POSTHARVEST DISEASES OF SOME SELECTED SPICES, THEIR CAUSES AND MANAGEMENT

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This is to certify that the thesis entitled, **"POSTHARVEST DISEASES OF SOME SELECTED SPICES, THEIR CAUSES AND MANAGEMENT"** submitted to the **DEPARTMENT OF PLANT PATHOLOGY**, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) IN PLANT PATHOLOGY**, embodies the result of a piece of bona-fide research work carried out by Sifat Hosen, Registration No. 18-09050 under my supervision and my guidance. No part of this thesis has been submitted for any other degree or diploma.

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

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POSTHARVEST DISEASES OF SOME SELECTED SPICES, THEIR CAUSES AND MANAGEMENT

ABSTRACT

A study was conducted to observe the prevalence of postharvest diseases of some selected spices (onion, garlic, ginger and chilli) and to identify their causes in storage. The experiment was conducted in the MS Laboratory of Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period of March, 2019- March, 2020. Spice samples were collected from five noted whole sale markets of Dhaka and from farmers field of Faridpur district. During each visit one (1) kg of samples of each spice was collected randomly from each of the market places. Sample collection was carried out thrice. Diseased spice samples were sorted out. The most frequent diseases found in the collected samples were black mould, blue mould, soft rot, *Fusarium* dry rot, anthracnose etc. Pathogens were isolated by tissue planting method and dilution plate method for fungi and bacteria, respectively. Several biochemical tests like Gram's staining, KOH solubility, starch hydrolysis, oxidase, catalase, motility, casein hydrolysate, citrate utilization, lactose, sucrose, dextrose tests were conducted to identify the bacteria isolated from collected samples. This study revealed that collected samples were infected by *Fusarium oxysporum* causing *Fusarium* dry rot of ginger and chilli (3.97%), *Aspergillus niger* causing black mould of onion and garlic (8%), *Aspergillus flavus* causing green mould of onion (2%), *Penicillium* sp causing blue mould of onion (5.90%), *Colletotrichum capsici* causing anthracnose of chilli (3.5%), *Bacillus subtilis* causing soft rot of onion (3.68%), *Pseudomonas aeruginosa* causing soft rot of garlic (5%), *Pseudomonas geniculata* causing soft rot of ginger (3%), *Serratia marcesens* causing soft rot of garlic (3%). Identification of the bacteria was confirmed by extracting DNA from the bacterial cultures. The DNA samples were subjected to PCR using primer 27F: AGA GTT TGA TCM TGG CTC AG and primer 1492 R: CGG TTA CCT TGT TAC GAC TT, which produced around 1465-bp amplicons that were purified and sequenced using the same primers. DNA sequences of D1/D2 domain of 16s rDNA sequences of isolated bacteria were submitted to National Center for Biotechnology Information (NCBI) for deposition in the GenBank and obtain accession number. The obtained accession no. MW404211 for *Pseudomonas aeruginosa* strain SAU-2, accession no. MW404212 for *Pseudomonas geniculata* strain SAU-3, accession no. MW404213 for *Serratia marcescens* strain SAU-4. However, further research needed to conduct to observe the diversity of the postharvest diseases of spices for the potential control of postharvest diseases of spices. Spices can store in paper bag up to 30 days at average 29^0C.

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LIST OF SYMBOLS AND ABBREVIATIONS

No. = Number

% = Percentage

et al. = And others

^{o}C = Degree Celsius

@ = At the rate

etc. = Etcetera

J. = Journal

Viz. = Namely

Cm = Centimetre

Cfu = Colony forming unit

& = And

ppm = Parts per million

Kg = Kilogram

G = Gram

ml = Millilitre

hr = Hour (s)

i.e. = That is

T = Treatment

cv. = Cultivar (s)

var. = Variety

mm = Millimetre

µl = Microliter

µm = Micrometre

SAU = Sher-e-Bangla Agricultural University

BBS = Bangladesh Bureau of Statistics

USA = United States of America

PDA= Potato Dextrose Agar (media)

NA = Nutrient Agar (media)

DAS= Days After Storage

Chapter I

INTRODUCTION

A spice is a seed, fruit, root, bark, or other plant substance primarily used for flavoring, coloring or preserving food. Many spices have antimicrobial properties. Studies (CAST 1984; Elias & Hossain, 1984) show that spices are more profitable compared with their competing crops. Spices are sometimes used in medicine, religious rituals, cosmetics or perfume production, or as a vegetable. There are about 27 varieties of spices grown in Bangladesh. Among them five major varieties of spice namely onion, garlic, chili, ginger and turmeric are common in Bangladesh cropping system. Annual productions of the five major varieties of spices namely onion, garlic, chili, ginger and turmeric are 12.25 lakh metric tonnes, which cover 63.15 percent of total demand. Rest of the demand is met up by importing 3.98 lakh metric tonnes per annum (Spices Research Centre, 2005).

Onion (*Allium cepa* L.) is one of the major spices crops in Bangladesh and is the queen of the kitchen (Selvaraj, 1976). Onion has many uses as folk medicine and recent reports suggests that onion plays an important role in preventing heart diseases and other ailments (Augusti, 1990). It is cultivated during kharif, late kharif and rabi seasons as an important crop and is used in raw form as salad and also cooked as vegetable (Singh *et al*. 1994). About 300.0 thousand acres of land in the country is under onion cultivation and the production is about 1052.0 M ton (BBS, 2011). However, the average yield rate is 3.33 M ton per acre, which is lower as compared to many other onions producing neighboring countries.

The most serious loss arises from storage rots due to bulb rotting microorganisms and also from unwanted sprouting (Jones and Mann, 2013). About 15 different fungal species are reported responsible for the onion diseases in the storage and transit all over the world for which the loss may go up to 40% (Aiyer, 2008). The most destructive diseases in storage are black mould rot (*Aspergillus niger*), blue mould rot (*Penicillium* spp.), *Fusarium* bulb rot (*Fusarium* spp.), basal rot (*Fusarium monilifome*), *Aspergillus* rot (*Aspergillus* spp.) etc. *Aspergillus niger* and *Aspergillus flavus* infect bulbs at high temperature with high relative humidity while *Penicillium* spp. may destroy bulbs even at

low temperature. Infections by *Penicillium* spp. may produce the mycotoxin Penitrem A, which has been recently implicated in tremorgenic toxicosis (Overy *et al.* 2005) in man and animals. Likewise, *Aspergillus flavus* infection may produce aflatoxin which is poisonous to human health. The objective of this experiment is to identify the pathogenic fungal isolates obtained from the naturally infected bulbs.

Garlic (*Allium sativum* L.) is a world's favorite, versatile horticultural commodity consumed for culinary, medicinal and antimicrobial purposes. The famous French chef, X. Marcell Boulestin said, "It is not really an exaggeration to say that peace and happiness begin, geographically where garlic is used in cooking." About one million hectares (2.5 million acres) of garlic produce about 10 million metric tons of garlic globally each year, according to the United Nations Food and Agriculture Organization (FAO). During the storage of garlic, major losses are weight loss (10-15%) and infection to diseases (15-20%). Major postharvest diseases of garlic are blue mould caused by *Penicilliumrory corymbiferum* and black mould caused by *Aspergillus* sp (Dhall *et al.* 2013). The most common storage fungi infecting garlic bulbs are *Aspergillus niger*, *Aspergillus ochraceus, Fusarium proliferatum*, and *Penicillium hirsutum* (Dugan, 2007). Recently, during both world wars I and II soldiers were prescribed to give garlic to prevent gangrene. Now-a-days people use garlic to help to prevent atherosclerosis, improve high blood pressure, and reduce colds, coughs, and bronchitis. (UMM, 2004).
Ginger (*Zingiber officinale*) is widely used around the world in food as a spice both in fresh and dried form. It is now cultivated throughout the humid tropics (Meadows, 1998) and is a most widely used spice worldwide. India is the highest producer of ginger and the annual production is about 2,63,170 tones from an area of about 77,610 hectares, contributing around 30 to 40% of the world production. (Ravindran and Nirmal, 2005). India ranks first with respect to ginger production contributing about 32.75% of the world's production followed by China (21.41%), Nigeria (12.54%) and Bangladesh (10.80%). Ginger accounted for 0.08 percent of total cropped area, occupied 7 thousand hectare and produce 42 thousand metric ton, (BBS, 2004). Estimation of financial profitability shown that ginger appeared to be highly profitable spice crops having net return tk. 2,82,011 per hectare followed by chilli tk. 71,441 per hectare, turmeric tk. 65,423 per hectare, garlic tk. 50,281 per hectare and onion tk. 38,282 per hectare

accordingly, (Huda *et al.* 2008). Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali *et al.* 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan & Vanitha, 2004). Ginger is used as a spice because of its aroma and pungency. Also has been used due to its medicinal properties. (Kubra and Rao, 2012). Ginger is also used in ayurvedic, tibbe-e-unani (Srivasta and Mustafa, 1989), allopathic (Fessenden *et al.* 2001), aromapethy (Shelly *et al.* 2004) and household medicines (Sloand and Vessey, 2001). Ginger rhizome can also be used as fresh paste, ginger tea, dried powder and preserved slices. (El-Ghorab *et al.* 2010). It can be utilized in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup. (Maxwell, 2008). Ginger is also valued for the extraction of oil, essences, oleoresin, tinctures etc. (Pruthi, 1998). In storage, ginger is severely infected by storage soft rot, (Meenu *et al.* 2017). During storage, different fungi have been found associated with the ginger rhizomes, which result in rotting and decaying of the rhizomes (Dohroo, 1993). These fungi include *Fusarium oxysporum* (Sharma and Jain, 1977), *Geotrichum candidum* (Mishra and Rath, 1989), *Aspergillus flavus* (Geeta and Reddy, 1990) and *Verticillium chlamydosporium* (Dohroo and Sharma, 1992).

Chilli (*Capsicum annuum* L.*)* is an important spice crop of Bangladesh is widely grown both in winter and summer seasons. Area under chilli cultivation was 93.55 thousand hectares producing about 102.25 thousand tons in the year 2012-13 (BBS, 2015). Chilli is used in green and dried forms. It is mostly liked for its pungency, spicy taste and the appealing color, it adds to the food (Mathukrishnan *et al.* 1993). Chilli is also used as pickles, sauces and other beverages (Purseglove *et al.* 1991). It has medicinal values too. Chilli is one of the principal ingredients in the Bangladesh kitchen as curry powder and paste. Among the large number of diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum* species and bacterial wilt by *Pseudomonas solanacearum* have been most detrimental to chilli production (Than *et al.* 2008). Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre and post-harvest fruit decay. (Hadden and Black, 1989; Bosland and Votava, 2003).

Postharvest losses of spices are due to fungi, bacteria, viruses and insects. Massive storage loss has occurred due to rotting fungal pathogens (Taskeen-un-Nisa, 2010). Bacterial soft rot is considered as one of the most destructive diseases of vegetables in storage and transit conditions. However, other causes are continuous metabolism and growth, water loss, physiological disorders and mechanical damage. The postharvest losses of spices, viz., sprouting, rotting and physiological loss in weight pose a great problem. It is reported that annual storage losses were over 40 percent (Bhagachandani *et al.* 2000) and between 40 to 60 per cent (Maini *et al.* 2000) in India. Significant losses in quality and quantity of spices occur during storage. Storage therefore, becomes a serious problem in the tropical countries like Bangladesh. Keeping this in view, efforts are being made to overcome these postharvest problems and to reduce the rate of deterioration. Physical treatments have been widely applied without impairing the keeping quality of spices and have gained great interest in recent years to control many postharvest diseases because the total absence of residues in the treated product and minimal environmental impact. Physical treatments, like use of cold storage, heat, hot water and hot air treatments, radio frequency and microwave, hypobaric and hyperbaric pressure and far ultraviolet radiation (UV-C light), are treated as promising control means, and controlled and modified atmospheres as complementary physical tools essential to reduce or delay the development of postharvest pathogens.

Objectives

The present research work was therefore carried out with the following specific objectives:

1. To isolate and identify the principle causes of postharvest diseases of selected spices.

2. To find out the efficacy of different storage bags in controlling postharvest diseases of selected spices.

3. To characterize the bacteria through molecular methods.

Chapter II

REVIEW OF LITERATURE

2.1. Present status of spices in Bangladesh

In Bangladesh, the total area under the spice's cultivation is 3.96 lakh hectares with annual production of 24.88 lakh metric tons. Spices cover almost 2.60 percent of total cropped area in Bangladesh. In recent year, the production rate of major spices like onion, garlic, chilli and ginger are 17.35, 3.82, 1.30 and 0.77 lakh tons respectively. In Bangladesh, the average price of onion is 27180 taka/ton, garlic is 110910 taka/ton, chilli is 195990 taka/ton and ginger is 74490 taka/ton. (BBS, 2016).

According to FAO (2014) annual report, annual production and consumption of spices has been growing rapidly.

Kumar *et al.* (2011) defined that spices are a broad term used to describe herbal by-products that add flavor and aesthetic, aromatic and therapeutic treatments to food, drink and other items.

Islam *et al.* (2011) conducted a research on profitability and resource use efficiency of producing major spices in Bangladesh and said that major spices are regularly used in daily diet at large amount like onion, garlic, chilli and ginger.

Miah *et al.* (2011) stated that the annual area, production and yield of spices were estimated at 7.14%, 9.90% and 2.76% during 1999-2000 to 2008-2009, respectively.

Ali and Haque (2011) reported that spice is one of the world's largest crop and also an important food crop in Bangladesh and has recently occupied an important place in the list of major crops in Bangladesh.

Hossain and Miah (2010) studied on storage behavior and drying characteristics of commercial cultivars of some spices and stated that Bangladesh ranks 11th in the world in terms of spice production in 2008.

Noor *et al.* (2008) conducted a research on integrated nutrient management for sustainable yield of major spice crops in Bangladesh and supported that there is a limited scope to increase production of spice in Bangladesh.

CAST (1984) a background report on production and marketing of spices in Bangladesh stated that the area and production of major spices remained constant or declined over the years in Bangladesh.

The imported cost of onion is 19300 taka/ton, garlic is 163980 taka/ton and ginger is 64460 taka/ton. The cultivation area under onion production is 439 thousand acre and annual production is 1735 thousand ton in 2015-16 (BBS, 2016).

Garlic is cultivated in 60728 ha land and total 82 thousand ton of it is produced with an average yield of 6.29 ton /ha (BBS, 2016).
Nowadays, world trade in garlic is dominated by the developing countries and their share of trade has been growing at the expense of that of the developed countries during the past ten years (FAO, 2004).

