# ANTIFUNGAL ACTIVITIES OF ETHYL ACETATE FRACTION OF *PSEUDOMONAS FLUORESCENS* FERMENTATION BROTH AGAINST PHYTO- PATHOGENIC FUNGI FROM ROTTEN YAM

**BY**

# RASHIDA DANLADI UMAR

**DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY**

# AHMADU BELLO UNIVERSITY, ZARIA NIGERIA.

**AUGUST, 2018**

# ANTIFUNGAL ACTIVITIES OF ETHYL ACETATE FRACTION OF *PSEUDOMONAS FLUORESCENS* FERMENTATION BROTH AGAINST PHYTO- PATHOGENIC FUNGI FROM ROTTEN YAM

**BY**

# Rashida Danladi UMAR, BSC. MICRBIOLOGY(UNIABUJA) 2012 P13PHPM8004

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA**

# IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE DEGREE (M.Sc.) IN PHARMACEUTICAL MICROBIOLOGY.

**DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL MICROBIOLOGY,**

# FACULTY OF PHARMACEUTICAL SCIENCES, AHMADU BELLO UNIVERSITY,

**ZARIA, NIGERIA.**

# AUGUST, 2018

# DECLARATION

I hereby declare that the work in this dissertation entitled **“Antifungal activities of ethyl acetate fraction of *Pseudomonas fluorescens* fermentation broth against phyto-pathogenic fungi from rotten yam.”** was performed by me in the Department of Pharmaceutics and Pharmaceutical Microbiology under the supervision of Prof. J.O. Ehinmidu and Dr. B.A. Tytler. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any institution.

|  |  |  |
| --- | --- | --- |
| Umar DANLADI RASHIDA |  | |
| ……………………… | …………………… | …..………… |
| **Name of Student** | **Signature** | **Date** |

# CERTIFICATION

This dissertation entitled “ANTIFUNGAL ACTIVITIES OF ETHYL ACETATE FRACTION OF *PSEUDOMONAS FLUORESCENS* FERMENTATION BROTH AGAINST PHYTO-

PATHOGENIC FUNGI FROM ROTTEN YAM*”* by Rashida Danladi UMAR meets the regulations governing the award of the degree of Master of Science in Pharmaceutical Microbiology of the Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

# ...……………………… ……………………..

Prof. J.O. Ehinmidu. **B.Sc (Micro); M.Sc; Ph.D. Date Chairman, Supervisory Committee**

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

**…..…….………………..** .**………………………** Dr. B.A. Tytler. **B.Sc (Pharm); M.Sc; Ph.D**. **Date**

# Member, Supervisory Committee

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria

# .....……...…………… ….…….......…………

Prof. B.O. Olayinka. **B.Pharm; M.Sc; Ph.D**. **Date Head of Department**

Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria.

# .…………….….…….. …..…..………………

Prof. S.Z. Abubakar **Date**

**Dean,** School of Postgraduate Studies Ahmadu Bello University, Zaria, Nigeria.

# DEDICATION

This work is dedicated to Allah (SWT) for giving me life and knowledge and to the Danladeez family.

# ACKNOWLEDGEMENTS

I hereby express my profound gratitude and sincere appreciation to my major supervisor Prof.

J.O. Ehinmidu who has tirelessly and tremendously supervised me throughout the course of this work; for all his time, encouragement, suggestions and constructive criticism. My appreciation also goes to my second supervisor Dr. B.A. Tytler, for his professional advice, support and commitments throughout the duration of this research work.

I would like to appreciate Prof. B. Olayinka, Head of Department Pharmaceutics & Pharmaceutical Microbiology, Prof. J. A. Onaolapo, Dr. A.K. Olowosulu and all lecturers of the department who have contributed to the success of my work one way or the other. I thank Mr. Ezekiel Dangana, Mr. Mike Ainoje, late Mrs Vicky Roman and Mallam Usman Umar & Mr. Williams Abban in pharmaceutical chemistry, Mallam Kabir Ibrahim in pharmacognosy may the Almighty bless you all for your assistance, concern, and valuable contributions toward the success of this work.

I specially want to acknowledge my family; my parents, Mallam Danladi Umar and Maryam Danladi Umar for their love, care, patience, understanding and financial support, my siblings, Abdulwalid, Abdulwahab, Rukayya, Abdulrahman, little Maryam and my cousin, Rukayya Hussaini for their love.

My sincere appreciation goes to my friends and collegues, Mrs Temi Otegwu, Saadatu Suleiman Baba, Isaiah Yusuf, Samira Garba, Simon Bitrus Gwary Akila, Joshua, Bashir Ishiaka, to mention but few, thank you for your support and making my work interesting.

# ABSTRACT

Yam tubers are prone to infestation by microorganism at all stages of growth and in storage. This rot reduces the quality of yam and affects food security. *Pseudomonas fluorescens* possess varieties of promising antifungal properties through production of secondary metabolites which makes it a biocontrol agent against many phyto-pathogens. In this study, the antifungal activities of metabolite produced by *Pseudomonas fluorescens* isolated in Zaria was evaluated. Rhizosphere soil was collected from different farms (sugarcane, rice and soybeans) in Zaria, Nigeria and *Pseudomonas fluorescens* was isolated and identified in accordance with standard microbiological procedure using cultural, microscopical and biochemical characteristics. The four isolate obtained were grown in three (3) fermentation media made from waste such as yam peel, banana peel and sugarcane shaft as carbon sources and groundnut cake as nitrogen source. Crude extract from these fermentations were tested for antifungal activities against *Aspergillus niger, Penicillium citrinum*, and *Rhizopus stolonifer* isolated from rotten yam using agar diffusion assay. Only four isolates labelled sugarcane A1, sugarcane A2, Rice B1 and Rice B2 were identified as Pseudomonas fluorescens and only fermentation broth extracts of Sugarcane A2, Rice B1 and Rice B2 had remarkable antifungal activities against the fungi with diameter zones of inhibition ranging from 14.00 – 39.00mm in two (2) of the fermentation media. The crude extracts from *P. fluorescens* fermentation broth were extracted in this study and were observed to inhibit the mycelial growth of the test fungi with zone of inhibition ranging from

19.50 - 33.50mm. The *in vitro* & *in vivo* MICs showed significant antifungal activities against test fungi spores. *Rhizopus stolonifer* was observed to be the most susceptible test organism *in vitro* & *in vivo* (0.15 – 5.00mg/ml). This study showed that the *Pseudomonas fluorescens*

isolated in Zaria produced potential antifungal metabolite which can arrest the growth of phyto- pathogenic fungi associated with yam tubers.

# TABLE OF CONTENTS

Cover page i

Title page i

[Declaration ii](#_TOC_250074)

[Certification v](#_TOC_250073)

[Dedication vi](#_TOC_250072)

Acknowledgement vii

[Abstract viii](#_TOC_250071)

Table of Content ix

[List of Tables xiii](#_TOC_250070)

[List of Figures xv](#_TOC_250069)

[List of Plates xvi](#_TOC_250068)

[List of Appendices xvii](#_TOC_250067)

Acronyms and Abbreviation xix

[CHAPTER ONE](#_TOC_250066)

* 1. INTRODUCTION… 1
  2. [Background… 2](#_TOC_250065)
  3. [Statement of problem 3](#_TOC_250064)
  4. [Justification of study 3](#_TOC_250063)
  5. [Aim and Objectives 4](#_TOC_250062)
     1. [Aim 4](#_TOC_250061)
     2. [Objectives of the study 5](#_TOC_250060)
  6. [Hypothesis 5](#_TOC_250059)
     1. [Null hypothesis 5](#_TOC_250058)
     2. [Alternate hypothesis 5](#_TOC_250057)

CHAPTER TWO

* 1. Literature Review 6
  2. [Yam 6](#_TOC_250056)
     1. [Description of yam 6](#_TOC_250055)
     2. [Species of yam 7](#_TOC_250054)
  3. [Yam Production in Nigeria 9](#_TOC_250053)
  4. [Yam Propagation and Harvesting 10](#_TOC_250052)
  5. [Yam Storage 11](#_TOC_250051)
     1. [Methods of Storage 12](#_TOC_250050)
        1. [Traditional Storage Methods 12](#_TOC_250049)
        2. [Modern Storage Methods 13](#_TOC_250048)
  6. [Yam Consumption in Nigeria and around the world 13](#_TOC_250047)
  7. [Nutritional Value of Yam 14](#_TOC_250046)
  8. [Importance of Yam Production 15](#_TOC_250045)
  9. [Problems Associated with Yam Production in Nigeria 16](#_TOC_250044)
     1. [Diseases and common pests affecting yam 17](#_TOC_250043)
        1. [Yam Field Diseases 17](#_TOC_250042)
        2. [Yam Storage Diseases 19](#_TOC_250041)
  10. [Management and Control of Pests and Diseases of Yams 20](#_TOC_250040)
  11. [Pseudomonas as Biocontrol agents 21](#_TOC_250039)
  12. [Pseudomonas fluorescens 26](#_TOC_250038)
      1. Bioactive metabolites of Pseudomonas fluorescens 27
      2. [Pyrrolnitrin (PRN) and Pyoluteorin (PLT) 27](#_TOC_250037)
         1. [Biosynthesis of Pyrrolnitrin 28](#_TOC_250036)
         2. Biosynthesis of Pyoluteorin 30
      3. [Phenazines (PHZ) 31](#_TOC_250035)
         1. [Phenazine Biosynthesis 33](#_TOC_250034)
      4. [2, 4-Diacetylphloroglucinol 35](#_TOC_250033)
         1. [Biosynthesis of DAPG 36](#_TOC_250032)
  13. [Use of agro-waste as part of media formulation for growth of microorganisms 37](#_TOC_250031)

CHAPTER THREE

* 1. MATERIALS AND METHODS 39
  2. [Materials 39](#_TOC_250030)
     1. [Equipment 39](#_TOC_250029)
     2. [Glass wares 39](#_TOC_250028)
     3. [Reagents and Chemicals 39](#_TOC_250027)
     4. [Culture media 40](#_TOC_250026)
  3. [Methods 40](#_TOC_250025)
     1. Isolation of Pseudomonas fluorescens 40
  4. Identification of Pseudomonas fluorescens 40
     1. [Microscopic characteristics 40](#_TOC_250024)
     2. [Cultural characteristics 40](#_TOC_250023)
     3. [Growth at 4°C and 42°C 41](#_TOC_250022)
     4. [Biochemical tests 41](#_TOC_250021)
        1. [Oxidase Test 41](#_TOC_250020)
        2. [Catalase Test 41](#_TOC_250019)
        3. [Indole Test 41](#_TOC_250018)
        4. [Gelatin Hydrolysis Test 41](#_TOC_250017)
        5. [Nitrate Reduction Test 42](#_TOC_250016)
        6. [Sugar Fermentation Test 42](#_TOC_250015)
        7. [Arginine Di-hydrolase Test 42](#_TOC_250014)
  5. [Isolation and Identification of Phyto-pathogenic fungi 43](#_TOC_250013)
  6. Screening for in vitro antifungal activity of crude extract of Pseudomonas fluorescens

[fermentation broth 44](#_TOC_250012)

* + 1. [Preparation of formulated fermentation media 44](#_TOC_250011)
    2. In vitro antifungal activities of crude extract of Pseudomonas fluorescens fermentation broth 45
  1. Extraction of the crude extract metabolite from Pseudomonas fluorescens in the fermentation broth 45
  2. [Quantitative Measurement of Metabolite Substance in the Extract 45](#_TOC_250010)
  3. [Determination of Minimum Inhibitory Concentration of the ethyl acetate fraction against test fungal spores 46](#_TOC_250009)
     1. [*In vitro* Minimum inhibitory concentration of ethyl acetate fraction against test fungal spores 46](#_TOC_250008)
     2. [*In vivo* minimum inhibitory concentration of ethyl acetate fraction against test fungal spores on yam tuber 46](#_TOC_250007)
  4. [Statistical analysis 47](#_TOC_250006)

CHAPTER FOUR

* 1. RESULTS 48
  2. Isolation and identification of Pseudomonas fluorescens 48
  3. [Isolation and identification of fungi from rotten yam 48](#_TOC_250005)
  4. Antifungal activities of crude extract of Pseudomonas fluorescens fermentation broth against the test fungi spores 52
  5. Antifungal activities of the ethyl acetate fraction of fermentation broth of Pseudomonas fluorescens 54
  6. In vitro Minimum inhibitory concentration (M.I.C.) values for the ethyl acetate fractions of Pseudomonas fluorescens fermentation broth on the test fungi spores 57
  7. [*In vivo* Minimum inhibitory concentration values for the ethyl acetate fractions of fermentation broth of *Pseudomonas fluorescens* on the test fungi isolates on yam tuber 60](#_TOC_250004)

CHAPTER FIVE

5.0 DISCUSSION 63

[CHAPTER SIX](#_TOC_250003)

* 1. SUMMARY, CONCLUSION AND RECOMMENDATION 67
  2. [Summary 67](#_TOC_250002)
  3. [Conclusion 67](#_TOC_250001)
  4. [Recommendations 67](#_TOC_250000)

# LIST OF TABLES

Table 2.1: World Yam Production 2014 9

Table 2.2: Nutritional Value of Raw yam (*Dioscorea* spp) Per 100g 14

Table 2.3: Antibiotics produced by biocontrol *Pseudomonas* spp and their target plant pathogens 22

Table 3.1: Composition of the fermentation media for growth of *Pseudomonas fluorescens* 44

Table 4.1: Cultural and Morphological Characteristics of *Pseudomonas fluorescens* isolates on Cetrimide agar 49

Table 4.2: Biochemical characterization of *Pseudomonas fluorescens* isolates from rice & sugarcane rhizosphere 50

Table 4.3: Morphological characteristics of isolated fungi species 51

Table 4.4: Antifungal activities against test fungi spores by the crude extract from

*Pseudomonas fluorescens* fermentation broth 53

Table 4.5.: Antifungal activities of ethyl acetate fraction of M1 fermentation broth culture of

*Pseudomonas fluorescens* 55

Table 4.6: Antifungal activities of ethyl acetate fraction of M2 fermentation broth culture of

*Pseudomonas fluorescens* 56

Table 4.7: *In vitro* Minimum Inhibitory Concentration of ethyl acetate fraction of M1 fermentation broth culture of *Pseudomonas fluorescens* on the test fungi spores 58

Table: 4.8 *In vitro* Minimum Inhibitory Concentration of ethyl acetate fraction of M2 fermentation broth culture of *Pseudomonas fluorescens* on the test fungi spores 59

Table: 4.9: *In vivo* Minimum Inhibitory Concentration of ethyl acetate fractions of M1 fermentation broth culture of *Pseudomonas fluorescens* 61

4.10: *In vivo* Minimum Inhibitory Concentration of ethyl acetate fraction of M2 fermentation broth culture of *Pseudomonas fluorescens* 62

# LIST OF FIGURES

Figure 1: Chemical structure of Pyrrolnitrin 27

Figure 2: Chemical structure of Pyoluteorin 28

Figure 3: Biosynthetic pathways for Pyrrolnitrin 29

Figure 4: Hypothetical pyoluteorin biosynthetic pathways 30

Figure 5: Structures of some common phenazines produced by fluorescent

*Pseudomonas* spp 32

Figure 6: Current understanding of core phenazine biosynthesis 34

Figure 7: Chemical structure of 2,4-Diacetylphloroglucinol 36

Figure 8: Proposed roles of PhlA, PhlB, PhlC, and PhlD in the biosynthesis of MAPG and

2,4-DAPG 36

# LIST OF PLATES

Plate 1: Zone of inhibition of the crude extract metabolite from Rice B1 in Medium 2 against

*Rhizopus stolonifer* (mm) 98

Plate 2: Zone of inhibition of the crude extract metabolite from Rice B1 in Medium 2 against

*Penicillium citrinum* (mm) 98

Plate 3: Zone of inhibition of the crude extract metabolite from Rice B1 in medium 2 against

*Aspergillus niger* (mm) 99

# LIST OF APPENDICES

Appendix 1: Antifungal activities against test fungi spores by the crude extract from

*Pseudomonas fluorescens* isolates in three (3) fermentation media 88

Appendix 2: Antifungal activities of ethyl acetate fractions of fermentation broth culture of

*Pseudomonas fluorescens* 89

Appendix 3: Two-way ANOVA for the antifungal activities of the crude extract of

*Pseudomonas fluorescens* isolates against the three test organism in medium 1 90

Appendix 4: Two-way ANOVA for the antifungal activities of the crude extract of

*Pseudomonas fluorescens* isolates against the three test organism in medium 2 91

Appendix 5: Two-way ANOVA for the antifungal activities of the crude extract of

*Pseudomonas fluorescens* isolates against the three test organism in medium 3 92

Appendix 6: Two-way ANOVA for the antifungal activities of the crude extract of

*Pseudomonas fluorescens* isolates in the three formulated media against *Aspergillus niger* 93

Appendix 7: Two-way ANOVA for the antifungal activities of the crude extract of

*Pseudomonas fluorescens* isolates in the three formulated media against *Penicillium citrinum*

.................................................................................................................................................. 94

Appendix 8: Two-way ANOVA for the antifungal activities of the cell free extract of

*Pseudomonas fluorescens* isolates in the three formulated media against *Rhizopus stolonifer*

.................................................................................................................................................. 95

Appendix 9: Two-way ANOVA for the antifungal activities of ethyl acetate fraction of M1 fermentation broth culture of *Pseudomonas fluorescens* 96

Appendix 10: Two-way ANOVA for the antifungal activities of ethyl acetate fraction of M2 fermentation broth culture of *Pseudomonas fluorescens* 97

Appendix 11: Appendix 11: Zones of inhibition of ethyl acetate fraction of Pseudomonas fluorescens fermentation broth in M2 98

Appendix 12: Growth curve for *Pseudomonas fluorescens* isolates in a standard medium containing glucose as carbon source, NH4NO3 as nitrogen source 100

Appendix 13: Growth curve for *Pseudomonas fluorescens* isolates in medium 1 containing yam peel as carbon source and groundnut cake as nitrogen source 101

Appendix 14: Growth curve for *Pseudomonas fluorescens* isolates in medium 2 containing banana peel as carbon source and groundnut cake as nitrogen source 102

Appendix 15: Growth curve for *Pseudomonas fluorescens* isolates in medium 3 containing sugar cane shaft as carbon source and groundnut cake as nitrogen source 103

# ACRONYMS AND ABBREVIATIONS

*In-vitro* Taking place outside living organism

*In-vivo* Within the living

DAPG Di-acetylphloroglucinol

SDA Sabouraud dextrose agar

DHHA 2,3-dihydro-3-hydroxyanthranillic acid

ADIC 2-amino-2-deoxyisochorismic acid

CaCO3 Calcium Carbonate

MgSO4 Magnesium sulphate

K2HPO4 di-potassium phosphate

ANOVA Analysis of variance

M.I.C. Minimum Inhibitory Concentration

S.D Standard Deviation

µg Micro gram

mm Millimeter

mg Milligram

ml Milliliter

RNA Ribonucleic acid

DNA Deoxyribonucleic acid

HCN Hydrogen cyanide

PRN Pyrrolnitrin

PLT Pyoluteorin

g grams

Kcal Kilocalories

PHZ Phenazines

PCA Phenazine-1-carboxylic acid

PCN Phenazine-1-carboxamide

PYO Pyocyanin

IU International Unit

RDA Recommended Dietary Allowance

# CHAPTER ONE

**INTRODUCTION**

# Background

Yam belongs to the genus Dioscorea (Family Dioscoreaceae) and is the second most important tropical root crop in West Africa after cassava (Adisa *et al*., 2015). The genus Dioscorea consists of about 600 species (Degras, 1993). The most cultivated species are the *Dioscorea rotundata* (white yam), *Dioscorea alata* (water yam), *Dioscorea dumetorum* (bitter yam), *Dioscorea esculentum* (loir), *Dioscorea cayenensis* (yellow or guinea yam) and *Dioscorea bulbifera* (aerial yam). White or guinea yam (D. rotunduata poir) is the most popular species in Africa (Lawal *et al*., 2014; Princewill-Ogbonna and Ibeji, 2015).

Yam plays significant roles in the social-cultural and economic wellbeing of thousands of people in Nigeria especially among the people of South Eastern Nigeria (Okigbo *et al*., 2013). Its cultivation is very profitable despite high costs of production and price fluctuations in the markets (IITA, 2013; Izekor and Olumese, 2010).

The quality of yam tubers is affected by rots, which makes them unappealing to consumers (Ogbo and Agu, 2014b; Agu *et al*., 2015). Most rots of yam tubers are caused by pathogenic fungi such as *Aspergillus flavus, Aspergillus niger* (Tiegh), *Fusarium oxysporum, Fusarium solani, Botryodiplodia theobromae, Penicilllium chrysogenum, Rhizoctonia spp, Penicillium oxalicum, Trichoderma viride* and *Rhizopus nodosus* (Okigbo and Ikediugwu, 2000; Okigbo *et al*., 2014; Frank and Kingsley, 2014b; Agu *et al*., 2014). Several methods have been adopted for controlling losses due to post harvest disease of yam; these include the use of chemicals, biological method of control (Okigbo, 2004) and curing by using natural plant extracts (Amusa *et al*., (2003).

Pseudomonas is one of the most ubiquitous bacterial genus and Migula designated and described the species associated with the genus in 1985 (Migula, 1985). Pseudomonads are

well adapted to colonizing roots and the rhizosphere, and they suppress disease by a wide variety of mechanisms (Pieterse *et al*. 2014; Weller 2007) but the most important are the production of antimicrobial metabolites. These characteristics makes Pseudomonas species good candidates for use as seed inoculant and root dips for biological control of soil borne pathogens.

