ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH THE SPOILAGE POSTHARVEST TOMATO FRUITS IN DIFFERENT LOCAL MARKETS IN DHAKA CITY

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This is to certify that the thesis entitled, **"ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH THE SPOILAGE POSTHARVEST TOMATO FRUITS IN DIFFERENT LOCAL MARKETS IN DHAKA CITY"** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, in partial fulfilment of requirements of the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY** embodies the results of a laboratory research work carried out by bearing **REGISTRATION NO. 1406237** under my supervision and guidance. No part of the thesis has been submitted of any other degree of deploma.

I further certify that such help or source of information, as has been avalied of during the course of the investigation has been duly acknowledged.

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DEDICATED
TO
MY PARENTS

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CULTURAL CHARACTERIZATION AND PATHOGENICITY OF FUNGI ASSOCIATED WITH THE SPOILAGE POSTHARVEST TOMATO FRUITS IN DIFFERENT LOCAL MARKETS OF DHAKA CITY

ABSTRACT

Tomato fruits are more susceptible to spoilage by fungi because it contains large amount of water. This study was conducted at Plant Pathology Laboratory, Sher-e-Bangla Agricultural University, Dhaka-1207 during October 2019 to September 2020 on cultural characterization and pathogenicity of fungi associated with the spoilage of postharvest tomato fruits in different local markets viz Kawran bazar, Farmgate, Taltola, Agargaon bazar, Townhall and Krishi market of Dhaka city. From infected tomato, post-harvest disease infecting fungi were incubated and isolated on moist chamber followed isolation on PDA media.The lowest percentage incidence found in Taltola bazar (20%) and the highest in Farmgate (48.89%). Both in winter season and summer season, highest percentage incidence occurred in Farmgate bazar (48.89%). Seven different genera of fungi were isolated and identified viz. *Aspergillus niger, Aspergillus flavus, Penicillium* spp., *Fusarium oxysporum, Alternaria solani, Rhizopus stolonifer* and *Geotrichum candidum*. In winter season, *Fusarium oxysporum* occurred most frequency (23.46%) *Geotrichum candidum* (2.04%) had the lowest percentage frequency. In summer season, *Alternaria solani* occurred most frequency (23.91%) while *Geotrichum candidum* (3.26%) and *Penicillium* sp (3.26%) had the lowest percentage frequency. The identified diseases were Fusarium rot of tomato, Aspergillus ear rot of tomato, Aspergillus black mold disease, Black spot disease of tomato, Blue mold disease, Rhizopus rot disease and sour rot disease of tomato. Pathogenicity test showed that all the isolated fungi were pathogenic to their respective host.

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List of Symbols and Abbreviations

et al. = And others

sp. = Species

J. = Journal

no. = Number

etc = Etcetera

˚C = Degree Celsius

/ = Per

kg = Kilogram

% = Percent

PDA = Potato Dextrose Agar

LSD = Least Significant Differences

CV% = Co-efficient of Variance

CHAPTER I

INTRODUCTION

Bangladesh is particularly an agrarian country. Agriculture sector play a part in 15% to the country`s Gross Domestic Product (GDP) and employs around 41% of the total labour force (BBS, 2018). Tomato is a widely consumed fruit eaten in both raw and processed forms (Moneruzzaman *et al,* 2008). Tomatoes (*Lycopersicum esculentum* L), one of the most popular and indivisible ingredients of human diet, are very popular use for vegetable and also fruit. They are highly produced Solanaceous vegetable crop only after potato all over the world. It is an annual and short-lived perennial herb, diocotic and angiospermic plant. Approximately 182.3 million tons of tomatoes are produced on 4.85 million ha. each year in the world (FAOSTAT, 2019) and tomato production in Bangladesh is 3,85,038 tonnes (FAO, 2019).

Tomato originated in the South America Andes, in the mountains of Peru (Shnain *et al*., 2017). It was taken to other parts of the world by the early travellers where it was planted as an ornamental curiosity but not eaten (Arah *et al.*, 2015). By 500 BC it had been moved to Mexico for the purposes of domestication. Tomato was brought to Europe in 1554 by the Spanish conquistadors. It was later cultured in the U.S. in 1710, and introduced from Europe into southern and eastern Asia, Africa and the Middle East. Thereafter, tomato became popular and was exported around the world by 1850 for commercial production (Shnain *et al*., 2017).

The water content of tomatoes is around 95%, 4% carbohydrates and less than 1% each of fat and protein contains in a tomato. Tomatoes are non-starchy and most significant source of dietary lycopene and ascorbic acid containing antioxidants,

lycopene, ascorbic acid and phenols (George *et al.* 2004) together with vitamin-carbohydrates, proteins, fats and potassium. (Talvas *et al.*, 2010). Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and its products. Lycopene being efficient quencher of single oxygen and free radicals provides protection against a broad range of epithelial cancers (Mascio *et al.*, 1989). Lycopene has been found to prevent prostate cancer, improve the skin's ability to protect itself against the harmful ultra violet rays, decrease the risk of breast, lungs, stomach, bladder, uterine, head and neck cancers, protect against neuro degenerative disease, lower urinary tract infections and reduce the cardiovascular risk associated with type 2 diabetes (Shidfar *et al*, 2010).

Fungi are the most important pathogens, infecting a wide range of fruits and causing destructive and economically important losses of the fruits during storage, transportation and marketing (Sommer, 1992). In storage condition, they are often infected by several species of fungi such as *Alternaria alternata, Collectrotrichum truncatum, Fusarium oxysporum, Curvularia spicifera, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Penicillum chrysogenum, Mucor mucedo,* etc. causing different diseases with distinct symptoms. They hinder the production of tomatoes leading to the severe economic loss. Moreover, they can lead to serious human health problem if consumed. Among them, tomato infected by *Fusarium* sp. is more dangerous to human health because they produce mycotoxins (Jofee, 1986; Nelson *et al*., 1990). Fusarium rot caused by *Fusarium oxysporum* is reported as the most destructive on ripened tomato in the U.S. (Benyal *et al.*, 2008). Further, Alternaria is main decay causing organism of postharvest tomato fruit (Agrawal *et al*., 1950*)* and Alternaria rot has been considered as most prevalent disease and causes huge losses to tomato thus making tomatoes unfit for

consumption (Douglas, 1922). *Alternaria solani*, *Rhizopus stolonifera* and *Aspergillus niger* are the most common pathogens and cause loss of 52.7%, 35.9% and 25% respectively in tomato fruits collectd from various market in Egypt (Mallek *et. al*., 1995). They can produce mycotoxin that is carcinogenic. Phytophthora rot is caused by *Phytophthora infestans* (Mills, 1940).

These postharvest losses are more severe in developing than in developed countries (Enyiukwu *et al.*, 2014). The magnitude of postharvest loses always vary from one country to another and one season to another and even one day to another (Mujib *et al.*, 2007). Huge losses has forced to researchers for simple effective and economic methods to control postharvest diseases and other losses in tomatoes (Wilson and Wisniewski, 1989). Postharvest diseases have not received the attention as a magnitude of the problem warrants which cause loss ranging from 20% to 50% between marketing and consumption (Kasso and Bekele, 2016). Postharvest practices include harvesting, storage, processing, packaging, transportation and marketing (Merema and Rolle, 2002). Both the biological and physical damages during the harvest and transportation phases, coupled with large amount of water and soft endocarp makes tomatoes more susceptible to spoilage by fungi (Asan and Ekmeki, 2002, Onuorah and Orji, 2015).

Postharvest food spoilages were threatening to food security (Madrid, 2011; Kumar and Bekele, 2016) and also the greatest losses were estimated in farming side. (Enyiukwu *et al*., 2014). it was reported that in the developing countries the of research efforts on agriculture sector (95%) was determine at pre-harvest field studies whilst at the same time few research attempts (5%) redesigned at postharvest studies (Madrid, 2011).

Considering the above facts the objectives were taken as follows:

1. To isolate and identify fungi associated with spoilage post-harvest tomato fruits.

2. To determine the cultural characteristics and pathogenicity of fungi associated with spoilage post-harvest tomato fruits.

CHAPTER II

REVIEW OF LITERATURE

Tomato is one of the most popular and widely grown vegetables of the world. It is a rich source of minerals and vitamins. Since the consumers purchase fruits on the basis of quality, the quality of tomato fruit is largely dependent on the stage of maturity of fruits and various ripening conditions. Changes in physiological characteristics during storage as well as ripening must be determining the fitness of tomato fruit for fresh consumption and marketing. The scientific literature does include a very few studies on physiological changes in tomato fruits but they are neither adequate nor conclusive. However, available literature and their findings on tomato and some other fleshy fruits that are related to the present study have been presented in the following section.

Mugao *et al.* (2021) studied an experiment with pathogens associated with tomato post-harvest losses in Mwea, Kenya. Disease causing micro-organisms that were suspected to cause the post-harvest damage were isolated, identified and re-inoculated to wounded surface sterilized fresh harvested ripe tomato fruits to establish pathogenicity. Six pathogens were isolated from infected tomato samples and they varied significantly ($p<0.001$) with *Furasium* spp. being the most prevalent (30%). Damage caused by the pathogens on tomato fruits also varied significantly ($p<0.001$) with *Rhizopus* spp. causing (100%) rot. The susceptibility of the tomato cultivars to the test pathogens differed significantly ($p<0.045$) with Kilele F1 being the most susceptible.

Abdulaziz *et al.* (2020) conducted an experiment on assessment of fungal species associated with tomato spoilage sold in Dutsin-ma metropolis, Katsina state,

Nigeria. This research revealed that fungal species are responsible for the spoilage of the tomato fruit in the study area. The fungi count was found to be $3.8x10^5$, $3.5x10^4$ and $4.5x10^4$ for *Aspergillus niger* at Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. While fungal count of *Fusarium oxysporum* was $3.9x10^3$, $4.8x10^3$ and $4.2x10^5$ for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively. The fungal count of *Rhizopus stolonifer* was $3.5x10^3$, $4.4x10^2$ and $5.1x10^4$ for Yara dole market, Wednesday Market and Tsohuwar kasuwa, respectively.

