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Isolation and Identification of the Fungi Causing Tomato Fruit Rot Disease in the Vicinity of Tandojam, Sindh

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ABSTRACT

Background: The post-harvest tomato fruit rot disease is common threat to the tomato fruit, causing huge economic loss as revealed by (GOP, 2018). The present study was conducted for isolation and identification of causative agent of tomato fruit rot in order to formulate the proper management stretegies.

Methods: Study was conducted in three phases. Phase one included collection of tomato fruit samples from vicinity of Tandojam. In phase two pathogens were isolated from the samples at laboratory, while in the phase three pathogens were identified using standard procedures.

Result: The experimental results indicated *Alternaria solani* as the main cause of post-harvest tomato fruit rot. The symptoms observed were presence of brown to black rot lesions on tomato fruits with distinct rings ranging from small pin-heads to whole surface of fruit. A total of six different fungi *viz., Alternaria alternata*, *Aspergillus niger*, *Alternaria solani*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* were found to be associated with post harvest tomato rot. Significantly higher infection was recorded for *A. solani* (53.667%) followed by *A. niger* (16.333%) and *G. candidum* (13.00%). The lowest infection percentage was observed for *F. oxysporum* (2.333%), followed by *A. alternata* (4.00%) and *R. stolonifer* (9.00%). *A. solani* produced aerial mycelium with yellowish to reddish diffusible pigments. *A. niger* cultures were typically black and colonies were initially whitish to yellow and later became brown to black in colour. *G. candidum* produced white and nonaerial colonies. *F. oxysporum* produced circular, aerial mycelium initially white, later changed to light pink. *R. stolonifer* produced whitish to grey fuzzy colonies.

Key words: Fruit rot, Fungi, Indentification, Tomato.

INTRODUCTION

The vegetable tomato has originated from South America and now it is widely grown vegetable throughout the world. Botanically, it is called as *Lycopersicon esculentum* L., belonging to the genus *Lycopersicon* and family Solanaceae (Wani, 2011). Since the ancient time, it has been consumed by peoples as a fruit in dishes as per evidence by the Aztecs of South America. It was first introduced to Europe by the Spanish conquistador Hernán Cortés and thereafter it became a popularly cultivated crop across Europe as well as other parts of the world by European explorers and colonists (Wee, 2017). Tomato is considered as second most important vegetable crop in the world after potato. Tomato is highly perishable crop. It has been stated that as high as 50% of the crop is lost between rural production and consumption channels in the tropical areas. One of the main reasons for production loss is post-harvest external damages that occurres during harvesting and handling (Mbuk *et al*., 2011).

The post harvest losses in fresh tomato fruit has been estimated around 25.80%. The magnitudes of post-harvest losses always vary with region, season and time (Mujib *et al*., 2007). It is also important to mention here that a tomato rot is also a major contributing factor to the post harvest loss worldwide including Pakistan (Thirupathi *et al*., 2006). Various micro-organisms are responsible for the post-harvest decay of tomatoes, of which fungi and bacteria are the most destructive (Obetta *et al*., 2011). It has been well established that the major post-harvest losses of fruits and vegetables are caused by species of pathogens such as *Alternaria, Botrytis, Diplodia, Monilinia, Penicillium, Phenopsis, Rhizopus Sclerotinia, Fusarium, Geotrichum, Heminthosporium, Curvularia* and of bacteria *Erwinia* and

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Pseudomonas (Sajad, 2017). Chohan *et al*. (2016) reported that tomato is vulnerable to attack by different fungal pathogens which are soilborne, airborne and seedborne and inflict heavy losses in production. In tomato fruit, there are several pathogens that are responsible for post-harvest decay. Among these, mycopathogens are the most destructive and one of the major causes of post-harvest deterioration in the humid regions (Nasir *et al*., 2015). Tomato fruit rots are mainly caused by several fungal species. *Alternaria* rot has been reported as the most common and severe fungal disease world-wide. The *Alternaria* rot was first reported from the California by Nowicki *et al*., (2012). The dead leaves of potato were used to isolate causal organism and later it was isolated from the decaying fruits of tomato. The alternaria spot size ranges from minute pin-heads to areas extending completely across the surface of the fruit giving it a flattened appearance (Pandey *et al*., 2017). World wide, several reports have mentioned that *Alternaria* is the main decay causing fungus of post harvest tomato fruits, however in Pakistan it still needs authentic confirmation. Specially, in the vicinity of Tandojam, Sindh, tomato fruit rot disease is more common. Therefore, current research was planned for the isolation and identification of specific fungi causing tomato fruit rot disease in order to formulate suitable integrated management stretegies.