*Pseudomonas fluorescens* is a gram negative, rod-shaped, and non-pathogenic bacterium that is known to inhibit primarily the soil, plants and water (Peix *et al.,* 2009). It derives its name from its ability to produce fluorescent pigments under iron-limiting conditions (Baysse *et al*., 2003). *P. fluorescens* are well characterized for their ability to produce antimicrobial compounds (Haas and De’ fago, 2005). These metabolites range from simple 2, 4-diacetyl phloroglucinol, phenazine-1-carboxylic acid and pyrrolnitrin [3-chloro-4-(2-nitro-3’- chlorophenyl)-pyrrole], as well as the complex macrocyclic lactone 2, 3-de-epoxy-2,3- didehydra-rhizoxin. 2,4-diacetylphloroglucinol (DAPG), is a major factor in control of a range of plant pathogens (Haas and De’ fago, 2005) while Pyrrolnitrin is active against *Rhizoctonia* spp, *Fusarium* spp, and other plant pathogen fungi and it has been used as a lead structure in development of a new phenyl pyrrole agricultural fungicide (Ligon *et al*., 2000).

Wastes are leftovers from production and consumption. It includes plant materials, agricultural, household, industrial and municipal wastes and residues (Okonkwo *et al*., 2006). The agricultural and agro-industrial activities generate a large amount of lignocellulosic by products such as bagasse, straw, stem, stalk, cobs, fruit peel and husk, among others (Saranraj and Anbu, 2017). These wastes contain simple and complex sugars that are metabolized by microorganism through secretion of extracellular products (Saheed *et al*., 2013).

# Statement of Problem

Large quantity of yam losses has been reported and these losses have been due to microbial rot diseases. Yam plants are prone to infection by fungi, bacteria, and viruses at all stages of growth and also during storage of tubers (Okigbo, 2002; Okigbo, 2005; Morse *et al*., 2000) Difficulties in yam preservation has compelled majority of the yam farmers are to either consume or sell all their yam products at low prices before the new harvesting season. Consequently, before new harvesting period, they are bound to suffer food security crises.

Chemical agents have been used to control this pre harvest and post-harvest losses in the past decades but these chemical agents are not only expensive for farmers, but they are not eco- friendly and micro-organisms are becoming resistant to them (Okigbo, 2004; Okigbo and Ikediugwu, 2000). This has lead scientist to divert their attention to the use of potential beneficial microbes. Biological agents as biocontrol is gaining ground and has proved to be safer, cheaper, effective and eco-friendly.

As a result of high consumption and industrial processing of the edible parts of fruit, fruit wastes (such as banana, grapes, dragon fruit, orange, strawberry, lemon, watermelon, citrus, Pomegranate, pineapple residues, sugarcane bagasse and other fruit) has become one of the main sources of municipal solid wastes which has become an increasingly tough environmental issue (Saranraj and Anbu, 2017). Insufficient and improper disposal of these solid waste result in scenic blights, increase in rodents and insect vectors of disease, and serious hazards to public health.

# Justification of Study

Nigeria is the largest producer of yam in the world (FAOSTAT, 2017) and yam production is regarded as a source of food security and employer of labour in many areas where it is cultivated. It generates employment for more than 50% of people in places of production either

through direct involvement in cultivation, marketing, processing, or indirectly through related services in production (i.e. labour both on the field of production and markets or transportation).

Yam tubers are nutritionally rich and a major source of dietary fiber, carbohydrates, vitamin C, and essential minerals (Charles *et al*., 2005; Polycarp *et al*., 2012).

The scope of developing microbial metabolites for commercial pesticides as an alternative to chemical control agent has gained importance due to increased concerns on environmental pollution, pathogens resistance and high plant protection cost (Reddy *et al*., 2007; Uppal *et al*., 2007)

The agricultural-based industries generate significant quantities of organic wastes including peels from cassava, plantain, banana, oranges and straw from cereals. Rather than allow these wastes to become solid municipal wastes, it is necessary to convert them to useful end products. These wastes could be utilized as cheap raw materials for some industries or as cheap substrates for microbiological processes (Nwabueze and Otowa, 2006). The need to develop alternative media has become imperative as the conventional media are either not readily available or expensive in most developing countries (Saranraj and Anbu, 2017).

# Aim and Objectives

# Aim

This study was designed to examine the antifungal activities of ethyl acetate fraction of

*Pseudomonas fluorescens* fermentation broth against some phyto-pathogenic test fungi.

# Objectives of the Study

1. To isolate & identify *Pseudomonas fluorescens* from rhizosphere of rice, sugar cane and soy beans soil in Zaria, Kaduna state.
2. To Isolate and identify three phyto-pathogenic organisms (*Aspergillus niger, Penicillium citrinum* and *Rhizopus stolonifer)* from rotten yam.
3. To formulate and prepare fermentation media using agricultural waste as different carbon and nitrogen sources for the production of active metabolite from *Pseudomonas fluorescens*.
4. To screen for antifungal activities of the crude extracts using agar well diffusion method.
5. To determine the *in vitro* and *in vivo* antifungal activities of the ethyl acetate fraction from *Pseudomonas fluorescens* fermentation broth on the test fungal spores.

# Hypothesis

# Null Hypothesis

The ethyl acetate fraction from *Pseudomonas fluorescens* fermentation broth does not have antifungal activities against phyto-pathogenic fungi spores of *Aspergillus niger, Penicillium citrinum, Rhizopus stonolifer.*

# Alternate Hypothesis

The ethyl acetate fraction from *Pseudomonas fluorescens* fermentation broth has antifungal activities against phyto-pathogenic fungi spores of *Aspergillus niger, Penicillim citrinum, Rhizopus stonolifer.*

# CHAPTER TWO LITERATURE REVIEW

# Yam

[Scientific classification](http://en.wikipedia.org/wiki/Biological_classification) – Kingdom: [*Plantae*](http://en.wikipedia.org/wiki/Plantae)Division: [*Magnoliophyta*](http://en.wikipedia.org/wiki/Flowering_plant)Class: *Liliopsida*

Order: *Dioscoreales* Family: *Dioscoreaceae* Genus: *Dioscorea* L

# Description of Yam

Yam is the name given to several plant species in the genus *Dioscorea* that are grown for their edible tubers. These species are not to be confused with the sweet potato, *Ipomoea batatas*, which is often referred to as a yam in the US. Yam plants are herbaceous annual or perennials with climbing or trailing vines. The vines can be smooth or prickly, reaching 10 m (32.8 ft) or more in length depending on the variety. The leaves of the plant are simple and usually oval to heart-shaped with petioles which are the same length, or slightly longer, than the leaf blade itself. Some varieties possess spikes at the bases of the leaves. The plant can produce one singular tuber or several tubers which extend from stolons from a central corm (up to 20) depending on the species. The tubers can be cylindrical, curved or lobed, with brown, grey, black or pink skin and white, orange or purplish flesh. Most yams are annual plants, harvested after one season, but some are perennial with tubers increasing in size each year with the vines dying back at the end of the growing season and re-growing on the return of favourable conditions. The origin of yams is uncertain and genetic information suggests that there may be more than one point of origin.

# Species of Yam

There are as many as 600 cultivars of yam throughout the humid tropics, among which there are *Dioscorea rotundata* (white yam); *Dioscorea alata* (soft or water yam); *Dioscorea cayenensis* (yellow yam), *Dioscorea dumetorum* (bitter yam), *Dioscorea nummularia* (wael yam); *Dioscorea esculenta* (Sweet Chinese yam); *Dioscorea trifida* (Africa yam); *Dioscorea bulbifera* (aerial yam); *Dioscorea pentaphila* and *Dioscorea transversa* (Strong yam) (National Advisory Board on Climate Change and Disaster Risk Reduction, 2013; FAO, 2013).

Out of these numerous species, the most important edible species are *D. rotundata* (white yam),

*D. alata* (water yam), *D. cayenensis* (yellow yam), *D. dumetorum* (bitter yam), *D. esculenta* (Chinese yam), and *D. bulbifera* (aerial yam) while *D. rotundata* is the most preferred and cultivated, accounting for a large proportion of yam production in West Africa, a region which produces 93 % of the world’s yam (FAO, 2013).

* + - 1. ***White yam*** (***Dioscorea rotundata)* and Yellow yam (*D. cayenensis):*** [*Dioscorea*](http://en.wikipedia.org/wiki/Dioscorea_rotundata)[*rotundata*](http://en.wikipedia.org/wiki/Dioscorea_rotundata), the "white yam", and [*Dioscorea cayenensis*](http://en.wikipedia.org/w/index.php?title=Dioscorea_cayenensis&action=edit&redlink=1), the "yellow yam", are native to Africa. They are the most important cultivated yams. In the past they were considered two separate species but most taxonomists now regard them as the same species. White yam's tuber is roughly cylindrical in shape, the skin is smooth and brown and the flesh usually white and firm. Yellow yam is named after its yellow flesh, a color caused by the presence of carotenoids. It looks similar to the white yam in outer appearance; its tuber skin is usually a bit firmer and less extensively grooved. The yellow yam has a longer period of vegetation and a shorter dormancy than white yam.
      2. **Water yam** ([***Dioscorea alata***](http://en.wikipedia.org/wiki/Dioscorea_alata)***):*** [*Dioscorea alata*](http://en.wikipedia.org/wiki/Dioscorea_alata), called "water yam", "winged yam" and "purple yam" was first cultivated in Southeast Asia. Although not grown in the same quantities as the African yams, it has the largest distribution world-wide of any cultivated yam, being grown in Asia, the Pacific islands, Africa, and the West Indies (Mignouna *et al*., 2003). Even in Africa, the popularity of water yam is second only to white yam. The tuber shape is generally cylindrical, but can vary. Tuber flesh is white and watery in texture. In the [Philippines](http://en.wikipedia.org/wiki/Philippines) it is known as "ube" and is

used as an ingredient in many sweet desserts. In [Indonesia](http://en.wikipedia.org/wiki/Indonesia) it is known as "ubi". In [Vietnam,](http://en.wikipedia.org/wiki/Vietnam) it is called *khoai mỡ* and is used mainly as an ingredient for soup. In [India,](http://en.wikipedia.org/wiki/India) it is known as *ratalu* or *violet yam*. In [Hawaii](http://en.wikipedia.org/wiki/Hawaii) it is known as *uhi*. In [Japan](http://en.wikipedia.org/wiki/Japan) it is called *daijo* or *beniimo*.

* + - 1. **Aerial yam** ([***Dioscorea bulbifera***](http://en.wikipedia.org/wiki/Dioscorea_bulbifera)***):*** Also called the "air potato", is found in both Africa

and Asia, with slight differences between those found in each place. It is a large vine, 6 meters (20 ft) or more in length. It produces tubers; however, the [bulbils](http://en.wikipedia.org/wiki/Bulb) which grow at the base of its leaves are

the more important food product. They are about the size of potatoes (hence the name "air potato"), weighing from 0.5 to 2 kilograms (1.1 to 4.4 lb). Some varieties can be eaten raw while some require soaking or boiling for detoxification before eating. It is not grown much commercially since the flavor of other yams is preferred by most people.

* + - 1. **Bitter yam** ([***Dioscorea dumetorum***](http://en.wikipedia.org/w/index.php?title=Dioscorea_dumetorum&action=edit&redlink=1)***)*:** [*Dioscorea dumetorum*](http://en.wikipedia.org/w/index.php?title=Dioscorea_dumetorum&action=edit&redlink=1), the bitter yam, is popular

as a vegetable in parts of West Africa; one reason being that their cultivation requires less labor than other yams. One marked characteristics of the bitter yam is the bitter flavour of its tubers. The wild forms are very toxic and are sometimes used to poison animals when mixed with bait. It is said that they have also been used for criminal purposes (Kay, 1987)

* + - 1. **Chinese yam** ([***Dioscorea esculenta)***](http://en.wikipedia.org/wiki/Dioscorea_opposita)***:*** It is called "Chinese yam" and is native to China.

The Chinese yam plant is somewhat smaller than the African, with the vines about 3 meters (10 feet) long. It is tolerant to frost and can be grown in much cooler conditions than other yams. It is now grown in China, Korea, and Japan. It was introduced to Europe in the 19th century when the [potato](http://en.wikipedia.org/wiki/Potato)

crop there was falling victim to disease, and is still grown in France for the Asian food market. The tubers are harvested after about 6 months of growth. Some are eaten right after harvesting and some are used as ingredients for other dishes, including noodles, and for traditional medicines (Kay, 1987).

# Yam Production in Nigeria

[Nigeria](https://en.wikipedia.org/wiki/Nigeria) is the largest world producer of yam with more than 45,004 million metric tonnes

annually (FAOSTAT, 2017). In 1985, Food and Agricultural Organization reported that Nigeria produced 18.3 million tonnes of yam from 1.5 million hectares, representing 73.8 percent of total yam production in Africa. According to 2008 figures, yam production in Nigeria has nearly doubled since 1985, with Nigeria producing 35.017 million metric tonnes with value equivalent of US$5.654 billion while the world's second and third largest producers of yams, [Côte d'Ivoire](https://en.wikipedia.org/wiki/C%C3%B4te_d%27Ivoire) and [Ghana,](https://en.wikipedia.org/wiki/Ghana) only produced 6.9 and 4.8 million tonnes of yams in 2008

respectively.

In 2014, worldwide production of yams was 68.1 million [tonnes](https://en.wikipedia.org/wiki/Tonne) where Nigeria farmed yams

on 5.4 million [hectares](https://en.wikipedia.org/wiki/Hectares) (FAOSTAT, 2017). Nigeria exported 72 tonnes of yam to Europe and US on June 29th 2017 as part of moves to diversify its oil-dependent economy and earn much needed foreign exchange.

# Table 2.1: World Yam production in 2014

|  |  |  |
| --- | --- | --- |
| *S/No* | *Countries* | *Production*  *(millions of tonnes)* |
| *1*  *2*  *3*  *4*  *5*  *6*  *7* | *Nigeria Ghana Ivory coast Benin Ethiopia Togo*  *Cameroun* | *45.0*  *7.1*  *5.8*  *3.2*  *1.4*  *0.8*  *0.6* |

Source: UN Food & Agriculture Organization (FAOSTAT, 2014)

The major yam producing area in Nigeria include; the middle belt (Benue, Kogi, Ilorin, Niger Nassarawa), Eastern part of Nigeria (Owerri, Onitsha, Porthacourt, Umuahia) and the Western part (Ondo, Oyo, Ibadan).

# Yam Propagation and Harvesting

1. **Propagation**

Yam crop begins when whole seed tubers or tuber portions are planted into mounds or ridges, at the beginning of the rainy season. The crop yield depends on how and where the seeds are planted, sizes of mounds, interplant spacing, provision of stakes for the resultant plants, yam species, and tuber sizes desired at harvest. Small-scale farmers in West and Central Africa often intercrop yams with cereals and vegetables. Large amounts of material (about 10,000 seed yams) are needed to plant one hectare. If farmers do not buy new seed yams, they must set aside 30% of their harvest for the next year planting (Kabeya *et al*, 2013). In addition, seed yams are bulky and perish quickly (Kabeya *et al*, 2013)

Yam typically grows for six to ten months and is dormant for two to four months, depending on the species. The growth and dormant phases correspond respectively to the wet season and the dry season. For maximum yield the yam requires a humid tropical environment, with an annual rainfall of over 1500 millimetres distributed uniformly throughout the growing season. White, yellow and water yams typically produce a single large tuber per year, generally weighing 5 to 10 kilograms (Calverly, 1998).

# Harvesting

Yams in West Africa are typically harvested by hand using sticks, spades or diggers (Okpara, 2003). Wood-based tools are preferred to metallic tools as they are less likely to damage the fragile tubers; however, wood tools need frequent replacement. Yam harvesting is labour intensive and physically demanding. For each 2-10kilogram tuber harvested, it involves standing, bending, squatting, and sometimes sitting on the ground, depending on the size of

mound, size of tuber or depth of tuber penetration. Care must be taken to avoid damage to the tuber, because damaged tubers do not store well and spoil rapidly. Some farmers use staking and mixed cropping, a practice that complicates harvesting in some cases.

In forested areas, tubers grow in areas where other tree roots are present. Harvesting the tuber then involves the additional step of freeing them from other roots. This often causes tuber damage. Aerial tubers or bulbils are harvested by manual plucking from the vine. Yields may improve and cost of yam production can be lower if mechanization were to be developed and adopted. However, current crop production practices and species used pose considerable hurdles to successful mechanization of yam production, particularly for small-scale rural farmers. Extensive changes in traditional cultivation practices, such as mixed cropping, may be required. Modification of current tuber harvesting equipment is necessary given yam tuber architecture and its different physical properties (Okpara, 2003).

# Yam Storage

In order to partly ensure food security in the region, yam storage is very important. In other words, yams are stored either for future consumption, higher market prices or for re-plantation (yam seeds) in another season. Due to the difficulties in yam preservation, the majority of the yam farmers are compelled to either consume or sell all their yam products at low prices before the new harvesting season. Consequently, before new harvesting period, they are bound to suffer food security crises.

Certain cultivars of yam store better than others. Easier to store yams are those that are adapted to arid climate, where they tend to stay in dormant low-respiration stage for much longer than yam breeds that are adapted to humid tropical lands where they do not need dormancy. The tubers of many varieties can be stored as long as six months without refrigeration (CGAIR 2006).

# Methods of Storage

Various traditional and modern storage techniques are practised in the country, depending on technical know-how, facilities available, climatic factors at the time of storage, risk of exposure to pest attack and the quantity of the farm produce. Although modern techniques are being developed for effective storage of these products in large quantities, most farmers still depend on the traditional methods since these new techniques are not within their reach.

# Traditional Storage Methods

Various traditional methods used include;

Storing the tubers on wooden platform in cool and well ventilated room to avoid direct sunlight which may cause drying and cracking. The tubers are arranged in irregular rows and covered with dry grass to allow air circulation.

They can also be stored by tying the tubers to a frame of living wooden poles (usually *Ficus* spp since they are not easily attacked by termites) which are fastened together and anchored firmly into the ground. Cross poles are fastened to these upright poles at intervals, usually about 30cm apart. The frames are set up in covered barns.

Yam tuber can be stored by leaving the yams in the soil for temporary storage after the stems have been cut off and by mixing/packing the tubers in ashes, heaping and covering with soil before storing at room temperature. The tubers may be stored in a thatched shed, huts, with a raised platform of about 0.6m-0.9m above the ground. The yams can be suspended singly from branches which shades them. This is practised with small quantities of yams.

Storing yam tubers in layers inside trenches. They are then covered with dry grass followed by a layer of soil. The trenches are normally made in areas where the soil is dry in order to avoid sprouting and decay.

# Modern Storage Methods

This method usually involves irradiating yam tubers with gamma rays at low dosage of 7.5 - 15 krads with in one months of harvest. This is followed by storage at ambient conditions. This will effectively control factors causing deterioration and keep the yams in good conditions for 8-9 months.

Yam tubers can be stored using chemicals, fungicides and pesticide to prevent and control rotting of stored tubers. Cold room storage at about 15ºC is also being tried in research stations. This has been found to be expensive for traditional farmers.

# Yam Consumption in Nigeria and Around the World

Yams of African species must be cooked to be safely eaten, because various natural substances in raw yams can cause illness if consumed. (Excessive skin contact with uncooked yam fluids can cause the skin to itch. If this occurs, a quick cold bath or application of red palm oil to the affected part of the body will stop the itching). Yams are used differently in different parts of the world. They are consumed after cooking by frying, boiling or roasting. The green parts of some plants can be cooked and consumed as a vegetable. Yams may also be used to produce flour or starch.

Preparing boiled yam involves cutting the tuber into five or so thick slices, then peeling the skin from each cut piece and boiling the whitish starchy flesh. The older the yam, the smaller the chunks must be cut and the more water it will need to boil. Boiled yam is traditionally consumed with palm oil, but is also commonly served with a sauce such as [pepper sauce](http://en.wikipedia.org/wiki/Pepper_sauce) or

[palaver sauce.](http://en.wikipedia.org/wiki/Palaver_sauce) In big cities and border towns in West Africa, fried yam and [pepper sauce](http://en.wikipedia.org/wiki/Pepper_sauce) is a

popular street food and has a similar position as [French fries](http://en.wikipedia.org/wiki/French_fries) and [ketchup](http://en.wikipedia.org/wiki/Ketchup) in [Western cuisine](http://en.wikipedia.org/wiki/Western_cuisine).

Yam balls (styled after [meat balls](http://en.wikipedia.org/wiki/Meat_ball)) have also gained some popularity in contemporary [West](http://en.wikipedia.org/wiki/West_African_cuisine)

[African cuisine.](http://en.wikipedia.org/wiki/West_African_cuisine)

Among the Akan of Ghana, boiled yam can be mashed with palm oil into *eto* in a similar manner to the plantain dish [*matoke*](http://en.wikipedia.org/wiki/Matoke), and is served with eggs. The boiled yam can also be

pounded with a traditional mortar and pestle to create a thick, starchy paste known as [*iyan*](http://en.wikipedia.org/w/index.php?title=Iyan&action=edit&redlink=1)

("pounded yam") or [*fufu*](http://en.wikipedia.org/wiki/Fufu) which is eaten with traditional sauces such as [egusi](http://en.wikipedia.org/wiki/Egusi) and [palmnut](http://en.wikipedia.org/wiki/Oil_palm) soup.

Another method of consumption is to leave the raw yam pieces to [dry in the sun](http://en.wikipedia.org/wiki/Drying_%28food%29). When dry,

the pieces turn a dark brown color. These are then milled to create a brown powder known in Nigeria as *elubo*. The powder can be mixed with boiling water to create a thick starchy paste, a kind of pudding known as [amala](http://en.wikipedia.org/wiki/Amala_%28food%29), which is then eaten with local soups and sauces.

# Nutritional Value of Yam

Nutritionally, yam is an essential source of carbohydrates for the consumers around the world. Many yam belt areas in Nigeria continuously proclaimed "yam is food and food is yam" (Maikasuwa and Ala, 2013). Some cultivars of yam tuber have been found to contain protein levels of 3.2-13.9% of dry weight. A yam meal could supply 100% of the energy and protein, 13% of the calcium and 80% of the iron requirement of an adult male (Knoth, 1993).