Kehinde and Oluwole (2020) worked on isolation of phytopathogenic fungi associated with the post-harvest deterioration of watermelon fruits and isolated fungi were *Fusarium oxysporum*, *Streptomyces* spp. and *Aspergillus flavus*.

Gadgile Dhondiram (2019) reported post-harvest fungal disease of tomato in palam dist-parbhani (M.S.) market in India and he surveyed during the July 2017 to February 2018 to assess the incidence of post-harvest fungal diseases of tomato fruits of fruit and vegetable market Palam Dist-Parbhani (Maharashtra state). *Alternaria* rot, *Rhizopus* rot, Black mould rot, *Fusarium* rot, Penicillium rot and *Colletotrichum* rot were major post-harvest fungal diseases of tomato fruits.

Lawrence *et al.* (2019) investigated on antimycotic potential of alum on postharvest deterioration of tomato and found poor storage system predisposes them to spoilage by a broad spectrum of mycoflora resulting in huge postharvest losses.The following species were identified; *Aspergillus niger*, *A. flavus*, *Fusarium* sp, *Penicillium* sp, *Rhizopus stolonifer*, *Geotrichum candidium* and *Saccharomyces cerevisiae*. *In vitro* antimycotic activity of alum (1% (w/v)) was determined on some of the isolates by agar well method (AWM).

Rodrigues and Kakde (2019) worked with tomato fruits and they isolated *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Colletotrichum* sp*; Rhizopus* sp, *Fusarium oxysporum, Botrytis cinerea, Penicillium digitatum, Penicillium chrysogenum, Phoma* sp; *Cladosporium* sp. and *Geotrichum candidum* fungi from tomato during the period of investigation.

Najada (2018) worked an experiment that isolation and characterization of post-harvest fungal species and pathogenicity assessment of spoilt tomato fruits and isolated the fungi were *Aspergillus niger*, *Fusarium oxysporum, Penicillium, Rhizopus, Cladosporium, Saccharomyces cerevisiae and Geotrichum candidum,* and *Aspergillus, Penicillium* and *Rhizopus* were the most prevalent fungi isolate from the samples.

Kator *et al*. (2018) conducted an experiment for isolation, identification and pathogenicity of fungal organisms causing postharvest spoilage of tomato fruits during storage and found five fungal pathogens like *Aspergillus flavus, Penicillium waksmanii, Botryodiplodia theobromae, Fusarium oxysporum* and *Colletotrichum asianum.*

Ahmed *et al*. (2017) carried out a survey of fresh-market tomato fruit and to determine fungal and bacterial pathogens which were most commonly associated with postharvest diseases. They found that *Alternaria, Botrytis, Colletotrichum, Fusarium, Geotrichum, Mucor, Stemphyllium, Rhizopus* and *Penicillium* were the most frequently isolated fungi and *Acetobactor, Glucono bacter, Klebsiella, Leucontoc* and *Pectobacterim* were the prevalent bacteria.

Sajad *et al.* (2017) carried out an experiment to find out fungi associated with the spoilage of postharvest tomato fruit and he observed that tomato fruit had been suffered by fruit rot caused by *Alternaria alternata*, *Aspergillus niger, Geotrichum*

candidum, Alternaria solani, Mucor racemosus, Aspergillus flavus, Fusarium oxysporum, Fusarium moniliforme, Penicillium digitatum, Rhizopus stolonifer, Colletotrichum lycopersici, Sclerotium rolfsii, Myrothecium rodium, Phoma destructiva and *Trichothecium roseum.*

Samuel *et al.* (2017) worked an experiment on isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Aspergillus niger* had the highest occurrence in pineapple, watermelon, oranges, pawpaw, and tomatoes with a frequency of 38%. *Fusarium avenaceum* followed with the frequency of occurrence of 31% in fruits such as pineapple, watermelon, oranges, pawpaw, and tomatoes, while *Penicillium digitatum* and *Rhizopus stolonifer* had the least frequency of 4% each in tomato. Other fungal species were identified as yeast *Saccharomyces* species (10%), *Fusarium solani* (8%), and *Aspergillus flavus* (5%). The highest prevalence rate was 70% of *A. niger* from orange followed by *F. avenaceum* of which 65% isolates were recovered from pawpaw. Other fungal organisms such as *Saccharomyces* species, *P. digitatum* and *R. stolonifer* were isolated with varying prevalence (40%, 20%, and 5%) from watermelon, tomato, and orange, respectively.

Dimphna (2016) conducted an experiment for isolation and identification of fungi associated with postharvest decay of *Lycopersicum esculentum* sold in Abakaliki, Nigeria and he indicated five species of fungi; *Penicillium, Aspergillus, Fusarium, Cladosporium and Rhizopus. Aspergillus, Penicillium and Fusarium* were more dominant.

Akinro *et al.* (2015) studied on isolation and identification of fungal species associated with the four spoilage fruits in Iree Town of Boripe Local Government, Osun State, Nigeria. They were pawpaw *(Carica papaya),* Pineapple *(Ananas comosus),* Orange *(Citrus sinensis)* and Tomato *(Lyeoptersieon esculentum)*. All

the fruits collected showed signs of spoilage by fungal species. The fungal species isolated were *Aspergillus flavus*, *Rhizopus stolonifer, Aspergillus niger*, *Candida tropicalis, Phytophthora* sp., *Fusarium oxysporum* and *Mucu*s sp. Among all, *Aspergillus* species had the highest rate of occurrence followed by *Rhizopus* and *Candida* species while *Phytophthora* sp., *Mucus* sp. and *Fusarium* sp. were the least.

Samuel and Orji (2015) conducted an experiment to isolate, identify, and characterize the fungi associated with spoilage of tomato fruits in Nigeria and they found the fungi *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum, Saccharomyces cerevisiae, Alternaria alternata, Penicillium digitatum* and *Geotrichum candidum.* The percentage occurrence of the isolates in the fruits from Eke Awka was the highest (32.73%) while that of the fruits from Nodu market was the least (12.73%). *Aspergillus niger* had the highest percentage occurrence (47.27%) in the fruits studied, with *Saccharomyces cerevisiae* and *Geotrichum candidum* having the lowest percentage occurrence (3.64%).

Lemma *et al*. (2014) carried out an experiment to identify the postharvest rotting microorganisms from tomato fruits in Ethiopia and they found *Erwinia carotovora, Clavibacter* sp, *Xanthomonas campestris, Ralstonia solanacearum, Pseudomonas aeruginosa*, *Alternaria* sp., *Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. as postharvest rotting microorganisms.

Wogu and Ofuase (2014) reported on microorganisms responsible for the spoilage of tomato fruits, sold in markets in four Benin City, southern Nigeria. The fungal isolates were *Penicilium* sp., *Mucor* sp., *Aspergillus niger*, *Fusarium* sp. and *Saccharomyces cerevisae*. Whereas *Mucor* sp. was the most prevalent with 57.7% and was found in fruit samples from all the markets. *Saccharomyces cerevisiae* had the least prevalence of 9.1% and occurred only in Vegetable and Santana markets.

The mean microbial count ranges were: $2.0 \times 10^4 - 35.0 \times 10^4$ for New Benin market; $1.0 \times 10^4 - 25 \times 10^4$ for Vegetable market; $2.0 \times 10^4 - 23.0 \times 10^4$ for Oba market and $1.1 \times 10^4 - 9.3 \times 10^4$ for Santana market.

Ignjatov *et al.* (2012) conducted an experiment to isolate, determine, and identify causal organisms of tomato wilt and fruit rot and he found *Fusarium oxysporum* was identified as causal agent.

Zhao *et al*. (2012) conducted an experiment about controlling of postharvest soft rot disease of tomato and they found *Erwinia carotovora* as a bacterial pathogen of soft rot disease of tomato.

Kakde and Kakda *et al*, (2012) carried out an extensive survey to investigate post-harvest disease due to the prevalence of air-borne fungi of vegetable and fruit market environment in India. They reported *Aspergillus niger*, *A. flavus, Penicillium chrysogenum, Curvularia* sp., *Alternaria sp., Fusarium sp*. The fungi like *Aspergillus, Penicillium, Cladosporium, Fusarium, Alternaria* etc. were the most frequent associated fungi isolated from the vegetable and fruits. These fungi were most prevalent in the air of market environment and also found to be responsible for most of the decay of the vegetables during storage. Hence, there is probably a cyclic relationship existing between the prevalence of fungal bioaerosols and spoilage diseases in market environments.

Rashad *et al.* (2011) studied investigated about the spoilage fruit fungi and their plant cell wall degrading enzymes of various fresh postharvest fruits sold in Jeddah city and share in establishment of a fungal profile of fruits. Ten fruit spoilage fungi were isolated and identified as follows *Fusarium oxysporum* (banana and grape), *Aspergillus japonicus* (pokhara and apricot), *Aspergillus oryzae* (orange), *Aspergillus awamori* (lemon), *Aspergillus phoenicis* (tomato), *Aspergillus*

tubingensis (peach), *Aspergillus niger* (apple), *Aspergillus flavus* (mango), *Aspergillus foetidus* (kiwi) and *Rhizopus stolonifer* (date).

Osakwe *et al*. (2010) identified three species of fungi viz., *Fusarium moniliforme, R. stolonifer* and *Geotrichum candidum* that were responsible for tomato rot and reported all the fungi were found to be pathogenic on different varieties of tomatos. They also tested severity of infection of fungal isolates on the three varieties and reported 100% severity of infection by *R. stolonifer* and 92% and 90% severity by *G. candidum* and *F. moniliforme*, respectively.

Ogaraku *et al*. (2010) carried out a work on storage decay of tomato and vitamin C content of infected fruits in Nigeria in four locations and they reported that out of 48 samples of tomatoes 34 samples had fungal associations. The species of fungi isolated and identified from deteriorated tomatoes were *Aspergillus niger, A.flavus*, *Alternaria alternataani* and *Fusarium oxysporum*.

Hadizadeh *et al.* (2009) reported that tomato is susceptible to various postharvest diseases caused by various pathogenic fungi. *Alternaria alternata* is a saprophytic pathogen of tomato causing *(Alternaria* rot) postharvest losses at high frequency.

