



Isolation and Identification of Fungi Associated with *Solanum lycopersicum* L. (Tomato) Leaves in Alapoti, Ogun State Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Authors TSE and ACO designed the study. Author ACO supervised the work. Author TSE carried out experimental analyses, managed literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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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. Therefore, this work was aimed at investigating the fungi associated with diseased 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 features. Pathogenicity test was conducted based on Koch's postulates. 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. Due to the effect of the leaf diseases of the overall productivity of tomato, it is important to put in place adequate control measures to mitigate the effect of the diseases.

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1. INTRODUCTION

Solanum lycopersicum L. (tomato) is commonly cultivated in most countries worldwide in outdoor fields, greenhouses and net houses [1]. It is the 2nd most essential vegetable on earth, belonging to the family Solanaceae [2]. The Solanaceae as well has some familiar species, for instance tobacco, potato, eggplant along with pepper [3]. Tomato is a fruit vegetable consumed extensively in Nigeria. Its production spreads all over the country [4]. Olaniyi et al. [5] 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.

In 2012, 17.938 million tonnes of tomato was produced in Africa. Egypt led the continent with 8.625 million tonnes [6]. More recently in 2016, the production increased to approximately 2.3 million tonnes and Nigeria was the 14th largest producer of tomato worldwide [7]. 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 [8]. 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 [6].

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. A lot of pests and diseases attack tomato. However, in many locations, cultivation of tomato is normally limited by diseases rather than pests [9]. 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 [10].

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 [11]. They are the mostly encountered diseases of vegetables throughout the world. They mainly affect leaf, stem, flower and fruit of annual plants, mostly vegetables and

ornamental plants [12]. 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 [13]. In a study conducted by Kumar [12] 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.

Leaf diseases of tomato which have been reported include *Septoria* leaf spot [14], anthracnose [15], early blight [16], late blight [17], *Fusarium* and *Verticillium* wilt [18,19] target leaf spot [20]. Other fungal pathogens that have been described to be associated with tomato diseases include *Pythium aphanidermatum* causing damping off [21], *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 [12]. Adequate knowledge of foliar diseases of tomato will assist farmers and plant breeders in improving its productivity. This study was undertaken to isolate and identify fungi causing leaf diseases of tomato.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Infected *S. lycopersicum* leaves (3 per plant, 30 plants per farm) were randomly collected in the morning between 8 to 10am 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° 1'53"E and Latitude 6° 37' 37.43" N. Samples were collected at the expression of disease symptoms. 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.

2.2 Preparation of Media

Potato Dextrose Agar (PDA) used for isolation of fungi was prepared according to standard procedures of Fawole and Oso [22].

2.3 Isolation of Fungi

Infected leaves were rinsed under running tap water to remove dirt, 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 $28 \pm 2^{\circ}\text{C}$. Sub-culturing of different fungal cultures on the same plates was done repeatedly until homogenous isolates were obtained.

2.4 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 slip to avoid formation of air bubbles. The slides were afterward viewed under the microscope. References were made to William and Dennis [23] and Olutiola et al. [24] 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 according to Smith et al. [25]. The final Identification report with reference YN3/14/H28 was obtained from CABI.

2.5 Pathogenicity Tests

To determine the pathogenicity of the fungi isolated from the diseased tomato leaves, Koch postulate [10] for establishing pathogenicity was followed. This was done both in the laboratory (*in-vitro*) and the screen house (*in-vivo*).

2.5.1 *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 haemocytometer. 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°C . 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.