The tuber is an excellent source of the B-complex group of vitamins. It provides adequate daily requirements of pyridoxine (vitamin B6), thiamin (vitamin B1), riboflavin, folates, pantothenic acid, and niacin. These vitamins mediate various metabolic functions in the body. Yam contains small amounts of vitamin-A, and β-carotene levels both of which are powerful antioxidants Carotenes convert into vitamin-A inside the body.

**Table 2.2: Nutritional value of raw yam (*Dioscorea spp*)per 100g**

|  |  |  |
| --- | --- | --- |
| Principle | Nutrient value | Percentage of RDA |
| **Energy Carbohydrates Protein**  **Total Fat Cholesterol Dietary Fiber** Vitamins **Folates Niacin**  **Pantothenic acid Pyridoxine Riboflavin Thiamin Vitamin C Vitamin A**  **Vitamin E** | 108 Kcal  27.88 g  1.53 g  0.17 g 0 mg  4.1 g  23 µg  0.552 mg  0.314 mg  0.293 mg  0.032 mg  0.112 mg  17.1 mg 138 IU  0.35 mg | 5%  21%  3%  0.5%  0%  11%  6%  3.5%  7%  23%  2.5%  9.5%  28.5%  5%  2% |

|  |  |  |
| --- | --- | --- |
| **Vitamin K** Electrolytes **Sodium Potassium** Minerals **Calcium Copper Iron Magnesium Manganese Phosphorus Selenium Zinc**  Phyto-nutrients  **Carotene-Beta**  **Crypto-xanthin Lutein-zeaxanthin** | 2.3 µg  9 mg  816 mg  17 mg  0.178 mg  0.54 mg 21 mg  0.397 mg 55 mg  0.7 µg  0.24 mg  83 µg  0 µg  0 µg | 2%  0.5%  17%  2%  20%  7%  5%  17%  8%  0.5%  2%  -----  -----  ----- |

(Source: [USDA National Nutrient data base](http://ndb.nal.usda.gov/ndb/foods/show/3313))

# Importance of Yam Production

Yams are one of the most highly regarded food products in tropical countries of West Africa where it also has a social and cultural benefit (Abdulsalam *et al*., 2016). It is a highly valued crop which forms, about 10% of the total roots and tuber produced in the world. Yam

production serves as a source of income generation to peasant farmers and the labourers who work on yam farms as well as for those that engage in its sale. Yam serves as source of food security for the country. Peelings and waste from yam are often used for feeding poultry and livestock and can also be used as a source of carbon for the growth of microorganism. According to Lawal *et al*. (2016) yam is considered an herbal medicine in Taiwan. Yam is known to contain a good quantity of bioactive compounds, such as phytochemicals in form of (Alkaloids, Tannis, flavonoids, saponins, glycoside steroids, Anthraquinones etc), polyphenols (flavonoids and phenolic acids), phenols which have been exploited for pharmaceutical products (Mignouna *et al*., 2008). Dioscorin and Diosgenin are the most predominant of the phytochemicals found in yam (Kanu *et al*., 2018)

Yam also plays a significant role in African socio-cultural traditions. There are many traditions associated with yams including ceremonies and festivals, which show their importance in traditional society. A new yam festival is celebrated where people offer yams to gods and ancestors first, before distributing them to the villagers. This is their way of giving thanks to the spirits above them. In Nigeria, the people of south eastern part celebrate. The celebration is followed by various cultural dances with the display of masquerades from different clans or groups. This usually lasts to very late night. During the occasion is also a moment of reuniting old friends and family members.

# Problems Associated with Yam Production in Nigeria

Zaknayiba and Tanko (2013), find that lack of access to farm inputs, high cost of inputs, poor producer prices, high incidences of pests and diseases and inadequate of storage facilities have negatively affected yam production. Similarly, Ike and Inoni (2006); Maikasuwa and Ala (2013) results indicate that the factors of production such as labour, finance and material inputs like fertilizer have influenced on yam production. In the same direction, studies by Etim *et al*.

(2013) suggest that farmers’ education, family labour, extension contact and experience of farmers have a positive effect on the farm level technical efficiency and yam output. Similarly, Shehu *et al*. (2010) found out that land, seed yam, family labour, education, and fertilizer are the drivers that influence yam production in Nigeria.

Yam production requires high labour. The crop has low yield per hectare compared to crops such as [cassava](http://en.wikipedia.org/wiki/Cassava) ([manioc](http://en.wikipedia.org/wiki/Manioc)) or [sweet potato](http://en.wikipedia.org/wiki/Sweet_potato). It is not an efficient [food staple](http://en.wikipedia.org/wiki/Food_staple) given the relatively

large amount of planting material that is required and its long growing season. Yam's labour requirement exceeds that of other comparable crops. Yam is also difficult to preserve and store over extended periods of time. Post-harvest food losses are one of the important sources of food insecurity in Africa.

# Diseases and Common Pests Affecting Yam

Yam microbial diseases can be classified into two:

* The field diseases and
* The storage diseases.

# Yam Field diseases

The field diseases are those diseases that cause economic damage to yam in the field from the seedling stage to the point of harvest (Osunde, 2008).

# Yam Anthracnose

Yam anthracnose is a fungal infection that can impact yam in any geographical area. This is caused mostly by the fungus *Colletotrichum gloeosporioides* (Amusa *et al*.,2003). *G. cingulata* is the perfect state of *C. gloeosporioides*, the form that is usually found causing field anthracnose disease.

On susceptible yam cultivars, symptoms appear as small, dark brown spots or black lesions on leaves which may be surrounded by a chlorotic halo; leaf necrosis; dieback of stem; withered

leaves and scorched appearance. *D. Alata* appears to be more susceptible than other yam species.

# Yam Mosaic Virus Disease

This disease is caused by an aphid-transmitted potyvirus that infects several species of Dioscorea*,* particularly *D. alata*, *D. cayenensis*, *D. rotundata* and *D. Trifida* ss (Amusa *et al*., 2003). Yam mosaic virus (YMV) is considered to cause the most severe losses in yams with symptoms on yam which include; mosaic, green vein banding, shoe string and stunting. These symptoms vary depending on host species and environmental conditions. Other yam viral diseases in West Africa include; yam mild mosaic virus (YMMV), *D. Dumetorum* virus, *D. Alata* bacilliform virus (DaBV), cucumber mosaic virus (CMV), D. Mottle virus (DMoV), and

*D. Sansibarensis* virus (DsBV) (Seal & Muller, 2007).

# Yam Beetles

The yam beetle *(Heteroligus meles*) is a major pest of yam in West Africa. It lays its egg in moist ground during the early part of the dry season. This means that the eggs are usually laid in swampy areas near rivers and streams and not necessarily in the yam field. Its adult beetles that migrate by flying to yam plots being visually attracted by the foliage of the yam plants.

Yam beetles rarely kill the yam plant but they do considerable damage to the tubers that can be harvested from it. Their feeding activity leaves holes in the body of the tuber. These holes not only make the tuber unsightly and unmarketable, but they may also predispose the tuber to rotting during spoilage.

# iv Yam Nematodes

Yam nematodes (*Scutellonema bradys*) and the root knot nematode (*Meloidogyne species*) are the most serious nematodes attacking yam (IITA, 2013; Amusa *et al*., 2003). The yam nematode inhabits the layer just beneath the skin of the tuber. The activity of the yam nematodes results in extensive browning of the sub surface layer of the yam tuber and the

resultant destruction of the meristematic zone. As such, tubers that have been infected with yam nematode may have difficulty sprouting if used as planting material.

The root knot nematode attacks growing yam tubers producing a characteristic warty appearance on the surface of the tubers. Yam nematodes reproduce and build up large population in stored tubers causing severe damage and facilitating fungal and bacterial attacks that cause anthracnose disease, dry rot, soft rot and wet rot.

# Yam storage diseases

In Storage diseases, during storage, tubers are subject to losses of up to 50% of the fresh matter. Here, the losses due to microbial attack play a predominant role. The fungal pathogens penetrate through wounds in the tubers and infect the inner tuber tissue. Such wounds are caused by insects, nematodes and poor handling before, during and after harvest (Amusa *et al*., 2003).

Morse *et al*. (2000) reported that most of the yam rot induced by fungi in specialized barns near Idah, Kogi State, Nigeria were predisposed by insect attack by mainly storage beetles (*Coleoptera*), mealy bug (*Planococcus citri*) and sclae insect (*Aspidiella hartii*) during storage.

# Yam insects

This is caused by insects such as Mealybug (*Planococcus dioscoreae*) and scale insect (*Aspidiella hartii*) and they attack stored yams. As they feed, these insects reduce the food reserves in the tubers. This both reduces the value of the yams as food, and also makes the yams too weak to sprout vigorously when used as planting setts. Other insect pests such as the yam Moth (*Blastobasis*) and Ginger Weevil (*Elytroteinus*) also damage yams in storage. They lay eggs on stored yams and when these eggs hatch, the larvae bore into the yam and eat the flesh. As the larvae feed, they leave piles of brown droppings which can be seen on the outside of the yam. The larvae mature in the rotten yam.

# Tuber rots

Tuber rots caused by mostly fungi are particularly problematic for yams during storage but may also occur in the field. It can be classified into three;

1. **Dry rots:** Dry is considered as the most devastating of all the storage disease of yam. Dry rots are caused by *Rossellinia* and *Sphaerostilbe.* All organisms which cause rots in the stored tuber are also responsible for rotting of setts after planting.
2. **Soft rot:** Infected tissues become soft ramified by the fungal mycelium. The causal fungi quickly ramified the tissue which turn brown and become soft and at times wet due to a rapid collapse of the cell walls. Fungi associated with this type of rot are Soft rots are caused by *Penicillium* species, *Fusarium* species *and Botrydiplodia* species. *Rhizopus* spp, *Mucor circinelloides, S.rolsii*, and *Rhizoctonia solani* and *Armillariella mellea* (Ikotun, 1983, 1989; Green *et al.,* 1995; Amusa and Baiyewu, 1999).
3. **Wet rot:** Wet rot characterized by the oozing of whitish fluid out of the tissue when pressed can also be increminated. this symptom is usually associated with a bacterium, *Erwinia carotovora* pv (Amusa and Baiyewu, 1999).

# Management and Control of Pests and Diseases of Yams

A number of treatments and techniques have been developed to reduce these physiological activities and also to protect the tuber from postharvest diseases. These include treatment with chemicals, plant extracts, palm wine and gamma irradiation; storage techniques used include cold storage, improved underground storage and improved yam barns (Osunde, 2008).

Management strategies adopted and advocated for combating the field disease includes;

1. the use of crop rotation, fallowing,
2. planting of healthy material: The most effective method of controlling the disease is to plant yam varieties that are resistant to anthracnose such as TDA 291 or TDA 297
3. The destruction of infected crop cultivars.
4. Control through careful selection of tubers for planting. Cut seed should be dipped in Bordeaux mixture or with a systemic fungicide

Management of the storage diseases include;

1. the use of tecto (thiabendazole),
2. locally made dry gins or wood ash before storage has been found to protect yam tubers against fungal infection in storage.
3. Treatments of the yam tubers with insecticide dust (Actellic 2% Dust; ai=pirimiphos- methyl) significantly reduced fungal infections and also ameliorated physical damages acquired during harvest resulting in significantly fewer fungal lesions (Morse *et al.,* 2000).
4. Mealy bugs can potentially be controlled by natural enemies; chemical pesticides may decrease populations of natural enemies leading to mealybug outbreaks; horticultural oils or soapy solutions can be used to treat heavy infestations

## Pseudomonas as biocontrol agents

The genus Pseudomonas is one of the most diverse gram negative bacterial genera and has been isolated from different environments such as water, soils, animals, plants and clinical samples. A total of 128 species is officially recognized for this genus (Peix *et al*., 2009). The members of the genus are the most studied bacterial biocontrol agents with potential to produce diverse range of bioactive secondary metabolites. Some isolates have been used in commercial products in the form of bio-fungicide and bio-fertilizers (Fravel, 2005). Pseudomonas spp have activity against a wide range of plant pathogens and they do so by both direct and indirect mechanisms. Antibiosis involving antibiotics and fungal cell wall degrading enzymes are direct mechanisms of biocontrol. On the other

hand, competition for space and nutrient resources in the rhizosphere and induction of disease resistance in plants are indirect mechanisms of a plant growth promotion.

Antibiotics such as DAPG, pyrrolnitrin, pyoluteorin, phenazines and lipopeptides (LPs) are commonly produced by some of the most effective biocontrol pseudomonads both in culture (Raaijmakers *et al*. 2002; Raaijmakers *et al*. 2009; Raaijmakers *et al*. 2010; Haas and Defago 2005; D’aes *et al*. 2010; Wang *et al.* 2014) and in the rhizosphere (Kwak *et al*. 2012; Mavrodi *et al*. 2012). Extracelluar diffusibles pigments such as pyoverdine, siderophores are responsible for the fluorescence of these organisms.

2,4-diacetylphloroglucinol, oomycin A, phenazine-1-carboxylic acid, phenazine-1- carboxamide, pyocanin, anthranilate, pyrrolnitrin, pyoluteorin, hydrogen cyanide, ammonia, viscosinamide and gluconic acid are the mostly reported antimicrobial metabolites of *Pseudomonas* spp (Jayaprakashvel & Mathivanan, 2011). Bacterial isolates that produce these secondary metabolites have biocontrol efficacy against wide range of phyto-pathogens. The list of some antimicrobial metabolites produced by the genera is shown in the Table 3.

# Table 2.3: Antibiotics produced by biocontrol *Pseudomonas* spp and their target plant pathogens

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *S/No* | *Compounds*  *Produced1* | *Producer organisms2* | *et*  *plant pathogens3* | *References* |
| *1* | *2,4-DAPG* | P. aeruginosa  P. brassicacearum  P. chlororaphis  P. fluorescens  P. protegens | Ac, Cg, Fo, Ggt Pc, Pu, Rs  St, Tb  Pst | *Chung* et al*. (2008) Haas and Keel (2003), Raajimakers* et al*. (2002), Ryu* et al*. (2000)*  *Weller* et al*. (2012) Ramette* et al*. (2011)* |
| *2* | *Phenazines (PCA, PCN, PYO)* | P. aeruginosa  P. chlororaphis | Bs, Cgr, Co, Fo  Ggt, Lk, Mp, Fo | *Haas and Keel (2003), Sharmuugaiah* et al*. (2010)*  *Kapsalis* et al*. (2008),*  *Morohoshi* et al*. (2013)* |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | P. fluorescens  P. oryzihabitans Pseudomonas spp  P. aureofaciens | Psp, Pu Phythium *spp* Rs, Sh, Ti  Fo | *Ligon* et al*. (2002) Powell* et al*. (2000) Raajimakers* et al*. (2002) Mazzola* et al*. (1992)* |
| *3* | *Pyrrolnitrin (PRN)* | P. fluorescens  Pseudomonas *spp* | Ggt, Alternaria spp  Bc, Fsa, Pe, Ptr, Rs, Ss, Tb, Vd | *Upadhyay and Srivastava (2011), Haas*  *and Keel (2003)*  *Ligon* et al*. (2000), Raajimaker* et al*. (2002), Athukorala* et al*. (2010)* |
| *4* | *Pyoluteorin (PLT)* | P. flourescens  Pseudomonas *spp* | Pu, Rs, Tb | *Haas and Keel (2003)*  *Raajimaker* et al *(2002)* |
| *5* | *Hydrogen cyanide (HCN)* | P. corrugata  P. aeruginosa  P. fluorescens Pseudomonas *spp*  P. brassicacearum | Bc, Tb Mj | *Gross and Loper (2009) Siddiqui* et al*. (2003) Guo* et al*. (2007)*  *Haas and Keel (2003)*  *Zhou* et al*. (2012)* |
| *6* | *Aerugine* | P. fluorescens | Co, Pu, Pc | *Lee* et al*. (2003)* |
| *7* | *Iturins (Cyclic lipopeptides)* | P. asplenii  P. fluorescens | Gc, Pd, Pi, Pim  Pu, Rp, Rs | *Hultberg* et al*. (2010)*  *Raajimakers* et al*. (2006)* |
| *8* | *Oomycin A* | P. fluorescens | Pu, Phythium *spp* | *Ligon* et al*. (2000)* |
| *9* | *Rhamnolipids* | P. aeruginosa  P. chlororaphis | Phythium *spp* | *Ligon* et al. *(2000)* |

Adapted from Someya *et al* (2013)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *10* | *Trigliceropeptides* | P. chlororaphis  P. putida | Aa, Fusarium *spp*,  Hs, Pf | *Chetverikov and Loginov*  *(2005)* |

1Compounds produced

2species that are responsible for the production of each antibiotics

3plant pathogen controlled by the antibiotics. Abbreviations: *Ac, Aphanomyces cochlioides, Cg Collectotrichum gleosporioides, Fo Fusarium oxysporum, Ggt Gaeumannomyces graminis var tritici, Pc Phytophthora capsici, Pu Phythium ultimum, Rs Rhizoctonia solani, St Septonia tritici, Tb Thielabiopsis basicola, Pst Pseudomonas syringae pv tomato, Bs Bipolaris sorokiniana, Cgr Collectotrichum graminicol, Co Collectotrichum orbiculae, Lk Leptosphaeria korrae, Mp Magnaporthe poae, Psp Phythium splendens, Sh Sclerotinia homoecarpa, Ti Typhyla incarnata, Bc Botrytis cinerea, Fsa Fusarium sambucinum, Pe Penicillium expansum, Ptr Pyrenophora triticirepentis, Ss Sclerotinia sclerotiorum, Vd Verticillium dahliae, Mj Meloidogyne javanica, Gc Geotrichum citriaurantii, Pd Penicilium digitatum, Pi Phytophthora infestans, Pim Phythium intermedium, Rp Rhodotorula pilimanae, Aa Alternaria alternata, Hs Helminthosporium sativum, Pf Penicillium funiculosum*

Although detailed mechanisms of action for most of these antibiotics are not known, the mechanism of biocontrol activity have been demonstrated for some. One of the better known antibiotics produced by biocontrol Pseudomonas isolates, DAPG, causes growth inhibition and excessive branching of hypha in fungi by disrupting the filamentous actin cytoskeleton (Islam and Fukushi, 2010). Hydrogen cyanide is generated due to oxidation of glycine that produces HCN and CO2 by a membrane-bound flavoenzyme (HCN synthase). HCN is a potent inhibitor of cytochrome C oxidase and other mettalloenzymes (Blumer and Haas, 2000). Cyclic lipopetide (CLP) causes immobilization and subsequent cell lysis of the entire zoosphere of *Phytophthora infestans* (de Bruijin *et al*., 2007). Pyrrolnitirn inhibits growth synthesis of protein, RNA, DNA and uptake of metabolites of pathogens (Pang *et al*., 2009)

## Pseudomonas fluorescens

*Pseudomonas fluorescens* encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. *Pseudomonas fluorescens* is a gram negative, rod-shaped, and non-pathogenic bacterium that is motile by means of multiple polar flagella.

It is an obligate aerobe, except for some strains that can utilize NO3 as an electron acceptor in place of O2.

*Pseudomonas fluorescens* is a widely known biocontrol agent against many soil borne plant pathogens because they are well adapted in soil. Some strains of the *P. fluorescens* have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases (Hoffland *et al*. 1996, Wei *et al*. 1996). *P. fluorescens* are well characterized for their ability to produce many antimicrobial compounds (Haas and De’ fago, 2005).

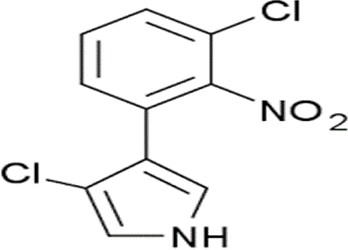
* + 1. **Bioactive Metabolites of *Pseudomonas fluorescens***

Some bioactive metabolites produced by *Pseudomonas fluorescens* and their synthesis are discussed as follows;

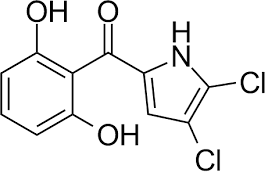
# Pyrrolnitrin (PRN) and Pyoluteorin (PLT)

Pyrrolnitrin and pyoluteorin are tryptophan-derived metabolites produced mainly by gram negative bacteria as *Pseudomonas* spp, *Burkholderia* spp., and *Serratia* spp. Pyrrolnitrin are monochlorinated heteroaromatic pyrrole rings while pyoluteorins possess dichlorinated rings (Pang *et al*., 2015; Schmidt *et al*., 2009). No mode of action has been published for pyoleutorin although pyrrolnitrin has been reported to inhibit respiratory electron transport and synthesis of proteins. They also combine with cell membrane phospholipids thus affecting transport (Haas and De fago 2005; Nose and Arima 1969; Tripathi and Gottlieb, 1969).