Pose (2009) reported that *Alternaria alternata* is a toxigenic fungus, predominantly responsible for black mould of ripe tomato fruits, a disease frequently causing substantial losses of tomatoes, especially those used for canning. The objective of this study was to determine the effect of water activity (aw, 0.904, 0.922, 0.954, 0.982) and temperature (6, 15, 21 and 35 °C) on germination and radial growth rate on a synthetic tomato medium of a cocktail inoculum of five strains of *A. alternata* isolated from tomato fruits affected by blackmould. The knowledge on the ecophysiology of the fungus in the substrate was necessary to elaborate future strategies to prevent its development and evaluate the consumer health risk.

Dal-Bello (2008) reported that the fungal pathogen *Botrytis cinerea* cause severe rots on tomato fruits during storage and reduced shelf life.

Howell *et al.* (2005) reported that Anthracnose of tomato was primarily a disease of ripe and over-ripe fruit. If left unchecked, the disease could cause serious losses in yield and marketability. Caused by several species of *Colletotrichum*, the disease was widespread and common in areas where moisture conditions promote disease development and also affects eggplant, pepper, and potato. C. *coccodes* was the most common pathogen of tomato fruit.

Tohamy *et al.* (2004) found that postharvest decay is the major limiting extension of shelf life in tomato fruits *(Lycopersicon esculentum* Mill).

Annonimous, (2003) worked that tomatoes can develop many postharvest diseases including Alternaria rot *(Alternaria alternata),* gray mold or Botrytis *(Botrytis cinerea),* rhizopus or hairy rot *(Rhizopus stolonifer),* and sour rot *(Geotrichum candidum).* Bacterial soft rot caused by *Erwinia* spp. can be a serious problem if good harvest and packing house sanitation practices are not implemented. Wounds and stems and stem scars provide potential points of entry for pathogens and decay organisms, so wounded fruit should be discarded immediately.

El-Essawy *et al.* (2003) studied that *Alternaria alternata* and *Botrytis cinerea* causing black and grey moulds are the two main fungi responsible for storage decay in Egypt.

Barnett and Hunter (1998) reported that *Cladosporium* species and *Alternaria alternata* identified by microscopic observations were the prevalent fungi (92%). Those pathogens were recovered following surface disinfection of the calyces, indicating that they had colonized the truss as latent disease already in the greenhouse. During storage (12°C), the mycelia expand gradually from the sepal

tips to the calyces and then to the peduncles and, finally, to the rachises, all of which eventually shrivel or dehydrate while uninfected trusses remain fresh and green. The severity of the PHECD syndrome increased during the warm seasons and declined in the winter. *Cladosporium* isolates from overtly infected calyces were identified as C. *sphaerospermum* (Penzig) and C. *tenuissimum* (Cooke) by the CBS Identification Service, Netherlands.

Abdel-Mallek *et al*. (1995) studied the microflora of tomato fruit from Assiut (Egypt) and they reported that *A. alternata, Rhizopus stolonifer* and *A. niger* were abundant with their maximum occurrence at 53%, 36% and 25%, respectively.

Howard *et al.* (1994) reported that symptoms were first noticeable on ripe fruit of tomato, although green fruits were infected. The latent infections on green fruits can become a serious post-harvest problem. They also reported that tiny lesions may also occur on leaves and stems, but are usually overlooked. Those lesions serve as initial source of inoculum for fruit infection. Small, circular, sunken spots appear on ripe fruit and are characterized by numerous submerged, black microsclerotia often in concentric rings. Spots can coalesce and involve large areas of the fruit. Under humid conditions, spots darken due to the production of hairs (setae) on the fruiting bodies of the pathogen and pink, gelatinous masses of conidia may ooze from lesions. Lesions may crack and become invaded by secondary soft-rotting organisms.

Jones *et al.* (1993) reported that tomato commercialization was limited by rotting caused by *Alternaria alternata* and *Botrytis cinerea. Alternaria alternata* is a saprophytic pathogen of tomato causing postharvest losses at high frequency.

Oladiran and Iwu (1993) stated that seven fungi associated with fruit rot of tomato. The fungi were *Fusarium equiseti, F. chlamydosporum, Alternaria solani,*

Geotrichum candidum, Aspergillus jlavus and *A. niger*. They were all pathogenic on tomato fruits, most pathogenic being *Geotrichum candidum* followed by *A. niger*. Least rot was caused by *Alternaria solani.* The optimum temperature for maximum rotting caused by G. *candidum, A. niger* and *A. jlavus* was 30°C. The relative humidity for maximum rot ranged from 70-90%. Tomato fruits stored well at 0-10°C and rather poorly at 20-30°C. Fruits stored at 35°C showed blemishes. The best RH for storage ranged between 60 and 90%.

Sommer *et al.* (1992) observed that *Alterneria* spp., *Cercospora* spp. *Colletotrichum* spp., *Aspergillus* spp. and *Fusarium* spp. were responsible for diseases. It is somewhat similar to postharvest diseases symptoms of tomato.

Narain and Rout (1981) reported that C. *tenuissimum* was associated twice with dry rot of tomato fruit. They also first time reported that two *Cladosporium* species and *A. alternata* promoted the shriveling of tomato calyces and rachises.

Fajola (1979) studied post-harvest fruit rot diseases of tomato in five states of Nigeria. During severe infections, the diseases could cause 25% loss at harvest and 34% loss of the remaining product in transit, storage and market stalls; thus, giving an overall 15% loss of about 50% of the product. Two types of rots, soft and dry were recognised. The soft rot was found to account for about 85% and the dry rot about 15% of the overall loss. *Erwinia carotovora, Rhizopus 01yzae. R. stolon (fer, Fusarium equiseti, F. nivale* and *F. oxysporwn* were established as the soft rot pathogens; while *Aspergillus aculeatus, A. jlavus, Cladosporium tenuissimum, Corynespora cassiicola, Curvularia lunata, Penicillium expansum P. multicolor* and *Rhizoctonia solani* were established as the dry rot pathogens of tomato fruits in Nigeria.

Srivastava *et al.* (1966) gave the systematic account of fungal diseases of tomatoes during storage. They reported many fungi viz., *Alternaria tenuis, Colletotrichum dematium, Cladosporium fulvum, Fusarium roseum, Malustela aeria, Myrothecium roridum, Oospora lactis, parasitica, Phoma* sp. and *Rhizopus nigricans.* Similar results were also registered in the present investigation.

CHAPTER III

MATERIALS AND METHODS

This chapter comprises the materials used and the methodologies followed during this study to isolate, identify and characterize the microorganisms responsible for post-harvest diseases of fruits where tomato was used as samples.

3.1. MATERIALS

3.1.1. For Sample Collection

- Sample collection bags
- Record keeping books.

3.1.2. For Laboratory Experiment

- **Media preparation:** Potato, carrot, dextrose powder, agar powder, lactic acid.
- **Glassware:** Conical flask, measuring cylinder, test tubes, cotton plug etc.
- **Machinary tools:** Laminar Air Flow, autoclave, oven, balance, stove, microscope, camera etc.
- **Sterilizer:** Alchohol or hexasol, tissue, mask etc.
- **Slide preparation:** Slide, cover slip, cotton blue, glycerine, nail polish etc.
- **Others:** Water, boiling pot, Bunsen burner, gas light, knife, filler, bowl, marker pen, cellophane, parafilm/alluminium foil, glass rod, test tube holder, color paper, forceps, needle, blade scissor, record book etc.

3.2. METHODS

3.2.1 Experimental site

The experiment was conducted in the Mycology Laboratory of the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.2.2 Experimental period

The experiment was conducted during the period from October 2019 to September 2020.

3.2.3 Study area

Six different locations were selected for collecting tomato samples. The study area was conducted in Kawran bazar, Krishi market, Town hall bazar, Agargaon bazar,Taltola bazar and Farmgate in the Dhaka city. Collection time was divided in two season two seasons such as winter and summer seasons. The winter season lasts between October 2019 to March 2020 and summer season lasts between July to September 2020.

3.2.4 Isolation and identification of infected fungi from tomato in winter season

3.2.4.1 Collection of tomato fruits

A total of 1kg tomato fruits were collected from each of six different markets in Dhaka city. There were some healthy fruits and few infected fruits. All the samples collected were placed in a sterile polythene bags separately and labelled appropriately and transported to Mycology Central Laboratory, Department of

Plant Pathology, Sher-e-Bangla Agricultural University. From each market place, total amount of tomatos were separate in three replications where each replication consists of 15 tomatoes (include healthy and infected).

3.2.4.2 Mycological studies: Glassware's, petriplates, conical flasks and other materials were properly washed in chromic acid solution and then sterilized in hot air oven at 160^0 C for one hour. Sterlized petriplates were used for PDA medium and were put in petriplates in appropriate concentration.

3.2.4.3 Isolation of fungi: The unwashed samples were kept in pre-sterilized moist blotters chambers separately. The samples then were incubated at 28^0 C for 3 to 5 days to allow the growth of fungi associated with it Also the diseased or decayed fruits sample was examined for the fungi by taking surface issues from the infected part or margins. Small sections of infected fruit were cut and surface sterilized individually in 2% sodium hypochlorite for 1 min and rinsed twice in sterile distilled water. Fungi were carefully isolated and the slide s were prepared in lactophenol cotton blue mounting on the glass slide. The microscopic slides were covered with a cover slip and were examined under the microscope for morphological examination.

3.2.4.4 Preparation of Potato Dextrose Agar (PDA) media

PDA media were used to stimulate sporulation (slide preparations), maintain stock cultures of certain dermatophytes and differentiate atypical varieties of dermatophytes by pigment production (Mac Faddin, 1985). Composition of PDA media preparation:

Peeled potato (decoction) : 200g

Dextrose : 20g

Agar : 20g

Distilled water : 1000g

At first, 200g potato was taken and cleaned followed by washing with tap water. Then the potato was peeled and cut in a small slice and boiled about 30-40 minutes in one-liter water. When potato was fully soft, it was sieved. After that, 20g dextrose and with a few minutes' interval, 20g Agar were mixed slowly with it and stirred properly so that it cannot be coagulated. Then the media was sterilized in an autoclave at temperature of 121^0 C temperature under 15 PSI pressure for about 30 minutes.