The cultivated area under chilli production is 252 thousand acres and the production is 130 thousand ton and the average yield is 1.32 ton/ha. (BBS, 2016).
The area cultivated with chilli is about 1 700 000 ha for producing fresh chilli, and around 1 800 000 ha for producing dried chilli; a total area of 3 729 900 ha with a total production of 20 000 000 ton. (FAO, 2003).

In Bangladesh, ginger occupies an area of about 23 thousand acres with the production of 77 thousand tons. (BBS,2016).
The world production of ginger is 20,95,056 tonnes from an area of 3,22,157 hectares, while Bangladesh's share is 7,03,000 tonnes from an area of 1,50,000 hectares (FAO, 2014).

2.2. Postharvest diseases of spices and their effects on spices quality

Nadia *et al.* (2013) carried out a survey in some selected spice growing districts in Bangladesh to know the status of postharvest bacterial diseases in terms of its incidence and severity. The results showed that the highest incidence was recorded in Munshigonj (22.65%); followed by Nilphamari (19.98%) and the lowest incidence was recorded in Jamalpur (9.07%). The highest severity was recorded in Munshigonj (3.80), while the lowest wilt severity was recorded in Jamalpur (2.90).

Spices are attacked by variety of pathogens in the field and as well as in the storage, which degrade its quality and yield. (Anonymous, 2011).

Taskeen-un-Nisa (2010) reported that massive storage loss of spices has occurred due to rotting fungal pathogens.

Fakir (2009) stated that, a few studies on economic aspect of cold storage have been conducted so far in Bangladesh. In a preliminary survey of the diseases of spices in cold storage in Bangladesh it was found that 2-9 percent of cold stored spices were lost in every year due to disease.

In developing countries, the post-harvest losses are more serious owing to postharvest diseases and storage facilities (Sharma *et al.* 2009).

Ross (2008) reported that, an estimated 22% of spices are lost worldwide per year to viral, bacterial and fungal diseases which is equivalent to an annual loss of over 65 million tones.

Ramachandran and Rathnamma, (2006) stated that significant losses have been reported from other parts of the world as well, like a significant amount of 20–80% loss has been accounted from Vietnam (Don *et al.* 2007) and about 10% from Korea (Byung, 2007).

Annual storage losses of spices were over 40%. (Bhagachandi *et al.,* 2000) and between 40-60%. (Maini *et al.* 2000).
A large number of diseases have been reported in onion and cause production loss in the field and also in storage (Munoz *et al.,* 2014; Ahmed and Hossain, 1985; Meah and Khan, 1987).

Most serious loss of onion arises from storage rots due to bulb rotting microorganisms and also from unwanted sprouting. Losses in the range of 10 – 50% due to bulb rot may occur in onion during storage within three months when varieties susceptible to rot organisms are handled. (Jones and Mann 2013; Matthananda 2012).
Tanaka (2011) reported that losses as high as 60% during storage due to moulds have been reported in onion.

Fungi are the main causal agent responsible for pre and postharvest losses in onion. Around 15 different fungal species are reported to be responsible for the onion diseases in the storage and transit all over the world for which the loss may go up to 40% (Currah and Proctor, 2010; Aiyer, 2008).
According to El-Nagerabi and Ahmed (2003), onion production is limited by fungal infections which cause considerable pre- and post-harvest losses.

Gupta and Verma (2002) revealed that around 35-40% onion is lost due to damage caused by different postharvest diseases.

Many postharvest pathogens of garlic may be carried in seed cloves and deteriorated quality. (Sumner, 2015), (Dugan *et.al.,* 2007) and (Schwartz and Mohan, 2006).
Postharvest losses of garlic have been estimated between 40 and 50% and most of them are caused by post-harvest diseases. (Kluge *et al.* 2007).

High humidity in the storages will favor mold growth and rooting of garlic. Mold growth can also be problematic if the garlic has not been well cured before storing (PTRIC, 2005).

Other than the losses due to the pests and pathogens, crop loss of chilli in post-harvest conditions further add in delimiting the yield and production of the crop (Prusky, 2011). Postharvest loss due to pathogen attack in chilli has been reported to be about 20% to about 100% of samples obtained from Turkey (Demircioglu and Filazi, 2010) and Malaysia (Reddy *et al.* 2011).

The loss is high owing to the post and pre harvest involvement of the pathogen causing a loss of 10–80% of the marketable yield of chilli fruits (Than *et al.* 2008).

Pakdeevaraporn *et al.* (2005) studied on inheritance of resistance to anthracnose caused by *Colletotrichum capsici* and recorded that chilli anthracnose may cause yield losses of up to 50%.

Ginger rhizome is affected by a number of postharvest diseases leading to great crop damage and yield loss. (Sharma and Jain, 2017).

Soft rot found in all the ginger growing countries, reported as the most dangerous and destructive disease of ginger which is responsible to reduce the production by 50–90%. (Sharma *et al.* 2009).

A number of fungal pathogens of ginger have been reported to be associated with the postharvest diseases from time to time. (Mitra and Subramaniam, 2008).

In Bangladesh, a calculated loss of 10–54% has been reported in yield of the crop due to the anthracnose disease (Lakshmesha *et al.* 2005).

2.3. Symptomology of different postharvest diseases of spices

Richard *et al.* (2017) studied on bacterial soft rot is a disease of stored onions and garlic and observed that shortly after the bacteria enter through neck tissues and/or wounds,

affected tissues become pale yellow and water soaked. Advanced cases of soft rot are associated with a watery rot and an unusually bad smell.

Prajapati *et al.* (2015). recorded that in case of black mould rot of onion and garlic clusters of black spores generally form along veins and on or between the outer papery scales of bulbs. Infected tissue first has a water-soaked appearance and over time will dry and shrivel.

In blue mould, symptoms of the disease start as pale blemishes, yellow lesions, and soft spots. A blue-green mold develops on lesions. When bulbs are cut open, one or more of the fleshy scales may be discolored and water-soaked. In advanced stages, bulbs may deteriorate into complete decay. (Mishra *et al.* 2014).

In case of black mold small masses of black spores appear under the outer-most scale of the bulb, and spread following the vein of the bulbs, starting from the base and heading to the neck parts. (Sinclair and Letham, 2006).

The *Penicillium* spp. have typical brush-like conidiophores with long chains of conidia which are blue or green in color. *Penicillium* spp. sometimes produce purple stains on the outer layer of garlic bulbs. Heavy sporulation on the basal part of the garlic bulb is followed by initial infection, with clearly visible massive blue-green spores. (McDonald *et al.* 2004).

The conidia of *Aspergillus niger* are black and spherical, whereas the wall of conidia may be rough or smooth. Conidiophores are long, thick-walled and brownish; black spore clusters are visible without magnification. (Klich, 2002).

Symptoms of soft rot of ginger first appear on rhizome in the form of watery, brown lesions. These lesions then enlarge and coalesce, causing stem rot and collapse (Dohroo, 2005).

Symptom of dry rot of ginger includes discoloration of rhizome surface by fungal mycelia accompanied by dry rotting. Due to the infection of soft rot, rhizomes appear soft, brown, water soaked, rotten, and decay gradually. It is not like bacterial rots, the soft rot caused by fungus does not produce offensive odors. (Agrios, 2005).

In storage fungal mycelia discolored the surface of ginger rhizome accompanied with dry rotting and decaying. (Prusky *et al.* 2005).

The disease is reported to cause fruit rot at both green and red stages primarily attacking ripe fruits, hence is also known by the name ripe fruit rot of chilli (Agrios, 2005).

The fruit lesion is the most economically important aspects of chilli anthracnose as sometimes, even a small lesion on the fruit is enough to lower its market value thereby affecting the yield of the crop (Manandhar *et al.* 2005).

Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 2002).

2.4. Isolation and identification of pathogen causing postharvest diseases

Sang *et al.* (2018) denoted that for the isolation of fungal pathogens from the diseased onions, the sterilized lesion pieces were lightly dried on a sterile filter paper and then transferred onto potato dextrose agar (PDA) media at 25 °C for 4 days or at 4 °C for 4–12 days. The isolated fungi were further purified with a mycelial tip culture using a stereomicroscope. They identified total 68 fungal pathogen, of which *Fusarium proliferatum* (36%), *Penicillium* sp (20%), *Rhizopus oryzae* (11%), and *Aspergillus niger* (33%) were responsible for diseases incidence.

Alternaria alternata, Alternaria porri, Botrytis allii, Botrytis cinerea, Cladosporium sp, Colletotrichum circinans, Drechslera (Pseudocochliobolus) australiensis, Fusarium solani, Fusarium moniliforme, Fusarium avenaceum, Fusarium moniliforme var.

subglutinans, Fusarium oxysporum, Mucor sp, *Pythium* spp., *Rhizoctonia solani, Rhizopus nigricans, Stemphylium botryosum, Stemphylium vesicarium,* and *Trichothecium roseum* were fungal species responsible for onion postharvest diseases. (Köycü and Özer, 2017).

Varga *et al.* (2012) investigated microbiota and mycotoxin contamination of onion bulbs in Hungary and found that all except one of the onion bulb samples tested were contaminated with black *Aspergilli,* which was identified as *Aspergillus* using calmodulin gene analysis. These are the three important fungi or fungal groups causing significant disease losses on onion and garlic.

Shehu and Muhammad (2011) reported *Aspergillus niger* as one of the main causal organisms of postharvest deterioration in onion bulbs in Sokoto, Nigeria.

Mohamed H. Abd-Alla *et al.* (2011) reported that the causal agent of onion soft rot of bulb was identified as *Pseudomonas* based on phenotypic characteristics. One representative isolate of these has been further identified as a *Pseudomonas aeruginosa* by sequencing of the 16S rRNA gene. Virulence of the *Pseudomonas aeruginosa* was also confirmed by the production and secretion of a large variety of enzymes capable of degrading the complex polysaccharides of the plant cell wall and membrane constituents. Scanning electron micrographs indicated that the *Pseudomonas aeruginosa* isolates were able to abundantly colonize the internal tissue of fleshy scales of onion bulbs. The virulence of the pathogen may be attributed to biofilm formation on the plant cell wall via fibrous materials as revealed by scanning electron microscopy as well as high activity of proteases, lipase, pectinases, and alkaline phosphates. This is the first evidence showing that a *Pseudomonas aeruginosa* strain E can cause soft rot of onion bulbs.

The major fungal disease of onion and garlic is black mold, which is caused by black *Aspergilli.* (Varga *et al.* 2008).

Rafika *et al.* (2006) investigated the microflora of red and white onions and found that *Botrytis* spp., *Aspergillus* spp., and *Penicillium* spp. were the major fungal groups.

The predominant fungal pathogens associated with the storage diseases of onions were *Aspergillus* sp, *Penicillium* sp, and *Fusarium* sp. (Velez *et al.,* 2004, Raju and Nail, 2006).

During storage various diseases destroy the onions such as Black mould rot (*Aspergillus niger*), Blue mould rot (*Penicillium* spp.), *Fusarium* bulb rot (*Fusarium* spp.), Basal rot (*Fusarium moniliforme), Aspergillus* rot (*Aspergillus* spp.), Dry rot (*Macrophomina phasiolina*), Soft rot (*Erwinia* spp.), Smudge (*Colletotrichum circinans*), Grey neck rot (*Botrytis allii)*, Green mold rot (*Penicillium* spp.), White rot (*Sclerotium cepivorum*) and Anthracnose (*Colletotrichum chardonianum*). (Rangaswami and Mahadevan, 2004).

Ko *et al.* (2002) evaluated onion cultivars for resistance to *Aspergillus niger* the causal organism of black mould. They stated that black mould caused by *Aspergillus niger* is a common postharvest disease of onion developed under hot and humid conditions.

Aspergillus niger, which infects both white and colored onions (Walker and Lindegren, 1924), has been found at different stages of development, from the germination stage of seeds to the storage of mature bulbs (Hayden and Maude, 2002).

J. Sadeghi-Seraji *et al.* (2018) identified the bacterium that causes soft rot of garlic bulb based on phenotypic and genotypic characteristics, the bacterium was identified as *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) Pcc. This is the first report of soft rot caused by Pcc on garlic from Iran. To further identify these pectolytic strains, a multi locus sequence typing (MLST) approach was employed. To this end, partial nucleotide sequences of the housekeeping genes, mdh (GenBank accession No. MG421001), recA (MG421002), gapA (MG421003) and 16S rRNA (MG388299) showed 99, 99.2, 98.9 and 99.8% similarity to the Pcc ATCC 15713 reference strain, respectively.

Dugan (2007) investigated that some species of fungal pathogens can remain quiescent in garlic tissues for one or more generations. Whereas, some fungal pathogens can make sclerotia and others survive in plant debris or soil.

The most common storage fungi infecting garlic bulbs, those are *Aspergillus niger, Aspergillus ochraceus, Fusarium proliferatum* and *Penicillium hirsutum*. Different *Penicillium* spp. such as *Penicillium aurantiogriseum, Penicillium expansum, Penicillium citrinum, Penicillium digitatum, Penicillium hirsutum, Penicillium funiculosum,* and *Penicillium oxalicum* cause blue mold on onion and/or garlic (McDonald *et al.,* 2004; Overy *et al.* 2005).

In India, primarily three important species, namely, *Colletotrichum capsici, Colletotrichum acutatum* and *Colletotrichum gleosporoides* have been reported to be linked with the disease, with *Colletotrichum capsici* causing major damage at the ripe fruit. (Ranathunge *et al.,* 2012; Saxena *et al.* 2014).

Colletotrichum spp. has been rated as one of the ten most notorious pathogens in the world, causing heavy crop losses of chilli worldwide, which is an asexual genus belonging to phylum Ascomycete and Coeleomycetes class of fungi imperfectii (Dean *et al.* 2012).

Anthracnose of chilli has been shown to be caused by more than one *Colletotrichum* spp. including *Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides* and *Colletotrichum coccodes*. (Simmonds, 1965; Johnston and Jones, 1997; Kim *et al.,* 1999; Nirenberg *et al.,* 2002; Voorrips *et al.,* 2004; Sharma *et al.*, 2005; Pakdeevaraporn *et al.*, 2005; Than *et al.* 2008).

Among the large number of postharvest diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum spp.* considered most detrimental to chilli production (Than *et al.* 2008).

In chilli anthracnose appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Bailey and Jeger, 2002).

Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits (Prusky and Plumbley, 1992).

In the *Colletotrichum* patho-system, different *Colletotrichum* spp. can be associated with anthracnose of the same host (Simmonds, 1965; Freeman *et al.,* 1998; Cannon *et al*. 2000).

The major causes of postharvest loss from decay of ginger rhizomes include *Fusarium oxysporum f.sp. zingiberi* was reported in Hawaii, Australia and Korea (Trujilo, 1964; Stirling, 2004; Farr and Rossman, 2010).
Prusky *et al*. (2005) reported that during storage, rhizomes rot is caused by fungi and bacteria.

