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Research Article

Fungal Pathogens Associated with Postharvest Rotting of Tomato (Lycopersicon esculentum Mill.) in Eastern Ethiopia

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Abstract

Tomato is a very perishable and highly susceptible to postharvest fungal diseases that cause rotting of the fruits in many parts of the world including Ethiopia. Investigations were conducted to isolate and identify fungal pathogens causing post-harvest rotting of tomato fruits. Samples were collected from stores and markets in Dire Dawa, Harar, Haramaya, and Bate towns and fungal pathogens were isolated and identified. Fourteen different fungal genera were found associated with the evaluated tomato fruits. These were Fusarium spp., Alternaria spp., Saccharomyces spp., A. niger, Geotrichum spp., Penicillium spp., Mucor spp., Phoma spp., Trichoderma spp., Cladosporium spp., Helminthosporium spp., Colletotrichum spp., Rizhoctonia spp., and Diplodia spp. Fusarium spp. had the highest (46.2%) frequency of occurrence, while Trichoderma spp. were the least (0.22%) encountered. Pathogenicity tests revealed that all of the isolated fungi except Trichoderma spp. were pathogenic. Penicillium was the most aggressive genus which produced lesion diameter of 42 mm in five days. A. niger was the least (9.5 mm) aggressive species. Fusarium was identified to species level and F. oxysporium, F. chlamydosporium, F. avenaceum, F. solani, F. acuminatum, and F. sporotrichiodes were recovered. F. oxysporium was the most frequently (54.21%) recorded one, while the lowest (0.93%) was F. acuminatum. Also the highest (28.25 mm) lesion diameter was produced by F. oxysporium, while the least (20 mm) was by F. avenaceum. In conclusion, the study revealed high occurrence and distribution of diverse fungi associated with post-harvest rotting of tomato fruits in eastern Ethiopia. However, further studies are important for identifing the isolated fungal pathogens to species level except fusarium and to develop appropriate manegement options for fruit rotting fungi.

Keywords

Fruit Rot, Lycopersicon Esculentum, Post Harvest

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) family Solanaceae, is a very important fruit vegetable in the world [12]. Tomatoes are rich in minerals, vitamins, essential amino acids, sugars and dietary fibres. Tomato contains much vitamin B and C, iron and phosphorus [15]. Tomato is grown in a wide range of climates [4]. In Ethiopia, tomato is produced mainly as a source of income and food. At present large scale production of tomato is being carried out in the Upper Awash Valley under irrigated and rain-fed conditions [11].

Nutritional values of tomato make it a widely accepted vegetable by consumers. Nevertheless, tomato is a very perishable vegetable with a short shelf-life and high susceptibil-

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ity to fungal disease. During prolonged storage, tomato is susceptible to postharvest disease caused by various pathogenic fungi [3]. Post-harvest diseases destroy 10-30% of the total yield of crops [2]. The improper handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological state of the fruits [28].

Hararghe is one of the important tomato producing areas in Ethiopia. Currently a lot of tomato supplied to local and export markets. However, in storage and during transportation to market, a lot of tomato is discarded due to damage by fungi. In Hararghie area fungal pathogens that cause post harvest disease of tomato are not yet identified. Thus, the objectives of this study were to isolate and identify fungal pathogens causing post-harvest rotting of tomato fruits.

