BIOASSAY OF PHYTOTOXIC METABOLITES OF FUNGI ASSOCIATED WITH *Solanum lycopersicum* L. (TOMATO) LEAVES

Tolulope Seun EWEKEYE

(MATRIC No. 129948)

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BY

Tolulope Seun EWEKEYE

(MATRIC No. 129948)

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ABSTRACT

Solanum lycopersicum (tomato) is an essential vegetable crop consumed worldwide. Major limiting factors in its production include fungal foliar diseases. Phytotoxic fungi play critical roles in the pathogenesis and expression of disease symptoms in the plant. An understanding of the phytotoxins produced in tomato leaves will enhance its optimal production. However, there is dearth of information on tolerance of tomato varieties to phytotoxins associated with their leaf diseases. Therefore, this work was aimed at investigating the phytotoxins produced by pathogenic fungi associated with tomato leaves.

Infected leaf samples (3 per plant, 30 plants per farm) of Kerewa variety were randomly collected at the expression of disease symptoms from 3 farms in Alapoti, Ogun State. Samples were cultured on Potato Dextrose Agar for fungal isolation. All isolates were identified using morphological and microscopic characteristics. Pathogenicity test was conducted based on Koch's postulates. Pathogenic fungi were cultured in Czapecks Dox broth using rotary shaker (96 rpm) for 28 days. Phytotoxins were extracted separately with Ethyl acetate and Diethyl ether and the yields determined and extracts measured in milligram (mg). Portions of the extracts were analysed by gas chromatography-mass spectrometry for identification of constituents. The pathogenicity of the extracts was evaluated using *in-vitro* and *in-vivo* leaf bioassays on eleven varieties of tomato (Kerewa, Ibadan local, LEMT3, LEMT25, LEMT39, LEMT47, LEMT49, Assila, Gem Pride, ROMA-VF and UC-82-B). Data were analysed using ANOVA and means were separated with Fisher's Least Significant Difference ($\alpha \leq 0.05$).

Identified symptoms on the leaf samples were chlorosis, leaf spot and wilt. Fungi isolated from diseased tomato leaves were *Aspergillus aculeatus, A. niger, A. tamarii, A. ustus, A. versicolor, Epicoccum nigrum, Fusarium oxysporum, Phialophora melinii, Phomopsis* sp. and *Trichodema asperellum. Fusarium oxysporum* and *Phomopsis* sp. were found to be the causal organisms of the leaf infections. Diethyl ether and ethyl acetate extracts of *Phomopsis* sp. produced 50.0mg and 41.0mg, respectively. *Fusarium oxysporum* extracted with ethyl acetate produced 54.5mg, while diethyl ether gave 39.0mg. Compounds identified from the extracts from *Phomopsis* were 1,2-Benzenedicarboxylic acid and Benzeneacetic acid, while 5-Butyl 2-Pyridinecarboxylic acid, 1,2-Benzenedicarboxylic acid and 3-butyl-pyridine were from *Fusarium oxysporum.* Treatment

of tomato varieties with phytotoxins from *Phomopsis* sp. for *in-vitro* assay showed LEMT39 and LEMT49 to be susceptible, while LEMT25 was highly resistant. For extracts from *Fusarium oxysporum*, LEMT3, LEMT25, LEMT39 and LEMT47 were susceptible; Kerewa and Ibadan local were highly resistant. For *in-vivo* leaf bioassay on Assila, there were significant differences by the fractions causing leaf spot and wilt and no significant difference for chlorosis. There were significant differences in the effect of the fractions causing wilt, spot and chlorosis in Gem Pride and Ibadan local, while for ROMA-VF, there was significant difference in leaf spot and none in wilt and chlorosis. On UC-82-B, wilt and chlorosis were significant in leaves treated with the extracts, while leaf spot was not significant.

The phytotoxins produced by the fungal pathogens induced foliar diseases on the tomato. Cultivation of varieties tolerant to these toxins is thus encouraged.

Keywords: Phytotoxin, *Fusarium oxysporum*, *Phomopsis* sp, screening, foliar diseases
Word count: 494

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DEDICATION

This project is dedicated to God, the source of wisdom and all good things and to the memory of my mother, Mrs. **Abigail Jolaade Ewekeye**

CERTIFICATION

I certify that this work was carried out by Mr. T. S. Ewekeye in the Department of Botany, University of Ibadan.

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Supervisor

A. C. Odebode

B.A. (Catholic), M.Sc. (Howard), PhD (Ibadan).

Professor of Pathology/Mycology, Department of Botany,

University of Ibadan, Nigeria.

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CHAPTER ONE

1.0 INTRODUCTION

Solanum lycopersicum L. (tomato) is commonly cultivated in most countries worldwide in outdoor fields, greenhouses and net houses (Adenuga *et al*., 2013). It is the 2nd most essential vegetable on earth, belonging to the family Solanaceae (Amuji *et al*., 2013). The Solanaceae as well has some familiar species, for instance tobacco, potato, eggplant along with pepper (Effiuwevwere, 2000). *S. lycopersicum* is said to have originated from the South America Andes in the contemporary Peru where it grew in the natural next to the base of hills (Naika *et al*., 2005). It was later conveyed to further areas of the globe by former explorers who planted it as ornamentals out of inquisitiveness (Arah *et al*., 2015). The expression "tomato" was deduced from the Nahuatl term, which precisely means "the swelling fruit." *S. lycopersicum* was probably innovated into Nigeria both by Portuguese trade missions to Africa and freed slaves from the West Indies, or by the later European merchants and colonizers (Ugonna *et al.*, 2015).

Phytotoxins are secondary metabolites formed by fungi as well as bacteria, though it might also be used when referring to noxious substances formed by advanced plants (Svabova and Lebeda, 2005). Kheder *et al*. (2012) also described phytotoxins as normally low molecular mass compounds exercising lethal consequences on host plants. In this study, the word "phytotoxin" is applied in reference to substances produced by fungi (or bacteria). According to Berestetskiy (2008), phytotoxins are capable of upsetting the vital activities of plants; they are formed by diverse organisms, particularly phytopathogenic fungi. Provided a phytotoxin is formed at an initial phase of plant infection progression and it induces part or the entire symptoms of the infection, it has a role in the pathological process. Graniti (1991), reported that phytotoxins known to take part in pathogenesis are referred to as pathotoxins or phytoaggresins.

Toxins are regarded as the unique missiles of plant pathogens which elude or surmount the in-built resistant schemes of host plants (Slavov, 2005). According to On-Line Glossary of Technical Terms

in Plant Pathology, a phytotoxin is described as a toxin produced by microorganisms and active against a plant or plant tissues. A toxin can be described as a substance of microbial origin involved in host pathogenesis (Mehrotra and Aggrarwal, 2004). On introduction of a noxious metabolite of a pathogen into a vulnerable host, the toxin should induce in the host the disease symptoms before it could be considered as a phytotoxins. Related host specificity should as well be expressed by the pathogen and the metabolite. Similar signs of disease must be elicited by both the pathogen and its toxin (Amusa, 2006).

Toxins are main determining factor of pathogenesis whenever they operate as the fundamental elements in infection trigger and symptom development. Whenever they only alter symptoms intensively, they are secondary causal factors (Wu *et al*., 2008). The usual symptoms of many plant diseases showed the contribution of phytotoxins, which could imply a function for poisonous metabolites produced through the pathogen in the infection progression. Metabolites of numerous fungi could cause undesirable impacts on plants. These include suppression of seed sprouting, distortion with slowing down of plantlet development (Slavov, 2005; Eziashi, *et al*., 2010). Other symptoms may include necrosis, chlorosis, wilt, blights, leaf spots, galls and water soaking (Türkkan and Dolar, 2008). According to Bronson (1991), not less than fifty metabolites of fungi have been accounted to be toxic to plants out of which about thirty have been implicated to contribute to plant diseases. Genes involved in syntheses of secondary metabolites have been reported to be clustered in phytopathogenic filamentous fungi (Kheder *et al*., 2012).

Chemopathogens have been referred to as lethal chemicals identified to induce plant infections and pathogens as “living organisms.” Although it is not safe to accept that all plant pathogens induce infection by the production of toxins, the use of microbial toxins in place of microbes holds huge expectation as instrument for studies of the type as well as trend of disease (Amusa, 2010). Toxins formed by infectious agents could be responsible for all or part of disease symptoms. Such toxins comprise different chemical types, including glycoproteins, peptides, polypeptides, organic acids, polysaccharides, fatty acids and derivatives, polyketides and terpenoids (Slavov, 2005).

Fusarium species are found everywhere, occurring worldwide as pathogenic and non-pathogenic strains (Rani *et al*., 2009). They have been implicated to be the cause of root rot and wilt diseases

(Gao *et al*,. 2016.) *Fusarium oxysporum* constitutes a species complex including many significant phytopathogens and toxigenic microorganisms (McGovern, 2015).

The genus *Phomopsis* (*Diaporthe*:teleomorph) contains a number of plant pathogens as well as endophytes and saprobes with a broad host and world-wide distribution. Many species identified as plant pathogens have been described as well as being endophytes from normal tissues of same or different plants and as saprobes from dead materials. *Phomopsis* species have been reported to cause canker, dieback, root rot, fruit rot, leaf spot, blight, decay and wilt on a broad host range comprising a number of economic plants globally. Several secondary metabolites that are biologically active have been reported from species of *Phomopsis* (Udayanga *et al*., 2011).

1. 2 Problem Statement and Justification

Fungi are responsible for about 70% of plant diseases resulting in global food scarcity. *S. lycopersicum* is an essential vegetable crop consumed worldwide. Major limiting factors in its production include fungal foliar diseases. Phytotoxic fungi play vital roles in the pathogenesis and expression of disease symptoms in the plant. An understanding of the phytotoxins produced in tomato leaves will enhance its optimal production. However, there is dearth of information on tolerance of tomato varieties to phytotoxins associated with their leaf diseases.

1.3 Aim and Objectives of the study

Aim

1. To extract phytotoxic metabolites of fungi isolated from infected tomato (*Solanum lycopersicum* L.) leaves

Objectives

The objectives of this study were to:

1. Isolate and identify causal organism(s) of leaf diseases of tomato,
2. Carry out pathogenicity tests to ascertain the causal organism(s) of the diseases,
3. Isolate and extract phytotoxic metabolites from the causal organism(s),
4. Confirm the phytotoxicity of the metabolites by screening different varieties of tomato for resistance to the diseases and establish their effects on the development of the diseases.

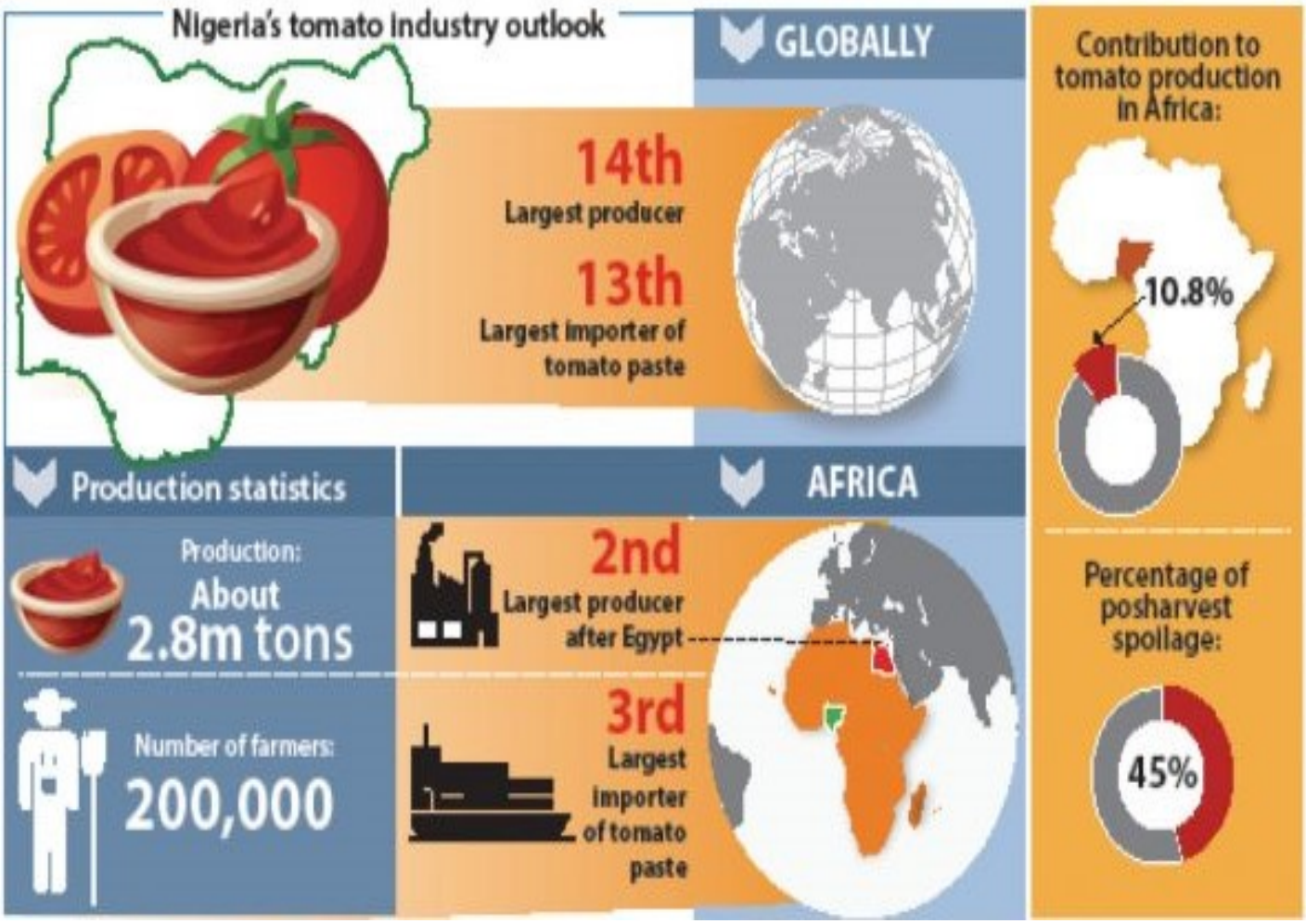
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Solanum lycopersicum*

Solanum lycopersicum L. (tomato) is a fruit vegetable consumed extensively in Nigeria. Its production spreads all over the country (Umeh *et al*., 2002). Olaniyi *et al*. (2010) described it as the most essential vegetable after onion and pepper. From Southern Nigeria, the crop probably dispersed through the Northern parts of the country. It has now become an integral part of the diet of most Nigerians and an important source of cash to a large number of farmers, middlemen and processors. Tomatoes generally range in size from 0.019m in diameter and 0.07kg in weight to 0.152m in diameter and 0.907kg. They range in colour from near white to red to greenish yellow and orange. Generally the lighter coloured tomatoes have a milder taste than the deep reddish coloured ones. The colour of tomato is as a result of the red pigment lycopene (Arah *et al*., 2015). Tomato is an essential and popularly cultivated horticultural crop in the universe. In global production by mass, it is 3rd in rank behind potato and sweet potato (Tan *et al*., 2010).

In 2010, Nigeria rated 16th among the tomato producing nations of the world (Ugonna *et al*., 2015). Nigeria‘s production of tomato then stood at about 1.8 million metric tonnes, this accounted for 68.4%, 10.8% and 1.28% of total outputs of West Africa, Africa and the world respectively (FAO, 2010). In 2012, 17.938 million tonnes of tomato was produced in Africa. Egypt led the continent with 8.625 million tonnes (Arah *et al*., 2015). More recently in 2016 (Figure 2.1), the production increased to approximately 2.3 million tonnes and Nigeria was the 14th largest producer of tomato worldwide (PwC, 2018). In Nigeria, *S. lycopersicum* takes about 18% of the mean intake of vegetables every day, this makes it an essential food crop to a typical Nigerian (Adepoju, 2014). The list of fifteen top producing nations in Africa is depicted in Table 2.1.



Adapted from PwC (2018)

Figure 2.1: Tomato Production in Nigeria

Table 2.1**: Tomato producing nations in Africa.**

Order	Nation	Production (tonnes)
1	Egypt	8,625,219
2	Nigeria	1,560,000
3	Morocco	1,219,071
4	Tunisia	1,100,000
5	Cameroon	880,000
6	Algeria	796,963
7	South Africa	564,740
8	Sudan (former)	529,200
9	Kenya	397,000
10	Ghana	321,000
11	Tanzania	255,000
12	Mozambique	250,000
13	Benin	244,742
14	Libya	225,000
15	Niger	188,767

(FAOSTAT, 2014)

2.2 Climatic data of major production areas

Tomato is majorly cultivated in the South West and North of Nigeria within latitudes 10^0N and $12^0$30N in the Northern Guinea and Sudan Savanna ecologic zones. These areas are characterized by a clear-cut wet period from either April or May to September or October and a clear-cut dry period from October to March when production is possible only under irrigation. Rainfall in the area varies from about 600 to 1300mm, with one peak in July or August. Temperatures are tropical throughout the area mostly with night temperatures above 21.1^0C and daytime temperature more than 32.2^0C. During the dry period there is a three to four month period when night temperatures may fall below 15^0C. It is generally well known, however, that yields are higher in the northern divisions of the nation than in the southwest where tomato is also grown. In the North where tomatoes are cultivated under channel irrigation, leaf infections are less, this account for better quality fruits and higher yields. Tomatoes are warm period crops and are responsive to elevated wetness and rain. Yield increments are generated in well-drained, sandy loam high in humus soil (Wokoma 2008; Ugonna *et al*., 2015). The major production of tomato is around the wet period. However, to facilitate its handiness all through the year, there should be improved turnout particularly at the dry periods (Adenuga *et al*., 2013). Tomato is the mainly crucial vegetable in a vigorous north-south trade in Nigeria. Its production offers employment to a large number of small-scale producers who sometimes make gross margins of up to ₦2,700 (US $3,000)/ha. A large number of people are also engaged in transportation, packing, sorting and sale to consumers.

2.3 Economic importance of tomato

In many areas of the globe, tomato has become a significant industrial crop due to its financial significance and dietary value to human nutrition and human health significance (Arah *et al*., 2015). Tomatoes provide substantial quantities of folate, potassium, ascorbic acid, vitamin A and tocopherols whilst supplying about two percent of the fiber and one percent of the protein mass (Tan *et al*., 2010). Processed tomatoes have higher levels of these nutrients because they are more concentrated. Next to oranges, tomatoes contribute a high amount of Vitamin C in most diets.

Tomato is a common delicacy in most homes in Nigeria. It can be prepared as vegetable sauce, stew and also in the preparation of various foods like jollof rice, stew for rice, boiled yam and potato. It is also a part of the ingredients in the preparation of other dishes like vegetable soup, melon soup and yam pottage and could be eaten fresh as salad (Ejale and Eikhuemelo, 2009). Canned and

dehydrated tomatoes are processed products which are economically vital. Tomatoes are used in a variety of untreated and processed foods like salads, beverages, paste, puree, ketchup, whole peeled tomatoes among others. The tomato fruit includes profuse and proportionate nourishment composed of minerals such as potassium, magnesium, calcium, iron, zinc, vitamins A, B_1, B_2, C and E, nutritional fibres, citric acid. Moreover, the licopene's red pigment, which tomato fruit includes in abundance, has lately drawn interest due to the elevated antioxidant capacity of the licopene against oxygen radicals, which is likely to cause cancer, aging. Eating of tomatoes has been linked with lessened threat of breast sarcoma (Zhang *et al*., 2009), head and neck sarcoma (Freedman *et al*., 2008) and prostate sarcoma (Tan *et al*., 2010). Tomato's elevated content of vitamin A and C is essential to prevent muscle deterioration and enhance vision. It is a potent blood cleanser and resolve infections of the urinary tract. *S. lycopersicum* is rich in fibre, making absorption easier and aiding to cut down weight. As a consequence of its many health advantages, tomato production globally is prominent (Arah *et al*., 2015). Table 2.2 provides fifteen major nutrients and their amounts which can be obtained from eating of 123g of mature tomatoes.

Table 2.2**: Fifteen main nutrients obtained from (123g mature) tomatoes**

Nutrient	Amount
Calcium	1.2mg
Carbohydrate	4.7g
Copper	0.073mg
Dietary fiber	1.5g
Fat	0.2g
Iron	0.33mg
Magnesium	1.4mg
Niacin	0.731mg
Pantothenic acid	0.109mg
Phosphorus	3mg
Potassium	292mg
Protein	1.0g
Thiamin	0.046g
Total sugars	3.23g
Vitamin C	16.9mg

Source: The USDA National Nutrient Database (2010)

2.4 Pests and Diseases of Tomato

Like many other plants, from the point of field planting to consumption, many procedures are required in tomato production. Each of these steps generates an avenue for entering or attaching microorganisms to the plant. Some of these organisms may be harmful to people. A lot of pests and diseases attack tomato. However, in many locations, cultivation of tomato is normally limited by diseases rather than pests. Cultivation of tomato in Nigeria is faced with many challenges, chief among which is unavailability of high quality seeds, inadequate storage facilities as well as losses due to pest and disease attack (Ugonna *et al.*, 2015). Pests and diseases is a critical constraint causing reduced production of tomato. *S. lycopersicum* is attacked by a wide range of plant pathogens including fungi, bacteria, viruses as well as plant parasitic nematodes (Agrios, 2005). About two hundred tomato diseases exist; out of these thirty are of economic significance.

The pests attacking tomato include flea beetles, tomato hornworms, cutworms, fruit worms, whiteflies and aphids (Arogundade *et al*., 2007). *T. absoluta* originated from South America (Aigbedion-Atalor *et al*., 2019) but was first detected in Nigeria in 2015 (Borisade *et al.*, 2017), it is extremely invasive and has a destructive effect on tomato production. From its point of detection in Kastina State, Nigeria, it has been reported to have spread to eleven other tomato producing States in Nigeria (Aigbedion-Atalor *et al*., 2019). It was referred to as "tomato ebola". The pest has reportedly led to about eighty percent loss of *S. lycopersicum* production in its first cycle. *T. absoluta* attack tomato at the larval stage and completely affect the plant by destroying all the productive parts of the plant. The parts of the plant affected include apical buds, flowers, fruits, stem and leaves. The pest feed on the green and mature fruits and the whole tomato plantation could be destroyed within 2 days (Sanda *et al*., 2018). The pest can be managed using a combination of measures including chemical control, use of Tuta trap tray, cultural means, biocontrol, resistant varieties and bio-pesticides.

2.4.1 Fungal diseases

Fungi are the most significant and widespread pathogens infecting a broad variety of host crops, resulting in either field or storage economic losses in tomatoes (Yahuza and Yahaya, 2015). They are the mostly encountered diseases of vegetables throughout the world. They mainly affect leaves, stems, flowers and fruits of annual plants, mostly vegetables and ornamental plants (Kumar, 2017).

Although *S. lycopersicum* is vulnerable to infection from other pathogen agents, it has been asserted that fungi constitute majorly to reduced yield as they attack the plant at every of its development stage and are borne by agents like air, water, soil and seed (Chohan, 2016). In a study conducted by Kumar (2017) in Niger State, Nigeria, 24 pathogens were associated with tomato diseases, these included; 17 fungi. Out of the diseases caused by fungi, 8 are foliage diseases, 3 fruit diseases, 2 each causing stem diseases and wilting and 1 each were root and seedling diseases.

2.4.1.1 Septoria leaf spot

One of the most prevalent foliar diseases of tomatoes is the Septoria leaf spot triggered by *Septoria lycopersici.* It appears first as tiny water-soaked spots that quickly turn into round spots with a diameter of about 1⁄8 inches. Gradually, the lesions create grayish white centres with dark boundaries. The light-coloured centres of these spots are the Septoria leaf spot's most characteristic symptoms. In the centers of the spots, fungal fruiting bodies appear as small black specks when environmental requirements are well-disposed. Spores extend through splashing rain to fresh leaves. Seriously infected leaves become yellow, droop, and drop off ultimately. Lower leaves are first infected, and if wet conditions continue, the disease move upward.

After extended hot, moist weather, defoliation can be serious. Infection can happen at any point of plant growth although mainly often appears subsequent to fruit setting by the crops. At any point of growth, the disease may impact crops (Arogundade *et al.*, 2007). Septoria leaf spot control is a mixture of multiple cultural methods that assist to decrease the threat of countless other diseases.

2.4.1.2 Anthracnose

The causal fungus is *Colletotrichum coccodes*, symptoms first become noticeable as tiny, spherical, depressed spots in the skin on mature or ripening fruit. While these spots enlarge, dark spots or concentrical bands of dark specks form, these are the spore-producing bodies of the fungi. These bodies give out huge number of spores in humid weather, giving to the diseased regions cream to salmon-pink colour. Dotted fruits can often rot entirely due to anthracnose spots being attacked by secondary fungi. Most frequently, anthracnose becomes visible on overripe fruits. Anthracnose may develop on foliar spots induced through another fungus or via wounds created while insect feeds weeks before the fruit ripens. Spores are mainly distributed through the splash of rain. Warm, moist

weather leads to the spread of the disease and symptoms development. Although insects or other injuries alleviate infection, if injuries are lacking, tomatoes can still become infected (Kilic-Ekici and Yuen, 2003).

Management of anthracnose is as observed for septoria leaf spot. Furthermore, at each harvest, select all ripe fruit, plant disease-free transplants sufficiently apart so as to avoid crowding after they have grown fully, this is to assist the foliage dry quickly. Watering of the plants at the base should be done earlier in the day to reduce the period when the leaves are moist. Plant rubble should be taken away immediately for burying or deep ploughing. Rotate plants so that it is only every three or four years that tomatoes are cultivated in the same soil. Keep away from working with crops when the leaves are moist to prevent the spread of microorganisms that cause diseases. Besides, harvest at regular intervals.

2.4.1.3 Early blight

The fungus *Alternaria solani* causes early blight (Alternaria leaf spot). The most evident symptom of the disease is premature loss of lower leaves. Brown to black spots emerge on lower leaves having dark borders. Often spots merge and form uneven blotches. There are often dark, concentric rings in leaf spots (Glandorf *et al*., 2001). Seldomly, the fungus invades fruit at the stem end, inducing big, depressed areas with concentrical bands and a smooth black outlook. Rapid distribution of early blight is supported by moist weather. Potatoes can also be infected by *A. solani*, crops could be infected with early blight at any phase during the growing season, but generally infection advances more quickly after fruit setting. During the hotter months, the symptoms become widespread. On older leaves, the infection develops brown to black, target-like spots. In acute cases, stems and fruit are also attacked by the fungus. The leaves affected may turn yellow, and fall, leaving the fruit subjected to sunburn. The greatest control is sanitation. All diseased plant should be gotten rid of. Avoid planting tomatoes at the same location for two consecutive years. To enhance air circulation, space plants further apart. Avoid working with crops when the leaves are moist to prevent the spread of microorganisms. Avoid overhead irrigation. Sulfur dust may assist to safeguard fresh leaves from infection if the infestation is severe.

2.4.1.4 Late blight

The causal organism of Late blight is *Phytophthora infestans*. Either young or old leaves can be infected with the disease. It initially shows as water-soaked portions that quickly enlarge, forming

uneven greenish black streaks, giving a frost-damaged appearance to the plant. In humid weather, the abaxial surfaces of the leaves often depict a downy white growth. Green or ripe fruit infection shows big brown blotches that are unevenly formed. Infected fruits degenerate quickly into stinking masses. *P. infestans* can spread from potatoes to tomatoes and cause comparable symptoms on potatoes (Masinde *et al*., 2011). Late blight can be controlled with measures similar to Septoria leaf spot. Furthermore, do not rotate tomato with potato.

