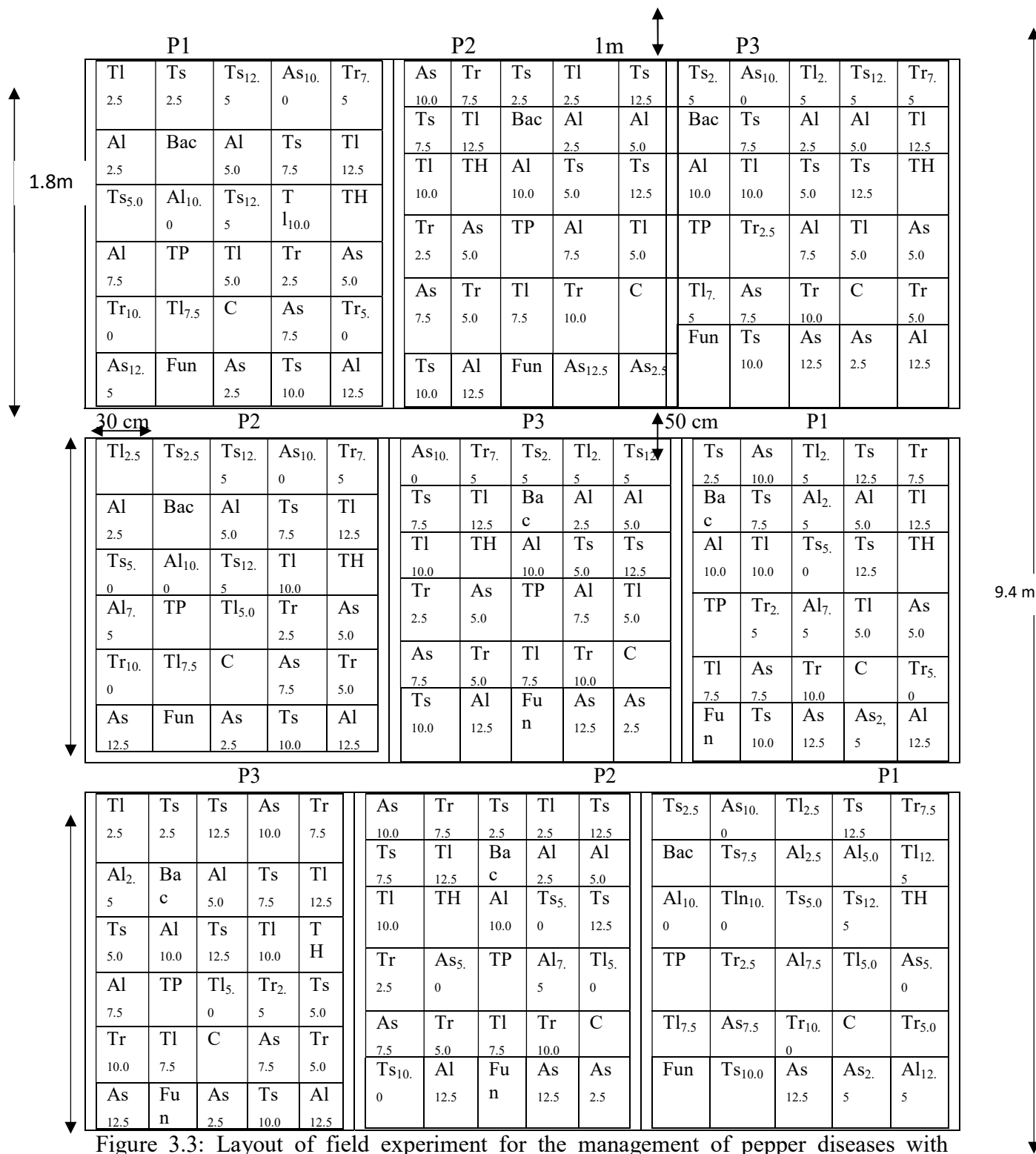


Figure 3.3: Layout of field experiment for the management of pepper diseases with biopesticides

P1- *Colletotrichum capsici*, P2-*Pyrenochaeta lycopersici*, P3- *Colletotrichumcoccodes*,Tl- *Thevetia nerifolia* leaf extract AZ-*Azadirachta indica* leaf extract AS- *Azadirachta indica* Seed extract, Tr- *Tagetes erecta* root extract, TS- *Tagetes erecta* shoot extract,TP-*Trichoderma pseudokonnigii*, Bac- *Bacillus subtilis*,Fun- fungicide, C-control, TH- *Trichoderma hazianum*

Treatments were as follows; *Thevetia neriiifolia* leaf, *Azadirachta indica* leaf, *Azadirachta indica* seed, *Tagetes erecta* root and *Tagetes erecta* shoot at the concentrations of 2.5 g, 5.0 g, 7.5 g, 10.0 g and 12.5 g, *Bacillus subtilis* at  $2.0 \times 10^7$  cfu, *Trichoderma harzianum* at  $2.0 \times 10^6$  spores, *T. pseudokonnigii* at  $2.0 \times 10^6$  spores, fungicide at 0.5 g and control (no pathogen and biopesticides). These treatments were used to spray the leaves of the inoculated plants using high-pressure sprayer containing the treatments at seven days post inoculation with the pathogens at a spore concentration of  $2.0 \times 10^6$  spores/mL as previously described (Section 3.8) while the non-inoculated plants served as control. Data were taken on plant height, number of leaves, number of fruit and weight of fruit per plant. Disease severity was determined as previously described in section 3.10.1. All cultural practices such as weeding were done on weekly basis and watering was done at 2-3 day intervals.

### **3.15. Data Collection**

Plant height was taken with meter rule from the soil line to the meristem, number of leaves and number of fruits was counted using visual observation. Weight of fruits per plant was determined by placing the fruits on a weighing balance then recorded the weight.

Disease Severity rating was taken per plant using a scale of 1-5 as described by Akinbode (2013), where;

1 = no symptom, 2 = 1-25 % of plant showing symptoms, 3 = 26-50 % of plant showing symptoms, 4 = 51-75 % of plant showing symptoms, 5 > 75 % of plant showing symptoms or complete death of the plant.

### **3.16. Phytochemical Analyses of Neem, Marigold and Milk bush**

The phytochemical analyses of the plant samples were carried out at KAPPA Biotechnology Laboratories, Bodija, Ibadan with the procedures of Trease and Evans (1989) and Sofowora (1993), Harborne(1973); Chhabro *et al.*(1984). Neem fruits and leaves, marigold shoot and root and milk bush leaves were air-dried for four weeks, ground to powder and the powdered forms were taken to the laboratory for phytochemical analysis.

### 3.16.1 Determination of Tannin Total Content

Presence of tannin in the botanical used was carried out according to the procedure of Padamaja (1989). One gram sample of powdered plants parts were dissolved with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The solution was centrifuged at 3000 rpm for 20 minutes. The supernatant (0.1 mL) was taken and 7.5 mL of distilled water was added into the tube, then 0.5 mL of Folin-Dennis reagent and 1 mL of 35% sodium carbonate solution was added thereafter 10 mL water distilled was then added. This solution was then thoroughly mixed and kept at 27°C for 30 minutes. The absorbance of the mixed solution was measured under 760 nm to compare with a blank which was prepared with 300 µl of water. The tannin content was determined by tannic acid equivalent (TAE) in mg/g material while the calibration equation for tannic acid was  $Y = 0.069x + 0.0175$  (Regression coefficient = 0.9978) (Trease and Evans, 1989).

### 3.16.2. Determination of Total Saponin

Total saponin (TS) was determined using a modified method of Hiai *et al.* (1976) as described by Makkar *et al.* (2007). Powdered samples of 0.5 g of each were dissolved with 25 mL of 80% methanol (aqueous) and then shaken for two hours. Thereafter the tube containing the mixture was centrifuged for 10 minutes at 3000 rpm. Then 0.25 mL of the supernatant was dispensed into a glass tube. Vanillin reagent (8%) with 2.5 mL of 72% aqueous sulphuric acid was added to the tube. These mixtures in the tubes were heated for 60°C for 10 minutes in a water bath. Thereafter the tubes were allowed to cool using ice for four minutes and left at 27°C. The mixture absorbance was determined with UV/visible spectrophotometer at 544 nm. The powder with diosgenin was used as a standard. The results that were obtained were expressed as mg diosgenin equivalent per g of sample dry matter (Makkar *et al.*, 2007).

### 3.16.3. Determination of Total Alkaloid

Total alkaloid present in the plant samples (*Azadirachta indica*, *Tagetes erecta* and *Thevetia neriiifolia*) was determined with a modification of the procedure described by Singh *et al.* (2004). One gram each of the powder was dissolved in 10 mL of 80% ethanol



and centrifuged for 10 minutes at 5000 rpm. The supernatant was then used to estimate total alkaloids. The absorbance was determined at 510 nm with a blank solution of 300  $\mu$ l of sterile distilled water. The alkaloid contents were quantified and calculated with quinine standard curve (0.1 mg/ mL, 10 mg dissolved in 10 mL ethanol and diluted to 100 mL with distilled water). The values were expressed as g. 100 g<sup>-1</sup> of dry weight. (Singh *et al.*, 2004)

#### **3.16.4. Determination of Total Flavonoid Content (TFC)**

Total flavonoid was carried out with the procedure of Kale *et al.* (2010) using aluminium chloride. A mixture of 5 mL ethanol with 2 g of each powder were made and shaken. Thereafter 0.5 mL of the mixture was taken and dispensed into a tube (Chan *et al.*, 2006). Then 1.5 mL methanol was added, 0.1 mL of 10% aluminium chloride with 0.1 mL 1 M potassium acetate and 2.8 mL sterile distilled water. They were left for 30 minutes and the absorbance was read at 514 nm using a spectrophotometer. The TFC was expressed as quercetin equivalent (QE) in mg/g. The calibration equation for quercetin was  $Y = 0.0395x - 0.0055$  (Regression coefficient = 0.9988).

#### **3.16.5. Determination of Total Phenolic Content (TPC)**

The total phenolic content of *A. indica*, *T. erecta* and *T. neriifolia* was determined with the method of Folin-Ciocalteu (Chan *et al.*, 2006). Volume of 5ml ethanol was added to 2 g of the sample and shaken. A 300  $\mu$ l of the solution was taken and dispensed into test tube in three replications (Chan *et al.*, 2006). Thereafter 1.5 mL of 10% Folin-Ciocalteu reagent was added to the mixture and 7.5w/v of sodium carbonate solution was added to the preparation. The mixture was left for 30 minutes at 27<sup>0</sup>C. Absorbance was determined at 765 nm with a blank of 300  $\mu$ l of sterile distilled water. Total phenolic content (TPC) was expressed as gallic acid equivalent (GAE) in mg/g material. The calibration equation for gallic acid was  $Y = 0.0645x - 0.0034$  (Regression coefficient = 0.999 (Chan *et al.*, 2006).

### **3. 17. Data Analyses**

Inferential and descriptive statistics were used to analyse the data. Descriptive statistics used include frequencies, percentages and means while inferential statistics used was Chi-square to test for relationships between personal characteristics and pesticides usage.

All data from fields, pots and laboratory experiments were subjected to Analysis of Variance using SAS (2008). Means with significant differences were separated using Fishers Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT) at  $P < 0.05$ . The GPS locations were used to draw a map of the disease incidence and locations visited using Google Map (Google Map, 2015).

## **CHAPTER FOUR**

### **4.0**

### **RESULTS**

#### **4.1 Characteristics and Perception of Pepper Growers in the Three Selected Southwestern States**

##### **4.1.1 Personal Characteristics of Respondents**

The age distribution of respondents fell within the range of 31-60 years(49.5%) while 33.9% were between the age range of 18-30 years and 16.5% were above 60 (Table 4.1). Table 4.1 equally revealed that 77.4% of the respondents were male while only 22.6% were female. The majority (85.9%) of the respondents in the study areas were married while 13.2% were single and 0.9% were divorced (Table 4.1). Most (66.5%) of the respondents had primary education while 23.5% had tertiary education and 10.0 % had their level of education up to secondary level (Table 4.1). Majority of the respondents (56%) had between 11-30 years of farming experience while 18.8% had less than 10 years of farming experience while 15.6% had between 31-40 years of farming experience and only9.4% of the respondents had above 40 years of farming experience (Table 4.1).

##### **4.2 Crops Grown and Farmers' Perception of Factors affecting Productivity of their Pepper Farms**

Information on farming activities of respondents on different types of crops grown during the dry season is given in Table 4.2. The results indicate that respondents in Ogun state produced more cereal with 22.3% during the dry season while Ekiti state had the least cereal production (1.1%). Oyo state had the highest number of respondents that produce vegetables (79.9%) with least from Ogun State (66.7%). For tuber crops grown during the dry season, Ekiti state had the highest percentage of respondents that produced tuber crop (30.5%) compared with Ogun State with least

**Table 4. 1: Personal Characteristics of Pepper Farmers in Southwestern States of Nigeria, 2014**

| <b>Personal characteristics</b>    | <b>Frequency</b> | <b>Percentage (%)</b> |
|------------------------------------|------------------|-----------------------|
| <b>Age (years)</b>                 |                  |                       |
| 18-30                              | 72               | 33.9                  |
| 31-60                              | 105              | 49.5                  |
| Above 60                           | 35               | 16.5                  |
| <b>Sex</b>                         |                  |                       |
| Male                               | 164              | 77.4                  |
| Female                             | 48               | 22.6                  |
| <b>Marital status</b>              |                  |                       |
| Married                            | 182              | 85.9                  |
| Single                             | 28               | 13.2                  |
| Divorced                           | 2                | 0.9                   |
| <b>Level of Education</b>          |                  |                       |
| Primary                            | 141              | 66.5                  |
| Secondary                          | 21               | 10.0                  |
| Tertiary                           | 50               | 23.5                  |
| <b>Years of Farming Experience</b> |                  |                       |
| ≤10                                | 40               | 18.8                  |
| 11-30                              | 119              | 56.0                  |
| 31-40                              | 33               | 15.6                  |
| Above 40                           | 20               | 9.4                   |

Source: Field survey 2014

**Table 4.2: Perception of Pepper Growers in the Three Selected States**

| Production Resources                    | Oyo (%) | Ekiti (%) | Ogun (%) |
|---|---------|-----------|----------|
| Crop grown during dry season            |         |           |          |
| <b>Cereals</b>                          | 5.0     | 1.1       | 22.3     |
| <b>Vegetables</b>                       | 79.9    | 68.4      | 66.7     |
| <b>Tubers</b>                           | 15.1    | 30.5      | 11.7     |
| Crop grown during raining season        |         |           |          |
| <b>Cereals</b>                          | 60.0    | 54.3      | 56.0     |
| <b>Vegetables</b>                       | 25.2    | 11.5      | 22.7     |
| <b>Tubers</b>                           | 14.8    | 34.2      | 21.3     |
| Production constraints                  |         |           |          |
| <b>Land</b>                             | 1.0     | 5.0       | 8.8      |
| <b>Funds</b>                            | 31.4    | 34.1      | 25.2     |
| <b>Pest and Diseases</b>                | 67.6    | 60.9      | 65.9     |
| Profitable crops                        |         |           |          |
| <b>Cereals</b>                          | 34.0    | 40.0      | 30.1     |
| <b>Vegetables</b>                       | 50.0    | 40.0      | 44.7     |
| <b>Tubers</b>                           | 16.0    | 20.0      | 25.2     |
| Respondents ranking rate for vegetables |         |           |          |
| <b>Pepper</b>                           | 50.0    | 40.0      | 42.0     |
| <b>Tomato</b>                           | 30.0    | 22.0      | 38.2     |
| <b>Cucumber</b>                         | 18.0    | 16.0      | 13.0     |
| <b>Garden egg</b>                       | 2.0     | 22.0      | 6.8      |

Source: Field survey 2014

(11.0%). During the rainy season farmers produced more of cereals than tuber with little or no vegetables. Cereal production in Oyo State was the highest (60.0%) followed by Ogun state (56%) while Ekiti state had the lowest percentage of production. Oyo state had the highest percentage vegetable production (25.0%), followed by Ogun state (22.7%), while Ekiti state had the least with 11.5%. Tuber crop production was highest in Ekiti state and lowest in Oyo state during rainy season.

Factors affecting productivity of pepper in their farm(s) as stated by the respondents include land, funds and more often, pests and diseases. The highest factor affecting production of vegetable crops across all the states were pests and diseases. Many of the respondents indicated that pests and diseases reduced the production of vegetables followed by funds as land is available to them most of the time. Table 4.2 also indicates that vegetable crops were more profitable despite the factors affecting their production. Although some of the farmers indicated that vegetables and cereals were more profitable than tuber crops. Many (50%) of the respondents in Oyo state, 42.2% in Ogun state and 40.0% in Ekiti state ranked pepper as their most preferred vegetable. Tomato was ranked second among other vegetables.

#### **4.3. Disease Description Symptoms associated with Pepper Plants in Farmers Fields**

Diseases encountered by pepper farmers are stated in Table 4.3. All the respondents observed leaf spot, chlorosis (yellowing), necrosis (blackening) and wilting symptoms across the states surveyed indicating these symptoms as the major problem faced by the farmers. All (100%) of respondents in Ekiti state indicated that leaf spot, yellowing, stunting, fruit rot and wilting, blackening were the major problems in vegetable production while only 32.6% of the respondents reported galling symptoms. Stunting was the least (66.1%) in Ogun state while 70.9% was reported in Oyo state. Galling of roots was also noticed across the states although with less than 50% of farmers indicated the presence of the disease across the states surveyed.

Monetary loss per hectare as indicated by respondents due to pests and diseases was between ₦200,000 - ₦500,000 in Oyo (75%), Ogun (65.3%) and Ekiti state (60.2%). Those that indicated greater than ₦500,000 were 27.8%, 27.5% and 20.0% in Ogun, Ekiti

and Oyo state respectively. Respondents that indicated less than ₦200,000 were 12.9%, 6.9% and 5.0% in Ekiti, Ogun and Oyo state respectively (Table 4.3).

**Table 4.3: Percentage of Diseases Indicated and Constraints Observed by Pepper Farmers in the Three Selected States**

| Constraints   | Oyo  | Ekiti | Ogun |
|---|------|-------|------|
| <b>Symptoms Observed by Respondents</b>                   |      |       |      |
| Leaf spot   | 100  | 100   | 100  |
| Yellowing   | 100  | 100   | 100  |
| Stunting  | 70.9 | 100   | 66.1 |
| Wilting   | 100  | 100   | 100  |
| Galls   | 40.5 | 32.6  | 36.9 |
| Blackening  | 100  | 100   | 100  |
| Fruit rot   | 100  | 100   | 100  |
| <b>Monetary loss due to Diseases per hectare annually</b> |      |       |      |
| > 500,000 Naira   | 20.0 |       |      |
| 200, 000-500,000 Naira                                    | 75.0 | 27.5  | 27.8 |
| < 200,000 Naira   | 5.0  | 60.2  | 65.3 |
|   |      | 12.3  | 6.9  |
| <b>Changes in Climate Condition</b>                       |      |       |      |
| Rain fluctuation  |      | 100   | 100  |
| Rain caseation  | 100  | 100   | 100  |
| Lengthened dry season                                     | 100  | 100   | 100  |
|   | 100  |       |      |

Source: Field survey 2014

Respondents stated that climate change which focused on rain fluctuation; lengthened dry season and rain cessation are some of the factors they indicated as environmental changes in the states surveyed.

#### **4. 4. Disease Management Practices of Pepper Farmers**

Respondents in Oyo state had highest number of farmers practicing crop rotation (4.9%) while Ogun state had fewer farmers practicing crop rotation with 1.1% (Figure 4.1). Farmer's sources of planting materials over the years have been through the use of old seeds as shown in Figure 4.2 because farmers preferred not to spend money to buy certified seeds. The highest (73%) percentage of respondents stated that they used the seeds from previous planting season while 16.0% of the respondents get their seeds from extension agents and only 11% of the total respondents bought their seeds from seed outlets (Figure 4.2).

#### **4.5. Percentage of Respondents Using Pesticides in Pepper Farms across Selected States**

The percentage of respondents using pesticide was high across all the sampled states. Oyo state had the highest percentage (80.0) pesticides users among the pepper farmers followed by Ogun state (77.6%) while Ekiti state had the lowest (50.2%) percentage of respondents that used pesticides (Figure 4.3).

##### **4.5.1 Relationship between personal characteristics of respondents' and the level of pesticide use**

The Chi-square analysis on Table 4.4 reveals that there is a relationship between the respondents' age and the level of pesticide usage ( $\chi^2 = 30.188$ ,  $P < 0.05$ ). There was significant difference in the age of respondents compared to their level of pesticides usage. Respondents level of education was showed significant relationship to the level of pesticides usage ( $\chi^2 = 20.006$ ,  $P < 0.05$ ). The years of farming experience was significantly ( $\chi^2 = 59.278$ ,  $P < 0.05$ ) related to the level of pesticide usage. Gender and marital status was not significantly related to the level of pesticide used (Table 4.4).



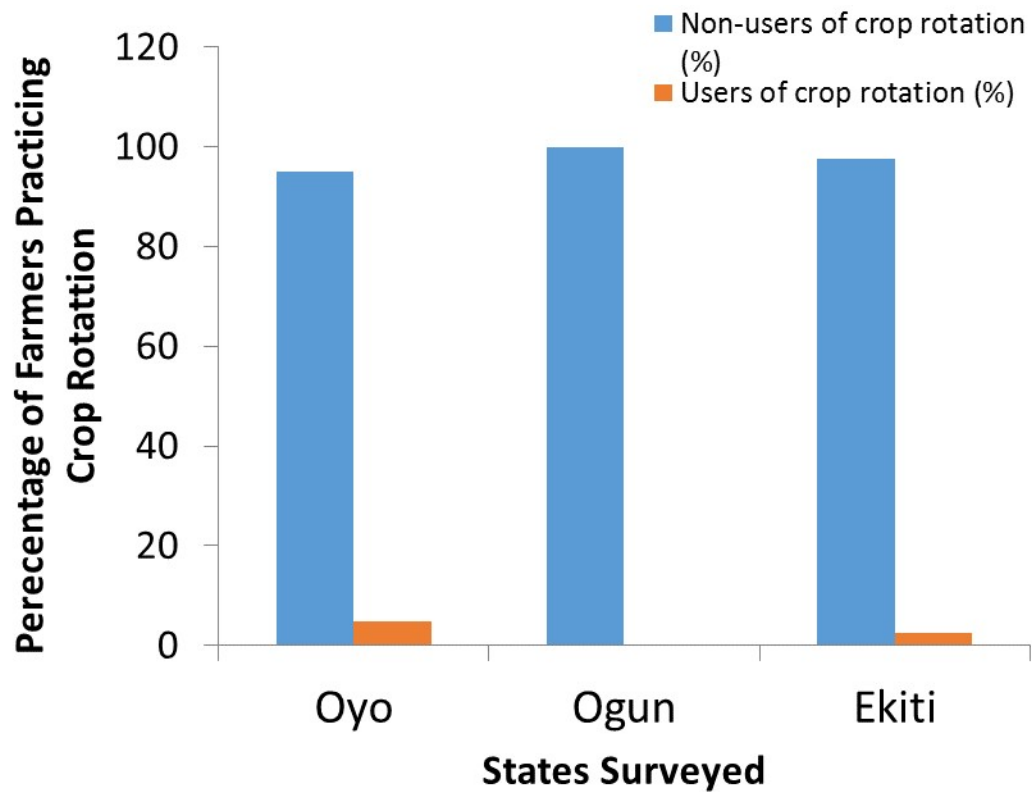


Figure 4.1: Percentage Respondents using Crop Rotation as a Management practice for Reducing Pepper Fungal Diseases in the Three Selected Southwestern, Nigeria, 2014

Source: Field survey 2014

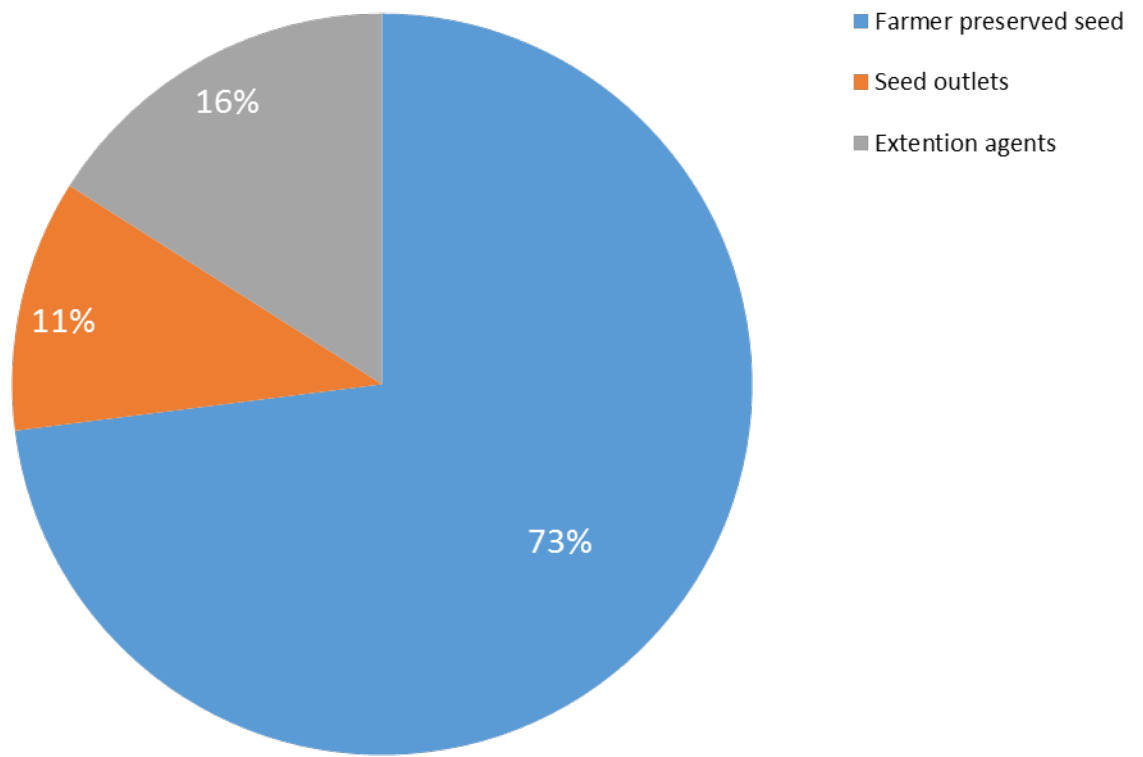


Figure 4.2: Farmer's Sources of Planting Materials in Selected Southwestern States  
Source: Field survey 2014