2. Materials and Methods

2.1. Isolation and Identification of Fungi

Samples of tomato fruits were collected in different parts of eastern Ethiopia which were Bate, Haramaya, Harar, and Dire Dawa. Fruits having disease symptom were surface sterilized by dipping them in 10% sodium hypochlorite for 1 min and then rinsed in sterile water. Small segments (1- 2 cm) of tissues from the healthy margins of the rotting areas were cut out with a sterile scalpel and placed on potato dextrose agar (PDA) in petridishes, one at the center and four at the margin with a total of five in one petridish. This was done in laminar air flow cabinate to reduce contamination. The plates were incubated at 27 ℃ in an incubator as suggested by [13]. Developing fungi were then sub-cultured on fresh media and identified based on macroscopic and microscopic examinations according to [6]. Some fungi were identified based on spore shape, size, color and ornamentation. However, as many fungi have similar appearing spores, it was not always possible to identify each fungus based on spore characteristics. For accurate identification of such fungi the spores produced in fruiting bodies were observed. Some fungi did not sporulate easily and to induce sporulation they were kept in alternative 12 hour light and 12 hour dark, and then exposed to UV light. *Fusarium* was identified to species level based on morphological characteristics like the presence or absence of microconidia, the shape of microconidia, the presence or absence of chlamydospores, the shape of the macroconidia, the shape of basal or foot cells on the macroconidia, the number of septation in the macroconidia, and the length and width of macroconidia [16, 6].

2.2. Pathogenicity Test

Fresh and healthy looking tomato fruits of the variety Roma VF were collected from green house grown tomato plants and surface sterilized by dipping them in 10% sodium hypochlorite for 1 min and then rinsing them with four successive changes of sterile water. Working area were cleaned by 90% alcohol and flamed before the work started. Fruits were then wounded with a sterile needle. Mycelial discs were lifted from pure cultures of the different isolates and introduced directly into the wounded tissues. The inoculation was done in a laminar air flow cabinet. Four tomato fruits were inoculated with each of the isolates. Another set of the same number of fruits was surface sterilized and wounded with a sterilized needle and inoculated with sterile water serving as the control. The inoculated fruits and the controls were placed separately in sterilized beakers lined with a moist filter paper and covered with aluminum foil and incubated at 27 ℃. After 72 hours, the fruits were observed for symptom development. The causal agents were reisolated from the infected tomatoes and compared with the original isolates [13].

2.3. Aggressiveness Test

Aggressiveness was determined based on the size of lesion and number of spores produced by each isolate after five days of inoculation. Lesion diameters in mm and spore concentration per ml on each fruit inoculated were measured. To determine spore concentration, spores from the inoculated fruits were washed with sterile water and twine 20 was added to keep uniformity of spores after shaking. Spores in four corner larger squares and in the middle one of hemocytometer were counted. Finally number of spores per ml was calculated as average spore of five squares multiplied by 10^4.

3. Results and Discussion

A total of 459 fungal isolates were recovered, identified and classified into 14 genera namely *Fusarium* spp., *Alternaria* spp., *Saccharomyces* spp., *A. niger, Geotrichum* spp., *Penicillium* spp., *Mucor* spp., *Phoma* spp., *Trichoderma* spp., *Cladosporium* spp., *Helminthosporium* spp., *Colletotrichum* spp., *Rizhoctonia* spp. and *Diplodia* spp. (Table 1). The results showed that the occurrence of these fungal species was heterogeneous in that *Fusarium* spp. had the highest (46.62%) frequency of occurrence followed by *Alternaria* spp. (24.4%) and yeast (7.19%). The frequency of occurrence of the other genera ranged from 0.22% to 4.79%.

The pathogenicity test of the fungal isolates on tomato fruits showed that all identified genera except *Trichoderma* spp. were virulent and able to cause lesions of varying sizes after five days of incubation at 25 ^{o}C. This experiment was repeated and the same result was obtained. Although almost all the isolates were pathogenic, aggressiveness of each isolate varied greatly (Table 1). Of these, *Penicillium* spp. consistently produced and developed the largest (42 mm) lesion diameter followed by *Colletotrichum* spp. (37 mm), while the least aggressive isolate was *A. niger* which produced lesion diameter of 9.5 mm. The result of amount of spores per ml showed that *Diplodia* spp. produced the highest (4.82×10^6 spores per ml). The lowest (3.30×10^5) number of

spores was produced by *Phoma* spp.