2.4.1.5 Fusarium and Verticillium wilts

Crops in the Solanaceae family like *S. lycopersicum*, *S. melongena*, *S. tuberosum* and *Capsicum annuum* could be infected with the fungi anytime during the period of growth. Of wilt diseases, Fusarium wilt is most widespread amid local varieties of tomatoes that are more vulnerable. Usually the microbes causing wilt penetrate the plant via juvenile roots and afterward develop into and up the roots and stem's water-conducing vessels. The supply of water to the leaves is obstructed as the vessels are plugged and break down. Leaves start to wilt on sunny days with a restricted supply of water and recover at night. Wilting may become visible earlier on the lower or upper leaves. The course of infection may proceed till the whole plant wilts or dies. *S. lycopersicum* and *S. tuberosum* might pick up fairly, however they are generally fragile, and turn out lesser value fruit. Symptoms of fusarial wilt start in *S. lycopersicum* as minor vein clearing on peripheral leaflets and limping of leafstalks. Subsequently, often before the plant matures, the leaves beneath droop, become yellowish and drop dead, and could result in killing the entire plant mostly prior to attaining maturity (Wokoma, 2008; McGovern, 2015).

Verticillium wilt symptoms are comparable to that of Fusarium wilt on tomato. Frequently, symptoms are not encountered till the plant bears strongly or there is arid phase. The base leaves turn light coloured, afterward ends and borders end up dying and dropping off. Lesions that are V in shape at the ends of the leaf are characteristic of tomato Verticillium wilt. Infected crops generally pull through the season however they are slightly underdeveloped and based on the severity of the attack, yields as well as fruits may be little. A light brown staining can be discovered within the stalk comparable to the one induced by Fusarial wilt, but is generally limited to lower areas of the plant. Characteristically, the discolouration is lighter than with Fusarial wilt. Symptoms are only occasionally seen on one side of the plant (Miller *et al*., 2010).

A number of different kinds of *Fusarium oxysporum* are causal organisms of Fusarial wilt in solanaceous plants. These include: *F. oxysporum* f. sp. *lycopersici (Fol)* in *S. lycopersicum*, *F. o.* f. sp. *melongenae (Fom)* in *S. melongena* and *F. oxysporum* f. sp. *vasinfectum (Fov)* in *Capsicum annuum*. All pathogens causing Fusarial wilt are usually hosts-specific. They are humid weather organisms. *Verticillium albo-atrum* and *V. dahliae* cause Verticillium wilt. They affect a wide variety of plant, covering weeds as well as cultivated crops (Miller *et al*., 2010).

2.4.1.6 Target leaf spot

Target leaf spot is a prevalent infection in almost all regions around the world where tomatoes are grown (Kurt, 2004). The disease has been discovered in both tropical and subtropical regions on a wide spectrum of hosts. The disease presents a severe menace particularly during the dry season in Southern Nigeria. In the seedling phase and soon before and during fruit formation, attacks on the crops are more frequent. At first, symptoms appear as minute pinpoint, water-soaked lesions on the adaxial leaf surface and then grow into light tan lesions surrounded by prominent yellow halos. Lesions appear on the fruit as dark, depressed, tiny, tan spot with a light brown centre. The causal organism, *Corynespora cassiicola*, is a pathogen of worldwide importance with a very wide host range (Kurt, 2004).

Other fungal pathogens that have been described to be associated with tomato diseases include *Pythium aphanidermatum* causing damping off (Bharathi, 2004), *Stemphylium solani* causing gray leaf spot, *Botrytis cinerea* causing gray mold, *Cercospora fuligena* causing Cercospora leaf mold, *Cladosporium fulvum* causing leaf mold, *Sclerotium rolfsii* causing southern blight (Kumar, 2017).

2.4.2 Viral diseases

2.4.2.1 Cucumber mosaic virus (CMV)

Cucumber mosaic virus induces yellowing, makes tomato bushy and stunted. Leaves could be mottled. Tomato seeds mainly frequently transmit the virus. There may also be mechanical transmission by workers touching crops and movement through aphid vectors, however, this is much less prevalent in tomatoes than in cucurbits. There are no chemical controls, affected plants should be removed and destroyed (Paulitz and Belanger, 2001).

2.4.2.2 Tomato Spotted Wilt Virus (TSWV)

In commercial tomato production, TSWV has conventionally been an issue. Symptoms start on the leaves as dark brown to purple spots. The dark regions extend to stems and form cankers. Stem mottling might be observed as well. As the disease proliferates, wilting symptoms steadily advance. The leaf tissue, though, is rigid, not limp. Yellow rings or spots on fruit are the mainly conspicuous symptoms. Fruits could also be deformed. TSWV is transmitted by western flower thrips from plant to plant. Uprooting and eradicating affected crops is the only way to manage the disease. Thrips are very hard to handle. Treatment with soaps, oils and sulfur dust have not produced excellent outcomes.

2.4.2.3 Tomato Yellow Leaf Curl Virus (TYLCV)

Tomato Yellow Leaf Curl Virus has been identified in many tropic and sub tropic nations (including Nigeria) as a severe virus with an extensive spread. Infected plants are dwarfed forming small chlorotic leaflets and curled leaf blade. The severity of leaf symptoms and reduction of yield is determined by the age and the period of development at which the plant becomes infected. Transmission of the virus is usually by the white fly, *Bemisia tabaci*. It can also be by grafting and in recent times, transmission via infected seeds and *S. lycopersicum* seedlings has been described. Symptoms of infection are yellowing, puckering, reduction in size of terminal leaves, curling of lower leaves and dwarfing.

Control of the vector has been proven to be ineffective; however, control of TYLCV can be achieved efficiently through breeding for varieties tolerant or resistant to the virus and manipulating cultural practices (Abraham *et al*., 2019).

2.4.2.4 Tomato Mosaic Virus (ToMV)

Tomato Mosaic Virus occurs globally and has been described as a prevalent tomato disease in Nigeria. The main hosts of ToMV include *S. lycopersicum, Capsicum annum, Abelmoschus esculentum, Solanum tuberosum.* Symptoms of infection include curling of leaves, mottling and chlorosis, stunted growth and reduced fruit production. Transmission is by grafting and via seeds. Management strayegies include planting virus-free seeds and resistant varieties (Ayo-John and Odedara, 2017). Other viral diseases of *S. lycopersicum* which have been reported in Nigeria include Tomato mosaic virus, tomato bunch top virus (Arogundade *et al*., 2007), Tomato Bushy

Stunt Virus (TBSV), Tomato Aspermy Virus (TAS), Potato Virus Y (PVY), Pepper Veinal Mottle Virus (PVMV) (Ayo-John and Odedara, 2017).

2.4.3 Bacterial diseases

2.4.3.1 Bacterial wilt

Bacterial wilt of tomato caused by *Raulstonia solanacearum* is one of the most significant bacterial diseases of plants occurring globally with a broad host spectrum of above two hundred species (Popoola *et al.*, 2015). The disease is commonly encountered in places where *S. lycopersicum* is grown. The disease spread is favoured by humid climate with plantation of only tomato or in combination with other Solaneceous crops. Control of the disease is through soil amendment, use of resistant varieties and biological control (Adebayo, 2011). The pathogen is basically soil-borne and water-borne. Plants are infected mainly via wounds on the roots created by lateral emergence of root or through damage resulting from soil-borne organisms (Fajinmi and Fajinmi, 2010). Symptoms in affected plants appear at first as showing drooping of terminal leaves and then rapid and persistent wilting. Control include phytosanitory measures and adjusting cultural measures such as rotating crops with non-hosts like grasses, inter-cropping, control of weed and root knot nematodes population , timing of planting to avoid heat, deep ploughing of crop residues that could harbour inoculums (Fajinmi and Fajinmi, 2010).

2.4.3.2 Bacterial canker

Bacterial canker: Bacterial canker occurs occasionally, symptoms start with lower leaves turning downward. Dark to light brown streaks can grow on the midribs of the leaf and ultimately spread to form a canker on the stem down the leafstalk. Vascular discoloration can occur. To control the disease, eradicate the crops if discovered. Avoid composting with plant materials. For two to three years, do not plant tomatoes, potatoes or eggplants in the same soil.

2.4.3.3 Bacterial spot

Bacterial spot is occurs globally. Host plants attacked by the disease are members of the family Solanaceae especially *Solanum* and *Capsicum* species. It has been reported (Potnis *et al*., 2015) that different species of *Xanthomonas* are associated with bacterial spot, however, according to Kumar

(2017) *Xanthomonas vesicatoria* is the causal organism of bacterial spot in Nigeria. The seeds as well as transplants serve as the major sources of inoculums. In both tropical and subtropical regions, the bacterium survives for a few months in crop debris. The dissemination of the bacterium in the field can be through rain drops carried by wind and wounds caused during cultural practices. Symptoms of infection include necrotic lesions on all aerial parts of plants. On the leaves, spots start as small, water-soaked areas that later turn dark brown and raised on leaf undersides. On the fruit, the blemish starts as small, water-soaked spots which enlarge and become blister-like. Fruit decay may result via the activities of other opportunistic pathogens. Measures to manage the disease include planting of disease resistant varieties, use of disease-free seeds, ensuring proper sanitary procedures and crop rotation (Potnis *et al*., 2015)

2.5 Phytotoxins and Plant Diseases

In all major taxonomic groups of fungi, toxigenic pathogen species are in existence. They are found in some of the genera: *Aspergillus, Penicillium, Fusarium, Claviceps, Alternaria, Stachybotrys, Myrothecium, Phoma, Diplodia, Verticllium, Colletotrichum, Septoria* and *Phytophthora. Xanthomonas, Pseudomonas* and *Clavibacter* are also toxin-producing bacteria (Svabova and Lebeda, 2005). A number of phytotoxic metabolites linked with bacterial and fungal pathogens have been discovered, causing symptoms comparable to the ones induced by the pathogens. These noxious metabolites comprise pinolidoxin from *Ascochyta pinodes*, nectriapyrone and altersolanols A and J, and macrosporin from *Diaporthe angelicae* (anamorph Phomopsis foeniculi) (Evidente *et al*., 2011), thaxtomin A from *Streptomyces scabies,* deoxyradicin and maculosin from *Alternaria alternata*. Described metabolic substances from more infective agents comprise piricularin from *Piricularia oryzea*, Product 1 (PR I) and Product II (PR II) from *Ascochyta pisi* (Abouzeid and El-tarabily, 2003), victorin from *Cochliobolus victoriae*, phaseolotoxin from *Pseudomonas syringae* pv. *phaseolicola*, toxin from *Periconia circinata*, saccharitoxin from *Helmithosporium sacchari,* cercosporin from *Cercospora* spp. Most of the phytotoxins produced by these pathogens have been accounted as having an important function in pathogenesis (Amusa *et al.*, 1993; Abouzeid and El-tarabily, 2003; Evidente *et al*., 2011).

Girish *et al*. (2009) indicated that *Phomopsis azadirachtae*, causal organism of neem die-back, (*Azadirachta indica*) produced phytotoxic compounds that suppressed seed germination and decreased seed quality. Tsantrizos *et al*. (1992) also revealed isolation of ergosterol, ergosterol

peroxide, phthalides, convolvularic acid A, convolvulic acid B, convolvulol, α-pyrone convolvulopyrone and other fungal metabolites from *Phomopsis convolvulus*, causative organism of leaf spots and anthracnose of *Convolvulus arvensis.*

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Toxins of microbial origin are formed by plant pathogens which could be fungi or bacteria. They take part in host-pathogen relations as well as in appearance of disease. Being substances of low molecular mass generated by certain pathogens that capable of replicating symptoms comparable to those detected in natural plant diseases. Phytotoxins are an output of microbial pathogens that are expected to cause apparent harm to plant tissue and need to be known for involvement in disease progression. Phytotoxins operate freely on cell protoplasts, while other pathogen metabolites, for example high molecular mass polysaccharides released by bacteria inducing wilt, blocking passage of solution in xylem vessels and could cause plant death, are non-toxic (Amusa, 2006).

The role of a toxin as a disease-causing factor is demonstrated by the presence of the toxin in a diseased plant and its capacity to produce singly as a minimum portion of the symptom of the disease (Slavov, 2005). A range of symptoms like necrosis, wilting, chlorosis, water soaking and eventual death may be elicited by phytotoxins produced by pathogenic fungi on their host plants.

Nevertheless, in some cases, fungal toxins may operate at the biochemical level and not cause noticeable effects. In many host-pathogen interactions, phytotoxins have been described as pathogenicity or virulence factors (Doohan, 2005). Pathogen like *Verticillium* generates metabolites not required for infection, but shows the manifestations. The greatest proof for a causal function for such toxins is to duplicate whole symptoms or those which cause predominantly distinguishing ones (Mansoori and Smith, 2005). Necrosis and chlorosis have been reported to be among the usual symptoms that toxin impacts on naturally infected crops.

Plant pathogenic fungi in the genus *Alternaria* have been known to cause diseases of cultivated as well as wild plants. *Alternaria* are known to produce phytotoxins which take part in a major function in plant infectivity. Zinniol is formed by various plant pathogenic species of *Alternaria* and is a causative agent of diseases of tomato, potato, carrot and onions (Berestetskii *et al*., 2010).
Besides the production of one or more toxins, some pathogens could also send out enzymes which cause collapse of the cell wall, resulting to tissue disarrangement, and could also upset the regular

functioning of the plant hormone resulting in unusual plant development. Through their abundant increase or through polysaccharide formation, the pathogen may physically obstruct the host's water conducting vessels (Slavov, 2005).

It is essential to ascertain the function of phytotoxic compounds in disease or virulence initiation and progress. Some authentication is given by indicating that the toxin replicates at least some of the disease's symptoms and that the toxin is formed by all virulent pathogen isolates. The connection of the quantity of toxin generated by distinct pathogen isolates by its virulency and the connection of toxin sensitiveness of the host's distinct genotypes with vulnerability includes additional proof of toxin's contribution in causing disease (Strange, 2007).

2.6 Detection and assessment of Phytotoxins

The production of phytotoxic metabolites is generally in liquid media (Strange, 2007). Bioassays are employed to identify phytotoxins in liquid cultures as well as to assess their phytotoxicity. In order to select for bioassay, the ecology and biology of the fungi are considered. For instance, in case of a phytotoxins originating from a soil fungus or a causal agent of root rot disease, the plant seedling will be used for the biological assay while the level of inhibition of growth of roots treated with the Culture Filtrate (CF) or the purified toxin is determined with untreated roots as control. For symptoms caused by phytotoxins produced by pathogens attacking plant leaves, the CF is applied to the leaves wounded with a needle; this is done because symptoms of toxicoses are not as pronounced in intact leaves. It is likely to separate phytotoxins from infected plant tissues and germinating conidia of fungi, however this method is not helpful because of the low content of the compound of interest. Hence, to be able to separate phytotoxins in quantities enough to study their biological and chemical characters, the fungi are cultured in liquid media (Berestetskiy 2008). Production of phytotoxins is affected by a couple of factors including the component of the medium, its acidity, the length and conditions of culturing. Many biological assays have been employed for detection and quantification of phytotoxins. These include seed, seedling, parts of plants or whole plant. As soon as the biochemical lesions resulting from applying the toxin is discovered, it could form the basis of the assay. Phytotoxins are usually determined by physico-chemical techniques like mass spectrometry, High Performance Liquid Chromatography (HPLC), ultra violet, infra red or Nuclear Magnetic Resonance (NMR) (Strange, 2007).

2.7 Classification of Toxins

A number of fungi generate toxicants which severely harm or otherwise destroy plant cells also they vary from low-molecular weight metabolites to proteins (Doohan, 2005). Many features have been used for classifying plant-influencing toxins. These consist of chemistry: some phytotoxins are thought to be low relative molecular mass peptides, polypeptides, polysaccharides, polyketides and terpenoids (Slavov, 2005). Another means of classification relies on the producing organism which may be either a fungus or bacterium. However, the widely accepted means of classification relies on toxic selectiveness to genotypes of plant and on the general role in development of infection (Yoder, 1980).

2.7.1 Non host-specific/selective toxins

These are toxins affecting a broad variety of host plants. It has been shown that several toxic substances generated by phytopathogenic microorganisms generate all or portion of the syndrome of the disease on the host as well as additional plants which naturally are not affected by the pathogen. These toxins raise the gravity of infection produced by a pathogen, impact the pathogen's virulence, but are not vital to cause disease for the pathogen, they do not determine the pathogenicity. Phytotoxins, such as tabtoxin and phaseolotoxin, suppress typical enzymes of the host, resulting in increased toxic substrate levels or reduction of the compounds required. Besides being phytotoxic, some non-host-specific toxins such as trichothecenes, fumonisins and fusarium acid are also categorized as mycotoxins, in other words, harmful to human as well as animal health (Abbas *et al*., 1999; Doohan, 2005; Rani *et al*., 2009).

2.7.1.1 Fusaric acid

Fusaric Acid (FA) is one of the most researched contents in *Fusarium oxysporum* isolates and their culture filtrates (Svabova and Lebeda 2005). FA's elevated output was associated with the virulence of *Fusarium* spp. in plant pathogenic strains. Yabuta *et al*. (1937) first isolated the fungal toxin, FA (5-butylpicolinic acid), during laboratory culture of *F. heterosporum* as a compound that inhibited rice seedling development and is poisonous to different crops, fungi and bacteria. Many *Fusarium* spp. are producing FA. It has a natural contaminant or mycotoxin that amasses in corn and cereal grains during infection, is highly noxious to livestock and humans by increasing the virulence of other metabolites of *Fusarium*. Not only is it mildly poisonous to livestock, it has antibiotic, insecticidal and pharmacological actions as well. It is used in plant selection of wilt resistance.

Fusarium spp. produce mycotoxins such as beauvericin, fumonisin and moniliformin or phytotoxins such as fusaric acid and gibberellic acid (Nur Ain Izzati *et al*., 2011), enniatin and trichothecenes (Abbas *et al*., 1999). *Fusarium oxysporum* or its special forms *F*. spp. *lycopersici* is the most expansive producer of FA (Rani *et al*., 2009). Species of *Fusarium* are widely spread from temperate to tropical areas globally. The species are also all-pervading fungi that occur as plant, animal and human saprophytes, endophytes or pathogens. They are generally pathogenic to a broad variety of plants in natural environments including *S. lycopersicum*, *Capsicum annuum*, *Sorghum bicolor, Zea mays, Ananas comosus*, *Musa* spp., *Oryza sativa, Saccharum officinarum, Mangifera indica*, the Fabaceae and Poaceae (Rani *et al.*, 2009; Nur Ain Izzati *et al*., 2011). Large concentrations of FA reduce growth of root and root hair and stimulate rapid hyperpolarization of transient membranes. By reducing cell viability, FA can be implicated in fungal pathogenicity. Common early defense response such as reactive O_2 species could be induced. It has been reported that FA has been produced by *Fusarium moniliforme, F. oxysporum, Gibberella fujikuroi*, *F. verticilloides* as well as *F. arthrosporioides*. HPLC, Thin layer Chromatography (TLC), mass spectroscopy, NMR are used to detect FA (Rani *et al*., 2009).

2.7.1.2 Tentoxin

Tentoxin is formed by *Alternaria alternata*, which in many crop species causes spots and chlorosis. More than one-third of the leaf region of seedlings dies and become chlorotic. It is a cyclical tetrapeptide which connects and deactivates a protein involving the transport of energy within chloroplasts. However, molecular location of tentoxin's action and the precise mechanisms it produces are still unknown (Agrios, 2005). Tentoxin is unspecific because it is discovered in many plant kinds at its delicate spot (Yoder, 1980).

2.7.1.3 Cercosporin

Cercospora along with a number of other fungi produces Cercosporin. It causes many crop plants' destructive leaf spot and blight diseases for example grey leaf spot of maize. It is distinctive amid toxins of fungi because it is triggered via light and turns to be poisonous to crops by producing excited oxygen species, especially single oxygen. This destroys the membrane of plants and offers the pathogen with nutrients. The capacity of the spores of the fungi and its mycelium to endure cercosporin's overall toxicity is due to the production of pyridoxine. There is a reaction between pyridoxine and single atoms of oxygen and it is nullified through this response (Agrios, 2005).

2.7.1.4 Other Non host-Specific/selective Toxins

Many other non-host-specific toxic substances were separated from the cultures of infectious fungi and bacteria and were involved as contributive agents in the pathogen-caused disease progression. Fumaric acid, generated by *Rhizopus* species is one of these toxins generated by fungi. Oxalic acid by *Sclerotium* and *Sclerotinia* species. Zinniol, alternariol and alternaric acid generated through *Alternaria* species in leaf spot infections in multiple crops. Ceratoulmin generated by *Ophiostoma ulmi*; fusicoccin, generated by *Fusicoccum amygdale*; ophiobolins, formed by several *Cochliobolus* species. Pyricularin, generated in rice blast disease by *Pyricularia grisea*. Coronatin, produced by *Pseudomonas syringae* pv. *atropurpurea* is another non-host specific toxins generated by bacteria. Others include tagetitoxin by *P. syringae* pv. *tagetis* and syringomycin by *P. syringae* pv. *syringae* (Doohan, 2005).

2.7.2 Host-Specific/Selective Toxins

Host-specific toxins at physiological levels, are generally needed for pathogenicity (Agrios, 2005), they are only lethal to a pathogen's host plants, and demonstrate no or low toxicity to other crops (Doohan, 2005). Most host-specific toxins must be present in order to cause disease by the producing microorganism. There have been reports to date that host-selective toxins are being formed by simply selected fungi such as *Corynespora, Cochliobolus, Periconia, Hypoxylon Alternaria,* and *Phyllosticta*. In addition, some polysaccharides from the bacteria *Pseudomonas* and *Xanthomonas* were shown as host selective (Markham and Hille, 2001).

2.7.2.1 Victorin

Cochliobolus victoriae produces Victorin, or Hv-toxin. A single gene controls the development of toxin in the fungus. Resistance and vulnerability to the fungi, in addition to tolerance with toxin reactivity, are moderated through two identical allelomorphs, though in cases of intermediate resistance, dissimilar batches of these allelomorphs could be concerned. Not only does the toxin produce all of the pathogen-induced disease's internal symptoms, it also generates comparable histochemical and biochemical modifications in the host, for instance modifications in the composition of the cell wall, loss of cell electrolytes, enhanced respiration, and reduced development and protein synthesis. In addition, only fungal isolates producing the toxin in culture are infectious in oats, while the ones producing no toxin are non-pathogenic. Victorin has been

purified and a complex chlorinated, partly cyclic pentapeptide has been determined to be its chemical structure (Agrios, 2005).

2.7.2.2 *Alternaria alternata* toxins

Several *Alternaria alternata* pathotypes attack various host crops and each produces one of several various types of associated compounds that are toxic only to each pathotype's specific host plant. Some of the toxins and hosts they are produced on and influence are, AAL toxins produced by *A. alternata* f. sp. *lycopersici* that cause stem canker on tomatoes, AF toxins (produced by *A. alternata* f. sp. *fragariae*) on strawberries, AM toxins generated by produced by *A. alternata* f. sp. *mali* on apples, ACT toxins (produced by *A. alternata* f. sp. *citri tangerine*) on tangerine. For sensitive varieties of apples, the toxin is highly selective, while resistive varieties without symptoms could withstand over ten thousand times as much toxin with no symptoms. The cell membranes of sensitive cells are induced to grow introversions and cells demonstrate substantial electrolyte loss. At the boundary between the cell membrane and cell wall, the toxin's original toxic effect appears to happen. The AM toxin, however, also stimulates fast chlorophyll loss, implying that the toxin has over one point of operation (Markham and Hille, 2001).

2. 7.2. 3 *Helminthosporium carbonum* toxin (HC-toxin)

HC-toxin is a host-selective toxin comprising of a family of 4 associated compounds, the most profuse of which is a cyclic peptide composed of D-proline, L-alanine, D-alanine, and L-Aeo, where Aeo is 2-amino-9,10-epoxy-8-oxodecanoic acid. Formation of toxin is determined by numerous genes. HC-toxin is not stored in the fungus ' resting spores, but is concurrently synthesized with spore germination, which is matched by HTS1 transcription. In addition, toxin formation and appressoria production seem to be controlled in a coordinated manner, as spores incubated under conditions that do not stimulate appressoria also do not synthesize toxin (Strange, 2007).

2.7.2.4 Other Host-Specific Toxins

There are a number of other familiar host-specific toxins produced by fungi. *Periconia circinata* forms peritoxin (PC toxin), causing sorghum rot; *Mycosphaerella* (*Phyllosticta*) *zeae-maydis* forms PM toxin in maize and *Pyrenophora tritici-repentis* forms Ptr toxin, causing tan spot in wheat.

Corynespora cassiicola, forms the tomato CC toxin that causes the leaves and tomato fruits target leaf place (Kurt, 2004; Agrios 2005).

2.8 Phytotoxin and Plant Disease Development

Different evidence indicates the significance of phytotoxins in symptoms of diseases. First, a cell-free toxin preparation should replicate some of the symptoms of disease. The toxin is formed by virulent pathogen isolates while non virulent do not and there might be a productive connection between the pathogen's toxin formation and its virulency. A favourable correlation between the host's toxin sensitivity and its pathogen predisposition may also exist (Strange, 2007). A pathogen's capacity for invading and infecting a well-matched plant might be aided by producing toxins causing death of cell near the incursive microbe (Baker *et al*., 1997). These toxins have also been revealed to participate in a major function in suppressing the physiological procedures in cells about the position of infectivity, allowing proliferation of the infection (Feys and Parker, 2000).

Phytotoxin now and again acts as a stimulating agent for effective development of disease. A number of fungal pathogen spores had been correlated with the formation of phytotoxins that is likely to kill or predispose host cells paving way for germ tube incursion. Quite a lot of phytotoxins are currently recognized as the determining element in pathogenesis, beyond reasonable doubt. It is known that distinct phytotoxins have distinct modes of action. Most phytotoxic metabolites function by altering the host plant's metabolism, while some once accumulated are toxic to plant tissues thereby poisoning them (Balasubramanian, 2003). Plants have a restricted measure of ways to respond to pathogens' intrusion. Distinctive and irregular symptoms may develop depending on inoculation and incubation circumstances. Also, the type of symptoms triggered by toxin depends on assay procedures and concentration. Various researches have been carried out to genetically evaluate the function of plant pathogenic fungi in disease development (Yoder, 1980; Bronson, 1991).

2.9 Phytotoxin and Plant Disease Management

As a consequence of numerous studies on phytotoxins along with their function in pathogenesis, there have been notable scientific breakthroughs in disease and weed control. Where toxins are the primary causal factors of plant diseases, knowledge of such phytotoxins can be used to manage such infections (Amusa, 2006). In vitro plant screening for resistance is regarded an additional tool

to conventional selection in breeding resistant cultivars based on numerous benefits such as quick testing of large number of individual crops in a little space, easier management and accurate assessment of quantitative variations by avoiding unfavorable weather circumstances (Slavov, 2005).