A. M. Stirling (2004) stated that the bacterium responsible for soft rot of ginger in Australia was identified as *Erwinia chrysanthemi*. This pathogen is part of a disease complex that has been responsible for poor establishment of ginger planting material.

Chapter III

MATERIALS AND METHODS

3.1. Experimental site

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

3.2. Experimental period

The experiment was conducted during the period of March, 2019 to March 2020.

3.3. Collection of samples

Samples were collected from different whole sale markets of Dhaka namely Kawran bazar, Town hall market, Mirpur-1, Mirpur-10 and Khilkhet bazaar and also from the farmers field of Faridpur district during the time of March-April 2019. During each visit 1 kg of each samples were collected by random sampling from each market place and samples were collected thrice. Total samples were investigated carefully and diseased samples were separated from healthy ones.

3.4. Measurement of prevalence of disease occurrence

Disease incidence of infected samples was determined by the following formula

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected samples}}{\text{No. of total samples observed}} \times 100$$

3.5. Preservation of samples

The collected samples were washed properly to remove the soils and dust. After that samples were surface sterilized with 70% ethanol, dried and kept in a poly bag and stored in the refrigerator at 5^0-6^0 C for further study.

3.6. Isolation of causal organisms

3.6.1. Isolation of fungal pathogen from infected samples

The previously collected samples were first washed in tap. Then the infected portion along with the healthy portion of the samples were cut into small pieces (0.5-1.0 cm) and surface sterilized with 70% ethanol for 2-3 minutes. Then the pieces were washed with sterilized water thrice and placed on sterilized filter paper to remove excess water adhering to the samples. The pieces were plated in moist chambers aseptically maintaining equal distance. The plates were incubated for 7 days at 25+ 1^0C. After incubation period, the fungal mycelia that grew over moist chamber were taken with the help of sterilized needle and transferred on PDA plates in three replications. Then the plates were incubated for 7 days for 12 hours alternating cycles of light and darkness at 25+ 1^0C temperature. After incubation pure culture of the fungus was identified and preserved in PDA slants at 4+ 1^0C in refrigerator as stock culture for future use.

3.6.2. Preparation of Potato Dextrose Agar (PDA) media

Potato dextrose agar (PDA) medium was prepared as described by Hussain *et al.,* (2012). 200 g peeled potato extract, 20 g dextrose and 20 g agar were taken in a conical flask containing 1000 ml distilled water and mixed well for the preparation of 1liter PDA medium. The opening was sealed with cotton plug. Then it was autoclaved for 20 minutes at 121^0C under 15 PSI pressure. For 250 ml medium 20 drops of 50% lactic acid was added to avoid the contamination of bacteria.

3.6.3. Preparation of Dilution of Bacteria from Samples

Then the infected portion along with the healthy portion of the samples were cut into small pieces (0.5-1.0 cm) and surface sterilized with 70% ethanol for 2-3 minutes. Then the pieces were washed with sterilized water thrice and placed on sterilized filter paper to remove excess water adhering to the samples. The cut pieces were taken in a test tube containing 9ml of sterile water. The samples were macerated and kept for 30 minutes for bacterial streaming to get stock solution. Five test tubes, each with 9 ml of distilled water were taken and marked as 1: 10, 1: 100, 1: 1000, 1: 10000 and 1: 100000 and were kept in a test tube holder sequentially. From stock solution 1 ml of properly mixed sample was drawn into the pipette and transferred to the first tube (1: 10) to make the total volume of 10 ml. This provided an initial dilution of 1: 10 dilution. The dilution was thoroughly mixed by using vortex mixture. Then, 1 ml of mixture was taken from the 1: 10 dilution and transferred into the second tube to get 1:100 dilution. The same process was then repeated for the remaining tube to get 1:100000 dilution.

3.6.4. Preparation of Nutrient Agar (NA)

In a conical flask 28g dehydrated NA medium was added in 1000 ml distilled water for the preparation of 1liter NA medium. The medium was shaken thoroughly for few minutes to mix the components properly. The opening was sealed with cotton plug. It was then autoclaved at 121^0 C under 15 PSI pressure for 15 minutes.

3.6.5. Isolation of bacteria on NA plates through spread plate method

NA plates were marked as 1: 10, 1: 100, 1: 1000, 1: 10000 and 1: 100000 and 0.1 ml of each dilution was spread over NA plates with the help of a sterile glass rod at three replications as described by Goszczynska and Serfontein (1998). The inoculated NA plates were kept in an incubation chamber at $30^0 \pm 1^0$C. The plates were observed after every 24 hours. Then single colony grown over NA plate was selected to re streak on fresh NA plate with the help of a sterile loop to get pure colony.

3.6.6. Growth of bacteria on *Bacillus* agar

Holbrook and Anderson developed *Bacillus Cereus* Agar, which is a highly specific and selective medium for the isolation (Holbrook *et al.* 1980). For *Bacillus* identification 20.5grams of *Bacillus Cereus* Agar was mixed in 475ml distilled water. The mixture was heated to boiling to dissolve it completely. Then the media was sterilized by autoclaving at 15 Ibs pressure and 121^0 for 15 minutes. Then the medium was poured into sterile petridishes. Bacteria were streaked for isolation with a sterile loop and incubated the plates aerobically at $30^0 \pm 1^0$C. for 24-72 hours. Examined the plates daily for colony morphology and growth characteristics.

3.6.7. Growth of bacteria on *Pseudomonas* agar

For *Pseudomonas* identification 46.7grams of Cetrimide Agar was mixed in 1000ml distilled water. The mixture was heated to boiling to dissolve it completely. Then the media was sterilized by autoclaving at 15 Ibs pressure and 1210for 15minutes. Then the medium was poured into sterile petridishes. Bacteria were streaked for isolation with a sterile loop and incubated the plates aerobically at $25+1^0$C for 24-72 hours. Examined the plates daily for colony morphology and growth characteristics.

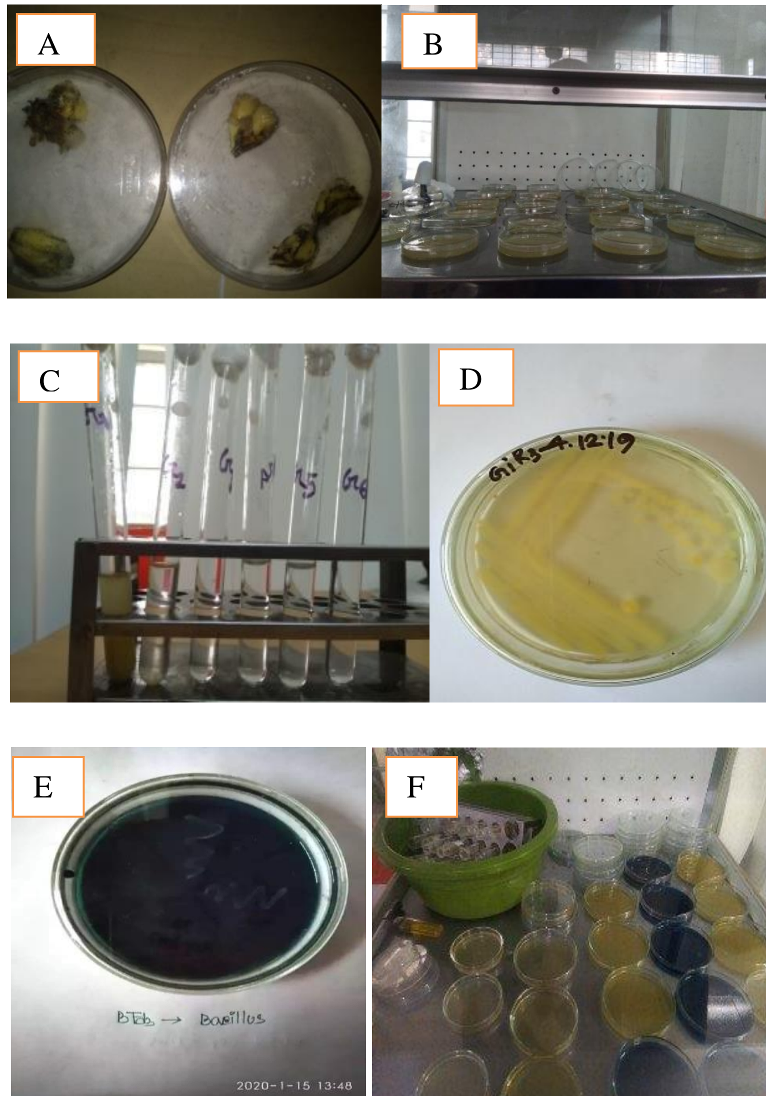

Plate 1. Isolation of causal organisms; A. Incubation of infected parts of samples in moist chamber B. Preparation of PDA media C. Dilution Method for isolating bacteria D. Streaking of Bacteria on NA Medium E. Growth of bacteria on *Bacillus* agar medium F. Preparation of *Bacillus* and Cetrimide Agar

3.6.8. Pathogenicity test

Regarding pathogenicity test apparently healthy samples of uniform size were procured. These samples were washed with tap water to remove dust particles dried. These samples were surface sterilized by dipping in 70% ethanol solution for one minute followed by three times washing in sterile distilled water to remove excesses 70% ethanol. The samples subjected to pathogenicity test were inoculated by following method:

Bacterial cell suspension (10^8 cfu/ ml) was prepared previously from 48hrs old cultures and the surface sterilized selected samples were inoculated by puncturing the sample with the help of tooth pick containing bacterial cell suspension. In case of control the tooth pick was dipped in sterile water then the sample was punctured with that and incubated at room temperature. Observations were recorded on the development of symptoms by constantly observing each and every inoculated samples daily. Any visual changes observed during incubation were recorded.

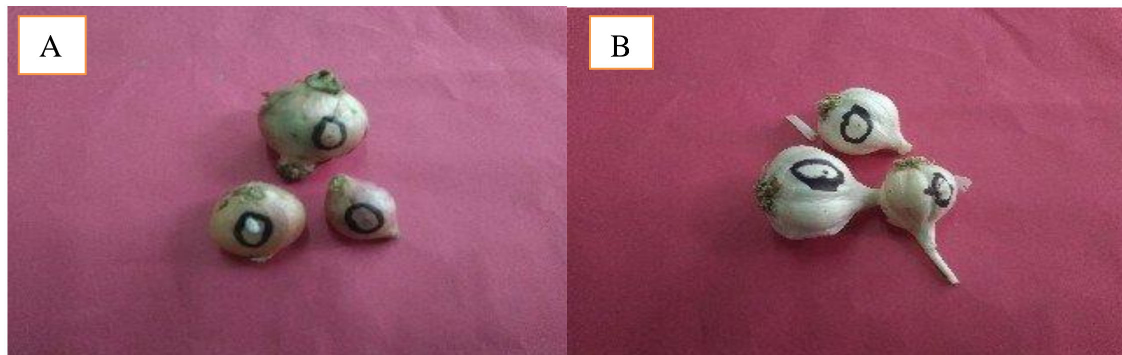

Plate 2. Pathogenicity Test, A. Onion B. Garlic

3.7. Biochemical tests

3.7.1. Gram's staining test

For this test at first on a clean microscopic slide a small drop of distilled water was mounted. Then a minute portion of a young colony (24 hours old) was removed with the help of a sterile loop from the nutrient agar medium and then the bacterial smeare was made on the slide. The thinly spread bacterial film was air dried. Underside of the glass

slide was heated by passing it two times through the flame of a sprit lamp to fix the bacteria on it. Then the slide was flooded with crystal violet solution for about 1 minute. It was rinsed under running tap water for few seconds and excess water was removed by air. Then it was flooded with lugol's iodine solution for about 1 minute. After that it was decolorized with 95% ethanol for 30 seconds and again rinsed with running tap water and air dried. Then it was counterstained with 0.5% safranine for about 10 seconds. It was rinsed under running tap water for few seconds and excess water was removed by air. Then the glass slide was examined under 40x and 100x magnification using oil immersion.

3.7.2. KOH solubility test or Gram differentiation test

KOH solubility test is a rapid method for Gram differentiation of plant pathogenic bacteria without staining (Suslow *et al.,* 1982). For this test two drops of 3% KOH solution were placed at the centre of a clean glass slide. One loopful of young colonies of bacterial pathogen (grown NA medium) were added to the KOH solution and homogenized with a nichrome loop with rapid circular movement of about 10 seconds. Viscous strand formation was observed and on drawing it with a loop it formed a fine thread of slime, 0.4 to 2.5 cm in length.

3.7.3. Catalase test

A few drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) was added with 48 hours old pure culture of bacteria grown on NA plate and observed whether it produced bubbles within a few seconds or not.

3.7.4. Oxidase test

For oxidase test Aqueous solution of (1%) of tetra methyl-p-phenylene-diamine dihydrochloride was used as test reagent. A strip of Whatman filter paper was soaked

with 3 drops of 1% aqueous solution of freshly prepared tetra methyl-p-phenylene-diamine dihydrochloride (color indicator). A loopful of young bacterial culture (24 hours) of each isolate was rubbed separately on the surface of the filter paper by a sterile platinum loop. Development of purple color within 10 seconds, which indicated positive reaction of oxidase test.

3.7.5. Motility test

Motility by bacteria is mostly demonstrated in a semi solid agar medium. In semi-solid agar media, motile bacterium 'swarms' and give a diffuse spreading growth that can be easily recognized by the naked eye. The medium mainly used for this purpose is SIM medium (Sulphide Indole Motility medium). For this test a straight needle was touched to a colony of a young (24 hours) culture growing on agar medium. Then stabbed once to a depth of only 1/3 inch in the middle of the tube and incubated at 35°-37°C and examined daily for up to 7 days to observe for a diffuse zone of growth flaring out from the line of inoculation. Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque indicate positive result. Whereas, growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent indicate negative result.

3.7.6. Casein test

Skim milk agar was used in casein test. For this, inoculated the organism on the plate in a zig-zag manner. Incubated the plate at 30+1°C. Then examined the milk agar plate cultures for the presence or absence of a clear area, or zone of proteolysis, surrounding the growth of each of the bacterial test organisms. Positive Test indicates clearing colony growth around and/or beneath colony growth. Whereas, negative test indicates no clear colony growth around and/or beneath the inoculum.