Among these isolates species of *Alternaria*, *Fusarium*, *Aspergillus*, *Penicillium* and *Geotrichum* have been reported as common post-harvest fungi [1]. Some of them could produce mycotoxins in infected fruits even during refrigeration [27]. Some are reported to be pathogenic to humans causing infections or allergies in susceptible individuals [9]. In this study, besides common post-harvest fungi, yeast or *Saccharomyces* spp., *Mucur* spp., *Phoma* spp. *Cladosporum* spp., *Helminthosporium* spp., *Colletotrichum* spp., *Rizhoctonia* spp., and *Diplodia* spp. were found to be associated with post-harvest rotting of tomato. *Fusarium* spp. was the most frequently isolated from tomato fruits. In earlier investigations *Fusarium* spp. were isolated from decaying tomato fruits [5]. *Fusarium* spp. are one of the most important plant pathogenic fungi infecting economically important crops [18]. Some species produce mycotoxin which affects human and animal health if the mycotoxin enters the food chain [17]. *Alternaria* spp. were the second most isolated species. It was also isolated by [8, 5, 19, 24] from diseased tomato fruits. It occurs wherever tomatoes are grown. Yeast or *Saccharomyces* spp. were reported by [8]. *Colletotrichum* spp. causes a disease called anthracnose. Most infection takes place on ripe or overripe fruit which was isolated by [24] from diseased tomato fruits. Besides causing postharvest disease on tomato *A. niger* was known to produce Aflatoxins on several plants produce [7] which are a group of highly toxic, mutagenic and carcinogenic polyketide compounds. *Rhizopus* spp. isolated from diseased tomato by [8, 5] which causes Soft Rot on Cherry Tomato [10]. The soft rot on the succulent tissues of vegetables, fruits and ornamentals caused by *Rhizopus* spp. occurs throughout the world [10]. *Geotrichum* spp. are a common soil-borne fungus that causes sour-rot of tomatoes, citrus fruits and vegetables, and is a major contaminant on tomato processing equipment [25]. *Cladosporium* spp. frequently isolated from many fruits and vegetables like okra, turnip, melon, pumpkin, carrot, bitter gourd, round gourd and radish [5]. It was also isolated from tomato fruits by [24]. Various species of *Penicillium* cause the blue mold and the green mold, also known as *penicillium* rots. They are the most common and the most destructive post-harvest diseases, occurring on most fruits and vegetables during storage or transport. The ubiquitous causal fungus *Penicillium* mostly enters tissues through wounds [2]. *Mucor* spp. has been reported to cause decay of numerous fruits and vegetables but usually have been considered of minor importance as storage pathogens [14]. *Helminthosporius* spp. were isolated by [13] and *Phoma* spp. by [24] from tomato fruits.

Table 1. *Type and Frequency of Identified Fungal Species, Size of Lesion and Number of Spores/ml They Produced on Fruits.*

Types of fungal genera identified	Frequency (%)	Lesion diameter (mm)	Spore count/ml
Alternaria spp.	24.4	10.00	4.06×10^{6}
Aspergillus niger	4.36	9.50	4.30×10^{5}
Cladosporium spp.	1.74	28.00	4.18×10^{6}
Colletotrichum spp.	4.79	37.00	3.58×10^{6}
Diplodia spp.	0.87	14.28	4.82×10^{6}
Fusarium spp.	46.62	25.37	2.99×10^{6}
Geotrichum spp.	1.96	16.00	1.36×10^{6}
Helminthosporium spp.	0.87	18.00	4.74×10^{5}
Mucor spp.	1.09	22.57	8.93×10^{5}
Penicillium spp.	1.53	42.00	1.12×10^{6}
Phoma spp.	0.44	30.67	3.30×10^{5}
Rizhoctonia spp.	3.92	34.00	2.34×10^{6}
Trichoderma spp.	0.22	-------	---------
Yeast	7.19	26.00	2.49×10^{6}
Total	100		

As shown above out of the total 459 fungal isolates, 214 were identified as *Fusarium* spp. These were further identified to species level based on morphological characteristics. Six *Fusarium* spp were identified namely, *F. oxysporum*, *F.*

chlamydosporum, *F. avenaceum*, *F. solani*, *F. acuminatum and F. sporotrichioides*. The most common species isolated was *F. oxysporum* with the highest frequency of 54.21% followed by *F. chlamydosporum* (18.69%), *F. acuminatum* was the least (0.93%) frequently encountered isolate (Table 2).