It is noted that inoculation of a number of phytopathogenic fungi with culture filtrates can generate disease-like symptoms and could be also used to screen for resistance. Culture filtrates are generated mainly through cultivation of fungi in liquid medium and successive partition of solid and liquid components of the culture (Svabova and Lebeda, 2005, Wagh *et al*., 2013). The means of selection is the liquid portion of the culture. This strategy produces a range of selection agents of constituents ranging from those without any toxin in the filtrate (meaning that the symptoms are stimulated by other metabolites of fungi) to filtrates containing uncharacterized dynamic toxins.

Crude culture filtrates permit uncomplicated bioassay the host species to easily test and screen for noxious impacts on crops, cutting, leaf disks or still cell suspensions. In countless disease resistance research in which they display phytotoxic activity, crude culture filtrates were used as selective agents (Chen and Swart, 2002; Svabova and Lebeda, 2005; Wagh *et al.*, 2013). Plant tissue culture treated with pathogenic fungal toxins or culture filtrates is a helpful mechanism for studying relationships between plant and pathogen. For *in vitro* and *in vivo* host resistance research, toxic metabolites generated by pathogens have often been used as selective agents. Crude culture filtrate has been employed as a selective agent in countless disease resistance research, particularly when phytotoxic activity was demonstrated in culture filtrate (Jin *et al*., 1996; Chen and Swart, 2002). Two requirements must be met before a phytotoxin is used as a screening agent, according to Slavov (2005), the toxin generated by the pathogen must be involved in disease progression and act directly at the cellular stage.

Various studies by Hell and Weber, (1986); IITA (1987); Vidhyasekaran *et al.* (1990); Slavov (2005); Amusa (2006); Amusa (2010) have shown that phytotoxins produced by different pathogens have been used in screening different cultivars of crops to evaluate them for resistance to infections by the pathogens. *Manihot esculenta* (cassava) and *Dioscorea* spp (yam) cultivars have

been screened for anthracnose disease resistance by the use of phytotoxic metabolites extracted from different species of *Colletotrichum* (Amusa, 2010). Ireland and Leath (1987) also reported the use of various fungal and bacterial filtrates and or toxins to screen plants for disease resistance. Filtrates from *Verticillium dahlia* have been tested on tobacco, cotton and alfalfa. In vitro selection for improved resistance has been successfully applied for; *Pisum sativum* using culture filtrate of *Fusarium*, Grapevine using *Elsinoe ampelina; Triticum aestivum* using *Fusarium culmorum*, and *F. graminearum*; Oilseed rape using PL toxin produced by *Leptosphaeria maculans*; Tobacco using culture filtrate of *Phytophthora parasitica* var *nicotianae*; *Sclerotinia sclerotiorum* fungal pathogen of Sunflower, canola and soybean (Slavov, 2005).

Some scientists have actually achieved effective selections in systems where no identified toxin has been recognized and where culture filtrates have been used to select soybean, alfafa, corn, potato resistant plant materials (Chen and Swart, 2002). Eziashi *et al*. 2010 as well described using *Trichoderma* species-produced metabolites in inhibiting mycelial growth of *Ceratocystis paradoxa* isolated from oil palm sprouted seeds from Nigerian Institute for Oil Palm Research (NIFOR), Edo State, Nigeria. In addition, Adebesin *et al.* (2009) recorded the use of *Trichoderma* species culture filtrates to control post-harvest pathogens causing banana fruit rot.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3. 1 Collection of samples

Infected *S. lycopersicum* leaves (3 per plant, 30 plants per farm) were randomly collected from three farms in Olorunnisola area of Alapoti Village (via Lusada), in Ado-Odo Ota Local Government area of Ogun State, Nigeria located on Longitude 3^0 1'53"E and Latitude 6^0 37' 37.43" N. Samples were collected at the expression of disease symptoms (Plates 3.1 and 3.2) Symptoms observed were wilting; leaf spots and yellowing Sterile black polythene bags were used for the collection of diseased tomato leaves. These were conveyed to the laboratory for isolation of causal organisms.

3.2 Sterilisation of materials

Glasswares used were first soaked in detergents for few hours and were then washed thoroughly and rinsed with tap water after which they were also rinsed with several changes of sterile distilled water. They were later air-dried. Glass Petri dishes were put inside canisters and then sterilised in an oven at 160^0C for 2 hours. Inoculating needles, scalpels and forceps were sterilised by dipping in 96% ethanol and flamed to red hot. Distilled water and the media used were sterilised in conical flasks, with the mouth plugged with cotton wool and wrapped in Aluminium foil and autoclaved at 121^0C and 1.1 kg/cm^2 pressure for 15minutes.

3.3 Preparation and Sterilisation of Culture Media

3.3.1 Potato Dextrose Agar (PDA)

The medium utilised for isolating and culturing fungi associated with the diseased *S. lycopersicum* leaves was PDA. The PDA was compounded by using 200 g of peeled Potato, 20 g of Dextrose and 20 g of Agar-Agar. Potato tubers were peeled, washed and diced and boiled gently for 1 hour in 1000 mL sterile distilled water. It was allowed to cool and the supernatant was filtered through a sterile muslin cloth. The filtrate was made up to 1000 mL by adding sterile distilled water. Dextrose

and agar-agar (20g each) were added and the mixture was divided into 500 mL flasks, the mixture in each flask was then homogenised to ensure even distribution of the agar and dextrose before autoclaving at 121^0C and 1.1 kg/cm^2 pressure for 15 minutes. Fifteen mL of medium to be used as slants for future storage were dispensed into McCartney bottles before sterilisation.

3.3.2 Czapecks Dox Broth (CDB)

Czapecks Dox Broth consisted of: Saccharose 30 g, $NaNO_3$ 3 g, K_2HPO_4 1 g, $MgSO_4$ 0.5 g, KCl 0.5 g, $FeSO_4$ 0.01 g in 1 litre of distilled water at pH 7.2. The different salts were mixed in a 1500 mL conical flask and thoroughly dissolved by heating in a water bath, the mixture was then dispensed, 100 mL each into 250 and 300 mL conical flasks before being sterilised in the autoclave. The medium was then allowed to cool on the laminar flow before the inoculation of the isolates.

3.4 Isolation of fungi

Infected leaves were rinsed under running tap water to remove dirts, the affected portions were excised into little pieces approximately 2 mm in diameter and surface sterilised by rinsing in seventy percent ethanol for 10 seconds and then rinsed in several changes of Sterile Distilled Water (SDW) to get rid of residues of ethanol. The excised pieces of leaves were then blotted dry with sterile filter papers before they were inoculated onto previously sterilized PDA plates and incubated at 28 ± 2^0C. Sub-culturing of different fungal cultures on the same plates was done repeatedly until homogenous isolates were obtained.

3.5 Preservation of pure isolates

Prepared PDA was then dispensed (15 mL in each bottle) in an aseptic condition into already sterilised McCartney bottles. The bottles were slanted on a rack and allowed to solidify. A little portion of the pure isolate was picked with sterilised inoculating needle and placed at the centre of the solidified slanted medium inside the McCartney bottles. Slants were incubated at 28 ± 2^0C for five day, after which they were kept in a refrigerator for further use. This was done for all pure cultures.

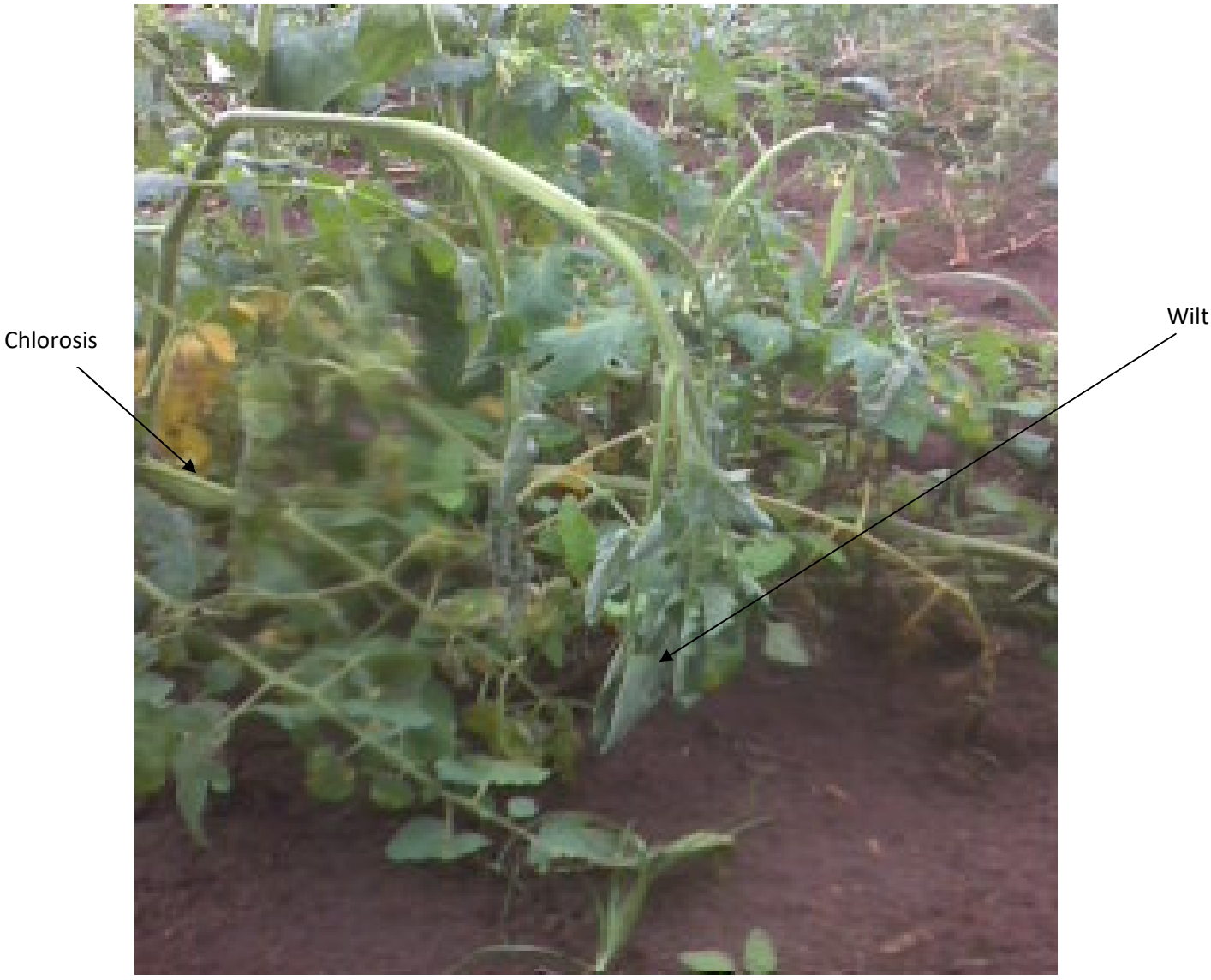


Plate 3.1: Diseased tomato plants on the field showing wilt and chlorosis symptoms.

Plate 3.2: Diseased tomato plants on the field showing leaf spot symptoms

3.6 Identification of isolated fungi

A precise description of each fungus on the medium was noted from the growth of the pure isolates and examined for colonial or cultural features at frequent periods. Microscopic morphology was studied by staining with a drop of lactophenol cotton blue stain. A sterile inoculating needle was used to pick a tiny portion from the mycelial growth of a seven day old culture onto a clean, grease-free glass slide, this was properly teased out. The preparation was carefully covered with a cover sip to avoid formation of air bubbles. The slides were afterward viewed under x10, x20 and x40 microscope objectives. References were made to William and Dennis, (1990); Olutola *et al*. (2000) for identification. Some of the fungal isolates were sent to Centre for Agriculture and Bioscience International (CABI), UK for confirmation of identity. The isolates were processed using ITS rDNA sequencing analysis. The final Identification report with reference YN3/14/H28 was obtained from CABI.

3.7 Pathogenicity tests

To determine the pathogenicity of the fungi isolated from the diseased tomato leaves, Koch postulate (Agrios, 2005) for establishing pathogenicity was followed. This was done both in the laboratory (*in-vitro*) and the screen house (*in-vivo*).

3.7.1 *In-vitro*

Healthy tomatoes leaves obtained from farms in Alapoti village of Ogun State were rinsed under slow running tap water. Sterile distilled water (15 mL) was dispensed into the pure fungi in culture plates to prepare suspension. The spores of the fungi were then dislodged from the plates using a sterile glass rod. The spores of the fungi were quantified using the heamocytometer. Suspension of each isolate has an average volume of about 3×10^4 conidia per cm^3.The leaves were left to stay in the suspension for five minutes. The leaves were afterward moved into sterile plates containing dampened sterile Whatman Filter paper No 1 (Whatman, United Kingdom) and incubated in a humid chamber at a temperature of 25^0C. Sterile distilled water was used instead of spore suspension to serve as control. The leaves on the plates were observed daily for any changes and the observation were recorded.

3.7.2 *In-vivo*

To confirm the pathogenicity of isolated fungi, disease-free seeds of tomato were raised in one of the green houses of Nematology unit, IITA, Ibadan. The soil used for planting was sterilized, stones and debris were removed. The seeds were planted and watered daily. Four Weeks After Planting (WAP), the leaves were surface sterilised and wounded with sterile needle after which they were inoculated by spraying to the point of runoff with fungal spore suspensions. For the control experiment, sterile distilled water was used to spray the leaves. The inoculated leaves were covered with sterile polyethene nylons to create a humid environment around the leaves as well as to prevent contamination by other pathogens. Daily observations were made after inoculation.

3.8 Phytotoxin production and isolation

The fungi found to be pathogenic were cultured for phytotoxins production and isolation. They were cultured on Czapecks Dox Broth (CDB). Three mycelial plugs (5 mm cork borer) of each isolate were removed from the margins of actively growing 7-day old fungi and inoculated into the liquid medium in conical flasks. Five conical flasks for each isolate were incubated on a rotary shaker at 96 rpm at 28 ± 2^0C for 28 days. After the period of incubation, the liquid medium for each isolate in the conical flasks were pooled together (Amusa, 2010; Matsumoto *et al*., 2010).

After incubation, the liquid medium was percolated over a four-layered muslin cloth to reduce the fungal biomass (Evidente *et al.,* 2011) and centrifuged at 8000 rpm for 20 minutes to precipitate mycelium and conidia (Matsumoto *et al*., 2010). The supernatant of the culture filtrate was sterilized by filtration through a 0.45µm membrane filter to remove the remaining fungal material. The pH of the culture filtrate was adjusted to 3.0 using a pH meter by adding drops of 1 M HCl (Khan *et al*., 2004). Culture filtrates were extracted 3 times successively into half the volume of ethyl acetate and diethyl ether separately (Khan *et al*., 2004; Berestetskii *et al*., 2010). The

extraction was done by vigorously shaking the aqueous and organic mixtures in a separating funnel. Two distinct layers were formed, the upper layer being the organic solvent and the lower layer the aqueous portion. pH of the extracted metabolites was readjusted to pH 7 using 1 M NaOH (Zivkovic *et al*., 2007). The organic phases were dried over anhydrous sodium sulphate (Na_2SO_4) filtered through Whatman Filter paper No 1 (Whatman, United Kingdom), evaporated to dryness at 30^0C under reduced pressure using a rotary evaporator (Cole-Parmer, Laboratory Consumables and

Chemical Supplies Co. Ltd, China). Yield and colour of extractable metabolites from each isolate and culture medium were observed and recorded (Amusa, 2010).

3.9 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed on Agilent Technologies 6890 N Network GC System and Agilent Technologies 5973 Network Mass Selective Detector matched with 7683B Series Injector. Agilent 122-5533 capillary column with specification: DB-5 ms, 0.25 mm*30 m*1 μm was the model number of the column employed. The carrier gas utilized was Helium at a flow rate of 1.2 mL/min while injection volume was 1μ. The inlet temperature was sustained at 230^0C. The oven temperature was programmed originally at 50^0C for 5 minutes, then programmed to increase to 300^0C at a rate of 10^0C ending with 25 minutes. Entire run time was 45 minutes. The MS transfer line was maintained at a temperature of 300^0C. The source temperature was maintained at 230^0C and the MS Quad at 150^0C. The ionization mode used was electron ionization mode at 70eV. Total Ion Count (TIC) was used to assess for compound detection and quantization. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software. The GC-MS analysis was performed at Central Science Laboratory, Usmanu Danfodiyo University, Sokoto.

3.10 Bioassay of phytotoxins

The phytotoxicity of the extracted metabolites was determined by testing them on different varieties of tomato to evaluate the sensitivity of such varieties. Two different sets of varieties were used including two local varieties, “Kerewa” and Ibadan local and five hybrid varieties, “LEMT3”, “LEMT25”, “LEMT39”, “LEMT47”, “LEMT49” were used. The seeds of “Kerewa,” were obtained from a local farmer in Alapoti. Ibadan local was obtained from the Nematology Laboratory, IITA, Ibadan while LEMT3, 25, 39, 47 and 49 were collected from Prof. S.C.O. Makinde a Plant Breeder in the Department of Botany, Lagos State University, Ojo, Lagos. The characters of the five hybrid varieties are shown in Table 3.1. The other set of hybrid varieties, ROMA-VF and UC-82-B were purchased from Premier Seeds Ibadan, while Assila and Gem-Pride (Seminis Brand) were purchased from Jubaili-Agros.

3.10.1 *In-vitro* leaf bioassay

The petioles of leaves of tomato (6 WAP) were cut with a razor blade. The leaves were surface sterilised and wounded with sterile needle (Berestetskiy, 2008). The leaves were then placed in 2 mL of the crude filtrate of each test isolate previously applied to 9 cm filter paper inside sterile Petri dishes according to Mansoori and Smith (2005). The plates were incubated in a humid chamber at 25 ± 2^0C and at 70% relative humidity. The separated aqueous portions of culture filtrates after extraction with solvents were used for the bioassay. The leaves were observed daily for manifestation of symptoms; data obtained were recorded until the 7th day. Three leaves per variety were used.

Toxicity scores according to Mansoori and Smith (2005) as modified was adopted. Where;

0 = no visible symptoms,

1 = chlorosis/wilting/leaf spots at the base of the lamina,

2 = one side of lamina showing chlorosis/wilting/leaf spots,

3 = marginal necrosis/wilting, and spots on the lamina,

4= general necrosis/wilting, and spots on the lamina

From the average values obtained, the levels of resistance/susceptibility of the varieties were rated thus:

0.00 – 0.32; highly resistant (HR),

0.33 – 1.00; resistant (R),

1.01 – 2.00; moderately susceptible (MS).

2.01 – 4.00; susceptible (S).

Table 3.1: Characters of hybrid varieties

Variety/ Characters	GH	FD	LA	LT	LC
LEMT3	3 (Semi Determinate	5 (Intermediate)	3 (Semi-erect)	3 (Standard)	3 (Light green)
LEMT25	2 (Semi Determinate	3 (Sparse)	3 (Semi-erect)	3 (Standard)	7 (Dark green)
LEMT39	3 (Semi determinate	7 (Dense)	3 (Semi-erect)	6 (Hirsutum)	3 (Light green)
LEMT47	2 (Determinate)	3(Sparse)	3 (Semi-erect)	1 (Dwarf)	3 (Light green)
LEMT49	1 (Dwarf)	3 (Sparse)	3 (Semi-erect)	3 (Standard)	3 (Light green)

Key: GH = Growth Habit, FD = Foliage Density, LA = Leaf Altitude, LT = Leaf Type, LC = Leaf Colour.

Adapted from Makinde *et al*. (2019)

3.10.2 *In-vivo* bioassay

The leaves of tomato (8 weeks old) were used for the bioassay. The tomato varieties were raised in the nursery of the Department of Botany, University of Ibadan. Seeds were sown in sterilised sandy loam-soil. Transplanting into polythene bags were done after emergence of seedlings. The varieties were screened for possible resistance to the effects of the metabolites. The crude extracts of the culture filtrate were used for the bioassay. This was done by adding 10 mL of sterile distilled water to extracts of AID –*Phomopsis* sp. extracted with diethyl ether; AIE – *Phomopsis* sp. extracted with ethyl acetate; FSE- *Fusarium oxysporum* extracted with ethyl acetate and FPIE - *Fusarium oxysporum* extracted with diethyl ether. The leaves were sprayed to the point of runoff. SDW without the extracts was used as control. The treatment of leaves per variety was done in three replicates. The mean values for the toxicity ratings were recorded according to the modified methods of Khan *et al*. (2004), Mansoori and Smith (2005).

3.11 Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) (SPSS for Windows version 17.0) and Fisher's Least Significant Difference (LSD) was applied at $\alpha = 0.05$ where significant variability exists. Data were expressed as mean ± standard error.

CHAPTER FOUR

4.0 RESULTS

4.1 Isolated fungi

The following fungi were isolated from diseased tomato leaves collected from the field:
Aspergillus aculeatus, A. niger, A. tamarii, A. ustus, A. versicolor, Epicoccum nigrum, Fusarium oxysporum f. sp. *lycopersici* (*Fol*)*, Phialophora melinii, Phomopsis* sp. and *Trichodema* sp.

4.1.1 Colonial and microscopic description of fungi

Aspergillus aculeatus – Colonies growing rapidly, felty, purplish-black (Plate 4.1a). Microscopically, conidial heads spherical to radiate, splitting into well-defined, divergent columns, black. Conidiophore stipes smooth-walled, hyaline or slightly pigmented at the apex. Vesicles brown, subspherical, Conidiogenous cells uniseriate. Conidia hyaline to brown, conspicuously (Plate 4.1a).

Aspergillus niger - colonies growth spread rapidly well with velvety and fluffy in texture with aerial mycelium white at first frequently developing into dark brown to black with conidia formation. Rear is light yellow and growth may produce radial fissures in the agar (Plate 4.1a{iii}). Conidial heads in round or globose, large and also radiate or as they grow splitting into, loose columns of conidial chains with age. Conidiophores arising from the substratum mostly colourless to brown, smooth, splitting when crushed like pieces of cane. Vesicules globose while phialides borne directly on the vesicles, metulae are usually present (Plate 4.1a).

Aspergillus tamari - colonies growth spreads within 3-4 days. Colonies floccose with aerial mycelia low and brown in colour, reverse dark brown (Plate 4.1a). Conidial head large, radiate, single chain of conidia visible under low magnification, thin walled. Metulae normally present, phialides also present, metulea occasionally longer, conidia dark, cylindrical to pyriform when young becoming globode in age and coarsely roughened or tuberculate from nodules of brown colouring matter (Plate 4.1a).

Aspergillus ustus - colonies growth spreads moderately well within 3-4 days. Colonies slightly floccose and brownish yellow. texture is woolly to cottony to somewhat granular, with reverse yellow (Plate 4.1b). Conidial heads radiate to loosely columnar, condiophores short, small, smooth, pale brown arising from prominent foot cells. Vesicles globose, metulae and phialides both always present. Conidia globose, rough, hulle cells abundant rarely globose, mostly ovate shaped and frequently bent and twisted (Plate 4.1b).

Aspergillus vesicolor - colonies growth spreading with floccose to velvety texture. Colonies often starting as a deep yellowish cream to yellow greenish brown to brown reverse light brown (Plate 4.1b). Conidial heads variable radiate to loosely column, conidiophores colourless or slightly brown, smooth, vesicles orate to elliptical fertile over medullae. Metulae normally presents. Phialides also present. Metulae occasionally longer. Hulle cells present and globose. Conidia usually globose as it age and coarsely roughened or tuberculate from nodules of brown colouring matter (Plate 4.1b).

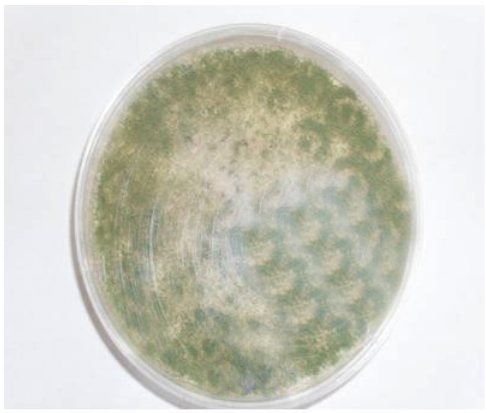
Epicoccum nigrum develops fast and forms flocculent to matted colonies on PDA at 25°C. The colonies are at first yellow to orange, orange to red or pink from the front and with age they turn greenish brown to black. The same colour is seen from the rear, but is generally more vivid than in the front view. Black dots may be observed macroscopically on the colony surface. These are the clumps of hyphae which have conidiophores on their surface (Plate 4.1b). Conidia are produced individually on heavily compacted, non-specialised, determinant, slightly pigmented conidiophores. Conidia are globose to pyriform, with a funnel-shaped base and broad attachment scar, often seceding with a protuberant basal cell. Conidia become multicellular (dictyoconidia), darkly pigmented and have a verrucose external surface (Plate 4.1b).

Fusarium oxysporum f. sp. *lycopersici* - colonies are pigmented with a reddish purple colour and surmounted by a pinkish white aerial mycelium (Plate 4.1c). Microconidia are cylindrical to oval and produced from long phialides which arise from complex branched conidiphores. Macrocondia are septate. Globose chlamydosphores are produced (Plate 4.1c).

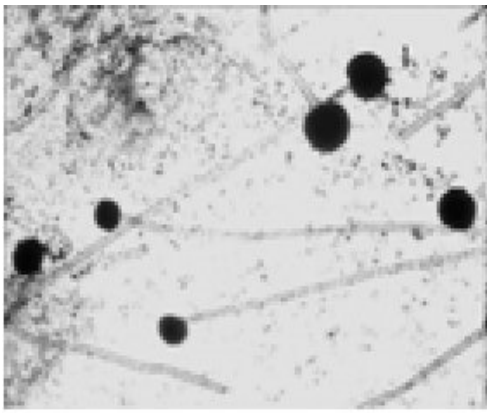
Phialophora melinii - colonies growth spread moderately well within 3-4 days with matted floccose or almost velvety or tuft texture dark gray and reverse black (Plate 4.1c). Conidigenous cells dark colour phialides which arise directly from aerial hyphae or simple branched or penicillate organisation and septate. Conidiophores variable sometimes branched bearing single phialides. Phialides pale brown with slightly swollen base tapering to a branch neck conidia pale brown, variable in shaped, septate, hyaline or often ellipsoidal (Plate 4.1c).

Phomopsis sp. - colonies growth is restricted, slow and matted. Colonies at first white, turning dark grey or black in patches (Plate 4.1c). Conidia are hyaline, non septate, containing elongate cylindrical phialides (Plate 4.1c).

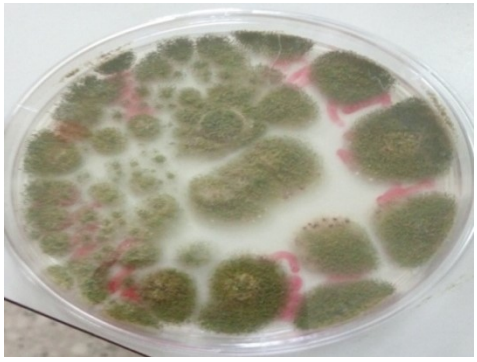
Trichoderma asperellum colonies develops with rapidity and becomes fully grown in five days. At a temperature of 25°C when on PDA, the colonies are woollen and get compacted. Colonies are within in colour from the fore. As the conidia are produced, dispersed yellow-green spots are noticeable. These make concentrical bands. Reverse is pale (Plate 4.1d). Conidiophores carrying branches or phialides unequal or in verticulus at a broad angle to the major stipe. Conidiogenous cells hyaline, phialides, conidia septate, hyaline, almost globose with truncate base and usually green (Plate 4.1d).