Arima *et al.* (1964) described it for the first time as antifungal substance produced by *Burkholderia pyrrocina.* However, its production has also been reported from *P. fluorescens*, *Pseudomonas chlororaphis, Pseudomonas aureofaciens, Burkholderia cepacia,* and *Myxococcus fulvus.*



**Figure 1: Chemical Structure of Pyrrolnitin (**[**https://pubchem.ncbi.nlm.nih.gov/compound/Pyrrolnitrin**](https://pubchem.ncbi.nlm.nih.gov/compound/Pyrrolnitrin)**)**

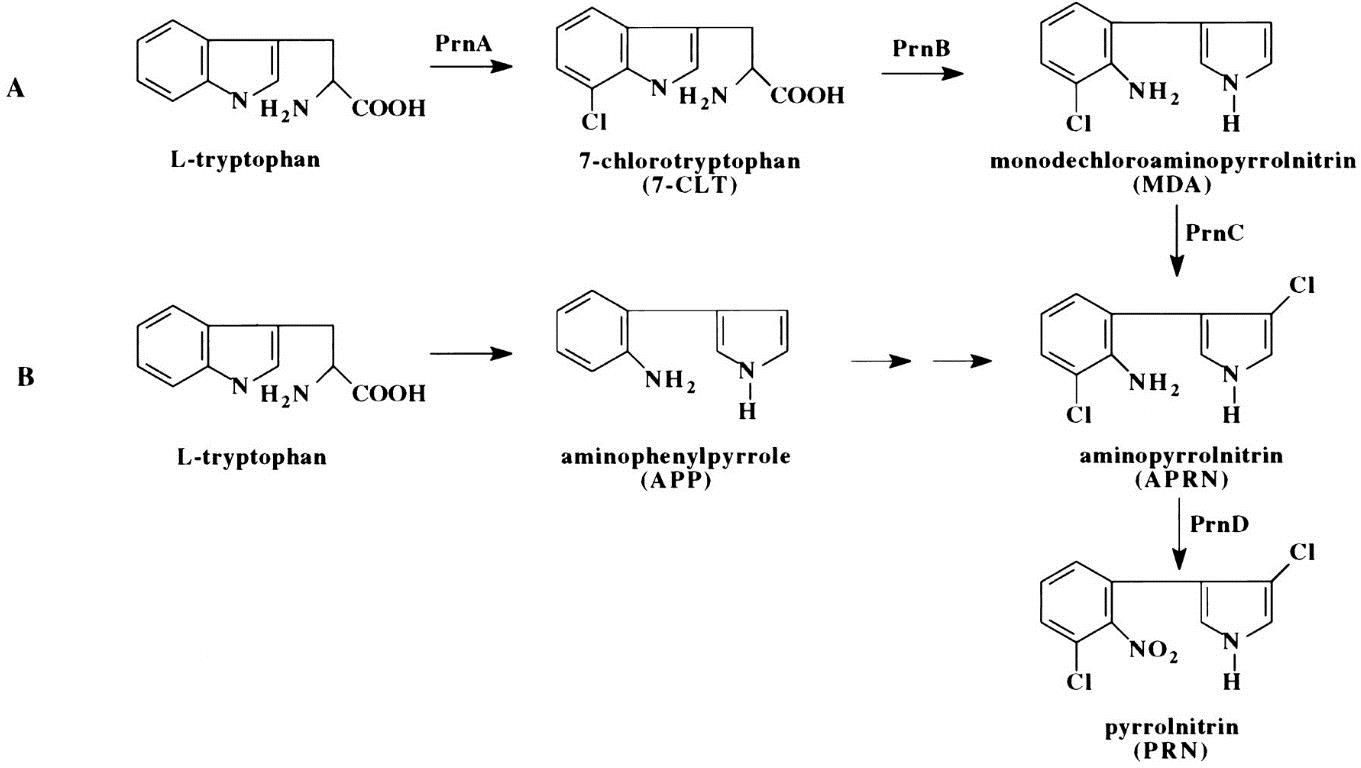


**Figure 2: Chemical Structure of Pyoluteorin (**[**https://pubchem.ncbi.nlm.nih.gov/compound/Pyoluteorin**](https://pubchem.ncbi.nlm.nih.gov/compound/Pyoluteorin)**)**

# Biosynthesis of Pyrrolnitrin

Synthesis of PRN occurs from aromatic amino acid, tryptophan, in a multistep process mediated by various enzymes encoded by prnA, prnB, prn C, and prnD genes of PRN operon (Hamill *et al.,* 1970). Product of prnA gene catalyzes the chlorination of l-tryptophan to 7- chlorotryptophan

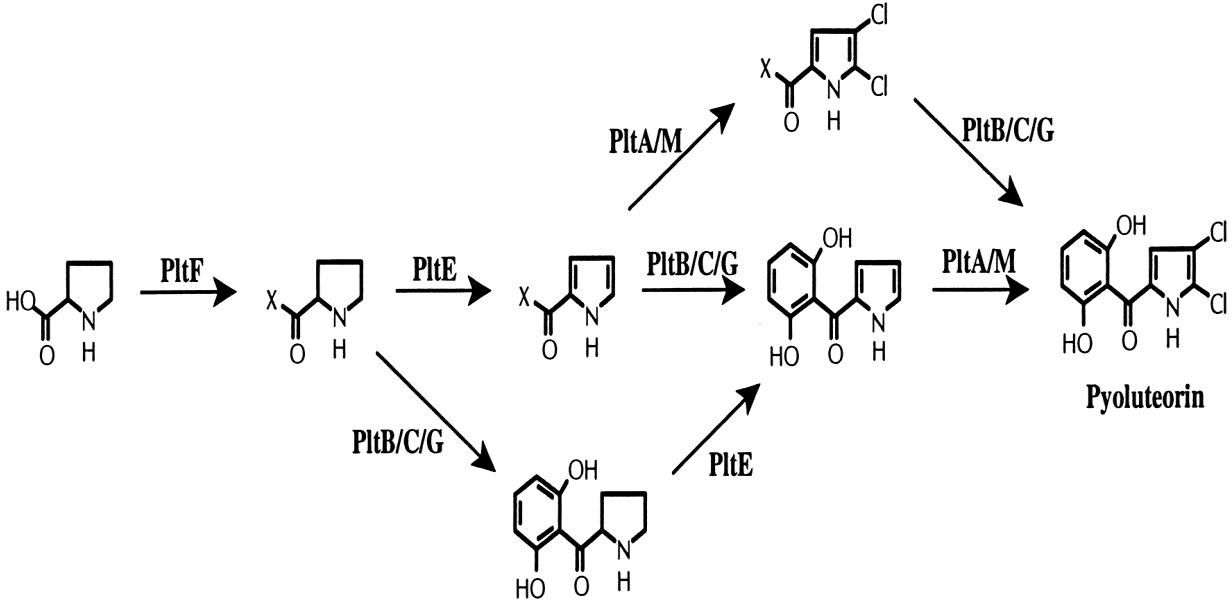
followed by its conversion to aminopyrrolnitrin (APRN) via, monodechloroamino pyrrolnitrin (MDA) byproduct of prnB and prnC genes while as product of prnD gene catalyzes the final step of oxidation of APRN to PRN. PRN operon is regulated by a global regulator gene, gac A (gac is an acronym for global activator of antibiotic and cyanide synthesis) (Duffy and Defago 2000; Haas and Keel, 2003). Howell and Stipanovic (1979) reported pyrrolnitrin as an effective metabolite produced by the soil-inhabiting *P. fluorescens* responsible for imparting protection to cotton seedlings against infection by *Rhizoctonia solani*. Tazawa *et al.* (2000) reported that pyrrolnitrin produced by *P. fluorescens* is associated with decline in takeoff disease of wheat.



# Figure 3: Biosynthetic pathways for PRN as proposed by Van Pee *et al*. (1980) (A) and by Chang *et al*. (1981) (B). Adapted from Sabine Kirner *et al*.

* + - 1. **Biosynthesis of Pyoleutorin**

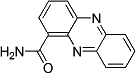
This occurs from amino acid proline in a series of coupled condensation reactions followed by chlorination and oxidation at different stages of the multistep reaction catalyzed by at least 10 enzymes encoded by 10 genes (plt L, M, R, A, B, C, D, E, F, G) of PLT operon (Kraus and Lopez 1995; Nowak-Thompson *et al.,* 1997). Among them, plt B and plt C encode Type I polyketide synthetase; plt A, plt D, and plt H encode three halogenases; and plt G encodes thioesterase. PLT operon is regulated by plt R gene, which acts as transcriptional activator of the operon. It shows resemblance with Lys R family of transcriptional factors. Production of PLT is mainly associated with the inhibition of oomycete class of fungus including *Phythium ultimum* causing Phythium damping disease (Nowak-Thompson *et al.,* 1999).



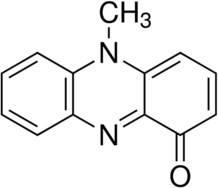
**Figure 4: Hypothetical pyoluteorin biosynthetic pathways as described by** Brian Nowak- Thompson *et al*. (1999).

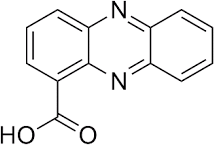
# Phenazines (PHZ)

Phenazines are nitrogen containing heterocyclic pigmented compounds, known for their broad spectrum antifungal activity, synthesized by a wide range of bacterial genus including *Pseudomonas, Burkholderia, Brevibacterium, Nocardia, Erwinia, Vibrio, Pelagiobacter* and some actinomycetes like Streptomyces (Kennedy *et al*., 2015; Mavrodi *et al*., 2010; Morohoshi *et al*., 2013). The core structure of phenazines is a pyrazine ring (1,4- diazabenzene) exhibiting two annulated benzenes. Fluorescent Pseudomonad members including strains of *P. fluorescens, P. chlororaphis,* and *P. aeruginosa* are best known PHZ producers. *P. aeruginosa*, a common soil inhabitant and opportunistic human pathogen, is the first PHZ-producing micro-organism reported to produce characteristic blue green pigment pyocyanin (PYO, 5-N-methyl-1-hydroxyphenazine). Besides

producing PYO, it also produces several other PHZs like 1-hydroxy-phenazine, phenazine-1- carboxylic acid (PCA), phenazine-1-carboxamide (PCN), aeruginosin A (5-methyl-7- amino-1- carboxymethylphenazinium betaine), and aeruginosin B (5-methyl-7-amino- 1- carboxy-3- sulfophenazinium betaine) (Holliman 1969; Byng *et al.,* 1979). Phenazines, such as PCA, PCN and PYO are toxic to a wide range of organisms. More than 6,000 phenazines derivatives have been described with one or other bioactivity (Mavrodi *et al*., 2006; Pierson & Pierson, 2010). Several modes of actions of phenazines have been identified, which include the reduction of molecular oxygen to reactive oxygen species (ROS), (Mahajan *et al*., 1999), the facilitation of energy generation (Glasser, *et al*., 2014; Wang *et al*., 2010; Prince-Whelan, 2007), involvement in iron homeostasis via Fe(III) reduction (Hernandez *et al*., 2004; Wang and Newman, 2008), participation as signal molecules via the activation of the Fe-containing transcription factor SoxR (Dietrich *et al*., 2008; Dietrich *et al*., 2006; Gu and Imlay, 2011) DNA p–p interaction and intercalation (Hollstein and vanGemert, 1971) and biofilm morphogenesis (Dietrich *et al*., 2013) through influencing the intracellular redox state. Phenazines are structurally related compounds but PCN and PCA have distinct effects on *Fusarium oxysporum f.sp. radicis-lycopersici*, which illustrate the difficulty of structure based prediction of antifungal activity (Chin-A-Woeng *et al*., 2001). Differences between phenazines in their functional groups also likely change their solubility and capacity for electron shuttling, especially at different pH levels (Cezairliyan et al., 2013; ChinA-Woeng et al., 2003; Pierson and Pierson, 2010).

# Phenazine-1-carboxamide (PCA)

**Pyocyanin**



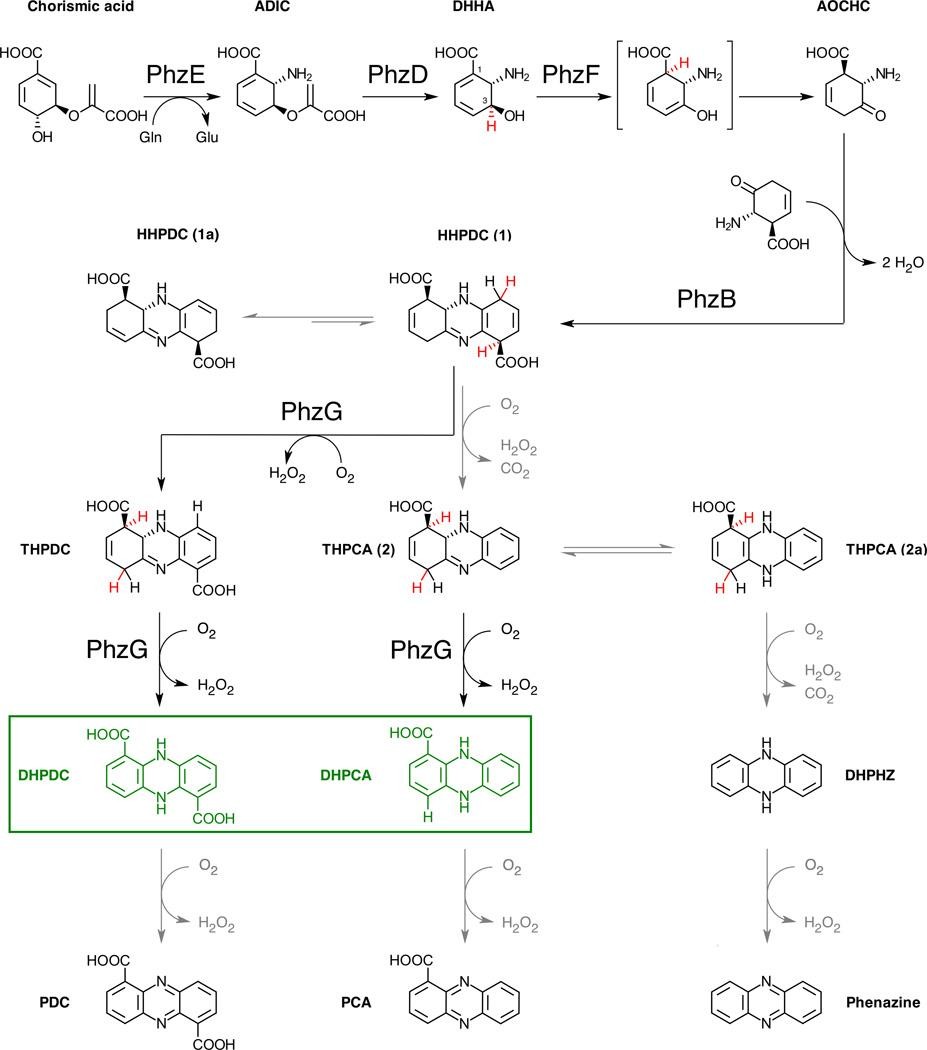
# Phenazine-1-carboxylic acid (PCN)

**Figure 5: Structures of some common phenazines produced by fluorescent *Pseudomonas* spp (Pierson and Pierson, 2010)**

# 2.11.3.1 Phenazine Biosynthesis

The phenazines are a class of over 150 bacterial secondary metabolites with redox properties that enable them to act as broad specificity antibiotics as well as virulence and survival factors e.g. in infections caused by the opportunistic pathogen *Pseudomonas aeruginosa* (Okegbe *et al*., 2012; Guttenberger *et al*., 2017). The five enzymes responsible in phenazine synthesis namely phzB, phzD, phzE, phzF and phzG are conserved among all phenazine-producing bacteria. Phenazine synthesis was shown to proceed via the shikimic acid pathway with chrosmic acid as the branch point intermediate and glutamine the source of nitrogen in the tricyclic moiety (Mentel *et al*., 2009; Turner and Messenger, 1986). Their biosynthesis involves the condensation of two chorismate-derived

precursors into the symmetrical phenazine scaffold (Fig. 6)). In this pathway, PhzF was found to act as an isomerase of (2 *S*,3 *S*)-2,3-dihydro-3-hydroxyanthranilic acid (DHHA) (Parsons *et al*., 2004; Blankenfeldt *et al*., 2004) The reaction is believed to require two chemical steps, namely a hydrogen shift from C3 to C1 of the substrate and a subsequent tautomerization of the enol intermediate. Two molecules of the ensuing highly reactive ketone (1 *R*,6 *S*)-6-amino-5-oxo-2-cyclohexene-1- carboxylic acid (AOCHC) then undergo condensation to hexahydro-phenazine-1,6-dicarboxylic acid (HHPDC), followed by oxidations to dihydro-phenazine-1,6-dicarboxylic acid (PDCH2) and/or dihydro-phenazine-1-carboxylic acid (PCAH2) as precursors for strain-specific derivatives (Mentel *et al*., 2009; Blankenfeldt *et al*., 2014).

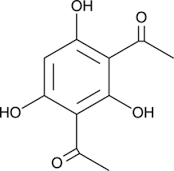


**Figure 6: Current understanding of core phenazine biosynthesis** (Blankenfeldt and Parson, 2014)

# 2, 4-Diacetylphloroglucinol

Among the phloroglucinols, 2,4-diacetylphloroglucinol (2,4-DAPG) is well known. DAPG, is a benzenetriol in which two of the ring hydrogen are replaced by acetyl group. 2,4- Diacetylphloroglucinol (DAPG) is a polyketide antibiotic that has antiviral, antibacterial, antifungal, antihelminthic, and phytotoxic properties (Bangera and Thomashow, 1999; de Souza *et al*., 2003a, b; Haas and Keel, 2003; Isnansetyo, *et al*., 2003; Kamei and Isnansetyo, 2003; Keel *et al*.,1992). It has been known as an excellent bio-control agent to suppress the take-all, a serious soil borne disease of wheat all over the world (Weller *et al*., 2002; Kwak *et al*., 2012).

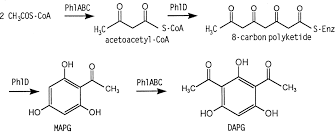
The mechanism of action of 2, 4-DAPG against phytopathogens is not fully elucidated, but it was reported to damage membranes and zoospores of *Pythium* spp. (de Souza *et al.,* 2003a). (Troppens *et al* 2013) demonstrated that DAPG produced by *Pseudomonas fluorescens* alters mitochondrial morphology with loss of the membrane potential and increase of cytotoxic Ca2+ in *Neurospora crassa*. Besides the direct activity against pathogens (Keel *et al.,* 1992; Ramette *et al.,* 2001), the antibiotic also protects plants indirectly by inducing resistance in *Arabidopsis thaliana* against *Peronospora parasitica* (Iavicoli *et al.,* 2003) and *Pseudomonas syringae* pv. *tomato* (Weller *et al.,* 2004). 2, 4-DAPG also affects root physiology directly by blocking amino acid uptake (Phillips *et al.,* 2004). 2, 4-DAPG is an auto-inducer of its own synthesis and can function as a molecular signal (Schnider-Keel *et al.,* 2000), influencing antibiotic expression in neighboring strains (Maurhofer *et al.,* 2004). However, its production is affected by a large number of abiotic factors, such as Fe3+, Zn2+ Cu2+, Mo2+, and glucose.



**Figure 7: Chemical Structure of 2,4-diacetylphloroglucinol (**[**https://pubchem.ncbi.nlm.nih.gov/compound/2\_4-Diacetylphloroglucinol**](https://pubchem.ncbi.nlm.nih.gov/compound/2_4-Diacetylphloroglucinol)**)**

# 2.11.4.1 Biosynthesis of DAPG

DAPG is synthesized by condensation of three acetyl-CoenzymeA molecules with one molecule of malonyl-CoenzymeA to produce the precursor monoacetylphloroglucinol, which is subsequently transacetylated to generate PHL utilizing a CHS-type enzyme (Shanahan *et al*., 1992a).



**Figure 8: Proposed roles of PhlA, PhlB, PhlC, and PhlD in the biosynthesis of MAPG and 2,4- DAPG (**Bangera and Thomashow, 1999**)**

The biosynthetic locus includes the five-gene operon phlACBDE (Bangera and Thomashow,1999). Particularly noteworthy is phlD, which is required for the synthesis of phloroglucinol, a precursor of monoacetylphloroglucinol (MAPG) and 2,4-DAPG (Achkar *et al*., 2005). PhlD has remarkable homology with members of the chalcone synthase/stilbene synthase (CHS/STS) family of plant enzymes (Bangera and Thomashow,1999; Ramette *et al*., 2001).

# Use of agro-waste as part of media formulation for growth of microorganisms

Large amount of wastes is generated every year from the industrial processing of agricultural raw materials. Most of these wastes are used as animal feed or burnt as means for elimination. However, such wastes usually have a composition rich in sugars, minerals and proteins, and therefore, they should not be considered “wastes” but raw materials for other industrial processes.

Wastes derived from agricultural activities include materials such as straw, stem, stalk, leaves, husk, shell, peel, lint, seed/stones, pulp or stubble from fruits, legumes or cereals (rice, wheat, corn, sorghum, barley), bagasses generated from sugarcane or sweet sorghum milling, spent coffee grounds, brewer’s spent grains, and many others. The presence of carbon sources, nutrients and moisture in these wastes provides conditions suitable for the development of microorganisms. Additionally, the agro-industrial wastes may be used in these processes as solid support, carbon, nitrogen and/or mineral sources, which would allow obtaining more economical fermentation processes avoiding the use of expensive chemical components in the media formulation.

Media are used for selective and differential cultivation of microorganisms (Seddon and Boriello, 1989; Taylor and Holland, 1989; Pelczar *et al*., 1993). Culture media used in the laboratory for the cultivation of microorganism supply the nutrients required for their growth and maintenance. Microorganisms can obtain energy directly from sunlight (autotrophs) while carbon can be made available in organic forms such as carbohydrates or inorganic forms such as carbon dioxide (Madigan *et al*., 2000).

Plant materials have been used to recover both fungi and bacteria from different sample sources such as Groundnut, Sorghum extracts, local food stuff waste, cassava whey, three – leaf yam, African oil bean, maize, beans and pigeon pea (Famurewa and David, 2008).

Different media for the growth and isolation of organisms have been reported from different substrates. Nkene (2015) used cassava peel, maize corn powder, banana peel and malt powder as carbon sources while soybeans and groundnut cake were used as nitrogen sources for the production of bioactive metabolites by *Pseudomonas fluorescens* against *Erwinia carotovora* isolates from rotten yam. The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported (Tharmila *et al*., 2011; Arulanantham *et al*., 2012; Ravimannan *et al*., 2014).

Laleye (1990) reported a modification of potato medium supplemented with cow dung, soy milk and other growth factors. These media supported the growth of the test organisms luxuriantly when compared with growth on conventional Nutrient (Oxoid) and Potato Dextrose Agar (Oxoid). Also Adesemoye and Adedire (2005) studied the feasibility of developing alternative media to Potato Dextrose Agar (PDA) using local cereal species as the basal media.