After autoclaving, the media was kept 20-30 minutes for cooling in laminar air flow cabinet. Then 25-30 drops lactic acid and Phenoxymethyl Penilillin tablet were added with the media in order to maintain slightly acidic condition for the growth of the fungus and preventing bacterial contamination. The works were done aseptically inside the laminar air flow cabinet.

3.2.4.5 Identification of Fungal pathogen

Identification was done macroscopically and microscopically. For macroscopic identification, colony characteristics such as conidial morphology and pigmentation were observed. The technique of Oyeleke and Manga (2003) was also adopted for the identification of the isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial

mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelia were well spread on the slide with the needle. A cover slide was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under 40x objective of the light microscope. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Adebayo-Tayo *et al,* Onuorah *et al,* Klich, Samson and Varga. These were *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Alternaria solani, Rhizopus stolonifer* and *Penicillium* sp.

3.2.4.6 Pathogenicity test

Healthy tomato fruits were collected from market. Surface of the fruits were sterilized by 70% ethanol. Firstly, the cork borer was flamed red hot with a spirit lamp and confessed to cool before use. A hole was prepared by the pressing with sterilize cork borer around 4 mm mycelial discs of 5 days old cultures of the isolates consequently. The cork borer was restored and then covered with vaseline jelly to make it air tight and mixture moisture level. Then they were placed in incubation chamber for growing the pathogen. The samples were examined every day to determine the effect of the pathogens on them. The fungi re-isolation was done and recorded the characteristic with the first one and then confirmed (Ewekeye *et al.*, 2016).

3.2.4.7 Fungal growth on PDA media

Using a sterile forcep, 2 slices were cut from each of the samples and incubated in separate plates containing freshly prepared PDA. The plates were kept in an incubator preset at 30^0 C for 48 hours (or more, depending on growth of the individual plate). To obtain pure cultures of the fungal isolates, developing fungal cultures were aseptically sub cultured repeatedly into freshly prepared PDA plates

until cultures consisting of only one type of fungus was obtained. To identify each of the fungus, a small portion of fungal growth from each pure plate was teased with a sterile inoculating loop into 1-2 drops of Lacto phenol in-cotton blue on a clean slide. A cover slip was placed on it and this was examined under a light microscope. Fungal identification was done by comparing the morphological features of each of the prepared fungal slide as examined under the microscope as well as their corresponding pure plate with the descriptions given by Talbot (1971) and Deacon (1980).

3.2.5 Isolation and identification of fungi from infected tomato in summer season

3.2.5.1 Collection of tomato fruits: Worked same local markets same as before (3.2.4.1.)

3.2.5.2 Mycological studies: Worked same as before (3.2.4.2.)

3.2.5.3 Isolation of fungi: Worked same procedure same as before (3.2.4.3.)

3.2.5.4 Preparation of Potato Dextrose Agar (PDA) media: Worked same as before (3.2.4.4.)

3.2.5.5 Identification of fungal pathogen: Worked same as before (3.2.4.5.)

3.2.5.6 Pathogenicity test: Worked same as before (3.2.4.6.)

3.2.5.7 Fungal growth on PDA media: Worked same as before (3.2.4.5.)

3.3. Data Collection: The following parameters of data were recorded during experimental study. They were as follows-

- No. of healthy tomato fruit
- No. of infected fruit
- Percent incidence
- Percent frequency
- Percent occurrence of fungi

3.4 Incidence of postharvest diseases of tomato fruits: Percent diseases incidence was calculating by using following formula (Samuei Orji, 2015):

$$\text{Percent disease incidence} = \frac{Number\ of\ infected\ fruits}{Total\ number\ of\ fruits} * 100$$

3.5 Frequency of occurrence: Percent frequency of occurrence was calculating by using following formula (Sajad *et al.* 2017):

$$\text{Percentage frequency of occurrence} = \frac{Number\ of\ times\ a\ fungus\ counted}{Total\ number\ of\ fungal\ isolates} \times 100$$

3.6 Data analysis: The data obtained were statistically analysed to observe the significant difference among the treatment by using the MSTAT-C program. The mean values of all the data were calculated and analyses of variance were calculated and to separate the means within the parameters at 5% level of probability.

CHAPTER IV

RESULTS

This chapter comprises the presentation of the results obtained from the experiments where tomato fruits were used as samples. The results of different parameters have been presented in figures and tables under the following headings and sub-headings.

4.1 Experiment 1: Isolation and identification of fungi associated with postharvest diseases of tomato fruits.

4.1. Percentage of incidence of spoilage fruits of tomato in winter season

Healthy fruits, infected fruits and percent incidence of infected fruits showed significant variation which were collected from different local markets are shown in Table 1. In case of healthy tomato fruit the highest healthy fruits were found in Taltola bazar (12.00) and Krishi market (11.33), followed by Kaoran bazar (9.33) and Town Hall (9.00), whereas the lowest infected fruits were in Farmgate (7.66) and Agargaon bazar (8.33).

In case of infected tomato fruits, the highest infected fruits were found in Farmgate (7.33) and Agargaon bazar (6.33) followed by Tawn hall (6.00) and Kaoran bazar (5.66) whereas the lowest in Taltola bazar (3.00) and Krishi market (3.66).

For percentage incidence of fungal infected tomato fruits, the highest PDI was found in Farmgate (48.89%) and Agargaon bazar (42.22%), followed by Town hall (40.00%) and Kaoran bazar (37.78%), whereas the lowest in Taltola bazar (20.00%) and Krishi market (24.45%).

Table 1. Percentage incidence of fungal isolates of postharvest tomato in winter season

Location	**Healthy tomato**	**Infected tomato**	**% incidence**
Kawran bazar	9.33 b	5.66 b	37.78 b
Farmgate	**7.66 d**	**7.33 a**	**48.89 a**
Taltola bazar	12.00 a	3.00 c	20. 00 c
Agargaon bazar	8.33 cd	6.33 b	42.22 b
Tawn Hall	9.00 bc	6.00 b	40. 00 b
Krishi market	11.33 a	3.66 c	24.45 c
CV (5%)	5.26	9.06	9.06
DF	10	10	10
EMS	0.2560	0.2330	10.37
Lsd	0.9205	0.8782	5.860

4.2. Percentage of incidence of spoilage fruits of tomato in summer season
Healthy fruits, infected fruits and percent incidence of infected fruits showed significant variation which were collected from different local markets are shown in Table 2. In case of healthy tomato fruits, healthy fruits were found the highest in Taltola bazar (12.00) and Agargaon bazar (10.67), followed by Kaoran bazar (10.00) and Krishi market (9.33) whereas the lowest in Farmgate (7.66) and Townhall (8.66).

In case of infected tomato fruits, the highest infected fruits were found in Farmgate (7.33) and Townhall (6.33) followed by Krishi market (5.66) and Kaoran bazar (5.00), whereas the lowest in Taltola bazar (3.00) and Agargaon bazar (4.33).

The highest percentage incidence of fungal infected tomato fruits were found in Farmgate (48.89%) and Tawnhall (42.22%), followed by Krishi market (37.78%) and Kaoran bazar (33.33%), whereas the lowest PDI were found in Taltola bazar (20.00%) and Agargaon bazar (28.89%).

Table 2. Percentage incidence of fungal isolates in summer season

Location	Healthy tomato	Infected tomato	% Incidence
Kawran bazar	10.00 bc	5.00 bc	33.33 bc
Farmgate	7.66 d	7.33 a	48.89 a
Taltola bazar	12.00 a	3.00 d	20.00 d
Agargaon bazar	10.67 ab	4.33 cd	28.89 cd
Townhall	8.66 cd	6.33 ab	42.22 ab
Krishi market	9.33 b-d	5.66 a-c	37.78 a-c
CV (5%)	5.26	18.84	18.84
EMS	0.9890	0.9890	43.94
lsd	1.809	1.809	12.06

4.3 Comparison between percentage incidence of fungal isolates in winter and summer season

Comparison between percentage incidence of fungal isolates in winter and summer season are shown in figure-1. There was no difference in Farmgate and Taltola bazar which showed more or less similar infection in both seasons. But other markets showed variation in infection. Compare to all market, in winter season the infection rate was higher than summer season and as well as Farmgate market showed the highest incidence compare to other markets.

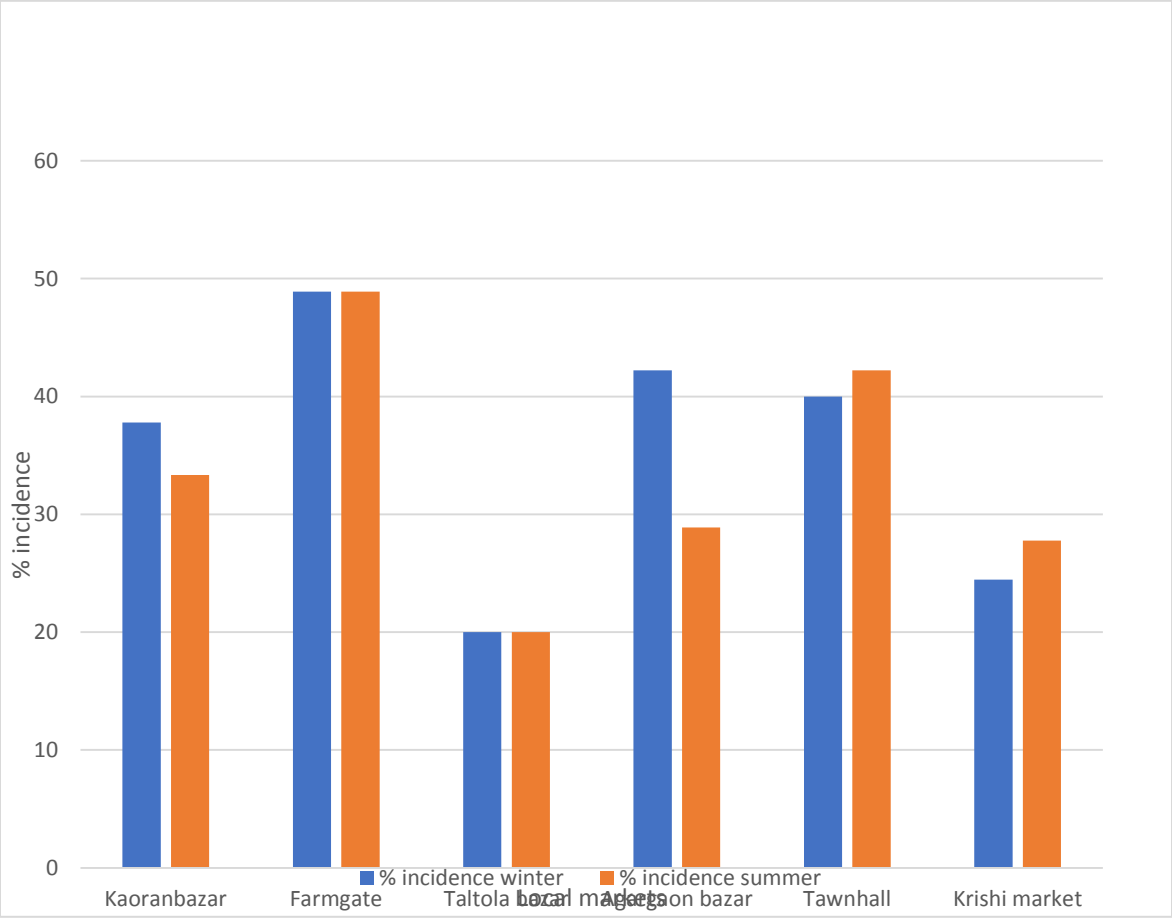


Figure 1. Comparison between percentage incidence of fungal isolates in winter and summer season