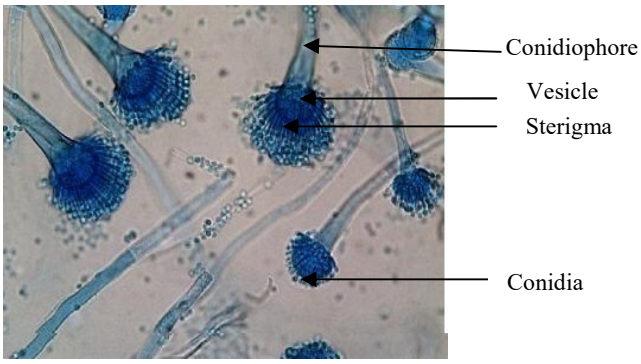
(i) *Aspergillus aculeatus* on PDA



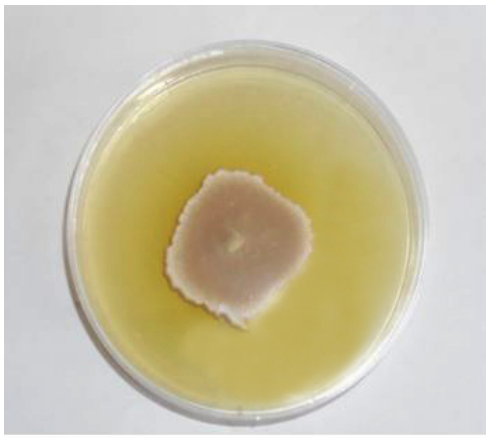
(ii) Photomicrograph of *Aspergillu aculeatus* (X100)



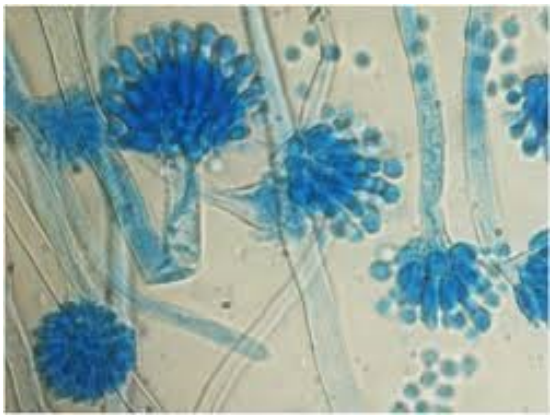
(iii) *Aspergillus niger* on PDA



(iv) Photomicrograph of *Aspergillus niger* (X400)

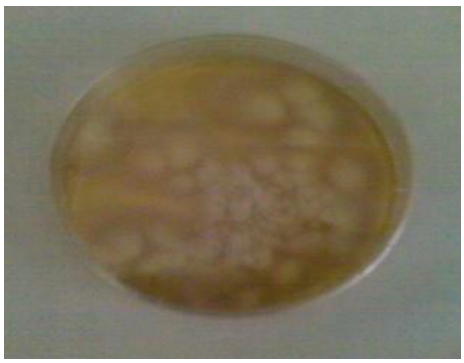


(v) *Aspergillus tamarii* on PDA

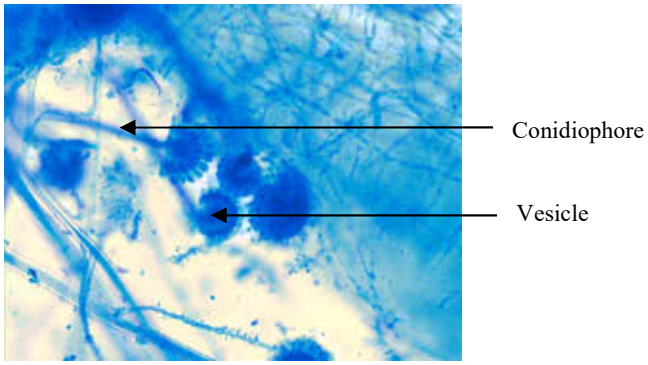


(vi) Photomicrograph of *Aspergillus tamari* (X400)
Conidiophore with uniseriate phialides

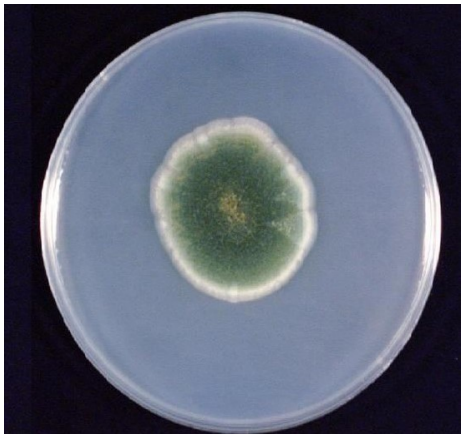
Plate 4.1a: Colonial and microscopic morphology of isolated fungi



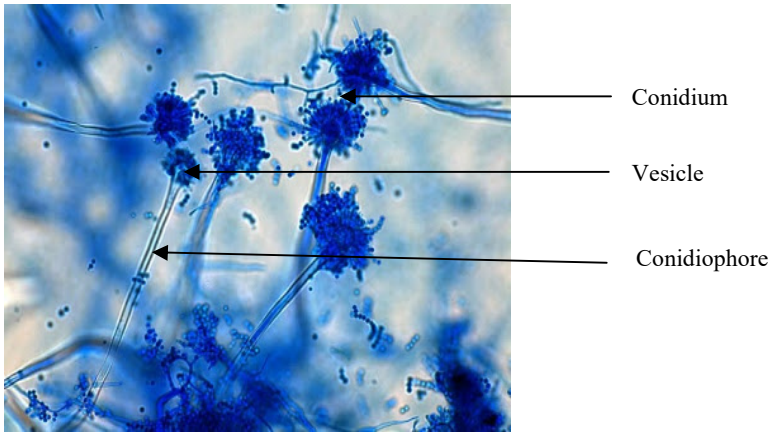
(i) *Aspergillus ustus* on PDA



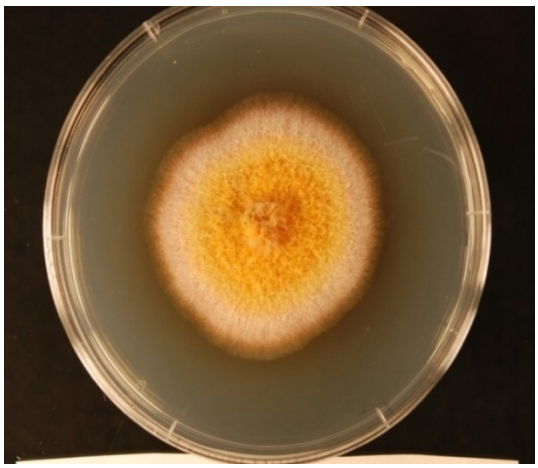
(ii) Photomicrograph of *Aspergillus ustus* (400)



(iii) *Aspergillus versicolor* on PDA



(iv) Photomicrograph of *A. versicolor* (X400)

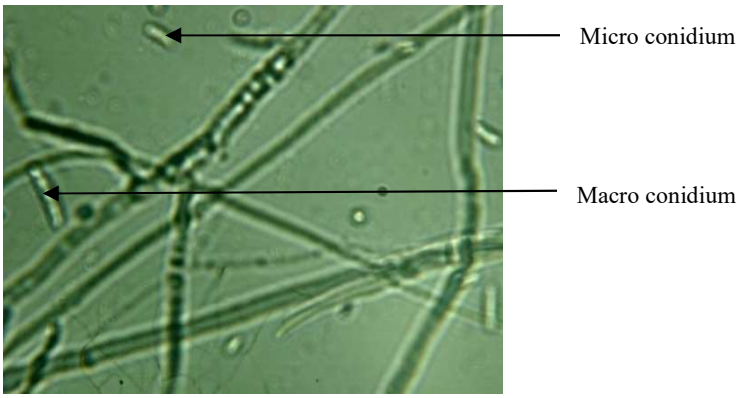


(v) *Epicoccum nigrum* on PDA

(vi) Conidia of *Epicoccum nigrum*

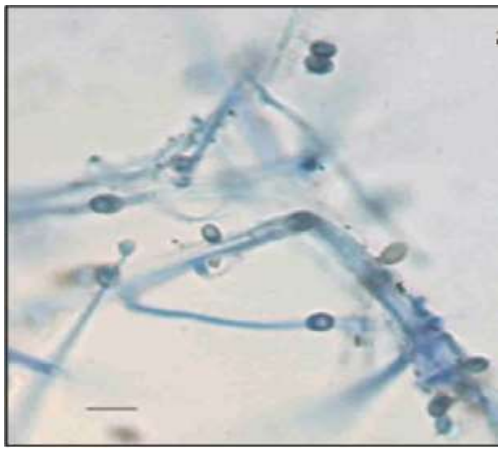
Plate 4.1b: Colonial and microscopic morphology of isolated fungi

(i) *Fusarium oxysporum* f. sp. *lycopersici* on PDA



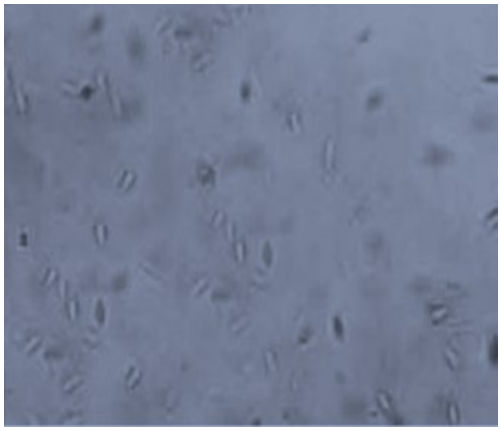
(ii) Photomicrograph of *Fusarium oxysporum* f. sp. *lycopersici* (X100)

(iii) *Phialophora melinii* on PDA



(iv) Photomicrograph of *Phialophora melinii*

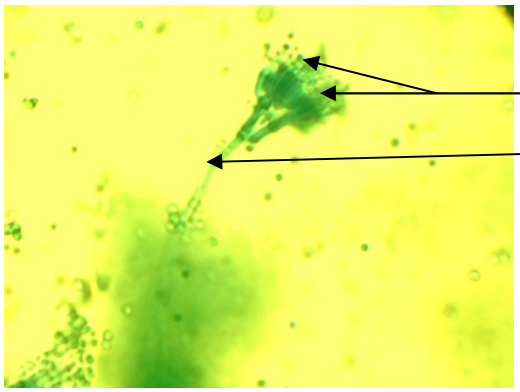
(v) *Phomopsis* sp. on PDA



(vi) Conidia of *Phomopsis* sp.

Plate 4.1c: Colonial and microscopic morphology of isolated fungi

(i) *Trichoderma asperellum* on PDA



(ii) Photomicrograph of *Trichoderma asperellum* (X400)

Plate 4.1d: Colonial and microscopic morphology of isolated fungi

4.2 Pathogenicity tests

The result of the pathogenicity tests (Table 4.1) indicated that on inoculation of healthy tomato leaves with all isolated fungi, *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) and *Phomopsis* sp. showed symptoms similar to the previously diseased tomato leaves collected from the field (Plate 4.2). Symptoms observed on the healthy leaves used for the pathogenicity after five days of inoculation included leaf spots, chlorosis/yellowing and wilting.

4.3 Phytotoxin production

After the incubation, the pH of the CF of *Phomopsis* sp. was 6.51, while the CF of *Fusarium oxysporum* f. sp. *lycopersici* had a pH of 7.87. The result in Table 4.2 showed the yield and colour of the crude extracts. *Phomopsis* sp. extracted with diethyl ether (AID) was 50.0mg and the colour was deep yellow while ethyl acetate extract (AIE) weighed 41.0mg and was deep brown in colour. *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate (FSE) was 54.5mg and colour was yellow, metabolite obtained from *Fol* extracted with diethyl ether was 39.0mg and the colour was orange.

4.4 Results of GC-MS analysis

Tables 4.3 - 4.6 show the names of compound isolated from the cultured filtrate from *Phomopsis* sp. and *Fusarium oxysporum*. The peaks number, Retention Time (RT), % composition, mass spectra (MS) data for the compounds with quality above 50% are presented. Compounds isolated from *Phomopsis* sp. extracted with diethyl ether (Table 4.3) were Benzeneacetic acid, 4-hydroxy-benzeneethanol, Butylated Hydroxytoluene, 4-(2-Methoxyethyl)phenol, 5-butyl-2-pyridine carboxylic acid, N-butyl-benzenesulfonamide, n-Hexadecanoic acid, (Z) 9-Octadecenamide and (2-ethylhexyl)1,2-Benzenedicarboxylic acid. The percentage composition of (2-ethylhexyl)1, 2-Benzenedicarboxylic acid was highest (62.31) and that of 4-(2-Methoxyethyl)phenol was lowest (0.86).

Isolated compounds from *Phomopsis* sp. extracted with ethyl acetate (Table 4.4) included Phenylethyl Alcohol, 4-hydroxy-benzeneethanol, 2,4-bis(1,1-dimethylethyl) phenol, 3,4-dihydro -8-hydroxy-3-methyl-1H-2-Benzopyran-1-one, (E)-2-tetradecene, Hexadecane, (E)-3-Eicosene, Octadecane, n-Hexadecanoic acid, 1-Docosene, Eicosane, (Z)-9-Octadecenamide, Bis (2-

ethylhexyl) phthalate. The compound with the least (0.90) % composition was (E)-2-tetradecene while the one with the highest (75.91) was Bis (2-ethylhexyl) phthalate.

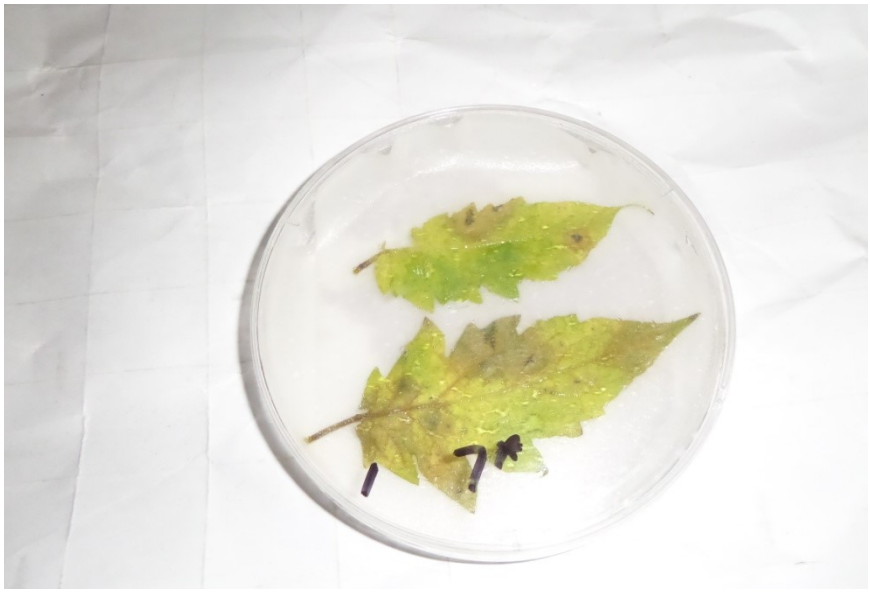
From Table 4.5, compounds isolated from *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate, were 2-hydroxy-3-methyl-butanoic acid, 3-butyl-pyridine, Benzeneacetic acid, Hexadecane, 5-Butyl 2-Pyridinecarboxylic acid, Octadecane, n-Hexadecanoic acid, Eicosane, 1-Heptadecanol, 1-Nonadecene, (2-ethylhexyl)1,2-Benzenedicarboxylic acid. The compound with highest % composition (82.42) was (2-ethylhexyl) 1,2-Benzenedicarboxylic acid while that with the lowest (0.45) composition was Benzeneacetic acid.

The following compounds: Benzeneacetic acid, 2-Coumaranone, Phthalic anhydride, 3-butyl-pyridine, 5-Butyl 2-Pyridinecarboxylic acid, N-Butyl Benzenesulfonamide and (Z)-9-Octadecenamide were isolated from diethyl ether extract of *Fusarium oxysporum* (Table 4.6). The compound with the highest percentage composition was 5-Butyl 2-Pyridinecarboxylic acid (45.80) while the lowest percentage of 1.12 was recorded for Phthalic anhydride.

4.5 *In-vitro* leaf bioassay

From the results of the bioassay on detached leaves, the responses of the first seven set of varieties to metabolites from *Phomopsis* sp. are presented in Table 4.7. Scoring of leaves with wounds showed that Kerewa, Ibadan local, LEMT47, LEMT49 were moderately susceptible (MS), LEMT3 and LEMT25 were susceptible (S), LEMT39 was resistant (R). Scoring on leaves without wounds revealed Kerewa and Ibadan local, moderately susceptible, however, LEMT3 was resistant, LEMT25 highly resistant, LEMT39 and LEMT49 were susceptible while LEMT47 was moderately susceptible.

The result of the response of the inoculated varieties with extracts from *Fusarium oxysporum* is presented in Table 4.8. Kerewa and LEMT25 were highly resistant, Ibadan local and LEMT49 were moderately susceptible, LEMT3 and LEMT39 were susceptible and LEMT47 was resistant. Leaves not wounded showed Kerewa and Ibadan local to be highly resistant, LEMT3, LEMT25, LEMT39 and LEMT47 to be susceptible while LEMT49 was moderately susceptible.



(i) Healthy tomato leaves inoculated with *Fusarium oxysporum* f. sp. *lycopersici* after 5 days

(ii) Healthy tomato leaves inoculated with *Phomopsis* sp. after 5 days

Plate 4.2: *In-vitro* Pathogenicity test

Table 4.1**:** Result of pathogenicity tests on tomato leaves

Fungi/Treatment	**Symptoms Observed**		
	Leaf spot	**Wilt**	**Chlorosis**
Aspergillus aculeatus	-	-	-
A. niger	-	-	-
A. tamarii	-	-	-
A. ustus	-	-	-
A. versicolor	-	-	-
Epicoccum nigrum	-	-	-
Fusarium oxysporum	+	+	+
Phialophora melinii	-	-	-
Phomopsis sp.	+	+	+
Trichoderma asperellum	-	-	-
Control	-	-	-

Table 4.2: Yield and colour of crude extracts

Extract	**Yield (mg)**	**Colour**
AID	50.0	Deep yellow
AIE	41.0	Deep brown
FSE	54.5	Yellow
FPIE	39.0	Orange

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

Table 4.3: Compounds isolated from *Phomopsis* sp. extracted with diethyl ether

Peak No	RT (mins)	Compound name	% Composition	Mass Spectra data	Method of Identification	Quality
1	17.66	Benzeneacetic acid	3.41	65, 91, 129, 136	MS, RI	90
2	20.40	4-hydroxy-benzeneethanol	1.07	77, 107, 120, 138	MS, RI	91
3.	20.71	1-Methylpentyl cyclopropane	3.66	83, 98, 114, 124	MS, RI	47
4	20.93	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	1.88	57, 137, 165, 180	MS, RI	62
5	21.57	Butylated Hydroxytoluene	1.82	57, 145, 205, 225	MS, RI	96
6	21.78	4-(2-Methoxyethyl)phenol	0.86	77, 107, 152, 205	MS, RI	87
7	23.04	5-butyl-2-pyridine carboxylic acid	1.48	65, 92, 135, 207	MS, RI	58
8	25.03	N-butyl-benzenesulfonamide	1.10	77, 141, 170, 281	MS, RI	97
9	26.57	n-Hexadecanoic acid	0.87	73, 129, 207, 281	MS, RI	90
10	28.06	2-Propenoic acid	1.31	139, 207, 281, 355	MS, RI	47
11	29.92	2-Furancarboxylic acid	1.73	169, 241, 326, 355	MS, RI	22
12	30.50	(Z) 9-Octadecenamide	7.97	59, 126, 281, 429	MS, RI	91
13	30.99	3-Butanone	6.65	123, 145, 207, 429	MS, RI	40
14	32.28	(2-ethylhexyl)1,2-Benzenedicarboxylic acid	62.31	149, 167, 279, 429	MS, RI	91
15	35.43	Silane 1,4-phenylenebis trimethyl-	1.07	113, 167, 275, 429	MS, RI	43
16	35.81	Silane 1,4-phenylenebis trimethyl-	2.82	73, 207, 251, 429	MS, RI	53
Total			100.01			

Key: RT – Retention Time, MS – Mass Spectrometry, RI – Retention Index

Table 4.4: Compounds isolated from *Phomopsis* sp. extracted with ethyl acetate

Peak No	RT (mins)	Compound name	% Composition	Mass Spectra data	Method of Identification	Quality
1	15.79	Phenylethyl Alcohol	1.61	91, 65, 103, 122	MS, RI	97
2	20.40	4-hydroxy-benzeneethanol	3.33	77, 107, 120, 138	MS, RI	91
3	21.50	2,4-bis(1,1-dimethylethyl) phenol	2.37	57, 115, 191, 206	MS, RI	97
4	22.36	3,4-dihydro -8-hydroxy-3-methyl-1H-2-Benzopyran-1-one	1.15	134, 160, 178, 207	MS, RI	98
5	22.52	(E)-2-tetradecene	0.90	55, 111, 152, 196	MS, RI	98
6	22.61	Hexadecane	1.75	57, 71, 207, 226	MS, RI	97
7	24.86	(E)-3-Eicosene	1.34	99, 113, 149, 169	MS, RI	95
8	24.93	Octadecane	1.45	57, 99, 254, 281	MS, RI	96
9	26.56	n-Hexadecanoic acid	0.94	73, 129, 256, 281	MS, RI	93
10	26.97	1-Docosene	1.04	83, 171, 207, 281	MS, RI	93
11	27.03	Eicosane	0.95	57, 85, 207, 281	MS, RI	97
12	30.49	(Z)-9-Octadecenamide	6.44	59, 207, 281, 429	MS, RI	90
13	30.69	1,3-Bis(trimethylsilyl)benzene	0.81	97, 207, 281, 341	MS, RI	53
14	32.26	Bis (2-ethylhexyl) phthalate	75.91	133, 191, 344, 429	MS, RI	92
Total			99.99			

Key: RT – Retention Time, MS – Mass Spectrometry, RI – Retention Index

Table 4.5: Compounds isolated from *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate

Peak No	RT (mins)	Compound name	% Composition	Mass Spectra data	Method of Identification	Quality
1	13.82	2-hydroxy-3-methyl-butanoic acid	3.21	55, 73, 76, 99	MS, RI	83
2	15.50	Isopropyl phosphine	0.61	57, 69, 76, 87	MS, RI	84
3	16.55	3-butyl-pyridine	0.55	65, 92, 105, 135	MS, RI	94
4	17.62	Benzeneacetic acid	0.45	51, 65, 91, 136	MS, RI	86
5	21.78	4-hydroxy-Benzeneacetic acid	0.50	92, 107, 135, 152	MS,RI	58
6	22.61	Hexadecane	1.99	57, 71, 135, 226	MS, RI	96
7	23.05	5-butyl 2-Pyridinecarboxylic acid	4.48	77, 92, 135, 207	MS, RI	94
8	24.92	Octadecane	1.27	57, 113, 225, 281	MS, RI	91
9	26.56	n-Hexadecanoic acid	0.70	73, 213, 256, 281	MS, RI	95
10	27.03	Eicosane	0.61	57, 85, 207, 281	MS, RI	96
11	27.88	1-Heptadecanol	0.57	55, 207, 281, 355	MS, RI	93
12	28.36	6-Octadecenoic acid	0.50	97, 135, 281, 341	MS,RI	50
13	28.93	Eicosane	0.55	57, 207, 281, 429	MS, RI	93
14	29.79	1-Nonadecene	0.46	55, 207, 281, 355	MS, RI	83
15	32.29	(2-ethylhexyl)1,2-Benzenedicarboxylic acid	82.42	149, 167, 279, 391	MS, RI	91
16	43.53	Nonadecane	1.14	207, 281, 341, 429	MS,RI	64
Total			100.01			

Key: RT – Retention Time, MS – Mass Spectrometry, RI – Retention Index

Table 4.6: Compounds isolated from *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether

Peak No	RT (mins)	Compound name	% Composition	Mass Spectra data	Method of Identification	Quality
1	17.62	Benzeneacetic acid	4.60	65, 91, 118, 136	MS, RI	86
2	17.88	2-Coumaranone	1.70	78, 91, 106, 136	MS, RI	91
3	19.06	Phthalic anhydride	1.12	76, 92, 104, 148	MS, RI	78
4	20.38	4-hydroxy-Benzeneethanol	1.13	84, 92, 107, 138	MS, RI	52
5	21.70	3-butyl-pyridine	1.57	92, 121, 135,164	MS, RI	83
6	21.77	3-butyl-pyridine	1.29	92, 107,135, 152	MS, RI	76
7	23.04	5-Butyl 2-Pyridinecarboxylic acid	45.80	92, 135, 179, 207	MS, RI	92
8	24.54	2-[4-(Methoxymethoxymethyl)cyclohe x-3-enyl]propan-2-ol	1.35	109,135,207,281	MS,RI	37
9	25.02	N-Butyl Benzenesulfonamide	2.53	119,147,193,207	MS, RI	81
10	26.56	n-Hexadecanoic acid	2.59	129,154,207,221	MS,RI	56
11	27.07	Benzonitrile, m-phenethyl-	1.41	129,142,170,256	MS,RI	22
12	27.92	N-Methyl-1-adamantaneacetamide	1.02	135,170,191,261	MS,RI	53
13	28.09	1H-Indole-3-acetamide	1.03	147,174,207,281	MS,RI	42
14	28.37	1,4-phenylenebis[trimethyl Silane	2.38	163,191,221,281	MS,RI	50
15	29.82	1,3-Bis(trimethylsilyl)benzene	3.81	191,207,249,281	MS,RI	53
16	30.49	(Z)-9-Octadecenamide	23.23	207,267,325,341	MS, RI	91
17	32.25	Tetrasiloxane, decamethyl-	3.42	207,281,341,355	MS,RI	43
Total			99.98			

Key: RT – Retention Time, MS – Mass Spectrometry, RI – Retention Index

Table 4.7: Response of seven tomato varieties to *in-vitro* inoculation with culture filtrate of *Phomopsis* sp.

Varieties	Disease scoring on leaves (with wounds)	Disease scoring on leaves (without wounds)
Kerewa	1.00	1.33
Ibadan local	2.00	1.33
LEMT3	3.00	0.33
LEMT25	3.00	0.00
LEMT39	0.33	2.00
LEMT47	2.00	1.33
LEMT49	1.33	3.66

Key:

HR – Highly resistant (0.00 – 0.32),

R – Resistant (0.33 – 1.00),

MS – Moderately susceptible (1.01 – 2.00),

S – Susceptible (2.01 – 4.00).

Table 4.8: Response of seven tomato varieties to *in-vitro* inoculation with culture filtrate of *Fusarium oxysporum* f. sp. *lycopersici*

Varieties	**Disease scoring on leaves (with wounds)**	**Disease scoring on leaves (without wounds)**
Kerewa	0.00	0.00
Ibadan local	1.33	0.00
LEMT3	2.00	4.00
LEMT25	0.00	2.66
LEMT39	2.66	2.00
LEMT47	0.33	4.00
LEMT49	1.66	1.00

Key:

HR – Highly resistant (0.00 – 0.32),

R – Resistant (0.33 – 1.00),

MS – Moderately susceptible (1.01 – 2.00),

S – Susceptible (2.01 – 4.00).