2.5.2 *In-vivo*

To confirm the pathogenicity of isolated fungi, disease-free seeds of tomato were raised in the green house. 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. RESULTS

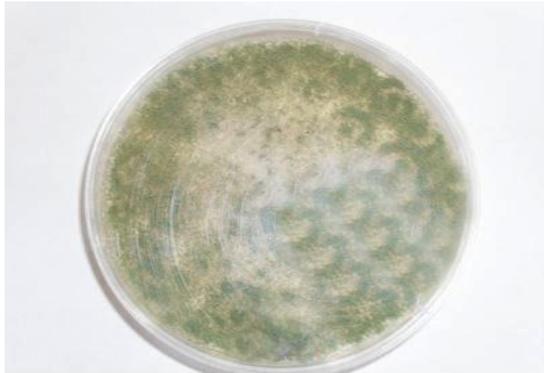
3.1 Isolated Fungi

The following fungi were isolated from diseased tomato leaves collected from the field:

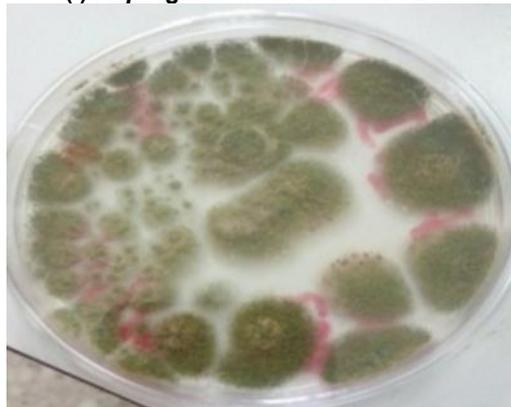
Aspergillus aculeatus, *A. niger*, *A. tamarii*, *A. ustus*, *A. versicolor*, *Epicoccum nigrum*, *Fusarium oxysporum* f. sp. *lycopersici* (Fol), *Phialophora melinii*, *Phomopsis* sp. and *Trichodema* sp. The colonial morphology on PDA and photomicrographs of isolated fungi are shown in Plates 1 (i-xx).

3.2 Pathogenicity Tests

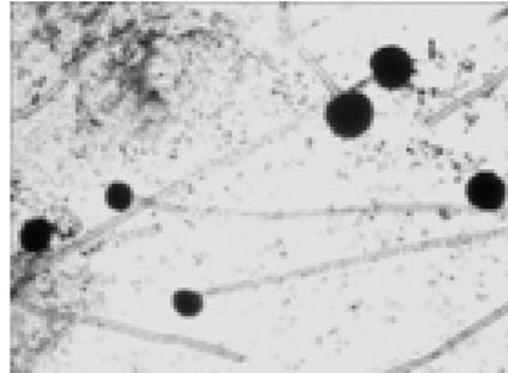
The result of the pathogenicity tests (Table 1) indicated that on inoculation of healthy tomato