MATERIALS AND METHODS

Study area

The present study was conducted at the Department of Plant Protection, Sindh Agriculture University, Tandojam, Pakistan for the isolation and identification of fungi causing tomato fruit rot disease.

Sample collection

Tomato fruit samples were randomly collected from the vicinity of Tandojam based on the typical symptoms of fruit rot. Samples were placed in perforated polythene bags and brought to the laboratory of Plant Protection department for further analysis.

Isolation of pathogen (s)

The collected samples were thoroughly washed with tap water. Small pieces of infected portion (about 2-3mm in length) were cut at the junction of diseased and healthy tissues with the help of alcohol sterilized sharp blade. These pieces were surface sterilized in 0.1 per cent mercuric chloride solution ($HgCl_2$) for 30 seconds followed by three washing with sterilized distilled water in beakers under aseptic conditions using laminar air flow. The pieces were then completely dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the Petri plates containing sterile potato dextrose agar (PDA) medium amended with an antibacterial agent and filled up to quarter strength. The inoculated plates were incubated at 25±20°C. All the plates were monitored regularly and growing colonies were subjected to different laboratory codes for frequency percentage and further analysis. The frequency of the fungi in the collected specimens was recorded using the following formula given below (Kirk *et al*., 2008):

$$\text{Frequency (\%)} = \frac{\text{Number of pieces colonized}}{\text{Total number of pieces studied}} \times 100$$

The culture, thus obtained was subjected to purification. A single spore culture technique was used to purify the isolates. Sub-culturing of isolates were made time to time to maintain the fresh culture for further analysis until the end of experiments.

Identification of pathogens

Temporary slides of fungal isolates from pure cultures were made and observed under light microscope at 100X. Morphological and cultural characters of isolated fungi were recorded and compared with standard keys for establishing their identity (Barnett and Hunter, 1972; Nelson *et al.,* 1983). In addition, already published reports on indentification features of *Alternaria* were also used to compare the morphological characteristics of isolates.

Statistical analysis

The data obtained was statistically analyzed by using the computer software Statistix 8.1 (Gomez and Gomez, 1984). Standard procedures were applied for calculating the analysis of variance, ANOVA (linear model). However, some data were analyzed using MS Excel Programme of MS Office 2007.

RESULTS AND DISCUSSION

Identification of fungi causing tomato fruit rot disease

A total of six different fungi *viz*., *Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* were found to be associated with tomato post harvest rot. All these fungi were isolated and then identified based on the morphological characterisitcs.

Symptoms produced by fungi on tomato fruit

Alternaria solani Sorauer was found as the main decay causing fungus of post harvest tomato fruits. The symptoms observed in current study showed brown to black rot lesions on tomato fruits, depressed and usually with distinct rings. The spot size ranged from minute pin-heads to areas extending completely across the surface of the fruit giving it a flattened appearance. The attacked fruit parts turned black and wrinkled, ultimately shrivelled and dried up (Fig 1). Fusarium rot caused by *Fusarium oxysporum* developed fluffy whitish mycelial growth along with pinkish to purple shades. While in case *Geotrichum* rot, sunken to water-soaked spots were found on tomato fruit during the observation of study (Fig 1).

Morphological characteristics

Morphologial features of different species casuing tomato fruit rot in tomatoes were observed under the microscope

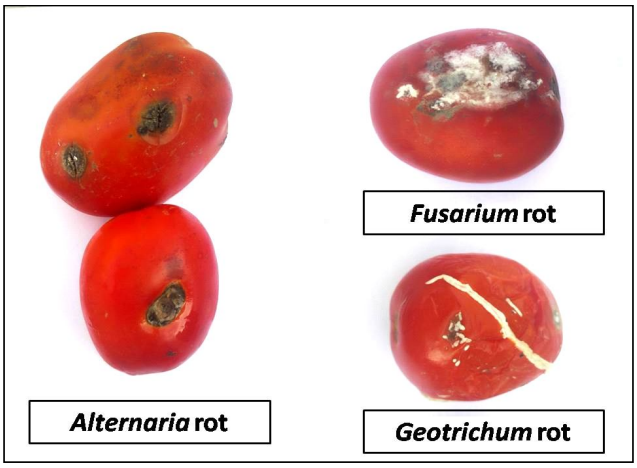


Fig 1: Symptoms of tomato post harvest rot casued by *Alternaria solani* and other fungi.

at 100X magnification and their size were measured using microscopic scale. Results for each observation are depicted in the respective sections below.