4.4. Isolation of fungi in winter season

On an average, seven fungi were isolated from six different local markets of Dhaka city in winter season. They were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum, Alternaria solani, Penicillium* sp., *Rhizopus stolonifer* and *Geotrichum candidum.* These fungi were identified comparing with the standard literature by preparing slide and seeing in compound microscope. The fungal isolates from the infected tomato fruits are shown in Table 3 in winter season.

Table 3. Fungal species isolates isolated from infected tomato fruits in winter season

Markets	Fungi isolates
Kawran bazar	*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Alternaria solani, Penicillium* sp
Farmgate	*Aspergillus niger, Fusarium oxysporum, Alternaria solani, Rhizopus stolonifer*
Taltola bazar	*Aspergillus flavus, Fusarium oxysporum, Geotrichum candidum, Rhizopus stolonifer*
Agargaon bazar	*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Alternaria solani, Penicillium* sp, *Rhizopus stolonifer*
Town Hall	*Aspergillus niger, Fusarium oxysporum, Geotrichum candidum, Alternaria solani, Rhizopus stolonifer*
Krishi market	*Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Alternaria solani*

4.5. Isolation of fungi in summer season

On an average, seven fungi isolated from six different local markets of Dhaka in summer season. They were *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Alternaria solani, Penicillium* sp, *Rhizopus stolonifer* and *Geotrichum candidum.* The fungal isolates from the infected tomato fruits are shown in Table 4 in summer season.

Table 4. Fungi isolates isolated from the infected tomato fruits in summer season

Markets	**Fungi isolates**
Kawran bazar	*Geotrichum candidum, Fusarium oxysporum, Penicillium* sp, *Aspergillus flavus, Alternaria solani*
Farmgate	*Alternaria solani, Rhizopus stolonifera, Fusarium oxysporum, Aspergillus niger*
Taltola bazar	*Fusarium oxysporum, Alternaria solani, Rhizopus stolonifera, Peniciliium* sp
Agargaon bazar	*Aspergillus niger, Fusarium oxysporum, Alernaria solani, Penicillium* sp, *Rhizopus stolonifer*
Townhall	*Fusarium oxysporum, Alternaria solani, Rhizopus stolonifer*
Krishi market	*Fusarium oxysporum, Alternaria solani, Aspergillus niger, Aspergillus flavus, Geotrichum candidum*

4.6. Percentage occurrence in winter season

The occurrence of the fungi from infected tomato fruits collected from different local markets in winter season are shown in Table 5. The highest occurrence of fungi was in the samples collected from Agargaon bazar (22.44%) followed by Farmgate (20.40%), whereas the lowest occurrence in fruits collected from Krishi market (10.20%) followed by Taltola bazar (12.24%).

4.7. Percentage occurrence in summer season

The occurrence of the fungi in relation to the markets in summer season is shown in Table 6. The highest occurrence of fungi was in the samples collected from Farmgate (25.55%), followed by Tawnhall (21.11%) whereas the lowest occurrence in the fruits from collected Taltola bazar (10%) followed by Krishi market (12.22%).

Table 5: Occurrence of fungal genera in different wholesale markets of Dhaka city in winter season.

Markets	***Aspergillus niger***	***Aspergillus flavus***	***Fusarium oxysporum***	***Geotrichum candidum***	***Alternaria solani***	***Penicillium* sp**	***Rhizopus stolonifer***	**%Occurrence**
Kawran bazar	3	4	4	0	5	1	0	17.34
Farmgate	3	0	8	0	3	0	6	20.40
Taltola	0	7	2	1	0	0	2	12.24
Agargaon bazar	8	2	3	0	6	2	1	22.44
Tawnhall	3	0	5	1	1	0	7	17.34
Krishi market	5	1	1	0	3	0	0	10.20

Table 6. Occurrence of fungal genera in different wholesale markets of Dhaka city in summer season.

Markets	***Aspergillus niger***	***Aspergillus flavus***	***Fusarium oxysporum***	***Geotrichum candidum***	***Alternaria solani***	***Penicillium* sp**	***Rhizopus stolonifer***	**%Occurrence**
Kawranbazar	1	1	5	0	6	0	2	16.66
Farmgate	5	3	4	1	4	0	6	25.55
Taltola	3	0	1	0	1	0	4	10
Agargaon bazar	0	2	3	1	6	1	0	14.44
Tawnhall	2	3	5	1	1	1	6	21.11
Krishi market	4	2	1	0	4	0	0	12.22

4.8. Percentage frequency of the fungal isolates in winter season

The percentage frequency of the fungi from infected tomato fruits are presented in Table **7.** From the Table 7 *Fusarium oxysporum* occurred with most frequency (23.46%) followed by *Aspergillus niger* (22.44%), while *Geotrichum candidum* (2.04%) had the lowest percentage occurrence followed by *Penicillium* sp (3.06%).

Table 7. Percentage frequency of occurrences on infected tomato fruits in different local markets in Dhaka city in winter season

Fungi	**No. of isolates**	**% Frequency**
Aspergillus niger	22	22.44
Aspergillus flavus	14	14.28
Rhizopus stolonifer	16	16.32
Penicillium sp	3	3.06
Alternaria solani	18	18.36
Fusarium oxysporum	23	23.46
Geotrichum candidum	2	2.04

4.9. Percentage frequency of the fungal isolates in summer season

The percentage frequency of the fungi infected tomato fruits in summer season are presented in Table 8. *Alternaria solani* occurred the most frequency (23.91%) while *Geotrichum candidum* (3.26%) and *Penicillium* sp (3.26%) had the lowest percentage frequency compared to other fungi.

Table-8. Percentage frequency of occurrences of infected tomato fruits in different local markets in Dhaka city in summer season

Fungi	**No. of isolates**	**% Frequency**
Aspergillus niger	15	16.30
Aspergillus flavus	11	11.95
Rhizopus stolonifer	19	20.65
Penicillium sp	3	3.26
Alternaria solani	22	23.91
Fusarium oxysporum	19	20.65
Geotrichum candidum	3	3.26

4.10. Comparison between frequency of fungal isolates in winter and summer season

Comparison between frequency of fungal isolates in both seasons are shown in figure 2. The figure showed in both seasons, the highest percentage frequency of fungi was *Aspergillus niger*, *Alternaria solani* and *Fusarium oxysporum* in both seasons. On the other hand, the lowest percentage frequency of fungi was *Penicillium* sp and *Geotrichum candidum* in both seasons. Compare to both seasons, winter was highly susceptible to spoilage of post-harvest diseases.

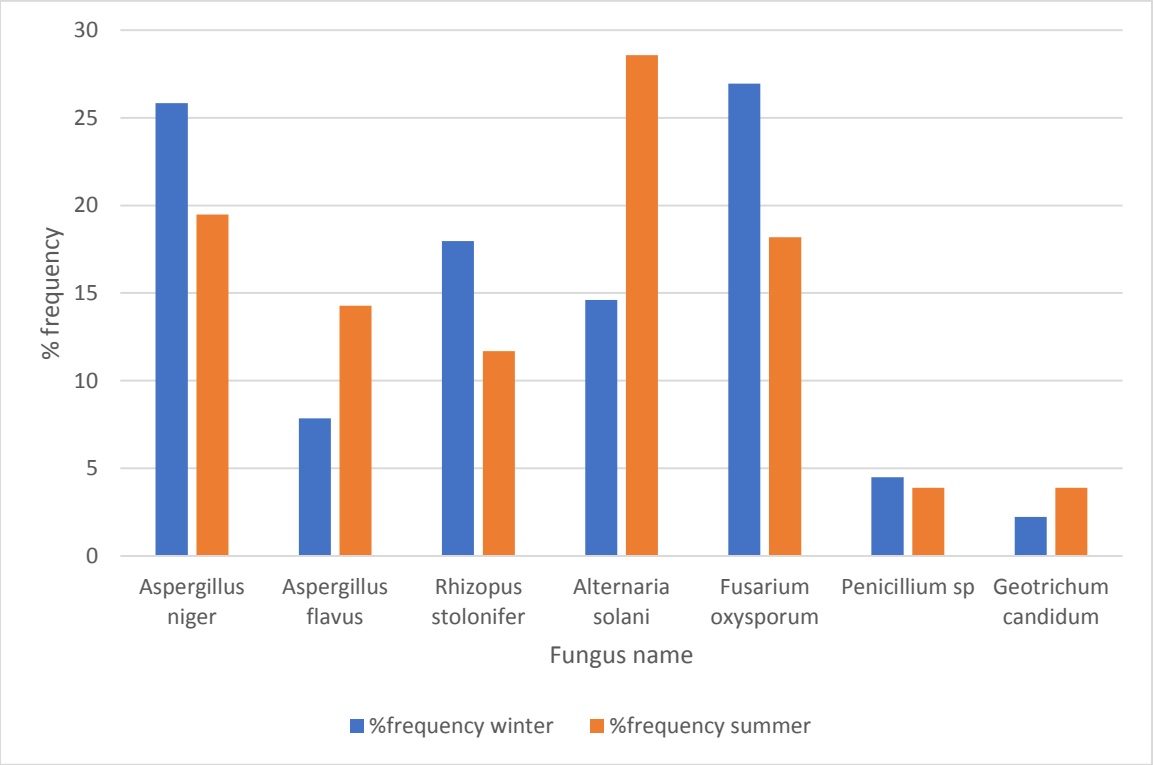


Figure 2. Comparison between % frequency of fungal isolates in winter and summer season

4.11. Identification of postharvest diseases of infected tomato fruits in both seasons

The diseases were identified by visual observations and investigations and the name of identified diseases were presented in Table 9. The identified spoilage post-harvest diseases were Fusarium rot disease, Aspergillus ear rot, Aspergillus black mold disease, Black spot disease, Blue mold disease, Rhizopus rot disease and sour rot disease of tomato.