3.7.7. Citrate test

The Simmon's citrate agar medium was poured into test tubes and prepared slant. For this test agar was inoculated lightly on the slant by touching the tip of a needle to a colony that is 24 hours old. Then incubated at 35°C to 37°C for 24 hours. Observed the development of blue color; denoting alkalinization. In citrate positive growth will be visible on the slant surface and the medium will be an intense prussian blue. The alkaline carbonates and bicarbonates produced as by-products of citrate catabolism raise the pH of the medium to around 7.6, causing the bromothymol blue to change from the original green color to blue. In case of citrate negative trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the un inoculated agar. Only bacteria which can utilize citrate as the sole carbon and energy source will be able to grow on the Simmo's citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

3.7.8. Lactose test

The purple broth consists of peptone with the pH indicator bromcresol purple was used in this test. For this test medium was allowed to warm to room temperature prior to inoculation. Then inoculated the purple broth (with carbohydrate of choice) with isolated colonies from a 24 hours pure culture of the bacteria. At the same time a control tube of purple broth base in parallel with the carbohydrate-based media was also inoculated. Incubated inoculated media aerobically at 35-37ºC for 3 days. This was observed daily for development of a yellow color in the medium. Positive test indicates the development of a yellow color in the medium is indicative of a positive carbohydrate fermentation reaction. Whereas, in negative test lack of yellow color development is indicative of a negative carbohydrate fermentation reaction.

3.7.9. Sucrose test

Several media are available for this. We used phenol red sucrose broth. The medium is a nutrient broth to which 0.5-1.0% sucrose is added. The pH indicator phenol red is red at neutral pH but turns yellow at pH < 6.8. It also changes to magenta or hot pink at pH >8.4. An inoculum from a pure culture was transferred aseptically to a sterile tube of phenol red sucrose broth. The inoculated tube was incubated at 35-37^0 C for 24 hours and the results were determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic.

3.7.10. Starch hydrolysis test

Nutrient Agar plate containing 0.2% soluble starch was inoculated with pure young colonies of bacteria. Then it was incubated at 30^{0}C for 48 hours in incubation chamber. The plates were flooded with lugol's iodine solution and observed whether a clear zone appeared around the colony or not.

Plate 3. Biochemical tests of bacteria

3.8. Observation of the development of postharvest diseases in storage

One kg fresh sample of each spices were collected. The collected samples were then washed properly to remove the soils and dust. Healthy, disease free samples were separated from diseased ones. After that healthy samples were surface sterilized with 70% ethanol, dried and kept in different types of bags (namely, polythene bag, paper bag and net bag) and basket (plastic basket) to observe the development of diseases and determine which bag is more suitable to store samples long. These bags were kept at room temperature. The samples were observed and the temperature was recorded daily up to 1 month.

Plate 4. Storage of samples in different types of bags; A. Onion in polythene bag, B. Garlic in paper bag and C. Chilli in net bag

3.9. Isolating genomic DNA from bacteria

Materials used

i) 1.5ml microcentrifuge tubes

ii) Water bath, 80°C

iii) Water bath, 37°C

iv) Isopropanol, room temperature

v) 70% ethanol, room temperature

vi) Water bath, 65°C (optional; for rapid DNA rehydration)

vii) 50mM EDTA (pH 8.0) (for Gram positive bacteria)

viii) 10mg/ml lysozyme (Sigma Cat. # L4919) (for Gram positive bacteria)

ix) 10mg/ml lysostaphin (Sigma Cat. # L7386) (for Gram positive bacteria)

Procedure used

1ml of an overnight culture was added to a 1.5ml microcentrifuge tube. Then centrifuged it at 16,000 × g for 2 minutes to pellet the cells. Supernatant was removed. 600µl of nuclei lysis solution was added and gently pipetted until the cells were resuspended. Incubated at 80°C for 5 minutes to lyse the cells; then cool to room temperature. 3µl of RNase solution was added to the cell lysate and inverted the tube 5 times to mix. Incubated at 37°C for 60 minutes, then the sample was cooled to room temperature. 200µl of protein precipitation solution was added to the RNase-treated cell lysate and vortex vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. Incubated the sample on ice for 5 minutes. Then centrifuged at 16,000 × g for 3 minutes. The supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol. Gently mixed by inversion until the thread-like strands of DNA formed a visible mass.

Centrifuged at 16,000 × g for 2 minutes. Then carefully poured off the supernatant and drained the tube on clean absorbent paper. 600µl of room temperature 70% ethanol was added and gently inverted the tube several times to wash the DNA pellet. Centrifuged at 16,000 × g for 2 minutes. Carefully aspirated the ethanol. Drained the tube on clean absorbent paper and allow the pellet to air-dry for 15 minutes. 100µl of DNA rehydration solution was added to the tube and rehydrated the DNA by incubating at 65°C for 1 hour. Periodically mixed the solution by gently tapping the tube. Alternatively, rehydrated the DNA by incubating the solution overnight at room temperature and stored the DNA at 8°C.

Determination of phylotype in PCR

Phylotype identification of each isolate was done by PCR. Phylotype specific PCR was carried out in 25 µl final volume of reaction mixture, containing 1x Hot Start Green Master Mix, (dNTPs, Buffer, MgCl2, Taq Pol), Cat: M7432, Origin: Promega, USA, genomic DNA, distilled water and specific forward and reverse primers of targeting specific bands. The following cycling program was used in a thermal cycler: 95^0C for 3mins, 95^0C for 30s, 48^0C for 30s, 72^0C for 90s, 72^0C for 5mins followed by a final extension period of 4^0C overnight. A 5 µl aliquot of each amplified PCR product was subjected to electrophoresis on 2% agarose gel, Cat: V3125, Origin: Promega, USA. The PCR amplification was targeted 1465-bp.

Chapter IV

RESULTS

4.1. Visual symptoms of collected samples

4.1.1. Black mould of onion (*Aspergillus niger*)

In case of black mould small masses of black spores were appeared under the outer-most scale of the bulb, and spread following the vein of the bulbs, starting from the base and heading to the neck parts. The conidia of *Aspergillus niger* were black and spherical. Conidiophores were long and brownish; black spore clusters were visible without magnification. The fungus on PDA medium grew in blackish color, (Plate 5; A, B & C).

4.1.2. Green mould of onion (*Aspergillus flavus*)

Black spores were appeared under the outer-most scale of the bulb. The conidia of *Aspergillus flavus* were brownish and spherical. The fungus on PDA medium grew in greenish color, (Plate 5; D, E & F).

4.1.3. Soft rot of onion

After cutting some portion of the infected sample a foul odour came out. When finger was pressed against the soft and rotted portion of the infected samples watery exudates came out. Large, more or less round growth was observed on PDA medium. Raised, whitish growth was observed on NA medium, (Plate 5; G, H & I).

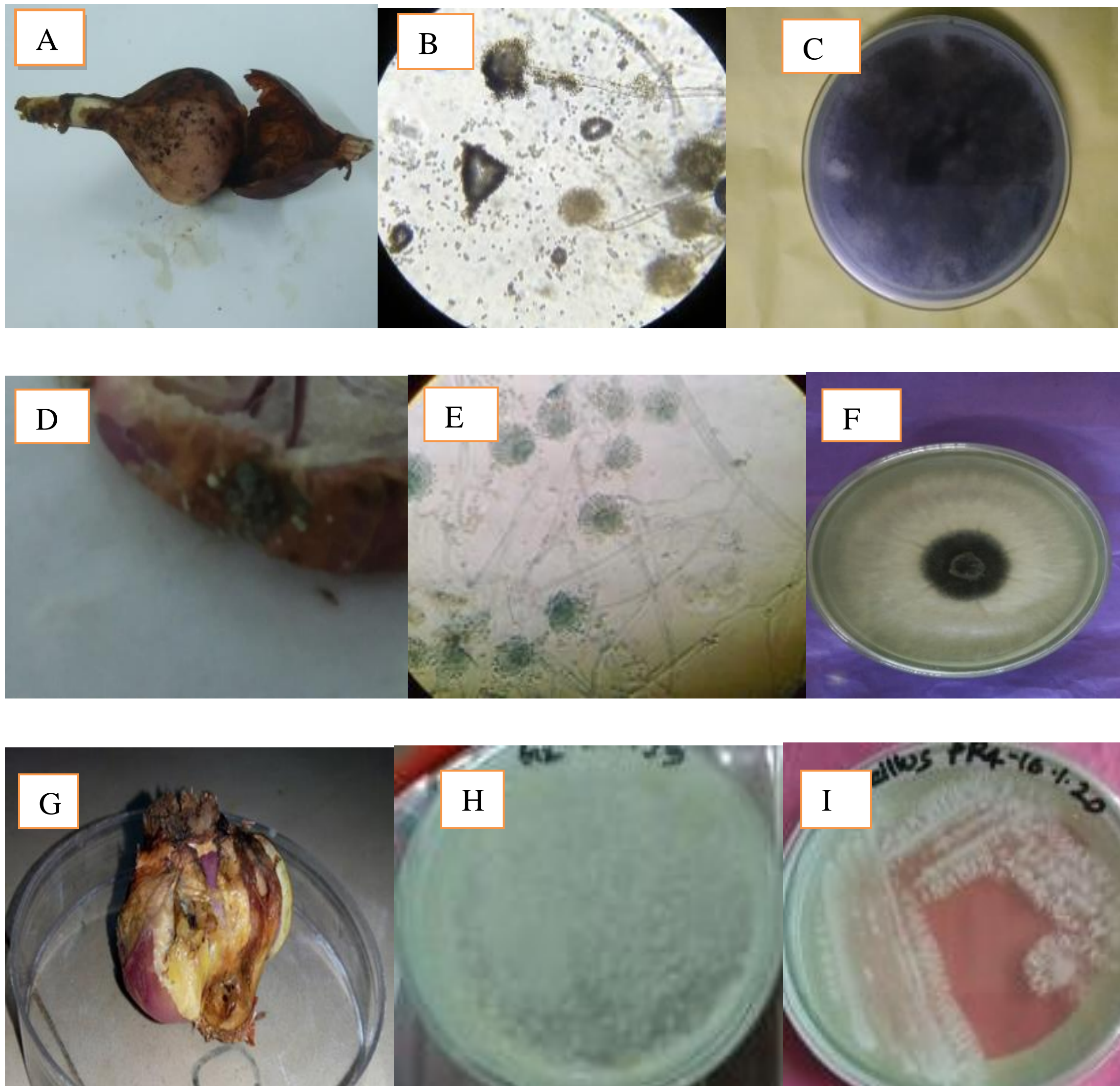

Plate 5. Symptoms of postharvest diseases of onion, their causes and pure culture of the pathogen; A. Black mould of onion; B. Microscopic view of *Aspergillus niger;* C. Growth of *Aspergillus niger* on PDA, D. Green mould of onion, E. Microscopic view of *Aspergillus flavus,* F. Growth of *Aspergillus flavus* on PDA, G. Soft rot of onion, H. Dilution plate of *Bacillus subtilis,* I. Growth of *Bacillus subtilis* on NA.

4.1.4. Black mould of garlic (*Aspergillus niger*)

In case of black mold small masses of black spores appeared under the outer-most scale of the bulb, and spread following the vein of the bulbs, starting from the base and heading to the neck parts. The conidia of *Aspergillus niger* were black and spherical, Conidiophores were long, thick-walled and brownish; black spore clusters were visible without magnification. The fungus on PDA medium grew in blackish color, (Plate 6; A, B & C).

4.1.5. Soft rot of garlic

After cutting some portion of the infected sample a foul smell came out because of the breakdown of cells by the bacteria. When finger was pressed against the soft and rotted portion of the infected samples watery exudates came out. Symptoms of soft rot of garlic first appeared on outer scales in the form of watery, brown lesions. These lesions then enlarge and coalesce, causing bulbs to rot and collapse. Yellowish, flat growth was observed on NA medium. The organism was identified as *Pseudomonas aeruginosa.* Reddish, flat growth was observed in case of *Serratia marcesens* (Plate 6; D, E & F).

4.1.6. *Fusarium* rot of ginger

Rhizomes were discolored brown and had a whitish dry rot of the cortex tissues. Few macros and a lot of micro conidia were found. White, cottony growth was observed on PDA medium, (Plate 6; G, H & I).

4.1.7. Soft rot of ginger

After cutting some portion of the infected rhizome a foul smell came out because of the breakdown of cells by the bacteria. When finger was pressed against the soft and rotted portion of the infected samples watery exudates came out. Yellowish, large growth was observed on PDA medium. Yellowish, flat growth was found on NA medium, the organism was identified as *Pseudomonas geniculata* (Plate 6; J, K & L).

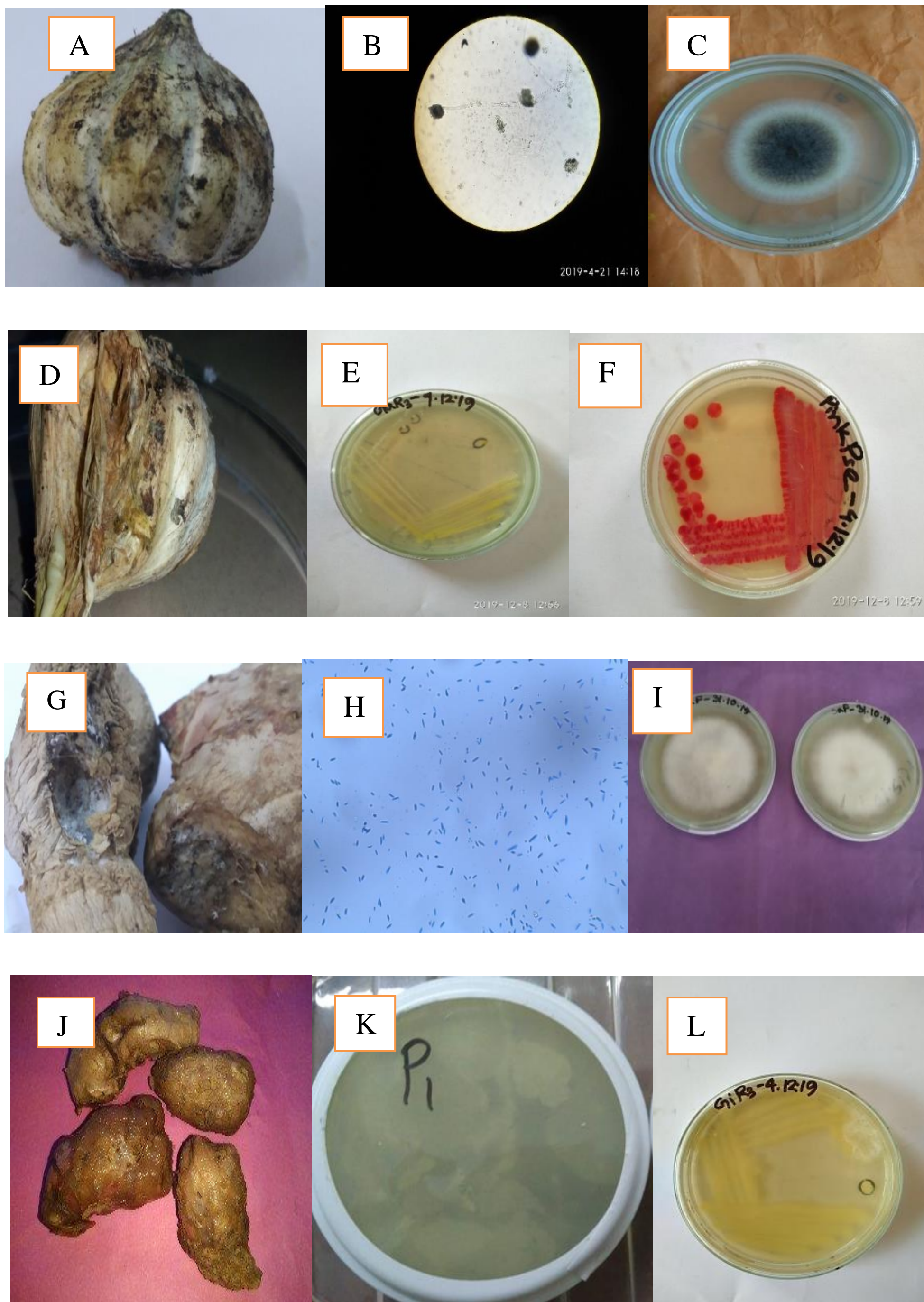

Plate 6. Symptoms of postharvest diseases of garlic and ginger, their causes and pure culture of the pathogen; A. Black mould of garlic; B. Microscopic view of *Aspergillus niger;* C. Growth of *Aspergillus niger* on PDA, D. Soft rot of garlic, E. Growth of *Pseudomonas aeruginosa* on NA*,* F. Growth of *Serratia marcescens* on NA, G. *Fusarium* rot of ginger, H. Microscopic view of *Fusarium* sp*,* I. Growth of *Fusarium* sp on PDA, J. Soft rot of ginger, K. Dilution plate of *Pseudomonas geniculata*, L. Growth of *Pseudomonas geniculata* on NA.