(i) *Aspergillus aculeatus* on PDA



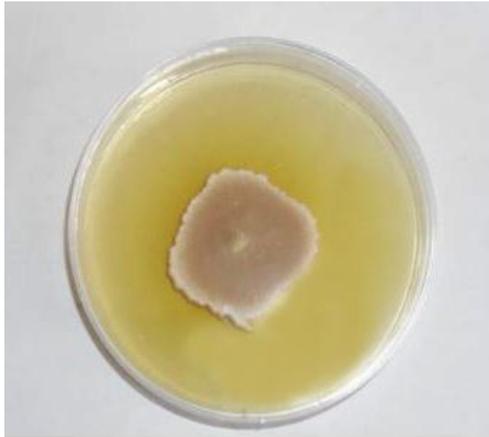
(iii) *Aspergillus niger* on PDA



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



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



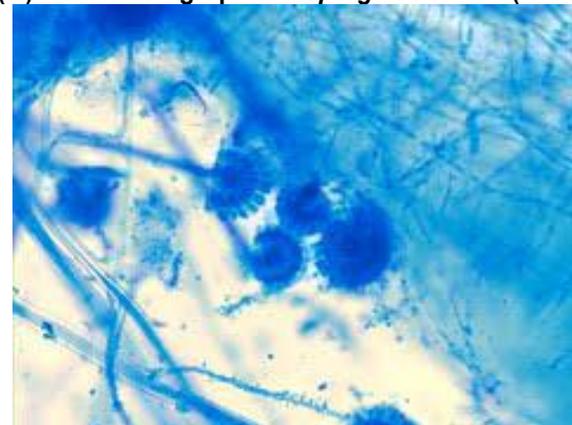
(v) *Aspergillus tamarii* on PDA



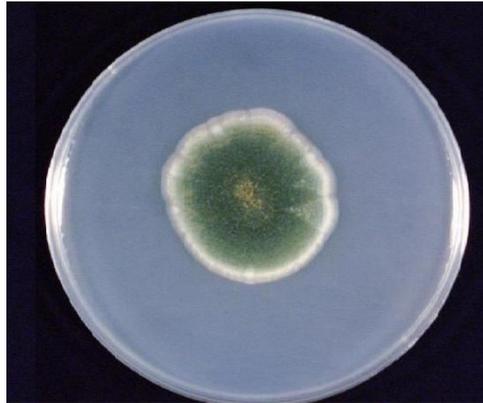
(vi) Photomicrograph of *Aspergillus tamarii* (X400)



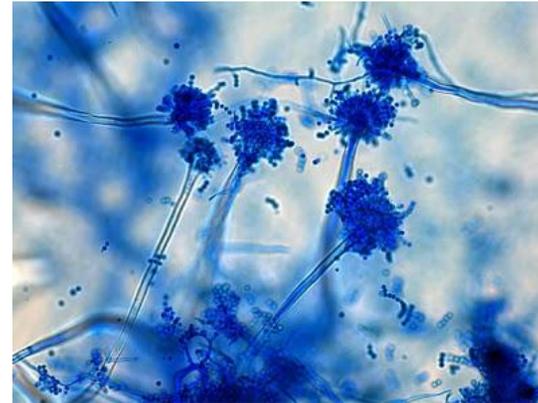
(vii) *Aspergillus ustus* on PDA



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



(ix) *Aspergillus versicolor* on PDA



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



(xi) *Epicoccum nigrum* on PDA



(xii) Conidia of *Epicoccum nigrum*



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



(xiv) Photomicrograph of *Fusarium oxysporum* f. sp. *Lycopersici* (X100)



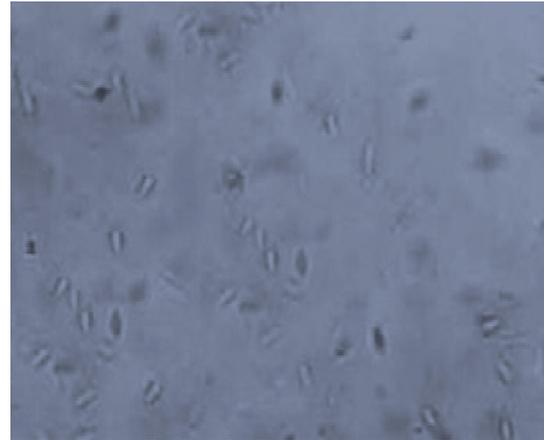
(xv) *Phialophora melinii* on PDA



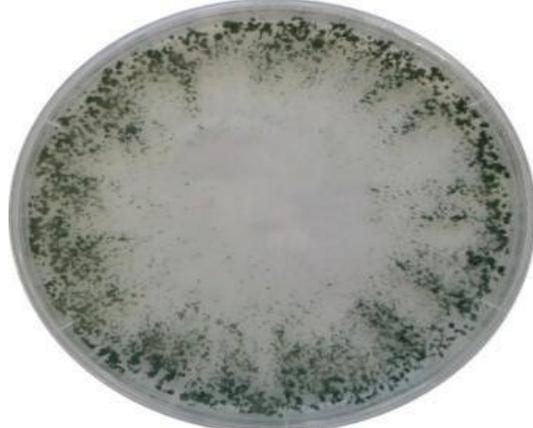
(xvi) Photomicrograph of *Phialophora melinii*