# CHAPTER THREE MATERIALS AND METHODS

# Materials

# Equipment

Autoclave (Portable 230V and 1850W Adelphi MFG CO. Ltd, England), incubator (National Appliance Co. Ltd, Oregon, USA: model 1630, 240V and 2340W), hot air oven (Baird and Tatlock (London) Ltd, Chad Well Health Essex, England), refrigerator (Haier Thermocool: Model No. HRF-688-FF/A), microscope (Wild Heerbrugg M11, made in Switzerland), bunsen-burner, UV-Visible spectrophotometer (Eppendorf Biophotometer 8,5mm, Lichtstrahihohe), UV iluminator (Vilberb Lourmat TFX-35-M serial no NoV02 8104), centrifuge (Eppendorf 5417R), micropipette, microwave oven (HINARI Life Style 800watts model MX310TCSL), Electronic weighing balance (QT 600), Vortexing machine (Touch plate Super Mixer, CAT No 1291 Lab-line Instrument Inc USA) were the equipment used for this study

# Glass wares

Glass wares such as Petri-dishes, beakers, bijou bottles, universal bottles, glass slides, test tubes, measuring cylinder, separating funnel, Eppendorf tube, magnetic stirrer (W and T Avery Ltd., England) were used for this study.

# Reagents and Chemicals

All reagents and chemicals that were used in this study include; Methyl red, Phenol red, Kovac’s reagent, calcium carbonate, magnesium sulfate, α-napthylamine, sulphanilic acid, zinc dust, buffer, potassium hydroxide, sodium hydroxide, ethanol, ethyl acetate, dipotassium hydrogen phosphate, glycerol, peptone mixture from BDH Chemical Ltd., England.

# Culture media

The media that were used in this study include; Nutrient agar, King’s B Agar, Cetrimide agar, SDA (Sabourand Dextrose Agar) from Oxoid Ltd, England.

# Methods

* + 1. **Isolation of *Pseudomonas fluorescens***

The isolation of *Pseudomonas fluorescens* was carried out on Cetrimide agar. Briefly, one gram (1g) of soil sample from rice farm, sugar cane farm and soybeans farm was suspended in 9ml of sterile distilled water. 0.1ml of sample was spread on plates containing the medium and incubated at 37°C for 24hours.

* 1. **Identification of *Pseudomonas fluorescens***

Standard microbiological tests were conducted for rapid identification of *Pseudomonas* colonies on the plates. Two parameters were used for the identification and confirmation of *P. fluorescens*, namely; cultural characteristics and biochemical test.

# Microscopic characteristics

Gram staining of the isolate was carried out as described by Chessbrough (2006). A smear of the isolate was made on a slide. The smear was heat fixed and stained with the primary stain crystal violet and allowed to stand for 60 seconds. The stain was rinsed gently with water. The culture was fixed with Lugol’s iodine solution for c60seconds, decolourized with ethanol and then counterstained with safranin. The stained slide was examined under an oil immersion lens and the isolate was classified as gram positive or gram negative.

# Cultural characteristics

The colony morphology and whether there was production of pigment was observed by growing the isolate on cetrimide agar.

# Growth at 4°C and 42°C

The ability of the isolates to grow at this temperature was determined by growing them in Cetrimide agar medium at respective temperatures.

# Biochemical tests

Biochemical tests such as oxidase, catalase, gelatin hydrolysis, and nitrate reduction test (Reynold, 2004) was carried out to confirm the organism.

# Oxidase Test

The oxidase test for the suspected organism was carried out as described by Chessbrough (2006). Bacterial inoculum was smeared on a moist filter paper and a drop of 1% tetramethyl- p-phenylenediaminedihydrochloride was added to the inoculum. Immediate colour change to purple gave a positive result.

# Catalase Test

Two (2) Millilitres of the hydrogen peroxide solution was poured into a test tube and a sterile glass rod (not a nichrome wire loop) was used to remove several colonies of the test organism and immerse in the hydrogen peroxide solution (Chessbrough, 2006). Formation of bubbles indicated a positive reaction, while the ones without any bubbles showed negative reaction.

# Indole Test

The indole test was carried out as described by Chessbrough (2006). Bacterial inoculum was inoculated in 5ml of peptone water in Bijou bottle and it was incubated at 30°c for 24hours after which some drops of Kovac’s reagent was added. Formation of red ring indicated indole positive.

# Gelatin Hydrolysis Test

This test was performed by stabbing the inoculum of the isolate into a tube containing Nutrient broth with gelatin used as the solidifying agent. Liquefied gelatin showed a positive result, while solid gelatin or non-liquefied showed negative result.

# Nitrate Reduction Test

This test was conducted to determine the ability of the isolates to reduce nitrate to nitrite or further to free nitrogen gas. Peptone Nitrate broth with Durham tube was prepared in a screw- cap tube. The medium was inoculated with a loop-full of test culture and the inoculated broth was incubated for 24 hours at about 37° C. After incubation, 0.5 ml of reagent A which is Sulphanilic acid was added and then reagent B α- napthylamine was added and the test tube was observed for development of red colour.

# Sugar Fermentation Test

An inoculum from a 24hours culture of the bacteria was transferred aseptically to a tube containing 5ml of nutrient broth tube containing phenol red indicator and different sugars; glucose, lactose, galactose, fructose, mannitol, arabinose, maltose and sorbitol. The inoculated tube was incubated at 37°C for 24 hours. A positive test result for the sugar fermentation showed a colour change from red to yellow, indicating a pH change to acidic.

# Arginine Di-hydrolase Test

An inoculum from a 24hours culture was transferred aseptically to a sterile tube of arginine di- hydrolase broth. The inoculated tube was incubated at 37° C for 24 hours and the preliminary results was determined. The microbe first uses the glucose present to cause the pH to drop. This was indicated by a change from purple to yellow. Once the medium has been acidified, the enzyme arginine di-hydrolase is activated. The culture was incubated an additional 24 hours at 37°C to allow the microbe to now use the arginine. The final results were obtained by observing the tube at 48 hours. Change back to purple from yellow indicated a positive test for

arginine di-hydrolase. Failure to turn yellow at 24 hours or to revert back to purple at 48 hours indicated a negative result.

# Isolation and Identification of Phyto-pathogenic fungi

The three (3) test organisms (*A. niger, P. citrinum*, *Rhizopus stolonifer)* were isolated on Sabourand Dextrose Agar (SDA) medium from rotten yam using the method described by Okigbo (2005). The rotten yam tubers were first surfaced sterilized by swapping with cotton wool soaked in 70% ethanol. A sterilized knife was used to cut open the yam and portion of the rotten part was crushed & mashed into small pieces and teased out in 10ml of sterile distilled water in a bottle and this was shaken to distribute the pathogen from the yam evenly. A loopful of the inocula was collected from the suspension of the test sample and cultured on the SDA medium.

The medium was prepared according to manufacturer’s instruction. A measured quantity of the powdered medium was dispensed into 1L of distilled water in a conical flask and mixed thoroughly. This was sterilized by autoclaving at 121°C for 15minutes. The sterile medium was allowed to cool and it was poured into sterile petri dishes and allowed to cool and gel. The inoculated plates with the test samples were then incubated at room temperature for 7 days and on establishment of growth, the plates were examined for distinct mycelia and from such mycelia, the test organisms were collected and sub-cultured on SDA slants.

The mycelial growth was identified as the test fungi based on morphology (such as, colour, texture, presence of visible spores on the culture plate and microscopy. Microscopic examination was done by observing their asexual or sexual reproductive structures (conidia, spores and hyphae) using lacto phenol dye to stain the isolates and examine under microscope.

A drop of 70% alcohol was dropped on a microscope slide and the fungal inoculum was dropped on the alcohol. A drop of the lactophenol was added before the alcohol dried out. A cover slip was placed in a way to avoid air bubbles and this preparation was viewed under a microscope using an oil immersion lens.

* 1. **Screening for in vitro antifungal activity of crude extract of *Pseudomonas fluorescens***

# fermentation broth

# Preparation of formulated fermentation media

The compositions of the formulated fermentation media for *P. fluorescens* used in the study is shown in table 3.1

**Table 3.1: Composition of the fermentation media for growth of *Pseudomonas fluorescens***

|  |  |  |  |
| --- | --- | --- | --- |
| Compound | Amount(g/L)  M1 | M2 | M3 |
| **K2HPO4 MgSO4 CaCO3**  **Yam Peel Banana Peel Sugarcane Shaft**  **Inorganic Nitrogen source** | 0.98  0.40  0.40  20.00  -  - 2.00 | 0.98  0.40  0.40  - 20.00  -  2.00 | 0.98  0.40  0.40  -  - 20.00  2.00 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Groundnut cake** | 20.00 | 20.00 | 20.00 |

The carbon sources and nitrogen source used i.e. yam peel, banana peel, sugar shaft were peeled, dried, blended and sieved to get a fine powder. They were weighed according to the quantity needed for the media preparations.

The media prepared was autoclaved at 121°C for 15mins and the fermentation was carried out in a 250ml of conical flasks. The flasks were inoculated with fresh culture of *P. fluorescens* and was incubated at 25°C under agitation (120rpm) for 72 hours.

* + 1. ***In vitro* antifungal activities of crude extract of *Pseudomonas fluorescens***

# fermentation broth

The screening for the *in vitro* antifungal activities of *P. fluorescens* was carried out by using agar well diffusion method. The cultures were centrifuged at 10,000rpm for 15mins to obtain the cell free filtrate (Tripathi and Johri, 2002). 0.1ml of the cell free extract was introduced into the well made on Sabourand Dextrose Agar plate flooded with the spores of *Aspergillus niger, Penicillium citrinum, Rhizopus stolonifer* and the plates was incubated at 25°C for 24h. this was done in duplicate and the zone of inhibition was measured and recorded.

* 1. **Extraction of the crude extract metabolite from *Pseudomonas fluorescens* in the fermentation broth**

The method used was described by Bonsali *et al*. (1997). Broth cultures including bacterial cells were acidified with 10% Trifluoroacetic acid (TFA) to pH 2.0 and then extracted with ethyl acetate.

The ethyl acetate and broth were shaken to achieve a homogenous mixture and to extract the metabolite from bacterial cell for 20 minutes. A separating funnel was used to remove the

aqueous phase from the organic phase. A rotary evaporator was used to remove the ethyl acetate and thus the extract was obtained.

# Quantitative Measurement of Metabolite Substance in the Extract

The concentration of the unknown metabolite in the sample was determined by using a spectrophotometer. A standard solution of phloroglucinol was made in different concentrations (100, 50, 25, 12.5, 6.25 to 0.391mg/ml) with sterile distilled water as blank. The absorbance of

different concentrations of the standard phloroglucinol solution was measured using UV light spectrophotometer at wavelength of 200nm to 900nm to generate a standard curve. The standard curve was used to determine the concentration of the metabolite in the sample.

# Determination of Minimum Inhibitory Concentration of the ethyl acetate fraction against test fungal spores

# *In vitro* Minimum inhibitory concentration of ethyl acetate fraction against test fungal spores

Ten millilitres (10ml) volume of double strength SDA was melted and mixed aseptically with 10mls volume of varying concentration of the test antifungal crude extract metabolite. Each mixture was aseptically poured into sterile plates and allowed to set. The standardized spores of the test fungi was inoculated (10.0µl) in duplicates on sterile filter paper discs and was placed equidistance on the SDA test antifungal plates. The inoculated organisms were allowed pre-diffusion period of 30minutes. The plates were then incubated at 30°C for 48hours.

The first lowest concentration that showed no growth of the inoculated test fungi spores was considered as the MIC of the test antifungal metabolites.

# *In vivo* minimum inhibitory concentration of ethyl acetate fraction against test fungal spores on yam tuber

This test was carried out on yam tuber. Healthy yam tubers were selected, surfaced sterilized with 70% ethanol and peeled with sterilized knife. The yam was sliced into cube shape and surface sterilized with 70% ethanol and rinsed with sterile distilled water and allowed to dry. The sliced yam was treated with different concentration of the crude extract metabolite by dipping the yam slice in a beaker containing prepared concentration of the extract. The sliced yam was allowed to dry and then a well was made using a sterile 10mm cork borer and this well was inoculated with 0.1ml of adjusted suspension (106 CFU/ml) of the phyto-pathogenic fungi *Aspergillus niger, Penicillium citrinum* and *Rhizopus stolonifer* using a micro pipette.

Yam slices treated with extracted metabolite with no organism inoculated served as negative control while yam slices treated with distilled water and inoculated with the organism served as positive control. The treated yam slices were placed in a desscicant cubic glass cabinet disinfected with 70%(v/v) alcohol. The cabinet was provided with 90% relative humidity by placing a sterile petri dish containing sterile normal saline. The treated yam slices were incubated for 7 days.

The minimum inhibitory concentration of the crude extract that inhibited visible rot was recorded as the *in vivo* MIC

# Statistical analysis

All the data obtained from the studies were expressed as Mean ± SEM (Standard Error of Mean) and ANOVA was used to as the statistical tool. Data were also presented in the form of figures and tables.

# CHAPTER FOUR RESULTS

* 1. **Isolation and identification of *Pseudomonas fluorescens***

The cultural, morphological and biochemical characteristics of *P. fluorescens* isolates from rhizosphere of sugarcane, rice and soybeans farms in Zaria was done and the result is in the Table 4.1 and 4.3. Out of six (6) soil samples of the rhizosphere of the plants such as 2 from sugarcane, 2 from rice and 2 soybeans, only the sugarcane and rice farm isolates were confirmed *Pseudomonas fluorescens* isolates.

# Isolation and identification of fungi from rotten yam

The morphological characteristics of the fungi species isolated as seen on the plate is presented/shown in Table 4.3. The fungi organism was confirmed after microscopic identification using Bergey’s Manual for identification.

**Table 4.1: Cultural and Morphological Characteristics of *Pseudomonas fluorescens***

# isolates on Cetrimide agar

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Soil samples** | **Growth on Cetrimide** | **Elevation** | **Surface** | **Gram staining** | **Shape** | **Growth at**  **4ºC 41ºC** |
| **Sugarcane A1 Sugarcane A2 Rice B1**  **Rice B2**  **Soybeans C1 Soybeans C2** | +  +  +  +  -  + | Flat Flat Flat Flat  -  Flat | Circular Circular Circular Circular  -  Circular | -  -  -  -  -  + | Rod Rod Rod Rod  -  Rod | + -  + -  + -  + -  - -  - - |

Key: + growth, - no growth, Gram staining; + Gram positive, - Gram negative

**Table 4.2: Biochemical characterization of *Pseudomonas fluorescens* isolates from rice & sugarcane rhizosphere**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolates** | **Oxidase test** | **Catalase test** | **Indole test** | **Gelatin hydrolysis** | **Nitrate reduction** | **Arginine dihydrolase test** | **Sugar fermentation**  **Glu Lac Suc Mal**  **test** |
| **Sugarcane A1**  **Sugarcane A2**  **Rice B1 Rice B2** | +  +  +  + | +  +  +  + | -  -  -  - | +  +  +  + | +  +  +  + | +  +  +  + | + - - +  + - + -  + - + +  + - + + |

Key: + positive to test, - Negative to test, Glu- Glucose, Lac- Lactose, Suc- Sucrose, Mal- Maltose

# Table 4.3: Morphological characteristics of isolated fungi species

|  |  |  |  |
| --- | --- | --- | --- |
| **Source of fungi** | **Mycelial colour** | **Reverse pigmentation** | **Organism** |
| **Rotten yam**  **Rotten yam**  **Rotten yam** | Initially white, quickly becoming black when matured with conidial production  Initially white when matured has a central gray colour with a white outer edge  Has a white fluffy cottony growth  covering the entire plate | Pale yellow  Pale yellow  Cream | *Aspergillus niger*  *Penicillium citrinum*  *Rhizopus stolonifer* |

* 1. **: Antifungal activities of crude extract of *Pseudomonas fluorescens* fermentation broth against the test fungi spores**

The antifungal activities of crude extract of *P. fluorescens* isolates from rhizosphere of sugarcane and rice farms against phyto-pathogenic fungi from rotten yam tubers using the formulated media (M1, M2 and M3) is as shown in Table 4.4.

# Table 4.4: Antifungal activities against test fungi spores by the crude extract from

***Pseudomonas fluorescens* fermentation broth**

# Keys: M1= Medium 1 containing same quantity of yam peel and groundnut cake as carbon & nitrogen sources

**M2= medium 2 containing same quantity of banana peel and groundnut cake as carbon & nitrogen sources**

# M3= medium 3 containing same quantity of sugarcane shaft and groundnut cake as carbon & nitrogen sources

There was statistically significant difference (p< 0.05) in antifungal activities of the crude extract of *Pseudomonas fluorescens* isolate on the test organisms in M1. The crude extract of isolate SGA1 had the lowest mean of activity with no activity against the three (3) test organisms. Either the organism

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Diameter of Zones of Inhibition (Mean±SEM) (mm)** | | | | | | | | | |
|  | ***Aspergillus niger***  **M1 M2** | | **M3** | ***Penicillium citrinum***  **M1 M2** | | **M3** | ***Rhizopus stolonifer***  **M1 M2 M3** | | |
| ***Pseudomonas***  ***fluorescens***  **isolates** |  | | |  | | |  | | |
| **Sugarcane A1** | 0 | 0 | 0 | 0 | 20.50±0.71 | 27.00±1.41 | 0 | 0 | 0 |
| **Sugarcane A2** | 20.50±0.71 | 24.50±0.71 | 0 | 19.00±1.41 | 25.00±1.41 | 24.50±0.71 | 14.00±1.41 | 31.50±2.12 | 0 |
| **Rice B1** | 34.00±1.41 | 0 | 19.00±1.41 | 24.00±1.41 | 0 | 29.50±0.71 | 20.50±0.71 | 39.00±1.41 | 20.50±0.71 |
| **Rice B2** | 24.50±0.71 | 30.50±0.71 | 0 | 29.00±1.41 | 33.50±2.12 | 20.50±0.71 | 21.50±2.12 | 31.00±1.41 | 0 |

doesn’t produce metabolite substance or the metabolite substance produced is too small to inhibit the test organisms. Rice B1 had the best mean of activity against the test organisms (Appendix 3)

There was no statistically significant difference (p> 0.05) in the antifungal activities of the crude extract metabolite of *P. fluoresens* isolates on the test organism in M2. SGA1 had the lowest activity compared to the remaining *P. fluorecens* isolates. Rice B2 had the best mean activity against the test organism (Appendix 4)

There was statistically significant difference (p< 0.05) in the antifungal activities of the crude extract of *P. fluorescens* isolates on the test organism in M3. Rice B1 had the best mean activity against the test organisms (Appendix 5).

# Antifungal activities of the ethyl acetate fraction of fermentation broth of

## Pseudomonas fluorescens

Only three *Pseudomonas fluorescens* isolates i.e. sugarcane A2, rice B1 rice B2 were used with two media formulation (M1 &M2) for the extraction of the crude extract metabolite. A total of 6 crude metabolite extract were collected after the complete removal of the aqueous phase with a separating funnel and the solvent ethyl-acetate with rotary evaporator.

The results of their antifungal test are presented in Tables 4.5 & 4.6.

**Table 4.5: Antifungal activities of ethyl acetate fraction of M1 fermentation broth culture of *Pseudomonas fluorescens***

*Diameter of Zone of Inhibition (mm) (Mean±SEM)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |
| ***2***  ***B1 B2*** | *0±0.71*  *0±0.71*  *0±1.41* | *0±2.12*  *0±1.41*  *0±1.41* | *0±1.41*  *0±1.41*  *0±1.41* |

*:M1= Medium 1 containing same quantity of yam peel and groundnut cake as carbon and plex nitrogen sources*

There was no statistical significant difference (p>0.05) in antifungal activities of the crude extract metabolite of *Pseudomonas fluorescens* isolate on the test organism in M1 and crude extract from Rice B1 had the highest mean antifungal activity against the test organisms (Appendix 9).

**Table 4.6: Antifungal activities of ethyl acetate fraction of M2 fermentation broth culture of *Pseudomonas fluorescens***

*Diameter of Zone of Inhibition (mm) (Mean±SEM)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |
| ***2***  ***B1 B2*** | *0±0.71*  *0±0.71*  *0±2.12* | *0±0.71*  *0±2.12*  *0±1.41* | *0±1.41*  *0±2.12*  *0±1.41* |

*ys:M2= Medium 2 containing same quantity of yam peel and groundnut cake as carbon and complex nitrogen source respectively*

There was no statistically significant difference (p>0.05) in antifungal activities of the crude extract metabolite of *Pseudomonas fluorescens* isolate on the test organism in M2 and crude extract from SGA2 had the highest mean antifungal activity against the test organisms (Appendix 10).

* 1. ***In vitro* Minimum inhibitory concentration (M.I.C.) values for the ethyl acetate fractions of *Pseudomonas fluorescens* fermentation broth on the test fungi spores**

The *in vitro* minimum inhibitory concentrations (M.I.C) of the crude extract metabolite on *Aspergillus niger*, *Penicillium citrinum* and *Rhizopus stolonifer* were found and the results are shown in the Tables 4.7 & 4.8

The Six (6) crude extract metabolite that was extracted were used for the *in vitro* MIC.

*Rhizopus stolonifer* was inhibited at lower concentrations by all the ethyl acetate fractions.

# Table: 4.7 *In vitro* MIC of ethyl acetate fraction of M1 fermentation broth culture of

***Pseudomonas fluorescens* on the test fungi spores**

In vitro *Minimum Inhibitory Concentration (mg/ml)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |

***2***

***B1 B2***

*ys:M1= Medium 1 containing same quantity of yam peel and groundnut cake as carbon and*

*complex nitrogen sources*

# Table: 4.8 *In vitro* MIC of ethyl acetate fraction of M2 fermentation broth culture of

***Pseudomonas fluorescens* on the test fungi spores**

In vitro *Minimum Inhibitory Concentration (mg/ml)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |

|  |  |
| --- | --- |
| ***2***  ***B1 B2*** | *00* |

*ys:M2= Medium 2 containing same quantity of banana peel and groundnut cake as carbon*

*and complex nitrogen sources*

# *In vivo* Minimum inhibitory concentration values for the ethyl acetate fractions of fermentation broth of *Pseudomonas fluorescens* on the test fungi isolates on yam tuber.

