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ISOLATION AND IDENTIFICATION OF MICROORGANISMS ASSOCIATED WITH LATE BLIGHT DISEASE OF TOMATO (*SOLANUM LYCOPERSICUM L.*) IN NIGER STATE, NIGERIA

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ABSTRACT
Late blight disease remains one of the major constraints affecting tomato production worldwide due to its rapid spread and destructive effects on infected plants. This study was undertaken to isolate and identify microorganisms associated with late blight disease of tomato plant in Niger state. A total of thirty diseased tomato samples showing symptoms of late blight were collected from selected tomato farmlands from Bida, Lapai and Agaie Local Government Area of Niger State, Nigeria. The samples were analyzed using standard microbiological and biochemical techniques. Fungal isolates were identified. based on their cultural and microscopic characteristics, while bacterial isolates were identified using biochemical tests. The fungi isolated included *Phytophthora infestans* (40.0%), *Alternaria solani* (25.3%), *Fusarium oxysporum* (18.7%), *Aspergillus flavus* (10.7%), and *Phoma destructiva* (5.3%). The bacterial isolates recovered *were Pseudomonas syringae* (38.5%), *Xanthomonas campestris* (30.8%), *Ralstonia solanacearum* (20.5%), and *Bacillus subtilis* (10.2%). *Phytophthora infestans* and *Pseudomonas syringae* were the most predominant fungal and bacterial isolates, respectively. The findings of this study indicate that late blight-infected tomato plants harbor diverse fungal and bacterial species that may collectively contribute to disease severity and tissue deterioration. The study therefore emphasizes the importance of accurate identification of microorganisms associated with tomato diseases for effective disease monitoring and management in tomato production.

Keywords: Late blight, *Phytophthora infestans*, *Solanum lycopersicum*, Fungi, Bacteria.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops in the world after potato. According to Ramada *et al.* (2022), tomato is cultivated on approximately five million hectares globally, with an annual production of about 187 million tons. The leading tomato-producing countries include China, United States, India, Turkey, and Egypt. This vital vegetative crop can be cultivated in greenhouses, home gardens, and open fields, making it an economically important crop worldwide.

Tomato fruits are highly valued for their nutritional and health benefits. They are rich sources of vitamins A, B, C, and K, as well as essential minerals such as copper (Cu), potassium (K), manganese (Mn), magnesium (Mg), phosphorus (P), iron (Fe), and zinc (Zn) (Zirak and Ahmed, 2022). In addition, tomato and its processed products contain significant amounts of carotenoids, particularly lycopene, alongside folate, flavonoids, ascorbic acid, and potassium, all of which contribute to antioxidant activity and disease prevention (Raza *et al.,* 2022). The fruits can be consumed raw, cooked, or processed into various products due to their high palatability and nutritional value.

In spite of its economic importance, tomato production is severely constrained by several diseases caused by fungi and bacterial species. These pathogens significantly reduce growth, yield and fruit quality worldwide. Major tomato diseases include late blight, bacterial wilt, leaf spot, bacterial stem rot, Alternaria stem canker, and anthracnose. Important causative organisms associated with these diseases include *Phytophthora infestans* (oomycete), *Alternaria solani*, *Phoma destructiva*, *Septoria lycopersici*, *Fusarium oxysporum*, *Aspergillus niger*, *Pseudomonas syringae*, *Clavibacter michiganensis*, *Xanthomonas campestris*, *Bacillus subtilis*, and *Bacillus cereus* (Ajiboye and Sobowale, 2022; Anakaa *et al.,* 2025). These microorganisms possess adaptive mechanisms that enable them to thrive under cool and humid environmental conditions, thereby rapidly invading plant tissues and causing necrotic lesions, wilting, and eventual plant death within a short period.

Among these pathogens, *Phoma destructiva* is known to infect the aerial parts of tomato plants, particularly the leaves and stems, where it causes dark lesions that progressively enlarge and destroy plant tissues, eventually leading to plant death (Abdullahi *et al.,* 2023; Bawa *et al.,* 2022). Similarly, *Phytophthora infestans*, are regarded as one of the most destructive pathogens of tomato globally because of its rapid spread and devastating effects on crop productivity.