4.6 *In-vivo* leaf bioassay

Tables 4.9 to 4.12 show the effects of isolated fungal metabolites extracted with diethyl ether and ethyl acetate on five tomato varieties; Assila, Gem Pride, ROMA-VF, UC-82-B and "Ibadan local". The symptoms originally observed from diseased samples on the field where the fungi were isolated were leaf spot, wilt and chlorosis. The symptoms were manifested when the crude extracts/metabolites were administered on healthy leaves of tomato raised in the nursery. The symptoms were observed after seven days (Plates 4.23a and b).

From the result of *Phomopsis* sp. extracted with diethyl ether (Table 4.9), there were considerable variation ($p< 0.05$) in the effects of the metabolite in causing leaf spot, wilt and chlorosis between the five varieties and Control. For leaf spot, the maximum value was gotten in Gem Pride (3.50 ± 0.50) while the lowest was in Ibadan local (0.50 ± 0.50). Also, wilt was highest in Gem Pride (2.50 ± 0.50) and lowest in Assila, ROMA-VF and Ibadan local (0.00 ± 0.00). Chlorosis was highest in Gem Pride (3.50 ± 0.50) and lowest in ROMA-VF (0.00 ± 0.00).

In Table 4.10, the effects of the metabolite from *Phomopsis* sp. when extracted with ethyl acetate revealed there was a considerable variation ($p< 0.05$) in the leaf spot, wilt and chlorosis symptoms on the screened varieties and control. The highest mean value of 3.00 ± 0.10 was observed for leaf spot from Gem Pride while the lowest (0.75 ± 0.25) was from Assila and UC-82-B. For wilt, Ibadan local had the highest value (2.00 ± 0.41) while UC-82-B had the lowest value (0.25 ± 0.25). Gem Pride had the highest value (2.25 ± 0.48) for chlorosis, whereas, Assila and UC-82-B had the lowest values (0.00 ± 0.00).

Table 4.9**:** Effect of *Phomopsis* sp. extracted with diethyl ether on five tomato varieties

Tomato Varieties	**Symptoms**		
	Leaf spot	**Wilt**	**Chlorosis**
Assila	1.50 ± 0.50^{a}	0.00 ± 0.00^{a}	0.50 ± 0.50^{ad}
Gem Pride	3.50 ± 0.50^{b}	2.50 ± 0.50^{b}	3.50 ± 0.50^{b}
ROMA-VF	1.00 ± 0.10^{ac}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
UC-82-B	1.50 ± 0.50^{d}	0.50 ± 0.50^{a}	1.50 ± 0.50^{d}
Ibadan local	0.50 ± 0.50^{ad}	0.00 ± 0.00^{a}	1.00 ± 0.10^{ad}
Control	0.00 ± 0.00^{cde}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Table 4.10**:** Effect of *Phomopsis* sp. extracted with ethyl acetate on five tomato varieties

Tomato Varieties	**Symptoms**		
	Leaf spot	**Wilt**	**Chlorosis**
Assila	0.75 ± 0.25^{a}	1.25 ± 0.25^{a}	0.00 ± 0.00^{a}
Gem Pride	3.00 ± 0.10^{b}	1.75 ± 0.48^{a}	2.25 ± 0.48^{b}
ROMA-VF	1.50 ± 0.29^{c}	0.75 ± 0.48^{a}	1.00 ± 0.58^{ad}
UC-82-B	0.75 ± 0.25^{a}	0.25 ± 0.25^{ab}	0.00 ± 0.00^{a}
Ibadan local	2.00 ± 0.10^{dc}	2.00 ± 0.41^{ad}	1.50 ± 0.29^{bcd}
Control	0.00 ± 0.00^{e}	0.00 ± 0.00^{ac}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Table 4.11**:** Effect of *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate on five tomato varieties

Tomato Varieties	**Symptoms**		
	Leaf spot	**Wilt**	**Chlorosis**
Assila	1.00± 0.10^{ab}	0.00± 0.00^{a}	0.00 ± 0.00^{a}
Gem Pride	2.50 ± 0.50^{a}	2.00 ± 0.10^{b}	2.50 ± 0.50^{b}
ROMA-VF	2.00 ± 0.10^{a}	1.00 ± 1.00^{ab}	1.00 ± 1.00^{ab}
UC-82-B	1.00± 1.00^{ab}	0.00± 0.00^{a}	0.00 ± 0.00^{a}
Ibadan local	1.50 ± 0.50^{ab}	1.00 ± 0.10^{ab}	1.50 ± 0.50^{ab}
Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

For the effects of the metabolite from *Fol* extracted with ethyl acetate (Table 4.11), there was substantial variation ($p< 0.05$) in leaf spot, wilt and chlorosis between the varieties and the control. For all the symptoms, the highest values (Leaf spot; 2.50 ± 0.50, wilt; 2.00 ± 0.10, chlorosis; 2.50 ± 0.50) were obtained in Gem Pride while the least values (Leaf spot; 1.00 ± 0.10, wilt; 0.00 ± 0.00, chlorosis 0.00 ± 0.00) were recorded in Assila and UC-82-B respectively.

The result in Table 4.12 shows the effects of extracts from *Fol* extracted with diethyl ether differed significantly ($p < 0.05$) in causing leaf spot, wilt and chlorosis on the varieties and Control. Leaf spot was more pronounced (2.00 ± 0.10) in Gem Pride than other varieties while it was least encountered (0.50 ± 0.50) in UC-82-B. Both UC-82-B and Ibadan local had the highest values of 2.50 ± 0.15; 2.50 ± 0.50 for wilt while the lowest (0.00 ± 0.00) was in ROMA-VF. Gem Pride had the highest value (2.50 ± 0.50) for chlorosis, while least values (0.00 ± 0.00) were in Assila and UC-82-B.

The effects of extracts from *Phomopsis* sp. (AID and ASE) and *Fusarium oxysporum* f. sp. *lycopersici* (FPIE and FSE) in causing leaf spot, wilt and chlorosis on the five varieties is shown in Tables 4.13– 4.17. The mean values for the control was significantly different from the effects of the symptoms throughout the experiment. There was considerable variation ($p < 0.05$) in the effects of the four extracts in causing leaf spot in Assila (Table 4.13). AID had the highest values of 1.50± 0.50 while AIE was the lowest (0.75± 0.25). For wilt, there was also a considerable variation ($p < 0.05$) among the extracts and the control. However, the maximum value (1.25± 0.25) for wilt was in AIE and the lowest (0.00± 0.00) in both AID and FPIE. Notably, there was no substantial variation ($p > 0.05$) in the effects of the four extracts in causing chlorosis in Assila.

The effects of the four extracts on leaf spot of Gem Pride in Table 4.14 was significantly different ($p< 0.05$) with AID showing the highest (3.50 ± 0.50) spot and FSE the least (2.00 ± 0.10). There was also significant variation ($p< 0.05$) in wilt with AID having the highest value (2.50 ± 0.50) and FSE the least (1.50 ± 0.50). Chlorosis on Gem Pride had the highest value (3.50 ± 0.50) on leaves treated with AID and the least (2.25 ± 0.48) on those treated with AIE.

Table 4.12**:** Effect of *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether on five tomato varieties

Tomato Varieties	**Symptoms**		
	Leaf spot	**Wilt**	**Chlorosis**
Assila	1.00 ± 0.10^{abc}	0.50 ± 0.50^{ab}	0.00 ± 0.00^{a}
Gem Pride	2.00 ± 0.10^{a}	1.50 ± 0.50^{ab}	2.50 ± 0.50^{b}
ROMA-VF	1.50 ± 0.50^{ab}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
UC-82-B	0.50 ± 0.50^{bde}	2.50 ± 1.50^{b}	0.00 ± 0.00^{a}
Ibadan local	1.50 ± 0.50^{ad}	2.50 ± 0.50^{b}	2.00 ± 0.10^{b}
Control	0.00 ± 0.00^{ce}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

(i) FPIE on Gem Pride

(ii) AIE on Assila

(iii) FSE on Assila

(iv) AID on Assila

(v) Control

(vi) Control

Plate 4.3a: *In-vivo* leaf bioassay

(i) AIE ON UC82B

(ii) AIE on Gem Pride

(iii) AID on Gem Pride

(iv) FSE on UC82B

(v) AID on UC82B

(vi) AIE on ROMAVF

Plate 4.3b: *In-vivo* leaf bioassay

Table 4.13**:** Effects of different extracts on Assila variety of Tomato

Extracts	Symptoms		
	Leaf spot	**Wilt**	**Chlorosis**
AID	1.50 ± 0.50^{a}	0.00 ± 0.00^{a}	0.50 ± 0.50^{a}
AIE	0.75 ± 0.25^{ac}	1.25 ± 0.25^{bc}	0.00 ± 0.00^{a}
FPIE	1.00 ± 0.10^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
FSE	1.00 ± 0.10^{a}	0.50 ± 0.50^{ac}	0.00 ± 0.00^{a}
Control	0.00 ± 0.00^{bc}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

Table 4.14**:** Effects of different extracts on GEM PRIDE variety of Tomato

Extracts	Symptoms		
	Leaf spot	**Wilt**	**Chlorosis**
AID	3.50± 0.50[a]	2.50± 0.50[a]	3.50 ± 0.50[a]
AIE	3.00± 0.10[ac]	1.75 ± 0.48[a]	2.25 ± 0.48[a]
FPIE	2.50 ± 0.50[bc]	2.00 ± 0.10[a]	2.50 ± 0.50[a]
FSE	2.00± 0.10[b]	1.50± 0.50[ab]	2.50± 0.50[a]
Control	0.00 ± 0.00[d]	0.00 ± 0.00[b]	0.00 ± 0.00[b]

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

The effects of the extracts on ROMA-VF were determined (Table 4.15). The result showed a substantial variation ($p< 0.05$) in leaf spot between the extracts and the control. Though, there was no substantial variation ($p >0.05$) in wilt and chlorosis between the extracts and control, but leaf spot was lowest (1.00 ± 0.10) in AID and highest (2.00 ± 0.10) in FPIE.

Treatment of UC–82–B with the extracts in Table 4.16 showed that there was no substantial variation ($p >0.05$) in leaf spot between the extracts and the control whereas, there were substantial variation ($p <0.05$) in wilt and chlorosis. Highest value of 2.50 ± 1.50 for wilt was in FSE and the least (0.00 ± 0.00) in FPIE. Chlorosis had mean value of 1.50 ± 0.50 in AID while it was 0.00 ± 0.00 for all the other extracts and control.

For Ibadan local (Table 4.17), there was substantial difference ($p <0.05$) in leaf spot, wilt and chlorosis between the extracts and control. AIE had highest value (2.00 ± 0.10) for spot while AID had the least (0.50 ± 0.50), the highest value (2.50 ± 0.50) for wilt was from FSE while AID had the least (0.00 ± 0.00) value. Chlorosis was highest (1.50 ± 0.29) on Ibadan local in the extracts of AIE and FPIE while it was lowest (0.00 ± 0.00) in FSE.

Table 4.15**:** Effects of different extracts on ROMA–VF variety of Tomato

Extracts	Symptoms		
	Leaf spot	**Wilt**	**Chlorosis**
AID	1.00 ± 0.100^{ab}.	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
AIE	1.50 ± 0.29^{a}	0.75 ± 0.48^{a}	1.00 ± 0.58^{a}
FPIE	2.00 ± 0.10^{a}	1.00 ± 1.00^{a}	1.00 ± 1.00^{a}
FSE	1.50 ± 0.50^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Control	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

Table 4.16**:** Effects of different extracts on UC-82-B variety of Tomato

Extracts	Symptoms		
	Leaf spot	**Wilt**	**Chlorosis**
AID	1.50 ± 0.50^{a}	$0.\ 50\pm 0.50^{ab}$	1.50 ± 0.50^{a}
AIE	0.75 ± 0.25^{a}	0.25 ± 0.25^{a}	0.00 ± 0.00^{b}
FPIE	1.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}
FSE	0.50 ± 0.50^{a}	2.50 ± 1.50^{b}	0.00 ± 0.00^{b}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

Table 4.17**:** Effects of different extracts on Ibadan local variety of Tomato

Extracts	Symptoms		
	Leaf spot	**Wilt**	**Chlorosis**
AID	0.50 ± 0.50^{ad}	$0.\ 00\pm 0.00^{a}$	1.00 ± 0.10^{ab}
AIE	2.00 ± 0.10^{bc}	2.00 ± 0.41^{bc}	1.50 ± 0.29^{a}
FPIE	1.50 ± 0.50^{ac}	1.00 ± 0.10^{ac}	1.50 ± 0.50^{a}
FSE	1.50 ± 0.50^{ac}	2.50 ± 0.50^{b}	0.00 ± 0.00^{a}
Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}

Note: Means having corresponding superscript alphabets and in a matching line do not vary significantly ($p>0.05$)

Key: AID- *Phomopsis* sp. extracted with diethyl ether;
AIE – *Phomopsis* sp. extracted with ethyl acetate;
FSE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with ethyl acetate;
FPIE- *Fusarium oxysporum* f. sp. *lycopersici* extracted with diethyl ether.

CHAPTER FIVE

5.0 DISCUSSION

Diseases represent a major restraining component to crop production in Nigeria. Fungi cause majority of plant diseases, accounting for about two thirds of all plant diseases. *Solanum lycopersicum* is predisposed to several diseases which diminish its output. Losses incurred ranging from minor to as high as one hundred percent (Kumar, 2017). Leaf diseases of tomato could be serious and can cause leaves to defoliate and kill the plant if not managed.

The most severe of these diseases are vascular wilts induced by *F. oxysporum* f. sp. *lycopersici* (*Fol*)*, Verticillium albo-atrum, Pseudomonas solanacearum* and early blight induced by *Alternaria solani* (Wokoma, 2008). Leaf spots of *S. lycopersicum* in this country have been linked to *Sclerotium rolfsii*, *Alternaria solani, Septoria lycopersici, Pseudomonas syringae* and *Xanthomonas vesicatoria* (Erinle, 1986). *Fusarium oxysporum* was reported by Wokoma (2008). He isolated the fungi and two others (*Verticillium albo-atrum* and *Rhizoctonia solani*) from roots and stems of wilted tomato plants in Choba, Rivers State. Although he did not isolate from tomato leaves, leaf spots were encountered in his work and he stated that several other fungi could be the cause of the noticed leaf spots.

Gao *et al*. (2016) also reported the isolation of *Fusarium proliferatum* causing leaf spots of tomato in China. Amuji *et al*. (2013) isolated *Fusarium oxysporum* and *Rhizopus stolonifer* from diseased leaves and fruits of tomato. McGovern (2015) described Fusarium wilt induced by *Fol* as one of the mainly studied diseases of tomato. Other pathogen described to have been associated with considerable reduction in yield and financial losses include *Botrytis cinerea, Alternaria solani* and *Phytophthora infestans* (Gao *et al*., 2016)

Symptoms of diseases observed on tomato leaves in this study included chlorosis/yellowing, leaf spots and wilting. Previous studies by Arogundade *et al*. (2007), Wokoma (2008), Amuji *et al*. (2013) also reported those symptoms as the most common on tomato leaves.

Phomopsis sp. has earlier been reported on eggplant, also a member of Solanaceae but not on tomato (Schwartz and Gent, 2007). *Phomopsis convolvulus* was reported (Tsantrizos *et al*., 1992) as a pathogen that caused leaf spot and anthracnose lesions on *Convolvulus arvensis* (field bindweed). Die-back of neem (*Azadirachta indica*) was also caused by *Phomopsis azadirachtae* (Girish *et al*., 2009). Other diseases caused by *Phomopsis* included Phomopsis cane spot of grapevines by *P. viticola;* Phomopsis leaf spot by *P. viticola*, Stem canker of sunflower by *P. helianthi*, Twig canker on *Prunus persica* by *P. amygdali* (Udayanga *et al*., 2011). Schwartz and Gent (2007) also stated that Phomopsis blight caused by *P. vexans* is a major disease of eggplant. However, tomato and pepper are not affected by the fungus. Although *Phomopsis* has been described as a universal genus of fungi which include plant pathogens as well as endophytes (Udayanga *et al*., 2011), to the best of my knowledge and from literature searches, this is the first report of the association of *Phomopsis* sp. as the causal agent of leaf diseases of tomato in Nigeria.

Employing the use of phytotoxic metabolites produced by pathogens is of more advantage as it allows for regulating temperature and humidity conditions which may be impossible with living organisms. Living organisms may have strict requirements for specific environmental conditions to establish infection (Chen and Swart, 2002). The treatment of plants with phytotoxins and culture filtrates of pathogenic fungi which were used in this study to test for the resistance of the selected tomato varieties both *in-vitro* and *in-vivo* is a helpful tool in plant-pathogen interactions. The use of liquid medium for phytotoxins extraction was employed in this study. Berestetskiy (2008) had stated that fungi are to be cultured in liquid nutrient media to isolate phytotoxins for chemical and biological properties. He further commented on the constraint of isolating low content of target phytotoxic compounds. He asserted that the regular yield range from a fungus when cultured in liquid medium is between one to fifty milligrams in a one litre liquid culture. In this study, the range of yield of the metabolites was from 39.0 to 54.5mg.

In a research conducted by Girish *et al*. (2009), culture filtrate of *Phomopsis azadirachtae* showed phytotoxic activity. It was established that toxic compounds were present in *P. azadirachtae*. From the findings of this work, it could be established that *Phomopsis* sp. produced phytotoxic metabolites.

Fusarium oxysporum f. sp. *lycopersici* in this research had 3-butylpyridine among the compounds isolated from the extracts. Fusaric Acid (FA) has been used for selecting for resistance in barley plant. The production of phytotoxic metabolites by plant pathogens in liquid medium has been well established. Plant pathogens generally do not synthesize only one compound with phytotoxic characteristics but instead a family of correlated compounds that differ in their toxicity (Strange, 2007). Due to the fact that toxins are required by pathogen to cause disease, if the host is made resistant to the toxin, it is invariably made resistant to the disease (Svabova and Lebeda, 2005). Hence the use of extracted metabolites for screening varieties of tomato instead of the pathogens producing them. Rani *et al*., (2009) stated that decarbonylation of Fusaric acid (FA) gives CO_2(C-7) and 3- butylpyridine.

There was substantial difference in the responses of the tomato varieties to the effects of the metabolites from both *Phomopsis* sp. and *Fusarium oxysporum* f. sp. *lycopersici* in this study. Culture filtrates of *Fusarium oxysporum* have been used to determine the resistance of five genotypes of *Amaranthus hybridus* in South Africa. *Fusarium oxysporum* was reported as the cause of stem decay and root rot of Amaranth. The five varieties tested showed significant variation in their response to the culture filtrate of the pathogen (Chen and Swart, 2002).

In a study by Masinde *et al*. (2011) evaluating the tolerance of tomato varieties to foliage disease in Kenya, it was reported that one of the means of combating losses due to diseases is the development of varieties that are tolerant. This leads to higher outputs. Productive tomato cultivation is dependent primarily on selection of appropriate varieties for a specific place. However, there are no varieties with complete resistance; partial resistance/tolerance is an indication that the varieties are good candidates for cultivation. The most common and devastating pathogens reported on tomato leaves were *Alternaria solani* causing early blight and *Phytophthora infestans* causing late blight.

Some fungal species like *Aspergillus, Fusarium* and *Penicillium* can degrade phthalates. There are many bacteria as well that are involved in this, they majorly include *Arthrobacter, Pseudomonas, Serratia, Acinetobacter*. Di-2-ethylhexylphthalate which has been widely used in plastics has been considered as an environmental pollutant due to its industrial origin. However, this view is changing steadily because it has been discovered that the compound is produced by some organisms like plants, bacteria and fungi and many studies have shown diverse bio-activities for this compound. Of these organisms, fungi are the largest group producing the compound (Ortiz and Sansinenea, 2018). *Curvularia senegalensis,* a fungal pathogen of some economic plants (Lucas *et al*., 2007) and *Aspergillus awamori* both produced phthalate in culture (Lotfy *et al*., 2018). Dibutyl phthalate was also previously isolated from the bacterium *Streptomyces albidoflavus* (Roy *et al*., 2006). Although phthalates are a group of substances encountered in pesticides, cosmetics and are mostly associated with plastics (Saillenfait and Laudet-Hesbert, 2005), they can also be produced in certain bacteria and fungi. Bis (2-ethylhexyl) phthalate was isolated from *Phomopsis* sp. in this study.

It has been reported by Ivanovic and Sinclair (1989) that culture filtrates of various unidentified isolates of *Phomopsis* spp. have varying ability to induce wilting in soybeans seedlings and also inhibit germination of various vegetables and wheat seeds. Some phytotoxic metabolites like ergosterol and ergosterol peroxide, pthalides convulvulanic acid A, convulvulanic acid B, convolvulop and α-pyrone convolvulopyrone were isolated from *P. convolvulus* (Tsantrizos *et al*., 1992). The compound with the highest percentage composition from *Phomopsis* in this study was Bis (2-ethylhexyl) phthalate. The compounds were quantified based on their abundance in the extracts; some are major constituents while others are trace.

In earlier studies by Yang *et al.* (2012), Reddy and Das (2014), a novel phytotoxic nonelide, 6,7-dihydroxy-9-propylanon-4-eno-9-lactone was isolated from *Phomopsis* sp. HCCB03520. Also fungal strains *Phomopsis* sp., *Phomopsis viticola* Sacc. and *Phomopsis viticola* complex associated with grapevine trunk diseases in Switzerland produced different phytotoxins with varying degrees of phytotoxicity (Goddard *et al*., 2014).

In the *in-vitro* leaf bioassay, LEMT25 was highly resistant to culture filtrate of *Phomopsis* sp., while LEMT39 and LEMT49 were susceptible. Kerewa and Ibadan local were highly resistant to culture filtrate of *Fol* whereas LEMT3, LEMT25, LEMT39 and LEMT47 were susceptible. There were varying degrees of responses of the varieties to the extracts in the *in-vivo* bioassay. In a study carried out in Ogbomoso, Oyo State by Olaniyi *et al*. (2010), seven varieties of tomato: DT97/162A(R), DT97/215A, Tropical, ROMA VF, UC82B, Ibadan local and Ogbomoso local were tested for growth, fruit yield and nutritional quality. DT97/162A(R) had the maximum yield while Ogbomoso local showed the maximum figure of foliage at six weeks after transplant. Superior fruit output was shown by UC82B succeeded by Ibadan and Ogbomoso local. Even though, the seven varieties were tested against pathogens, their performance may be an indication of how resistant they could be to pathogenic attack. This shows that their resistance to disease differs according to the variety (Arogundade *et al*., 2007).

The result of the *in-vitro* bioassay of tomato leaves showed that there was no marked difference in the effect of the wounding on the leaves. Although, Berestetskiy (2008) posited that symptoms of toxicoses are generally more noticeable in leaves wounded rather that in unscathed leaves. Also, in a study by Shanmugapackiam *et al*. (2017), injury was created on finger millet leaves in an *in-vitro* detached leaf bioassay.

Some metabolites are particularly produced by certain species of fungi. However, majority are produced by more than one species. For instance, AAL toxin, a host specific toxin which is generally produced by different species of *Alternaria* (Markham and Hille, 2001), has been reported (Lou *et al*., 2013) to be obtained from *Fusarium moniliforme* and *F. verticillioides* as well. Fumonisin, a non-host specific toxin generally isolated from *Fusarium* spp. has also been obtained from *Alternaria alternata*. Furthermore, Octadecane, a compound obtained from both *Fusarium oxysporum* and *Phomopsis* sp. in this present study was recorded in *Alternaria* sp. by Lou *et al*. (2013). In the same vein, 2-ethylhexylphthalate, was the compound with the highest composition from *Phomopsis* sp in this study, phthalides were also described in *Alternaria* (Lou *et al*., 2013).

n-Hexadecanoic acid, Hexadecane, 1,2-Benzenedicarboxylic acid and Octadecane were among the compounds obtained from the GC-MS analysis of the extracts from *Phomopsis* sp. and *Fusarium oxysporum* during this study. These compounds were reported (Shanmugapackiam *et*

al., 2017) among the phytotoxic compounds produced by *Magnaporthe grisea* causing leaf blast of finger millet.

Screening for resistance in crops via *in-vitro* and *in-vivo* studies by treating with toxins and culture filtrates of phytopathogenic fungi have shown to be a functional means in breeding to determine varietal resistance to major pathogens (Chen and Swart, 2002).

CONCLUSION AND RECOMMENDATION

The phytotoxicity of the ethyl acetate and diethyl ether extracts from culture filtrates of both *Fusarium oxysporum* f. sp. *lycopersici* and *Phomopsis* sp. was an indication of their role in pathogenesis and establishment of disease. The metabolites induced foliar diseases on different varieties of tomato. This study further confirms that phytotoxins could be used as substitute for pathogenic fungi. Cultivation of tomato varieties tolerant to these phytotoxins is thus advised. Furthermore, more efforts could be made to purify the identified metabolites.

Contribution to knowledge

To the best of my knowledge, this is the first isolation of *Phomopsis* as a causal agent of leaf diseases of tomato in Nigeria. The study proved that phytotoxic metabolites could be used in screening plants for resistance without the pathogens. The results of the reactions of the varieties to phytotoxins from *Fusarium oxysporum in-vitro* showed that the local varieties were resistant while the hybrids were susceptible. The role of phytotoxins generally in disease expression and development and specifically in fungal pathogens associated with foliar diseases of tomato has been further established from this study, hence addressing the paucity of information in this regard.

REFERENCES

Abbas, H. K., Boyette, C. D. and Hoagland, R. E. 1999. Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid, and moniliformin to jimsonweed. *Phytoprotection* 76.1: 17-25.

Abouzeid, M. A. and El-tarabily, K. A. 2003. Production of Phytotoxins by *Ascochyta pisi* Lib., the Causal Agent of Leaf Spot Disease of Pea. *International Journal of Agriculture and Biology* 5.2:145-149.

Abraham, P., Banwo, O. O., Kashina, B. D. and Alegbejo, M. D. 2019. Status of Tomao Viruses in Nigeria. *FUDMA Journal of Sciences* 3.3: 482-494.

Adebayo, O. S. 2011. Control of Bacterial Wilt Disease of Tomato: a Review of Research Efforts in Nigeria. *Acta Horticulturae* 914: 35-37. Doi:10.17660/actahortic.2011.914.2

Adebesin, A. A., Odebode, A. C. and Ayodele, A. M. 2009. Control of postharvest rots of banana fruits by conidia and culture filtrates of *Trichoderma asperellum*. *Journal of Plant Protection Research* 49.3: 302-308.

Adenuga, A. H., Muhammad-Lawal, A. and Rotimi, O.A. 2013. Economics and Technical Efficiency of Dry Season Tomato Production in Selected Areas in Kwara State, Nigeria. *Agrios on-line Papers in Economics and Informatics* 5.1: 11-19.

Adepoju, A. O. 2014. Post-harvest losses and welfare of tomato farmers in Ogbomosho, Osun State, Nigeria. *Journal of Stored Products and Postharvest Research* 5.2:8-13.

Agrios, G. N. 2005. *Plant Pathology*.5th Edition. Elsevier Academic Press, Inc. New York. 922pp.