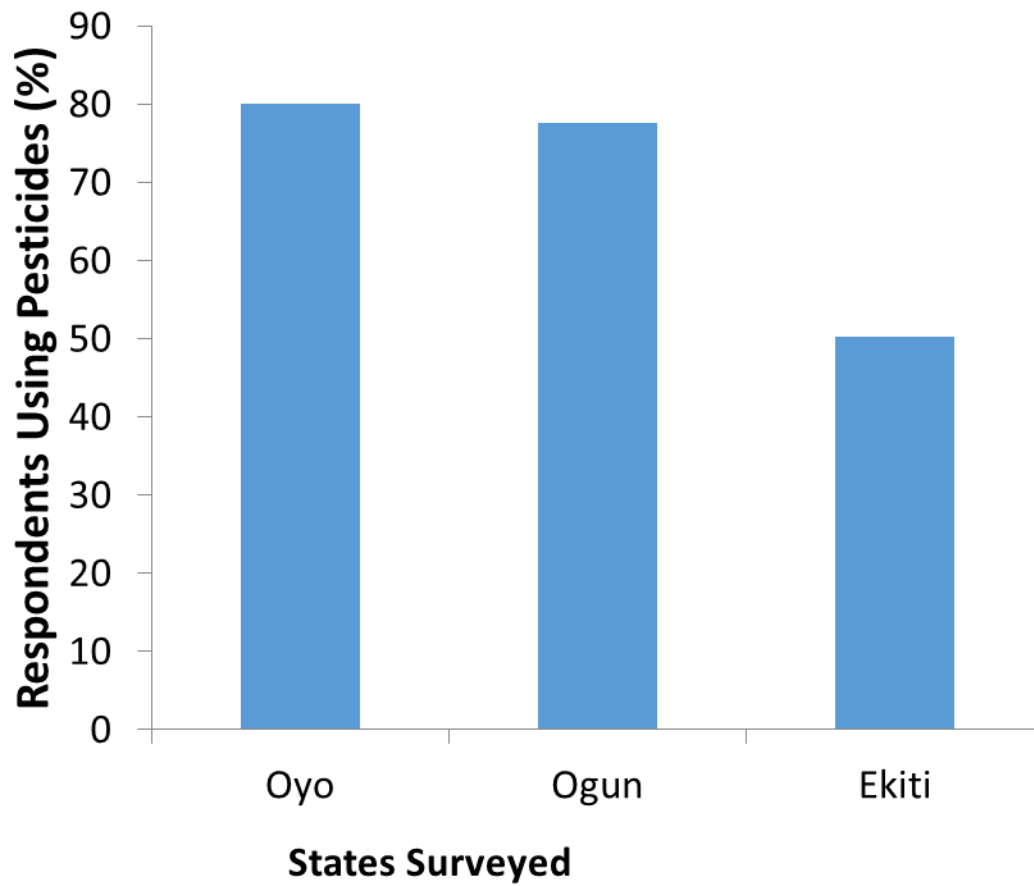


Figure 4.3: Percentage of Respondents Using Pesticides for Management of Disease in Selected Southwestern Nigeria, 2014

Source: Field survey 2014

**Table 4.4: Chi-square Analysis Showing the Relationship between Respondents' Personal Characteristics and their Level of Pesticides Usage**

| Variables           | $\chi^2$ Value | Df | P     |
|---------------------|----------------|----|-------|
| Sex                 | 21.097         | 1  | 0.323 |
| Age                 | 30.188         | 2  | 0.000 |
| Marital status      | 87.329         | 2  | 0.238 |
| Level of education  | 20.006         | 2  | 0.001 |
| Years of experience | 59.278         | 3  | 0.003 |

## **4.6 Symptoms Expression and Distribution of Fungal Diseases**

### **4.6.1. Symptom Observation from Pepper Farms**

The diseases observed from sampled fields in Southwestern Nigeria were mainly foliar diseases, wilting and fruit diseases of pepper plants. The symptoms include wilting of plant, fruit rot, and leaf spots (Plate 4.1a). The leaf spots were necrotic spots surrounded by yellow discolouration (chlorotichalo) extending out from the spots (Plate 4.1 c). This symptom was followed by leaf necrosis causing death of such leaves. The necrotic part of the leaves were tan in colour, dry and leathery and carried dark, semi-concentric rings in which pycnidia of the fungus were formed at the centre of the leaves. The fruit rots started with black spots on the fruits which were sometimes water-soaked. The spots coalesced and spread through to the whole fruit until the fruits became rotten (Plate 4.1 b).

Mosaic patterns were also observed on leaves and fruits of infected plants. This resulted in stunting and fruit distortion. (Plate 4.1d). Leaf mottle observed started with leaves becoming rosette with ring-spots and later showing mottling, mosaic, bronzing and terminal necrosis on infected plants (Plate 4.1e). Leaf curling which started with alternation of light-green areas on the leaves later resulted in leaf distortion and curled (Plate 4.1f).

## **4.7 Diseases Severity and Incidence in Pepper Farms in Southwestern Nigeria**

Disease incidence on pepper farms visited ranged between 60% and 100 % while severity of low to high was observed in the fields. In Oyo, Ogun and Ekiti States, 100 % incidence were observed on sampled pepper fields while Osun and Ondo had 65% and 60% disease incidence respectively (Table 4.5). Disease severity was high in Oyo, Ogun and Ekiti States with > 60% of the plants damaged. In Osun state disease severity was medium (31%-60% of the plant damaged) and low in Ondo State with 1%-30% plant damaged (Figure 4.4).

## **4.8 Fungi Isolated from Diseased Pepper Plants**

Six fungi of four genera were isolated and identified from the infected pepper plant samples collected from farms in Southwestern states. These fungi were *Colletotrichum coccodes*, *Colletotrichum capsici*, *Penicillium* species, *Trichoderma*

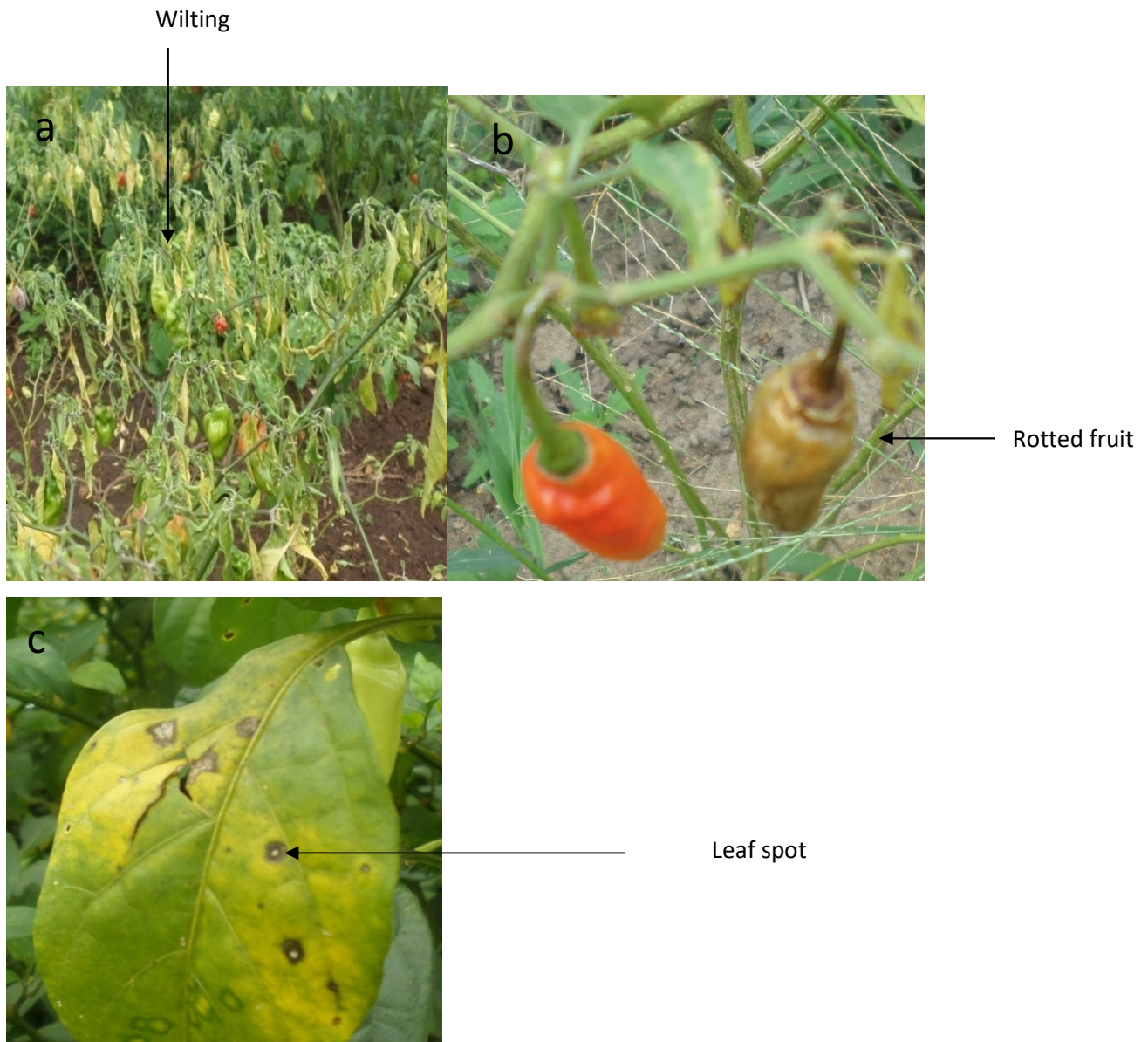


Plate 4.1. Symptoms observed on infected pepper plant from the survey (a) Wilting (b) Fruit rot (c) Leaf spot/chlorosis

Leaf Mosaic

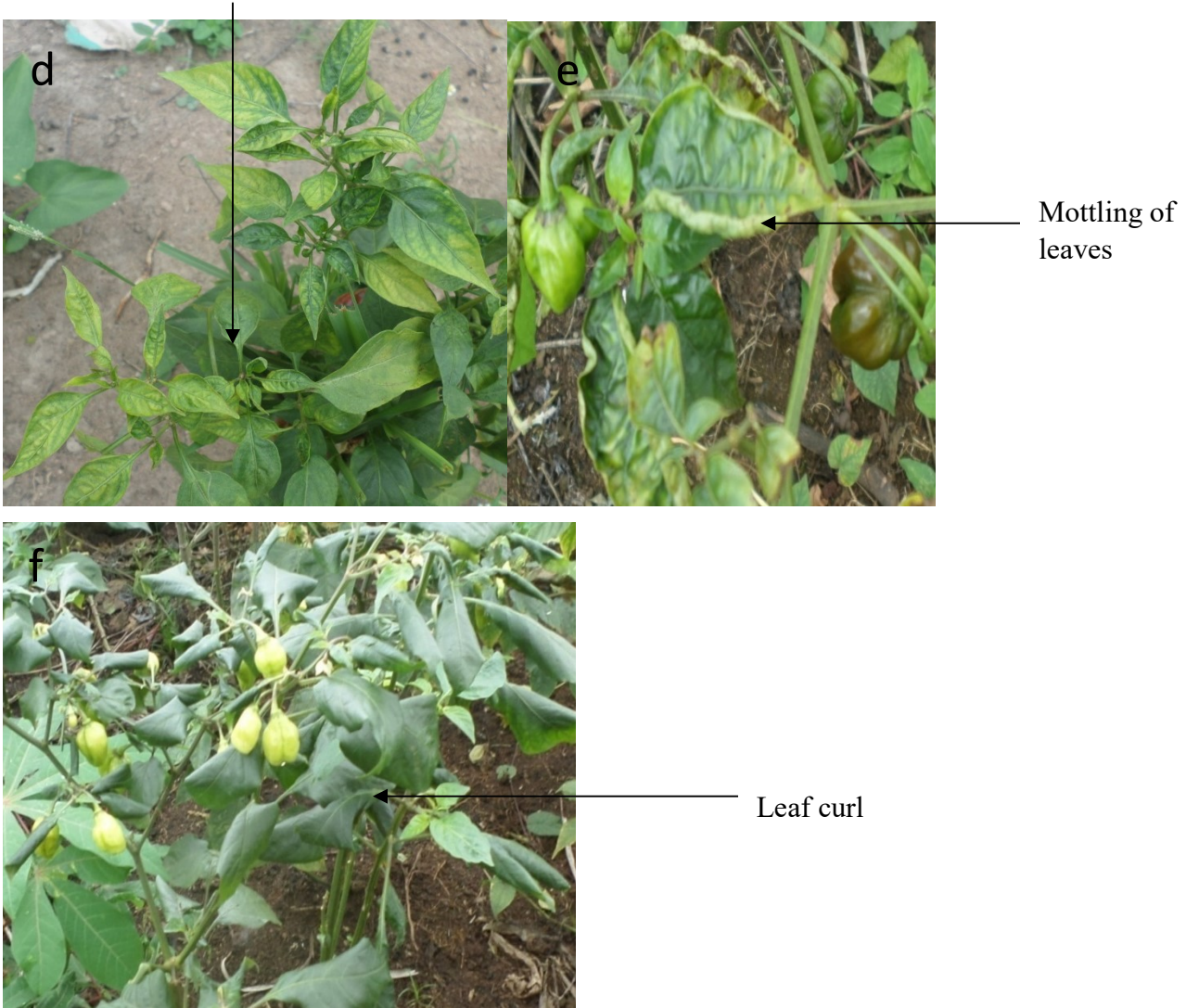


Plate 4.1contd: Viral diseases of pepper plants observed on infected pepper plants the survey (d) Mosaic on Leaves (e) Mottling of leaves(f) Leaf curl

**Table 4.5: Percentage Disease Incidence from Infected Pepper Fields in Southwestern Nigeria**

| States | Disease Incidence (%) |
|--------|-----------------------|
| Oyo    | 100.0                 |
| Ogun   | 100.0                 |
| Ekiti  | 95.0                  |
| Osun   | 65.0                  |
| Ondo   | 60.0                  |

n=100



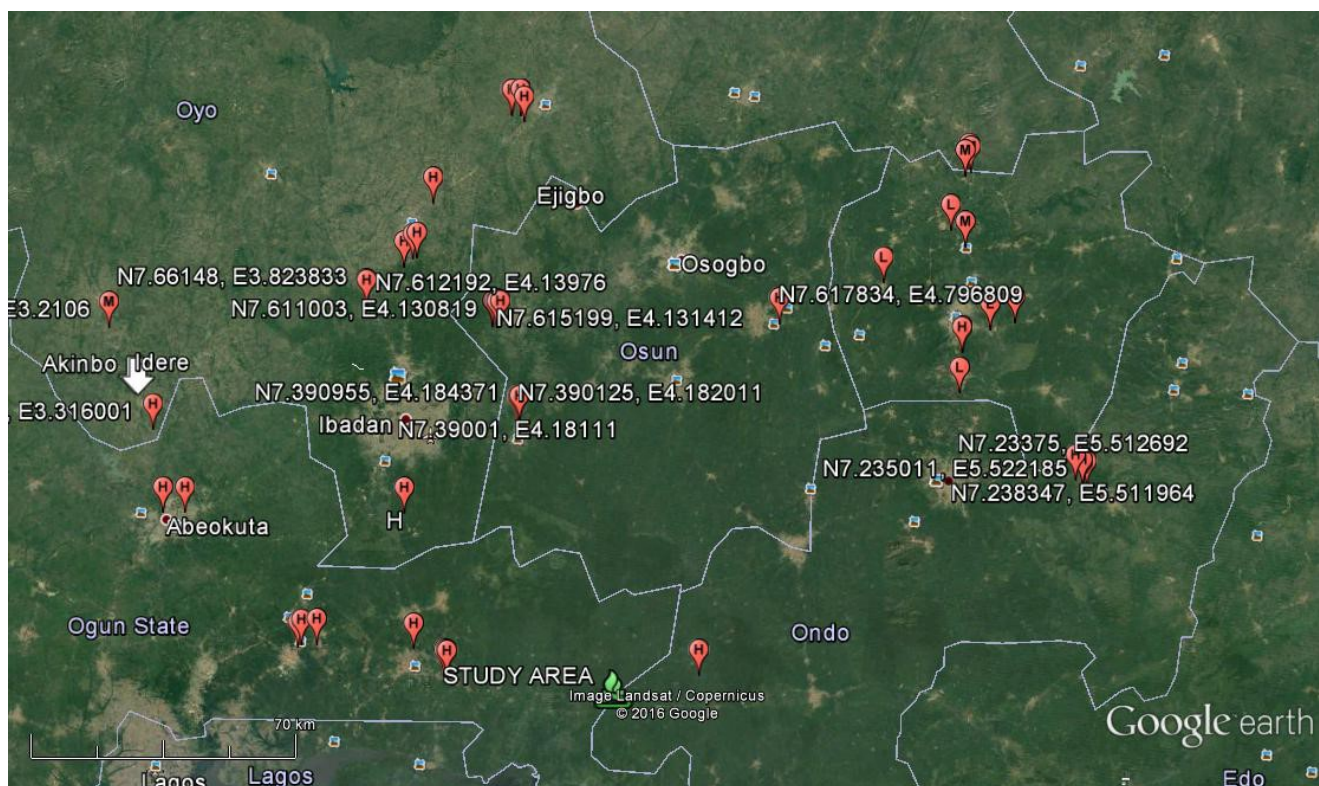


Figure 4.4: Disease Severity of Pepper Plants in the Southwestern States(Google Earth, 2015)

H=High incidence (> 60 % of plants damaged)

M=Medium incidence (31-60% of plants damaged)

L=Low incidence (1- 30% of plants damaged)

*pseudokoningii*, *Trichoderma harzianum* and *Pyrenochaeta lycopersici* (Plate 4.2-4.7). The mycelial diameter of *C.coccodes*, *C. capsici*, *Penicillium* sp, *T.pseudokoningii*, *T.harzianum* and *P. lycopersici* was measured 7 days after incubation and ranged from 23.67 to 89.15 mm (Table 4.6). Maximum mycelial diameter of 81.17 and 89.15 mm were observed in isolates of *C.coccodes*, *Penicillium* species and *T. harzianum*, *T. pseudokoningii*, proving to be the fastest growing isolates (Table 4.6) while *Colletotrichum capsici* showed minimum diametric growth and was rated as a slow growing isolate with an average growth of 23.67 mm. *P. lycopersici* showed intermediate growth (Table 4.6).

*Colletotrichum coccodes* had the fastest growth, occupying the whole surface of the Petri dish in 7 days (Plate 4.2a). The culture had a dense texture with sections of light and dark gray mycelium. The central part of the isolate was grey while the reverse side of the plates were filled with dark grey mycelium. *C. coccodes* produced large number of conidia, which developed on simple, short, erect conidiophores with setae. The spores were hyaline and one-celled before maturity.

The mature spores were brownish, two-celled, with ovoid to elongate shape (Plate 4.2b). The conidia of *Penicillium* species isolated were recognized by the dense brush-like light green mycelium spore-bearing structures. They dispersed in Petri dish in 7 days (Plate 4.3a). The central part of the isolate was covered by a white mycelium while the reverse plates were dark cream in colour. The conidiophore was simple or branched and was terminated by clusters of flask-shaped phialides. The conidia are produced in chains from the tips of phialides, with the youngest spore at the base of the chain (Plate 4.3b).

*T. pseudokoningii* and *T. harzianum* were also fast growing fungi. *T. pseudokoningii* growth was initially whitish and later appeared as green, distinct concentric rings on Petri dish (Plate 4.4a). Its conidia appeared dry and were ellipsoidal in shape (Plate 4.4b). *T. harzianum* was whitish and later turned green in Petri dish (Plate 4.5a). The conidia were long and branched (Plate 4.5b). The growth pattern of *C. capsici* was irregular with fluffy mycelium surface on PDA. The isolate produced cottony, fluffy colonies with cream pigments (Plate 4.6a). The conidia of *C. capsici* were one-celled, smooth walled, hyaline, falcate and tapered towards both ends (Plate 4.6b). *P. lycopersici* produced colonies which were flat, spreading with sparse aerial mycelium.

**Table 4.6. Isolated Fungi Categorized into Three Classes on the Basis of Diametric Mycelial Growth**

| Category       | Diametric Growth | Isolated Fungi   |
|----------------|------------------|--|
| Fast growing   | > 80 mm          | <i>Colletotrichum coccodes</i> , <i>Penicillium</i> sp, <i>Trichoderma harzianum</i> and <i>Trichoderma pseudokoningii</i> |
| Medium growing | 61-80 mm         | <i>Pyrenochaeta lycopersici</i>  |
| Slow growing   | < 61 mm          | <i>Colletotrichum capsici</i>  |

fast growing pathogens - > 80 mm; medium growing pathogens- 61-80 mm; slow growing pathogens- < 61 mm

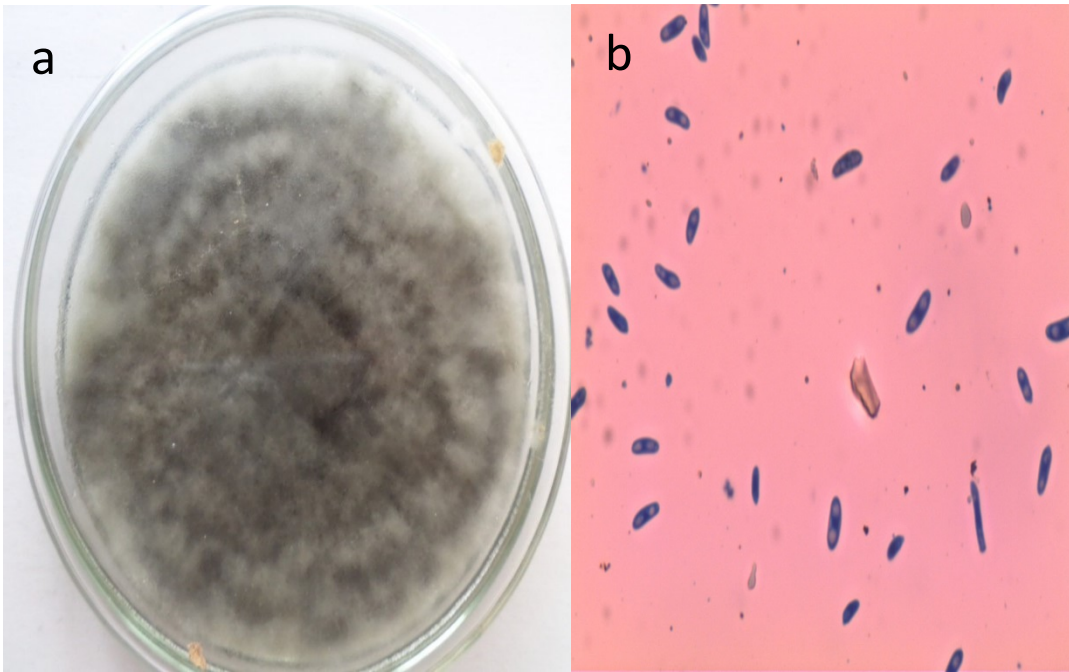


Plate 4. 2: (a) Seven day old culture of *Colletotrichum coccodes* (b) Conidia of *Colletotrichum coccodes* isolated from infected pepper plants collected during the survey conducted in Southwestern Nigeria. MAG  $\times$  400

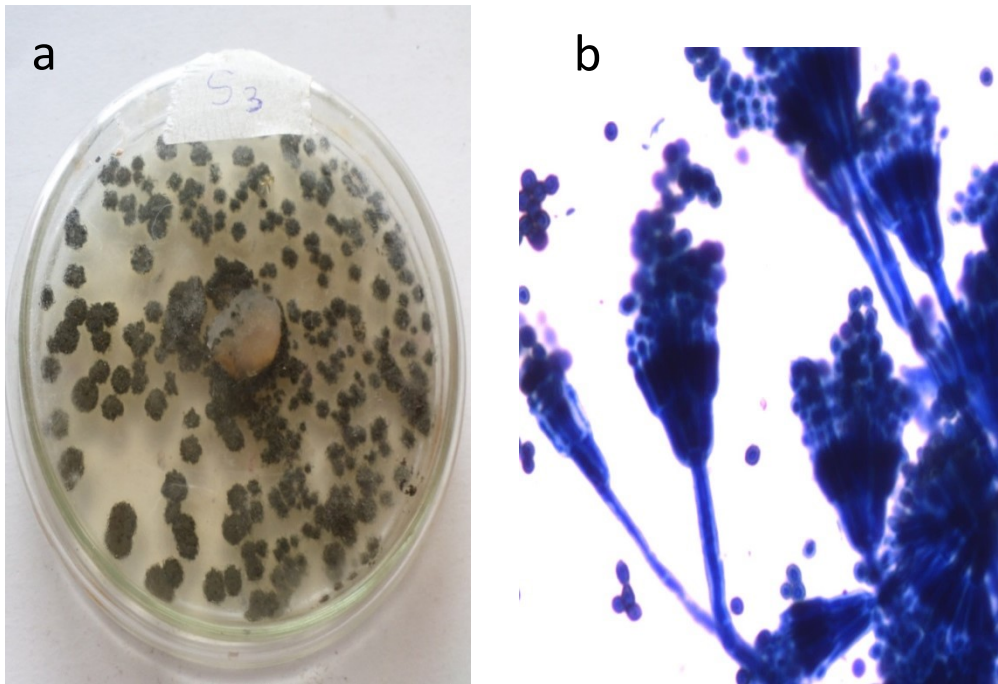


Plate 4.3 : (a) Seven day old culture of *Penicillium* species (b) Conidiphore bearing conidia of *Penicillium* species isolated from infected pepper plants collected during the survey conducted in Southwestern Nigeria. MAG  $\times$  400