In Nigeria, tomato is considered one of the major vegetable crops; however, its productivity and postharvest quality are greatly affected by fungal and other microbial diseases, including late blight and fruit rots (Lengai *et al.,* 2022). Yield losses caused by these diseases threaten food security, reduce farmers' income, and pose public health concerns because some associated microorganisms are capable of producing toxins and may possess antibiotic resistance traits. Although several studies have reported tomato spoilage and foliar diseases in different parts of Nigeria, there remains limited location-specific information on the microorganisms associated with late blight-like symptoms and fruit decay in many tomato-producing areas, including Niger State.

Understanding the microorganisms associated with tomato late blight disease within a specific agro-ecological zone is essential for developing effective and sustainable disease management strategies. Such information will provide a scientific basis for improving integrated disease management practices and promoting the use of plant-derived biocontrol agents as environmentally friendly alternatives to synthetic chemicals. Furthermore, the climatic conditions of Niger State, particularly high

humidity and moderate temperatures, favour the growth and spread of microorganisms associated with tomato diseases. Despite this, there is inadequate information regarding the occurrence and distribution of these pathogens in the state. Therefore, this study aims to isolate and identify microorganisms associated with late blight disease of tomato (*Solanum lycopersicum* L.) in Niger State, Nigeria (Ishieze *et al.,* 2024).

MATERIALS AND METHODS

Study Area

The samples used in this study were collected from Lapai Bida and Agaie Local Government Areas of Niger State, Nigeria. Diseased tomato plant samples were collected from selected tomato farmlands between November 2025 and May 2026. Ten tomato plants exhibiting characteristic late blight symptoms were randomly selected from each location, giving a total of 30 diseased plants. From each plant, three tissue types leaves, stems, and roots were harvested, yielding a total of 90 tissue specimens for laboratory analysis. Samples were collected using sterile gloves, placed in sterile polythene bags, clearly labelled, and transported immediately to the Department of Biology, Ibrahim Badamasi Babangidda University, Lapai, for further processing.

Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared by dissolving 39 g of PDA powder in 1,000 mL of distilled water, then autoclaved at 121°C for 20 minutes and used for fungal isolation. Nutrient Agar (NA) was prepared by dissolving 28 g of NA powder in 500 mL of distilled water, autoclaved at 121°C for 15 minutes, allowed to cool to 45–50°C, and poured into sterile Petri dishes for bacterial isolation (Hamzat, and Mamman, 2025**).**

Isolation of Fungi

Diseased tomato plant tissues were thoroughly washed under running tap water to remove surface debris. Small segments (approximately 1 cm) were removed from the margins of infected regions of roots, stems, and leaves. The tissue samples were surface sterilized in 1% sodium hypochlorite solution for 2 minutes, rinsed several times with sterile distilled water, and blotted dry using sterile filter paper (Kabiru *et al,.*2024)). The sterilized tissue pieces were then inoculated onto Potato Dextrose Agar (PDA), supplemented with streptomycin (50 mg/mL) to suppress bacterial growth, while samples intended for bacterial isolation were inoculated onto Nutrient Agar (NA). The inoculated Petri dishes were incubated at 28 ± 2°C for fungal growth and at 37°C for bacterial growth for 3–7 days. Emerging distinct colonies were repeatedly sub-cultured to obtain pure cultures (Rashid, 2016).

Identification of Fungal Isolates

A drop of lactophenol cotton blue was placed on a grease-free glass slide. A small amount of fungal or oomycete mass was extracted from the culture using aseptic needle and placed on a Slide, with a drop of lactophenol cotton blue cover with a coverslip and examined under a compound microscope using X40 magnification lenses to view the phialides arrangement, conidial shape, and colony morphology septate hyphae, right angle branching by comparing to mycological atlas as described by Jamal *et al*. (2021).

Isolation of Bacteria

Using forceps, the sterilized plant pieces prepared in the previous step were hold and placed on nutrient agar (NA) plates and incubated at 37°C for 3–7 days. The plates were examined visually for colony change. The and colonies showing different morphological characteristics were streaked separately on fresh nutrient agar plates using sterile wire loop and incubated at 37°C for 3 days. This step was repeated until pure bacterial colonies were obtained (Rashid 2016, and Al-Fadhal *et al.* 2019). These pure isolated are used in this study.