***Alternaria solani* Sorauer**

The species *A. solani* was indentified based on the morphological characteristics. The purified culture of the *A. solani* on PDA produced aerial mycelium, yellowish to reddish diffusible pigments later changed to greyish black with black reverse. Microscopic examination revealed septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. The conidia were 12-20 X 120-296 µm and found singly or in chains of two. Conidia were 9–11 transverse septa (cross walls) and long beaks. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts (Fig 2).

***Aspergillus niger* van Tiegh**

Colonies of *A. niger* on agar cultures were typically homogeneously black. *Aspergillus* colonies were found fast growing. Colonies were initially whitish to yellow, yellow-brown, then became brown to black in colour and they mostly consisted of a dense felt of erect conidiophores. Conidiophores terminated in a vesicle covered with either a single palisade-like or layer of phialides. Conidiophores were hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles. Conidia were phialosporous, brown, black in mass, globose andminutely echinulate. Conidia were single-celled, smooth or rough walled usually hyaline or pigmented and were basocatenate, forming long dry chains which were either divergent (radiate) or aggregated in compact columns (columnar).

***Alternaria alternata* (Fr.) Keissler**

The species *A. alternate* was indentified based on the morphological characteristics. Microscopic examination (100X) revealed septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia were catenulate, mostly up to 9 in a chain, often branched. Conidia were prosperous, acropetally developed, dark brown, cylindrical or spindle-shaped, often with cylindrical beaks, muriform composed of 3-4 transverse walls and 1-2 longitudinal walls.

***Geotrichum candidum* Link**

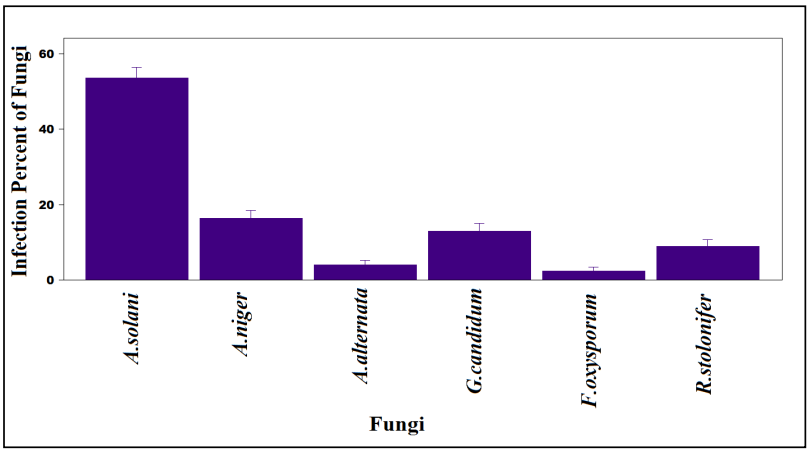
Geotrichum candidum produced white and nonaerial colonies. Conidiophores were usually absent. However, conidia was arthrosporous, aerial on the agar surface forming creeping hyphae. Cylindrical to barrel-shaped or subglobose and hyaline hyphae was developed with single cell, often guttulate. Chlamydospores were subglobose and were solitarily borne at the sterigmata on the hyphae.

***Fusarium oxysporum* (Schl.) Snyder and Hansen**

F. oxysporum produced circular, aerial mycelium initially white, changed to light pink in colour on PDA medium. Microscopic examination revealed that the hyphae were septate and hyaline, along with the presence of macro and micro conidia. Conidiophores were hyaline, simple, short or not well differentiated from hyphae, bearing spore masses at the apexes. Conidia were phialosporous, hyaline and of two kinds: acroconidia boat-shaped, with slightly tapering



Fig 2: Morphological characteristics of *Alternaria solani* isolated from tomato post harvest fruit rot (100X).



SE = 2.6909

LSD (P<0.05) = 5.2922

Fig 3: Infection per cent of different fungi associated with tomato post harvest rot.

apical cells and hooked basal cells, 4-celled; and microconidia ellipsoidal, 1-celled. Chlamydospores were brown, globose and usually solitary.

Rhizopus stolonifer

Colonies of *R. stolonifer* on agar cultures were whitish to grey fuzzy in colour. Many haploid sporagiospores were developed within the sporangia structure. *Sporangia* were bulbous structures that sprout from the vegetative hyphae and hold the haploid spores.