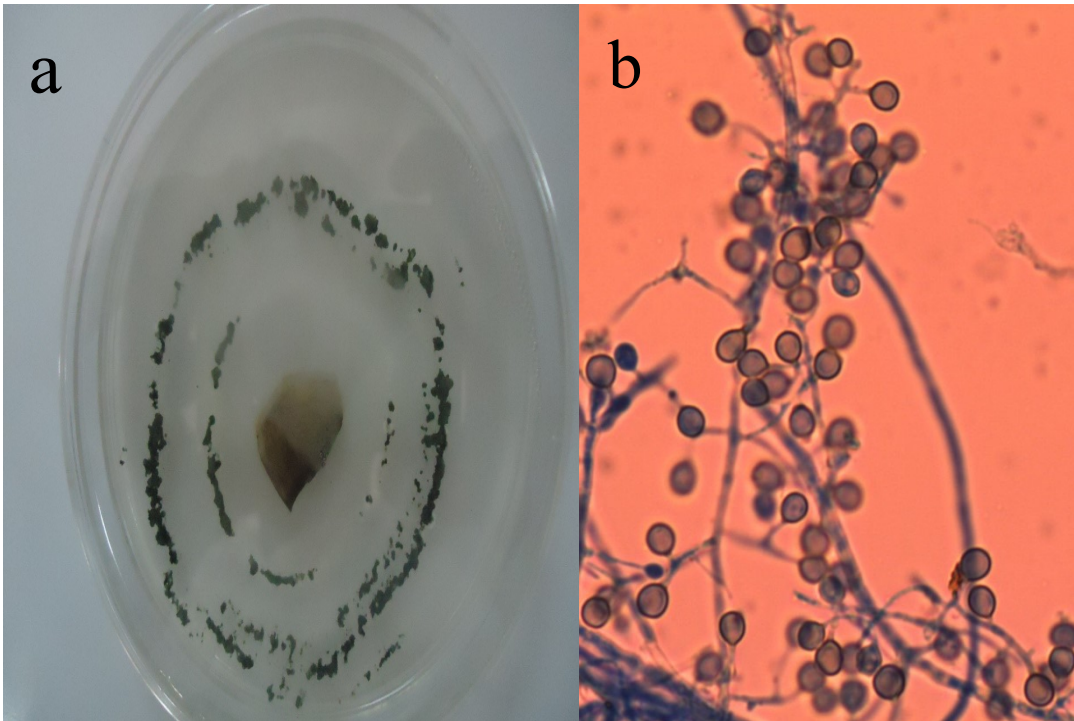


Plate 4.4: (a) Seven day old culture of *Trichoderma pseudokoningii* (a) Conidia of *Trichoderma pseudokoningii* isolated from infected pepper plants collected during the survey conducted in Southwestern Nigeria. MAG  $\times$  400

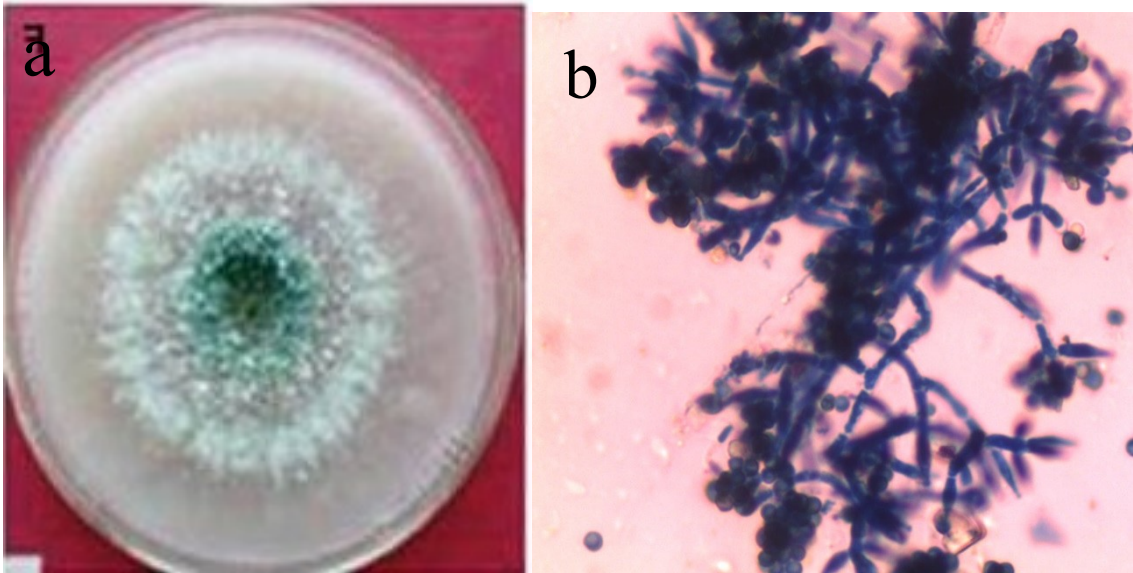


Plate 4. 5: (a) Seven day old culture of *Trichoderma harzianum*(b) Conidia of *Trichoderma harzianum* isolated from infected pepper plants during survey conducted in Southwestern Nigeria. MAG  $\times$  400

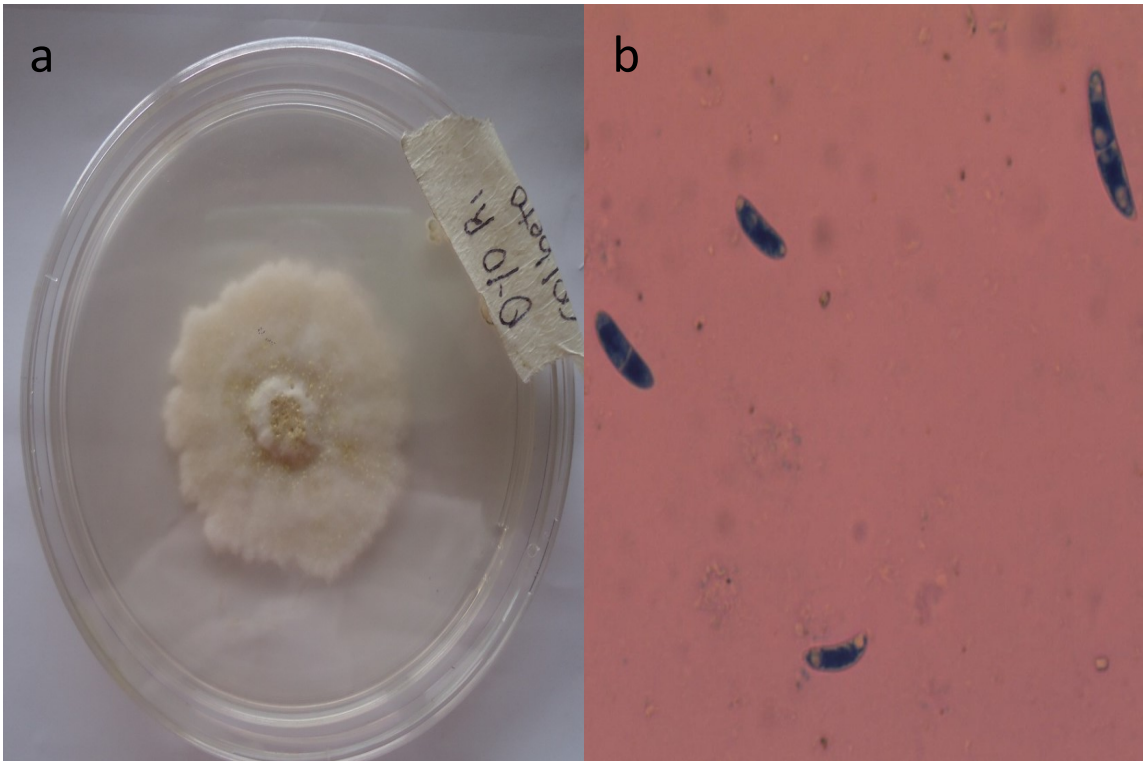


Plate 4.6: (a) Seven day old culture of *Colletotrichum capsici* (b) Conidia of *Colletotrichum capsici* isolated from infected pepper plants collected during the survey conducted in Southwestern Nigeria. MAG  $\times$  400



On PDA it was light grey on surface and reverse of the plate (Plate 4.7a). Mycelium consist of hyaline to pale brown hyphae, forming intercalary chains of brown, ellipsoid chlamydospores, 8–15 µm diameter. Conidia were thick-walled, hyaline, smooth, aseptate guttulate or not, ellipsoid with obtuse end (Plate 4.7b).

#### **4.9 Percentage Frequency of Occurrence of Fungi Isolated from Three Pepper Cultivars Sampled in Southwestern States**

Generally, *C. coccodes* had the highest frequency of occurrence on *C. chinense* across sampled states followed by *Penicillium* sp and *Trichoderma pseudokoningii*. Frequency of occurrence of *C. coccodes* was highest (91%) in *C. frutescens* followed by those of *T. pseudokoningii* and *Penicillium* spp. *C. coccodes* was also observed to be the highest in *C. annum* cultivars followed by *P. lycopersici* (Table 4.7).

*Colletotrichum coccodes* was the highest (81%) occurring fungi on *C. chinense* in Oyo state. *C. coccodes* was also the most encountered fungi on *C. chinense* in Ogun (64%), Osun (60) and Ekiti (54.3%) (Table 4.8). However, *T. pseudokoningii* was the most frequently isolated fungi in Ondo state (55.5%). *Trichoderma harzianum* was least occurring fungi on *C. chinense* across all the five States.

In Table 4.9 for chilli pepper (*C. frutescens*), *C. coccodes* was also the most frequently occurring fungi in all the five states followed by *T. pseudokoningii* in Oyo (29.3%), Osun (45.6%), Ogun (33.6%) and Ekiti (44.8%). In Ondo state, the second most important fungi encountered was *Penicillium* spp. *T. harzianum* was the least isolated fungi in Oyo (0%), Ogun (0%), Ondo (0%) and Ekiti (0%). However, *P. lycopersici* had the lowest (0%) percentage frequency of occurrence of fungi isolated from *C. frutescens* in Osun state. The frequency of occurrence (%) of fungal pathogens isolated from *C. annum* was highest in Oyo state (72%) for *P. lycopersici*. *P. lycopersici* was also the most encountered pathogen on *C. annum* in Osun (51%), Ogun (49.2%) and Ekiti (54.3%) (Table 4.10). However, *T. pseudokoningii* was the most frequently isolated pathogen in Ondo (61%) and Ekiti (45%). Lowest frequency of occurrence of fungal pathogens isolated from *C. annum* was *Penicillium* species in Oyo, Osun, Ondo and Ekiti (0%). In Ogun, state *Penicillium* sp was the least occurring fungi from all the varieties of infected pepper isolated (Table 4.10)

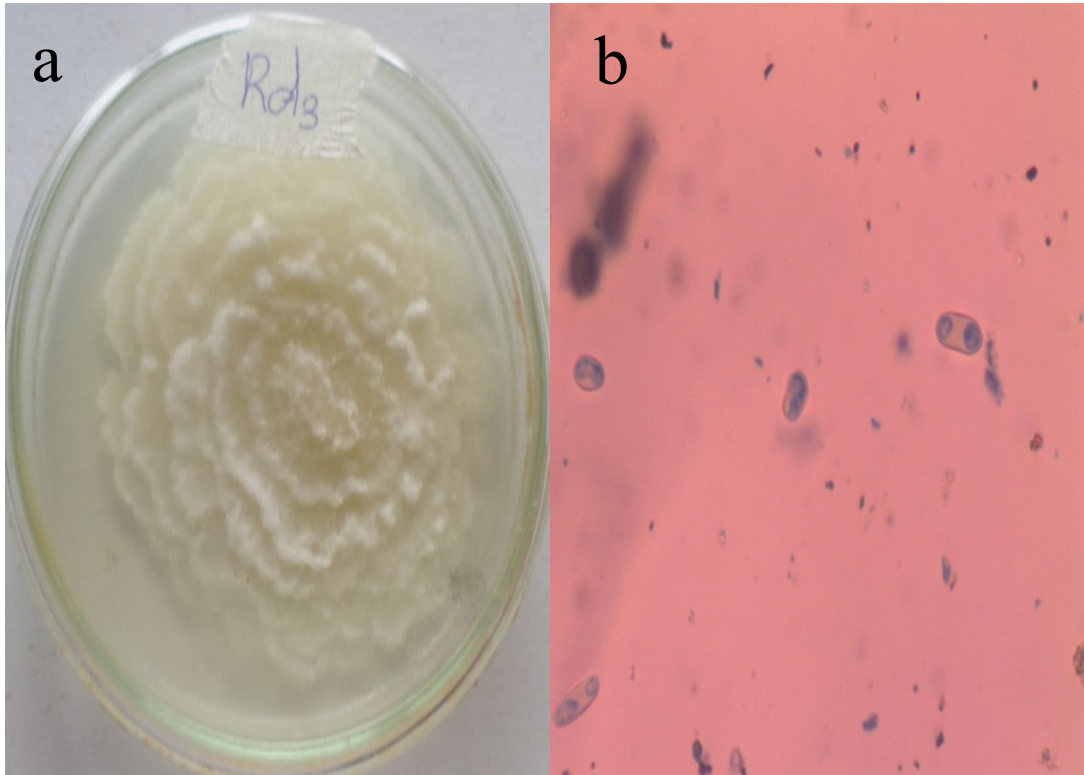


Plate 4. 7: (a) Seven day old culture of *Pyrenochaeta lycopersici* (b) Conidia of *Pyrenochaeta lycopersici* isolated from infected pepper plants collected during the survey conducted in Southwestern Nigeria. MAG  $\times$  400

**Table 4. 7: Frequency of Occurrence (%) of Isolated Fungi from Three Pepper Cultivars Sampled in Southwestern States, Nigeria, 2014**

| Isolated Fungi                    | <i>Capsicum chinense</i> | <i>Capsicum frutescens</i> | <i>Capsicum annum</i> |
|-----------------------------------|--------------------------|----------------------------|-----------------------|
| <i>Colletotrichum coccodes</i>    | 81.3                     | 91                         | 49.1                  |
| <i>Colletotrichum capsici</i>     | 21.0                     | 2.0                        | 0.0                   |
| <i>Penicillium</i> species        | 38.7                     | 47.7                       | 2.2                   |
| <i>Trichoderma pseudokoningii</i> | 38.1                     | 65.1                       | 14.5                  |
| <i>Pyrenochaeta lycopersici</i>   | 2.0                      | 4.2                        | 31.2                  |
| <i>Trichoderma harzianum</i>      | 1.0                      | 1.0                        | 2.0                   |

**Table 4.8: Frequency of Occurrence (%) of Isolated Fungi from Hot Pepper (*Capsicum chinense*) in Southwestern States Nigeria, 2015**

| Isolated Fungi                    | OYO  | OSUN | OGUN | ONDO | EKITI |
|-----------------------------------|------|------|------|------|-------|
| <i>Colletotrichum coccodes</i>    | 81.0 | 60.0 | 64.0 | 22.0 | 54.3  |
| <i>Colletotrichum capsici</i>     | 12.0 | 2.0  | 7.0  | 0.0  | 0.0   |
| <i>Penicillium</i> species        | 1.0  | 2.0  | 2.0  | 22.5 | 11.2  |
| <i>Trichoderma pseudokoningii</i> | 2.1  | 35.0 | 11.0 | 55.5 | 34.5  |
| <i>Pyrenochaeta lycopersici</i>   | 0.9  | 0.0  | 1.1  | 0.0  | 0.0   |
| <i>Trichoderma harzianum</i>      | 0.0  | 1.0  | 0.0  | 0.0  | 0.0   |

**Table 4.9: Frequency of Occurrence (%) of Fungi Isolated from Chilli Pepper (*Capsicum frutescens*) in Southwestern States, Nigeria, 2015**

| Isolated Fungi                    | OYO  | OSUN | OGUN | ONDO | EKITI |
|-----------------------------------|------|------|------|------|-------|
| <i>Colletotrichum coccodes</i>    | 61.0 | 54.1 | 54.0 | 67.9 | 54.0  |
| <i>Colletotrichum capsici</i>     | 1.0  | 0.0  | 1.0  | 0.0  | 0.0   |
| <i>Penicillium</i> species        | 6.7  | 10.3 | 8.2  | 21.3 | 1.2   |
| <i>Trichoderma pseudokoningii</i> | 29.3 | 45.6 | 33.6 | 11.8 | 44.8  |
| <i>Pyrenochaeta lycopersici</i>   | 1.0  | 0.0  | 3.2  | 0.0  | 0.0   |
| <i>Trichoderma harzianum</i>      | 0.0  | 1.0  | 0.0  | 0.0  | 0.0   |

**Table 4. 10: Percentage Frequency of Occurrence of Fungi Isolated from Bell Pepper (*Capsicum annum*) in Southwestern States, Nigeria, 2015**

| Isolated Fungi                    | OYO  | OSUN | OGUN | ONDO | EKITI |
|-----------------------------------|------|------|------|------|-------|
| <i>Colletotrichum coccodes</i>    | 26.3 | 44.6 | 45.2 | 14.0 | 19.0  |
| <i>Penicillium</i> species        | 0.0  | 0.0  | 2.2  | 0.0  | 0.0   |
| <i>Trichoderma pseudokoningii</i> | 0.7  | 4.4  | 3.4  | 61.0 | 45.0  |
| <i>Pyrenochaeta lycopersici</i>   | 72.0 | 51.0 | 49.2 | 24.0 | 35.0  |
| <i>Trichoderma harzianum</i>      | 0.0  | 0.0  | 0.0  | 1.0  | 1.0   |

#### 4.12 Pathogenicity Test of Isolated Fungi on Three Cultivars of Pepper

The pathogenicity test on the six isolated fungi, (*C. coccodes*, *C. capsici*, *Penicillium* species, *T. harzianum*, *T. pseudokoningii* and *P. lycopersici*) showed that three fungi, *C. coccodes*, *C. capsici* and *P. lycopersici* were pathogenic. They indicated causing disease on healthy seedlings of hot pepper (local rodo), sweet pepper (local bawa) and bell pepper (local tatashe). The leaves of pepper showed symptoms six days after inoculation. The most virulent among the six test fungi was *C. coccodes* causing leaf spot resulting in 84% diseased leaves, followed by *C. capsici* (72%) and *P. lycopersici* with necrotic spot of 50% damage (Table 4.11). Severity rating was 4.6 with the damage greater than 75% on pepper plant inoculated with *C. coccodes* while *C. capsici* was rated 4 for causing 72% damage. Plants inoculated with *P. lycopersici* were rated 3.3 as only 50% of leaves were infected as shown in Table 4.11.

Plants inoculated with *C. coccodes* showed initial symptoms of chlorotic foliage followed by wilting (Plate 4.8a). At 42 days after inoculation, symptoms on fruits of infected plants were evident with fruits showing soft, sunken, round or slightly elongated lesions bearing acervuli (Plate 4.8b). Pepper plants inoculated with *C. capsici* showed small brownish spots and lesions on leaves six days after inoculation which later became necrotic (Plate 4.9a). Fruit lesions appeared elliptical to circular with presence of acervuli on pepper fruits with white masses (Plate 4.9b). Pathogenicity study using *P. lycopersici* in Plate 4.10a showed symptoms of the leaf spots which include yellowing of leaves and death of the plant in few cases while Plate 4.10b showed rot symptoms on fruits caused by *C. coccodes*. *Penicillium* species, *T. harzianum*, *T. pseudokoningii* were not pathogenic on pepper plants resulting into healthy leaves and fruits (Plate 11a, 11b and 11c). All tested isolates were again re-isolated from infected plants following Koch's postulate and showed similar characteristics as previously observed from the field. The pathogenicity test carried out showed that *C. coccodes*, *C. capsici* and *P. lycopersici* were capable of causing leaf spot, chlorosis, blight and fruit rot on pepper plants.

**Table 4.11: Number of Diseased Leaves (%) Inoculated with Six Isolated Fungi on Healthy pepper plants**

| Isolates                        | Percentage Number of Diseased Leaves | Severity Rating |
|---------------------------------|--------------------------------------|-----------------|
| <i>Colletotrichum coccodes</i>  | 84.0                                 | 5               |
| <i>Colletotrichum capsici</i>   | 72.0                                 | 4.0             |
| <i>Pyrenochaeta lycopersici</i> | 50.0                                 | 3.3             |
| <i>Penicillium</i> species      | 0.0                                  | 1.0             |
| <i>T. harzianum</i>             | 0.0                                  | 1.0             |
| <i>T. pseudokoningii</i>        | 0.0                                  | 1.0             |

1 = no symptoms, 2 = 1- 25% of plant showing symptoms, 3 = 26-50% of plant showing symptoms, 4 = 51- 75% of plant showing symptoms, 5 >75 % of plant showing symptoms or complete death of the plant.



Chlorotic leaves



Acervuli masses  
on fruits

Plate 4. 8: Chlorotic foliage and wilting (a) Acervuli masses on the fruits of bell pepper (*Capsicum annuum*) caused by *Colletotrichum coccodes* from inoculated pepper plant (b)

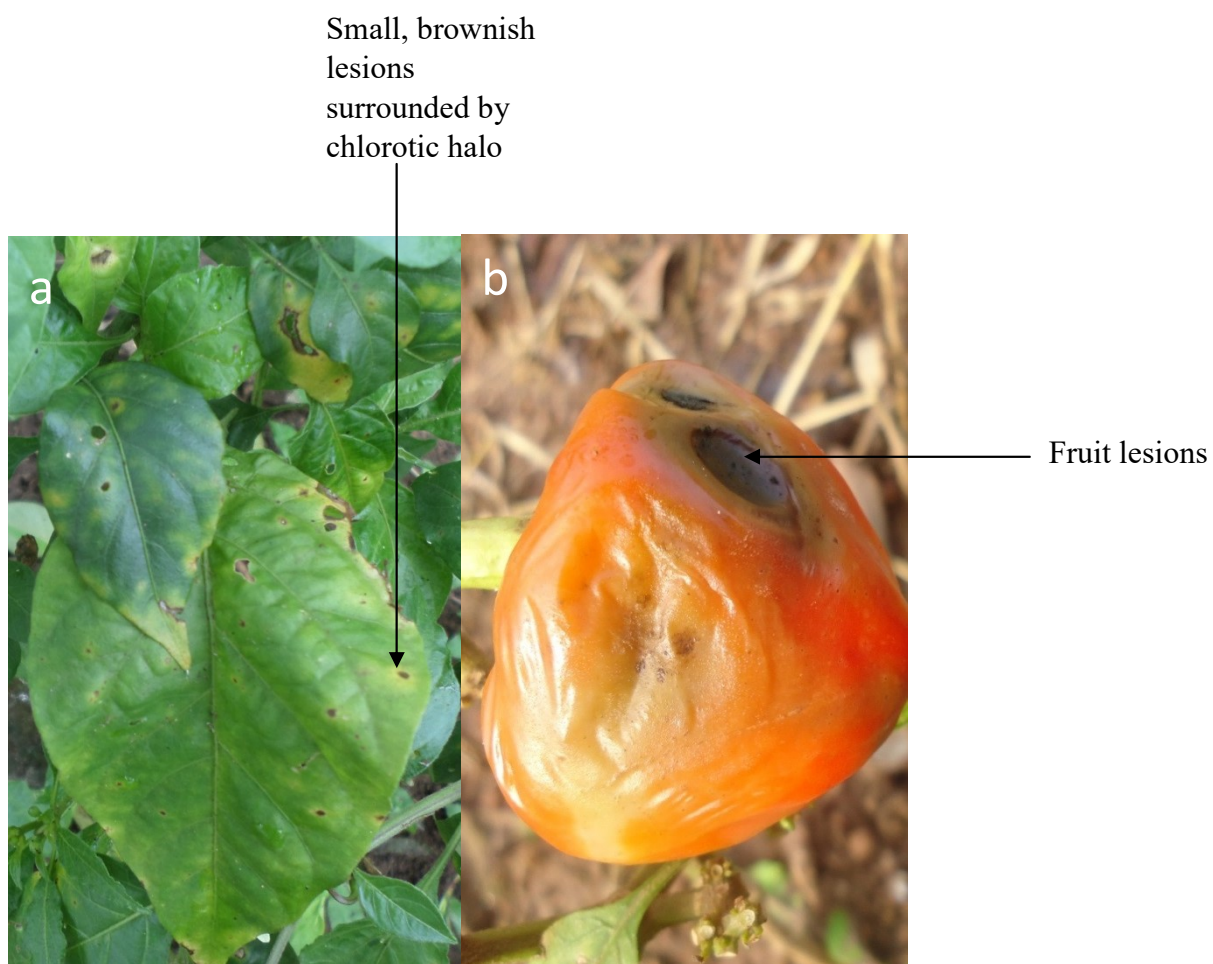


Plate 4.9: Small, brownish lesions surrounded by chlorotic halo on the leaves of inoculated pepper plant (a). Fruit lesions on bell pepper (*Capsicum annuum*) concentrically arranged on fruit caused by *Colletotrichum capsici* from inoculated pepper plant (b)

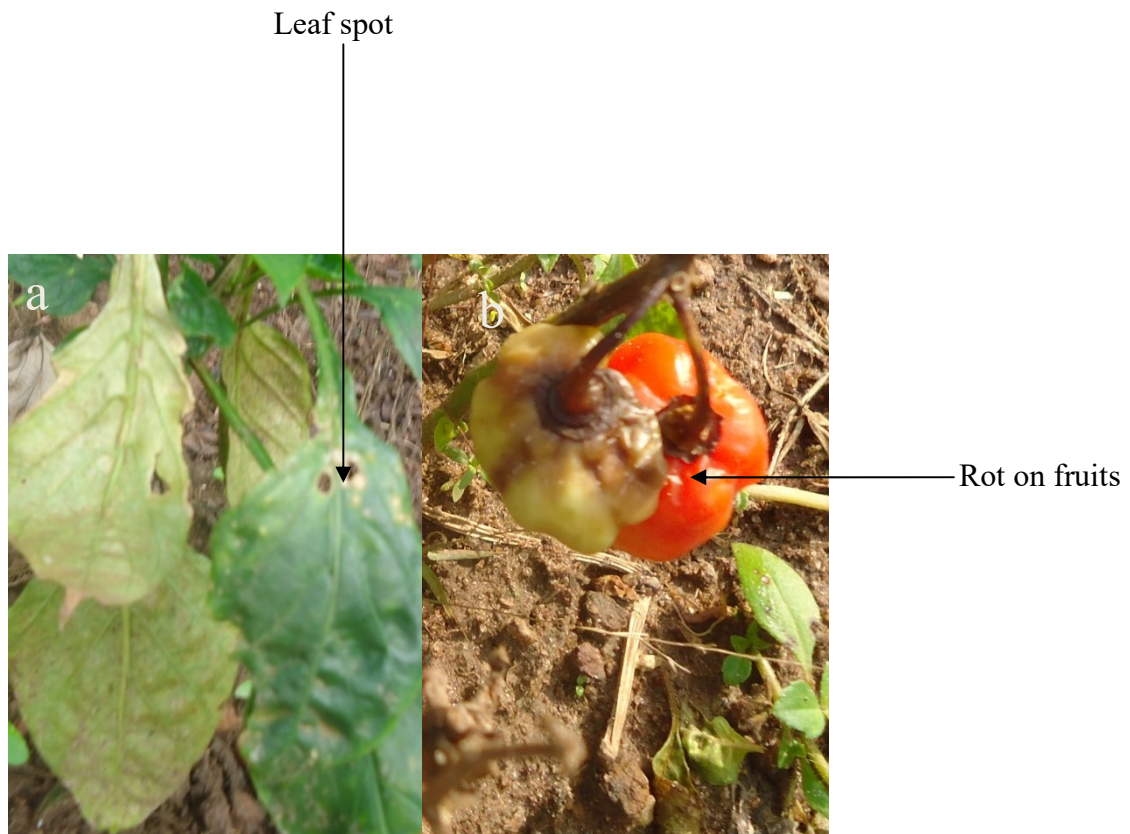


Plate 4.10: Leaf spot which include yellowing of leaves (a) Rot symptoms on hot pepper (*Capsicum chinense*) fruits caused by *Colletotrichum capsici* from inoculated pepper plant (b).