Aigbedion-Atalor, P. O., Oke, A. O., Oladigbolu, A. A., Layade, A. A., Igbinosa, I. B. and Mohamed, S. A. 2019. *Tuta absoluta* (Lepidoptera: Gelechiidae) invasion in Nigeria: first report of its distribution. *Journal of Plant Diseases and Protection* https://doi.org/10.1007/s41348-019-00255-3

Amuji, C. F., Uguru, M. I., Ogbonna, P. E., Ugwuoke, K. I., Eze, I. E. and Mbadianya, J. I. 2013. Isolation and Identification of Fungal Pathogens Associated with Tomato Genotypes/Lines in Nsukka South-Eastern Nigeria. *International Journal of Scientific Research* 2.6: 5-8.

Amusa, N. A., Ikotun, T. and Asiedu, R. 1993. Extraction of a phytotoxic substance from *Colletotrichum gloeosporioides* infected yam leaves. *International Journal of Tropical Plant Dis*ease 11: 207-211.

Amusa, N. A. 2006. Microbially produced phytotoxins and plant disease management. *African Journal of Biotechnology* 5.5: 405-414.

Amusa, N. A. 2010. Mass screening techniques of some tropical crops for resistance to anthracnose diseases using phytotoxic metabolites. In: *Mass screening techniques for selecting crops resistant to diseases*. International Atomic Energy Agency, Vienna, Austria. Pp 173-188.

Arah, I. K., Kumah, E. K., Anku, E. K. and Amaglo, H. 2015. An Overview of Post-Harvest Losses in Tomato Production in Africa: Causes and Possible Prevention Strategies. *Journal of Biology, Agriculture and Healthcare* 5.16: 78-87.

Arogundade, O., Balogun, O. S. and Fawole, O. B. 2007. Incidence and severity of common viral and fungal diseases of dry season tomato crop in a Southern Guinea Savannah Agro-ecology. *Agrosearch* 9.1&2: 53-60.

Ayo-John, E. I. and Odedara, O. O. 2017. Serological detection of viruses infecting tomato and pepper in Southwest Nigeria and their distribution. *Nigerian Journal of Biotechnology* 33:78-82.

Baker, B., Zambryski, P., Staskawicz, B. and Dinesh-Kumar, S. P. 1997. Signaling in Plant-Microbe Interactions. *Science* 276: 726-733.

Balasubramanian, P. 2003. Biotechnology in Plant Disease Control. Foundation for Biotechnology Awareness and Education. http://www.fbae.org/channels/biotechinplantdiseasecontrl/biotechnologyinplantdisease.html.

Berestetskii, A. O., Yuzikhin, O. S., Katkova, A. S., Dobrodumov, A. V., Sivogrivov, D. E. and Kolombet, L. V. 2010. Isolation, Identification, and Characteristics of the Phytotoxin Produced by the Fungus *Alternaria cirsinoxia Applied Biochemistry and Microbiology* 46: 75- 79.

Berestetskiy, A. O. 2008. A Review of Fungal Phytotoxins: from Basic Studies to Practical Use. *Applied Biochemistry and Microbiology* 44.5: 501-514.

Bharathi, S. 2004. Developing botanical formulations for the management of major fungal diseases of tomato and onion. PhD Thesis Tamil Nadu Agricultural University, Coimbatore. 177pp.

Borisade, O. A., Kolawole, A. O., Adebo, G. M. and Uwaidem, Y. I. 2017. The tomato leafminer (*Tuta absoluta*) (Lepidoptera: Gelechiidae) attack in Nigeria: effect of climate change on over-sighted pest or agro-bioterrorism? *Journal of Agricultural Extension and Rural Development* 9.8: 163-171. DOI: 10.5897/JAERD2017.0856

Bronson, C. R. 1991. The genetics of phytotoxins production by plant pathogenic fungi. *Experientia* 47: 771-776.

Chen, W. Q. and Swart, W. J. 2002. The *in vitro* phytotoxicity of culture filtrates of *Fusarium oxysporum* to five genotypes of *Amaranthus hybridus*. *Euphytica* 127: 61-67.

Chohan, S., Perveen, R. Mahmood, M. A. and Rehman, A. U. 2016. Fungi colonizing different parts of tomato plant (*Lycopersicon lycopersicum* (L.) Karst. in Pakistan. *Pakistan Journal of Phytopathology* 28.01: 25-33.

Doohan, F. 2005. Fungal Pathogens of Plants. *Fungi: Biology and Applications.* Kavanagh, K. (Ed). John Wiley and Sons, Ltd, England. pp 219-250.

Effiuwevwere, B. J. O. 2000. *Microbial Spoilage Agents of Tropical and Assorted Fruits and Vegetables.* Paragraphics Publishing Company, Port Harcourt, Nigeria, 39pp.

Ejale, A. U. and Eikhuemelo, A. O. 2009. Preservation of fresh tomato (*Lycopersicon esculentum* M.) fruits with leaf extracts of (*Momordica charantia* L.) bitter melon. *Nigerian Journal of Botany* 22.2: 337-347.

Erinle, I. D. 1986. Tomato Diseases in the Northern States of Nigeria. Extension Bulletin 11, Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria.

Evidente, A., Rodeva, R., Andolfi, A., Stoyanova, Z., Perrone, C. and Motta, A. 2011. Phytotoxic polyketides produced by *Phomopsis foeniculi*, a strain isolated from diseased Bulgarian fennel. *European Journal of Plant Pathology* 130: 173-182.

Eziashi,E. I., Uma, N. U., Adekunle, A. A., Airede, C. E. and Odigie, E. E. 2010. Evaluation of lyophilized and non lyophilized toxins from *Trichoderma* species for the control of *Ceratocystis paradoxa. African Journal of Agricultural Research* 5.13: 1733-1738.

Fajinmi, A. A. and Fajinmi, O. B. 2010. An Overview of Bacterial Wilt Disease of Tomato in Nigeria. *Agricultural Journal* 5.4: 242-247.

FAOSTAT 2014.Global tomato production in 2012. Rome, FAO.

Food and Agricultural Organisation of the United Nations (FAO). 2010. FAOSTAT. Available http://faostat.fao.org/

Feys, B. J. and Parker, J. E. 2000. Interplay of signaling pathways in plant disease resistance. *Trends in Genetics* 16.10: 449-455.

Freedman, N. D., Park, Y. and Subar, A. F. 2008. Fruit and vegetable intake and head and neck cancer risk in a large United States prospective cohort study. *International Journal of Cancer* 122.10: 2330-2336.

Gao, Mei-Ling, Luan, Yu-Shi, Yu, Hai-Ning and Bao, Yong-Ming 2016. First report of tomato leaf spot caused by *Fusarium proliferatum* in China, *Canadian Journal of Plant Pathology* DOI: 10.1080/07060661.2016.1217277

Girish, K, Shankara, B. S. and Raveesha, K. A. 2009. Crude toxin extract from culture filtrate of *Phomopsis azadirachtae* infecting neem and its phytotoxicity. *International Journal of Integrative Biology* 6.2: 79-84.

Glandorf, D. C., Verheggen, P., Jansen, T., Jorritsma, J. W., Smit, E., Leefang, P., Wernars, K., Thomashow, L. S., Laureijs, E., Thomas-Oates, J. E., Bakker, P. A. and Van Loon, L. C. 2001. Effect of genetically modified *Pseudomonas putida* WCS358r on the fungal rhizosphere microflora of field-grown wheat. *Applied Environ*mental *Microbiology* 67: 3371-3378.

Goddard, M., Mottier, N., Jeanneret-Gris,N., Christen,D., Tabacchi,R. and Abou-Mansour, E.. 2014. Differential Production of Phytotoxins from *Phomopsis* sp. from Grapevine Plants Showing Esca Symptoms. *Journal of Agricultural and Food Chemistry* 62: 8602−8607. dx.doi.org/10.1021/jf501141g

Graniti, A. 1991. Phytotoxins and their involvement in plant diseases – Introduction. *Experientia* 47: 751-755.

Hell, W. H. and Weber, D. J. 1986. Assay for determining resistance and susceptibility of onion cultivars to Pink root diseases. *Phytopathology* 78: 115-117.

IITA. 1987. Annual Report, International Institute of Tropical Agriculture, Ibadan, Nigeria.

Ireland, K. F. and Leath, K. T. 1987.Potential of Using Culture Filtrates from *Verticillium albo-atrum* to Evaluate Alfalfa Germplasm for Resistance to Verticillium Wilt. *Plant Disease* 71.10: 900-903.

Ivanovic, M. and Sinclair, J. B. 1989. Comparison of possible phytotoxic metabolites in culture filtrates of the *Diaporthe / Phomopsis* complex of soybeans. *Mycopathologia* 108: 59-63.

Jin, H., Hartman, G. L., Nickell, C. D. and Widholm, J. M. 1996. Characterization and purification of a phytotoxin produced by *Fusarium solani*, the causal agent of soybean sudden death syndrome. *Phytopathology* 86: 277-282.

Khan, I. A., Alam, S. S. and Jabbar, A. 2004. Purification of Phytotoxin from culture filtrates of *Fusarium oxysporum* f. sp. *ciceris* and its biological effects on chickpea. *Pakistan Journal of Botany* 36.4: 871-880.

Kheder, A. A., Akagi, Y., Tsuge, T. and Kodama, M. 2012. Functional Analysis of the Ceramide Synthase Gene *ALT7,* A Homolog of the Disease Resistance Gene *Asc1*, in the Plant Pathogen *Alternaria alternata*. *Journal of Plant Pathology and Microbiol*ogy S2:001. doi:10.4172/2157-7471, S2-001.

Kilic-Ekici, O. and Yuen, G. Y. 2003. Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. *Phytopathology* 93: 1103-1110.

Kumar, N. 2017. Occurrence and distribution of tomato diseases and evaluation of bio-efficacy of *Trichoderma harzianum* on growth and yield components of tomato. *Nigerian Journal of Agriculture, Food and Environment* 13.2: 37-44.

Kurt, Ş. 2004. Host-Specific Toxin Production by the Tomato Target Leaf Spot Pathogen *Corynespora cassiicola*. *Turkish Journal of Agriculture Forestry* 28: 389-395.

Lotfy, M. M., Hassana, H. M., Hetta, M. H., El-Gendy, A. O. and Mohammed, R. 2018. Di-(2-ethylhexyl) Phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from River Nile derived fungus *Aspergillus awamori*. *Beni-Suef University Journal of Basic and Applied Sciences* 7: 263–269.

Lou, J., Fu, L., Peng, Y. and Zhou, L. 2013. Metabolites from *Alternaria* Fungi and Their Bioactivities. *Molecules* 18: 5891-5935. Doi10.3390/molecules/8055891.

Lucas, E. M. F., Abreu, L. M., Marriel, I. E., Pfenning, L. H. and Takahashi, J. A. 2007. Phthalates production from *Curvularia senegalensis* (Speg.) Subram, a fungal species associated to crops of commercial value. *Microbiological Research* 163: 495-502.

Makinde, S. C. O., Olatunji, O., Ogunba, A., Onyemeka, R. M., Ezenwata, I. S. and Asuni, M. A. (2019). Assessment of genetic divergence in mutant lines of tomato (*Solanum lycopersicum* L.). *International Journal of Engineering Applied Sciences and Technology,* 4.7: 204-210.

Mansoori, B. and Smith, C. J. 2005. Verticillium-toxins: Their role in Pathogenesis. *Journal of Agricultural Science Technol*ogy 7: 103-114.

Markham, J. E. and Hille, J. 2001. Host-selective toxins as agents of cell death in plant-fungus interactions. *Molecular Plant Pathology* 2.4: 229-239.

Masinde A. O. A., Kwambai K. T, and Wambani N. H. (2011). Evaluation of tomato (*Lycopersicon esculentum* L.) variety tolerance to foliar diseases at Kenya Agricultural Research Institute Centre-Kitale in North west Kenya**.** *African Journal of Plant Science* 5.11: 676-681.

Matsumoto, K., Barbosa, M. L., Souza, L. A. C. and Teixeira, J. B. 2010. In vitro selection for resistance to Fusarium wilt in Banana. In: "*Mass screening techniques for selecting crops resistant to diseases*."International Atomic Energy Agency, Vienna, Austria.Pp 101-113.

McGovern, R. J. 2015. Management of tomato diseases caused by *Fusarium oxysporum*. *Crop Protection* 73: 78–92.

Mehrotra, R. S. and Aggrarwal, A. 2004. *Plant Pathology*. 2nd Edition. Tata McGraw-Hill Publishing Company Limited. New Delhi. 845pp

Miller, S. A., Rowe, R. C., and Riedel, R. M. 2010. Fusarium and Verticillium wilts of Tomato, Potato, Pepper and Eggplant. The Ohio State University Extension Factsheet.

Naika, S. Juede, J., Goffau, M., Hilmi, M., Dam, V. 2005. "Cultivation of Tomato" Production, processing and marketing, Agronomisa/CTA. Revised Edition, Agrodokseries No. 17.

Nur Ain Izzati, M.Z., Azmi, A.R., Siti Nordahliawate, M.S. and Norazlina, J. 2011.Contribution to the knowledge of diversity of *Fusarium* associated with maize in Malaysia. *Plant Protection Science* 47: 20–24.

Olaniyi, J. O., Akanbi, W. B., Adejumo, T. A. and Akande, O. G. 2010. Growth, fruit yield and nutritional quality of tomato varieties. *African Journal of Food Science* 4.6: 398- 402.

Olutola, P.O., Famurewa, O. and Somtag, H.G. 2000. *An Introduction to General Microbiology: A practical approach*, Bolabary publications, Nigeria. 124pp.

Ortiz, A. and Sansinenea, E. 2018. Di-2-ethylhexylphthalate may be a natural product rather than a pollutant. *Journal of Chemistry*. https://doi.org/10.1155/2018/6040814.

Paulitz, T. C. and Belanger, R. R. 2001. Biological control in greenhouse systems. *Annual Review Phytopathology* 39: 103-133.

Popoola, A. R.1, Ganiyu, S. A.1, Enikuomehin, O. A., Bodunde, J. G., Adedibu, O. B., Durosomo, H. A. and Karunwi, O. A. 2015. Isolation and Characterization of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Nigeria. *Nigerian Journal of Biotechnology* 29: 1-10.

Potnis, N., Timilsina, S. Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E. and Jones, J.B. 2015. Bacterial spot of tomato and pepper: Diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology* 16.9:907-920.

PricewaterhouseCoopers (PwC) 2018. X-raying the Nigerian tomato industry. Focus on reducing tomato wastage. 16pp.

Rani, T. D., Rajan, S., Lavanya, L. and Kamalalochani, S. and Bharathiraja, B. 2009. An overview of Fusaric acid production. *Advanced Biotech* 4:18-22.

Reddy, C. R. and Das, B. 2014. Stereoselective total synthesis of the (Z)-isomer of a novel phytotoxic nonenolide from *Phomopsis* sp. HCCB03520 and its C-6 epimer. *Tetrahedron Letters* 55: 67- 70.

Roy, R. N., Laskar, S., Sen, S. K. 2006. Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus. Microbiological Research* 161.2: 121-126. DOI:10.1016/j.micres.2005.06.007

Saillenfait, A. M. and Laudet-Hesbert, A. 2005. Phthalates. *Emc-toxicologie Patologie* 2: 1-13. Doi: 10.1016 / S1155-1925 (05) 43864-1.

Sanda, N. B., Sunusi, M., Hamisu, H. S. Wudil, B. S., Sule, H. and Abdullahi, A. M. (2018). Biological Invasion of Tomato Leaf Miner, *Tuta absoluta* (Meyrick) in Nigeria: Problems and Management Strategies Optimization: A Review. *Asian Journal of Agrictural and Horticultural Research* 1.4: 1-14.

Schwartz, H. F. and Gent, D. H. 2007. Phomopsis Fruit Rot (Phomopsis Blight). High Plants IPM Guide, a cooperative effort of the University of Wyoming, University of Nebraska, Colorado State University and Montana State University.

Shanmugapackiam, S., Parthasarathy, S. and Raguchander, T. 2017. Detection of Phytotoxin Produced from Leaf, Neck and Finger Blast Disease Causing *Magnaporthe grisea* through GCMS analysis. *International Journal of Biochemistry Research and Review* 19.3DOI:10:9734/IJBCRR/201733353

Slavov, S. 2005. Phytotoxins and in Vitro Screening for Improved Disease Resistant Plants. *Biotechnology & Biotechnological Equipment* 19.3:48-55. DOI:10.1080/131028 18.2005.10817285.

Strange, R. N. 2007. Phytotoxins produced by microbial plant pathogens. *Natural Product Reports* 24: 127–144.

Svabova, L. and Lebeda, A. 2005. In Vitro Selection for Improved Plant Resistance to Toxin-Producing Pathogens. *Journal of Phytopathology* 153: 52-64.

Tan, H., Thomas-Ahner, J.M., Grainger, E.M., Wan, L., Francis, D.M., Schwartz, S.J., Erdman Jr J.W. and Clinton, S.K. 2010. Tomato-based food products for prostate cancer prevention: What have we learned? *Cancer Metastasis Reviews* 29: 553–568.

Tsantrizos, Y. S., Ogilvie, K. K. and Watson, A. K. 1992. Phytotoxic metabolites of *Phomopsis convolvulus*, a host-specific pathogen of field bindweed. *Canadian Journal of Chemistry* 70: 2276-2284.

Türkkan, M. and Dolar, F. S. 2008. Role of Phytotoxins In Plant Diseases. *Tarim Bilimleri Dergisi* 14.1: 87-94.

Udayanga, D., Liu, X., McKenzie, E. H. C., Chukeatirote, E., Bahkali, A. H. A. and Hyde, K. D. 2011. The Genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. *Fungal Diversity* 50: 189-225.

Ugonna, C. U., Jolaoso, M. A. and Onwualu, A. P. 2015. Tomato Value Chain in Nigeria: Issues, Challenges and Strategies. *Journal of Scientific Research and Reports* 7.7: 501-515.

Umeh, V.C., Kuku, F. O., Nwanguma, E.I., Adebayo, O. S. and Manga, A.A. 2002. A Survey of the Insect Pests and Farmers' Practices in the Cropping of Tomato in Nigeria. *Tropicultura* 20.4: 181-186.

USDA National Nutrient Database for Standard Reference 2010. SR23 - Reports by Single Nutrients. Release # 23. Pp 1-26. US Dept. of Agric. Agric. Research Service

Usman, H. 2006. Impact of the Hadeja Valley Irrigation project on Agricultural activities in Hadeja Emirate, Nigeria. A Paper presented at a 2-day conference to commemorate the Centenary of Hadeja's Resistance against the British Colonial Invasion.

Vidhyasekaran, P., Ling, D. H., Borromeo, E.S., Zapata, F. J. and Mew, T. W. 1990. Selection of brown spot-resistant rice plants from *Helmithosporium oryzea* toxic-resistant calluses. *Annals of Applied Biology* 117: 515-523.

Wagh, P., Sinha, S., Singh, H. K. and Khare, U. K. 2013. Pathogenic behaviour of *Alternaria alternata* and phytotoxicity of its culture filtrates on *Lepidium sativum*: a medicinal herb of immense pharmacological potential. *The Bioscan* 8.2: 643-647.

William, C. F. and Dennis, C. W. 1990. Food Microbiology Ed. 4th. Tata McGraw-Hill Publishing Company Ltd. Pp 412-416.

Wokoma, E. C. W. 2008. Preliminary Report on Diseases of Tomato in Choba, Rivers State. *Journal of Applied Sciences and Environmental Management* 12.3: 117-121.

Wu, H. S., Bao, W., Liu, D. Y., Ling, N., Ying, R. R., Raza, W. and Shen, Q. R. 2008. Effect of fusaric acid on biomass and photosynthesis of watermelon seedlings leaves. *Caryologia* 61.3: 258-268.

Yabuta, T., Kambe, K. and Hayashi, T. 1937. Biochemistry of the bakanae fungus. I. Fusarinic acid, a new product of the bakanae fungus. *Journal of the Agricultural Chemical Society of Japan* 10: 1059-1068.

Yahuza, A. and Yahaya, S. M. 2015. Comparison between water washing and detergent washing on reduction of post harvest losses of tomato (*Lycopersicum esculentum*). *Indian Journal of Plant Sciences* 4.4: 22-29.

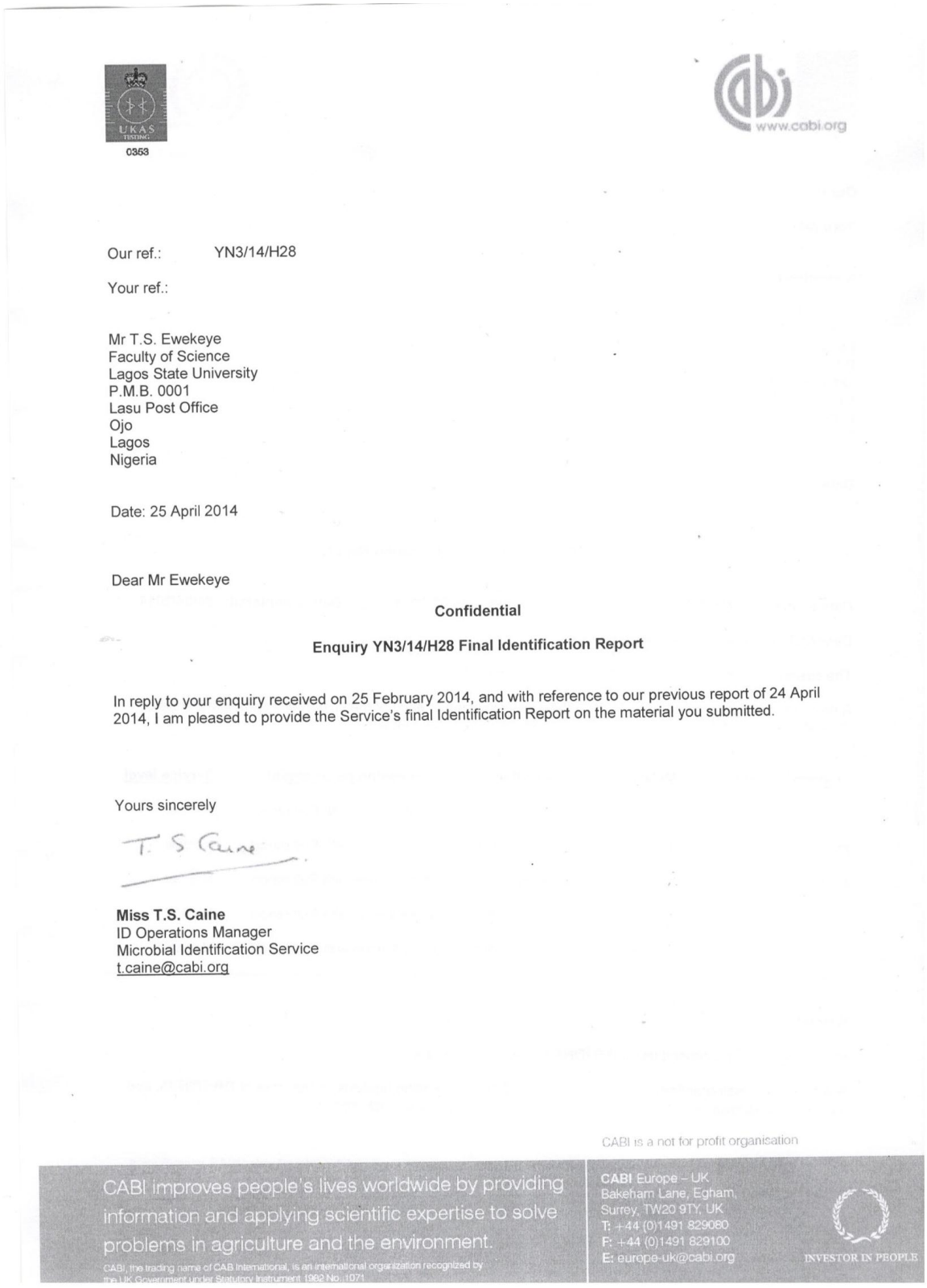
Yang, Z., Geb, M., Yinb, Y., Chen, Y., Luo, M. and Chen, D. 2012. A Novel Phytotoxic Nonenolide from *Phomopsis* sp. HCCB03520. *Chemistry & Biodiversity* 9: 403-408.

Yoder, O. C. 1980. Toxins in pathogenesis. *Annual Review of Phytopathol*ogy 18: 103-129.

Zhang, C. X., Ho, S. C., Chen, S. Z. and Lin, F.Y. 2009. Greater vegetables and fruit intake is associated with a lower risk of breast cancer among Chinese women. *International Journal of Cancer* 125.1:181-188.

Zivkovic, S. T., Stojanovia, S. D., Balay, J. and Gavrilovia, V. P. 2007. Characteristics of *Phomopsis* sp. isolates of plum trees origin. *Proc. Nat. Sci*., 113: 83-91.

Appendix 1: CABI Identification Report



0353

www.cabi.org

Our ref.: YN3/14/H28

Your ref.:

Mr T.S. Ewekeye
Faculty of Science
Lagos State University
P.M.B. 0001
Lasu Post Office
Ojo
Lagos
Nigeria

Date: 25 April 2014

Dear Mr Ewekeye

Confidential

Enquiry YN3/14/H28 Final Identification Report

In reply to your enquiry received on 25 February 2014, and with reference to our previous report of 24 April 2014, I am pleased to provide the Service's final Identification Report on the material you submitted.

Yours sincerely

T S Caine

Miss T.S. Caine
ID Operations Manager
Microbial Identification Service
t.caine@cabi.org

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CABI Europe – UK
Bakeham Lane, Egham,
Surrey, TW20 9TY, UK
T: +44 (0)1491 829080
F: +44 (0)1491 829100
E: europe-uk@cabi.org

INVESTOR IN PEOPLE

CABI IDENTIFICATION REPORT

Our ref: YN3/14/H28

Your ref:

Reporting to:

Mr T.S. Ewekeye
Faculty of Science
Lagos State University
P.M.B. 0001
Lasu Post Office
Ojo
Lagos
Nigeria

Date: 25 April 2014

CONFIDENTIAL

Enquiry YN3/14/H28 Final Identification Report

Date received: 25/02/2014 **Date started: 26/02/2014** **Date completed: 25/04/2014**

Description of material received:

The customer submitted 5 samples for microbial identification.

A unique CABI reference number (IMI number) was assigned to each of the customer's samples. Details of the samples received and the customer's requirements are listed below.

Customer sample	IMI Number	Description	Processing requirement	Service level
L	503744	Microbial culture	Identification with Full report	Normal
7*	503745	Microbial culture	Identification with Full report	Normal
4*14	503746	Microbial culture	Identification with Full report	Normal
T1	503747	Microbial culture	Identification with Full report	Normal
T.L.	503748	Microbial culture	Identification with Full report	Normal

Methods:

All samples were processed using ITS rDNA sequencing analysis.

Additional processing undertaken was partial TEF rDNA sequencing analysis, in the case of **IMI** 503745, and partial endochitinase (chi18-5) gene sequence analysis in the case of **IMI** 503747.

Opinions and interpretations based on test results are outside the scope of this laboratory's UKAS accreditation.
Tests marked with an asterisk are not UKAS accredited.

All procedures were validated and processing undertaken in accordance with CABI's in-house methods as documented in TPs 72-80 for filamentous fungi.

Procedures involved the following steps:

The original samples were subjected to a purity check.