The *in vivo* minimum inhibitory concentrations of the ethyl acetate fractions were carried out on yam tuber inoculated with the test fungi spores and the results are shown in the Tables 4.9 & 4.10

Based on the results obtained, all the crude extract had active against *Rhizopus stolonifer* isolate at different concentrations as compared to *Aspergillus niger and Penicillium citrinum* isolates.

# Table 4.9: *In vivo* MIC of ethyl acetate fractions of M1 fermentation broth culture of

## Pseudomonas fluorescens

In vivo *Minimum Inhibitory Concentration (mg/ml)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |

|  |  |  |
| --- | --- | --- |
| ***2***  ***B1 B2*** | *00* | *00* |

*ys:M1= Medium 1 containing same quantity of yam peel and groundnut cake as carbon*

*and complex nitrogen sources*

# 4.10: *In vivo* MIC of ethyl acetate fraction of M2 fermentation broth culture of

## Pseudomonas fluorescens

In vivo *Minimum Inhibitory Concentration (mg/ml)*

|  |  |  |  |
| --- | --- | --- | --- |
| domonas fluorescens  *tes* | ergillus niger | cillium citrinum | opus stolonifer |

|  |  |  |  |
| --- | --- | --- | --- |
| ***2***  ***B1 B2*** | *00*  *00* | *00*  *00* | *0* |

*ys:M2= Medium 2 containing same quantity of banana peel and groundnut cake as carbon*

*and complex nitrogen sources*

# CHAPTER FIVE DISCUSSION

*Pseudomonas fluorescens* are aggressive colonizers of the rhizosphere of plants and have been isolated from many rhizospheric plant soils from diverse geographical regions. Jayashree *et al* (2000) isolated fluorescent pseudomonads from the rhizosphere of black gram, carrot, banana, pepper, rice & forest trees grown in several geographical areas of Tamil nadu. In this study, *Pseudomonas*

*fluorescens* strains were isolated from the rhizosphere of rice and sugar cane farms in Samaru, Zaria of Kaduna state. This is in agreement with the work of Nkene (2015) who isolated *Pseudomonas fluorescens* strains from rice, soybeans and groundnut farms in Keffi, Nassarawa state. Also, (Prakash *et al*.,2011; Reddy & Reddy 2009) both isolated *Pseudomonas flourescens* from rhizosphere of rice plants.

*Pseudomonas fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources (Palleroni, 1984) and waste materials contain minerals and nutrients that can meet the nutritional requirements of microorganism, Hence an alternative media using agro-waste consisting of yam peel, banana peel, sugarcane shaft as sources of carbon and groundnut cake as source of nitrogen were studied in this work for antifungal activities of the isolated *Pseudomonas fluorescens* strains for the production of metabolite substance. Although previous studies on activities of bioactive metabolites of *P. fluorescens* against fungal pathogens of other plants using lactose, glucose, molasses and malt extract as carbon source as well as yeast extract, peptone, corn steep liquor and soy bean extract as complex nitrogen source has been reported by (Heidari-Tajabadi *et al.,* 2013).

The antimicrobial activity of *P. fluorescens* have been reported against several phytopathogens. *P. fluorescens* was shown to effectively inhibit *R. solani* and *P. oryzae* by agar plate method (Rosales *et al* 1995). The results in this study showed that crude extract from the fermentation broth of four strains of *Pseudomonas fluorescens* in three formulated media (M1, M2 and M3) inhibited mycelial growth of *Aspergillus niger*, *Penicillium citrinum* and *Rhizopus stolonifer* isolates from rotten yam. A total of 36 crude extracts from fermentation broth of *Pseudomonas fluorescens* were obtained and screened for antifungal activities (Table 4.4). 24 crude extract presented antifungal activities against the fungi test organisms showing inhibition range of (19.00mm-39.00mm) (Table 4.4.). the results show that the crude extract of SGA2, RiceB1, Rice B2 had antifungal activities against at least two

(2) of the test fungal organisms. However, crude extract of SGA1 showed no antifungal activity

against *Aspergillus niger* and *Rhizopus stolonifer* in M1, M2 and M3. This finding agrees with a study conducted by Raajimaker *et al.* (2002) that many antibiotics produced by *P. fluorescens* isolate has varying activity against different phyto-pathogenic micro-organism. On the other hand, *Penicillium citrinium* was the most susceptible test organism as it was inhibited by almost all the crude extracts (Table 4.4). Based on these results, only three (3) *Pseudomonas fluorescens* isolates SGA1, Rice B1 and Rice B2 were chosen for the extraction of metabolites.

In this study, fermentation of *Pseudomonas fluorescens* isolate in M1 and M2 with carbon source yam peel and banana peel supported the production of secondary metabolite as both media had higher inhibition averages (17.20mm and 19.60mm) against the test fungi organisms compared to those obtained in M3 (11.75mm) which sugarcane shaft was carbon source. It was observed that mycelial growth was reduced due to the production of secondary metabolites which inhibited growth of the test organisms, and production of secondary metabolites depends mostly on nutrients available in the medium (Higgs *et al*., 2001). This makes it possible for different species of Pseudomonas to generate secondary metabolite of different composition during their growth (Robles-Huízar *et al*., 2017). Polanski *et al*. (2013) found that the production of extracellular antibiotics produced by *P. fluorescens* CL0145A against *B. subtilis* depends on the content of carbon sources as well as the presence of amino acids and vitamins, since when glycerol, hydrolyzed soy, tryptophan, glutamine, biotin and riboflavin were added to the medium, the antibiotic was generated. However only carbon sources were varied in this media formulated as other components were the same.

*In vitro* evaluation of antifungal activities of ethyl acetate fractions of fermentation broth of *Pseudomonas fluorescens* in formulated media M1 reveal that the ethyl acetate crude fraction completely inhibited the growth of *A. niger, P. citrinum, R. stolonifer*. Maximum inhibition was observed in extracts of Rice B1 (29.00mm) against *P. citrinum* and SGA2 (29.00mm) against *R. stolonifer* (Table 4.5). Crude fraction from Rice B1 had the highest mean antifungal activities against the test fungi organisms (Appendix 9). Similarly, *in vitro* evaluation of antifungal activities of ethyl

acetate fractions of fermentation broth of *Pseudomonas fluorescens* in formulated media M2 also reveal that the ethyl acetate crude fraction completely inhibited the growth of *A. niger, P. citrinum,*

*R. stolonifer*. Maximum inhibition was observed in extracts of Rice B1 (33.50mm) against *P. citrinum* and SGA2 (31.00mm) against *R. stolonifer* (Table 4.6). Crude fraction from SGA2 had the highest mean antifungal activities against the test fungi organisms (Appendix 10). *P. flourescens* was shown to have highest antifungal activity against *Penicillium italicum* (94%) and was moderately effective against *Aspergillus niger* (61%) (Mushtaq *et al*., 2010). Reddy *et al*. (2007) reported that the crude compounds from *P. fluorescens* isolates metabolites completely inhibited the growth of *Magnaporthe grisea*, *Dreschelaria oryzae*, *R. solani* and *Sarocladium oryzae*. The antifungal activity of the three solvent extracts of secondary metabolites of Pf 1, Pf 2, Pf 4, Pf 5, Pf 6, Pf 7, Pf 9, Pf 10 and Pf 11 showed that ethyl acetate extracts showed highest antifungal activity, suggesting that the antifungal inhibitory compound is better extracted with ethyl acetate than methanol and hexane (Alemu and Alemu, 2013). Similarly, the metabolite extracted from *P. fluorescens* with ethyl acetate effectively inhibited *P. oryzae* and *R. solani* (Battu and Reddy, 2009).

Observations from the *In vitro* MIC of the ethyl acetate fraction of fermentation broth of *Pseudomonas fluorescens* in M1 against the test fungi organisms in this study reveal that the ethyl acetate crude fraction inhibited all the test organism at different concentrations (0.15mg/ml- 6.49mg/ml). *Rhizopus stolonifer* was the most susceptible organism as it was inhibited at lower concentrations compared to *A. niger* and *P. citrinum. A. niger* needed a x4 concentrations to be inhibited by all the crude fractions (Table 4.7). Similarly, the *in vitro* MIC of the ethyl acetate fraction of fermentation broth of *Pseudomonas fluorescens* in M2 show that *Rhizopus stolonifer* was inhibited at lower concentrations. However, ethyl acetate fraction from Rice B2 didn’t have activity against *A. niger* even at the highest prepared concentration (>12.00mg/ml) (Table 4.8). In most cases, antibiotics showing *in vitro* activity are generally active *in vivo* against plant diseases.

Results from the *in vivo* MIC of the ethyl acetate fraction of fermentation broth of *Pseudomonas fluorescens* in M1 show that ethyl acetate fraction from SGA2 had no activity against *A. niger* and

*P. citrinum* at the highest prepared concentrations (>20.00mg/ml) expect against *R. stolonifer*

(5.00mg/ml) while the ethyl acetate fraction of Rice B1 and Rice B2 had activity against *R. stolonifer*,

*P. citrinum* and *A. niger* ranging from (1.00mg/ml-5.00mg/ml) (Table 4.9). the *in vivo* MIC of the ethyl acetate fraction from SGA2 in M2 had activity against *A. niger*, *P. citrinum*, *R. stolonifer* (4.00mg/ml-5.00mg/ml). However, ethyl acetate fraction from Rice B1 and Rice B2 had no activity against *A. niger* and *P. citrinum* at the highest prepared concentrations (>20.00mg/ml and

>30.00mg/ml) respectively but had *in vivo* activity against *R. stolonifer* (3.37mg/ml and 20.00mg/ml) respectively. The result from this study show that *P. fluorescens* strain from zaria has great potential for the biocontrol of yam rot fungi *in vitro* and *in vivo* conditions.

# CHAPTER SIX

**SUMMARY, CONCLUSION AND RECOMMENDATION**

# Summary

*Pseudomonas fluorescens* strains were isolated from the rhizosphere of rice & sugarcane farms in samaru, Zaria. Alternative media using agro waste consisting of yam peel, sugarcane shaft as sources of carbon and groundnut cake as source of nitrogen was studied in this work for antifungal activities

of the isolated *P. fluorescens* strains for the production of metabolite substance. Observation from this study showed that *Rhizopus stolonifer* was the most susceptible test organism as it was inhibited at a lower MIC in vitro (0.15-2.01 mg/ml) & in vivo (1-20 mg/ml) irrespective of the crude extract metabolite compared to the other test organisms.

# Conclusion

The result in this work showed that *Pseudomonas fluorescens* produce antifungal substances which justify their use as biocontrol agents against fungi pathogens of different crops in the field. This secondary metabolite when probably engaged will promote/enhance food security by reducing the loss of yam tubers to rot in Nigeria.

This study showed that fermentation with medium containing yam and banana peels as carbon source led to the production of more bioactive secondary metabolite by *Pseudomonas fluorescens* isolates from rice and sugar cane rhizospheres.

Furthermore, the use of waste products like yam and banana peel with groundnut cake to produce useful antibiotics demonstrated in this work has shown that local waste can indeed be utilized as laboratory media.

# Recommendations

Antibiotic production by fluorescent *Pseudomonas* spp. is now recognized as an important feature in plant disease suppression by some strains and the presented data exhibit the antifungal activities of Pseudomonas strains and indicate the possibility of using *Pseudomonas fluorescens* as a biological control agent against some plant pathogenic fungi of yam

it is however recommended that;

* + 1. There should be further screening of a large number of Pseudomonas strains from different region in Nigeria that can produce active antifungal agents.
    2. Further studies on the isolation and characterization of the secondary metabolites of *P. fluorescens* from the crude extract of the formulated media should be carried out.
    3. Since *Pseudomonas fluorescens* are known to produce more than one bioactive metabolite, further studies should be carried out to know which of the secondary metabolite is responsible for the biocontrol.
    4. Also continuous application of these organism to disease control will help select and produce better candidates for reliable commercial products.

# References

Achkar, J., Xian, M., Zhao, H. and Frost, J.W. (2005). Biosynthesis of phloroglucinol. *Journal of America Chemical Society.* 127:5332-5333.

Adesemoye, A.O. & Adedire, C.O. (2005). *World journal of microbiology and biotechnology*, 21; 329-336.

Adisa, R.S., Adefalu, L.L., Olatinwo, L.K., Balogun, K.S., and Ogunmadeko O.O. (2015).

Determinants of postharvest losses of yam among yam farmers in Ekiti state, Nigeria. *Bulletin of the Institute of Tropical Agriculture, Kyushu University*, 38: 73-78.

Agu, K.C., Awah, N.S., Sampson, P.G., Ikele, M.O., Mbachu, A.E., Ojiagu, K.D., Okeke, C.B., Okoro, N.C.N. & Okafor, O.I. (2014). *International Journal of Agriculture Innovations and Research,2(6)*: 2319-1473.

Agu, K.C., Nweke, G.U., Awah, N.S., Okeke, B.C., Mgbemena, I.C.C., Okigbo, R.N. & Ngenegbo,

U.C. (2015). *International Journal of Research Studies in Biosciences*, 3 (9), 32-37

Aidoo, K.A. (2007), Identification of yam tuber rot fungi from storage systems at the Kumasi Central market. A dissertation submitted to Faculty of Agriculture, K.N.U.S.T.; 18-19 pp.

Alemu, F. and Alemu, T. (2013). Antifungal activity of secondary metabolites of Pseudomonas fluorescens isolates as a biocontrol agent of chocolate spot disease (Botrytis fabae) of faba bean in Ethiopia. *African Journal of Microbiology Research*, Vol. 7(47), pp. 5364-5373.

Amusa, N.A. & Baiyewu, R. A. (1999). Storage and market disease of yam tubers in southwestern Nigeria Ogun. *Journal of Agricultural Research.* (Nig) 11: 211- 225.

Amusa, N.A., Adegbite, A.A., Muhammed, S. & Baiyewu, R.A. (2003). Yam diseases and their management in Nigeria. *African Journal of Science Biology* 2(12): 497-502.

Anitha, A. & Das, M.A. (2011). Activation of rice plant growth against *Rhizoctonia solani* using

*Pseudomonas fluorescens*, Trichoderma and salicylic acid. *Research in Biotechnology* 2:07-12.

Arima, K., Imanaka, I., Kousaka, M., Fukuta, A. & Tamura, G. (1964). Pyrrolnitrin, a new antibiotic substance, produced by Pseudomonas*. Agricultural and Biological Chemistry 28*:575–576.

Arulanantham, R., Pathmanathan, S., Ravimannan, N. and Kularajany, N. (2012). Alternative culture media for bacterial growth using different formulation of protein sources. *Natural Product Plant Resource*, 2: 697-700.

Athukorala, S.N., Fernando, W.G.D., Rashid, K.Y. & de Kievity, T. (2010). The role of volatile and non-volatile antibiotics produced by *Pseudomonas chlororaphis* strain PA23 in its root colonization and control of *Sclerotinia sclerotiorum*. *Biocontrol Science Technology 20*:875– 890.

Bangera, M.G., and L.S. Thomashow. 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *Journal of Bacteriology*, 181:3155-3163.

Battu, P.R. and Reddy, M.S. (2009). Isolation of secondary metabolites from *Pseudomonas fluorescens* and its characterization. *Asian Journal of Research Chemistry*, 2:26-29.

Baysse, C., Matthijs, S., Schobert, M., Layer, G., Jahn, D. & Cornelis, P. (2003). Coordination of iron acquisition, iron porphyrin chelation and ironprotoporphyrin export via the cytochrome c biogenesis protein CcmC in *Pseudomonas fluorescens*. *Microbiology*, 12: 3543-52.

Blumer, C. & Haas, D. (2000). Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Archives of Microbiology*, 173:170–177.

Bonsall, R.F., Weller, D.M., and Thomashow, L.S. (1997). Quantification of 2,4- diacetylphloroglucinol produced by fluorescent Pseudomonas spp. in vitro and in the rhizosphere of wheat. *Applied and Environmental Microbiology 63*, 951–955.

Calverly (1998). "Storage and Processing of Roots and Tubers in the Tropics". Food and Agriculture Association of the United Nations.

Cartwright, D.K., Chilton, W.S. & Benson, D.M. (1995). Pyrrolnitrin and phenazine production by *Pseudomonas cepacia* strain 55B, a biological agent of *Rhizoctonia solani. Applied Microbiology and Biotechnology*, 43: 211-221.

Charles, A. L., Sriroth, K. & Huang, T. C. (2005). Proximate compostion, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes. *Food Chemistry* 92, 615-620.

Cheesbrough, M. (2006). *District Laboratories Practice in Tropical Countries, Part 2*, Second Edition, Cambridge University Press, UK, pp. 105-108.

Chetverikov, S.P. & Loginov, O.N. (2005). Triglyceropeptides from pseudomonads: new agents for biological control of phytopathogenic fungi. *Applied Biochemistry and Microbiology 41*:79– 82.

Chin-A-Woeng, T.F.C., Bloomberg, G.V. & Lugtenberg, B.J.J. (2003). Phenazines and their role in Biocontrol by Pseudomonas bacteria. *New Phytologist 157*:503–523.

Chin-A-Woeng, T.F.C., Thomas-Oates, J.E., Lugtenberg, B.J.J. & Bloemberg, G.V. (2001). Introduction of the phzH gene of *Pseudomonas chlororaphis PCL1391* extends the range of biocontrol ability of phenazine-1-carboxylic acid-producing Pseudomonas spp. strains. *Molecular Plant Microbe Interaction 14*:1006–1015.

Chung, B.S., Aslam, Z., Kim, S.W., Kim, G.G., Kang, H.S., Ahn, J.W. & Chung, Y.R. (2008). A

bacterial endophyte, *Pseudomonas brassicacearum YC5480* isolated from the root of Artemisia sp. producing antifungal and phytotoxic compounds. *Plant Pathology Journal 24*:461–468.

Consultative Group on International Agricultural Research (CGIAR). 2006. Yam. Retrieved June 8, 2007.

D’aes, J., Maeyer, K. D., Pauwelyn, E., & Höfte, M. (2010). Biosurfactants in plant Pseudomonas interactions and their importance to biocontrol. *Environmental Microbiology Reports*, 2, 539– 572.

David, N.D. & O’Gara, F. (1994). Metabolites of Pseudomonas involved in biocontrol in the biocontrol of plant diseases. *Trends in Biotechnology*, 12: 133-141.

de Bruijn, I., de Kock, M.J.D., Yang, M., de Waard, P., van Beek, T.A. & Raaijmakers, J.M. (2007). Genome-based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. *Molecular Microbiology 63*:417–428.

de Souza, J.T., Arrnould, C., Deulvot, C., Lemanceau, P., Gianinazzi-Pearson, V. & Raaijmakers,

J.M. (2003a). Effect of 2,4-diacetylphloroglucinol on *Pythium*: cellular responses and variation in sensitivity among propagules and species. *Phytopathology 93*:966–975.

Degras, L. (1993). *The yam, a tropical root crop*. Macmillan press, London and Basinstoke,

Delaney, S.M., Mavrodi, D.V., Bonsall, R.F. & Thomashow, L.S. (2001). phzO, a gene for biosynthesis of 2-hydroxylated phenazine compounds in *Pseudomonas aureofaciens* 30–84. *Journal of Bacteriology 183*:318–327.

Dietrich, L.E.P., Okegbe, C., Price-Whelan, A., Sakhtah, H., Hunter, R.C. and Newman, D.K. (2013). *Journal of Bacteriology,*195:1371–1380.

Dietrich, L.E.P., Price-Whelan, A., Petersen, A., Whiteley, M. and Newman, D.K. (2006).

*Molecular Microbiology*, 61:1308–1321.

Dietrich, L.E.P., Teal, T.K., Price-Whelan, A. and Newman, D.K. (2008). *Science*, 321:1203–1206.

Duffy, B.K. & Défago, G. (1999). Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Applied and Environmental Microbiology 65*:2429–2438.

Duffy, B.K. & Défago, G. (2000). Controlling instability in gacS-gacA gacS-gacA regulatory genes during inoculant production of *Pseudomonas fluorescens* biocontrol strains. *Applied and Environmental Microbiology 66*:3142-3150.

Duffy, B.K., Keel, C. & Défago, G. (2004). Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. *Applied and Environmental Microbiology 70*:1836–1842.

Famurewa, O. & David, O.M. (2008). Formulation and Evaluation of Dehydrated Microbiological Media from Avocado Pea (Peasea Americana Cmill). *Research Journal of Microbiology 3(5)*, 326-330

FAO (2013). Food and Agricultural Organization of the United Nations. [www.fao.org/statistics/en/](http://www.fao.org/statistics/en/) FAO, Rome

FAO, (1998). Food and Agricultural Organization. Production Year Book. Trade 13 (16): 32 39. FAOSTATS. (2000). Crop statistics of FAO, URL<[http://fao.org](http://fao.org/)>Rome, Italy.

FAOSTATS. (2017). [*Yam production in 2014; Crops/Regions/World/Production Quantity; from*](http://www.fao.org/faostat/en/%23data/QC)[*pick lists"*](http://www.fao.org/faostat/en/%23data/QC). FAOSTAT, Statistics Division of the UN Food and Agriculture Organization. 2017. Retrieved 31 May 2017.

Fravel, D.R. (2005). Commercialization and implementation of biocontrol. *Annual Review of Phytopathology 43*:337–359.

Glasser, N.R., Kern, S.E. and Newman, D.K. (2014). *Molecular Microbiology*, 92:399–412.

Gomez-Pazos, J.M., Cuoto, S.R. & Sanroman, M.A. (2005). Chestnut and barley bran as potential substrate for lactase production by *Coriolopsis rigida* under solid state conditions. *Journal of Food Enginerring, 68*:315-319.

Green, K.R., Sangoyomi, A.T. & Amusa, N.A. (1995). The importance of *Rhizoctonia solani* as a pathogen of yam (*Dioscorea spp)* in Nigeria. *In: Proceedings of the 6th symposium of the International Society for Root and Tuber Crops African Branch*, pp. 412-418.