4.1.8. *Fusarium* rot of chilli

Samples were discolored brown and had a whitish dry rot of the surface tissues. Few macros and a lot of micro conidia were found. White, cottony growth was observed on PDA medium, (Plate 7. A, B & C).

4.1.9. Anthracnose of chilli

The disease was characterized by very dark, sunken lesions, containing spores. Brush like growth was observed under microscope. Whitish, flat growth was found on PDA medium, (Plate 7. D, E & F).

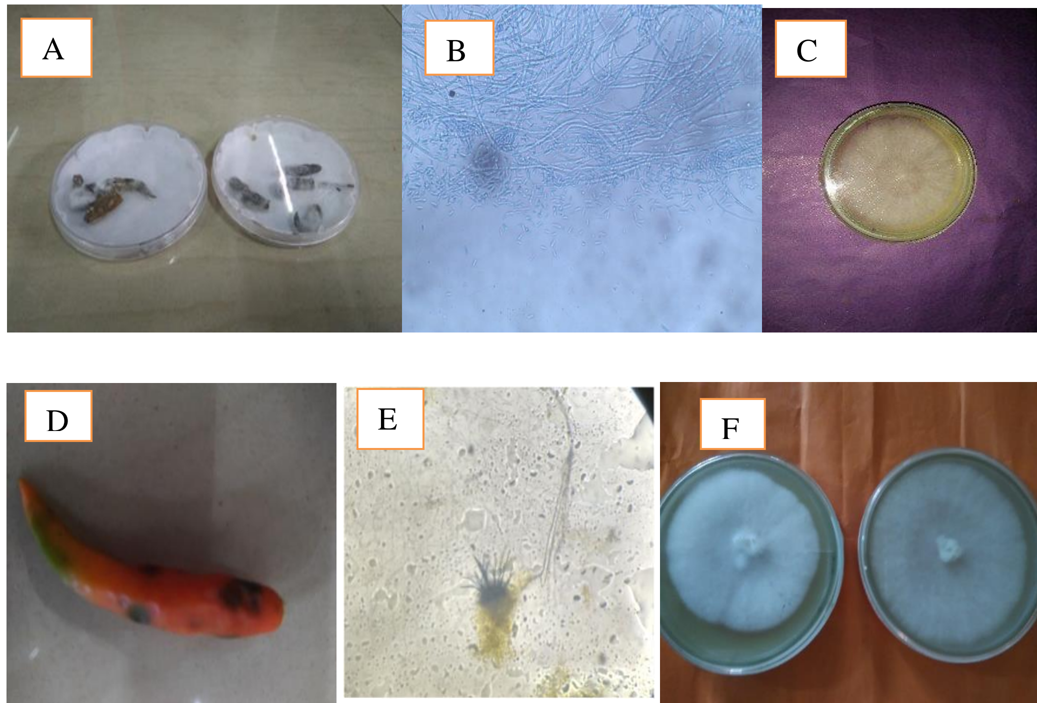

Plate 7. Symptoms of postharvest diseases of chilli, their causes and pure culture of the pathogen; A. *Fusarium* rot of chilli, B. Microscopic view of *Fusarium oxysporum,* C. Growth of *Fusarium oxysporum* on PDA, D. Anthracnose of chilli, E. Microscopic view of *Colletotrichum capsici,* F. Growth of *Colletotrichum capsici* on PDA.

4.2. Identification of *Fusarium oxysporum*

Fusarium oxysporum was identified based on the cultural characteristics on PDA medium and the conidia (macro and micro) produced.

Characteristics of *Fusarium oxysporum* on PDA (Potato dextrose agar) medium:

Mycelia were floccose and abundant. White mycelial color was observed on PDA medium. Some isolates of *Fusarium oxysporum* produced light purple pigment in the agar medium and some isolates produced no pigment at all.

Characteristics of Macroconidia:

Relatively wide, straight and stout. Apical cells are blunt and rounded. Basal cells are foot shaped, straight to almost cylindrical. Number of septa present were 4 to 6 septa. Septa were abundance, usually abundant in sporodochia.

Characteristics of Microconidia:

Shape/septations were oval, ellipsoid, and fusiform with 1 to 2 septa. Aerial mycelium presentation was false heads**,** abundant in the aerial mycelia.

4.3. Isolation and Identification of Different Bacteria

Different cultural, physiological and biochemical tests were conducted and some selective and semi-selective media were also used to identify and differentiate the bacteria.

4.4. Disease incidence

This study revealed that collected samples were infected by *Fusarium oxysporum* causing *Fusarium* dry rot of ginger and chilli (3.97%), *Aspergillus niger* and *Aspergillus flavus* causing black mould and green mould of onion and garlic (10%), *Penicillium* sp causing blue mould of onion (5.90%), *Colletotrichum capsici* causing anthracnose of chilli (3.5%), *Bacillus subtilis* causing soft rot of onion (3.68%), *Pseudomonas geniculata* causing soft rot of ginger (3%), *Pseudomonas aeruginosa* causing soft rot of garlic (5%) and *Serratia marcesens* causing soft rot of garlic (3%). The study revealed the fact that spices are subjected to various postharvest diseases in storage.

4.5. Effect of different storage bags and basket on disease development in storage

Effects of different types of storage bags were observed and recorded on postharvest disease development of selected samples. The samples were observed and the temperature was recorded daily up to 1 month. The temperature was varied from 26-31^{0}C. The study found that paper bag was more suitable than the others to keep the samples in storage. It also absorbed the moisture which was produced by the samples. This helped to prevent the rotting of samples. In paper bags spices can store 1 month or more without any rotting except mould development. But the samples got shriveled as the average temperature during storage was 29^0 C.

Table 1. Effects of different bags on development of post-harvest diseases of selected spices

Spices	Time (Damaged or showed pathogenic growth)		
	Net bag	**Polythene bag**	**Paper bag**
Onion	At 20DAS	At 17DAS	After 30DAS
Garlic	At 26DAS	At 19DAS	
Ginger	At 12DAS	At 10DAS	
Chilli	At 16DAS	At 10DAS	

Table 2. Disease incidence at before and after storage of spices

Spices	%Disease incidence									
	Before storage	**After storage**								
		AN	AF	P sp	BS	PA	SM	FO	PG	CC
Onion	Fresh samples	5%	2%	5.90%	3.68%					
Garlic		3%				5%	3%			
Ginger								2%	3%	
Chilli								1.97%		3.5%
Total		8%	2%	5.90%	3.68%	5%	3%	3.97%	3%	3.5%

*AN= *Aspergllus niger,* AF=*Aspergillus flavus*, P sp= *Penicillium* sp, BS= *Bacillus subtilis,* PA= *Pseudomonas aeruginosa,* SM= *Serratia marcesens,* FO= *Fusarium oxysporum*, PG= *Pseudomonas geniculata*, CC= *Colletotrichum capsici.*

4.6. Cultural characterization of different bacteria on NA plates and different biochemical test results

Cultural characteristics are tools of bacteria as an identifying and classifying bacteria into various taxonomic groups. When grown on a variety of medium, bacteria exhibit differences in the microscopic appearances of their growth. These differences, called cultural characteristics and are used as the basis for separating bacteria into different taxonomic group. Growth of different bacteria on NA plates are shown in below and cultural characteristics of different bacteria are shown in Table 1. Whereas, different biochemical tests results are shown in Table. 4 and in plate 8.

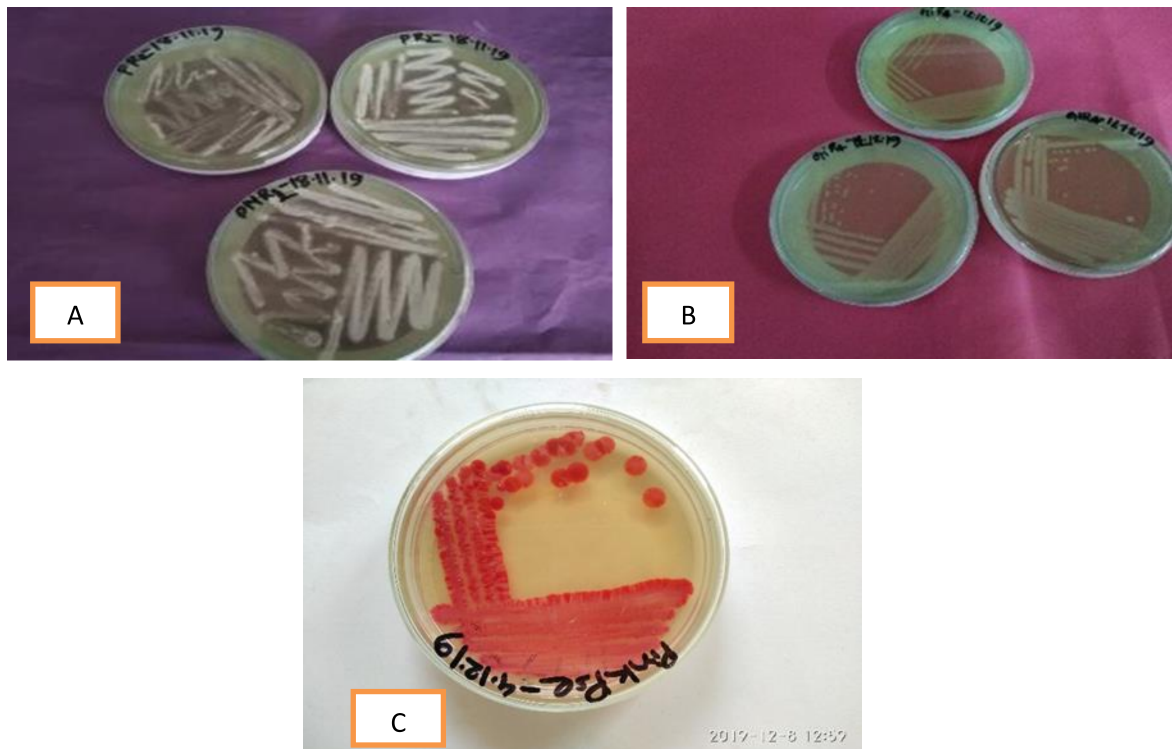

Plate 8. Cultural characteristics of different bacteria, A. *Bacillus* subtilis B. *Pseudomonas aeruginosa* and C. *Serratia marcescens*

Table 3. Cultural characteristics of different bacteria on NA (Nutrient Agar) plates

Isolates	Size	Pigment	Form	Margin	Elevation
Pseudomonas geniculata	Small	Yellowish	Short rod	Entire	Flat
Pseudomonas aeruginosa	Small	Yellowish	Short rod	Entire	Flat
Bacillus subtilis	Large	Whitish	Cocci	Serrate	Raised
Serratia marcescens	Small	Red	Short rod	Entire	Flat

Isolate No. 1. *Pseudomonas* geniculata

Isolate No. 2. *Pseudomonas* aeruginosa

Isolate No. 3. *Bacillus subtilis*

Isolate No. 4. *Serratia* marcescens

Table 4. Biochemical tests for identification of different bacteria

Bacteria with sample	Motility test	Casein test	Citrate test	Lactose test	Sucrose test	Dextrose test	Oxidase test	Catalase test	Gram reaction test	KOH test
Pseudomonas geniculata (Ginger)	+	-	-	-	-	-	-	+	Short rod Gram (-) ve	(+) ve
Pseudomonas aeruginosa (Garlic)	-	-	-	-	-	-	-	+	Gram (-) ve	(+) ve
Bacillus subtilis (Onion)	+	-	-	Alkaline	Alkaline	Acid no gas	-	+	Gram (+) ve	(-) ve
Serratia marcescens (Garlic)	+	+	+	+	+	+	-	+	Gram (-) ve Short rod	(+) ve

4.7. Pathogenicity test

This study reveal that the micro-organisms isolated from infected samples were pathogenic but with varied pathogenicity level. When inoculated into healthy samples, inoculated samples were completely rotten by the end of the third day after inoculation in case of most rapid infection by the pathogens. After inoculation all of the samples were showing their individual symptoms slowly. Within 5 days inoculated samples got completely rotted. From the rotted samples pathogens were isolated and identified as described earlier to confirm pathogenicity.