Identification of Bacterial Isolates

Bacterial isolates were identified through a combination of macroscopic examination, Gram staining, and biochemical tests. All tests were performed and interpreted following standard microbiological methods described by Cheesbrough (2006) and Cowan & Steel (1993).

DATA ANALYSIS

Data generated from the isolation and identification of fungal and bacterial pathogens associated with tomato late blight disease were analysed using descriptive statistical. The occurrence and distribution of the identified microorganisms were presented as frequencies and percentages to determine the predominance of each isolate within the sampled tomato tissues. This approach facilitated the interpretation and comparison of the different microbial isolates associated with the disease condition in the study area.

Fungal Species from Late Blight-Infected Tomato Plants

The result from late blight-infected tomato plants, (Table 1) comprises of *Phytophthora infestans, Alternaria solani*, *Fusarium oxysporum*, *Aspergillus flavus*, and *Phoma destructiva*, with occurrence frequencies of 40.0%, 25.3%, 18.7%, 10.7%, and 5.3%, respectively.

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Table 1: Cultural, Microscopic Characteristics, Fungal Species from Late Blight-Infected Tomato Plants

Fungal Species	Cultural Characteristics	Microscopic Features	Source of tissue	Frequency (%)
***Phytophthora infestans* (Oomycetes)**	White cottony colonies, aerial mycelium, water-soaked appearance	Non-septate hyphae, biflagellate zoospores, lemon-shaped sporangia	Leaves and stem	40.0
Alternaria solani	Greenish-grey colonies with dark brown reverse pigmentation	Septate muriform conidia with transverse and longitudinal septa	Leaves and stem	25.3
Fusarium oxysporum	White to salmon-pink, cottony colonies	Sickle-shaped macroconidia, microconidia, chlamydospores	Root and stem base	18.7
Aspergillus flavus	Green powdery spores with visible sporulation	Septate hyphae, conidiophores bearing vesicles and phialides	Root	10.7
Phoma destructive	Greyish-brown colonies with visible pycnidia	Unicellular, hyaline, oval conidia; flask-shaped pycnidia	Fruit and leaves	5.3

Bacterial Species from Late Blight-Infected Tomato Plants

Four bacterial species were isolated from tomato roots, leaves, stems, and fruit portions collected from the study area on Nutrient Agar medium. Colonies displaying distinct morphological characteristics were further characterised (Table 2). Of the four isolates, one was Gram-positive and three were Gram-negative. Biochemical characterisation revealed the presence of *Bacillus subtilis*, *Xanthomonas campestris*, *Ralstonia solanacearum*, and *Pseudomonas syringae*, as presented in Table 2.

Table 2: Biochemical and Microscopic characteristics of bacterial isolated from late blight-infected tomato plants

Isolate	Gram Rxn	Catalase	TSI	Oxidase	VP	Indole	Urease	Citrate	Freq. (%)
Pseudomonas syringae	Gram (–)	+	K/K	+	–	–	–	+	38.5
Xanthomonas campestris	Gram (–)	+	K/K	+	–	–	–	–	30.8
Ralstonia solanacearum	Gram (–)	+	K/K	+	–	–	–	+	20.5
Bacillus subtilis	Gram (+)	+	A/A	+	+	–	–	+	10.2

Key: + = Positive; – = Negative; TSI = Triple Sugar Iron; K/K = Alkaline slant/alkaline butt (non-fermenter); A/A = Acid slant/acid butt (glucose/lactose fermenter); VP = Voges–Proskauer

DISCUSSION

The study undertaken reveal the presence of fungal, and bacterial species associated with diseased tomato plants showing symptoms of late blight. Among the species recovered, *Phytophthora infestans* had the highest frequency of occurrence (40.0%), confirming its strong association with late blight disease of tomato. The organism was identified based on the presence of white cottony colonies, non-septate hyphae, and lemon-shaped sporangia observed during cultural and microscopic examination. Its high occurrence in the infected samples may be associated with favourable environmental conditions such as high humidity and moisture, which support rapid growth and spread in tomato fields. The predominance of *P. infestans* observed in this study is consistent with the findings of Karadzhova and Ganeva (2024), who identified it as the principal organism associated with tomato late blight in infected field samples. Similarly, Deweer *et al.* (2023) reported that humid environmental conditions favour the rapid multiplication and dissemination of *P. infestans* in tomato crops, resulting in severe disease outbreaks.