Infection frequency of different fungi associated tomato fruit rot

The results regarding the association of fungi with tomato post harvest fruit rot indicated a significant variation among the different fungal isolates. Total of six different fungi *viz., Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* were found to be associated with tomato post harvest rot. Significantly highest frequency percentage of tissue infection was recorded for *A. solani* (53.667%) followed by *A. niger* (16.333%) and *G. cadidum* (13.00%). The lowest infection percentage was observed for *F. oxysporum* (2.333%), followed by *A. alternata* (4.00%) and *R. stolonifer* (9.00%), respectively. However, no significant difference was observed between *F. oxysporum* and *A. alternata; A. alternata* and *R. stolonifer*; *G. cadidum* and *R. stolonifer* causing infection in tomato fruits (Fig 3).

Numerous mycopathogens were reported as cause of post- deterioration of tomatoes (Abdel-Kader *et al*., 2011). However, the dominancy and infection percentage varies greatly according to areas. It has been well established that the major post-harvest losses of fruits and vegetables are caused by species of pathogens such as *Alternaria, Botrytis, Monilinia*, *Penicillium*, *Phenopsis, Rhizopus Sclerotinia, Fusarium*, *Geotrichum, Heminthosporium, Curvularia* and of bacteria *Erwinia* and *Pseudomonas* (Adekalu *et al.,* 2009). In our study, a total of six different fungi *viz., Alternaria solani*, *Aspergillus niger*, *Alternaria alternata*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* were found to be associated with tomato post harvest rot. Significantly highest frequency percentage of tissue infection was recorded for *A. solani* followed by *A. niger* and *G.candidum.* Lowest infection was found for *F. oxysporum*, *A. alternate* and *R. stolonifer* with no significant difference among themselves. Similar to current study, Sajad *et al.* (2017) observed that most of the tomato fruits have been suffered by fruit rot disease caused by *Alternaria alternata, Aspergillus niger, Geotrichum candidum, Alternaria solani*, Mucor racemosus*, Aspergillus flavus, Fusarium oxysporum*, *Fusarium moniliforme*, *Penicillium digitatum*, *Rhizopus stolonifer*, *Alternaria alternata*, *Colletotrichum lycopersici, Sclerotium rolfsii, Myrothecium roridum, Phoma destructiva* and *Trichothecium roseum*. Percentage frequency of occurrences on all tomato fruits were found maximum for *Alternaria alternata* 16.51%. *Alternaria* rot has been considered as the most common disease of tomato fruits and causes heavy losses in quality of the fruits, thus rendering large quantity of tomato fruits unfit for consumption. Awan *et al*. (2012) have reported *Alternaria* as the main decay causing organism of post harvest tomato fruits producing black rot lesions on tomato fruits. The magnitude of post-harvest losses always vary from one country to another country and one season to another and even one day to another (Mujib *et al.*, 2007; Sajad, 2017).

Further, in present study different fungi causing post harvest tomato fruit rot were isolated and identified from study samples using species specific morphological features. *A. solani* was found to produce purified culture on PDA, aerial mycelium, yellowish to reddish diffusible pigments which later changed to greyish black with black reverse. Colonies of *A. niger* on agar cultures were typically homogeneously black. *Aspergillus* colonies were found fast growing. Their colonies were initially whitish to yellow, yellow-brown, then become brown to black in colour and they mostly

consist of a dense felt of erect conidiophores. Microscopic examination of *A. alternate* revealed septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts. However, colonies of *R. stolonifer* on agar cultures produced whitish to grey fuzzy colour. Similar study has also been reported by (Bashir *et al*., 2014) who studied the association of *Alternaria* sp. with tomato fruits based on morphological characters; Ayala-Zavala *et al*. (2010) also supported our study. They found similar identification features as observed in the current study. Similar findings have also been stated by Britt and Kristin, (2011) who investigated tomato fruit rots caused by *Geotrichum candidum, Rhizopus* stolonifer, *Alternaria* sp. and *Fusarium sp*. Charchar *et al*. (2003) and Hassan, (1996) have also reported that *Alternaria* is the main decay causing organism of post harvest tomato fruits while responsible for black rot lesions on tomato fruits.

CONCLUSION

The present studies concludes that six different fungi *viz., Alternaria solani*, *Alternaria alternata*, *Aspergillus niger*, *Geotrichum candidum*, *Fusarium oxysporum* and *Rhizopus stolonifer* were found be associated with tomato post harvest rot. Further, significantly highest frequency percentage of tissue infection was recorded for *A. solani*. The results from the present study will form a base for better understanding of tomato post harvest fruit rot and for desiging suitable management practices of tomato post harvest rot disease.

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