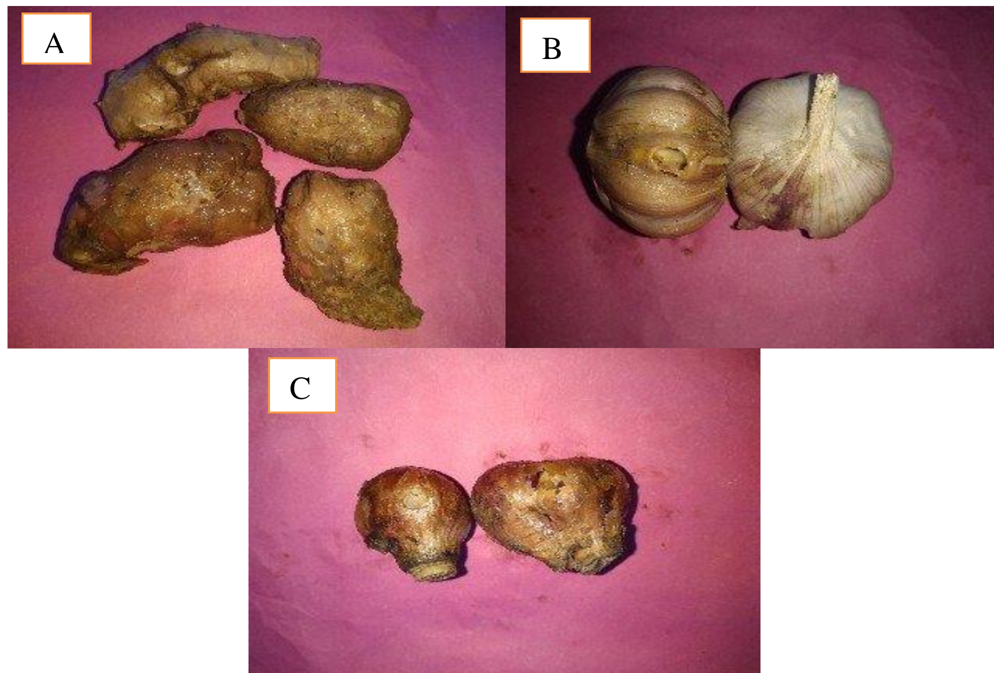

Plate 9. Pathogenicity test's result; A. Pathogenicity test for *Pseudomonas geniculata* on ginger, B. Pathogenicity test for *Pseudomonas aeruginosa* on garlic, C. Pathogenicity test for *Bacillus subtilis* on onion

4.8. DNA sequencing for bacteria

For the sequencing PCR product of around 1465 base was used as a template. Sequence analysis by using Basic Local Alignment Search Tool (BLAST) with the existing NCBI GenBank database entries. The comparison of the sequenced gene from garlic showed 98.89% homology with *Pseudomonas aeruginosa* strain SAU-2, another sequenced gene from garlic showed 98.73% homology with *Serratia marcescens* strain SAU-4. The comparison of the sequenced gene from ginger showed 94.87% homology with *Pseudomonas geniculata* strain SAU-3.

Deposition of nucleotide sequences in GenBank of NCBI

DNA sequences of D1/D2 domain of 16s rDNA sequences of isolated bacteria were submitted to National Center for Biotechnology Information (NCBI) for deposition in the GenBank and obtain accession number. The obtained accession no. MW404211 for *Pseudomonas aeruginosa* strain SAU-2, accession no. MW404212 for *Pseudomonas geniculata* strain SAU-3, accession no. MW404213 for *Serratia marcescens* strain SAU-4.

```
1      CAGTCTACAA TAGCAAGTAC GAGCGGATGA GAGGAGCTTG CTCTTCGATT CAGCGGCGGA
61     CGGGTGAGTA ATGCCTAGGA ATCTGCCTAG TAGTGGGGGA CAACGTTTCG AAAGGAACGC
121    TAATACCGCA TACGTCCTAC GGGAGAAAGT GGGGGATCTT CGGACCTCAC GCTATTAGAT
181    GAGCCTAGGT CGGATTAGCT AGTTGGTAGG GTAAAGGCCT ACCAAGGCGA CGATCCGTAA
241    CTGGTCTGAG AGGATGATCA GTCACACTGG AACTGAGACA CGGTCCAGAC TCCTACGGGA
301    GGCAGCAGTG GGGAATATTG GACAATGGGC GAAAGCCTGA TCCAGCCATG CCGCGTGTGT
361    GAAGAAGGCC TTCGGGTCGT AAAGCACTTT AAGTTGGGAG GAAGGGCTCA TAGCGAATAC
421    CTGTGAGTTT TGACGTTACC AACAGAATAA GCACCGGCTA ACTTCGTGCC AGCAGCCGCG
481    GTAATACGAA GGGTGCAAGC GTTAATCGGA ATTACTGGGC GTAAAGCGCG CGTAGGTGGC
541    TTGATAAGTT GGATGTGAAA TCCCCGGGCT CAACCTGGGA ACTGCATCCA AAACTGTCTG
601    GCTAGAGTGC GGTAGAGGGT AGTGGAATTT CCAGTGTAGC GGTGAAATGC GTAGATATTG
661    GAAGGAACAC CAGTGGCGAA GGCGACTACC TGGACTGACA CTGACACTGA GGTGCGAAAG
721    CGTGGGGAGC AAACAGGATT AGATACCCTG GTAGTCCACG CCGTAAACGA TGTCAACTAG
781    CCGTTGGGAT CCTTGAGATC TTAGTGGCGC AGCTAACGCA TTAAGTTGAC CGCCTGGGGA
841    GTACGGCCGC AAGGTTAAAA CTCAAATGAA TTGACGGGGG CCCGCACAAG CGGTGGAGCA
901    TGTGGTTTAA TTCGAAGCAA CGCGAAGAAC CTTACCTGGC CTTGACATGC TGAGAACTTT
961    CCAGAGATGG ATTGGTGCCT TCGGGAACTC AGACACAGGT GCTGCATGGC TGTCGTCAGC
1021   TCGTGTCGTG AGATGTTGGG TTAAGTCCCG TAACGAGCGC AACCCTTGTC CTTAGTTACC
1081   AGCACGTTAT GGTGGGCACT CTAAGGAGAC TGCCGGTGAC AACCGGAGGA AGGTGGGATG
1141   ACGTCAAGTC ATCATGGCCT TACGGCAGGG CTACCACGTG CTACATGGTC GGTCAAAGGG
1201   TTGCCAGCCG AGGTGAGCTA TCCCTAAACC GATCTAGTCG GATCCATTTG CACTCCATGC
1261   TGAATCGAAT CCTAATATCG GAATCAAACT CCCGGGGAAA CTTTCCGGCC TTTACC
```

Figure 1. Nucleotide sequence of 16S rDNA from *Pseudomonas aeruginosa* strain SAU-2 (Accession number: MW404211).

```
1       CGGAAGTCGT ACAAGGTAGA GTTTGCTCTT ATGGGTGGAG AGTGGCGCGA CGGGTGAGGA
61      GTACGTCGGA ATCTACTTTT TCGGGGGGGA TAACGTGGGG AAACTTACGC TAATACCGCA
121     TACGACCTAC GGGTGAAAGC AGGGGACCTT CGGCCCTTGC GCGATTAATG AGCCGATGTC
181     GGATTAGCTA GTTGGCGGGG TAAAGGCCCA CCAAGGCGAC TATCCGTATC TGGTCTGAGA
241     GGATGATCAG CCACACTGAA ACTGAGACAC GGCCCACACT CCTACGGGAG GCAGCAGTGG
301     GGAATATTGG ACAATGGGCG CAAGCCTGAT CCAGCCATAC CGCGTGGGTG AAGAAGGCCT
361     TCGGGTTGTA AAGCCCTTTT GTTGGGAAAG AAATCCAGCC GGCTAATACC TGGTTGGGAT
421     GACGGTACCC AAAGAATAAG CACCGGCTAA CTTCGTGCCA GCAGCCGCGG TAATACGAAG
481     GGTGCAAGCG TTACTCGGAA TTACTGGGCG TAAAGCGTGC GTAGGTGGTT GTTTAACTCT
541     GTTGTGAAAG CCCTGCTCTC AACCTGGGAA CTGCAGGAAA CTGGACAACT AGAGTGTGGT
601     AGAGGGTAGC GGAATTCCCG GTGTAGCAGT GAAATGCGTA GAGATCGGGA GGAACATCCA
661     TGGCGAAGGC AGCTACCTGG ACCAACACTG ACACTGAGGC ACGAAAGCGT GGAGAGCAAA
721     CAGGATTAGA TACCCTGGTA GTCCACGCCC TAAACGATGC GAACTGGATG TTGGGTGCAA
781     TTTGGCACGC AGTATCGAAG CTAACGCATT AAGTTCGCCG CCTGAGTACG GTCGCAAGAC
841     TGAAATTCAA AAGAATTGAC TGGGCCCCCG CACAGCTGTT GTATTATGTG TTTTAATTCT
901     ATGCAACACG AAGAACCTTA CCTTGCCTTG ACATGTCGAG AACTATACAA AGAATGAATT
961     GTTGCCTTTC GGCAACTCGA AACCCAGGAC TGCATGGCTG TCGGTCGCCT CCTCTCATCA
1021    AAATGATGGG ATAAATCCCC CAACGAGCGC CACCCCTTTC CTTATTTTTC CAGCCGCGTA
1081    ATGGGCCCTA ATAAAGAAAC CCCCCCTCCA ACCCCAA
```

Figure 2. Nucleotide sequence of 16S rDNA from *Pseudomonas geniculata* strain SAU-3 (Accession number: MW404212).

```
1       AAGGACAGCT TACACATGCA AGTCGAGCGG TAGCACAGGG GAGCTTGCTC CCTGGGTGAC
61      GAGCGGCGGA CGGGTGAGTA ATGTCTGGGA AACTGCCTGA TGGAGGGGGA TAACTACTGG
121     AAACGGTAGC TAATACCGCA TAACGTCGCA AGACCAAAGA GGGGGACCTT CGGGCCTCTT
181     GCCATCAGAT GTGCCCAGAT GGGATTAGCT AGTAGGTGGG GTAATGGCTC ACCTAGGCGA
241     CGATCCCTAG CTGGTCTGAG AGGATGACCA GCCACACTGG AACTGAGACA CGGTCCAGAC
301     TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC GCAAGCCTGA TGCAGCCATG
361     CCGCGTGTGT GAAGAAGGCC TTCGGGTTGT AAAGCACTTT CAGCGAGGAG GAAGGTGGTG
421     AACTTAATAC GTTCATCAAT TGACGTTACT CGCAGAAGAA GCACCGGCTA ACTCCGTGCC
481     AGCAGCCGCG GTAATACGGA GGGTGCAAGC GTTAATCGGA ATTACTGGGC GTAAAGCGCA
541     CGCAGGCGGT TTGTTAAGTC AGATGTGAAA TCCCCGGGCT CAACCTGGGA ACTGCATTTG
601     AAACTGGCAA GCTAGAGTCT CGTAGAGGGG GGTAGAATTC CAGGTGTAGC GGTGAAATGC
661     GTAGAGATCT GGAGGAATAC CGGTGGCGAA GGCGGCCCCC TGGACGAAGA CTGACGCTCA
721     GGTGCGAAAG CGTGGTGAGC AAACAGGATT AGATACCCTG GTAGTCCACG CTGTAAACGA
781     TGTCTATGGA GGTTGTGCCC TTGAGGCGTG GCTTCCGGAG CTAACGCGTT AAATCGACCG
841     CCTGGGGAGT ACGGCCGCAA GGTTAAAACT CACATGAATT GACGGGGGCC CGCACAAGCG
901     GTGGAACATG TGGTTATTCA ATGCACCGCG AAGACCTACC TACTCTGACC TCCCAGAAAT
961     TTCAAAATAT TTGTTCCTTC GAAAATCTGA AAAGTACCG CATGGTTGTC CTCGC
```

Figure 3. Nucleotide sequence of 16S rDNA from *Serratia marcescens* strain SAU-4 (Accession number: MW404213).

Chapter V

DISCUSSION

The experiment was conducted to isolate and identify the organisms associated with postharvest deterioration of some selected spices namely, onion, garlic, chilli and ginger. The pathogenicity of the isolated organisms was tested and the molecular characterization of few bacterial isolates were carried out. Finally, effect of different containers on the post-harvest deterioration of selected spices during storage were also observed.

Black mould and blue mould of onion and garlic, fusarium rot of ginger and chilli, anthracnose of chilli and soft rot of onion, garlic and ginger were found most common spice's diseases in the market places. Physiological disorders were also very frequent. In the present study the disease symptoms were identified by investigating them visually and then the disease-causing agents were isolated following tissue planting method and dilution plate method for fungi and bacteria, respectively. The isolated fungi were identified by studying cultural, morphological and microscopic characteristics. In this present study *Bacillus, Pseudomonas* and *Serratia* were differentiated by using different biochemical tests following the protocol described by (Kumar *et al*. 2007). Five fungal species and four bacterial strains were isolated from selected spices as post-harvest pathogens. They were *Aspergillus niger*, *A. flavus, Fusarium oxysporum., Penicillium* sp, *Colletotrichum capsici, Bacillus subtilis*, *Pseudomonas aeruginosa* and *Serratia marcescens* and *Pseudomonas geniculata*. Among them *A. niger, A. flavus*, *Penicillium* sp and *Bacillus subtilis* were found to be associated with the onion causing post-harvest deterioration, where their prevalence was 5%, 2%, 5.9% and 3.68%, respectively. From garlic, A. niger*, P. aeruginosa*, and *Serratia marcescens* were found responsible for the post-harvest deterioration and their prevalence were 3%, 5% and 3%, respectively. *F. oxysporum* (2%) and *P. geniculata* (3%) were responsible for deterioration of ginger. And *F. oxysporum* (1.97%) and *C. capsici* (3.5%) were found responsible for post-harvest rotting of chilli after storage. This finding was supported by Than *et al.,* 2008; Hadden and Black, 1989; Bosland and Votava, 2003. Several researches have been found similar with the findings of the present study. The predominant fungal pathogens associated with the storage diseases of onions were *Aspergillus* sp*, Penicillium* sp, and

Fusarium sp. (Velez *et al.,* 2004, Raju and Nail, 2006). During storage various diseases destroy the onions such as Black mould rot (*Aspergillus niger*), Blue mould rot (*Penicillium* spp.*)*, *Fusarium* bulb rot (*Fusarium* spp.), Basal rot (*Fusarium moniliforme), Aspergillus* rot (*Aspergillus* spp.), Dry rot (*Macrophomina phasiolina*), Soft rot (*Erwinia* spp.), Smudge (*Colletotrichum circinans*), Grey neck rot (*Botrytis allii)*, Green mold rot (*Penicillium* spp.), White rot (*Sclerotium cepivorum*) and Anthracnose (*Colletotrichum chardonianum*). (Rangaswami and Mahadevan, 2004). *Aspergillus niger*, which has been found at different stages of development, from the germination stage of seeds to the storage of mature bulbs (Hayden and Maude, 2002). *Aspergillus niger* was responsible for black mould development in storage. *Pseudomonas* spp. were the causal organisms of soft rot of ginger and garlic (Dhital *et al*. 2001) and they found the similar result in their study. Soft rot is considered the most destructive disease of vegetables. It occurs worldwide wherever fleshy storage tissues of vegetables and ornamentals are found. The disease can be found on crops in the field, in transit and in storage or during marketing resulting in great economic losses. Thirty-five bacterial isolates were recovered from 15 onion bulb samples (Mohamed *et al.,*2011). Twenty-five bacterial isolates were classified as putative *Pseudomonas aeruginosa*. Ten other unidentified bacteria were isolated at the same time and designated as Gram negative bacteria and non-flourescents. The soft rot of onion is caused by *E. carotovora* subsp. *carotovora* (Shing, 1985). This organism is a common cause of loss in storage (Sherf and Macnab, 1986). Postharvest diseases caused by bacterial pathogens include the species of soft rotting genera *Erwinia, Pseudomonas, Xanthomonas, Cytophaga* and *Bacillus* (Liao and Wells, 1987). Bacterial soft rot is mainly a postharvest disease (Coplin 1980; Tegene and Korobko, 1985). The finding was supported by Overy *et al.* (2005). *Bacillus* sp was responsible for soft rotting of onion.