Table 9. Post-harvest tomato fungal diseases and their causal organisms

Fungal diseases	**Causal organisms**
Aspergillus ear rot	*Aspergillus flavus*
Black mold	*Aspergillus niger*
Fusarium rot disease	*Fusarium oxysporum*
Black spot disease	*Alternaria solani*
Blue mold disease	*Penicillium italicum*
Rhizopus rot disease	*Rhizopus stolonifer*
Sour rot disease	*Geotrichum candidum*

4.11.1. Visual symptoms of postharvest diseases of tomato in both seasons

i. **Aspergillus ear rot:** The infected tomatoes appeared with olivaceous or greenish colored pathogenic growth, developing soft rot and water-soaked tissue (Plate 1; A- B)

ii. **Aspergillus black mold disease:** The infected tomatoes appeared with black colored pathogenic growth resulting water soaked decayed tissue (Plate 1; C- D).

iii. **Fusarium rot disease of tomato:** The infected fruits had an earthy, musty odor, caused soft rot in infected area with white cottony pathogenic growth (Plate 1; E-F).

iv. **Black spot disease of tomato:** The infected tomatoes were brown to black circular lesions (Plate 1; G- H).

v. **Blue mold disease*:*** Caused distension of the fruit surface, infected area covered with blue colored pathogenic growth, spread through the surface having inward progress of infection, infected tissue was shrinked (Plate1; I- J).

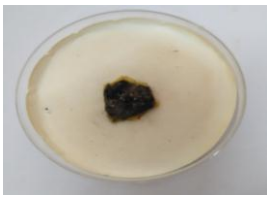
vi. **Rhizopus rot disease of tomato:** Fully developed lesions on fruit in high humidity have extensive surface coverage by a coarse gray to white mold. Younger lesions develop near wounds, stem scars, or open blossom pores as water-soaked areas that rapidly enlarge. The lesion is relatively soft but has some consistency (Plate 1; K- L).

vii. **Sour rot disease of tomato*:*** The fruit skin was ruptured and creamy white mycelia were found inside the tomato. The pulp was found very soft in infected areas (Plate 1; M- N).

A.Aspergillus ear rot

B. Moist chamber

C. Black mold

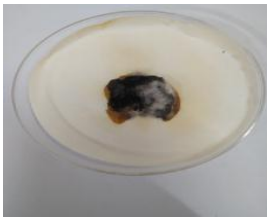


D. Moist chamber

E. Fusarium rot

F. moist chamber

G. Black spot



H. Moist chamber

I. Blue mold

J. Moist chamber

K. Rhizopus rot

L. Moist chamber

M. Sour rot disease

N. Moist chamber

Plate 1. Visual symptoms of postharvest diseases of tomato and infected part in moist chamber

4.11.2. Cultural and microscopic characteristics of identified fungi

During experiment, identified fungi were kept on PDA media. After growth in the culture all the fungi were again isolated on fresh PDA medium to get pure culture. This culture media characterized by visual observation and fungi were identified basal on cultural characteristics and microscopic view.

a. Cultural and microscopic characteristics of *Aspergillus niger*

The fungus colonies on PDA exhibit whitish mycelia growth. At later stage, the colonies turned into blackish velvety appearance (Plate 2, C)

***Aspergillus niger*:** Colorless conidiophores were observed under the microscope. Conidial heads of the organism were globose and dark brown in color. Two series (biseriate) phialide s were found covering the entire vesicle (Plate 2, D).

b.Cultural and microscopic characteristics of *Fusarium oxysporum*

The fungus colonies on PDA exhibit fluffy, pinkish mycelium around with whitish mycelium (Plate 2, E).

Fusarium oxysporum: Macro conidia observed under microscope were Relatively wide, straight and stout, gradually curved and pointed towards the end. Apical cell morphology was found blunt and rounded. Basal cell morphology was found straight to almost cylindrical. 3 to 5 septation was present which was observed under microscope. Larger sized conidia were present**.**

The smaller sized conidia were present. 1 to 2 septation present, oval, ellipsoid and fusiform. Aerial mycelium was false headed (Plate 2, F).

c. Cultural and microscopic characteristics of *Alternaria solani*

The fungus colonies on PDA exhibit aerial mycelium, yellowish to reddish diffusible pigments later changed to greyish black with black reverse (Plate 2, G).

Alternaria solani: Septate brown hyphae, with septate and brown conidiophores bearing conidia in chains. Conidia were 9-11 transverse septa (cross walls) and long beaks. Conidiophores were pale brown, simple and branched, bearing conidia at the apex and apical fertile (Plate 2, H).

d. Cultural and microscopic characterictics of *Rhizopus stolonifer*

The fungus colonies on the PDA exhibit whitish to grey fuzzy in color (Plate 2, K).

***Rhizopus stolonifer*:** Many haploid spore agiospores were developed within the sporangia structure. Sporangia were bulbous structures that sprout from the vegetative hyphae and hold the haploid spores (Plate 2, L).

e. Cultural and microscopic characteristics of *Penicillium* sp.

The fungus on the PDA exhibit prolific production of colonies with filamentous, cottony, velvety, flat or woolly in texture (Plate 2, I).

***Penicillium* sp:** From a specialized conidiogeny, chains of single–celled conidia (Ameroconidia) were produced in basipetal succession called a phialide (Plate 2, J).

f. Cultural and microscopic characteristics of *Aspergillus flavus*

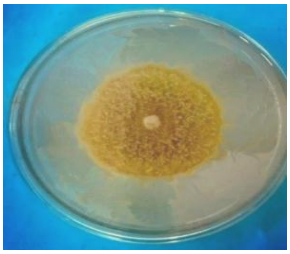
The surface color of the colony is greyish green and the margins are entire. The reverse is hyaline. The colony growth is moderate to rapid (Plate 2, A).

***Aspergillus flavus*:** The vesicle is hemispherical, conidia is globose covering the entire vesicle.Conidiophore is smooth, long and hyaline (Plate 2, B).

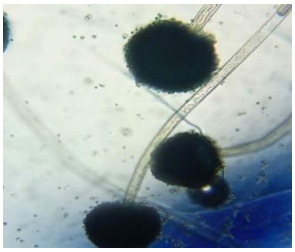
g. Cultural and microscopic characteristics of *Geotrichum candidum*

Colonies were fast growing, flat, white to cream, dry and finely leather like whose surface has been made slightly rough and soft (Plate 2, M).

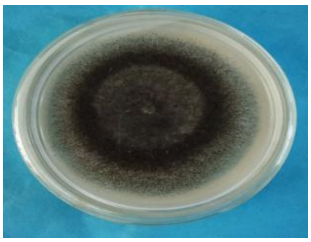
Geotrichum candidum: Hyphae were hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled. Conidia were cylindrical shape or barrel shape. Conidiophore were not produced (Plate 2, N).



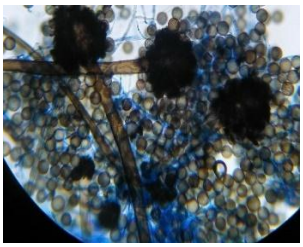
A.*Aspergillu flavus*



B.*Aspergillus flavus* (40X)

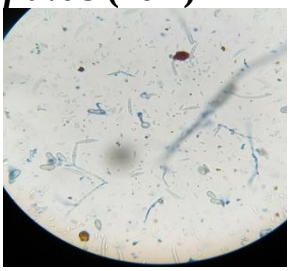


C.*Aspergillus niger*

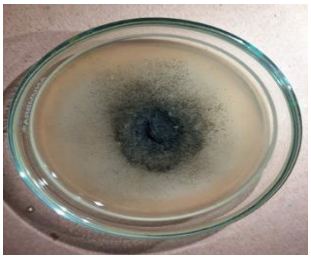


D.*Aspergillus niger* (10X)

E.*Fusarium oxysporum*



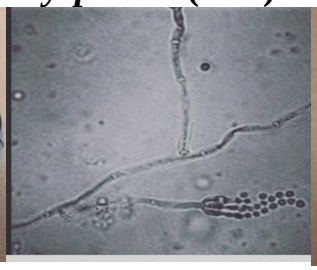
F.*Fusarium oxysporum* (40X)



G.*Alternaria solani*

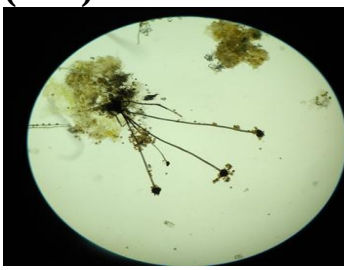
H.*Alternaria solani* (40X)

I.*Penicillium* sp.