Alternaria solani was the second most frequently species organism, accounting for 25.3% of total isolates. It was characterised by dark septate hyphae and muriform conidia possessing both transverse and longitudinal septa. Its occurrence alongside *P. infestans* indicates that more than one pathogenic organism may be involved in the deterioration of infected tomato plants, potentially contributing to increased leaf damage, tissue necrosis, and reduction in plant vigour. The occurrence of *A. solani* alongside *P. infestans* in the present study is consistent with the observations of El-Ganainy *et al.* (2021), who reported mixed infections involving both organisms in diseased tomato plants.

Fusarium oxysporum accounted for 18.7% of species recovered, mainly from root and stem tissues, and was characterised by sickle-shaped macroconidia, microconidia, and chlamydospores. Its occurrence in diseased tomato plants suggests its involvement in root and vascular tissue deterioration, which may contribute to wilting symptoms and poor plant development. The isolation of *F. oxysporum* from root and stem tissues corroborates the findings of Fatah *et al.* (2022), who associated the fungus with vascular discolouration and wilting symptoms in tomato plants.

Aspergillus flavus represented 10.7% of the fungal species. Its isolation from diseased tomato tissues suggests its possible involvement in seed decay, damping-off, and deterioration of weakened plant tissues, consistent with the findings of Kumhar *et al.* (2022). *Phoma destructiva* was the least frequently isolated fungus (5.3%), identified based on greyish-brown colonies, flask-shaped pycnidia, and hyaline unicellular conidia. Its occurrence in infected tissues supports the report of Pernezny (1998), who identified the organism as a contributor to rot and tissue deterioration in tomato plants.

Among the bacterial species recovered, *Pseudomonas syringae*

had the highest frequency of occurrence (38.5%), demonstrating a Gram-negative reaction, positive oxidase and citrate tests, and a K/K reaction on triple sugar iron agar. Its predominance is consistent with the observations of Savriddinov (2025), who described *P. syringae* as one of the major bacterial species associated with tomato leaf and fruit lesions. *Xanthomonas campestris* accounted for 30.8% of bacterial isolates; its presence in infected samples is consistent with the findings of Ishieze *et al.* (2024), who identified the organism as an important cause of bacterial spot disease in tomato.

Ralstonia solanacearum represented 20.5% of the bacterial species; its occurrence in infected tomato plants suggests involvement in wilting and vascular discolouration, corresponding with the work of Maji and Chakrabartty (2014). *Bacillus subtilis* was the least occurring bacterial isolate (10.2%), identified as a Gram-positive bacterium with positive Voges–Proskauer and citrate reactions. Its presence in diseased tissues is consistent with the findings of Sundin *et al.* (2016), who described the frequent occurrence of *Bacillus* species in association with weakened or stressed plant tissues.

Collectively, the findings of this study confirm that late blight-infected tomato plants harbour a diverse assemblage of oomycete, fungal, and bacterial organisms that may act synergistically to exacerbate disease severity, promote tissue deterioration, and reduce tomato productivity. The study thereby underscores the importance of microorganisms for effective development of targeted disease management and interventions in tomato production systems.

Conclusion

The findings of this study revealed that *Phytophthora infestans* was the most frequently isolated organism associated with late blight-infected tomato plants, followed by the true fungi *Alternaria solani*, *Fusarium oxysporum*, *Aspergillus flavus*, and *Phoma destructiva*. The bacterial isolates recovered included *Pseudomonas syringae*, *Xanthomonas campestris*, *Ralstonia solanacearum*, and *Bacillus subtilis*. The occurrence of these organisms in diseased tomato tissues confirms that tomato late blight disease may involve complex, multi-pathogen associations across distinct biological kingdoms that collectively increase tissue deterioration and reduce crop productivity.

These findings highlight the necessity of importance of microbiological identification, including the taxonomic distinction of isolates as a prerequisite for selecting appropriate and effective disease management strategies. Future studies should incorporate molecular identification techniques (e.g ITS and 16S rRNA gene sequencing) to confirm species identity and explore the epidemiological dynamics and management of the recovered pathogens under Niger State agro-ecological conditions.

Conflict of Interest

The authors declare no conflict of interest.

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