The colonies of *Bacillus* were found to be whitish in color, the bacterium was gram positive. (Olivieri *et al.,* 2004 and Mahmoud *et al.* (2008) in their separate works reported the similar result. Smear culture with a drop of hydrogen peroxide (H_2O_2) produced bubbles indicating positive result for catalase tests. Similar biochemical test results were found by Ashmawy *et al.* (2015). Positive results were recorded in motility and catalase

test, whereas, negative in casein, citrate, lactose, sucrose and oxidase test. Same result was reported by Bradbury (1986).

Large size of circular colonies was formed on NA medium after 48-72 hours of incubation by *Pseudomonas aeruginosa.* Same description was stated by Kelman and Person (1954). After the Gram's staining under the compound microscope at 100x magnification with oil immersion, *Pseudomonas aeruginosa* was seen rod shaped with rounded ends, cells appeared singly and also in pairs (Creamy yellowish color), the bacterium was Gram negative. Razi (2012) recorded the same result of Gram-negative bacteria under compound microscope. The result is also supported by Sutton (2006). *Pseudomonas* showed positive results in motility and catalase test, whereas, negative in casein, citrate, lactose, sucrose and oxidase test. This was partially supported by Dhital *et al.* (2001).

Serratia marcescens was red in color, showed positive results in motility test, catalase test, casein test, citrate test, lactose test, sucrose test and negative result in oxidase test. This result was partially supported by Ahmed *et al.* (2013).

For the rhizomes sampled after harvest, the mean incidence of pathogens was *Fusarium oxysporum* 74%, *Fusarium solani* 21%, *Nigrospora oryzae* 5%, *Fusarium semitectum* and *Nigrospora sphaerica* 6%, *Alternaria tenuissima* 4%, *Penicillium commune*, *Verticillium* sp and *Verticillium* sp 3%, *Aspergillus luteo-albus*, *Aspergillus niger*, *Chaetomium* sp and *Epicoccum* sp 2% and *Curvularia geniculata* and *Mucor hiemalis* 1%. The mean incidence of bacteria that cause soft rot was *Enterobacter cloacae* subsp. *cloacae* 4% and *Pseudomonas fluorescens* 1% (Silvino Intra Moreira *et al.,* 2013).

In case of ginger *Fusarium oxysporum (Fusarium* rot*)* and *Pseudomonas geniculata* (Soft rot*)* were found responsible for postharvest disease development in storage. Sharma and Jain, 1977 found the similar result for postharvest disease development of ginger in storage.

Among the large number of diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum* species, bacterial wilt by *Psuedomonas solanacearum* and viral

diseases like chilli veinal mottle virus (CVMV) infection and cucumber mosaic virus (CMV) infection have been most detrimental to chilli production (Than *et al*., 2008).

Fruit rot of chilli caused by *Colletotrichum capsici* is one of the important postharvest disease in chilli. The disease is favoured by high moisture content in chillies and improper storage during post-harvest condition resulting in deterioration and loss of quality of the chilli beside aflatoxin contamination (Mesta *et al.,* 2013).

In case of chilli *Fusarium oxysporum* (*Fusarium* rot) and *Colletotrichum capsici* (Anthracnose) were found most prominent diseases. This finding was supported by Than *et al.,* 2008; Hadden and Black, 1989; Bosland and Votava, 2003. Macro and micro conidial characteristics of *Fusarium oxysporum* were determined by the descriptions of Leslie *et al.* (2006).

Different containers showed variation in developing post-harvest deterioration during storage of onion, garlic and chilli. Paper bags was the most suitable for storage of selected spices. Spices did not show any rotting up to thirty days after storing in paper bags followed by net bags.

Plastic net sack was more suitable package used to store both of red and yellow onion bulbs compared with other used package materials. On the other hand, sacks made from polyethylene encouraged the bacterial soft rot of onion bulb. This is due to increase RH around the bulbs associated with less ventilation that reduced the activity of oxidative enzymes specially polyphenol oxidase. Sacks from gunny and plastic were between in their effect to use in stored onion bulb. In this regard, packing material was reported to be influence against bacterial soft rot and manifest the potential for controlling *Pectobacterium carotovorum* subsp. *carotovorum* soft rot as reported by Wright *et al.* (1993) and Bhat *et al.* (2012).

Packaging of garlic seed bulbs that produce plants with growth and the best results is the use of plastic nets with a shelf life of 57 days and damage of 9.6%. The optimum storage temperature for garlic buds ranges between 5 and 10°C. Cold storage combination 7°C duration for 30 days has a higher growth percentage (Nurmalia *et al.,* 2019).

DNA sequences of D1/D2 domain of 16s rDNA sequences of isolated bacteria were submitted to National Center for Biotechnology Information (NCBI) for deposition in the GenBank and obtain accession number. It had been observed that isolated *Pseudomonas* strain SAU-2 (Accession number: MW404211) closely related to *Pseudomonas aeruginosa* (98.89% homology) strain CZ-45 16S ribosomal RNA gene with accession no. MT184863.1, *Pseudomonas* strain SAU-3 (Accession number: MW404212) closely related to *Pseudomonas geniculata* (94.87% homology) strain JS19 16S ribosomal RNA gene with accession no. HQ857772, *Serratia* strain SAU-4 (Accession number: MW404213) closely related to *Serratia marcescens* (98.73% homology) strain NPK_2_2_32 16S ribosomal RNA gene with accession no. MN691687.1.

In Bangladesh so far, many postharvest diseases in spices have been recorded. In this present study five fungal species and four bacterial strain were identified, so there might be some other pathogens present in the market places which were not identified through this experiment. Further study is required to identify those pathogens and diseases caused by them.

Chapter VI

SUMMARY and CONCLUSION

A spice is a seed, fruit, root, bark, or other plant substance primarily used for flavoring, coloring or preserving food. Many spices have antimicrobial properties. Studies (CAST 1984; Elias & Hossain 1984) show that spices are more profitable compared with their competing crops. Spices are sometimes used in medicine, religious rituals, cosmetics or perfume production, or as a vegetable. There are about 27 varieties of spices grown in Bangladesh. Among them five major varieties of spice namely onion, garlic, chilli, ginger and turmeric are common in Bangladesh cropping system. Annual productions of the five major varieties of spices namely onion, garlic, chilli, ginger and turmeric are 12.25 lakh metric tonnes, which cover 63.15 percent of total demand. Rest of the demand is met up by importing 3.98 lakh metric tonnes per annum (Spices Research Centre, 2005). The laboratory experiment was conducted in the MS Laboratory of Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University during the period of March, 2019- March, 2020.at MS laboratory to observe the prevalence of postharvest diseases of some selected spices (onion, garlic, ginger and chilli) and to identify their causes in storage. Pathogenicity test of each organism was also carried out and molecular characterization of few bacterial strains were performed. Spice samples were collected from five noted whole sale markets of Dhaka and from farmers' field of Faridpur district. During each visit one (1) kg of samples of each spice was collected randomly from each of the market places. Sample collection was carried out thrice. Then diseased spice samples were sorted out. Total samples were investigated carefully and diseased samples were separated from healthy ones. Healthy samples were kept in different storage containers and stored at room temperature and investigation was carried out up to one and half months. The organisms responsible for post-harvest storage rot were isolated following standard laboratory procedures. The fungi were isolated on PDA medium following tissue planting method and the bacteria were isolated following dilution plate method on NA medium.

Black mould, blue mould, *Fusarium* rot, anthracnose and bacterial soft rot were primarily identified by visual observation. Five fungal species from four genera and four bacterial

strains were isolated and identified. *Aspergillus niger, A. flavus, Penicillium* sp and *Bacillus subtilis* were isolated from onion. The prevalence of black mould of onion caused by *A. niger* was 5% where the prevalence of *A. flavus* causing green mould was 2%. And the prevalence of *Penicillium* sp and *Bacillus subtilis* were 5.9% and 3.68%, respectively. *Aspergillus niger* (3%), *Pseudomonas aeruginosa* (5%) and *Serratia marcescens* (3%) were isolated from garlic. In case of ginger *Fusarium oxysporum* (3.97%) and *Pseudomonas geniculata* (3%) were found to be responsible for postharvest disease development in storage. *Fusarium oxysporum* and *Colletotrichum capsici* (5.3%) were isolated from chilli where they were responsible for *Fusarium* rot and anthracnose development, respectively.

Bacillus subtilis produced whitish, raised large colonies on NA plates. The bacterium was gram positive. Positive results were recorded in motility and catalase test, whereas, negative in casein, citrate, lactose, sucrose and oxidase test. Large size of circular colonies were formed on NA medium after 48-72 hours of incubation by *Pseudomonas aeruginosa* After the Gram's staining under the compound microscope at 100x magnification with oil immersion, *Pseudomonas aeruginosa* was seen circular shaped with rounded ends, cells appeared singly and also in pairs (Creamy yellowish color) and this indicates the bacterium was Gram negative. Positive results were recorded in motility and catalase test, whereas, negative in casein, citrate, lactose, sucrose and oxidase test. The colonies of *Serratia marcescens* were red in color, showed positive results in motility test, catalase test, casein test, citrate test, lactose test, sucrose test and negative result in oxidase test.

In pathogenicity tests all the isolated organisms showed positive results. Effects of different types of storage bags on post-harvest deterioration during storage were observed and recorded. The samples were observed and the temperature was recorded daily up to 1 month. The average temperature was 29^0 C during storage. The study found that paper bag was more suitable than the others to keep the samples in storage. It also absorbed the moisture which was produced by the samples. This helped to prevent the rotting of samples. In paper bags spices can store 1 month or more without any rotting except

mould development. But the samples got shriveled as the average temperature during storage was 29^0 C.

The genomic DNA was extracted for Gram-negative bacteria and used as template (1ml of an overnight culture was added to a 1.5ml microcentrifuge tube. Then centrifuged it at 16,000 × g for 2 minutes to pellet the cells. Supernatant was removed. 600µl of nuclei lysis solution was added and gently pipetted until the cells were resuspended. Incubated at 80°C for 5 minutes to lyse the cells; then cool to room temperature. 3µl of RNase solution was added to the cell lysate and inverted the tube 5 times to mix. Incubated at 37°C for 60 minutes, then the sample was cooled to room temperature. 200µl of protein precipitation solution was added to the RNase-treated cell lysate and vortex vigorously at high speed for 20 seconds to mix the protein precipitation solution with the cell lysate. Incubated the sample on ice for 5 minutes. Then centrifuged at 16,000 × g for 3 minutes. The supernatant containing the DNA was transferred to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol. Gently mixed by inversion until the thread-like strands of DNA formed a visible mass. Centrifuged at 16,000 × g for 2 minutes. Then carefully poured off the supernatant and drained the tube on clean absorbent paper. 600µl of room temperature 70% ethanol was added and gently inverted the tube several times to wash the DNA pellet. Centrifuged at 16,000 × g for 2 minutes. Carefully aspirated the ethanol. Drained the tube on clean absorbent paper and allow the pellet to air-dry for 15 minutes. 100µl of DNA rehydration solution was added to the tube and rehydrated the DNA by incubating at 65°C for 1 hour. Periodically mixed the solution by gently tapping the tube. Alternatively, rehydrated the DNA by incubating the solution overnight at room temperature and stored the DNA at 8°C.

DNA sequences of D1/D2 domain of 16s rDNA sequences of isolated bacteria were submitted to National Center for Biotechnology Information (NCBI) for deposition in the GenBank and obtain accession number. The obtained accession no. MW404211 for *Pseudomonas aeruginosa* strain SAU-2, accession no. MW404212 for *Pseudomonas geniculata* strain SAU-3, accession no. MW404213 for *Serratia marcescens* strain SAU-4.

It had been observed that isolated strain, strain *Pseudomonas* SAU-2 (Accession number: MW404211) closely related to *Pseudomonas aeruginosa* (98.89% homology) strain CZ-45 16S ribosomal RNA gene with accession no. MT184863.1, strain *Pseudomonas* SAU-3 (Accession number: MW404212) closely related to *Pseudomonas geniculata* (94.87% homology) strain JS19 16S ribosomal RNA gene with accession no. HQ857772, strain *Serratia marcescens* SAU-4 (Accession number: MW404213) closely related to *Serratia marcescens* (98.73% homology) strain NPK_2_2_32 16S ribosomal RNA gene with accession no. MN691687.1.

In Bangladesh so far as many twelve postharvest diseases in spices have been recorded. In this present study five species of fungal and four types of bacterial strain were identified, so there might be some other pathogens present in the market places which were not identified through this experiment. This research study was conducted based on the fungi and bacteria responsible for postharvest diseases of some selected spices in storage. However, further research needed to conduct to observe the diversity and causes of postharvest diseases of spices and to identify potential control of the diseases in the storage.

Chapter VII

REFERENCES

Agrios G. N. (2005) Plant Pathology. St. Louis, MO: Academic Press.

Ahmed, N.N., Islam, M.d., Hossain, M.A., Meah, M.B. and Hossain, M.M. (2013). Determination of Races and Biovars of Bacteria Causing Bacterial Rot of Garlic. *J. Agril. Sci*. **5** (6):1916-9760.

Ahmed, C. and Hossain, H.B. (1985). Contribution to the postharvest study of stored onion (*Allium cepa*). *Ochrana Rostlin*. **10**: 149-153.

Aiyer, A.K.N. (2008). Field crops of India with special reference to Karnataka, (7th ed.). Bangalore. BAPPCO.

Ali, M.A. and Haque, S.S. (2011). Research on Postharvest Fungal Diseases of Spices in Bangladesh. *Bangladesh Journal of Training and Development.* **15** (2): 245-250.

Anonymous, (2011). Annual progress report for kharif, 2008. Division of Plant Pathology, S. K. Univrsity of Agricultural Science and Technology (Kashmir). 34pp.