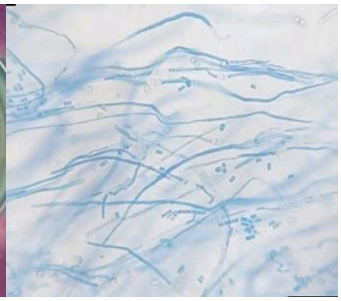
J.*Penicillium* sp. (40X)

K.*Rhizopus stolonifer*



L.*Rhizopus stolonifer* (10X)

M.*Geotrichum candidum*



N.*Geotrichum candidum* (10X)

Plate 2. Cultural and microscopic view of identified fungi

4.12. Pathogenicity test

Pathogenecity test was done on apparently healthy mature tomato fruits. In this test, all the fungal isolates were observed positive for causing spoilage in fruits. All of the isolated organisms were found to be pathogenic as they successfully performed the ability to cause decay in healthy fruits. In most cases pathogenic growth was developed on inoculated fruits after or within 7 days post inoculation at 25°C and 30°C. Characteristics of each isolates were present at the individual inoculation sites of tomato fruits. On Visual inspection, a sharp margin between infected and healthy tissue could easily be seen and the decayed tissue felt out leaving a proof of pathogenicity. But no symptoms were observed on the control fruits. The symptoms on inoculated fruits were very similar to those of natural infection. Re-isolated fungi showed the same morphological characteristics as original isolates, thus fulfilling pathogenicity test.

The Various disease symptoms caused by each of the isolates were observed and recorded as follows:

A. *Aspergillus niger*: Fruits appeared with water soaked and soft decayed area with black powdery colonial growth. Developed rapidly through fruit tissue resulting in total rot and exudation of liquids (Plate 3; C).

B. *Aspergillus flavus*: Soft rot spoilage, the infected area turned brown and water soaked with olivacious colored colony growth, colony texture was powdery, scattered or velvety (Plate 3; F).

C. *Fusarium oxysporum*: Caused soft rot, infected area covered with white to browny cottony pathogenic growth (Plate 3; E).

D.*Penicillium* sp (Blue mold): Fruits decayed gradually with lesions having blue colored mats structure of pathogen around inoculated area and rotten tissue was soft and watery (Plate 3; B).

E.*Rhizopus stolonifer:* Symptoms originate at an inoculation area in the tomato and consist of a soft, black spore mass that progresses quickly under favourable conditions and can result in full decay of an infected fruit within three days. White fungal mycelium produces black sporangia similar to the isolates as shown in Plate 3; A.

F. *Geotrichum candidum*:Soft, watery, colourless decay were found on inoculation side of the tomato. Decay area covered with dull, white spores of pathogen. Vinegar like odour may developed on the infected part of tomato fruit and the tissue of the fruit became dull which was similar to the isolates as shown in Plate 3; G.

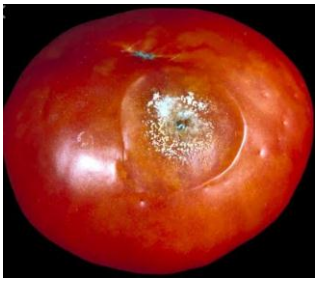
G. *Alternaria solani*: Infected area found at the inoculation side. Once infection occurs, it slowly enlarges in all directions. Wrinkled and shrink aged area were found over the infected area. Soft, watery, decay was found on inoculation side of the tomato. Black lesions were developed at the inoculation side then black spore mass covered on the infected tomato (Plate 3; D).

A.Rhizopus rot disease

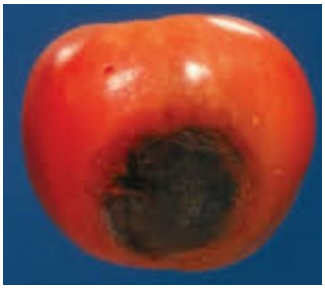
B. Blue mold disease

C. Black mold disease

D.Black spot disease



E. Fusarium rot disease



F. Aspergillus ear rot

G. Sour rot disease

Plate 3. Typical disease symptoms and contamination of pathogenicity on inoculated tomato fruits.

CHAPTER V

DISCUSSION

A study was conducted in the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 to study on cultural characterization and pathogenicity of the fungi associated with the spoilage postharvest tomato fruits in different local markets in Dhaka city. In this study, fruit samples having typical symptoms were collected from different local markets in Dhaka and the causal organisms were isolated on PDA media. After isolating pure culture of fungi, pathogenicity test was done for each pathogen. The pathogens were identified on the basis of morphological, cultural and microscopic studies. There was therefore a need to evaluate the fungi associated with their spoilage hence in work, the fungi associated with the spoilage of postharvest tomatoes sold in local markets in Dhaka city.

In this present study, the highest fungi percentage incidence both in the winter and summer season was in the samples collected from local markets in Farngate (48.89%) and Taltola bazar (20%) had the lowest fungi percentage incidence both in winter and summer season. Similar work done by Samuel *et al.*(2015), the percentage occurrence of the fungi in relation to the markets showed that the fungi had the highest percentage occurrence of 32.73% in the samples from Eke Awka market while their percentage occurrence was lowest (12.73%) in the fruits from Nodumarke.

Seven genera of fungi such as *Fusarium oxysporum, Penicillium* sp, *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Alternaria solani* and *Geotrichum candidum* had been identified from tomato fruits collected from six different local

markers in Dhaka city. These pathogens were also identified by Samuel *et al*. (2015). They also isolated *A. niger, P. digitatum, A. flavus, F. solani, Rhizopus stolonifer, A. alternata* and *Geotrichum candidum.* Ibrahim *et al*. (2011) isolated *Aspergillus niger* as one of the major fungus responsible for the production of volatile compounds in spoilt tomatoes. Baker (2006) also isolated *Aspergillus niger* from rotten tomato fruits and reported that they are pathogenic on tomato fruits. Akinmusire (2011) reported that *Rhizopus* spp were associated with the spoilage of tomatoes. Wogu and Ofuase (2014) isolated *Aspegillius* spp, *Penicilium* spp, *Fusarium* spp. and *Saccharomyces* spp. from spoilt tomato fruits. Mbajiuka and Enya (2014) also isolated *Aspergillius* spp., *Penicilium* spp. and *Saccharomyces cerevisiae* from spoilt tomatoes while Fatih *et al* (2005) reported the presence of *Alternaria alternata* and *Fusarium oxysporum* in the spoilt tomato fruits they studied. Ghosh (2009) also isolated *Fusarium oxysporum, Aspergillius niger* and *Rhizopus stolonifer* from the spoilt tomato fruits studied. *Penicilium* spp. were found next to *Aspergillus* in abundance. Mbajiuka and Enya (2014) found abundant presence of *Penicilium nalgiovense, Penicillium notatum* and *Penicilim expansium* among other fungi species involved in deterioration of tomato fruits. *Penicillium* and *Fusarium* are among the most important genera of mycotoxigenic fungi (Zain, 2011).

Alternaria solani were already reported by Agarwal *et al*, (1950) in local markets of Jabalpur. Kajansoon and Mathur, (1961) also reported it on tomato fruit. *Alternaria solani* were also reported from Tikamgarh MP India by Chaurasia *et al*., (2013). Fungal rot by *Alternaria solani* were also reported by Hasssan, (1996) from different parts of India. *Aspergillus niger* also causes black mold, and causes huge losses to tomato fruits in Jabalpur market. Anwer *et al*., (2013) also reported *Aspergillus niger* on tomato fruit. *Fusarium oxysporum* mostly reported in wet

conditions reported already by Ansari *et al*., (2012). *Sclerotium rolfsii* forms yellowish lesions and on tomato fruit, Banyal *et al*., (2008). *Phytophthora infestans* were also reported from Jabalpur market and other parts of India.

In case of fungal frequency, *Fusarium oxysporum* has the highest frequency (23.46%) followed by *Aspergillus niger* (22.44%) and *Alternaria solani* (18.36%), while *Geotrichum candidum had* the lowest frequency (2.04%) in winter season. On the other hand, in summer season, *Alternaria solani* has the highest frequency (23.91%) and *Penicillium* sp. and *Geotrichum candidum* both had the lowest frequency (3.26%).

Bukar *et al.* (2009) showed, the frequency of occurrence of each of fungal isolate where *Aspergillus* sp. which had the highest occurrence of 32.5%, followed by *Penicillium* sp and *Rhizopus* sp (15%), *Fusarium* sp. (7.5%), with *Alternaria* sp having the least occurrence of 5%. The study shows that all the fungal isolates were able to infect the healthy oranges with the exception of *Aspergillus* spp, which was not able grow and produce disease condition on the inoculated healthy oranges. A total of 793 fungi isolate were recorded, namely *Aspergillus* 300 (38.8 %), *Penicillium* 144 (17.2 %), *Fusarium* 212 (26.9 %), *Cladosporium* 63 (7.6 %) and *Rhizopus* 74 (9.5 %) in decereasing order of dominance. All identified fungi occurred in all rotten tomatoes procured from five markets in Abakaliki, though with varied frequency (Dimphna *et al*. 2016). They found, *Mucor* sp. was the most prevalent fungal isolate with 52.7% while *Fusarium* sp. was the least prevalent with 5.5%. The finding in this study of *Mucor* sp. and *Aspergillus* sp. as the most prevalent tomato fruit spoilage fungi is similar to an earlier report of Dennis *et al*, 1980.

Aspergillus niger had the highest percentage occurrence of 47.27% in the spoilt tomato fruits examined, while *Saccharomyces cerevisiae* and *Geotrichum candidum* each had the lowest percentage occurrence of 3.64% in the fruits studied. The result agreed with the work of Akinmusire *et al*, 2013 and Ibrahim *et al*, 2015. They reported that *Aspergillus niger* had the highest rate of occurrence in the tomato fruits and concluded that the fungus may be the major organism responsible for the spoilage of tomato fruits. Tafinta *et al* (2013) reported a frequency of occurrence of 36%, 25%, 22%, and 17% for *R. stolonifer, A. flavus, A. fumigatus,* and *A. niger*, respectively, from sweet oranges.

The similar work done by Tafinta *et al.* (2013) where they reported that *A. fumigatus, A. niger, A. flavus* and *R. stolonifer* and some yeasts were found in the spoilt sweet orange fruits sold in Sokoto State, Nigeria. Some of these pathogens have been reportedly isolated from Pawpaw fruits in Nigeria (Baiyewu *et al.,* 2007; Chukwuka *et al*., 2010). Out of the fungi isolated, *R. stolonifer* had the highest frequency of occurrence (36%) followed by *A. flavus* (25 %) then *A. fumigatus* (22%) and *A. niger* with 17 % frequency of occurrence. This is however in agreement with Ifeanyi, (1995) and Bello (2010) whom both isolated about seven different fungal genera from different fruits including sweet orange fruits.