Molecular assays were carried out on the samples using nucleic acid as a template. A proprietary formulation [microLYSIS®-PLUS (MLP), Microzone, UK)] was subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA).

Following DNA extraction, Polymerase Chain Reaction (PCR) was employed to amplify copies of the partial ITS fragment of rDNA in vitro for all samples, and in the case of **IMI** 503745 and **IMI** 502747, also to amplify copies of the partial TEF and partial endochitinase (chi18-5) genes respectively.

The quality of the PCR product was assessed by undertaking gel electrophoresis.

PCR purification step was carried out to remove unutilised dNTPs, primers, polymerase and other PCR mixture compounds and obtain a highly purified DNA template for sequencing. This procedure also allowed concentration of low yield amplicons.

Sequencing reactions were undertaken using BigDye® Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilises fluorescent labelling of the chain terminator ddNTPs, to permit sequencing.

Removal of excess unincorporated dye terminators was carried out to ensure a problem-free electrophoresis of fluorescently labelled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK) modules containing prehydrated gel-filtration resin were optimized for clean-up of sequencing reactions containing BigDye® terminators. Dye removal was followed by suspension of the purified products in highly deionised formamide Hi-Di™ (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation.

The samples were loaded onto the AB 3130 Genetic Analyzer and sequencing undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide.

Following sequencing, identification was undertaken by comparing the sequences obtained with those available at the European Molecular Biology Laboratory (EMBL) database via the European Bioinformatics Institute (EBI).

Results:

Customer sample	IMI Number	Identification and comments
L	503744	**Identified as: Phomopsis sp.** **Process:** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. **Result:** The sequence from **IMI** 503744 showed 98-99% identity to multiple ITS sequences reported from largely unidentified species of *Phomopsis* or the *Diaporthe* teleomorph. Best matches include sequences published in peer reviewed literature (eg Sim JH *et al.* (2010). Molecular diversity of fungal endophytes isolated from *Garcinia mangostana* and *Garcinia parvifolia*. *J. Microbiol. Biotechnol.* **20**: 651-

Customer sample	IMI Number	Identification and comments
		658). **Comment:** Since the ITS sequence of this strain does not match any from authentically identified species, it cannot be identified further. Members of this genus are assigned by ACDP (UK) to hazard group 1, a biological agent most unlikely to cause human disease. **Destination:** This material will be discarded.
7*	503745	**Identified as: Fusarium oxysporum aggregate.** **Process:** This sample was identified by ITS and partial TEF rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI and confirmed using the *Fusarium*-ID identification database. **Result:** Top matches of >98% for ITS and >99% for TEF were to sequences assigned to this genus and predominantly to the *Fusarium oxysporum* species aggregate. The top matches to authenticated material included 99.8% to *Fusarium oxysporum f. sp. spinaciae* TEF sequence DQ837687 (= NRRL 26871). **Comment:** Whilst ITS is considered to be the DNA "barcode" of choice for fungi, in *Fusarium* the species delimitation with ITS is not ideal, owing mainly to the presence of non-orthologous copies of the ITS which can confound analyses. TEF offers better resolution and is used widely within the genus for species identification. *Fusarium oxysporum* is a very widespread, often soil-borne species aggregate that causes diseases of an exceptionally wide range of plants. Numerous segregate taxa have been recognized, but many of the tropical forms remain poorly known and multilocus sequencing is often needed to distinguish between them. Members of this genus are assigned to hazard group 2 by ACDP (UK). **Destination:** This material will be discarded.
4*14	503746	**Identified as: Phomopsis sp.** **Process:** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. **Result:** The sequence from **IMI** 503746 showed 98-99% identity to multiple ITS sequences reported from largely unidentified species of *Phomopsis* or the *Diaporthe* teleomorph. Best matches include sequences published in peer reviewed literature (eg Sim J.H. *et al.* (2010). Molecular diversity of fungal endophytes isolated from *Garcinia mangostana* and *Garcinia parvifolia*. *J. Microbiol. Biotechnol.* **20**: 651-658). **Comment:** Since the ITS sequence of this strain does not match any from authentically identified species, it cannot be identified further. Members of this genus are assigned by ACDP (UK) to hazard group 1, a biological agent most unlikely to cause human disease.

Customer sample	IMI Number	Identification and comments
		Destination: This material will be discarded.
T1	503747	**Identified as: Trichoderma sp.** **Process:** This sample was identified by ITS rDNA and partial endochitinase (chi18-5) gene sequence analysis using the FASTA algorithm with the Fungus database from EBI and confirmed by screening the ITS sequence against the ISTH TrichOKEY identification tool. **Result:** ITS sequence gave top matches >99% to members of this genus, predominantly to *T. asperellum*, including the authenticated strains: *Trichoderma asperellum* strain GJS 99-6 (DQ109538) and *Trichoderma asperelloides* strain GJS 04-187 (JN133553), whilst the ISTH TrichoKEY identification tool gave a "high" reliability species identification to *T. asperellum* and to *T. koningiopsis*. The partial chi18-5 sequence gave >99% matches to several members of the genus including several sequences of *T. asperellum,* and 100% match to *Trichoderma asperellum* strain CBS 361.97 (AF188930). In the light of these results, this strain can be assigned to *Trichoderma* section *Trichoderma* but cannot be assigned definitively to a species within the section. Further resolution would require sequencing of additional loci however attempts to amplify the partial TEF gene were unsuccessful. **Comment:** *Trichoderma* species are widely distributed in all types of soil. They are also commonly isolated from plants, wood, paper and textiles. They are generally saprobes in the environment, but many can colonise, kill or inhibit other fungi. They are considered as potential biocontrol agents and some formulations have been developed for their application in the field. They are not normally considered to be human pathogens and are assigned by ACDP (UK) to hazard group 1, a biological agent that is most unlikely to cause human disease. Literature references include: Gams, W. and Bissett, J. (1998). *Morphology and identification of Trichoderma in Trichoderma and Gliocladium* Vol.1. (C.P. Kubicek and G.E. Harman, eds) pp. 1-34. Taylor and Francis, London. **Destination:** This material will be discarded.
T.L.	503748	**Identified as: Epicoccum nigrum.** **Process:** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI. **Result:** The sequence obtained from this sample showed 100% identity to sequences of *Epicoccum nigrum*. The best matches included sequences published in peer-reviewed literature e.g. FN646619 published in Lahlali R. and Hijri M. (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. *FEMS Microbiology Letters* **311**(2):152-9.

Customer sample	IMI Number	Identification and comments

Comment: This is species is a very common saprobe with a world-wide distribution. It is a secondary invader of dead plant tissues, and is also frequently isolated from soil, mouldy paper, air, textiles, dead insects etc. Optimum growth temperature is 23-28°C. Mycotoxins including flavipin and epicorazine are produced by some strains and allergic reactions to spores have been reported. It is assigned by ACDP to hazard group 1, a biological agent most unlikely to cause human disease. For additional information see Ellis, M.B. (1971) *Dematiaceous Hyphomycetes* (Kew: CMI): 72.

Destination: This material will be discarded.

Opinions and Interpretations:

Identification was undertaken by comparing the sequence obtained from each sample with those available from the European Molecular Biology Laboratory (EMBL) database via the European Bioinformatics Institute (EBI).

Where matches of 99-100% identity are obtained, identification is provided to species level, or where appropriate to species aggregate, provided that matches include a sequence derived from type or other validated culture and when there is a clear sequence distinction between taxa.

Where matches of 98-100% identity are obtained to more than one species within a genus, including matches to sequences published in peer-reviewed literature or matches to a validated type strain, and there is no clear sequence distinction between taxa, identification is given to genus level.

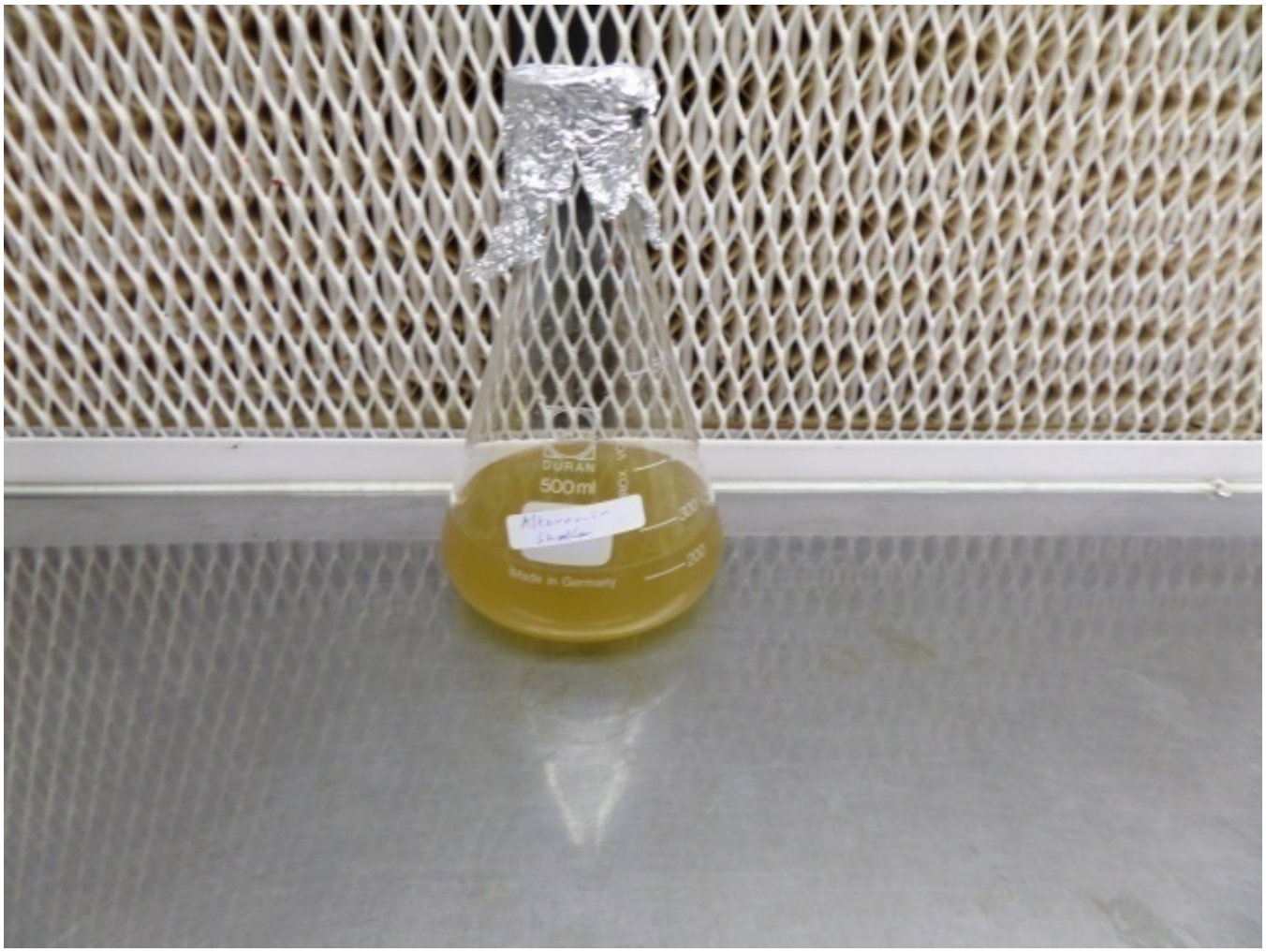
Authorisation:

I certify that this report has been checked and approved.

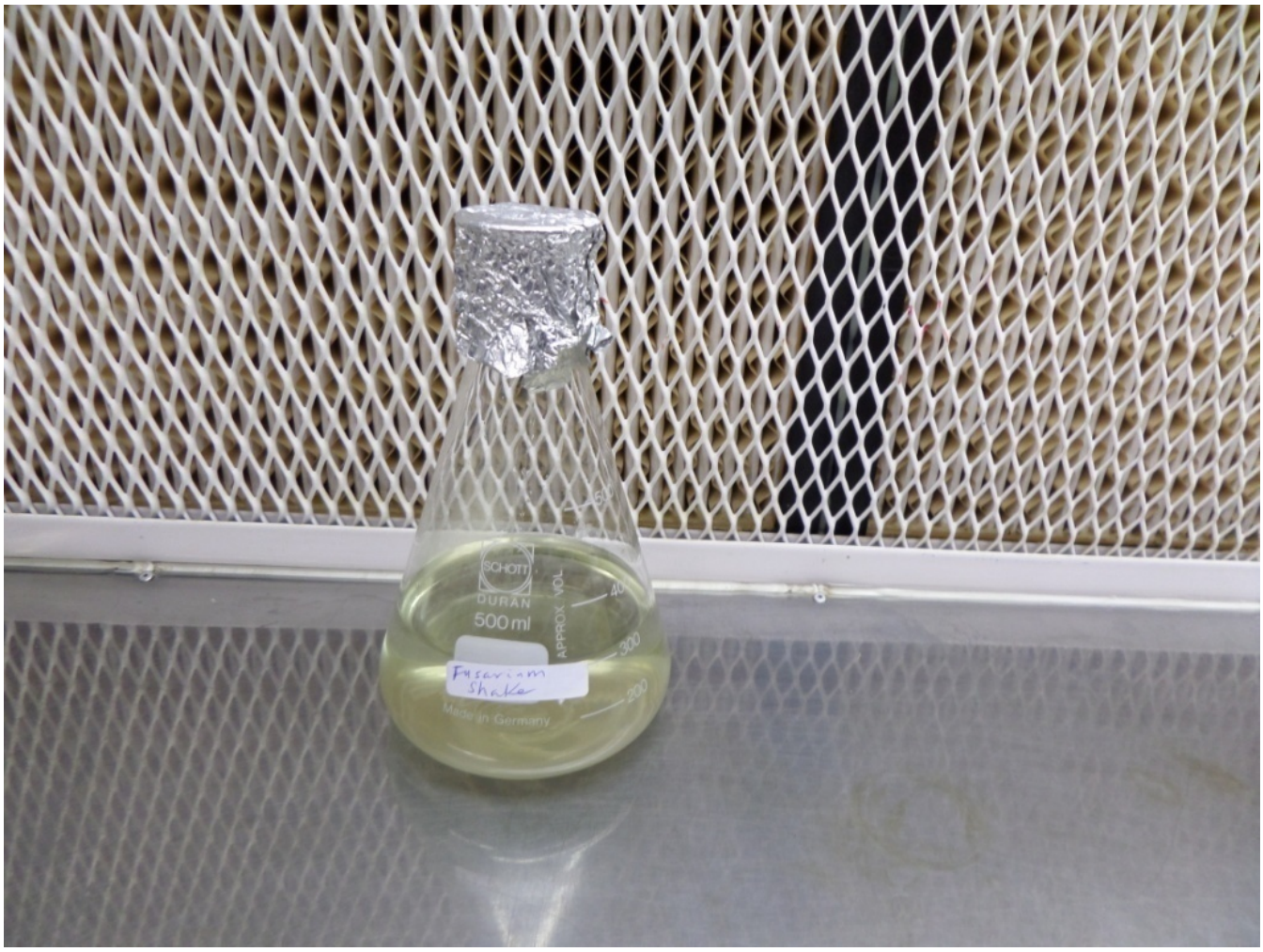
Signed:

T. S Caine

Miss T.S. Caine
ID Operations Manager
Microbial Identification Service
t.caine@cabi.org



Appendix 2: Culture filtrate of *Phomopsis* sp.



Appendix 3: Culture filtrate of *Fusarium oxysporum* f. sp. *lycopersici*

Library Search Report AID

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_AID.D
Acq On : 7 Sep 2014 20:30
Operator : Aminu
Sample : TS_AID
Misc :
ALS Vial : 6 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
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			Benzeneacetic acid	15708	000103-82-2	90
			Benzeneacetic acid	15709	000103-82-2	78
			Benzene, (2-methoxyethyl)-	15863	003558-60-9	72
2	20.40	1.07	C:\Database\NIST02.L			
			Benzeneethanol, 4-hydroxy-	16711	000501-94-0	91
			Benzeneethanol, 4-hydroxy-	16712	000501-94-0	91
			Benzeneethanol, 4-hydroxy-	16702	000501-94-0	83
3	20.71	3.66	C:\Database\NIST02.L			
			1-Methylpentyl cyclopropane	11213	006976-28-9	47
			1-Nonanol	20225	000143-08-8	43
			Nonyl chloroformate	59708	057045-82-6	38
4	20.93	1.88	C:\Database\NIST02.L			
			3-tert-Butyl-4-hydroxyanisole	42463	000121-00-6	62
			Phenol, 3-(1,1-dimethylethyl)-4-me thoxy-	42486	000088-32-4	62
			Phenol, 3-(1,1-dimethylethyl)-4-me thoxy-	42484	000088-32-4	62
5	21.57	1.82	C:\Database\NIST02.L			
			Butylated Hydroxytoluene	69511	000128-37-0	96
			Butylated Hydroxytoluene	69513	000128-37-0	96
			Butylated Hydroxytoluene	69514	000128-37-0	94
6	21.78	0.86	C:\Database\NIST02.L			
			4-(2-Methoxyethyl)phenol	24805	056718-71-9	87
			Benzeneacetic acid, 4-hydroxy-	24690	000156-38-7	86
			Benzeneacetic acid, 4-hydroxy-	24699	000156-38-7	83
7	23.04	1.48	C:\Database\NIST02.L			
			2,2-Dimethylpropanoic acid, 2-adam antyl ester	79830	1000282-85-0	64
			Methyl dithio-3-methylbenzoate	44623	005969-49-3	59
			2-Pyridinecarboxylic acid, 5-butyl	41832	000536-69-6	58
8	25.03	1.10	C:\Database\NIST02.L			

			Benzenesulfonamide, N-butyl-	64847	003622-84-2 97
			Benzenesulfonamide, N-butyl-	64848	003622-84-2 95
			N-(2-Cyano-ethyl)-benzenesulfonami de	63280	002619-21-8 83
9	26.57	0.87	C:\Database\NIST02.L		
			n-Hexadecanoic acid	92228	000057-10-3 90
			n-Hexadecanoic acid	92226	000057-10-3 89
			Tridecanoic acid	65566	000638-53-9 81
10	28.06	1.31	C:\Database\NIST02.L		
			2-Propenoic acid, 3-(2,2,6-trimeth yl-7-oxabicyclo[4.1.0]hept-1-yl)-, methyl ester, (E)-	72198	052298-37-0 47
			Silane, 1-hexynyltrimethyl-	26549	003844-94-8 35
			2-(4-Chlorophenyl)-2-oxoethyl 5-me thyl-3-phenylisoxazole-4-carboxyla te	143041	282730-83-0 30
11	29.92	1.73	C:\Database\NIST02.L		
			2-Furancarboxylic acid, trimethyls ilyl ester	45845	055887-53-1 22
			2-Furancarboxylic acid, trimethyls ilyl ester	45844	055887-53-1 22
			6-Octyloxy-naphthalene-2-carbonitr ile	106890	342414-42-0 14
12	30.50	7.97	C:\Database\NIST02.L		
			9-Octadecenamide, (Z)-	106876	000301-02-0 91
			7-Nonenamide	26840	090949-53-4 78
			9-Octadecenamide, (Z)-	106875	000301-02-0 72
13	30.99	6.65	C:\Database\NIST02.L		
			3-Butanone, 2-(2,6-dioxo-3-piperid yl)-2-methyl-	54015	198283-36-2 40
			1-Methyl-3-tetradecanoyl-pyrrolidi ne-2,4-dione	129369	095734-35-3 38
			9-Oxabicyclo[3.3.1]nona-2-ene, 6-a cethylthio-	54393	039869-51-7 27
14	32.28	62.31	C:\Database\NIST02.L		
			1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	105069	004376-20-9 91
			1,2-Benzenedicarboxylic acid, diis ooctyl ester	154183	027554-26-3 90
			1,2-Benzenedicarboxylic acid, dicy clohexyl ester	132732	000084-61-7 72
15	35.43	1.07	C:\Database\NIST02.L		
			Silane, 1,4-phenylenebis[trimethyl	70586	013183-70-5 43
			1,3-Bis(trimethylsilyl)benzene	70584	002060-89-1 43
			N-Methyl-1-adamantaneacetamide	60896	031897-93-5 43
16	35.81	2.82	C:\Database\NIST02.L		
			Hexestrol di-TMS	159179	070244-15-4 60
			Silane, 1,4-phenylenebis[trimethyl	70586	013183-70-5 53

1-Methyl-3-phenylindole 60944 030020-98-5 46

Area Percent Report
Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_AID.D
Acq On : 7 Sep 2014 20:30
Operator : Aminu
Sample : TS_AID
Misc :
ALS Vial : 6 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 2 Min Area: 1 % of largest Peak
Start Thrs: 0.1 Max Peaks: 200
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 0

Method : C:\MSDCHEM\1\METHODS\BAYAWA\AB_CRUDE OIL_ALI_SIM2.M
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	17.662	1193	1211	1237	rBV	298374	876312	5.46%	3.405%
2	20.396	1623	1648	1659	rBV	145424	276473	1.72%	1.074%
3	20.708	1679	1698	1717	rBV	378402	940901	5.87%	3.656%
4	20.933	1721	1734	1743	rVB	258781	483157	3.01%	1.878%
5	21.572	1819	1836	1845	rBV	240929	467211	2.91%	1.816%
6	21.778	1859	1869	1885	rBV	77507	221412	1.38%	0.860%
7	23.035	2061	2070	2089	rVB2	149155	380809	2.37%	1.480%
8	25.031	2379	2389	2415	rVB2	115004	283608	1.77%	1.102%
9	26.570	2623	2635	2651	rBV4	68726	223538	1.39%	0.869%
10	28.058	2861	2873	2897	rBV3	73161	337303	2.10%	1.311%
11	29.922	3161	3171	3197	rVB3	156143	445133	2.78%	1.730%
12	30.498	3249	3263	3289	rBV	791308	2051294	12.79%	7.971%
13	30.986	3329	3341	3383	rBV	654586	1711583	10.67%	6.651%
14	32.281	3531	3548	3591	rBV	5182408	16035278	100.00%	62.313%
15	35.427	4039	4051	4069	rBV	55310	274896	1.71%	1.068%
16	35.815	4099	4113	4157	rVB	53889	724445	4.52%	2.815%

Sum of corrected areas: 25733353

Library Search Report AIE

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_AIE.D
Acq On : 7 Sep 2014 19:33
Operator : Aminu
Sample : TS_AIE
Misc :
ALS Vial : 5 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	15.99	0.48	C:\Database\NIST02.L			
			4-Octanol, 2-methyl-	20251	040575-41-5	72
			1-Octyn-4-ol	10950	052517-92-7	72
			5-Nonanol	20228	000623-93-8	56
2	19.39	0.49	C:\Database\NIST02.L			
			Benzaldehyde, 4-hydroxy-	9578	000123-08-0	95
			Benzaldehyde, 4-hydroxy-	9581	000123-08-0	95
			Benzaldehyde, 4-hydroxy-	9582	000123-08-0	94
3	22.36	0.78	C:\Database\NIST02.L			
			1H-2-Benzopyran-1-one, 3,4-dihydro -8-hydroxy-3-methyl-, (R)-	40894	000480-33-1	99
			1H-2-Benzopyran-1-one, 3,4-dihydro -8-hydroxy-3-methyl-	40889	017397-85-2	96
			4-tert-Butylaniline	22313	000769-92-6	30
4	22.61	2.52	C:\Database\NIST02.L			
			Hexadecane	73964	000544-76-3	97
			Hexadecane	73968	000544-76-3	97
			Hexadecane	73966	000544-76-3	96
5	23.04	0.45	C:\Database\NIST02.L			
			2-Pyridinecarboxylic acid, 5-butyl	41832	000536-69-6	94
			(2-Oxazolidinylidene)malononitrile	14908	002733-51-9	64
			1-Adamantanecarboxylic acid, 2-ada mantyl ester	125181	1000282-94-3	64
6	24.93	1.82	C:\Database\NIST02.L			
			Octadecane	91035	000593-45-3	92
			Heptacosane	151556	000593-49-7	91
			Hexadecane	73968	000544-76-3	91
7	25.03	0.88	C:\Database\NIST02.L			
			Benzenesulfonamide, N-butyl-	64847	003622-84-2	95
			Benzenesulfonamide, N-butyl-	64848	003622-84-2	93
			N-(2-Cyano-ethyl)-benzenesulfonami de	63280	002619-21-8	83

8 26.57 0.92 C:\Database\NIST02.L
n-Hexadecanoic acid 92227 000057-10-3 94
Tridecanoic acid 65562 000638-53-9 91
n-Hexadecanoic acid 92228 000057-10-3 87

9 26.69 0.47 C:\Database\NIST02.L
1,2-Benzenedicarboxylic acid, buty 134722 000084-78-6 86
l octyl ester
Dibutyl phthalate 105064 000084-74-2 86
1,2-Benzenedicarboxylic acid, bis(105071 000084-69-5 78
2-methylpropyl) ester

10 27.03 1.04 C:\Database\NIST02.L
Eicosane 107651 000112-95-8 97
Eicosane 107655 000112-95-8 97
Heptacosane 151556 000593-49-7 91

11 27.88 0.66 C:\Database\NIST02.L
1-Heptadecanol 92320 001454-85-9 95
1-Nonadecanol 108955 001454-84-8 95
Bromoacetic acid, hexadecyl ester 145521 005454-48-8 94

12 28.38 0.71 C:\Database\NIST02.L
9-Octadecenoic acid, (E)- 107527 000112-79-8 59
Cyclotetradecane 53621 000295-17-0 42
Ethanol, 2-(tetradecyloxy)- 93396 002136-70-1 35

13 28.93 0.63 C:\Database\NIST02.L
Eicosane 107655 000112-95-8 97
Eicosane 107651 000112-95-8 96
Heneicosane 115570 000629-94-7 87

14 29.79 0.55 C:\Database\NIST02.L
2-Propenoic acid, pentadecyl ester 107542 043080-23-5 58
1-Docosene 121981 001599-67-3 55
Cyclotetracosane 135652 000297-03-0 55

15 30.74 0.64 C:\Database\NIST02.L
Eicosane 107653 000112-95-8 95
Eicosane, 9-octyl- 155179 013475-77-9 68
13-Methylhentriacontane 164419 1000131-19-4 68

16 32.35 85.65 C:\Database\NIST02.L
1,2-Benzenedicarboxylic acid, mono 105069 004376-20-9 91
(2-ethylhexyl) ester
1,2-Benzenedicarboxylic acid, diis 154183 027554-26-3 91
ooctyl ester
1,2-Benzenedicarboxylic acid, dihe 145658 003648-21-3 60
ptyl ester

17 43.53 1.29 C:\Database\NIST02.L
Eicosane 107653 000112-95-8 96
Eicosane 107655 000112-95-8 95
13-Methylhentriacontane 164419 1000131-19-4 58

Area Percent Report

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_AIE.D
Acq On : 7 Sep 2014 19:33
Operator : Aminu
Sample : TS_AIE
Misc :
ALS Vial : 5 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 2 Min Area: 0.5 % of largest Peak
Start Thrs: 0.1 Max Peaks: 200
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 0