Plate 4.11: Leaves of pepper plants Inoculated with (a) *Penicillium* species (b) *T. harzianum*(c) *T. pseudokoningii* from inoculated pepper plant (d) uninoculated plants

## **4.13: Screening of Ten Pepper Cultivars for Resistance to Pathogenic Fungi in Pot Experiment**

### **4.13.1 Effect of *Colletotrichum capsici* on Plant Height and Number of Leaves of Pepper Plants in Pot Experiment**

There was no significant ( $P \leq 0.05$ ) difference in the plant height of pepper plants inoculated with *C. capsici* compared to non-inoculated plants except for *Capsicum annuum* cv. tatashe-bell where the plants were significantly taller compared to non-inoculated plants (Table 4.12). There was no significant ( $P \leq 0.05$ ) difference in the number of leaves produced from pepper plants inoculated with *C. capsici* compared to non-inoculated in all the cultivars screened in pots (Table 4.12).

### **4.13.2 Effect of *Colletotrichum capsici* on Yield and Fungal Disease Severity of Pepper in Pot Experiment**

There was no significant ( $P \leq 0.05$ ) difference in the number of fruits produced by plants inoculated with *C. capsici* compared with non-inoculated plants except for *Capsicum frutescens* cv. Ijosi-Bird where the number of fruits was significantly higher in non-inoculated plants compared to the inoculated plants (Table 4.13).

The fruit weight of tatashe cultivar inoculated with *C. capsici* was significantly ( $P \leq 0.05$ ) more compared with the fruit weight of non-inoculated *Capsicum annuum* cv. tatashe-bell. There was no significant ( $P \leq 0.05$ ) difference in the fruit weight of other cultivars inoculated compared with non-inoculated (Table 4.13).

The fungal disease severity on pepper plants inoculated with *C. capsici* were significantly higher ( $P \leq 0.05$ ) compared to non-inoculated plants (Table 4.14). Disease severity of inoculated *Capsicum chinense* cv. Batassa-hot pepper had the mean value of 2.0 while other cultivars had the mean value of 3.5 – 4.8 severity (Table 4.14).

**Table 4.12: Effect of *Colletotrichum capsici* on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Plant Height (cm) |                    | No of Leaves |                    |
|--|-------------------|--------------------|--------------|--------------------|
|  | Control           | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 46.0              | 12.5*              | 33.8         | 10.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 21.3              | 18.8 <sup>NS</sup> | 37.3         | 12.8 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 11.0              | 9.8 <sup>NS</sup>  | 37.3         | 24.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 31.5              | 23.0 <sup>NS</sup> | 44.8         | 38.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 15.5              | 15.0 <sup>NS</sup> | 28.5         | 27.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 11.5              | 10.3 <sup>NS</sup> | 34.8         | 20.0 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 10.3              | 9.5 <sup>NS</sup>  | 13.3         | 6.0 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 15.3              | 8.5 <sup>NS</sup>  | 27.8         | 18.3 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 19.0              | 12.8 <sup>NS</sup> | 20.5         | 17.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 15.8              | 14.8 <sup>NS</sup> | 25.0         | 19.0 <sup>NS</sup> |
| LSD  | 13.5              | 10.5               | 27.2         | 27.6               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.13: Effect of *Colletotrichum capsici* on Number of Fruits and Weight of Fruits of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Number of fruits |                    | Weight of fruits (g) |                    |
|--|------------------|--------------------|----------------------|--------------------|
|  | Control          | Inoculated         | Control              | Inoculated         |
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 7.3              | 3.0 <sup>NS</sup>  | 16.8                 | 5.0*               |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 7.5              | 3.8 <sup>NS</sup>  | 13.5                 | 11.8 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 5.3              | 3.5 <sup>NS</sup>  | 8.5                  | 5.0 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 6.0              | 5.5 <sup>NS</sup>  | 8.8                  | 8.3 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 8.3              | 4.5 <sup>NS</sup>  | 13.5                 | 11.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 6.3              | 3.5 <sup>NS</sup>  | 7.8                  | 5.0 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 16.5             | 4.0*               | 5.3                  | 3.0 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 1.3              | 0.8 <sup>NS</sup>  | 17.0                 | 13.0 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.5              | 1.0 <sup>NS</sup>  | 9.3                  | 8.7 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 14.5             | 10.0 <sup>NS</sup> | 34.5                 | 27.5 <sup>NS</sup> |
| LSD  | 11.7             | 3.0                | 18.1                 | 7.3                |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.14: Effect of *Colletotrichum capsici* on Disease Severity of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Control | Inoculated | Status |
|--|---------|------------|--------|
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 1.0     | 4.0*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0     | 3.4*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0     | 3.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0     | 4.8*       | HS     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0     | 4.0*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0     | 4.0*       | HS     |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 1.0     | 4.0*       | HS     |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.0     | 4.0*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0     | 2.0*       | R      |
| LSD  | 1.0     | 2.3        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 = Highly susceptible (HS).- (Roane *et al.*, 1974)

NS= Not significant;\*=Significant.



#### **4.13.3. Effect of *Pyrenochaeta lycopersici* on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment**

There was no significant ( $P \leq 0.05$ ) difference in the plant height of pepper plants inoculated with *P. lycopersici* compared to non-inoculated plants except for *Capsicum annuum* cv. tatashe-bell where the height of non-inoculated plants was significantly higher compared to inoculated plants (Table 4.15). The number of leaves produced by *Capsicum annuum* cv. tatashe-bell, *Capsicum chinense* cv. Bawa-chilli, *Capsicum frutescens* cv. Ijosi-Bird and *Capsicum chinense* cv. Bendel-rodo plants inoculated with *P. lycopersici* were significantly ( $P \leq 0.05$ ) lowered compared to non-inoculated. The difference in the number of leaves produced by *Capsicum chinense* cv. Bendel-rodo was significantly ( $P \leq 0.05$ ) lower compared to rodo Avenir and Rodo (Cameroon pepper)(Table 4.15).

#### **4.13.4 Effect of *Pyrenochaeta lycopersici* on Yield and Disease Severity of Ten Pepper Cultivars in Pot Experiment**

There was no significant ( $P \leq 0.05$ ) difference in the number of fruits of pepper plants inoculated with *P. lycopersici* compared to non-inoculated plants in all the cultivars screened (Table 4.16). *Capsicum annuum* cv. Nikitta-bell and *Capsicum chinense* cv. Avenir-rodo had significantly ( $P \leq 0.05$ ) lower fruit weight when inoculated with *P. lycopersici* compared to non inoculated plants (Table 4.16).

The fungal disease severity of pepper plants inoculated with *P. lycopersici* was significantly ( $P \leq 0.05$ ) higher compared to non-inoculated plants (Table 4.17). *Capsicum chinense* cv. Batassa-hot inoculated had the lowest disease severity with the mean value of 2.0 while other cultivars had disease severity value of 2.5 – 4.5 (Table 4.17).

#### **4.13.5 Effect of *Colletotrichum coccodes* on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment**

There was no significant ( $P \leq 0.05$ ) difference in the plant height of pepper plants inoculated with *C. coccodes* compared to non-inoculated plants except for *Capsicum annuum* cv. tatashe-bell where the height of non-inoculated plants were significantly taller compared to inoculated plants (Table 4.18). Number of leaves produced by *Capsicum annuum* cv. Pizzaro-bell, *Capsicum frutescens* cv. Ijosi-Bird, *Capsicum*

**Table 4.15: Effect of *Pyrenochaeta lycopersici* on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Plant Height (cm) |                    | No of Leaves |                    |
|--|-------------------|--------------------|--------------|--------------------|
|  | Control           | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 31.0              | 9.4*               | 33.8         | 14.5*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 17.5              | 12.1 <sup>NS</sup> | 37.3         | 13.3*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 24.0              | 14.3 <sup>NS</sup> | 27.2         | 25.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 14.2              | 7.8 <sup>NS</sup>  | 34.8         | 18.5*              |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 21.3              | 19.8 <sup>NS</sup> | 18.5         | 13.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 15.1              | 10.6 <sup>NS</sup> | 34.8         | 26.8 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 12.3              | 10.0 <sup>NS</sup> | 44.0         | 13.3*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 11.0              | 9.3 <sup>NS</sup>  | 17.8         | 9.8 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 9.0               | 7.5 <sup>NS</sup>  | 20.5         | 11.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 14.8              | 13.3 <sup>NS</sup> | 25.0         | 13.3 <sup>NS</sup> |
| LSD  | 22.7              | 13.7               | 27.0         | 22.2               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.16: Effect of *Pyrenochaeta lycopersici* on Number of Fruits and Weight of Fruits of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Number of fruits |                    | Weight of Fruits (g) |                    |
|--|------------------|--------------------|----------------------|--------------------|
|  | Control          | Inoculated         | Control              | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 5.5              | 3.1 <sup>NS</sup>  | 21.2                 | 14.1 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 7.5              | 2.0 <sup>NS</sup>  | 11.5                 | 6.5 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 5.1              | 2.7 <sup>NS</sup>  | 10.5                 | 7.5 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Rodo-Hot      | 7.0              | 4.5 <sup>NS</sup>  | 12.3                 | 8.1 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Rodo   | 7.9              | 4.5 <sup>NS</sup>  | 13.9                 | 6.9 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 5.8              | 1.2 <sup>NS</sup>  | 9.0                  | 3.1 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 11.5             | 4.0 <sup>NS</sup>  | 15.3                 | 9.5 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 7.1              | 2.5 <sup>NS</sup>  | 25.0                 | 14.8*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 2.6              | 1.6 <sup>NS</sup>  | 18.7                 | 12.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 14.5             | 10.0 <sup>NS</sup> | 30.0                 | 9.5*               |
| LSD  | 11.8             | 3.1                | 18.1                 | 7.3                |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant;\*=Significant.

**Table 4.17: Effect of *Pyrenochaeta lycopersici* on Disease Severity of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Control | Inoculated | Status |
|--|---------|------------|--------|
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0     | 4.3*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0     | 3.3*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0     | 3.3*       | HS     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0     | 2.5*       | MR     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0     | 4.0*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0     | 3.3*       | HS     |
| <i>Capsicum annuum</i> cv. Nikitta-Bell    | 1.0     | 3.3*       | HS     |
| <i>Capsicum annuum</i> cv. Pizzaro-Bell    | 1.0     | 3.3*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0     | 2.0*       | R      |
| LSD  | 1.0     | 3.5        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 =Highly susceptible (HS). (Roane *et al.*, 1974)

NS= Not significant;\*=Significant

**Table 4.18: Effect of *Colletotrichum coccodes* on Plant Height and Number of Leaves of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Plant Height (cm) |                    | No of Leaves |                    |
|--|-------------------|--------------------|--------------|--------------------|
|  | Control           | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 21.1              | 10.0*              | 33.8         | 14.5*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 10.1              | 5.8 <sup>NS</sup>  | 37.3         | 19.0*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 12.7              | 8.0 <sup>NS</sup>  | 37.3         | 17.9*              |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 19.4              | 12.8 <sup>NS</sup> | 11.0         | 10.8 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 16.3              | 11.3 <sup>NS</sup> | 34.6         | 17.8*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 13.0              | 9.5 <sup>NS</sup>  | 41.0         | 19.5*              |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 11.4              | 9.5 <sup>NS</sup>  | 49.8         | 13.3*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 15.3              | 12.8 <sup>NS</sup> | 23.8         | 17.8*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 9.0               | 5.3 <sup>NS</sup>  | 20.5         | 12.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 15.7              | 12.8 <sup>NS</sup> | 15.0         | 14.0 <sup>NS</sup> |
| LSD  | 32.6              | 12.9               | 32.0         | 14.3               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

*chinense* cv. Bawa-chilli, *Capsicum frutescens* cv. Sombo-Bird, *Capsicum chinense* cv. Cameroon-rodo, *Capsicum chinense* cv. Bendel-rodo and *Capsicum chinense* cv. Avenir-hot inoculated with *C.coccodes* were significantly ( $P\leq 0.05$ ) lower compared to non-inoculated plants (Table 4.18). Rodo (Cameroon pepper) and rodo (Avenir) had significantly ( $P\leq 0.05$ ) higher number of leaves when inoculated with *C. coccodes* compared to other cultivars (Table 4.18).

#### **4.13.6 Effect of *Colletotrichum coccodes* on Yield and Fungal Disease Severity of Ten Pepper Cultivars in Pot Experiment**

There was no significant ( $P\leq 0.05$ ) difference in the number of fruits of pepper plants inoculated with *C. coccodes* compared to non-inoculated plants except for Ijosi cultivar where the number of fruits was significantly greater than inoculated plants (Table 4.19). There was no significant ( $P\leq 0.05$ ) difference in the number of fruits produced from inoculated plants among the cultivars. The fruit weight of non-inoculated tatashe, bawa and *Capsicum chinense* cv. Batassa-hot pepper were significantly ( $P\leq 0.05$ ) higher compared to inoculated plants with *C. coccodes* (Table 4.19).

The fungal disease severity was significantly ( $P\leq 0.05$ ) higher in pepper plants inoculated with *C. coccodes* compared to non-inoculated plants in all the cultivars screened (Table 4.20). Disease severity of inoculated *Capsicum chinense* cv. Batassa-hot had the mean value of 2.5 while other cultivars had the mean value of 4.3 – 5.0 (Table 4.20).

#### **4.14: Evaluation of Ten Pepper Cultivars for Resistance to Pathogenic Fungi in First and Second Field Trials in Year 2016 and 2017**

##### **4.14.1 Effect of *Colletotrichum capsici* on Plant Height and Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

The non-inoculated sombo cultivar was significantly ( $P\leq 0.05$ ) taller compared to inoculated in the first and second trial (Table 4.21). *Capsicum chinense* cv. Bawa-chilli, *Capsicum chinense* cv. Avenir-hot, *Capsicum annum* cv Nikitta-bell, *Capsicum annum* cv Pizzaro-bell and *Capsicum chinense* cv. Cameroon-rodo pepper non-inoculated had significantly ( $P\leq 0.05$ ) taller plants. There was no significant ( $P\leq 0.05$ )

**Table 4.19: Effect of *Colletotrichum coccodes* on Number of Fruits and Weight of Fruits of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Number of fruits |                   | Weight of Fruits(g) |                   |
|--|------------------|-------------------|---------------------|-------------------|
|  | Control          | Inoculated        | Control             | Inoculated        |
| <i>Capsicum annuum</i> cv. Tatashe-Bell    | 7.1              | 2.0 <sup>NS</sup> | 19.4                | 4.9*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 6.5              | 0.0 <sup>NS</sup> | 14.4                | 0.0*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 4.1              | 0.4 <sup>NS</sup> | 7.5                 | 1.9 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 5.2              | 1.0 <sup>NS</sup> | 7.9                 | 1.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 9.3              | 3.5 <sup>NS</sup> | 10.7                | 5.8 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 7.4              | 0.9 <sup>NS</sup> | 7.8                 | 2.2 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 12.5             | 1.3*              | 5.3                 | 4.7 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv. Nikitta-Bell    | 2.1              | 0.7 <sup>NS</sup> | 9.0                 | 6.0 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.5              | 0.5 <sup>NS</sup> | 4.2                 | 2.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 14.5             | 9.0 <sup>NS</sup> | 37.1                | 16.3*             |
| LSD  | 11.8             | 4.4               | 18.1                | 7.2               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.20: Effect of *Colletotrichum coccodes* on Disease Severity of Ten Pepper Cultivars in Pot Experiment**

| Cultivars                                  | Control | Inoculated | Status |
|--|---------|------------|--------|
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0     | 4.3*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0     | 4.3*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0     | 4.5*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0     | 4.5*       | HS     |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 1.0     | 5.0*       | HS     |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.0     | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0     | 2.5*       | MR     |
| LSD  | 1.0     | 2.3        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 =Highly susceptible (HS). (Roane *et al.*, 1974)

NS= Not significant; \*=Significant at  $P \leq 0.05$



**Table 4.21: Effect of *Colletotrichum capsici* on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second Trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 12.8        | 10.0 <sup>NS</sup> | 16.5         | 12.8 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 20.5        | 19.0 <sup>NS</sup> | 14.5         | 8.5*               |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 15.8        | 5.7*               | 12.0         | 8.3*               |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 17.8        | 13.0 <sup>NS</sup> | 12.5         | 9.5 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 12.8        | 12.5 <sup>NS</sup> | 20.3         | 13.5*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 11.3        | 8.5 <sup>NS</sup>  | 19.8         | 11.0*              |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 17.8        | 17.5 <sup>NS</sup> | 16.1         | 12.3 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 18.3        | 9.0 <sup>NS</sup>  | 17.3         | 10.5*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 13.8        | 7.0 <sup>NS</sup>  | 19.3         | 11.5*              |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 12.5        | 11.8 <sup>NS</sup> | 15.8         | 11.3 <sup>NS</sup> |
| LSD  | 10.8        | 11.3               | 16.3         | 8.7                |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.22: Effect of *Colletotrichum capsici* on Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 14.3        | 13.0 <sup>NS</sup> | 17.8         | 13.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 33.0        | 29.8 <sup>NS</sup> | 43.0         | 36.5 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 21.5        | 10.0*              | 19.8         | 17.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 27.3        | 14.5 <sup>NS</sup> | 16.0         | 15.8 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 17.8        | 7.8 <sup>NS</sup>  | 14.0         | 13.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 7.5         | 4.8 <sup>NS</sup>  | 14.3         | 9.8 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 36.5        | 33.5 <sup>NS</sup> | 21.9         | 13.5 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 9.5         | 6.5 <sup>NS</sup>  | 11.5         | 4.7 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 8.8         | 7.3 <sup>NS</sup>  | 10.8         | 4.3 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 14.8        | 9.0 <sup>NS</sup>  | 16.3         | 11.6 <sup>NS</sup> |
| LSD  | 25.2        | 14.3               | 17.0         | 12.9               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

difference in the number of leaves produced by pepper plants inoculated with *C. capsici* compared to non-inoculated plants in the first trial and second trial; however, in the first trial *Capsicum frutescens* cv. sombo-Bird cultivar significantly produced more leaves than inoculated (Table 4.22).

#### **4.14.2 Effect of *Colletotrichum capsici* on Yield and Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trials**

The number of fruits of inoculated pepper plants were significantly ( $P \leq 0.05$ ) lower compared to non-inoculated plants in the first trial and second trial except for *Capsicum frutescens* cv. Ijosi-Bird, *Capsicum chinense* cv. Cameroon-rodo and *Capsicum chinense* cv. Bendel-rodo (Table 4.23). The difference in the number of fruits produced by *Capsicum chinense* cv. Bendel-rodo was significantly ( $P \leq 0.05$ ) lower compared to the *Capsicum chinense* cv. Avenir-hot in the two trials. Similarly the number of fruits produced by *Capsicum chinense* cv. Avenir-hot was significantly ( $P \leq 0.05$ ) lower compared to *Capsicum chinense* cv. Batassa-hot in the two trials. There was no significant ( $P \leq 0.05$ ) difference in the number of fruits of inoculated pepper plants compared with non-inoculated (Table 4.23). The fruit weight was significantly ( $P \leq 0.05$ ) higher in non-inoculated compared to inoculated plants in the first and second trials except for *Capsicum chinense* cv. Bendel-rodo (Table 4.24).

Fungal disease severity was significantly ( $P \leq 0.05$ ) higher in inoculated cultivars compared to non-inoculated plants in the two trials. Inoculated *Capsicum chinense* cv. Batassa-hot had the least disease severity of 1.9 in the first trial and 2.0 in the second trial (Table 4.25) followed by *Capsicum frutescens* cv. Ijosi-Bird pepper (2.4) and *Capsicum chinense* cv. Bendel-rodo (2.5) while other cultivars had the highest disease severity in the first and second trial (Table 4.25).

#### **4.14.3 Effect of *Pyrenochaeta lycopersici* on Plant Height and Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

*Capsicum chinense* cv. Bawa-chilli, *Capsicum annuum* cv. Tatashe-bell, *Capsicum frutescens* cv. sombo-Bird, *Capsicum annuum* cv. Nikitta-bell and *Capsicum annuum* cv. Pizzaro-bell were significantly ( $P \leq 0.05$ ) taller in non-inoculated plants

**Table 4.23: Effect of *Colletotrichum capsici* on Number of Fruits of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 35.0        | 15.3*              | 32.0         | 9.9*               |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 25.8        | 8.5*               | 26.5         | 6.0*               |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 20.8        | 4.5*               | 17.3         | 3.8*               |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 14.3        | 7.0 <sup>NS</sup>  | 8.3          | 7.3 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 27.0        | 9.0*               | 29.0         | 14.0*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 19.0        | 12.8 <sup>NS</sup> | 14.0         | 7.3 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 21.8        | 20.0 <sup>NS</sup> | 12.0         | 12.3 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 14.3        | 4.0*               | 13.5         | 1.8*               |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 17.8        | 7.4*               | 14.0         | 4.0*               |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 35.0        | 16.0*              | 21.3         | 16.0*              |
| LSD  | 15.3        | 7.0                | 13.8         | 10.0               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.24: Effect of *Colletotrichum capsici* on Weight of Fruits (g) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 120.8       | 45.0*              | 178.3        | 44.0*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 179.8       | 20.0*              | 175.0        | 17.8*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 142.5       | 16.0*              | 131.5        | 18.8*              |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 47.8        | 29.8 <sup>NS</sup> | 36.3         | 38.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 97.0        | 11.3*              | 93.3         | 10.5*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 147.5       | 49.3*              | 115.0        | 32.0*              |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 56.0        | 17.3*              | 67.7         | 15.0*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 132.2       | 30.0*              | 111.0        | 50.9*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 113.7       | 36.3*              | 140.0        | 49.0*              |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 156.0       | 23.8*              | 123.8        | 28.6*              |
| LSD  | 97.8        | 29.1               | 79.5         | 35.9               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.25: Effect of *Colletotrichum capsici* on Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trial Experiments**

| Cultivars                                  | First trial |            |        | Second trial |            |        |
|--|-------------|------------|--------|--------------|------------|--------|
|  | Control     | Inoculated | Status | Control      | Inoculated | Status |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 1.0         | 4.5*       | HS     | 1.0          | 3.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0         | 4.5*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0         | 3.4*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0         | 2.2*       | MR     | 1.0          | 2.5*       | MR     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0         | 3.0*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0         | 4.3*       | HS     | 1.0          | 4.8*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0         | 2.4*       | HS     | 1.0          | 2.5*       | MR     |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 1.0         | 3.5*       | HS     | 1.0          | 4.0*       | HS     |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.0         | 3.0*       | HS     | 1.0          | 3.5*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0         | 1.9*       | R      | 1.0          | 2.0*       | R      |
| LSD  | 1.0         | 1.6        |        | 1.0          | 1.5        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1–2.5 = Moderately Resistant (MR); 2.6–3.0 = Susceptible (S), and >3.0 = Highly susceptible (HS).

NS= Not significant; \*=Significant at  $P \leq 0.05$

compared to plants inoculated with *P. lycopersici* in the first trial. Also, *Capsicum annuum* cv Tatashe-bell, *Capsicum chinense* cv. Cameroon-rodo and *Capsicum annuum* cv Nikitta-bell cultivars are significantly ( $P \leq 0.05$ ) taller in non-inoculated plants compared to plants inoculated with *P. lycopersici* in the second trial (Table 4.26). The number of leaves produced by *Capsicum annuum* cv Tatashe-bell, *Capsicum chinense* cv. Bawa-chilli, *Capsicum chinense* cv. Cameroon-rodo, *Capsicum chinense* cv. Batassa-hot and *Capsicum frutescens* cv. sombo-Bird when inoculated with *P. lycopersici* was significantly ( $P \leq 0.05$ ) less compared to non-inoculated plants in the first trial. *Capsicum annuum* cv Tatashe-bell, *Capsicum frutescens* cv. sombo-Bird and *Capsicum chinense* cv. Batassa-hot and *Capsicum annuum* cv Nikitta-bell non-inoculated cultivars significantly ( $P \leq 0.05$ ) had more leaves compared to inoculated plants in the second trial (Table 4.27).