Gross, H. & Loper, J.E. (2009). Genomics of secondary metabolite production by *Pseudomonas spp*. *Nature Product Reports, 26*:1408–1446.

Gu, M. and Imlay, J.A. (2011). *Molecular Microbiology,* 79:1136–1150.

Guo, Y., Zheng, H., Yang, Y. & Wang, H. (2007). Characterization of *Pseudomonas corrugata strain P94* isolated from soil in Beijing as a potential biocontrol agent. *Current Microbiology 55*:247–253.

Haas, D. & De´fago, G. (2005). Biological control of soil borne pathogens by ﬂuorescent Pseudomonads. *Nature Review Microbiol*ogy 3: 307-319.

Haas, D. & Keel, C. (2003). Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annual Reviews of Phytopathology, 41*:117–153.

Haeidari-Tajabadi, F., Masoud, A., Vahid, F. and Asma, M. (2013). Effect of carbon and Nitrogen sources of the medium used to grow Biocontrol UTPF61 of *Pseudomonas fluorescens* on its protective activity against Sclerotinia wilt of sun flower and its survival during and after the formulation process. *Insight Bacteriology*, 2 (1): 3-9

Hernandez, M.E., Kappler, A. and Newman, D.K. (2004). *Applied Environmental Microbiology*, 70:921–928.

Higgs, R.E., Zahn, J.A., Gygi, J.D., and Hilton, M.D. (2001). Rapid method to estimate the presence of secondary metabolites in microbial extracts. *Applied Environmental Microbiology*, 67(1):371-376.

Hollstein, U. and van Gemert, R.J. JR (1971). *Biochemistry*, 10:497–504

Howell, C.R. & Stipanovic, R.D. (1980). Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic pyoluteorin. *Phytopathology 70*:712–715

Howell, C.R. and Stipanovic, R.D. (1979). Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology, 69*: 480-482.

Hultberg, M., Alsberg, T., Khalil, S. & Alsanius, B. (2010a). Suppression of disease in tomato infected by *Pythium ultimum* with a biosurfactant produced by *Pseudomonas koreensis*. *Biological Control 55*:435–444.

Iavicoli, A., Bóutet, E., Buchala, A. & Métraux, J.P. (2003). Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Molecular Plant Microbe Interaction, 16*:851–868.

Ike, P.C., & Inoni, O.E. (2006). Determinants of yam production and economic efficiency among small-holder farmers in South-eastern Nigeria. *Journal of Central European Agriculture,* 7(2), 337- 342.

Ikotun, T. (1983). Post-harvest microbial rot of yam in Nigeria. *Fito Pathologia Brasileira*, 8: 1-7

Ikotun, T. (1989). Disaeses of yam tubers. *International Journal of Tropical. Plant Disease*, 7:1- 21.

International Institute for Tropical Agriculture (2013). *Healthy yam seed production*. IITA Publications.

Islam, M.T. & Fukushi, Y. (2010). Growth inhibition and excessive branching in *Aphanomyces cochlioides* induced by 2,4-diacetylphloroglucinol is linked to disruption of filamentous actin cytoskeleton in the hyphae. *World Journal of Microbiology Biotechnology, 26*:1163–1170.

Isnansetyo, A., Cui, L. Hiramatsu, K. and Kamei, Y. (2003). Antibacterial activity of 2,4- diacetylphloroglucinol produced by *Pseudomonas* sp. AMSN isolated from a marine alga, against vancomycin-resistant *Staphylococcus aureus*. *International Journal of Antimicrobial Agents,* 22:545-547.

Izekor, O. B., & Olumese, M.I. (2010). Determinants of yam production and profitability in Edo State, Nigeria. *African Journal of General Agriculture*, 6(4): 205-21.

Jayaprakashvel, M. & Mathivanan, N. (2011). Management of plant disease by microbial metaboites. *In: D.K. Maheshwari (ed.) Bacteria in Agrobiology: Plant Nutrient Management*, pp 237–265.

Johnson, L.F. & Carl, E.A. (1972). Methods for Research on the Ecology of Soil Borne Plant Pathogens. Burgees Minneopolis 32:74-79.

Kabeya, M. J., Kabeya, U. C., Bekele, B. D. and Kikuno, H. (2013). cuttings technology in food yam (Dioscorea Rotundata) production. *Asian Journal of Plant Science and Research*, 3(3):107-111.

Kamei, Y. & Isnansetyo, A. (2003). Lysis of methicillin-resistant *Staphylococcus aureus* by 2,4- diacetylphloroglucinol produced by *Pseudomonas* sp. AMSN isolated from a marine alga. *International Journal of Antimicrobial Agents 21*:71–74.

Kanu, N.A., Ezeocha, V.C. and Ogunka, P.N. (2018). A Review on Bioactive Compounds of Yam Varieties for Human Disease Management. *Asian Food Science Journal* 1(4): 1-10.

Kapsalis, A., Gravanis, F. & Gowen, S. (2008). Involvement of phenazine-1-carboxylic acid, siderophores and hydrogen cyanide in suppression of *Rhizoctonia solani* and *Pythium* spp. damping-off by *Pseudomonas oryzihabitans* and *Xenorhabdus nematophila. Journal of Food, Agriculture and Environment, 6*:168–171

Kay, D.E. (1987). *Root crops tropical development and research institute London*, pp 205-206.

Keel, C., Schnider, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., Wirthner, P., Haas, D. & Défago, G. (1992). Suppression of root diseases by *Pseudomonas fluorescens CHA0*: importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Molecular Plant Microbe Interaction, 5*:4–13.

Kennedy, R.K., Naik, P.R., Veena, V., Lakshmi, B.S., Lakshmi, P., Krishna, R. & Sakthivel, N. (2015). 5-methylphenazine-1-carboxylic acid: novel bioactive metabolite by a rhizosphere soil bacterium that exhibits potent antimicrobial and anticancer activities. *Chemico-biological interactions, 231*:71-82.

King, E.O., Ward, M.K. & Raney, D.E. (1954). Two simple media for the demonstration of pyocyanine and fluorescein. *Journal of Laboratory and Clinical Medicine*, 44:301-307.

Knoth, J. (1993). *Traditional storage of yams and cassava and its improvement, Deutsche Gesellschaftfür Technische Zusammenarbit (GTZ) post-harvest project*, Hamburg, Germany, pp. 9-43.

Kraus, J. and Loper, J.E. (1995). Characterization of a genomic region required for production of the antibiotic pyoluteorin by the biological control agent *Pseudomonas fluorescens Pf-5*. *Applied Environmental Microbiology 61*:849-854.

Kwak, Y. S., Bakker, P. A. H. M., Glandorf, D. C. M., Rice, J. T., Paulitz, T. C., & Weller, D. M. (2009). Diversity, virulence and 2,4-diacetylphloroglucinol sensitivity of Gaeumannomyces graminis var. tritici isolates from Washington state. *Phytopathology*, 99, 472–479.

Kwak, Y. S., Bonsall, R. F., Okubara, P. A., Paulitz, T. C., Thomashow, L. S., & Weller, D. M. (2012). Factors impacting the activity of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* against take-all of wheat. *Soil Biology and Biochemistry,* 54, 48–56.

Laleye, S.A. (1990). Modification of potato extract medium for the growth of some selected organisms. B.Sc. Thesis. Ado-Ekiti.

Lawal, B., Ossai, P., Shittu, O., Abubakar, A. and Ibrahim, A. (2014). Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam peel, maize chaff and bean coat. *International Journal of Applied Biological Research*, 6(2):21-37

Lee, J.Y., Moon, S.S. & Hwang, B.K. (2003). Isolation and antifungal and antioomycete activities of aerugine produced by *Pseudomonas fluorescens strain MM-B16*. *Applied Environmental Microbiology, 69*:2023–2031.

Ligon, J.M., Hill, D.S., Hammer, P.E., Torkewitz, N.R., Hofmann, D., Kempf, H.J. & VanPee, K.H. (2000). Natural products with antifungal activity from Pseudomonas bicontrol bacteria. *Pest management science*, 56: 688-695.

Madigan, M.T., Martinko, J.M. and Parker, J. (2000). Nutrition and Metabolism in Brock Biology of Microorganisms. 9th Edn., Prentice Hall, New Jersey, pp: 102-134.

Maikasuwa, M.A., & Ala, A.L. (2013). Determination of profitability and resource-use efficiency of yam production by women in Bosso local government area of Niger State, Nigeria. *European Scientific Journal,* 9(16), 196-205.

Maurhofer, M., Baehler, E., Notz, R., Martinez, V. & Keel, C. (2004). Cross talk between 2,4-- diacetylphloroglucinol-producing biocontrol pseudomonads on wheat roots. *Applied Environmental Microbiology, 70*:1990–1998.

Mavrodi, D. V., Mavrodi, O. V., Parejko, J. A., Bonsall, R. F., Kwak, Y.-S., Paulitz, T. C., Thomashow, L. S., & Weller, D. M. (2012). Accumulation of the antibiotic phenazine-1- carboxylic acid in the rhizosphere of dryland cereals. *Applied and Environmental Microbiology*, 78, 804–812.

Mavrodi, D.V., Blankenfeldt, W. & Thomashow, L.S. (2006). Phenazine compounds in fluorescent Pseudomonas spp. biosynthesis and regulation. *Annual Review Phytopathology, 44*:417–445.

Mavrodi, D.V., Bonsall, R.F., Delaney, S.M., Soule, M.J., Phillips, G. & Thomashow, L.S. (2001). Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. *Journal of Bacteriology, 183*:6454–6465.

Mavrodi, D.V., Ksenzenko, V.N., Bonsall, R.F., Cook, R.J., Boronin, A.M. & Thomashow, L.S. (1998) A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2–79. *Journal of Bacteriology,* 180:2541–2548.

Mavrodi, D.V., Peever, T.L., Mavrodi, O.V., Parejko, J.A., Raaijmakers, J.M., Lemanceau, P. & Thomashow, L.S. (2010). Diversity and evolution of the phenazine biosynthesis pathway. *Applied Environmental Microbiology,76*:866-879.

Mavrodi, O.V., Mc Spadden Gardener, B.B., Mavrodi, D.V., Bonsall, R.F., Weller, D.M. & Thomasshow, L.S. (2001). Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol- producing fluorescent *Pseudomonas* spp. *Phytopathology 91*:35–43.

Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M. & It, L.S.P. (1992). Contribution of phenazine biosynthesis to the ecological competence of fluorescent Pseudomonads in soil habitats. *Applied Environmental Microbiology, 58*:2616-2624.

Mazzola, M., Funnell, D.L. & Raaijmakers, J.M. (2004). Wheat cultivar-specific selection of 2,4- diacetylphloroglucinol-producing fluorescent Pseudomonas species from resident soil populations. *Microbial Ecology, 48*:338–348.

Mignouna, H. D., Abang, M. M. & Asiedu, R. (2008). Genomics of yams, a common source of food and medicine in the tropics: *In plant genetics and genomics: crop and models, eds P. Moore and R. Ming (Berlin: springer),* 549-570.

Mignouna, H.D., Abang, M.M. & Asiedu, R. (2003). Harnessing modern biotechnology for tropical tuber crop improvement: Yam (Dioscorea spp.) molecular breeding. *African journal of biotechnology, 2*: 478-485.

Migula, W. (1984). Arbeiten aus dem Bakteriologischen Institute der Technischen Hocheschule Zu Karlsruhe, 1:235-238.

Migula, W. (1985). Bacteriaceae (Stabbchennbakterien). *Die Naturlichen Pflanzenfamilien Ehgler*

*A. Pranti N (Eds). Teill, Abtla, W. Engelmann publishers Leipzig*. Pp 20-30.

Morohoshi, T., Wang, W., Suto, T., Saito, Y., Ito, S., Someya, N. & Ikeda, T. (2013). Phenazine antibiotic production and antifungal activity are regulated by multiple quorum sensing systems in Pseudomonas chlororaphis subsp. aurantiaca st FRB508. *Journal of Bioscience and Bioengineering, 116*:580-584.

Morse, S., Acholo, M., McNamara, N. & Oliver, R. (2000). Control of storage insects as a means of limiting yam tuber fungal rots. *Journal of Stored Product Research,* 36: 37-45.

Mushtaq, S., Ali, A., Khokhar, I. and Mukhtar, I. (2010). Antagonisitic potential of soil bacteria against food borne fungi. *World Applied Science Journal* ,11:966-969.

National Advisory Board on Climate Change and Disaster Risk Reduction (2013). Training Series on Agrometeorology and Climate Change Adaptation: *Yam Propagation Using Vine Cutting Technique*. Training materials prepared in cooperation with Vanuatu Department of Meteorology and Geo-Hazards; SPC/GIZ Coping with Climate Change in the Pacific Island Region with funding support from the EU Global Climate Change Alliance.

Nkene, I.H. (2015). Evaluation of bioactive metabolite from rhizobacteria against bacteria isolates from rotten yam tubers (Unpublished Master’s Dissertation). Ahmadu Bello University, Zaria.

Nose, M. & Arima, K. (1969). On the mode of action of a new antifungal antibiotic, Pyrrolnitrin.

*Journal of Antibiotics, 22*:135-143.

Nowak-Thompson, B., Chaney, N., Wing, J.S., Gould, S.J. & Loper, J.E. (1999). Characterization of the pyoluteorin biosynthetic gene cluster of *Pseudomonas fluorescens* Pf-5., *Journal of Bacteriology, 181*:2166–2174.

Nwabueze, T.U. & Otuwa, U. (2006). Effect of Supplementation of African bread fruit (Treculia africana) hulls with organic wastes on growth of Saccharomyces cerevisiae. *African Journal of Biotechnology, 5(16)*: 1494-1498.

Okigbo, R. N., Anukwuorji, C. A. and Ezeabara, C. A. (2013). Effects of leaf extracts of *Chromolaena odorata* (L.) and *Terminalia catappa* (L.) on mycelia growth of yam tuber pathogens. *Nigerian Journal of Plant Protection*, 27(1): 74 – 84.

Okigbo, R.N. (2004): A review of biological control methods for postharvest yams (Dioscorea spp.) in storage in South Eastern Nigeria. *Kmitl Science Journal,* 1: 207-215.

Okigbo, R.N. (2005). Biological control of postharvest fungal rot of yam (Dioscorea spp.) with

*Bacillus subtilis*. *Mycopathologia*, 156: 81-85.

Okigbo, R.N. and Ikediugwu, F.E.O. (2000). Studies on biological control of postharvest rot of yams (Dioscorea spp.) with *Trichoderma viride*. *Journal of Phytopathology, 148*: 351-355.

Okigbo, R.N. and Ikediugwu, F.E.O. (2002), Evaluation of water losses in different regions of yam (*Dioscorea* spp.) tuber in storage. *Nigerian Journal of Experimental and Applied Biology, 3***.** 320 pp.

Okigbo, R.N. and Ogbonnaya, U.O. (2006). Antifungal effects of two tropical plant extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam rot. *African Journal of Biotechnology 5(9)*: 727-731.

Okigbo, R.N., Anukwuorji, C.A. and Igweonu, M.Z. (2014). Fungitoxic effect of *Azadirachta indica* and *Vernonia amygdalina* extracts on *Aspergillus niger* and *Fusarium oxysporium* the causal organisms of yam (*Dioscorea rotundata*) rot. *The Bioscientist* Vol. 2(1): 70- 86.

Available online at [http://www.bioscientistjournal.com.](http://www.bioscientistjournal.com/)

Okonkwo, I.O., Oiabode, O.P. & Okeleji, O.S. (2006). The role of Biotechnology in the Socio- economic Advancement and National Development. *African Journal of Biotechnology, 5(19*): 2354-2366.

Okpara, L.U. (2003). *Yams: Post-harvest operation*. (AGST/FAO, D. Mejia, & F. (Technical editions), pp. 1–22.

Osunde, Z.D. (2008). *Minimizing Postharvest Losses in Yam (Dioscorea spp.)*: Treatments and techniques, pp. 1-12.

Palleroni, N.J. (1984). Pseudomonas. *Bergey’s manual of systematics of archea and bacteria*. 1.

Pang, A.H., Garneau-Tsodikova, S., Tsodikov, O.V. (2015). Crystal structure of halogenase PltA from the pyoluteorin biosynthetic pathway. *Journal of Structural Biology, 192*: 349-357.

Pang, Y., Liu, X., Ma, Y., Chernin, L., Berg, G. & Gao, K. (2009). Induction of systemic resistance, root colonization and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on N-acylhomoserine lactones. *European Journal of Plant Pathology*, 124:261–268

Peix, A., Ramirez-Bahena, M.H. & Velazquez, E. (2009). Historical evolution and current status of the taxonomy of genus Pseudomonas. *Infection, Genetics and Evolution,* 6:1132-1147.

Pelczar, J.M., Chan, L.E.A. and Krieg, N.R. (1993). Microbiology, Concept and Application. Int.

Edn., McGraw-Hill Inc., New Jersey, pp: 847.

Pierson, E.A. & Pierson, L.S. (2010). Metabolism and function of phenazines in bacteria: impacts on the behaviour of bacteria in the environment and biotechnological processes. *Applied Microbiology Biotechnology, 86*:1659-1670.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker,

P. H. A. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology,* 52, 347–375

Polanski-Cordovano, G., Romano, L., Marotta, L.L., Jacob, S., Soo Hoo, J., Tartaglia, E., Asokan, D, Kar, S. and Demain, A.L. (2013). Nutritional studies on production of antibacterial activity by the zebra mussel antagonist, *Pseudomonas fluorescens* CL0145A. *Journal of Microbiology and Biotechnology,* 23(5): 656-660.

Polycarp, D., Afoakwa, E. O., Budu, A. S., & Otoo, E. (2012). Characterization of chemical composition and anti-nutrition factors in seven species within the Ghanian yam (*Dioscorea*) germplasm. *International food Research Journal,*19: 985-992.

Powell, J.F., Vargas, J.M. Jr., Nair, M.G., Detweiler, A.R. & Chandra, A. (2000). Management of dollar spot on creeping bentgrass with metabolites of *Pseudomonas aureofaciens (TX-1). Plant Disease, 84*:19–24.

Price-Whelan, A., Dietrich, L.E.P. and Newman, D.K. (2007). *Journal of Bacteriology*, 189:6372– 6381.

Princewill-Ogbonna, I. and Ibeji, C. (2015). Comparative study on nutritional and ant- nutritional composition of three cultivars (red, green and yellow) of aerial yam (*Dioscorea bulbifera*).

IOSR *Journal of Environmental Science and Toxicological Food Technology*, 9:79-86

Raaijmakers, J. M., De Bruijn, I., Nybroe, O., & Ongena, M. (2010). Natural functions of lipopeptides from Bacillus and Pseudomonas: More than surfactants and antibiotics. *FEMS Microbiology Reviews*, 34, 1037–1062.

Raaijmakers, J. M., Paulitz, T., Steinberg, C., Alabouvette, C., & Moe¨nne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil*, 321, 341–361.

Raaijmakers, J.M., de Bruijn, I. & de Kock, M.J.D. (2006). Cyclic lipopeptide production by plant- associated Pseudomonas spp.: diversity, activity, biosynthesis, and regulation. *Molecular Plant Microbe Interaction, 19*:699–710.

Raaijmakers, J.M., Vlami, M. & de Souza, J.T. (2002) Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek 81*:537–547.

Ramette, A., Fraspolli, M., Saux, M.F., Gruffaz, C., Meyer, J., Défago, G. & Moenne-Loccoz, Y. (2011). Pseudomonas protegens sp nov. wide-spread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Systematic and Applied Microbiology, 34*:180-188.

Ravimannan, N., Arulanantham, R., Pathmanathan, S. and Kularajani, N. (2014). Alternative culture media for fungal growth using different formulation of protein sources, *Annals of Biological Research,* 5: 36-39.

Reddy, B.P, Reddy, M.S., and Kurma, K.V.K. (2009).Characterization of antifungal metabolites of *Pseudomonas fluorescens* and their effect on mycelial growth of *Magnaporthe grisea* and *Rhizoctonia solani*. *International Journal of Pharmaceutical Technology Research,* 1(4): 1490- 1493

Reddy, K.R.N., Choudary, K.A. & Reddy, M.S. (2007). Antifungal metabolites of *Pseudomonas fluorescens* isolated from rhizosphere of rice crop. *Journal of Mycology and Plant Pathology*, 37(2).

Robles-Huízar, M., De La Torre-Zavala, S., De La Garza-Ramos, M.A. and Galan-Wong, L.J. (2017). Antimicrobial activity of secondary metabolites obtained with different carbon sources at different stages of the Pseudomonas growth curve isolated from Fresnillo, Zacatecas, México mineral soils. *African Journal of Bacteriology Research,* Vol. 9(5), pp. 30- 36.

Rosales, A.M., Thomashow, L., Cook, R.J. & Mew, T.W. (1995). Isolation and identification of antifungal metabolites produced by rice associated antagonistic *Pseudomonas* spp*. Phytopathology,* 85:1029-1032.

Ryu, J.S., Lee, S.D., Lee, Y.H., Lee, S.T., Kim, D.K., Cho, S.J., Park, S.R., Bae, D.W., Park, K.H.

& Yun, H.D. (2000). Screening and identification of an antifungal Pseudomonas sp. that suppresses balloon flower root rot caused by *Rhizoctonia solani*. *Journal of Microbiology and Biotechnology, 10*:435–440.

Saheed, O.K., Jamal, P., Karim, M.I.A., Alam, Z. and Muyibi, S.A. (2013). Cellulolytic fruits wastes: a potential support for enzyme assisted protein production. *Journal Biological Science*, 13 (5), 379–385.

Saranraj, P. and Anbu, S. (2017). Utilization of Agroindustrial wastes for the cultivation of industrially important fungi – A Review. *International Journal of Innovations in Agricultural Sciences,* 1 (2): 59 – 71. DOI: 10.22192/ijias.2017.1.2.4.