Postharvest tomato diseases were identified by visual observations and investigations. The identified diseases were Fusarium rot of tomato, Aspergillus ear rot of tomato, Aspergillus black mold disease, Black spot disease of tomato, Blue mold disease, Rhizopus rot disease and sour rot disease of tomato.

The fungal pathogens associated with storage diseases of tomatoes were *Fusarium oxysporum, Aspergillus niger, Trichoderma* sp and *Geotrichum candidum* (Ogo-Oluwa and Kator, 2016; Samuel and Orji, 2015; Etebu *et al.,* 2013; Ignjatov, 2012). During storage various diseases destroy the tomatoes such as black mould

rot (*Aspergillus niger*), Fusarium rot (*Fusarium* sp), gray mold rot (*Botrytis cinerea*), Aspergillus rot (*Aspergillus flavus*), blue mold (*Penicillium* sp), Phoma rot (*Phoma* sp), sour rot (*Geotrichum candidum*), Anthracnose (*Colletotrichum* sp) (Rodrigues and Kakde, 2019).The soft rot of tomato caused by *Bacillus* sp (Al-Allaf, 2010). Postharvest diseases caused by bacterial pathogens include the species of soft rotting genera *Erwinia*, *Clavibacter*, *Xanthomonas*, *Ralstonia*, *Pseudomonas* (Lemma *et al.*, 2014).

The cultural characteristics of the fungi were also recorded. Pathogenicity test of some pathogen were also precisely Samues Orji, (2015). The inoculated tomato fruits and the controls were placed in sterile polythene bags (one fruit per bag). Each of the fruits was moistened with wet balls of absorbent cotton wool to create a humid condition. The fruits were thereafter incubated at 28℃ for five days and observed for spoilage. The fungi were re-isolated from the fruits and compared with the original isolates. The decay rate of each fungus in the healthy fruits was also determined by measuring its rot diameter after two weeks of its inoculation into the healthy tomato fruit.

The present study revealed that seven fungi viz. *Fusarium oxysporum*., *Aspergillus niger*, *Aspergillus flavus*, *Geotrichum candidum*, *Penicillium* sp., *Rhizopus stolonifer*, *Alternaria solani* have been found to cause postharvest disease of tomato. These pathogens lead to enormous loss of tomato fruits not only in terms of quantity but also reduce its economic and nutritive value. Some of these fungi are capable of producing mycotoxins which are hazardous to the health of consumers. Therefore, attention is required for the disease, thereby increasing the economic yield of the produce. This will ensure substantial contribution of the crop to food supply and national economy.

CHAPTER VI

SUMMARY AND CONCLUSION

The present study was conducted to isolate and to identify the fungi associated with the spoilage of postharvest tomato fruits in different local markets in Dhaka city. The experiment was conducted in the Mycology laboratory of Department of the Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period from October 2019 to September 2020. The diseased samples were collected from local market places of Dhaka city. Seven fungal species were identified in this study. The fungal pathogens were isolated from infected samples and were kept in the moist chamber. Then it transferred into PDA medium for their growth. The fungi namely *Aspergillus niger* (Aspergillus black mold disease), *Aspergillus flavus* (Aspergillus ear rot), *Fusarium oxysporum* (Fusarium rot), *Geotrichum candidum* (Sour rot), *Penicillium* sp (Blue mold disease), *Rhizopus stolonifer* (Rhizopus rot), and *Alternaria solani* (Black spot disease of tomato) were identified.

In this study, experiment conducted by two seasons such as winter and summer season. In the both of the winter and summer season, highest percentage incidence occurred in Farmgate bazar (48.89%), whereas the lowest percentage incidence occurred in Taltola bazar (20%). All the seven fungi were found in both the seasons. These were *Fusarium oxysporum, Aspergillus niger, Aspergillus flavus*, *Geotrichum candidum*, *Penicillium* sp, *Rhizopus stolonifer* and *Alternaria solani*.

In winter season, highest percentage frequency of occurrences on infected tomato fruits occurred in *Fusarium oxysporum* (26.96%) followed by *Aspergillus niger* (25.84%) and *Rhizopus stolonifer* (17.97%). Lowest percentage frequency of

occurrences on infected tomato fruits occurred in *Geotrichum candidum* (2.24%) followed by *Penicillium* sp (4.49%) and *Aspergillus flavus* (7.86%).

In summer season, *Alternaria solani* (28.57%) had the highest percentage frequency of occurrence in the infected tomato fruits examined followed by *Aspergillus niger* (19.48%) and *Fusarium oxysporum* (18.18%), while *Penicillium* sp and *Geotrichum candidum* each had the lowest percentage frequency occurrence of (3.89%).

In the present study the diseases were identified by visual observations and investigations. The diseases name was Fusarium rot of tomato, Aspergillus ear rot of tomato, Aspergillus black mold disease, Black spot disease of tomato, Blue mold disease, Rhizopus rot disease and sour rot disease of tomato.

The pathogens were identified on the basis of morphological, cultural and microscopic studies. All isolates showed remarkable variations in respect of their visual symptoms and morphological characteristics in different media. In respect of cultural characteristics, all the isolates of the causal agents showed variation in relation to mycelial growth, colony colour, shape, textures, subsurface colour. Pathogenicity test was done by fresh fruits inoculation which was compared with the isolated pathogen.

The present study revealed that seven fungi have been found to cause diseases during postharvest storage condition. These pathogens lead to enormous loss of the tomato fruits not only in terms of quantity but also reduce its economic and nutritive value. Some of these fungi are capable of producing mycotoxins which are hazardous to the health of consumers. Therefore, attention is required for the disease, thereby increasing the economic yield of the produce. This will ensure substantial contribution of the crop to food supply and national economy.

The present study was based only on isolation ans identification of fungi associated with the spoilage of postharvest tomato fruits in different local markets in Dhaka city. Further studies are needed to increase storage period and post-harvest disease management of tomato fruits.

From the above study, the following conclusions could be done:

1. Seven fungi were isolated and identified from collected infected tomato fruits from both the seasons. They were *Fusarium oxysporum*, *Penicillium* sp, *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifera*, *Alternaria solani* and *Geotrichum candidum*.
2. The highest incidence was found in samples collected from Farmgate (48.89%) and lowest in Taltola (20%) in the both seasons.

CHAPTER VII
RECOMMENDATIONS

Good quality control measures must therefore be employed in both seasons. Frequent inspection of the fruits for sale by food inspectors is also recommended. These will go a long way in preventing the consumption of contaminated tomato fruits thereby reducing the health hazards posed by the mycotoxins produced by these fungi isolated in this study.

More studies should be carried out of other local markets in Dhaka city to determine whether seasons correlate with pathogen occurrence. There is need for careful handling of the produce during and after tomato fruits harvesting to avoid injuries that allow penetration of pathogens. Consumers should be made aware of the effects of consuming cheap pathogen contaminated fruits.

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APPENDICES

Preparation of the culture media:

The composition of the media which is used in this thesis work are given below:

At 1210C for 20 minutes all media are autoclaved at 15 lb pressure.

Potato Dextrose Agar (PDA)

Peeled potato extracts	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	1000 ml

Variable 1: Healthy fruits in winter season

Analysis of variance table

Degree of prob	Sum of Source	Freedom	Squares	Mean Square	F-value
r	2	0.11	0.056	0.22	0.8083
1	5	43.61	8.722	34.13	0.0000

Error	10	2.56	0.256
Non-additivity	1	0.00	0.000
Residual	9	2.56	0.284
Total	17	46.28	

Grand Mean= 9.611 Grand Sum= 173.000 Total Count= 18

Coefficient of Variation= 5.26%

Variable 2: Infected fruits in winter season

Analysis of variance table

Degree of prob	**Sum of Source**	**Freedom**	**Squares**	**Mean Square**	**F-value**
r	2	0.33	0.167	0.71	0.5129
1	5	41.33	8.267	35.43	0.0000

Error	10	2.33	0.233
Non-additivity	1	0.00	0.004
Residual	9	2.33	0.02
Total	17	44.00	

Grand Mean= 5.333 Grand Sum= 96.000 Total Count= 18

Coefficient of Variation= 9.06%

Variable 3: Percentage incidence in winter season

Analysis of variance table

Degree of prob	Sum of Source	Freedom	Squares	Mean Square	F-value
r	2	14.81	7.404	0.71	0.5132
1	5	1837.01	367.401	35.42	0.0000

Error	10	103.74	10.374
Non-additivity	1	0.18	0.176
Residual	9	103.56	11.507
Total	17	1955.56	

Grand Mean= 35.556 Grand Sum= 640.010 Total Count= 18

Coefficient of Variation= 9.06%

Variable 4: Healthy fruits in summer season

Analysis of variance table

Degree of prob	Sum of Source	Freedom	Squares	Mean Square	F-value
r	2	0.78	0.389	0.39	0.6848
1	5	34.94	6.989	7.07	0.0045

Error	10	9.89	0.989
Non-additivity	1	0.70	0.695
Residual	9	9.19	1.022
Total	17	45.61	

Grand Mean= 9.722 Grand Sum= 175.000 Total Count= 18

Coefficient of Variation= 10.23%

Variable 5: Infected fruits in summer season

Analysis in variance table

Degree of prob	**Sum of Source**	**Freedom**	**Squares**	**Mean Square**	**F-value**
r	2	0.78	0.389	0.39	0.6848
1	5	34.94	6.989	7.07	0.0045
Error	10	9.89	0.989		
Non-additivity	1	0.70	0.695		
Residual	9	9.19	1.022		
Total	17	45.61			

Grand Mean= 5.278 Grand Sum= 95.000 Total Count= 18

Coefficient of Variation= 18.84%

Variable 6: Percentage incidence in summer season

Analysis of variance table

Degree of prob	**Sum of Source**	**Freedom**	**Squares**	**Mean Square**	**F-value**
r	2	34.61	17.305	0.39	0.6845
1	5	1553.29	310.658	7.07	0.0045

Error	10	439.40	43.940
Non-additivity	1	31.00	31.002
Residual	9	408.40	45.378
Total	17	2027.31	

Grand Mean= 35.185 Grand Sum= 633.330 Total Count= 18

Coefficient of Variation= 18.84%