All recovered *Fusarium* isolates were pathogenic (Table 2) but their aggressiveness varied. *F. oxysporum* produced the highest (28.25 mm) lesion followed by *F. chlamydosporum* that produced lesion of 26 mm. The smallest (20 mm) lesion was produced by *F. avenaceum*. When the amount of spore produced by each species is considered, *F. solani* produced the highest spore count of 4.83×10^6 spores per ml. The lowest (7.77×10^5 spore/ml) number of spores was produced by *F. acuminatum*.

Fusarium oxysporum was the most frequently isolated *Fusarium* species from tomato fruits. [23] reported that *F. oxysporum* was isolated from the decaying area of tomato which appeared to be water soaked and slightly sunken. Besides causing decay or rot on tomato, *F. oxysporum* also caused wilt disease on tomato plants [18].

F. chlamydosporum is mainly inhabitant of soils in warmer climates, and is not regarded as a plant pathogen or spoilage fungus. *F. chlamydosporum* was also isolated by [7] from tomato fruit. However, it is commonly isolated from grains in drier area. Its involvement in dry rot of potatoes has also been reported [20].

Fusarium solani was recovered from tomato. *F. solani* has been reported to cause damage during fruit development [22]. It is also a well known plant pathogen, causing root rot, stem rot, canker, wilting, fruit and seed rot and leaf diseases on a wide variety of agriculture crops such as soybean, peppers, potatoes and peas [1, 21, 6].

F. acuminatum has been isolated from a wide variety of plants through the world. *F. acuminatum* is generally regarded as saprophyte. *F. avenaceum* has a worldwide distribution whenever crops are grown, but is relatively uncommon in food commodities. It has occasionally caused spoilage of apples, pears, asparagus, tomatoes, eggplant and potatoes. *F. sporotrichiodes* is important because of its toxicity. It occurs more commonly in cool climates and, in foods, is almost entirely confined to grains. All this six *Fusarium* species produce different types of mycotoxins [20].

Contamination of *Fusarium* on tomato could be due to the soft and thin skin of tomato fruits that easily ruptured. Therefore, the vegetable fruits are more prone to injury and breakage of the epidermis, where *Fusarium* could enter the tissues [26]. Contamination of fungal spoilage on fruits and raw vegetables could occur in the field, postharvest storage, handling, during transportation and at consumer's hand [26]. Therefore, *Fusarium* contamination on vegetable fruits could occur due to the same reasons reported by [26].

The present study provides information on the presence of *Fusarium* species on tomato fruits. The prevalence of *Fusarium* species on tomato fruits should be of great concern because of their toxigenic potential as the six *Fusarium* species have been reported to produce different types of mycotoxins. High level of mycotoxin could affect human and animal health.

Table 2. *Frequency of Occurrence of Fusarium spp.*

Fusarium spp.	No. of isolates	Frequency (%)	Lesion size (mm)	Amount of spore/ml
F. acuminatum	2	0.93	25.40	7.77×10^5
F. avenaceum	29	13.55	20.00	1.34×106
F. chlamydosporum	40	18.69	26.00	4.76×106
F. oxysporum	116	54.21	28.25	3.85×106
F. solani	17	7.94	24.00	4.83×106
F. sporotrichioides	10	4.67	25.75	3.01×106
Total	214	100.00		

4. Conclusion

This study revealed that different fungi, especially *Fusarium* spp. are associated with post-harvest rotting of tomato. However, further research is needed to identify more tomato fruit rotting diseases in different partes of the country. In addition, further research is needed to identify all the recovered fungal pathogens except to species level.

Abrivations

LSD	Least Segnificant Defference
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
USDA	United States Department of Agriculture

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Author Contributions

Abdirshikur Reshid Jemal is the sole author. The author read and approved the final manuscript.

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Conflicts of Interest

The author declares no conflicts of interest.

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