#### **4.14.4 Effect of *Pyrenochaeta lycopersici* on Yield and Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trials**

The numbers of fruits produced were significantly ( $P \leq 0.05$ ) lower in inoculated plants compared with non-inoculated plants in the first trial and second trial. *Capsicum chinense* cv. Bendel-rodo, *Capsicum annuum* cv Pizzaro-bell, *Capsicum frutescens* cv. Ijosi-Bird and *Capsicum annuum* cv Nikitta-bell inoculated with *P. lycopersici* had less fruits compared to non-inoculated plants in the first and second trial (Table 4.28). The fruit weight of *Capsicum chinense* cv. Bendel-rodo, *Capsicum annuum* cv Nikitta-bell, *Capsicum frutescens* cv. Ijosi-Bird, *Capsicum chinense* cv. Cameroon-rodo, and *Capsicum chinense* cv. Batassa-Hot were significantly ( $P \leq 0.05$ ) lower in inoculated plants compared to non-inoculated plants in the first trial (Table 4.29). In the second trial, the weight of fruits of *Capsicum frutescens* cv. sombo-Bird, *Capsicum chinense* cv. Bendel-rodo, *Capsicum chinense* cv. Cameroon-rodo and *Capsicum chinense* cv. Batassa-hot was significantly ( $P \leq 0.05$ ) greater in non-inoculated plants compared with inoculated plants (Table 4.29).

Fungal disease severity was significantly ( $P \leq 0.05$ ) higher in inoculated pepper plants compared to non-inoculated plants in the first and second trials (Table 4.30). Inoculated *Capsicum chinense* cv. Batassa-hot had the least mean disease severity of 2.4

**Table 4.26: Effect of *Pyrenochaeta lycopersici* on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 16.6        | 5.2*               | 15.0         | 4.5*               |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 37.0        | 17.3*              | 14.3         | 7.3 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 21.5        | 9.0*               | 18.3         | 11.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 14.5        | 15.5 <sup>NS</sup> | 16.5         | 9.8 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 17.8        | 8.3 <sup>NS</sup>  | 20.3         | 12.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 9.3         | 4.8 <sup>NS</sup>  | 19.8         | 9.5*               |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 25.3        | 22.5 <sup>NS</sup> | 14.9         | 12.8 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 19.5        | 4.8*               | 17.3         | 7.1*               |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 18.8        | 6.7*               | 14.5         | 8.3 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 8.7         | 7.0 <sup>NS</sup>  | 11.3         | 12.0 <sup>NS</sup> |
| LSD  | 10.9        | 11.5               | 6.4          | 11.3               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.



**Table 4.27: Effect of *Pyrenochaeta lycopersici* on Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 38.3        | 21.5*              | 35.5         | 13.0*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 25.8        | 10.3*              | 29.3         | 9.5 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 20.8        | 8.0*               | 21.8         | 11.8*              |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 20.3        | 13.5 <sup>NS</sup> | 19.5         | 15.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 27.0        | 13.3 <sup>NS</sup> | 14.0         | 8.8 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 21.8        | 7.3*               | 24.3         | 16.8 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 12.0        | 8.5                | 23.5         | 16.5 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 14.3        | 13.0               | 21.5         | 9.3*               |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 17.8        | 13.3               | 13.3         | 10.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 39.0        | 17.3*              | 26.3         | 15.5*              |
| LSD  | 22.7        | 17.7               | 17.0         | 13.8               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.28: Effect of *Pyrenochaeta lycopersici* on Number of Fruits of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 33.3        | 17.8*              | 35.0         | 12.5*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 15.8        | 7.3*               | 25.8         | 6.8*               |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 19.1        | 9.0*               | 12.0         | 7.0*               |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 21.7        | 13.5 <sup>NS</sup> | 13.8         | 8.8 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 29.0        | 13.3*              | 29.0         | 16.5*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 22.6        | 7.3*               | 11.0         | 23.0*              |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 16.0        | 8.5 <sup>NS</sup>  | 12.0         | 13.5 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 4.3         | 3.0 <sup>NS</sup>  | 3.5          | 1.8 <sup>NS</sup>  |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 7.8         | 3.3 <sup>NS</sup>  | 3.0          | 1.5 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 39.0        | 17.3*              | 21.3         | 10.5*              |
| LSD  | 15.9        | 14.8               | 15.0         | 13.1               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.29: Effect of *Pyrenochaeta lycopersici* on Weight of Fruits (g) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                     | Second trial |                    |
|--|-------------|---------------------|--------------|--------------------|
|  | Control     | Inoculated          | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 101.8       | 70.0*               | 121.6        | 81.0*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 129.7       | 63.5*               | 123.1        | 69.8*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 112.5       | 62.5*               | 89.0         | 27.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 52.8        | 39.3 <sup>NS</sup>  | 68.3         | 59.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 111.8       | 58.5*               | 133.3        | 68.0*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 77.5        | 39.0 <sup>NS</sup>  | 61.8         | 24.5 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 56.0        | 33.5 <sup>NS</sup>  | 76.0         | 25.8*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 114.2       | 83.3 <sup>NS</sup>  | 92.7         | 50.0*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 113.7       | 37.5*               | 101.0        | 30.8*              |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 156.0       | 101.3 <sup>NS</sup> | 123.8        | 67.5 <sup>NS</sup> |
| LSD  | 101.1       | 59.6                | 119.6        | 56.5               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

followed by *Capsicum chinense* cv. Avenir-hot with 2.5 in the first trial (Table 4.30). *Capsicum chinense* cv. Batassa-hot, *Capsicum chinense* cv. Avenir-hot and *Capsicum frutescens* cv. Ijosi-Bird inoculated with *Pyrenochaeta lycopersici* had the least disease severity of 2.4 and 2.5 respectively in the first trial while in the second trial they had the mean disease severity of 2.5 and 2.4 (Table 4.30).

#### **4.14.5. Effect of *Colletotrichum coccodes* on Plant Height and Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

*Capsicum frutescens* cv. sombo-Bird, *Capsicum annuum* cv Nikitta-bell and *Capsicum annuum* cv Pizzaro-bell cultivars inoculated with *C. coccodes* had significantly ( $P \leq 0.05$ ) shorter plants compared to non-inoculated plants in the first trial. In the second trial there was no significant ( $P \leq 0.05$ ) difference in the plant height of pepper plants inoculated compared to non-inoculated plants except *Capsicum chinense* cv. Avenir-hot and *Capsicum annuum* cv Nikitta-bell where the plants were taller than inoculated (Table 4.31). There was no significant ( $P \leq 0.05$ ) difference in the number of leaves produced by inoculated pepper plants compared to non-inoculated plants in the second trial. However in the first trial number of leaves produced by non-inoculated *Capsicum frutescens* cv. sombo-Bird, *Capsicum annuum* cv Nikitta-bell and *Capsicum annuum* cv Pizzaro-bell were significantly ( $P \leq 0.05$ ) more than inoculated plants (Table 4.32).

#### **4.14.6 Effect of *Colletotrichum coccodes* on Yield and Fungal Disease Severity of Ten Pepper Cultivars in First and Second Field Trials**

The number of fruits of *Capsicum annuum* cv Nikitta-bell and *Capsicum chinense* cv. Bendel-rodo inoculated with *C. coccodes* significantly ( $P \leq 0.05$ ) reduce compared to non-inoculated plants in the first trial. The number of fruits of *Capsicum annuum* cv Nikitta-bell inoculated with *C. coccodes* were significantly lower compared to uninoculated plants in the second trial (Table 4.33).

The fruit weight was significantly ( $P \leq 0.05$ ) lower in all the cultivars inoculated with *C. coccodes* compared to non-inoculated plants except for *Capsicum chinense* cv. Bendel-rodo where the weight of fruits inoculated plant was not significantly ( $P \leq 0.05$ ) different compared with the non-inoculated plants in the two trials (Table 4.34).

**Table 4.30: Effect of *Pyrenochaeta lycopersici* on Disease Severity of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |            |        | Second trial |            |        |
|--|-------------|------------|--------|--------------|------------|--------|
|  | Control     | Inoculated | Status | Control      | Inoculated | Status |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 1.0         | 4.6*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0         | 4.3*       | HS     | 1.0          | 4.0*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0         | 3.3*       | HS     | 1.0          | 3.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0         | 3.5*       | HS     | 1.0          | 3.8*       | HS     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0         | 2.5*       | MR     | 1.0          | 2.4*       | MR     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0         | 4.3*       | HS     | 1.0          | 3.8*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0         | 3.4*       | HS     | 1.0          | 2.5*       | HS     |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 1.0         | 3.5*       | HS     | 1.0          | 4.0*       | HS     |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 1.0         | 4.3*       | HS     | 1.0          | 5.0*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0         | 2.4*       | MR     | 1.0          | 2.5*       | MR     |
| LSD  | 1.0         | 2.3        |        |              | 2.4        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 =Highly susceptible (HS).

NS= Not significant; \*=Significant at  $P \leq 0.05$

**Table 4.31: Effect of *Colletotrichum coccodes* on Plant Height (cm) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 14.3        | 9.3 <sup>NS</sup>  | 19.1         | 17.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 17.0        | 15.8 <sup>NS</sup> | 11.6         | 7.0 <sup>NS</sup>  |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 21.5        | 5.8*               | 12.3         | 5.0 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 15.5        | 12.5 <sup>NS</sup> | 18.5         | 12.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 17.8        | 14.5 <sup>NS</sup> | 20.3         | 9.5*               |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 13.8        | 9.8 <sup>NS</sup>  | 15.0         | 14.3 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 21.0        | 15.3 <sup>NS</sup> | 16.3         | 12.3 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 19.5        | 2.3*               | 17.8         | 13.0*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 17.8        | 5.5*               | 14.5         | 8.3 <sup>NS</sup>  |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 17.0        | 10.3 <sup>NS</sup> | 12.3         | 11.8 <sup>NS</sup> |
| LSD  | 11.2        | 13.4               | 6.3          | 12.0               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.32: Effect of *Colletotrichum coccodes* on Number of Leaves of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 24.3        | 19.0 <sup>NS</sup> | 12.5         | 11.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 37.2        | 35.9 <sup>NS</sup> | 28.0         | 20.0 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 31.5        | 16.1*              | 32.0         | 13.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 24.5        | 17.9 <sup>NS</sup> | 24.8         | 12.3 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 27.6        | 14.8 <sup>NS</sup> | 24.0         | 17.0 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 24.8        | 17.1 <sup>NS</sup> | 24.3         | 19.5 <sup>NS</sup> |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 35.3        | 31.0 <sup>NS</sup> | 36.5         | 16.0 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 17.5        | 12.0*              | 21.5         | 12.0 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 19.3        | 11.0*              | 15.3         | 13.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 27.0        | 20.3 <sup>NS</sup> | 16.3         | 10.0 <sup>NS</sup> |
| LSD  | 22.8        | 17.7               | 17.6         | 11.9               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

**Table 4.33: Effect of *Colletotrichum coccodes* on Number of fruits (g) of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                   | Second trial |                   |
|--|-------------|-------------------|--------------|-------------------|
|  | Control     | Inoculated        | Control      | Inoculated        |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 35.0        | 9.8*              | 31.0         | 7.0*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 25.8        | 2.5*              | 17.8         | 2.0*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 20.8        | 3.5*              | 17.0         | 2.3*              |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 9.5         | 7.0 <sup>NS</sup> | 13.9         | 2.8*              |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 27.0        | 7.3*              | 29.0         | 4.8*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 19.0        | 2.5*              | 14.0         | 1.3*              |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 12.0        | 4.0*              | 13.5         | 1.5*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 6.0         | 4.3 <sup>NS</sup> | 7.3          | 3.5 <sup>NS</sup> |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 11.8        | 1.6*              | 19.0         | 3.5*              |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 35.0        | 13.8*             | 31.3         | 14.5*             |
| LSD  | 16.8        | 6.2               | 19.5         | 10.2              |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.



**Table 4.34: Effect of *Colletotrichum coccodes* on Weight (g) of Fruits of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |                    | Second trial |                    |
|--|-------------|--------------------|--------------|--------------------|
|  | Control     | Inoculated         | Control      | Inoculated         |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 96.8        | 48.8*              | 89           | 36.5*              |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 79.6        | 44.3*              | 90.0         | 46.0*              |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 112.5       | 25.3*              | 104.8        | 22.0*              |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 39.8        | 32.0 <sup>NS</sup> | 40.8         | 30.5 <sup>NS</sup> |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 97.0        | 28.3*              | 93.3         | 18.8*              |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 67.0        | 24.5*              | 15.0         | 8.5*               |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 56.0        | 19.3*              | 49.0         | 12.0*              |
| <i>Capsicum annuum</i> cv Nikitta-Bell     | 92.2        | 19.5*              | 79.3         | 26.0*              |
| <i>Capsicum annuum</i> cv Pizzaro-Bell     | 88.8        | 28.5*              | 68.4.0       | 16.8*              |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 136.0       | 72.3*              | 123.8        | 59.8*              |
| LSD  | 97.7        | 27.6               | 79.5         | 35.8               |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

NS= Not significant; \*=Significant.

The disease severity was significantly ( $P \leq 0.05$ ) higher in inoculated pepper plants compared to non-inoculated plants in all the cultivars screened in the first trial and second trial (Table 4.35). Inoculated *Capsicum chinense* cv. Batassa-hot had the least mean disease severity value of 2.4 in the first trial (Table 4.35). Disease severity mean value of inoculated *Capsicum chinense* cv. Batassa-hot was the least with 2.2 followed by Ijosi with 2.5 in the second trial (Table 4.35).

#### **4.15: The Effect of Different Plant Crude Extracts on Three Fungal Isolates of Pepper *in vitro***

##### **4.15. 1: The Effect of Plant Crude Extract on *Colletotrichum capsici in vitro***

Seed extracts of *Azadirachta indica* at 5% (5000 mg/kg) as shown in Plate 4.12a reduced the mycelial growth of *C. capsici* when compared with untreated plates (Plate 4.12b). The concentration of 10, 000 and 12,500 mg/kg of *A. indica* seed powder significantly ( $P \leq 0.05$ ) reduced the mycelial growth of *C. capsici* compared to other concentrations of 2,500 mg/kg, 5,000mg/kg and 7,500mg/kg (Table 4. 36). Mycelial inhibition of *C. capsici* was higher in plates treated with *Thevetia neriifolia* leaf extract at a concentration of 10, 000 and 12,500 mg/kg than in the other concentrations. All plates incorporated with different concentrations of *A. indica* leaf extract showed mycelial inhibition with *C. capsici*. Plates incorporated with *C. capsici* and different concentrations of *A. indica* leaf extracts reduced mycelial growth of *C. capsici* in the plates (Table 4. 36). *Tagetes erecta* root recorded mycelial reduction in plates inoculated with *C. capsici* at 5000 mg/kg (20.6%), 7,500 mg/kg (47.9%), 10,000 mg/kg (49.7%) and 12,500 mg/kg (50.9%) concentrations. The mycelial inhibition of *C. capsici* by *T. erecta* shoot at 5000, 7,500 and 10,000 mg/kg concentration was significantly ( $P \leq 0.05$ ) lower compared to the mycelial inhibition at 12,500 mg/kg (50.9%) concentration (Table 4.36). *Colletotrichum capsici* tested with *T. erecta* shoot at different concentrations reduced the mycelia growth of *C. capsici* (Figure 4.6). The mycelial reduction of *C. capsici* by *T. erecta* shoot significantly ( $P \leq 0.05$ ) reduced the mycelia growth of 5.7%) conc 12,500 mg/kg (60.1%) when compared with those of other concentrations (Table 4.36).

**Table 4.35: Effect of *Colletotrichum coccodes* on Disease Severity of Ten Pepper Cultivars in First and Second Field Trials**

| Cultivars                                  | First trial |            |        | Second trial |            |        |
|--|-------------|------------|--------|--------------|------------|--------|
|  | Control     | Inoculated | Status | Control      | Inoculated | Status |
| <i>Capsicum annuum</i> cv Tatashe-Bell     | 1.0         | 4.0*       | HS     | 1.0          | 3.5*       | HS     |
| <i>Capsicum chinense</i> cv. Bawa-Chilli   | 1.0         | 4.1*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum frutescens</i> cv. Sombo-Bird  | 1.0         | 4.5*       | HS     | 1.0          | 4.2*       | HS     |
| <i>Capsicum chinense</i> cv. Bendel-Rodo   | 1.0         | 3.3*       | HS     | 1.0          | 3.8*       | HS     |
| <i>Capsicum chinense</i> cv. Avenir-Hot    | 1.0         | 3.5*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Cameroon-Rodo | 1.0         | 3.4*       | HS     | 1.0          | 4.6*       | HS     |
| <i>Capsicum frutescens</i> cv. Ijosi-Bird  | 1.0         | 3.4*       | HS     | 1.0          | 2.5*       | HS     |
| <i>Capsicum annuum</i> cv Nikitta –Bell    | 1.0         | 4.5*       | HS     | 1.0          | 5.0*       | HS     |
| <i>Capsicum annuum</i> cv Pizzaro –Bell    | 1.0         | 4.3*       | HS     | 1.0          | 4.5*       | HS     |
| <i>Capsicum chinense</i> cv. Batassa-Hot   | 1.0         | 2.4*       | MR     | 1.0          | 2.2*       | MR     |
| LSD  | 1.0         | 2.2        |        | 1.0          | 2.0        |        |

Values are means of four replicates. Means with significant differences were separated using Fishers Least Significant Difference along the row.

Disease host assessment status- 1.0–2.0 = Resistant (R); 2.1-2.5 = Moderately Resistant (MR); 2.6–3.0= Susceptible (S), and >3.0 =Highly susceptible (HS).

NS= Not significant; \*=Significant at  $P \leq 0.05$

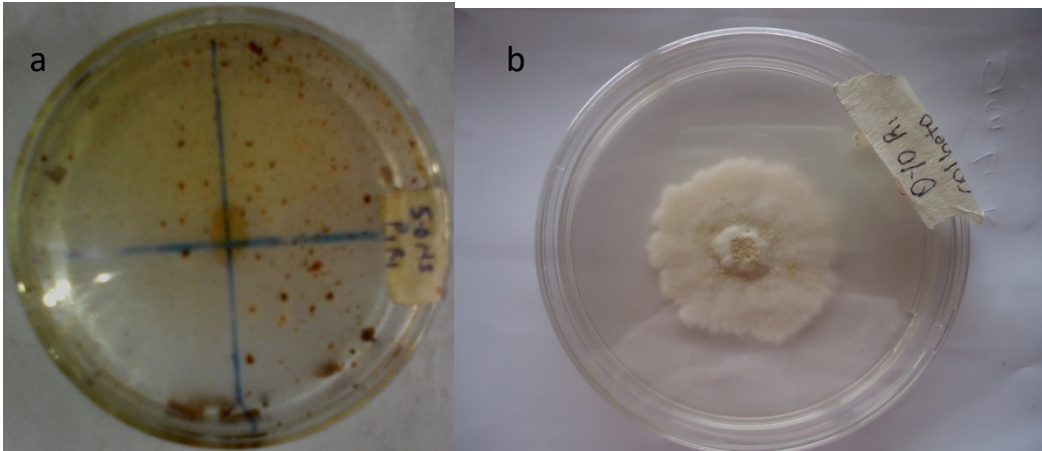


Plate 4. 12: Growth inhibition of *Colletotrichum capsici* on PDA media incorporated with *A. indica* seed extract at 5,000 mg/kg concentration (a), *Colletotrichum capsici* on PDA media seven days after Inoculation (b).

**Table 4. 36: *In vitro* Growth Inhibition of *Colletotrichum capsici* by Plant Crude Extracts**

| Plant Species                    | Concentrations (mg/kg) | (%) Mycelial Inhibition |
|----------------------------------|------------------------|-------------------------|
| <i>Azadirachta indica</i> (seed) | 0.0                    | 0.0c                    |
|                                  | 2,500                  | 11.6b                   |
|                                  | 5,000                  | 28.1a                   |
|                                  | 7,500                  | 33.1a                   |
|                                  | 10,000                 | 31.9a                   |
|                                  | 12,000                 | 31.9a                   |
|                                  | Fungicide              | 5.7c                    |
| <i>Azadirachta indica</i> (leaf) | 0.0                    | 0.0b                    |
|                                  | 2,500                  | 27.0a                   |
|                                  | 5,000                  | 34.9a                   |
|                                  | 7,500                  | 24.5a                   |
|                                  | 10,000                 | 24.7a                   |
|                                  | 12,000                 | 26.5a                   |
|                                  | Fungicide              | 5.7b                    |
| <i>Tagetes erecta</i> (root)     | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 0.0d                    |
|                                  | 5,000                  | 4.4cd                   |
|                                  | 7,500                  | 9.1bc                   |
|                                  | 10,000                 | 14.1ab                  |
|                                  | 12,000                 | 15.7ab                  |
|                                  | Fungicide              | 5.7cd                   |
| <i>Tagetes erecta</i> (shoot)    | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 9.2bc                   |
|                                  | 5,000                  | 14.3ab                  |
|                                  | 7,500                  | 16.6ab                  |
|                                  | 10,000                 | 16.6ab                  |

|  |           |       |
|--|-----------|-------|
|  | 12,000    | 60.1a |
|  | Fungicide | 5.7cd |
| <b><i>Thevetia neriifolia</i> (leaves)</b> | 0.0       | 0.0d  |
|  | 2,500     | 2.8c  |
|  | 5,000     | 32.2b |
|  | 7,500     | 57.9a |
|  | 10,000    | 67.1a |
|  | 12,000    | 73.3a |
|  | Fungicide | 5.7cd |

Values are means of four replicates. Values with the same letter are not significantly different along column at  $P \leq 0.05$  level of significance using DMRT. %inhibition of mycelia growth =  $\frac{Dc-Dt}{Dc} \times 100$

Dc

Where, Dc= diameter of control; Dt= diameter of test of pathogens

The leaf extract of *T. neriifolia* at different concentrations reduced the mycelial growth of *C. capsici* (Plate 4. 13). The concentrations of *T. neriifolia* at 12,500 mg/kg significantly ( $P \leq 0.05$ ) reduced the mycelial growth of *C. capsici* compared with control (0.0 mg/kg) (Table 4.36).

#### 4.15. 2: The Effect of Plant Crude Extract on *Pyrenochaeta lycopersici* in vitro

The maximum inhibition was obtained when *P. lycopersici* was exposed to 12,500 mg/kg (12.5 g) per 100 ml of water extract of *A.indica* seed which was similar to those inoculated with 10,000 mg/kg concentration (Table 4.37). There was no significant difference in the concentration of 12,500 mg/kg when compared with 10,000 mg/kg. The mycelia growth of *P. lycopersici* in the concentrations of 12,500 and 10,000 mg/kg were significantly ( $P \leq 0.05$ ) reduced compared to other concentrations of 2,500, 5,000 and 7,500 mg/kg (Table 4.37).

All concentrations of *A. indica* leaf extracts inhibited the mycelial growth of *P. lycopersici* (Table 4.37). Plate 4.14 showed the inhibition of *P. lycopersici* incorporated with *A. indica* leaf extract at the concentration of 7,500 mg/kg. The mycelial inhibition of *P. lycopersici* incorporated with *A.indica* leaf at different concentrations was significantly ( $P \leq 0.05$ ) higher compared with fungicide treated plates (Table 4. 37). The effect of *T. erecta* root extract at different concentrations significantly ( $P \leq 0.05$ ) reduced mycelial of *P. lycopersici* compared with non-treated plates (Table 4.37). Plates incorporated with 12,500 mg/kg of *T. erecta* shoot recorded the highest mycelial reduction of *P. lycopersici* (Table 4.37). Mycelial inhibition of *P. lycopersici* caused by 12,500 mg/kg were significantly higher compared with other concentrations. Plate 4.15 showed the inhibition of *P. lycopersici* incorporated with *T. erecta* shoot at the concentration of 7,500 mg/kg. The inhibition of *C. coccodes* incorporated with *A. indica* seed extracts at the lowest concentration (2,500 mg/kg) reduced the mycelial growth in plates (Plate 4.16). There was no significant ( $P \leq 0.05$ ) difference in mycelial inhibition of *C. coccodes* in plates inoculated with 12,500 and 10,000 mg/kg *A. indica* seed extracts compared with 7,500 and 5,000 mg/kg.