Method : C:\MSDCHEM\1\METHODS\BAYAWA\AB_CRUDE OIL_ALI_SIM2.M
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	15.992	935	944	963	rBV	84178	284107	0.56%	0.483%
2	19.388	1479	1487	1499	rVB	155238	289210	0.57%	0.491%
3	22.360	1953	1962	1971	rBV	244039	461326	0.92%	0.784%
4	22.610	1995	2002	2013	rVV	886523	1481061	2.94%	2.516%
5	23.035	2061	2070	2091	rVV	111793	264749	0.53%	0.450%
6	24.931	2367	2373	2381	rVB	630648	1073800	2.13%	1.824%
7	25.031	2381	2389	2399	rBV	275927	516323	1.02%	0.877%
8	26.570	2623	2635	2649	rBV	230916	540766	1.07%	0.919%
9	26.695	2649	2655	2661	rVV	144575	278778	0.55%	0.474%
10	27.026	2703	2708	2721	rVV	311971	615032	1.22%	1.045%
11	27.883	2837	2845	2859	rVV	189753	386453	0.77%	0.657%
12	28.377	2907	2924	2935	rBV4	94539	419131	0.83%	0.712%
13	28.934	2995	3013	3019	rBV	148474	372919	0.74%	0.634%
14	29.791	3145	3150	3163	rBV	146208	326149	0.65%	0.554%
15	30.736	3283	3301	3321	rBV4	82448	377547	0.75%	0.641%
16	32.349	3531	3559	3637	rBV3	7533518	50416062	100.00%	85.649%
17	43.528	5325	5346	5369	rVB3	98857	760310	1.51%	1.292%

Sum of corrected areas: 58863723

Library Search Report FSE

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_FSE.D
Acq On : 8 Sep 2014 10:59
Operator : Aminu
Sample : TS_FSE
Misc :
ALS Vial : 3 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	13.82	3.21	C:\Database\NIST02.L			
			Butanoic acid, 2-hydroxy-3-methyl-	8450	004026-18-0	83
			Carbon disulfide	958	000075-15-0	4
			Carbon disulfide	959	000075-15-0	4
2	15.50	0.61	C:\Database\NIST02.L			
			2-Butynedinitrile	951	001071-98-3	4
			Isopropyl phosphine	947	004538-29-8	4
			Carbon disulfide	958	000075-15-0	4
3	16.55	0.55	C:\Database\NIST02.L			
			Pyridine, 3-butyl-	15028	000539-32-2	94
			Pyridine, 3-butyl-	15023	000539-32-2	91
			Pyridine, 3-(2-methylpropyl)-	15058	014159-61-6	64
4	17.62	0.45	C:\Database\NIST02.L			
			Benzeneacetic acid	15708	000103-82-2	86
			Benzeneacetic acid	15707	000103-82-2	83
			Propanedioic acid, phenyl-	43208	002613-89-0	72
5	21.78	0.50	C:\Database\NIST02.L			
			Benzeneacetic acid, 4-hydroxy-	24690	000156-38-7	58
			Pyridine, 3,5-dimethyl-	5057	000591-22-0	53
			Benzeneacetic acid, 4-hydroxy-	24699	000156-38-7	53
6	22.61	1.99	C:\Database\NIST02.L			
			Hexadecane	73967	000544-76-3	96
			Hexadecane	73966	000544-76-3	95
			Hexadecane	73964	000544-76-3	95
7	23.05	4.48	C:\Database\NIST02.L			
			2-Pyridinecarboxylic acid, 5-butyl	41832	000536-69-6	94
			1-Adamantaneacetic acid	51764	004942-47-6	53
			Tricyclo[3.3.1.1(3,7)-]decane, 1-b romo-	65241	000768-90-1	53
8	24.92	1.27	C:\Database\NIST02.L			
			Octadecane	91035	000593-45-3	91

	Nonadecane	99477	000629-92-5	91
	Hexadecane	73967	000544-76-3	91
9	26.56 0.70 C:\Database\NIST02.L			
	n-Hexadecanoic acid	92228	000057-10-3	95
	n-Hexadecanoic acid	92227	000057-10-3	94
	n-Hexadecanoic acid	92226	000057-10-3	93
10	27.03 0.61 C:\Database\NIST02.L			
	Eicosane	107651	000112-95-8	96
	Eicosane	107655	000112-95-8	96
	Octadecane	91036	000593-45-3	87
11	27.88 0.57 C:\Database\NIST02.L			
	1-Heptadecanol	92320	001454-85-9	93
	Bromoacetic acid, octadecyl ester	154046	018992-03-5	93
	Bromoacetic acid, hexadecyl ester	145521	005454-48-8	93
12	28.36 0.50 C:\Database\NIST02.L			
	6-Octadecenoic acid, (Z)-	107523	000593-39-5	50
	9-Octadecenoic acid, (E)-	107527	000112-79-8	45
	Octadec-9-enoic acid	107520	1000190-13-7	38
13	28.93 0.55 C:\Database\NIST02.L			
	Eicosane	107655	000112-95-8	93
	Eicosane	107651	000112-95-8	90
	Tritetracontane	172667	007098-21-7	70
14	29.79 0.46 C:\Database\NIST02.L			
	1-Nonadecene	98170	018435-45-5	83
	2- Chloropropionic acid, octadecyl ester	144923	088104-31-8	50
	Dichloroacetic acid, heptadecyl es ter	146886	1000282-98-2	50
15	32.29 82.42 C:\Database\NIST02.L			
	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	105069	004376-20-9	91
	1,2-Benzenedicarboxylic acid, diis ooctyl ester	154183	027554-26-3	90
	1,2-Benzenedicarboxylic acid, diis ooctyl ester	154181	027554-26-3	74
16	43.53 1.14 C:\Database\NIST02.L			
	Nonadecane	99477	000629-92-5	64
	Cyclotrisiloxane, hexamethyl-	71175	000541-05-9	38
	Octacosane	155178	000630-02-4	35

Area Percent Report
Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_FSE.D
Acq On : 8 Sep 2014 10:59

Operator : Aminu
Sample : TS_FSE
Misc :
ALS Vial : 3 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 2 Min Area: 0.5 % of largest Peak
Start Thrs: 0.1 Max Peaks: 200
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 0

Method : C:\MSDCHEM\1\METHODS\BAYAWA\AB_CRUDE OIL_ALI_SIM2.M
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	13.821	573	597	635	rBV	227925	1136158	3.90%	3.211%
2	15.498	831	865	905	rBV	58117	214592	0.74%	0.606%
3	16.549	1011	1033	1073	rBV	40501	195147	0.67%	0.552%
4	17.625	1193	1205	1229	rBV	71558	159781	0.55%	0.452%
5	21.778	1861	1869	1883	rBV	65779	175155	0.60%	0.495%
6	22.610	1995	2002	2019	rVV	385537	703676	2.41%	1.989%
7	23.048	2061	2072	2121	rVB	483680	1586375	5.44%	4.483%
8	24.924	2367	2372	2381	rVV	262427	450008	1.54%	1.272%
9	26.557	2623	2633	2649	rBV	98393	248368	0.85%	0.702%
10	27.026	2703	2708	2713	rBV	123431	215213	0.74%	0.608%
11	27.883	2839	2845	2859	rBV2	99522	200305	0.69%	0.566%
12	28.359	2911	2921	2935	rBV8	41346	177283	0.61%	0.501%
13	28.934	2997	3013	3027	rVV2	65158	195266	0.67%	0.552%
14	29.791	3143	3150	3165	rBV2	59966	161245	0.55%	0.456%
15	32.293	3531	3550	3599	rBV	7245854	29163508	100.00%	82.420%
16	43.534	5327	5347	5371	rVB3	50324	402092	1.38%	1.136%

Sum of corrected areas: 35384172

Library Search Report FPIE

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_FPIE.D
Acq On : 8 Sep 2014 11:55
Operator : Aminu
Sample : TS_FPIE
Misc :
ALS Vial : 4 Sample Multiplier: 1

Search Libraries: C:\Database\NIST02.L Minimum Quality: 0

Unknown Spectrum: Apex
Integration Events: RTE Integrator - rteint.p

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	17.62	4.60	C:\Database\NIST02.L			
			Benzeneacetic acid	15708	000103-82-2	86
			Benzeneacetic acid	15709	000103-82-2	78
			Propanedioic acid, phenyl-	43208	002613-89-0	72
2	17.88	1.70	C:\Database\NIST02.L			
			2-Coumaranone	14725	000553-86-6	91
			2,4,6-Cycloheptatrien-1-one	4948	000539-80-0	87
			2,4,6-Cycloheptatrien-1-one	4949	000539-80-0	80
3	19.06	1.12	C:\Database\NIST02.L			
			Phthalic anhydride	22147	000085-44-9	78
			Phthalic anhydride	22146	000085-44-9	78
			Bicyclo[4.2.0]octa-1,3,5-triene-7, 8-dione	14004	006383-11-5	64
4	20.38	1.13	C:\Database\NIST02.L			
			Benzeneethanol, 4-hydroxy-	16711	000501-94-0	52
			Pyridine, 3,4-dimethyl-	5058	000583-58-4	49
			Pyridine, 3,5-dimethyl-	5057	000591-22-0	47
5	21.70	1.57	C:\Database\NIST02.L			
			Pyridine, 3-butyl-	15028	000539-32-2	83
			Pyridine, 3-butyl-	15023	000539-32-2	80
			Ketone, methyl 6-methyl-2-pyridyl	15006	006940-57-4	58
6	21.77	1.29	C:\Database\NIST02.L			
			Pyridine, 3-butyl-	15028	000539-32-2	76
			Pyridine, 3-butyl-	15023	000539-32-2	62
			Ketone, methyl 6-methyl-2-pyridyl	15006	006940-57-4	49
7	23.04	45.80	C:\Database\NIST02.L			
			2-Pyridinecarboxylic acid, 5-butyl	41832	000536-69-6	92
			1-Adamantanecarboxylic acid, 2-met hylphenyl ester	100765	1000293-75-3	59
			1-Adamantaneacetic acid	51764	004942-47-6	59
8	24.54	1.35	C:\Database\NIST02.L			
			2-[4-(Methoxymethoxymethyl)cyclohe	65441	1000192-12-2	37

x-3-enyl]propan-2-ol
Acetamide, 2-(4-tolyloxy)-N-(2-pyr 92060 332399-37-8 27
idylmethyl)-
9-Octadecenamide, (Z)- 106874 000301-02-0 27

9 25.02 2.53 C:\Database\NIST02.L
Benzenesulfonamide, N-butyl- 64847 003622-84-2 81
Benzenesulfonamide, N-butyl- 64848 003622-84-2 74
Diphenyl ether 36129 000101-84-8 53

10 26.56 2.59 C:\Database\NIST02.L
n-Hexadecanoic acid 92226 000057-10-3 56
n-Hexadecanoic acid 92227 000057-10-3 40
Dodecahydropyrido[1,2-b]isoquinoli 60900 108873-36-5 16
n-6-one

11 27.07 1.41 C:\Database\NIST02.L
Benzonitrile, m-phenethyl- 60948 034176-91-5 22
Benzene, (5-bromopentyl)- 73355 014469-83-1 12
1-Propanol, 2-benzyloxy- 32945 070448-03-2 12

12 27.92 1.02 C:\Database\NIST02.L
N-Methyl-1-adamantaneacetamide 60896 031897-93-5 53
Silane, 1,4-phenylenebis[trimethyl 70586 013183-70-5 43
1,3-Bis(trimethylsilyl)benzene 70584 002060-89-1 43

13 28.09 1.03 C:\Database\NIST02.L
1H-Indole-3-acetamide 38429 000879-37-8 42
1H-Indole-3-methanamine, N,N-dimet 38621 000087-52-5 30
hyl-
2-Phenylcyclohexanone 38651 001444-65-1 30

14 28.37 2.38 C:\Database\NIST02.L
Silane, 1,4-phenylenebis[trimethyl 70586 013183-70-5 50
1,3-Bis(trimethylsilyl)benzene 70584 002060-89-1 50
N-Methyl-1-adamantaneacetamide 60896 031897-93-5 49

15 29.82 3.81 C:\Database\NIST02.L
1,3-Bis(trimethylsilyl)benzene 70584 002060-89-1 53
Tetrasiloxane, decamethyl- 122472 000141-62-8 53
Benzene, 2-[(tert-butyldimethylsil 96840 330455-64-6 53
yl)oxy]-1-isopropyl-4-methyl-

16 30.49 23.23 C:\Database\NIST02.L
9-Octadecenamide, (Z)- 106874 000301-02-0 91
9-Octadecenamide, (Z)- 106876 000301-02-0 87
9-Octadecenamide, (Z)- 106877 000301-02-0 78

17 32.25 3.42 C:\Database\NIST02.L
Tetrasiloxane, decamethyl- 122472 000141-62-8 43
Methyltris(trimethylsiloxy)silane 122473 017928-28-8 43
Cyclotrisiloxane, hexamethyl- 71175 000541-05-9 38

Area Percent Report

Data Path : C:\MSDChem\1\DATA\SEPT.2014\Tolulope Seun\
Data File : TS_FPIE.D
Acq On : 8 Sep 2014 11:55
Operator : Aminu
Sample : TS_FPIE
Misc :
ALS Vial : 4 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 2 Min Area: 2 % of largest Peak
Start Thrs: 0.1 Max Peaks: 200
Stop Thrs : 0 Peak Location: TOP

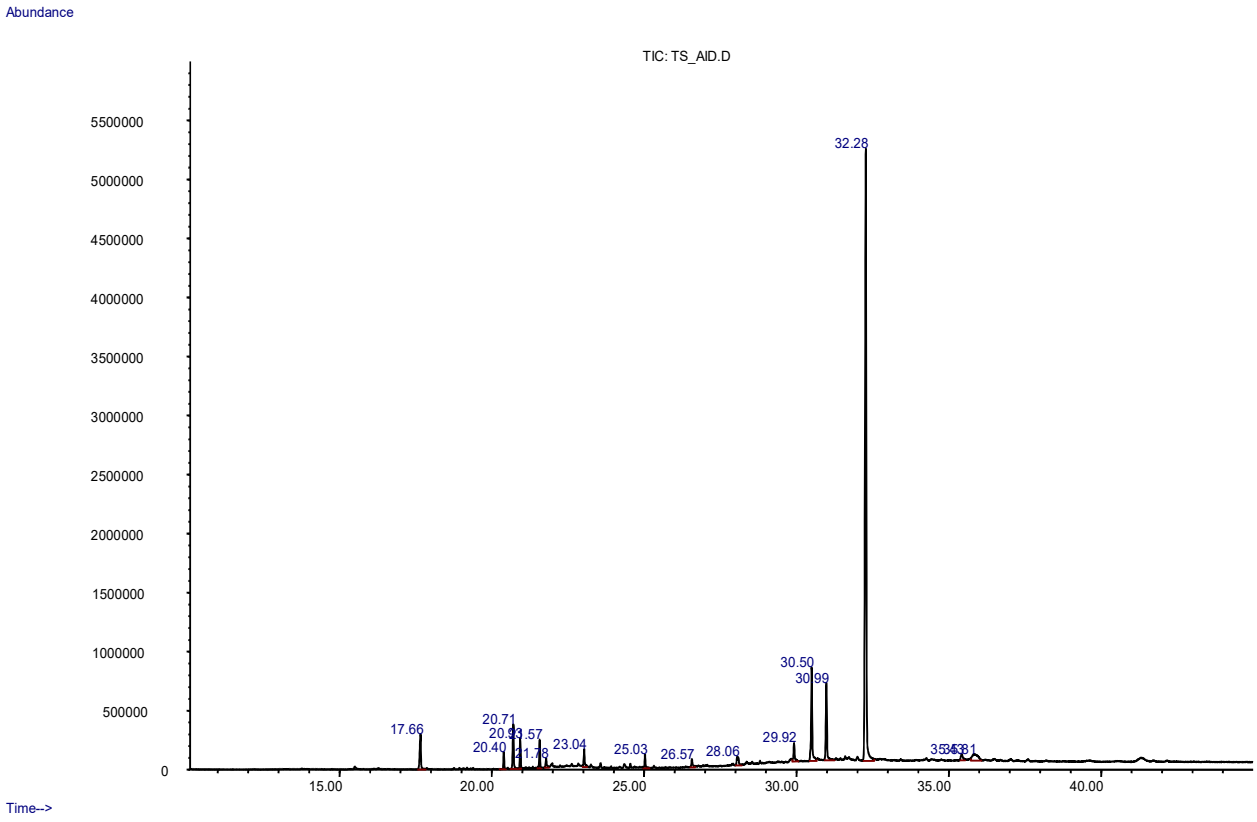
If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 0

Method : C:\MSDCHEM\1\METHODS\BAYAWA\AB_CRUDE OIL_ALI_SIM2.M
Title :

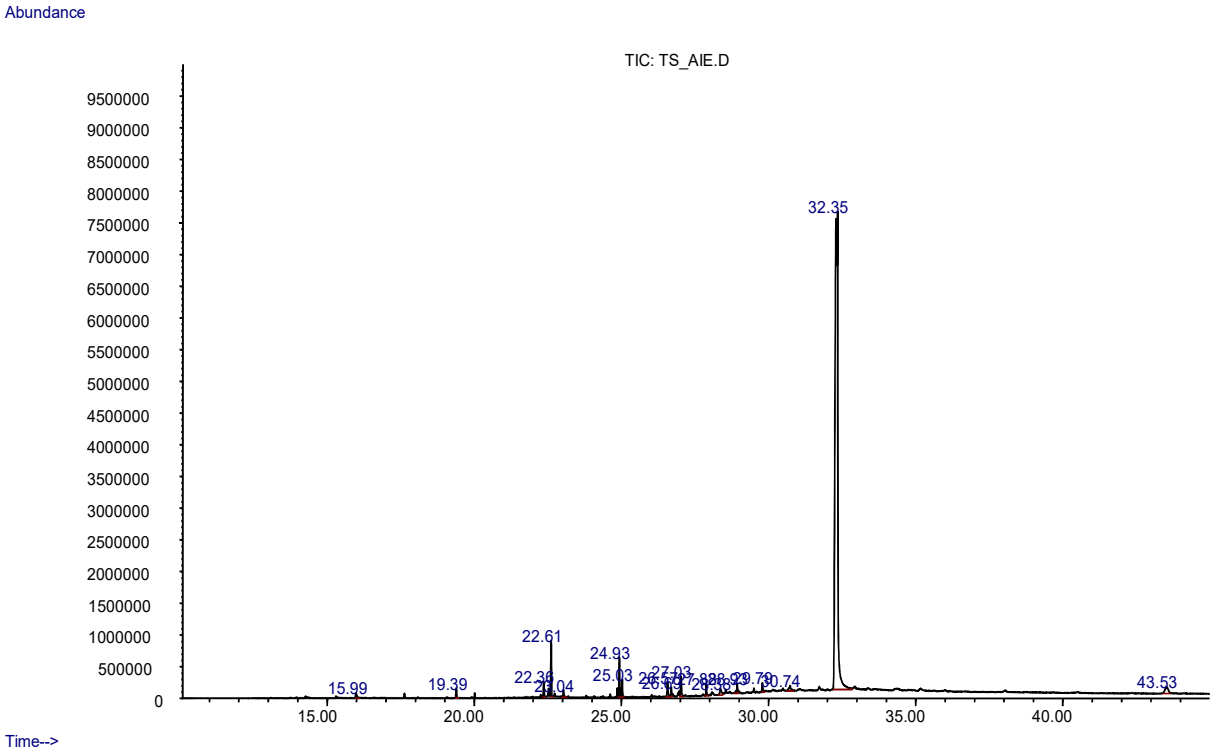
Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	17.618	1193	1204	1229	rBV	49031	117589	10.05%	4.603%
2	17.881	1235	1246	1273	rVB3	11300	43403	3.71%	1.699%
3	19.063	1419	1435	1457	rVB4	4717	28602	2.44%	1.120%
4	20.383	1625	1646	1657	rBV3	8281	28877	2.47%	1.130%
5	21.697	1835	1856	1861	rBV3	9437	40111	3.43%	1.570%
6	21.772	1861	1868	1875	rBV3	10597	33077	2.83%	1.295%
7	23.035	2059	2070	2119	rVB	402333	1170215	100.00%	45.804%
8	24.537	2305	2310	2319	rBV3	17109	34425	2.94%	1.347%
9	25.024	2379	2388	2401	rBV2	27780	64543	5.52%	2.526%
10	26.557	2623	2633	2649	rBV10	21761	66209	5.66%	2.592%
11	27.070	2709	2715	2723	rVV	17484	36106	3.09%	1.413%
12	27.921	2841	2851	2859	rBV	9310	26050	2.23%	1.020%
13	28.090	2873	2878	2893	rBV2	11140	26413	2.26%	1.034%
14	28.371	2911	2923	2937	rBV2	8857	60924	5.21%	2.385%
15	29.816	3145	3154	3169	rBV2	15341	97390	8.32%	3.812%
16	30.492	3251	3262	3281	rBV	229434	593479	50.72%	23.230%
17	32.249	3533	3543	3557	rBV2	27659	87431	7.47%	3.422%

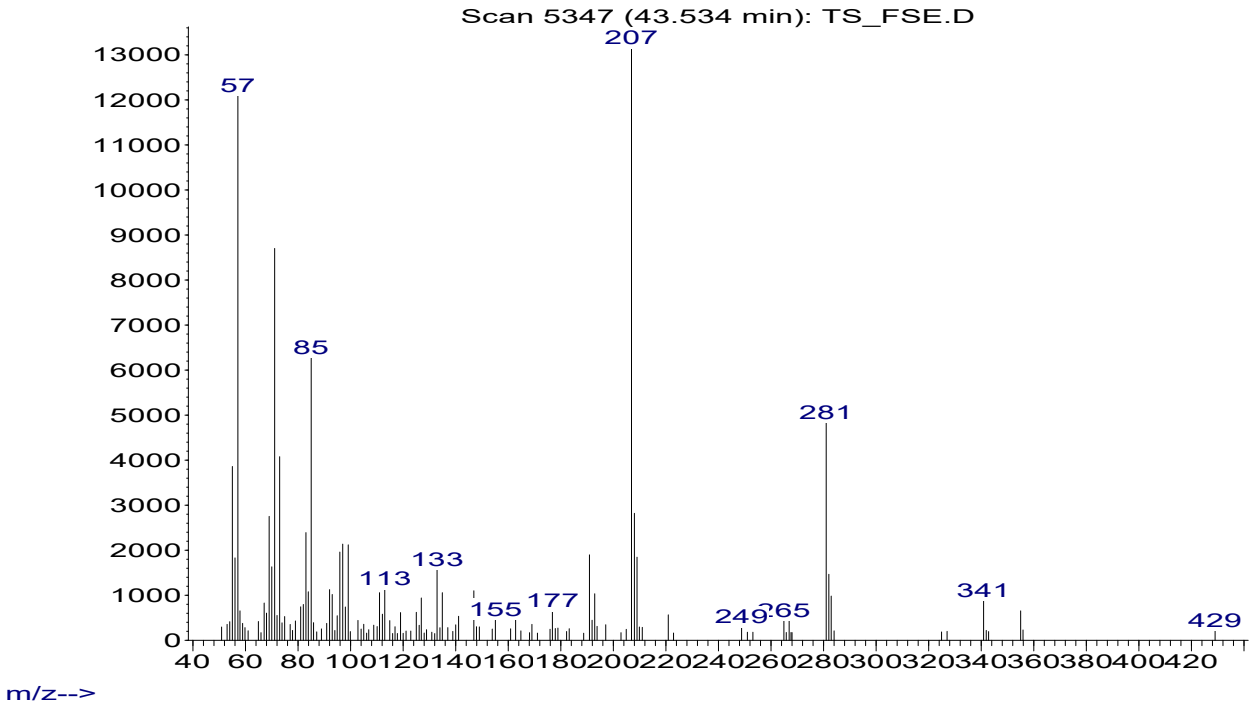
Sum of corrected areas: 2554844



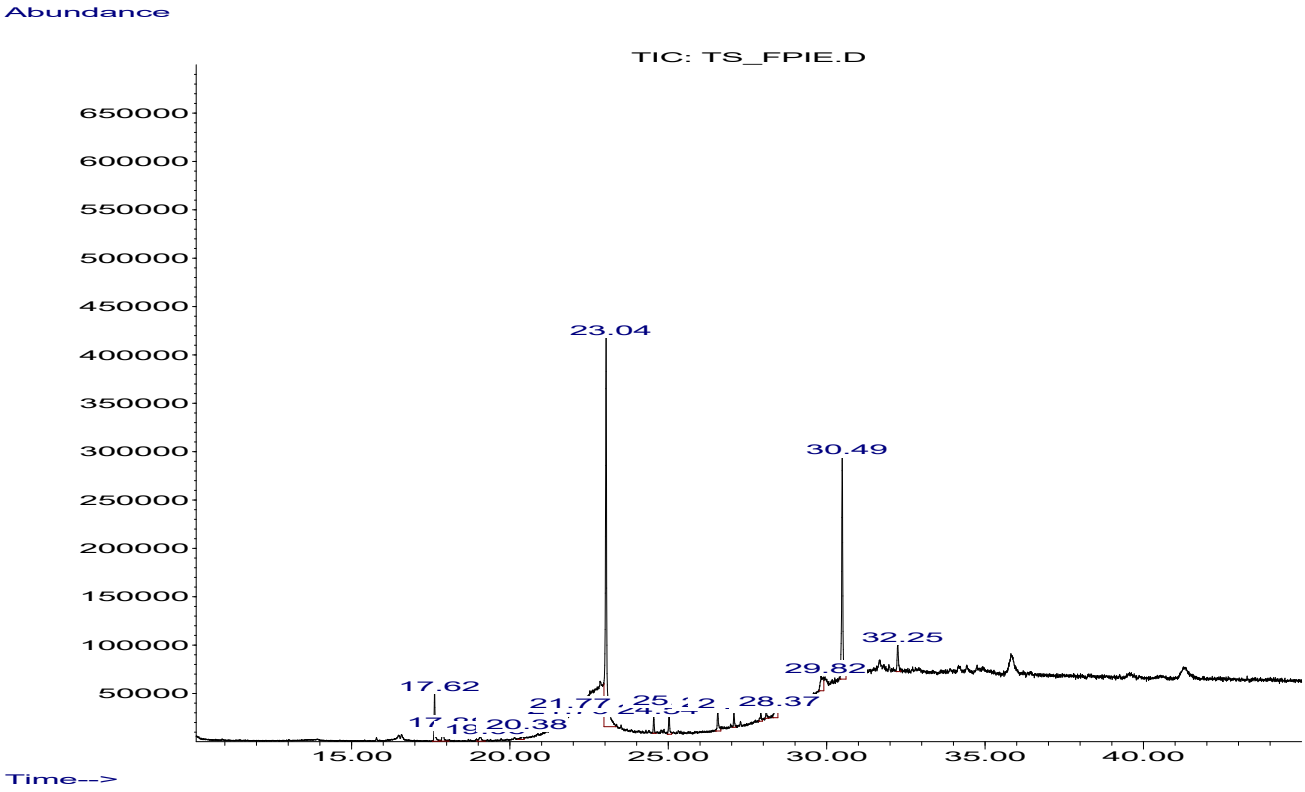
Appendix 8: GC-MS Chromatogram of *Phomopsis* sp. (diethyl ether extract)



Appendix 9: GC-MS Chromatogram of *Phomopsis* sp. (ethyl acetate extract)



Appendix 10: GC-MS Chromatogram of *Fusarium oxysporum* (ethyl acetate extract)



Appendix 11: GC-MS Chromatogram of *Fusarium oxysporum* (diethyl ether)