Schmidt, S., Blom, J.F., Pernthaler, J., Berg, G., Baldwin, A., Mahenthiralingam, E. & Eberl, L. (2009). Production of the antifungal compound pyrrolnitrin is quorum sensing- regulated in members of the *Burkholderia cepacia* complex. *Environmental Microbiology, 11*:1422-1437.

Schnider-Keel, U., Seematter, A., Maurhofer, M., Blumer, C., Duffy, B., Gigot-Bonnefoy, C., Reimmann, C., Notz, R., Défago, G., Haas, D. & Keel, C. (2000). Autoinduction of 2,4- diacetylphloroglucinol biosynthesis in the biocontrol agent Pseudomonas fluorescens CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *Journal of Bacteriology, 182*:1215–1225.

Seal, S. & Muller, E. (2007). Molecular analysis of a full length sequence of a new yam badnavirus from *Dioscorea sansibarensis*. *Archives of Virology. 152*, 819-825.

Seddon, S.V. and Boriello, S.P. (1989). A chemically defined and minimal medium for *Clostridium difficile*. *Letters in Applied Microbiology*, 9: 237-239.

Selin, S., Negrel, J., Wendehenne, D., Ochatt, S., Gianinazzi, S. & van Tuinen, D. (2010) Stimulation of defense reactions in *Medicago truncatula* by antagonistic lipopeptides from *Penibacillus* sp. strain B2. *Applied Environmental Microbiology,* 76:7420–7428.

Shanahan, P., O’Sullivan, D.J., Simpson, P., Glennon, J.D. & O’Gara, F. (1992). Isolation of 2,4-- diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Applied Environmental Microbiology 58*:353–358.

Shanmugaiah, V., Mathivanan, N. & Varghese, B. (2010). Purification, crystal structure and antimicrobial activity of phenazine-1-carboxamide produced by a growth-promoting biocontrol bacterium, *Pseudomonas aeruginosa MML2212*. *Journal of Applied Microbiology, 108*:703– 711.

Shehu *et al.* (2010). Determinants of yam production and technical efficiency among yam farmers in Benue State. *Nigeria Journal of Social Science,* 24(2), 143-148.

Siddiqui, I.A., Shaukat, S.S., Khan, G.H. & Ali, N.A. (2003). Suppression of *Meloidogyne javanica* by Pseudomonas aeruginosa IE-6S+ in tomato: the influence of NaCl, oxygen and iron levels. *Soil Biology and Biochemistry, 35*:1625–1634

Someya, N., Ikeda, S. & Tsuchiya, K. (2013). Pseudomonas inoculants as agents for plant disease management. *In: D.K. Maheshwari (ed.), Bacteria in Agrobiology: Disease Management*, pp

Taylor, D. and Holland, K.T. (1989). Amino acid requirements for the growth and production of some exocellular products of *Staphylococcus aureus*. *Journal of Applied Bacteriol*ogy, 66: 319- 329

Tazawa, J., Watanabe, K., Yoshida, H., Sato, M. & Homma, Y. (2000). Simple method of detection of the strains of fluorescent *Pseudomonas* spp. producing antibiotics pyrrolnitrin and phloroglucinol. *Soil Microorganism, 54*:61–67.

Tharmila, S., Jeyaseelan, E.C. and Thavaranjit, A.C. (2011). Preliminary screening of alternative culture media for the growth of some selected fungi. *Archive of Applied Science Research*, 3: 389-393

Thomashow, L. S., and Weller D. M. (1996). Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites, p. 187–235. *In G. Stacey and N. Keen (ed.), Plant-microbe interactions, vol. 1*. Chapman & Hall, New York, N.Y.

Tripathi, M. & Johri, B.N. (2002). In vitro antagonistic potential of fluorescent Pseudomonads and control of sheath blight of maize caused by *Rhizoctonia solani*. *Indian Journal of Microbiology.* 42:207-214.

Tripathi, R.K. & Gottlieb, D. (1969). Mechanism of action of the antifungal antibiotic pyrrolnitrin.

*Journal of Bacteriology, 100*:310-318.

Troppens, D.M., Chu, M., Holcombe, L.J., Gleeson, O., Gara, F.O., Read, N.D. & Morrissey, J.P. (2013). The bacterial secondary metabolite 2,4-Diacetylphloroglucinol impairs mitochondrial function and affects calcium homoestasis in *Neurospora crassa. Fungal Genetics and Biology, 56*:135-146.

Upadhyay, A. & Srivastava, S. (2011). Phenazine-1-carboxylic acid is a more important contributor to biocontrol in *Fusarium oxysporum* than pyrrolnitrin in *Pseudomonas fluorescens* strain psd. *Microbiological Research, 166*:323-335.

Wang, Y. and Newman, D.K. (2008). *Environmental Science Technology*, 42:2380–2386. Wang, Y., Kern, S.E. and Newman, D.K. (2010). *Journal of Bacteriology*, 192:365–369.

Wei, G., Kloepper, J. W. and Tuzum, S. (1996). Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology 86*:221-224.

Weller, D. M., and Thomashow, L. S. (1993). Use of rhizobacteria for biocontrol. *Current Opinion in Biotechnoloy,* **4:**306–311.

Weller, D.M., Mavrodi, D.V., van Pelt, J.A., Pieterse, C.M.J., van Loon, L.C., Bakker, P.A.H.M. (2012). Induced systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringe* pv tomato by 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens*. *Biological Control, 102*:403-412.

Zaknayiba, D.B., & Tanko, L. (2013). Costs and returns analysis of yam production among small scale farmers in Karu local government area, Nasarawa State, Nigeria. *PAT,* 9(1), 73-80.

Zehnder, G.W., Murphy, J.F., Sikora, E.J. & Kloepper, J.W. (2001). Application of Rhizobacteria for induced resistance. *European Journal of Plant Pathology, 107*:39–50.

Zhou, T., Chen, D., Li, C., Sun, Q., Li, L., Liu, F. & Shen, B. (2012). Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralsotonia solanacearum* and identification of its antimicrobial components. *Microbiological Research*, 167: 388-394.

# APPENDICES

**Appendix 1: Antifungal activities against test fungi spores by the crude extract from**

***Pseudomonas fluorescens* isolates in three (3) fermentation media**

# Zone of inhibition (mm) in duplicate for medium 1: yam peel + groundnut cake

|  |  |  |  |
| --- | --- | --- | --- |
| ***Pseudomonas fluorescens* isolates** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |
| SGA1 SGA2  Rice B1  Rice B2 | 0 0  20 21  35 33  25 24 | 0 0  20 18  25 23  30 28 | 0 0  15 13  20 21  20 23 |

**Zone of inhibition (mm) in duplicate for medium 2: Banana peel + groundnut cake**

|  |  |  |  |
| --- | --- | --- | --- |
| ***Pseudomonas fluorescens* isolates** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |

|  |  |  |  |
| --- | --- | --- | --- |
| SGA1 SGA2  Rice B1  Rice B2 | 0 0  25 24  0 0  30 31 | 20 21  26 24  0 0  35 32 | 0 0  30 33  40 38  30 32 |

# Zone of inhibition (mm) in duplicate for medium 3: sugarcane shaft + groundnut cake

|  |  |  |  |
| --- | --- | --- | --- |
| ***Pseudomonas fluorescens* isolates** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |
| SGA1 SGA2  Rice B1  Rice B2 | 0 0  0 0  20 18  0 0 | 28 26  24 25  30 29  20 21 | 0 0  0 0  20 21  0 0 |

**Appendix 2: Antifungal activities of ethyl acetate fractions of fermentation broth culture of *Pseudomonas fluorescens***

# Zone of inhibition (mm) in duplicate for SGA2

|  |  |  |  |
| --- | --- | --- | --- |
| **Media** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |
| M1  M2 | 20 19  30 31 | 25 22  30 31 | 30 28  30 32 |

**Zone of inhibition (mm) in duplicate for RiceB1**

|  |  |  |  |
| --- | --- | --- | --- |
| **Media** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |
| M1  M2 | 25 26  25 24 | 30 28  35 32 | 25 23  30 27 |

# Zone of inhibition (mm) in duplicate for RiceB2

|  |  |  |  |
| --- | --- | --- | --- |
| **Media** | ***Aspergillus niger*** | ***Penicillium citrinum*** | ***Rhizopus stolonifer*** |
| M1  M2 | 20 22  20 23 | 25 27  21 23 | 30 27  30 28 |

**Appendix 3: Two-way ANOVA for the antifungal activities of the crude extract of**

***Pseudomonas fluorescens* isolates against the three test organism in medium 1**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| SGA1 | 3 | 0 | 0 | 0 |  |  |
| SGA2 | 3 | 53.5 | 17.83333 | 11.58333 |  |  |
| Rice B1 | 3 | 78.5 | 26.16667 | 49.08333 |  |  |
| Rice B2 | 3 | 75 | 25 | 14.25 |  |  |
|  |  |  |  |  |  |  |
| A. niger | 4 | 79 | 19.75 | 205.4167 |  |  |
| P. citrinum | 4 | 72 | 18 | 160.6667 |  |  |
| R. stolonifer | 4 | 56 | 14 | 98.16667 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 1312.417 | 3 | 437.4722 | 32.67427 | 0.000411 | 4.757063 |
| Columns | 69.5 | 2 | 34.75 | 2.595436 | 0.154121 | 5.143253 |
| Error | 80.33333 | 6 | 13.38889 |  |  |  |

# Appendix 4: Two-way ANOVA for the antifungal activities of the crude extract of

***Pseudomonas fluorescens* isolates against the three test organism in medium 2**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| SGA1 | 3 | 20.5 | 6.833333 | 140.0833 |  |  |
| SGA2 | 3 | 81 | 27 | 15.25 |  |  |
| Rice B1 | 3 | 39 | 13 | 507 |  |  |
| Rice B2 | 3 | 95 | 31.66667 | 2.583333 |  |  |
|  |  |  |  |  |  |  |
| A. niger | 4 | 55 | 13.75 | 258.0833 |  |  |
| P. citrinum | 4 | 79 | 19.75 | 202.4167 |  |  |
| R. stolonifer | 4 | 101.5 | 25.375 | 299.5625 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 1220.729 | 3 | 406.9097 | 2.30444 | 0.17671 | 4.757063 |
| Columns | 270.375 | 2 | 135.1875 | 0.765603 | 0.505662 | 5.143253 |
| Error | 1059.458 | 6 | 176.5764 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 2550.563 | 11 |  |  |  |  |

# Appendix 5: Two-way ANOVA for the antifungal activities of the crude extract of

***Pseudomonas fluorescens* isolates against the three test organism in medium 3**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| SGA1 | 3 | 27 | 9 | 243 |  |  |
| SGA2 | 3 | 24.5 | 8.166667 | 200.0833 |  |  |
| Rice B1 | 3 | 69 | 23 | 32.25 |  |  |
| Rice B2 | 3 | 20.5 | 6.833333 | 140.0833 |  |  |
|  |  |  |  |  |  |  |
| A. niger | 4 | 19 | 4.75 | 90.25 |  |  |
| P. citrinum | 4 | 101.5 | 25.375 | 14.72917 |  |  |
| R. stolonifer | 4 | 20.5 | 5.125 | 105.0625 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 513.4167 | 3 | 171.1389 | 8.798286 | 0.012895 | 4.757063 |
| Columns | 1114.125 | 2 | 557.0625 | 28.6387 | 0.000853 | 5.143253 |
| Error | 116.7083 | 6 | 19.45139 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 1744.25 | 11 |  |  |  |  |

**Appendix 6: Two-way ANOVA for the antifungal activities of the crude extract of *Pseudomonas fluorescens* isolates in the three formulated media against *Aspergillus niger*.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| M1 | 4 | 79 | 19.75 | 205.4167 |  |  |
| M2 | 4 | 55 | 13.75 | 258.0833 |  |  |
| M3 | 4 | 19 | 4.75 | 90.25 |  |  |
|  |  |  |  |  |  |  |
| SGA1 | 3 | 0 | 0 | 0 |  |  |
| SGA2 | 3 | 45 | 15 | 172.75 |  |  |
| Rice B1 | 3 | 53 | 17.66667 | 290.3333 |  |  |
| Rice B2 | 3 | 55 | 18.33333 | 261.0833 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 456 | 2 | 228 | 1.378569 | 0.321638 | 5.143253 |
| Columns | 668.9167 | 3 | 222.9722 | 1.348169 | 0.344609 | 4.757063 |
| Error | 992.3333 | 6 | 165.3889 |  |  |  |

**Appendix 7: Two-way ANOVA for the antifungal activities of the crude extract of *Pseudomonas fluorescens* isolates in the three formulated media against *Penicillium citrinum*.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| M1 | 4 | 72 | 18 | 160.6667 |  |  |
| M2 | 4 | 79 | 19.75 | 202.4167 |  |  |
| M3 | 4 | 101.5 | 25.375 | 14.72917 |  |  |
|  |  |  |  |  |  |  |
| SGA1 | 3 | 47.5 | 15.83333 | 198.5833 |  |  |
| SGA2 | 3 | 68.5 | 22.83333 | 11.08333 |  |  |
| RiceB1 | 3 | 53.5 | 17.83333 | 246.0833 |  |  |
| Rice B2 | 3 | 83 | 27.66667 | 43.58333 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 118.7917 | 2 | 59.39583 | 0.405029 | 0.683914 | 5.143253 |
| Columns | 253.5625 | 3 | 84.52083 | 0.57636 | 0.651421 | 4.757063 |
| Error | 879.875 | 6 | 146.6458 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 1252.229 | 11 |  |  |  |  |

**Appendix 8: Two-way ANOVA for the antifungal activities of the crude extract of *Pseudomonas fluorescens* isolates in the three formulated media against *Rhizopus stolonifer***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | |  |  |  |  |
|  |  |  |  |  |  |  |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| M1 | 4 | 56 | 14 | 98.16667 |  |  |
| M2 | 4 | 101.5 | 25.375 | 299.5625 |  |  |
| M3 | 4 | 20.5 | 5.125 | 105.0625 |  |  |
|  |  |  |  |  |  |  |
| SGA1 | 3 | 0 | 0 | 0 |  |  |
| SGA2 | 3 | 45.5 | 15.16667 | 249.0833 |  |  |
| Rice B1 | 3 | 80 | 26.66667 | 114.0833 |  |  |
| RiceB2 | 3 | 52.5 | 17.5 | 252.25 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 824.2917 | 2 | 412.1458 | 6.08271 | 0.036034 | 5.143253 |
| Columns | 1101.833 | 3 | 367.2778 | 5.420519 | 0.038236 | 4.757063 |
| Error | 406.5417 | 6 | 67.75694 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 2332.667 | 11 |  |  |  |  |

# Appendix 9: Two-way ANOVA for the antifungal activities of ethyl acetate fraction of M1 fermentation broth culture of *Pseudomonas fluorescens*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Anova: Two-Factor Without Replication | | | | |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
| SGA2 | 3 | 72 | 24 | 22.75 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Rice B1 | 3 | 78.5 | 26.16667 | 6.583333 |  |  |
| Rice B2 | 3 | 74.5 | 24.83333 | 11.58333 |  |  |
| *A. niger* | 3 | 66 | 22 | 9.75 |  |  |
| *P. citrinum* | 3 | 78.5 | 26.16667 | 7.583333 |  |  |
| *R. stolonifer* | 3 | 80.5 | 26.83333 | 6.583333 |  |  |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 7.166667 | 2 | 3.583333 | 0.352459 | 0.722796 | 6.944272 |
| Columns | 41.16667 | 2 | 20.58333 | 2.02459 | 0.246954 | 6.944272 |
| Error | 40.66667 | 4 | 10.16667 |  |  |  |
| Total | 89 | 8 |  |  |  |  |

**Appendix 10: Two-way ANOVA for the antifungal activities of ethyl acetate fraction of M2 fermentation broth culture of *Pseudomonas fluorescens***

Anova: Two-Factor Without Replication

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| SGA2 | 3 | 92 | 30.66666667 | 0.083333 |  |  |
| Rice B1 | 3 | 86.5 | 28.83333333 | 20.33333 |  |  |
| Rice B2 | 3 | 72.5 | 24.16666667 | 17.58333 |  |  |
| *A. niger* | 3 | 76.5 | 25.5 | 21 |  |  |
| *P. citrinum* | 3 | 86 | 28.66666667 | 35.58333 |  |  |
| *R. stolonifer* | 3 | 88.5 | 29.5 | 1.75 |  |  |
| ANOVA | | | | | | |
| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 67.38888889 | 2 | 33.69444444 | 2.735062 | 0.178406 | 6.944272 |
| Columns | 26.72222222 | 2 | 13.36111111 | 1.084555 | 0.420412 | 6.944272 |
| Error | 49.27777778 | 4 | 12.31944444 |  |  |  |
| Total | 143.3888889 | 8 |  |  |  |  |

**Appendix 11: Zones of inhibition of ethyl acetate fraction of *Pseudomonas fluorescens***

# fermentation broth in M2



**Plate I: Zone of inhibition of the ethyl acetate fraction from Rice B1 in medium 2 against**

***Rhizopus stolonifer***



# Plate II: Zone of inhibition of the ethyl acetate fraction from Rice B1 in medium 2 against

***Penicillium citrinum***



# Plate III: Zone of inhibition of the ethyl acetate fraction from Rice B1 in medium 2 against

***Aspergillus niger***

# Appendix 12: Growth curve for *Pseudomonas fluorescens* isolates in a standard medium containing glucose as carbon source, NH4NO3 as nitrogen source.

ABSORBANCE

**Optical density values for *Pseudomonas fluorescens* isolates in standard medium**

0.25

Growth curve for *P. fluorescens* isolates in Standard medium

0.2

0.15

0.1

contro

l Rice B2

SGA1

0.05

0

6

12

18

24TIME (HR 30

S)

36

42

48

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Time (hrs)* | *Absorbance*  *Control* | *SGA1* | *SGA2* | *RICE B1* | *RICE B2* |
| ***6*** | *0.01* | *0.031* | *0.014* | *0.033* | *0.022* |
| ***12*** | *0.012* | *0.122* | *0.123* | *0.116* | *0.111* |
| ***24*** | *0.01* | *0.125* | *0.124* | *0.118* | *0.115* |
| ***30*** | *0.01* | *0.127* | *0.131* | *0.131* | *0.153* |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***36*** | *0.01* | *0.131* | *0.126* | *0.118* | *0.164* |
| ***48*** | *0.008* | *0.133* | *0.13* | *0.128* | *0.217* |

# Appendix 13: Growth curve for *Pseudomonas fluorescens* isolates in medium 1 containing yam peel as carbon source and groundnut cake as nitrogen source

Growth curve for *P. fluorescens* isolates in formulated medium1

0.89

0.79

0.69

0.59

0.49

control

Rice B2 SGA1 SGA2

Rice B1

0.39

6

12

18

24TIME (HRS 30

)

36

42

48

ABSORBANCE

**Optical density values for *Pseudomonas fluorescens* isolates in medium 1**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Time (hrs)* | *Absorbance*  *Control* | *SGA1* | *SGA2* | *RICE B1* | *RICE B2* |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***6*** | *0.401* | *0.633* | *0.643* | *0.612* | *0.436* |
| ***12*** | *0.399* | *0.702* | *0.703* | *0.718* | *0.535* |
| ***24*** | *0.4* | *0.836* | *0.819* | *0.814* | *0.84* |
| ***30*** | *0.399* | *0.784* | *0.856* | *0.82* | *0.817* |
| ***36*** | *0.398* | *0.807* | *0.86* | *0.724* | *0.67* |
| ***48*** | *0.399* | *0.804* | *0.862* | *0.802* | *0.664* |

# Appendix 14: Growth curve for *Pseudomonas fluorescens* isolates in medium 2 containing banana peel as carbon source and groundnut cake as nitrogen source

ABSORBANCE

**Optical density values for *Pseudomonas fluorescens* isolates in medium 2**

1

Growth curve for *P. fluorescens* isolates on formulated medium 2

0.9

0.8

0.7

0.6

0.5

control

Rice B2 SGA1 SGA2

Rice B1

0.4

0.3

6

12

18

24

TIME (HRS)

30

36

42

48

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Time (hrs)* | *Absorbance*  *Control* | *SGA1* | *SGA2* | *RICE B1* | *RICE B2* |
| ***6*** | *0.31* | *0.351* | *0.433* | *0.334* | *0.408* |
| ***12*** | *0.311* | *0.467* | *0.544* | *0.409* | *0.534* |
| ***24*** | *0.311* | *0.615* | *0.613* | *0.608* | *0.833* |
| ***30*** | *0.309* | *0.607* | *0.698* | *0.614* | *0.943* |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***36*** | *0.308* | *0.582* | *0.632* | *0.599* | *0.907* |
| ***48*** | *0.309* | *0.637* | *0.649* | *0.621* | *0.965* |

# Appendix 15: Growth curve for *Pseudomonas fluorescens* isolates in medium 3 containing sugar cane shaft as carbon source and groundnut cake as nitrogen source

0.8

Growth curve for *P. fluorescens* isolates on formulated medium 3

0.75

0.7

0.65

0.6

0.55

0.5

0.45

0.4

control

Rice B2 SGA1 SGA2

Rice B1

0.35

0.3

6

12

18

24TIME (HRS30

)

36

42

48

ABSORBANCE

**Optical density values for *Pseudomonas fluorescens* isolates in medium 3**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Time (hrs)* | *Absorbance*  *Control* | *SGA1* | *SGA2* | *RICE B1* | *RICE B2* |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ***6*** | *0.329* | *0.385* | *0.37* | *0.338* | *0.386* |
| ***12*** | *0.33* | *0.429* | *0.455* | *0.458* | *0.584* |
| ***24*** | *0.328* | *0.626* | *0.615* | *0.637* | *0.708* |
| ***30*** | *0.329* | *0.671* | *0.623* | *0.641* | *0.701* |
| ***36*** | *0.329* | *0.63* | *0.635* | *0.651* | *0.781* |
| ***48*** | *0.328* | *0.704* | *0.706* | *0.695* | *0.773* |