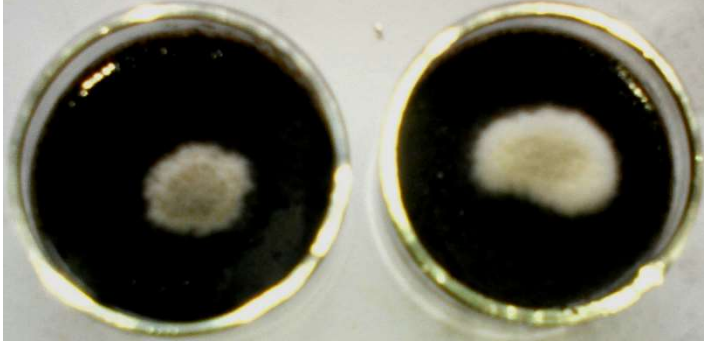


Plate 4.:13 : Growth inhibition by *Colletotrichum capsici* on PDA media incorporated with *T. neriifolia* leaf extract at concentrations of 12,500 mg/kg



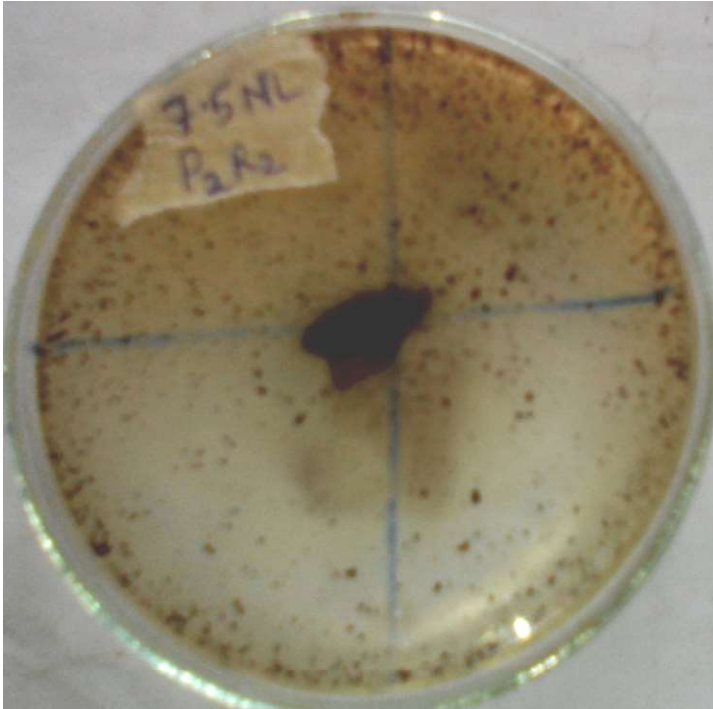


Plate 4.14: Growth inhibition of *Pyrenochaeta lycopersici* on PDA media incorporated with *A. indica* leaf plant extracts at 7,500 mg/kg concentration at seven days after Inoculation

**Table 4. 37: *In vitro* Growth Inhibition of *Pyrenochaeta lycopersici* by Plant Crude Extracts**

| Plant Species                    | Concentrations (mg/kg) | (%) Mycelial Inhibition |
|----------------------------------|------------------------|-------------------------|
| <i>Azadirachta indica</i> (seed) | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 0.0d                    |
|                                  | 5,000                  | 0.0d                    |
|                                  | 7,500                  | 0.0d                    |
|                                  | 10,000                 | 20.6b                   |
|                                  | 12,000                 | 26.4b                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Azadirachta indica</i> (leaf) | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 44.4a                   |
|                                  | 5,000                  | 46.7a                   |
|                                  | 7,500                  | 47.4a                   |
|                                  | 10,000                 | 49.9a                   |
|                                  | 12,000                 | 50.9a                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Tagetes erecta</i> (root)     | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 14.4bc                  |
|                                  | 5,000                  | 20.6b                   |
|                                  | 7,500                  | 47.9a                   |
|                                  | 10,000                 | 49.6a                   |
|                                  | 12,000                 | 51.3a                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Tagetes erecta</i> (shoot)    | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 10.4c                   |
|                                  | 5,000                  | 11.9c                   |
|                                  | 7,500                  | 11.3c                   |
|                                  | 10,000                 | 11.9c                   |

|                                      |           |        |
|--------------------------------------|-----------|--------|
|                                      | 12,000    | 22.1b  |
|                                      | Fungicide | 5.7d   |
| <i>Thevetia neriiifolia</i> (leaves) | 0.0       | 0.0d   |
|                                      | 2,500     | 17.4bc |
|                                      | 5,000     | 59.8a  |
|                                      | 7,500     | 40.5a  |
|                                      | 10,000    | 41.3a  |
|                                      | 12,000    | 44.4a  |
|                                      | Fungicide | 5.7d   |

Values are means of four replicates. Values with the same letter are not significantly different along column at  $P \leq 0.05$  level of significance using DMRT. %inhibition of mycelia growth =  $\frac{Dc-Dt}{Dc} \times 100$

Dc

Where, Dc= diameter of control; Dt= diameter of test of pathogens

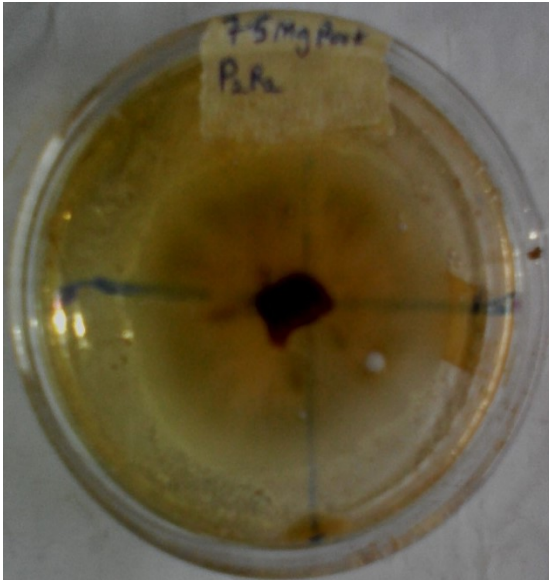


Plate 4:15 : Growth inhibition of *Pyrenochaeta lycopersici* on PDA media incorporated with *Tagetes erecta* shoot extract at concentrations of 7,500 mg/kg

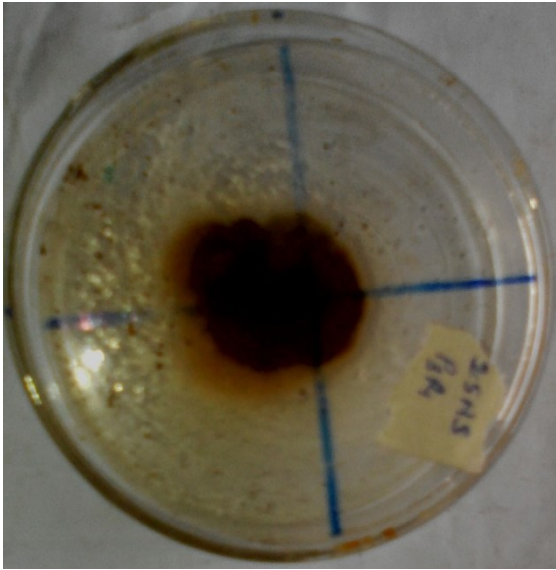


Plate 4.16: Growth inhibition of *Colletotrichum coccodes* on PDA media incorporated with *A. indica* seed extracts at 2,500 mg/kg concentration (b) seven days after Inoculation

The mycelial inhibition of *P. lycopersici* by *T. neriifolia* at the concentration of 12,500 mg/kg was significantly reduced on Petri-plates. The different concentrations of *T. neriifolia* inhibited the mycelial growth of *P. lycopersici*. There was no significant difference in plates incorporated with 0.5 g fungicide compared uninoculated plates (Table 4.37).

#### **4.15. 3: The Effect of Plant Crude Extracts on *Colletotrichum coccodes* in vitro**

The mycelial inhibition of *C. coccodes* was significantly higher in plates incorporated with 2,500 mg/kg *A. indica* seed extracts compared to plates without extracts. *Azadirachta indica* leaf extract at 10,000 mg/kg significantly reduced the mycelial growth of *C. coccodes* and this was similar with plates treated with 12,500 mg/kg concentrations (Table 4.38). There was no significant ( $P \leq 0.05$ ) difference in the mycelial inhibition of *C. coccodes* in all the concentrations of *A. indica* seed extracts treated plates except for plates incorporated with 2,500 mg/kg seed extract. The mycelial inhibition of *C. coccodes* was significantly higher in plates incorporated with 12,500 mg/kg *A. indica* seed extract compared tonon-incorporated plates

The mycelial inhibition of *C. coccodes* by *T. erecta* root and shoot were significantly higher in treated plates compared with untreated plates (Table 4.38). The mycelial inhibition of *C. coccodes* by *T. neriifolia* were significantly higher in treated plates compared with untreated plates (Table 4.38).

#### **4.16 : The Effect of Bio-control Agents on Fungal Pathogens of Pepper in vitro**

The effect of *Bacillus subtilis*, *Trichoderma harzianum* and *Trichoderma pseudokoningii* on *Colletotrichum coccodes*, *Colletotrichum capsici* and *Pyrenochaeta lycopersici* are shown in Table 4.39. Inhibition of *C. capsici* by *B. subtilis* was significantly ( $P \leq 0.05$ ) higher compared with the control (without bio-agent). There were significant differences in plates inoculated with *B. subtilis* + *C. capsici* compared with those inoculated with *C. capsici* alone. *C. coccodes* and *P. lycopersici* showed some level of growth inhibition by *T. harzianum* while *C. capsici* did not show any level of inhibition (Table 4.39). There was no significant ( $P \leq 0.05$ ) difference between the plates inoculated with *C. coccodes* + *B. subtilis* compared with the control plates

**Table 4 .38: *In vitro* Growth Inhibition of *Colletotrichum coccodes* by Plant Crude Extracts**

| Plant Species                    | Concentrations (mg/kg) | (%) Mycelial Inhibition |
|----------------------------------|------------------------|-------------------------|
| <i>Azadirachta indica</i> (seed) | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 11.6c                   |
|                                  | 5,000                  | 28.1b                   |
|                                  | 7,500                  | 31.9b                   |
|                                  | 10,000                 | 33.1b                   |
|                                  | 12,000                 | 33.1b                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Azadirachta indica</i> (leaf) | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 43.9ab                  |
|                                  | 5,000                  | 44.5ab                  |
|                                  | 7,500                  | 45.4ab                  |
|                                  | 10,000                 | 57.8a                   |
|                                  | 12,000                 | 56.3a                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Tagetes erecta</i> (root)     | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 68.3a                   |
|                                  | 5,000                  | 69.8a                   |
|                                  | 7,500                  | 77.8a                   |
|                                  | 10,000                 | 79.4a                   |
|                                  | 12,000                 | 79.4a                   |
|                                  | Fungicide              | 5.7d                    |
| <i>Tagetes erecta</i> (shoot)    | 0.0                    | 0.0d                    |
|                                  | 2,500                  | 31.8b                   |
|                                  | 5,000                  | 31.5b                   |
|                                  | 7,500                  | 61.6a                   |
|                                  | 10,000                 | 60.0a                   |

|                                      |           |       |
|--------------------------------------|-----------|-------|
|                                      | 12,000    | 64.0a |
|                                      | Fungicide | 5.7d  |
| <i>Thevetia neriiifolia</i> (leaves) | 0.0       | 0.0d  |
|                                      | 2,500     | 8.4cd |
|                                      | 5,000     | 8.9cd |
|                                      | 7,500     | 14.6c |
|                                      | 10,000    | 15.2c |
|                                      | 12,000    | 16.1c |
|                                      | Fungicide | 5.7d  |

Values are means of four replicates. Values with the same letter are not significantly different along column at  $P \leq 0.05$  level of significance using DMRT. %inhibition of mycelia growth =  $\frac{Dc-Dt}{Dc} \times 100$

Dc

Where, Dc= diameter of control; Dt= diameter of test of pathogens



**Table 4. 39: Growth Inhibition of Bio-control Agents on Pepper Fungal Pathogens *in vitro* using Slide Technique**

| Treatments                                       | % Mycelial Inhibition |
|--|-----------------------|
| <i>C. capsici</i> + <i>T. pseudokoningii</i>     | 95.2b(1.98)           |
| <i>C. capsici</i> + <i>T. harzianum</i>          | 0.0e(1.00)            |
| <i>C. capsici</i> + <i>B. subtilis</i>           | 92.5c(1.98)           |
| <i>C. capsici</i> + PDA                          | 0.0e(1.00)            |
| <i>P. lycopersici</i> + <i>T. pseudokoningii</i> | 96.7a(1.99)           |
| <i>P. lycopersici</i> + <i>T. harzianum</i>      | 96.3a(1.99)           |
| <i>P. lycopersici</i> + <i>B. subtilis</i>       | 0.0e(1.00)            |
| <i>P. lycopersici</i> + PDA                      | 0.0e(1.00)            |
| <i>C.coccodes</i> + <i>T. pseudokoningii</i>     | 94.4b(1.98)           |
| <i>C.coccodes</i> + <i>T. harzianum</i>          | 91.3d(1.90)           |
| <i>C.coccodes</i> + <i>B. subtilis</i>           | 0.0e(1.00)            |
| <i>C.coccodes</i> +PDA                           | 0.0e(1.00)            |

Values are means of four replicates. Means were separated by using Duncan Multiple Range Test (DMRT) at P≤0.05 level of probability. Values in parenthesis are transformed

$$\text{Mycelial Inhibition} = \frac{\text{Inhibition Zone}}{\text{Distance between pathogen-bioagent disc}} \times 100$$

(uninoculated). Zone of inhibition of *C. coccodes* on *T. harzianum* was 91.3% while *P. lycopersici* + *T. harzianum* caused inhibition of 96.4%. The inhibition zone of *C. coccodes* + *T. harzianum* was significantly ( $P \leq 0.05$ ) higher compared with control (without bio-agent) (Table 4.39). The zone of inhibition by *T. pseudokoningii* was significantly ( $P \leq 0.05$ ) higher with the three pathogens; *C. coccodes* (94.5), *C. capsici* (95.2) and *P. lycopersici* (96.7) compared with their control (0.0) (Table 4.39). *Colletotrichum coccodes* and *P. lycopersici* showed hyperparasitic interaction to the treatment of *T. pseudokoningii* (Plate 4.17a&b) while there was hyperparasitic interaction between of *C. capsici* by *T. pseudokoningii* (Plate 4.17c)

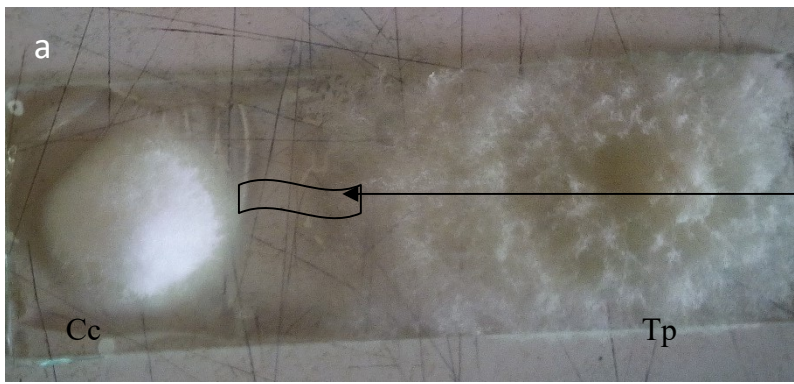
#### **4.17. Growth Parameters, Fungal Disease Severity and Yield of Three Fungal Isolates on Pepper Plants Treated with Biopesticides in the Field**

##### **4.17.1 Effect of Biopesticides on Plant Height and Number of Leaves of Pepper Plants Inoculated with *Colletotrichum capsici* in Field**

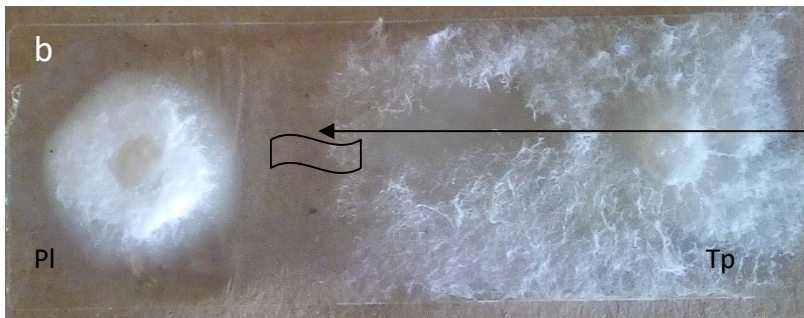
The non-inoculated plants were the tallest plants (32.0 cm) compared with other treatments (Figure 4.5). Inoculated pepper plants treated with *A. indica* leaf extracts at the concentration of 2.5 g per 100 mL of water with the mean plant height of 23.2 cm were significantly ( $P \leq 0.05$ ) taller compared to plants treated with other plant extracts except for the non-inoculated plants where the plant height was taller. The number of leaves produced by non-inoculated plants (26.2) were more compared with the other treatments (Figure 4.5). Plants treated with *A. indica* leaf and seed extracts had the highest plant height and number of leaves (Figure 4.5).

##### **4.17.2: Effect of Biopesticides on Number of Fruits and Fruit Weight of Pepper Plants Inoculated with *Colletotrichum capsici* in Field**

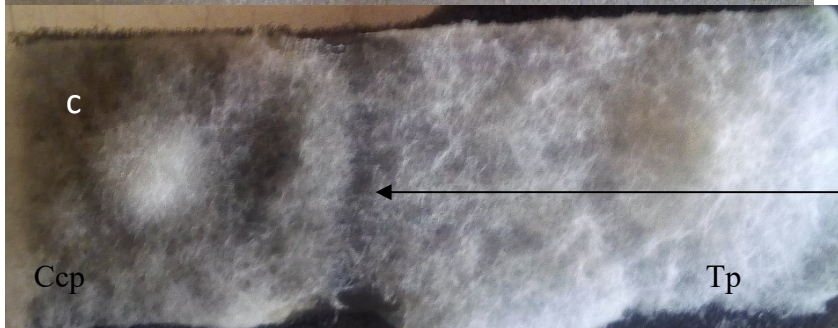
The plants treated with *T. nerifolia* leaf extract had the highest number of fruits (38.6) compared with other treatments (Figure 4.6). Plants treated with *T. erecta* root extract at the concentration of 7.5 g significantly ( $P \leq 0.05$ ) produced more fruits compared with plants treated with *T. erecta* roots at 2.5 g. Fruit weight was highest with plants treated with *A. indica* leaf extracts (161.3 g) compared with the other treatments (Figure 4.6). The incorporation of *A. indica* leaf extract significantly ( $P \leq 0.05$ ) increased



Hyperparasitism between *C. coccodes* (Cc) and *T. pseudokoningii* (Tp)

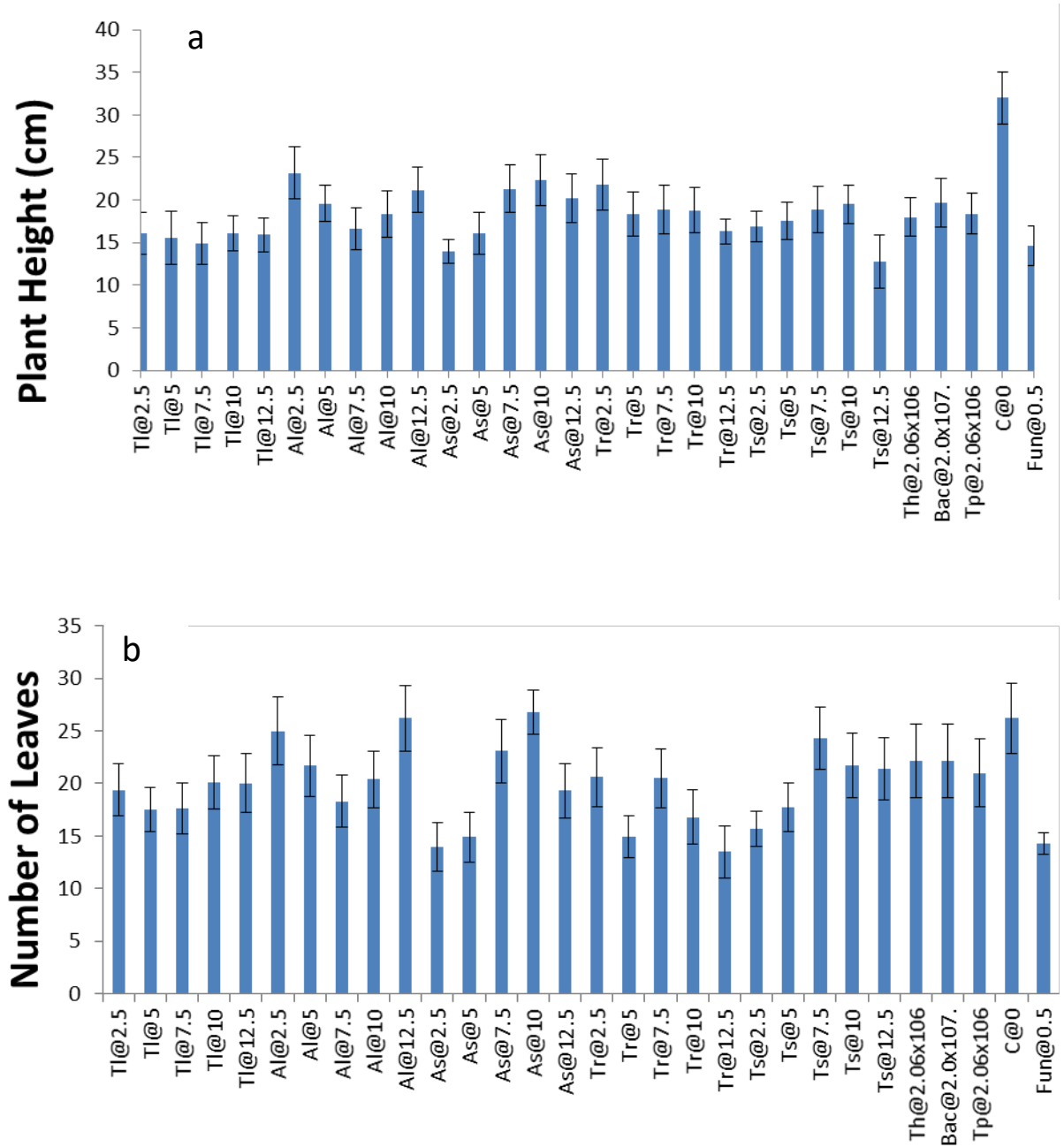


Hyperparasitism between *P. lycopersici* (Pl) and *T. pseudokoningii* (Tp)



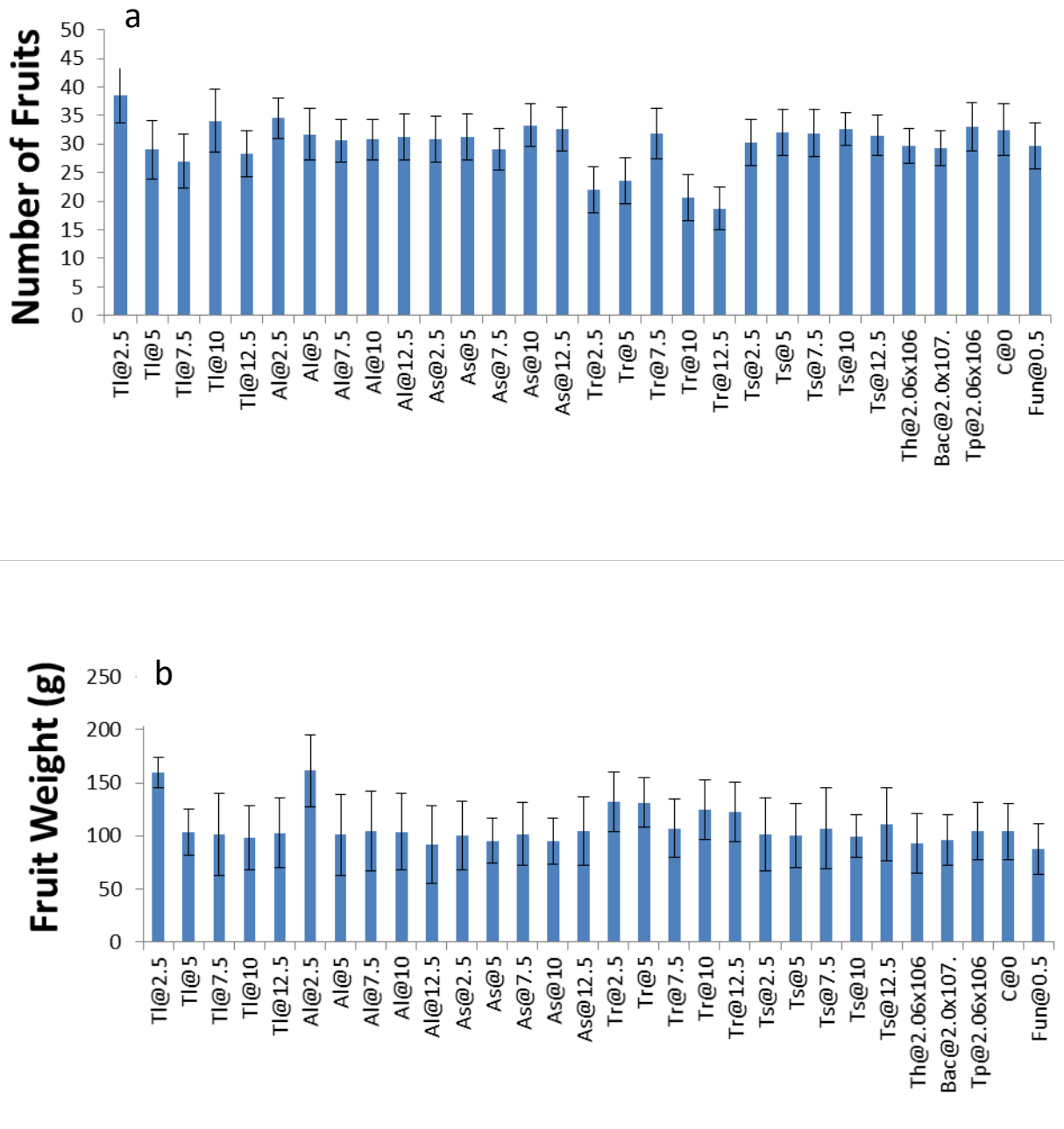
Hyperparasitism between *C. capsici* (Ccp) and *T. pseudokoningii* (Tp)

Plate 4.17: Growth inhibitions between (a) *C. coccodes* (b) *P. lycopersici* and (c) *C. capsici* and *Trichoderma pseudokoningii* in a dual culture 7 days after incubation



**Figure 4.5: Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants Inoculated with *Colletotrichum capsici* on the Field**

Values are means of four replicates. Bars are standard error. *Thevetia neriifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C



**Figure 4.6: Effect of Biopesticides on Number of Fruits (a) and Weight of fruits (b) of Pepper Plants Inoculated with *Colletotrichum capsici* on the Field**

Values are means of four replicates. Bars are standard error. *Thevetia neriifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C

the weight of fruits compared to other treatments (Figure 4.6). There was no significant ( $P \leq 0.05$ ) difference in fruit weight of plants treated with the bio agents compared with non-inoculated plants.