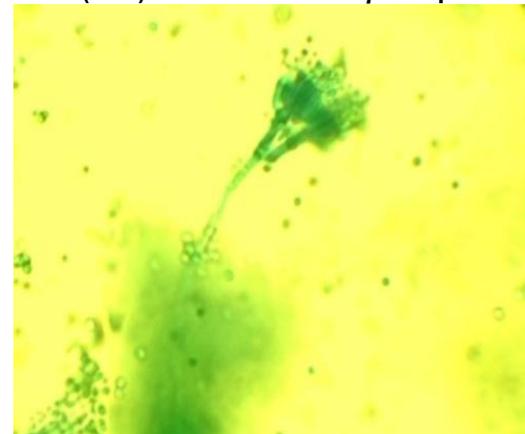
(xvii) *Phomopsis* sp. on PDA



(xviii) Conidia of *Phomopsis* sp.



(xix) *Trichoderma asperellum* on PDA



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

Plates 1 (i-xx). Colonial and microscopic morphology of isolated fungi

Table 1. Result of pathogenicity tests on tomato leaves

Fungi/Treatment	Symptoms observed		
	Leaf spot	Wilt	Chlorosis
<i>Aspergillus aculeatus</i>	-	-	-
<i>A. niger</i>	-	-	-
<i>A. tamaritii</i>	-	-	-
<i>A. ustus</i>	-	-	-
<i>A. versicolor</i>	-	-	-
<i>Epicoccum nigrum</i>	-	-	-
<i>Fusarium oxysporum</i>	+	+	+
<i>Phialophora melinii</i>	-	-	-
<i>Phomopsis</i> sp.	+	+	+
<i>Trichoderma asperellum</i>	-	-	-
Control	-	-	-

Key: + Present; - Absent

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. Symptoms observed on the healthy leaves used for the pathogenicity after five days of inoculation included leaf spots, chlorosis/yellowing and wilting.

4. 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 [12]. 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* [18]. Leaf spots of *S. lycopersicum* in this country have been linked to *Sclerotium rolfsii*, *Alternaria solani*, *Septoria lycopersici*, *Pseudomonas syringae* and *Xanthomonas vesicatoria* [26]. *Fusarium oxysporum* was reported by Wokoma [18]. She 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 she did not isolate from tomato leaves, leaf spots were encountered in her work and she stated that several other fungi could be the cause of the noticed leaf spots.

Gao et al. [27] also reported the isolation of *Fusarium proliferatum* causing leaf spots of tomato in China. Amuji et al. [2] isolated *Fusarium oxysporum* and *Rhizopus stolonifer* from diseased leaves and fruits of tomato. McGovern [19] described *Fusarium* wilt induced by *Fol* as one of the mainly studied diseases of tomato. Other pathogens described to have been associated with considerable reduction in yield and financial losses include *Botrytis cinerea*, *Alternaria solani* and *Phytophthora infestans* [27].

Symptoms of diseases observed on tomato leaves in this study included chlorosis/yellowing, leaf spots and wilting. Previous studies by Arogundade et al. [14], Wokoma [18], Amuji et al. [2] also reported those symptoms as the most common on tomato leaves.

Phomopsis sp. has earlier been reported on eggplant [28], also a member of Solanaceae but not on tomato. *Phomopsis convolvulus* was reported [29] 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* [30]. 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* [31]. Schwartz and Gent [28] 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 [31], to the best of our 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.

5. CONCLUSION

Symptoms of foliar diseases which were encountered in the study sites for this research included leaf spots, chlorosis and wilting. These diseases can adversely affect the yield and production of tomato. *F. oxysporum* f. sp. *lycopersici* has been previously reported to cause wilt of tomato; however, it is uncertain if *Phomopsis* sp. has earlier caused leaf diseases in tomato.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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