#### **4.17.3: Effect of Biopesticides on Disease Severity of Pepper Plants Inoculated with *Colletotrichum capsici* in Field**

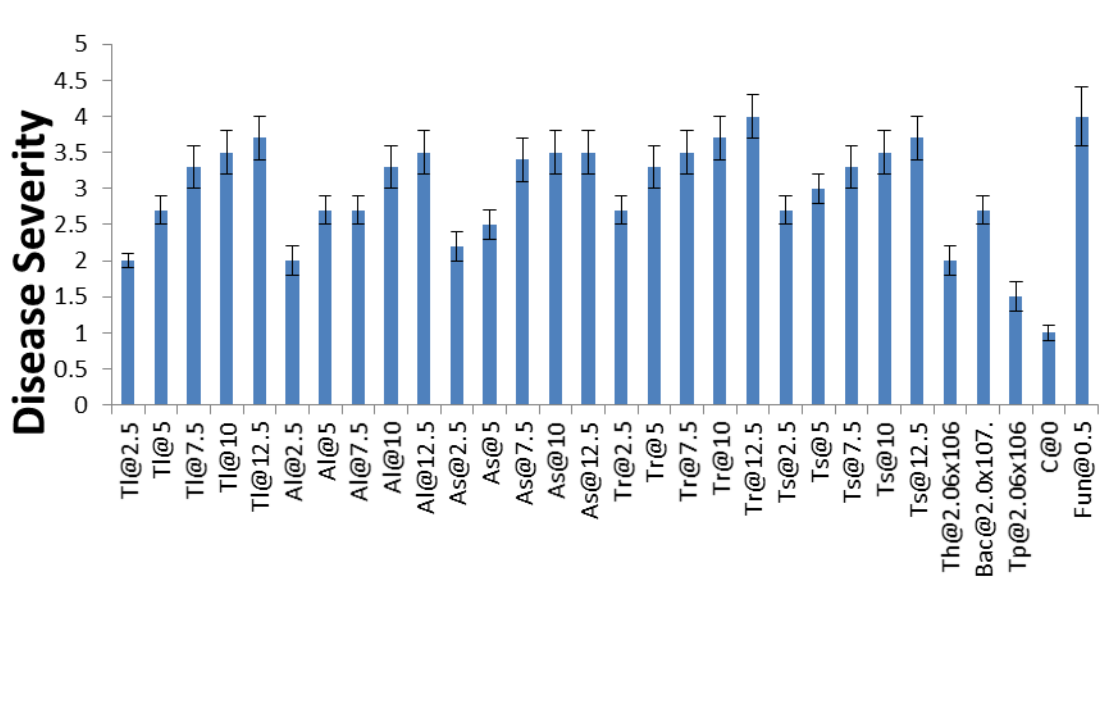
The fungal disease severity of pepper plants treated with fungicide at 0.5 g per 100 mL of water had the highest disease severity (4.0) compared with other treatments (Figure 4.7). The plants treated with *B. subtilis* significantly ( $P \leq 0.05$ ) had higher disease severity compared to plants treated with *T. harzianum* and *T. pseudokoninigi* (Figure 4.7).

#### **4.17.4 Effect of Biopesticides on Plant Height and Number of Leaves of Pepper Plants Inoculated with *Pyrenochaetalycopersici* in Field**

Plants treated with *A. indica* leaf extracts had the highest mean (23.0 cm) compared with other treatments (Figure 4.8). Plants treated with *A. indica* leaf extract at the concentration of 2.5 g were significantly ( $P \leq 0.05$ ) taller compared to plant treated with other extracts. The number of leaves produced by plants treated with *A. indica* leaf extract at the concentration of 2.5 g were greater compared with the other treatments (Figure 4.8). The plants treated with *A. indica* leaf extract had more leaves with the mean value of 47.0 followed by plants treated with *T. erecta* shoot extract (34.6) (Figure 4.8).

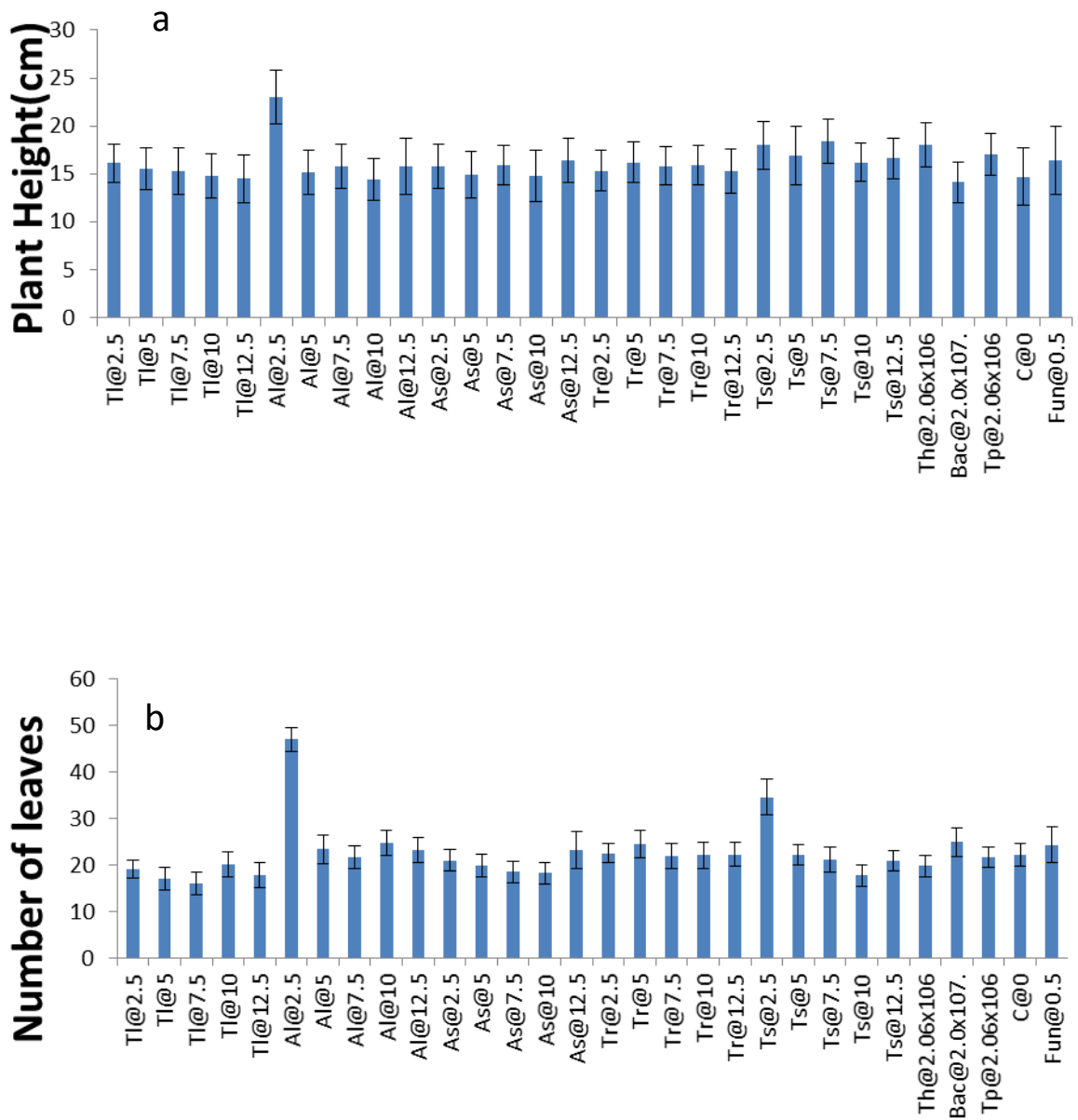
#### **4.17.5: Effect of Biopesticides on Number of Fruits and Weight of Fruits of Pepper Plants Inoculated with *Pyrenochaetalycopersici* in Field**

The inoculated plants treated with *T. erecta* shoot extracts at the concentration of 2.5 g significantly ( $P \leq 0.05$ ) produced more fruits compared with other concentrations (Figure 4.9). Plants treated with *T. erecta* root extracts at 12.5 g significantly ( $P \leq 0.05$ ) produce more fruits compared to *T. erecta* shoot extracts at 2.5 g. There was no significant ( $P \leq 0.05$ ) difference in the number of fruits produced by plants treated with biocontrol agents compared with non-inoculated plants. Fruit weight of plant treated with *Azadirachta indica* seed extract at the concentration of 2.5 g was significantly ( $P \leq 0.05$ ) higher compared to plant treated with *Tagetes erecta* shoot extracts at the concentration of 2.5 g (Figure 4.9).



**Figure 4.7: Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with *Colletotrichum capsici* in the Field**

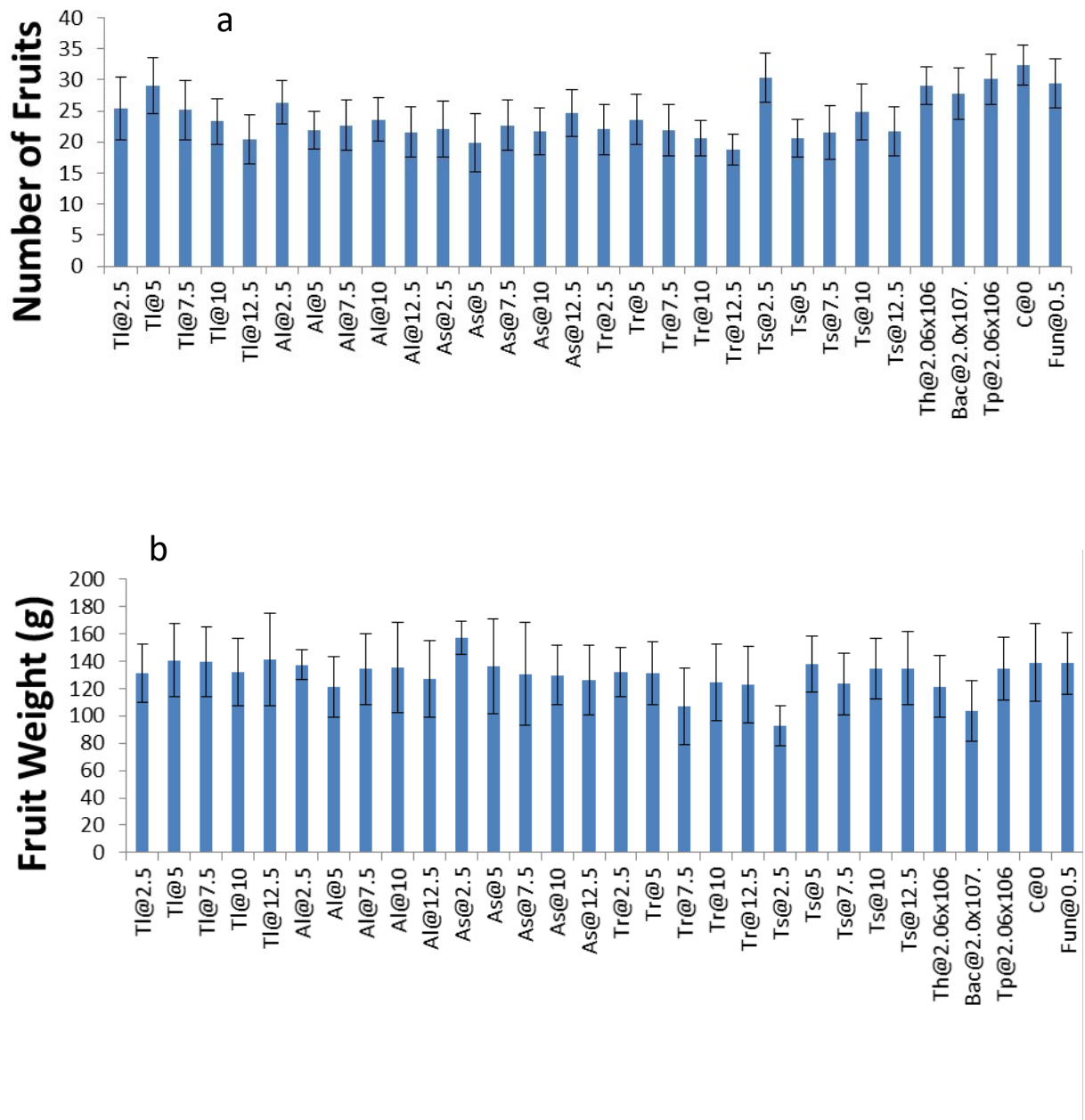
Values are means of four replicates. Bars are standard error. *Thevetia neriifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C



**Figure 4.8: Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants Inoculated with *P. lycopersici* in the Field**

Values are means of four replicates. Bars are standard error. *Thevetia nerifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*-Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract -Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C





**Figure 4.9: Effect of Biopesticides on Number of Fruits (a) and Fruit Weight (b) of Pepper Plants Inoculated with *P. lycopersici* in the Field**

Values are means of four replicates. Bars are standard error. *Thevetia neriifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C

#### **4.17.6: Effect of Biopesticides on Fungal Disease Severity of Pepper Plants**

##### **Inoculated with *Pyrenochaeta lycopersici* in Field**

The fungal disease severity of pepper plants treated with fungicide at 0.5 g per 100 mL of water had the highest disease severity of 4.5 compared with other treatments (Figure 4.10). The plants treated with *T.pseudokoningii* significantly ( $P \leq 0.05$ ) lowered disease severity compared to plants treated with *T.harzianum* and *B. subtilis* (Figure 4.10).

#### **4.17.7: Effect of Biopesticides on Plant Height and Number of Leaves of Pepper**

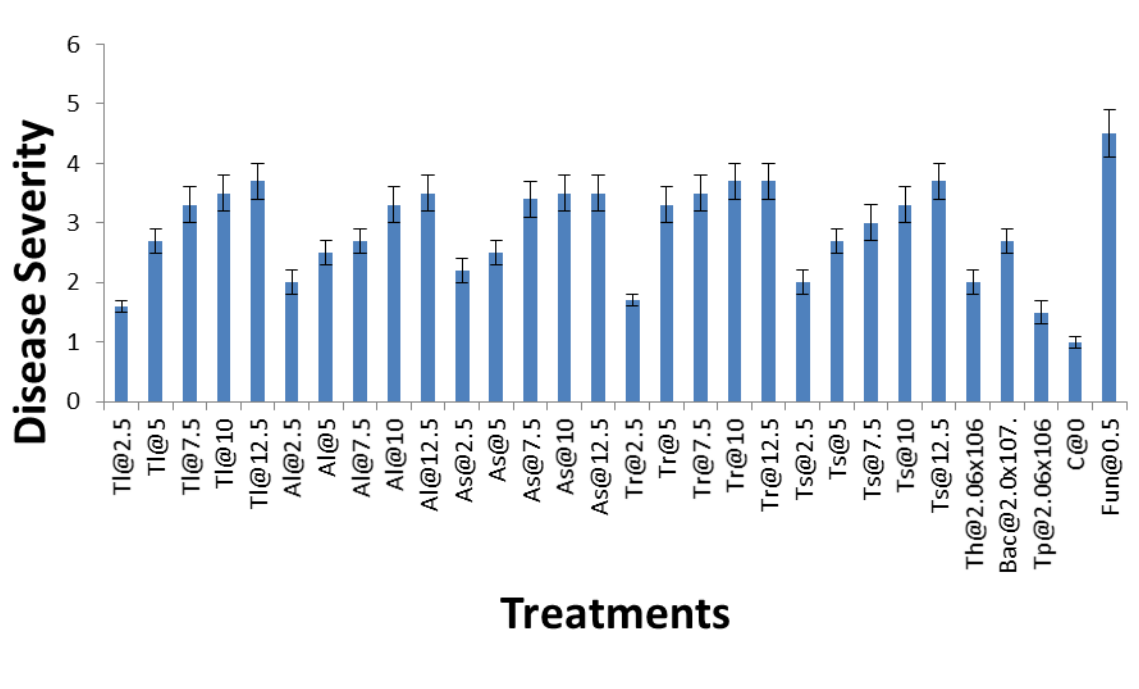
##### **Plants Inoculated with *Colletotrichum coccodes* in Field**

The plants treated with *A. indica* seed extracts at the concentration of 2.5 g was significantly ( $P \leq 0.05$ ) taller compared with *T. erecta* root extract at 10.0 g and 12.5 g concentrations (Figure 4.11). There was no significant ( $P \leq 0.05$ ) difference in plants treated with *A. indica* leaf extracts at the concentration of 2.5 g compared with other treatments. The number of leaves produced by plants treated with *A. indica* seed extracts were significantly ( $P \leq 0.05$ ) more compared with the other treatments (Figure 4.11). The number of leaves of inoculated plants treated with *A. indica* seed extract at the concentration of 5.0 g significantly ( $P \leq 0.05$ ) produced more leaves compared to *A. indica* leaf extract at the concentration of 10.0 g (Figure 4.11).

#### **4.17.8: Effect of Biopesticides on Number of Fruits and Weight of Fruits of Pepper**

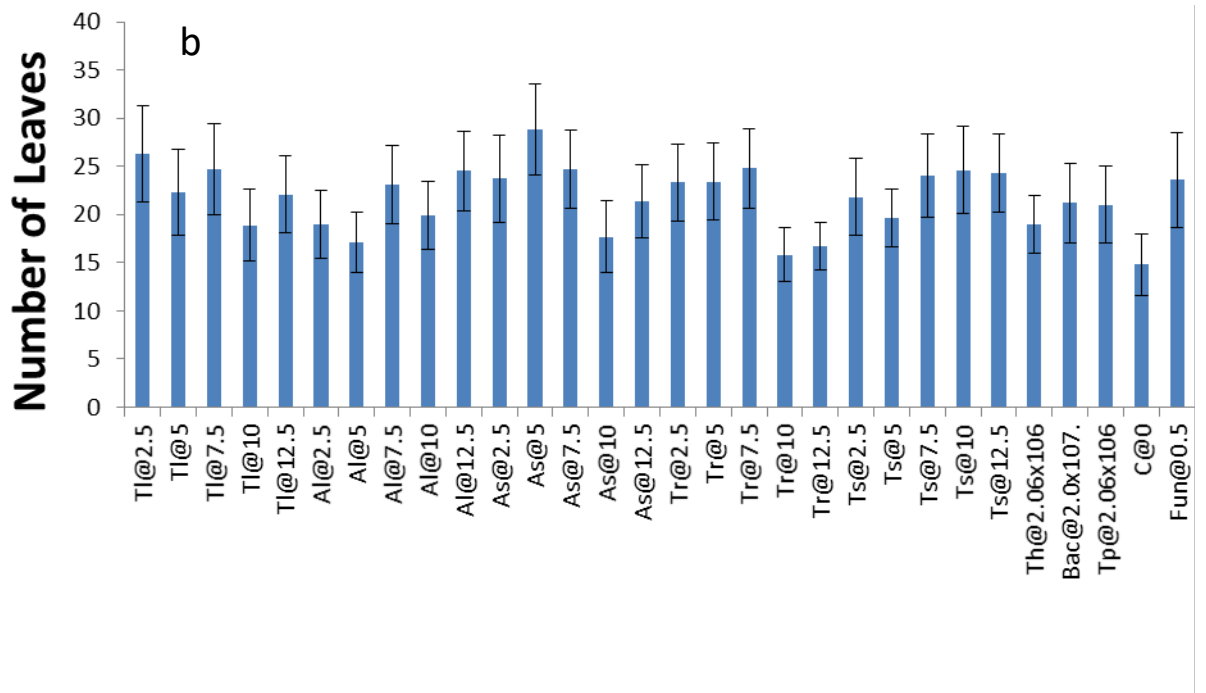
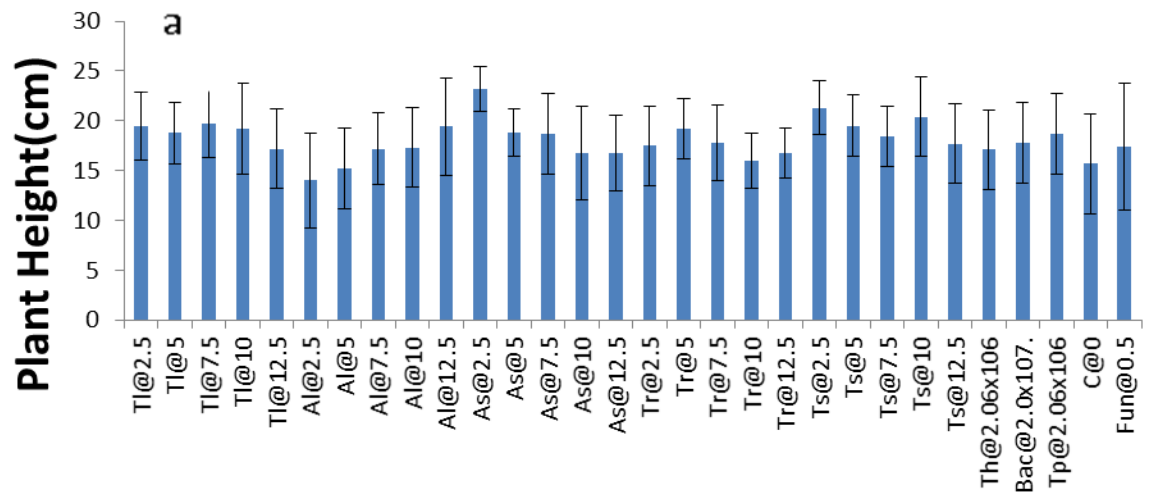
##### **Plants Inoculated with *Colletotrichum coccodes* in Field**

The plants treated with *A. indica* leaf extracts had the highest mean number of fruits (34.1) compared with other treatments (Figure 4.12). There was no significant ( $P \leq 0.05$ ) difference in the number of fruit produced among all the treatments. The weight of fruits of plants treated with *Azadirachta indica* leaf extracts at the concentration of 2.5 g was significantly ( $P \leq 0.05$ ) higher compared to weight of plant of other treatments (Figure 4.12).

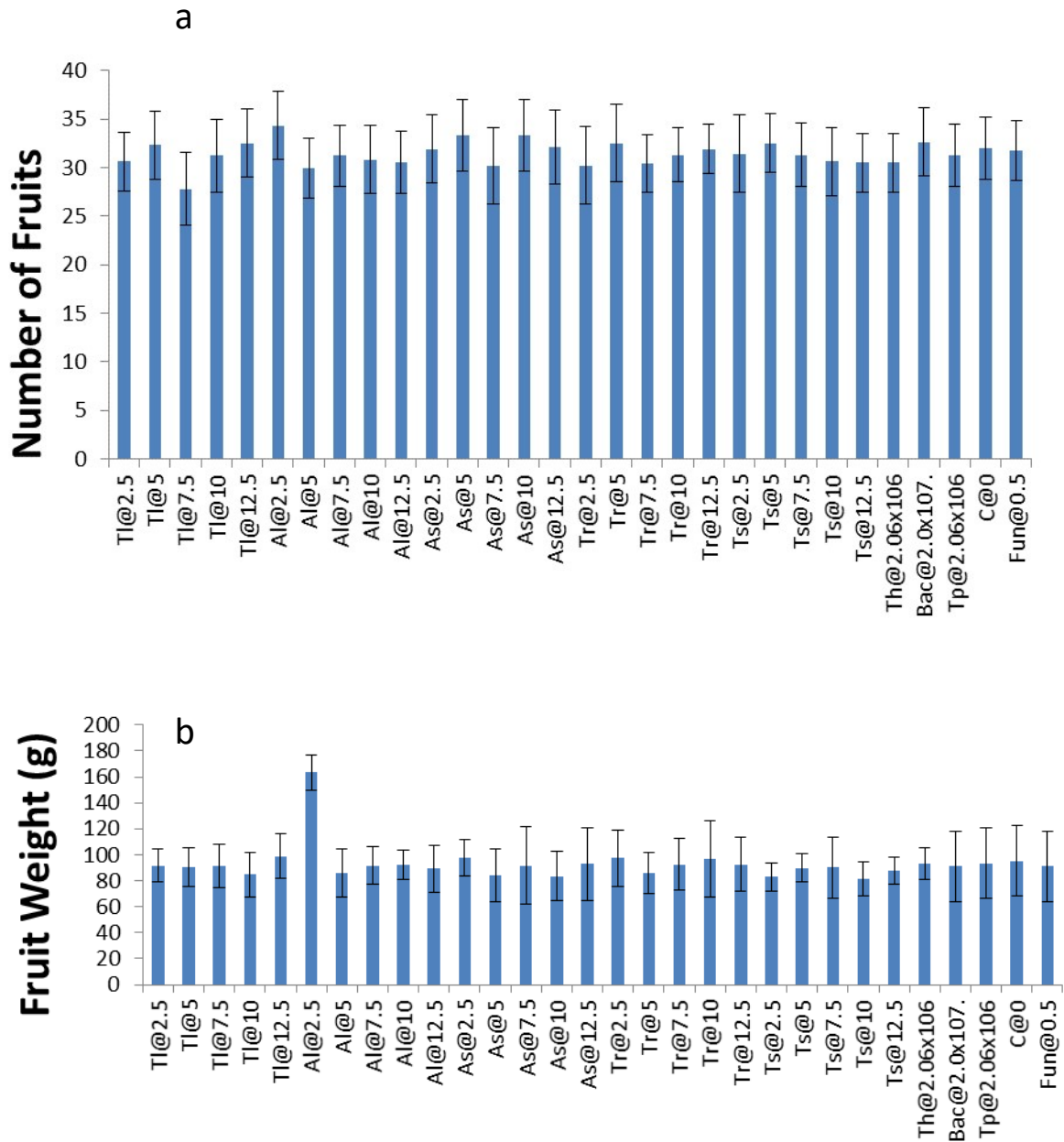


**Figure 4.10: Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with *P. lycopersici* in the Field**

Values are means of four replicates. Bars are standard error. *Thevetia neriifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*- Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C



**Figure 4.11: Effect of Biopesticides on Plant Height (a) and Number of Leaves (b) of Pepper Plants of Pepper Plants Inoculated with *Colletotrichum coccodes* on the Field**  
 Values are means of four replicates. Bars are standard error. *Thevetia nerifolia*-TI, *Azadirachta indica* leaf extract-AI, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C



**Figure 4.12: Effect of Biopesticides on Number of Fruits (a) and Fruit Weight (b) of Pepper Plants Inoculated with *Colletotrichum coccodes* on the Field**

Values are means of four replicates. Bars are standard error. *Thevetia nerifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*-Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C

#### **4.17.9: Effect of Biopesticides on Fungal Disease Severity of Pepper Plants**

##### **Inoculated with *Colletotrichum coccodes* in Field**

The fungal disease severity of pepper plants treated with fungicide at 0.5 g per 100 mL of water had the highest disease severity (5.0) compared with other treatments (Figure 4.13). The plants treated with *T.pseudokoningii* significantly ( $P \leq 0.05$ ) lowered disease severity compared to plants treated with *T.harzianum* and *B. subtilis* (Figure 4.13).

#### **4.18. Quantitative composition of Phytochemicals in *Azadirachta indica* seed, *Tagetes erecta* shoot, *Azadirachta indica* leaf, *Tagetes erecta* root and *Thevetia neriifolia* leaf**

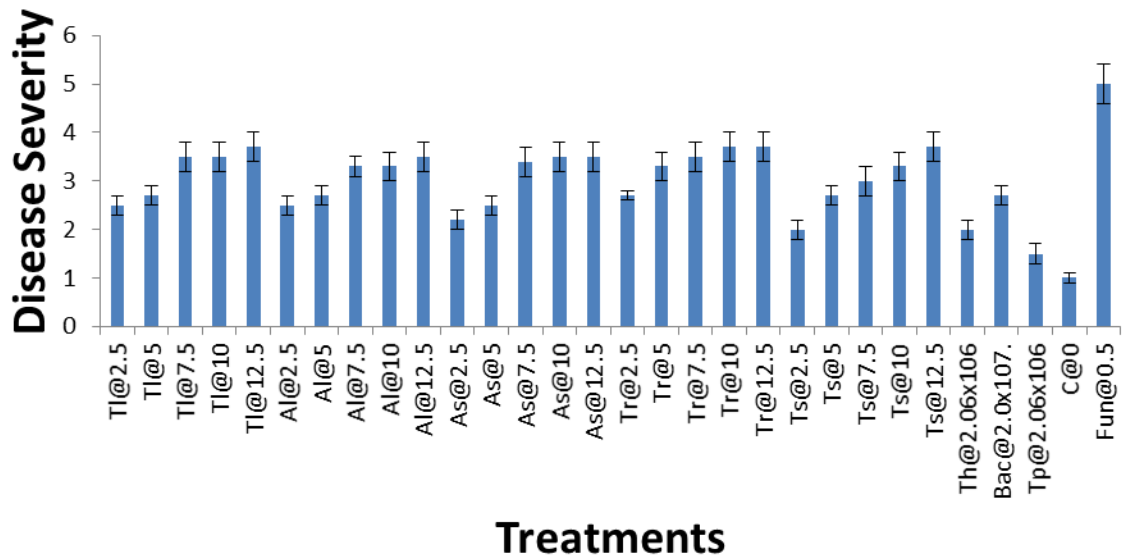
The quantity of selected phytochemicals (phenols, flavonoids, tannins, alkaloids and saponins) present are shown in Table 4.40. There was variation in the concentrations of phytochemicals across the plant parts screened.

*Azadirachta indica* leaves had the highest total phenol (64.5 mg/100 g) amongst all the plant extracts screened and this value was significantly ( $P \leq 0.05$ ) higher than the mean phenols of the other plant parts.

The quantity of flavonoids in *A. indica* seeds (541.6 mg/100 g) was significantly ( $P \leq 0.05$ ) higher when compared with the flavonoids in the other plant extracts namely; *A. indica* leaves (275.0 mg/100 g), *T. erecta* shoot (281.6 mg/100 g) and *T. erecta* roots (191.7mg/100 g). The least quantity of flavonoids was found in *T.neriifolia* leaves (128.3mg/100 g).

*Tagetes erecta* shoot had the highest quantity of tannin (1135 mg/100g) compared to the other extracts; *A. indica* leaves(653.3 mg/100g), *A. indica* seeds (480.0 mg/100 g), and *T. erecta* roots (246.6mg/100 g). The least concentration of tannin was found in *T.neriifolia* leaves (183.3mg/100 g).

The highest quantity of alkaloids was found in *A. indica* seeds (596.7mg/100 g), *A. indica* leaves (458.3mg/100 g), *T. erecta* roots (476.6 mg/100 g) and *T. neriifolia* leaves (513.3mg/100 g). The least quantity of alkaloid content was obtained in the *T. erecta* shoot (431.6.0 mg/100 g).



**Figure 4.13: Effect of Biopesticides on Fungal Disease Severity of Pepper Plants Inoculated with *Colletotrichum coccodes* in the Field**

Values are means of four replicates. Bars are standard error. *Thevetia nerifolia*-Tl, *Azadirachta indica* leaf extract-Al, *Azadirachta indica* seed extract-As, *Trichoderma pseudokoningii*- Tp, *Trichoderma harzianum*- Th, *Tagetes erecta* shoot extract - Ts, *Tagetes erecta* rootextract- Tr, *Bacillus subtilis*- Bac, Fungicide- Fun, Control (no biopesticides, no pathogen)- C

**Table 4.40: Quantitative Composition of Phytochemicals present in *Azadirachta indica* seed, *Tagetes erecta* shoot, *Azadirachta indica* leaf, *Tagetes erecta* root and *Thevetia nerifolia* leaf**

| Plant Parts                    | Total Phenols (mg/100g) | Flavonoids (mg/100g) | Tannins (mg/100) | Alkaloids (mg/100g) | Saponins (mg/100g) | Total Glycosides (mg/100g) | Total Steroids (mg/100g) |
|--------------------------------|-------------------------|----------------------|------------------|---------------------|--------------------|----------------------------|--------------------------|
| <i>Azadirachta indica</i> seed | 51.3b                   | 541.6a               | 480.0c           | 596.7a              | 311.7a             | 23.3b                      | 266.7b                   |
| <i>Azadirachta indica</i> leaf | 64.5a                   | 275.0b               | 653.3b           | 458.3c              | 148.3d             | 18.3b                      | 213.3c                   |
| <i>Tagetes erecta</i> root     | 26.3e                   | 191.7b               | 246.6d           | 476.6c              | 186.7c             | 33.3a                      | 206.6c                   |
| <i>Tagetes erecta</i> shoot    | 36.4d                   | 281.6b               | 1135.0a          | 431.6d              | 113.3c             | 21.6b                      | 123.3d                   |
| <i>Thevetia nerifolia</i> leaf | 43.4c                   | 128.3d               | 183.3e           | 513.3b              | 251.7b             | 38.3a                      | 313.3a                   |

Values are means of four replicates. Means were separated by using Duncan Multiple Range Test (DMRT) at  $P \leq 0.05$  level of probability.



The highest quantity of saponins was found in *A. indica* seeds (311.7mg/100 g), *A. indica* leaves (148.3mg/100 g), *T.neriifolia* leaves (251.7mg/100 g) and *T. erecta* roots (186.7 mg/100 g). The least quantity of saponins content was obtained in the *T. erecta* shoots (113.3 mg/100 g).

*Thevetia neriifolia* leaves had the highest total steroids (313.0 mg/100 g) amongst all the plant extracts screened and this value was significantly ( $P \leq 0.05$ ) higher than the mean of steroids in the other extracts. The mean concentration of *A. indica* seeds was 266.6 mg/100 g. The steroids content in *A. indica* leaves and *T. erecta* roots were not significantly ( $P \leq 0.05$ ) different from each other with the mean of 213.3mg/100 g and 206.7mg/100 g respectively. *T. erecta* shoot had the least concentration of 123.3mg/100 g. The least quantity of steroids was obtained in *T. erecta* root (120.0 mg/100 g).

*Thevetia neriifolia* leaf had the highest total glycosides (38.3mg/100 g) amongst all the plant extracts screened. The total glycosides in *T.neriifolia* leaf was significantly ( $P \leq 0.05$ ) higher than glycosides of other plant extracts; *T. erecta* root (33.3 mg/100 g), *A. indica* seed (23.3.0 mg/100 g) and *T. erecta* shoot (21.6mg/100 g). The least quantity of glycosides was obtained in *A. indica* leaf (18.3mg/100 g).

## CHAPTER FIVE

### 5.0

### DISCUSSION

Farmers in Oyo, Ekiti and Ogun States of Southwestern Nigeria grow arable crops such as maize, yam, cassava, tomatoes and pepper etc. Pesticides were used to control pests and diseases that attack pepper plants. Findings from this study showed that most of the respondents were actively engaged in subsistence farming. Majority of the respondents are the youth who seem to be young and ready to go into farming despite its risk compared to farmers who are relatively old. The highest percentage of respondents are married (86.2%). The marital status is an important factor that determines the per capita income of the farmers (Akeem and Sofoluwe, 2012). When a farmer has many wives with more children, the farmers per capita income reduces because large number of people will depend on him for survival hence reducing his real income (Akeem and Sofoluwe, 2012).

The result of farmers concerning pests and diseases as part of the major limiting factors for crop production supported with the work of Akeem and Sofoluwe (2012). However, other problems mentioned included lack of agricultural land and funds this is in agreement with the work of Akeem and Sofoluwe (2012) which stated land and fund are major challenges encountered by farmers

The report of farmers concerning the usage of pesticides supported the work of Akeem and Sofoluwe (2012) who stated that majority of the farmers had a high level of pesticides usage combined with low education this could have contributed to the misuse of pesticides. Akeem and Sofoluwe (2012) who also observed that farmer's level of education is low and the efficient usage of pesticides and farming in general required some knowledge and skills. Frequent usage of pesticide has possible effects to the environment unknowingly to the farmers. The increased usage of commercial pesticides have long term negative effects on fauna and flora, changes in soil characteristics and reduced production overtime according to Pimentel and Greiner (1997) and Edmeades

(2003). The Chi-square analysis revealed that there is a relationship between the respondent's personal characteristics and level of pesticides usage. It can be inferred from the findings that farmers' age has an influence on the level of pesticide usage. Similarly, there was negative relationship between respondents' level of education and the level of pesticides usage. The years of farming experience was also found to be related to the level of pesticide usage. These indicate that as farming experience, level of education increases, farmers tend to gather adequate technical knowledge which invariably influences the use of pesticide. However gender and marital status were not related to the level of pesticide used. This implies that respondents' gender and marital status do not influence the level of pesticide use.

Results obtained that there are reduction in crop loss during dry season, could be explained that there are less attack of pests and diseases during the dry season than wet season. These results are comparable to the findings of Ngowi *et al.* (2007) and Obopile *et al.* (2008) who stated that pests and diseases infestation are reduced in dry season while there is high infestation due to favourable environment in the rainy season which in-turn increases pesticide usage. This could be attributed to high moisture, high relative humidity favourable temperature during the wet season which encouraged disease development. Imran *et al.*, (2013) reported that humidity favours disease development, while the low disease attack during the dry season may be because environmental factor did not favour disease development. Observation showed that vegetable production is low during raining season this indicates that few farmers grow vegetables during rainy season due to high incidence of pest and diseases resulting in loss of produce (Imran *et al.*, 2013). The report of farmers on changes in climate such as long dry season could lead to high disease incidence in vegetable farming as a result, farmers tends to use more pesticides which are detrimental to humans health and the environment (Akeem and Sofoluwe, 2012). Although farmers report concerning profitable crops indicated that pepper is easier to manage than some crops this could be because it is hardy, drought tolerant and yield loss may not be 100% despite its production constraints compared to tuber crops which takes longer period to mature and there is no marketability or acceptance of consumer for some vegetables such as garden egg (Nonga *et al.*, 2011). From these findings, many of the farmers used preserved seeds, which could be

aninfected seeds from previous seasons and serve as sources of their planting materials across all the states sampled. This could lead to spread of diseases from infected seeds to disease free seeds and also serve as a source of inocula in the disease outbreak.

Pesticides are widely used in the study area by farmers. Respondents listed different example of pesticides such as insecticides; DDVP – Dimethyl- DichloroVenly Phosphate 1000 EC) and only few farmers used fungicides which include Red- force (Metalaxyl + copper (I) Oxide 60% WP). All the farmers established that the use of pesticides has been increasing over time resulting from disease outbreak and pesticides by their very nature are toxic and can be hazardous to the environments (Nonga *et al.*, 2011). Farmers also noted that their knowledge of biopesticides usage is very low. The frequent application of pesticides may result in accumulation of pesticides leading to health challenges (Nonga *et al.*, 2011). Improper usage of pesticides by farmers practice has resulted in yield loss, pollution in the environment as well as health concerns (Akeem and Sofoluwe, 2012).

The report from this study with those of Imran *et al.*, (2013) stated that fungal diseases are major factor limiting pepper production in Southwestern, Nigeria. These fungi include *C. coccodes*, *C. capsici*, *Penicillium* sp, *T. pseudokoningii*, *T. harzianum* and *P. lycopersici* as confirmed with the work of Imran *et al.*, (2013). From the findings, the most frequently occurring fungi were *C. coccodes*, *T. pseudokoningii* and *P. lycopersici* although, the occurrence and distribution of these fungal were not consistent as confirmed with the work of Pratt *et al.* (1994) because diseases lacked consistency in incidence and severity in different environment. The inconsistency of fungal distribution corresponded to the natural uneven occurrence and distribution of plant pathogenic fungi which is determined by the host plant, soil, climate, cropping patterns and cropping history (Imran *et al.*, 2013). Most of the fungi isolated from the three cultivars of pepper studied have earlier been reported as the causal agents of pepper disease on field and storage in some countries (Imran *et al.*, 2013). Although majority of these pathogens have not been reported in the states surveyed and distribution differ in this study.

*Colletotrichum coccodes*, *Colletotrichum capsici* and *Penicillium* sp were listed as fungi associated with vegetables such as pepper, tomato, okra etc (Terry-Kelley and Boyhan, 2009). *P. lycopersici* is commonly found associated with vegetable crops but its

damage to vegetable production has not been determined (Pedro and Johannes, 2011). There were more leaves infected in inoculated plants, and this could be attributed that these organisms, *Colletotrichum* species and *P. lycopersici* were pathogenic to pepper plants. These pathogens have been reported to cause diseases on range of crops including pepper, strawberry, grapevine and apple (Freeman and Katan, 1997). *T. pseudokoningii*, *Penicillium* species and *T. harzianum* are non-pathogenic fungi on pepper but also occur in infected tissue as opportunist fungi (Jeang *et al.*, 2010).

The disease severity scale indicated pathogenic infection of fungi in the cultivars screened. This result was similar to those of Sapnesh *et al.* (2012) who reported that during screening studies that no line was fully immune or resistant against anthracnose disease. Imran (2007) observed that *C. capsici* also infects pepper cultivars causing symptoms which appeared on fruits, leaves, immature and matured fruits leading to heavy reduction in yield of pepper plants. The varying response of the pepper cultivars to fungal pathogens had been reported in other crops such as tomato (Pedro and Johannes, 2011). This study has shown that fungal pathogens and their level of resistance differs from resistance to moderately resistance in some pathogens and highly susceptible in other pathogens. Although, *Capsicum chinense* cv. Batassa-hot was resistant to these pathogenic fungi affecting pepper.

Anthrachnose is one of the major diseases of pepper caused by *Colletotrichum* species which is responsible for more than 50% loss of the total annual pepper production (Manadhar *et al.*, 1995). Distinct symptoms of *Colletotrichum* species on pepper fruits include deep necrotic hole with ring form of acervuli and causes blemish on fruits thereby reducing the marketability (Manandhar *et al.*, 1995). This study found out that the influence of pathogenic fungi on different cultivars varied in pot and field experiments. There were significant reductions in plant height, number of leaves, and yield of inoculated pepper plants in pot and field experiments. The reduction in plant height of peppers in this study was similar to findings of Sapnesh (2012) on the reduction of plant growth caused by *Colletotrichum* species on some cultivars screened. Imran (2007) reported that inoculation of peppers with *Colletotrichum* species resulted in reduction in plant growth, number of leaves and yield of the peppers. *Colletotrichum* species reduced the growth of pepper as compared to the non-inoculated check plants. There was

reduction in plant height, number of leaves and emerging leaves of pepper cultivars as disease severity increased. Manandhar *et al.* (1995) also reported there is reduction in plant height and number of leaves of plants inoculated with *Colletotrichum* species as compared to non-inoculated plants in pepper cultivars. Infected plants showed yellowing, leaf spot, fruit rot and decline in growth. Jeang *et al.* (2010) reported that plant height and number of leaves of pepper per plant were reduced as a result of inoculation with *C. coccodes* and *C. capsici*. The increase in disease severity of inoculated plants resulted in reduced plant height and number of leaves which was similar to the findings of Than *et al.* (2008) who reported that higher disease incidence caused by *Colletotrichum* species increased exponentially with increase in disease occurrence. Imran (2007) also found out that *Colletotrichum* species is one of the most important diseases causing remarkable loss in yield of the pepper. The yield reduction was generally higher in the wet season. This could be due to low infection rate because dry environment reduce pathogen establishment and thereby limiting infection. Pepper production could be increased in dry season as long as environmental factors limit fungal infection and spread. The poor yield recorded in this study was similar to the findings of other workers that *Colletotrichum* species along other pathogens of pepper resulted in significant yield losses on pepper (Manandhar *et al.*, 1995; Than *et al.*, 2008). The rate of infection and degree of leaf spots and necrosis showed by *Colletotrichum* species on the pepper cultivars indicates the suitability of pepper as a host for these fungal diseases. It also demonstrated the pathogenic effect of *Colletotrichum* species on pepper and severe damage could occur if the crop is grown in field-infested with the fungal propagules.

In the *in vitro* tests, water extracts of *Azadirachta indica* leaf powder, *Tagetes erecta* root powder, *Azadirachta indica* seed powder, *Tagetes erecta* shoot powder and *Thevetia neriifolia* powder at concentrations of 2, 500 mg/kg, 5,000 mg/kg, 7,500 mg/kg, 10,000 mg/kg and 12,500 mg/kg extracts inhibited *C. coccodes*, *C. capsici* and *P. lycopersici*. An increase in concentrations of *Thevetia neriifolia* extract resulted in increased percentage mycelia inhibition of *C. coccodes*, *C. capsici* and *P. lycopersici* ranging from 2.8%-73.3%, which was similar to the findings of Ndukwe *et al.* (2005) who observed that increase in the concentration of plant botanicals reduce its activities. The anti-fungal activity of plant extracts on *Colletotrichum* species and *P. lycopersici*

with in this study indicated that these plant parts have potent antifungal substances that can control fungal diseases of pepper. Six different compounds tannins, saponins, flavonoids, phenols, alkaloids and glycosides present in the plant parts used were shown to be effective in the management of the pathogens. The broad antimicrobial activity of the plant species was shown to be related to the presence of saponin, alkaloid and tannin (Ndukwe *et al.*, 2005). Results indicated the presence of antifungal compounds in different plant extracts which was in agreement with the results of Sapnesh *et al.* (2012) on different pathogen.

As discussed by Kim *et al.* (2002), the efficacy of plant extracts on the management of plant pathogens in field experiments have been proven. The results of this experiment on the field indicated that extracts of lower concentrations reduced the disease severity of the fungi on inoculated plants this could result from method of extracting the active ingredients present in the plant extract at higher concentrations. All the treatments significantly suppressed fungal disease compared to the inorganic fungicides (Kim *et al.*, 2002).

The use of live biological agents such as *Trichoderma pseudokoningii*, *Bacillus subtilis* and *Trichoderma harzianum* have been reported to reduce disease severity (Chouaki *et al.*, 2002) The biological agents used (*Trichoderma pseudokoningii*, *Bacillus subtilis* and *Trichoderma harzianum*) were also effective *in vivo*. However, the isolate of *Trichoderma pseudokoningii* showed antagonistic activity against fungal plant pathogens. This could be attributed to toxins release by these organisms. *T. pseudokoningii* showed significant reduction of mycelia of the pathogens when compared with *B. subtilis* and *T. harzianum* this could be because of the presences of digestible casein and xylanases in *T. pseudokoningii* which explain the greater efficiency of this microorganism (Fleurence and Dumay, 2018).

In the slide culture a clear zone of inhibition was observed exhibiting antibiosis between pathogen and antagonist. Chet *et al.* (2007) reported that *Trichoderma* species are common inhabitant of rhizosphere and contribute to control of many soil borne plant diseases caused by fungi. *T. harzianum* was reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey, 2002; Dubey, 2003; Poddar *et al.*, 2004). The biological agents significantly

suppressed infection of the isolated fungi with about 60%. This correlated with the work of Mishra and Mukhopadhyay (2000) who stated that biocontrol agents are effective in managing fungal disease effectively as well as they are ecologically friendly.

Aqueous extracts of *Azadirachta indica* leaves, *Azadirachta indica* seeds, *Thevetia nerifolia* leaves resulted in significant suppression of disease severity. Plant height and number of leaves of treated plants were significantly higher compared to fungicide treated plants with necrotic portion on leaves and rots of fruits were significantly reduced in treated plants. All treatments except the *Tagetes erecta* root and *Tagetes erecta* shoot were significantly more effective than the application of the mancozeb (fungicide). The use of plant extracts of *Azadirachta indica* leaves, *Azadirachta indica* seeds, and *Thevetia nerifolia* leaves were able to reduce infection on pepper caused by *C.coccodes*, *C. capsici* and *P. lycopersici* *in vitro* and field trials. These findings were similar to earlier report that plant extracts, essential oils reduced the number of the soil borne pathogens and disease severity on crops (Kim *et al.* 2002). Both in the laboratory and field conditions, the highest inhibition of infection were achieved by *Azadirachta indica* and *Thevetia nerifolia*. Treatments with neem-based formulations improved plant growth and caused significant reduction in fungi infection as compared with other treatments. The findings of Ndukwe *et al.*(2005), inoculated plants treated with *Azadirachta indica* extracts of different plant parts increased the plant growth parameters such as height of plant, number of leaves and number of fruits and also reduced infection by fungi pathogens. These findings were similar to the findings of Cantrell *et al.* (2005) who reported that the extracts of leaves of *Azadirachta indica* proved better on fungal disease management compared to other plant species used. The differences in anti-fungal activity of *A.indica* and *T. erecta* plants in this study could be explained by Chaube and Pundhir (2005) who stated that the difference in anti-fungal activity exerted by the different plant species against *Colletotrichum* species on vegetables could be on the basis of the anti-fungal active principle(s) that they contained, which varied qualitatively and or quantitatively. The management of fungal diseases on pepper by the botanicals could be attributed to the combined actions of the phytochemicals- saponins, total phenols, tannins, flavonoids, glycosides and alkaloids. The differences in the concentrations of the phytochemicals in *A. indica* and *T.*



*erecta* plant parts might be responsible for the varying effects in reducing the infection on pepper. This might suggest that total phenols and alkaloids contributed more to the management of fungi on pepper than the other phytochemicals in the amendments.

The findings that biological control and botanicals resulted in reducing the disease severity of pathogenon infected pepper plants which support the growth of plant thereby there is higher plant height, number of leaves, improved yield of pepper plants were similar to works by other earlier workers. The results from this research could help to develop new natural fungicides different plant species have inhibitory effect on these fungi. According to Chitwood (2002) the plants with antifungal properties can be grown in a crop rotation program or be planted in a way to reduce phytopathogenic fungi and success in a long-term crop production program.

## CHAPTER 6

### 6.0 CONCLUSION AND RECOMMENDATIONS

Fungal diseases constitute a major constraint to pepper production. Chemical method is an effective approach, but they are expensive as well as their damage to the environment. There is therefore a need to search for alternative approach such as the development of cultivars with improved disease resistance and use of biopesticides in the management of fungal diseases in pepper. These plant pesticides can be sourced locally and are cheaper than the traditional synthetic pesticides. The toxic ingredients can be found in the leaves, roots, seeds or fruits of some plants.

The phytochemicals exhibit pest control abilities by increasing plant resistance to pests and pathogens possess antifeedant and repellent, can be found in the plants identified to possess pesticidal properties. The use of these plant pesticides, in addition to controlling pathogens, increases plant growth and yield of crops by the supply of plant nutrients. *Capsicum chinense* cv. Batassa-hot can be recommended to farmers for planting and Breeders can use this cultivar gene to develop new cultivars with resistance. *Capsicum chinense* cv. Batassa-hot can be also used as root stock for susceptible cultivars. Based on the result of this study, it is recommended that *A.indica* plants can be regarded as a plant with antifungal activity. Therefore, *A.indica* at 2.5 g/ 100 mL of water can be used to formulate biopesticides for farmers. Also, incorporating these plant species into the soil is a way to reduce phytopathogenic fungi and success in a long-term crop production program

The findings from this study include the following;

1. Confirmation that ₦200, 000- ₦500, 000 is the amount lost to fungal diseases of pepper per hectare annually through survey.
2. *Colletotrichum coccodes*, *Colletotrichum capsici* and *Pyrenochaeta lycopersici* were the pathogens associated with fungal diseases of pepper in Southwestern, Nigeria.

3. *Capsicum chinense* cv. Batassa-hot was resistant to moderately resistant *Colletotrichum capsici*, *Pyrenochaeta lycopersici* and *Colletotrichum coccodes* and can be used in breeding programs.
4. *Azadirachta indica* leaf, *Azadirachta indica* seed, *Thevetia neriifolia* leaf, *Tagetes erecta* root and *Tagetes erecta* shoot have the potential to manage fungal diseases in pepper. The three biological agents used; *Trichoderma pseudokoningii*, *Bacillus subtilis* and *Trichoderma harzianum* reduced the disease severity of fungal diseases while *Trichoderma pseudokoningii* was the most effective.

In retaining soil and environment health, there is need to reduce pesticides usage and finding alternatives management practices. The use of biopesticides should be encouraged as alternatives to fungicides. With regard to the research results, the resistant cultivars could be employed as breeding materials to develop cultivars that are resistant to fungal diseases.

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