Araji, S., Grammer, F.A., Gertzen, R., Anderson, S.D., Petkovsek, M., Veberic, R. Phu, M.L., Solar, A., Leslie, C.A., Dandekar, A.M. and Escobar, M.A. (2014). Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut. *Plant Physiol*. **164** (3):1191-1203.

Ashmawy, N. A., Jadalla, N. M., Shoeib, A. A. and El-Bebany, A. F. (2015). Identification and Genetic Characterization of *Bacillus spp.* Causing Soft Rot Diseases in Egypt. *J. Pure Appl. Microbiol.* **9** (3): 1847-1858.

Augusti, K.T. (1990). Therapeutic and medicinal values of onion and garlic. In Onion and Allied Crops. Ed. Brewster, J.L. and Rabinowith, H.D., Baco Ratani, Florida, CRC Press. **3**: 93-108.

Bailey JA, Jeger MJ, editors. Colletotrichum: Biology, Pathology and Control. Wallingford: Commonwealth Mycological Institute; 2002. p. 388.

Bangladesh Bureau of Statistics (BBS), (2016). Statistical Year Book of Bangladesh, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka.

Bangladesh Bureau of Statistics (BBS), (2015). Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Dhaka.

Bangladesh Bureau of Statistics (BBS), (2011). Statistical Yearbook of Bangladesh. Bangladesh Bureau of Statistics, Planning Division, Ministry of Planning, Dhaka.

Bangladesh Bureau of Statistics (2004). Year Book of agricultural Statistics, Statistics Division, Ministry of Planning, Dhaka.

Bhat, K.A., N.A. Bhat, F.A. Mohiddin, S.A. Mir and M.R. Mir (2012). Management of postharvest *Pectobacterium* soft rot of cabbage (*Brassica oleracea* var *capitata* L.) by biocides and packing material. *Afr. J. Agri. Res*. **7** (28): 4066-4074.

Bhagachandi, P.M., Pal, N., Singh, N. and Chaudhary, B. (2000). White onion for dehydration. *Indian Horticulture*. **24** (4): 7-9.

Bogala, M., Vishvambhar, D.M. and Padam, S.C. (2019). Storage of garlic bulbs (Allium sativul L.): A review. First published: 12 July 2019.

Bosland, P.W. and Votava, E.J. (2003). Peppers: Vegetable and Spice Capsicums. England: CAB International; p. 233.

Bradbury, J. F. (1986). Guide to Plant Pathogenic Bacteria. CAB International Mycological Institute, p. 329.

Byung S. K. (2007). Country report of Anthracnose research in Korea, in First International Symposium on Chili Anthracnose, Hoam Faculty House, Seoul National University (Seoul:), 24.

Canadian Agricultural Sector Team (1984): Production and marketing of spices in Bangladesh: A background report, Dhaka.

Cannon, P.F., Bridge, P.D. and Monte,E. (2000). Linking the Past, Present, and Future of *Colletotrichum* systematics. In: Prusky D, Freeman S, Dickman M, editors. *Colletotrichum:* Host specificity, Pathology, and Host-pathogen Interaction. St. Paul, Minnesota: APS Press; 2000. pp. 1–20.

Coplin, R.W. (1980). The incidence of decay and factors affecting bacterial soft rot of spices. *Am. Spice. J.* **50**: 398–407.

Currah, L. and Proctor, F. J. (2010). Onion in tropical region. Bullletin no.35, Natural Resources Institute, Chatam, Maritime, Kent, UK, 79p.

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A. and Spanu, P. D. *et al.,* (2012). The Top 10 fungal pathogens of spices in storage in molecular plant pathology. *Mol. Plant Pathol.* **13**: 414–430.

Dhall, R.K. and Ahuja, S. (2013). Postharvest Management of Garlic. Department of Vegetable Science, Punjab Agricultural University, Ludhiana.

Dhital, T. P. and Hayward, A. C. (2001). Identifying diseases of spices in Pennsylvania. Department of Plant Pathology. College of Agril. Science, Pennsylvania. pp. 50-56.

Don, L. D., Van, T. T., Phuong Vy, T. T. and Kieu, P. T. M. (2007). *Colletotrichum spp.* Attacking on chilli pepper growing in Vietnam, Country Report, in Abstracts of the First International Symposium on Chilli Anthracnose, eds Oh D. G., Kim K. T., editors. (Seoul: Seoul National University), 24.

Dohroo, A.M. (2005) The causes of dry rot in ginger (*Zingiber officinale*) in Australia. *Australian Plant Pathology.* **31**: 419-420.

Dohroo, N.P. and Sharma, M. (1992). New host records of fungi from India. *Indian Phytopath*. **45**: 280.

Dugan, F.M., Hellier, B. C. and Lupien, S. L. (2007). Pathogenic fungi in garlic seed cloves from the United State and China and efficacy of fungicides against pathogens in garlic germplasm in Washington State. *J. Phytopathol*. **155:** 437 - 445.

Dugan, F. M. (2007). Diseases and disease management in seed garlic: Problems and prospects. *Am. J. Plant Sci. Biotechnol.* **1**: 47-51.

EL-Ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S. and Nadeem, M.A. (2010). Comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J Agric Food Chem*. **58**: 8231-8237.

El-Marzoky, Hanan, A. and Shaban, W.I. (2013). Studies on some garlic diseases during storage in Egypt. *Journal of Applied Plant Protection*. **2**: 15-20.

El-Nagerabi, S. A. F. and Ahmed, A. H. M. (2003). Storability of onion bulbs contaminated by *Aspergillus niger* mould. *Phytoparasitica*. **31**: 515-523.

Elias, S. M. and Hossain, M. I. (1984). Chili cultiuation in Bangladesh: Agro-economic Survey and Constraints to its Higher Production at Farm Level, Gazipur: Bangladesh Agricultural Research Institute.

Fakir, B. A. (2009). Storage rot fungi and seed-borne pathogens of spices. *J. Sci. Technol*. **35**: 13-21.

FAO (2014). Food and Agriculture Organization, Rome. www.fao.org.in. Accession date: (15th Jan, 2014).

FAO (Food and Agriculture Organization of the United Nations) FAO Production Yearbook 2001. Rome: FAO; 2003. p. 333.

FAO. (2004). The market for non-traditional agricultural exports. FAO commodities and trade technical paper 3.

Farr, D.F. and Rossman, A.Y. (2010). Fungal Databases, Systematic Mycology and Microbiology Laboratory, USDA-ARS.

Fessenden, J.M., Wittenborn, W. and Clarke, L. (2001). Gingko biloba: A case report of herbal medicine and bleeding postoperatively from a laparoscopic cholecystectomy. *Am Surg.* **67** (1): 33-35.

Freeman, S. and Shabi, E. (1998). Cross-infection of subtropical spices by *Colletotrichum* species from various hosts. *Physiol. Mol. Plant Pathol.* **49**: 395–404.

Geeta, G.S. and Reddy, T.K.R. (1990). *Aspergillus flavus* and its occurrence in relation to other mycoflora on stored spices. *J. Stored Prod. Res.* **26**: 211-213.

Goszczynska, T. and Serfontein, J. J. (1998). Milk Tween agar, a semi selective medium for isolation and differentiation of *Pseudomonas syringae pv. Syringae, Pseudomonas syringae pv. Phaseolicola* and *Xanthomonas axonopodis pv. phaseoli*. *J. Microbiol. Method.* **32** (1): 6572.

Gupta, R.P. and Verma, L.R., (2002). Problem of diseases during storage in onion and garlic and their strategic management. In implication of plant diseases on produce quality. (Eds.singh, D.P.). pp 55-62. Kalyani publishers, Ludhiana.

Hadden, J.F. and Black, L.L. (1989). Anthracnose of pepper caused by *Colletotrichum spp.* proceeding of the international symposium on integrated management practices: Tomato and pepper production in the tropics; Taiwan: Asian Vegetable Research and Development Centre. pp. 189–199.

Hayden, N. J., Maude, R. B., Hassan, H. S. and Magid, A. A. (2002). Studies on the biology of black mould (*Aspergillus niger*) on temperate and tropical onions. 2. The effect of treatments on the control of seedborne *Aspergillus niger. Plant Pathol.* **43:** 570-578.

Holbrook, R. and Anderson, J. (1980). An improved selective and diagnostic medium for the isolation and enumeration of *Bacillus cereus* in foods. *Can. J. Microbiol.* **26** (7): 753-759.

Huda, F. A., Islam, M. S., Biswas, H. and Islam, M. S. (2008). Impact assessment study on selected spice crops under action plan in Bangladesh. *Progress Agric.* **19** (2): 229-241.

Hussain, M. Z., Rahman, M. A., Islam, M. N., Latif, M. A. and Bashar, M. A. (2012). Morphological and molecular identification of *Fusarium oxysporum* isolated from guava wilt in Bangladesh. *Bangladesh, J. Bot.* **41** (1): 49-54.

Isaac S. Fungal Plant Interaction. London: Chapman and Hall Press; 2002. p. 115.

Islam, M. S., Rahman, K. M. and Hasan, M. K. (2011). Profitability and Resource Use Efficiency of Producing Major Spices in Bangladesh. *Bangladesh Journal of Agricultural Economics*. **34:** 1-2.

Johnston, P.R. and Jones, D. (1997). Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycologia.* **89** (3): 420–430.

Jones, H.A. and Mann, L.K. (2013). Onions and their allies. Interscience publishers. Inc.New York.

Kim, K.D., Oh, B.J. and Yang, J. (1999). Differential interactions of a *Colletotrichum gloeosporioides* isolate with green and red pepper fruits. *Phytoparasitica*. **27**: 1–10.

Klich, M. M. (2002). Postharvest losses from delayed harvest and during common storage of short-day onions. *Hort Science.* **29**: 802-804.

Kluge, R.A., Aguila, J.S.D., Jacomino, A.P., Scarpare Filho, J.A. (2007). Colheita e Climatização de Banana. Piracicaba: ESALQ, Divisão de Biblioteca e Documentação, Boletim Série Produtor Rural, n. 35.

Köycü, N. D. and Özer, N. (2017). Determination of seedborne fungi in onion and their transmission to onion sets in storage. *Phytoparasitica.* **25**: 25-31.

Ko, S.S., Huang, J.W., Wang, J.F., Shanmugasundaram, S. and Chang, W.N. (2002). Evaluation of onion cultivars for resistance to *Aspergillus niger,* the causal agent of black mold. *J. Amer. Soc. Hort. Sci.* **127**: 697-702.

Kubra, I.R. and Rao, L.J.M. (2012). An impression on current developments in the technology, chemistry, and biological activities of ginger. (*Zingiber officinale*). *Crit Rev Food Sci Nutr.* **52** (8): 651–688.

Kumar, A.A., Kumar, V., Kaushal, S., Patil, C., Payal and Kumar, A. (2011). "Antibacterial potential of some spices against *staphylococcus aureus* isolated from various food samples of Himachal Pradesh (India)", *World Journal of Science and Technology*. **1** (10): 48-53.

Kelman, A. (1954). The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. *Phytopathol.* **44**: 693-695.

Lakshmesha K., Lakshmidevi K., Aradhya N. and Mallikarjuna S. (2005). Changes in pectinase and cellulase activity of *Colletotrichum capsici* mutants and their effect on anthracnose disease on Capsicum fruit. *Arch. Phytopathol. Plant Prot*. **38**: 267–279.

Leslie, G. A. and Salas, B. (2006). *Fusarium* dry rot and *Fusarium* wilt. In: Stevenson, W., Loria, R., Franc, G.D., Weingartner, D.P. 3rd Ed. Compendium of Spices Diseases. ASP Press, St. Paul, MN, pp. 23-25.

Liao, C.B. and Wells, J. M. (1987). Diversity of pectolytic, *Pseudomonas fluorescence* causing soft rot of fresh vegetables at produce market. *Phytopathology*. **77:** 673-677.

Mahmoud, D.A.R., Mahmoud, A.A. and Gomaa, A.M. (2008). Antagonistic activities of spices associated bacteria via their production of hydrolytic enzymes with special reference to pectinases. *Res. J. Agric. Biol. Sci.* **4** (5): 575 - 584.

Maini, S.B., Diwan, B. and Anand, J.C. (2000). Storage behavior and drying characteristics of commercial cultivars of onion. *Journal of Food and Science and Technology*. **21** (6): 417.

Manandhar, J. B., Hartman, G. L. and Wang, T. C. (2005). Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. *Plant Dis*. **79**: 380–383.

Mathukrishnan, C.R., Thangaraj, T. and Chatterjee. (1993). Chilli and Capsicum. Vegetable Crop, Naya Prakash, Calcatta, India, pp.334-374.

Matthananda, K. A. (2012). Varietal evaluation for storability of big onion. Seasonal Report, Maha 2011/2012, Agriculture Research Station. MahaIlluppallama.

Maxwell I. (2008). Let's make ginger beer. Dave's Garden.

McDonald, M. R., de los Angeles Jaime, M. and Hovius, M. H. (2004). Management of diseases of onions and garlic. In: Diseases of Spices and Vegetables: Volume II, ed. By S. A. M. H. Naqvi, pp. 149-200.

Meah, M. A. M., Khatun, M. L. and Ashrafuzzaman, M. (1987). Fungi causing rots in onions at storage and market. *J. Bangladesh Agril. Univ.* **6:** 245-251.

Meadows, A.B. (1998). Ginger processing for food and industry. In: Proceedings of first National ginger workshop. Umudike, Nigeria, pp. 34-42.

Meenu, G and Kaushal, M. (2017). Diseases infecting ginger (*Zingiber officinale Roscoe*): A review. *Agricultural Reviews*. **38** (1): 15-28.

Mesta, R.K., Shivaprasad, M., Nidoni, U., Kurubar, A.R. and Hegde, G.M. (2013). Postharvest management of fruit rot of chilli using solar tunnel dryer. *Acta Hortic*. **1012**: 755-758

Miah, S.B., Sagar, U.R., Chandan, S.S. and Rajeshkumar, (2011). Evaluation of different structures for storage of onions. *Vegetable Science*. **24** (1): 73-74.

Miah, S.B. and Hossain B. (2010). Storage behaviour and drying characteristics of commercial cultivars of some spices. *Journal of Food Science and Technology*. **21** (6): 417.

Mishra, R.K., Jaiswal, R.K., Kumar, D., Saabale, P.R. and Singh, A. (2014). Management of major diseases and insect pests of onion and garlic. *Journal of Plant Breeding and Crop Science*. **6** (11): 160-170.

Mishra, B. and Rath, G.C. (1989). *Geotrichum* rot of stored ginger. *Indian J. Mycol. Plant Pathol*. **18**: 213.

Mitra, M. and Subramaniam, L. S. (2008). Soft rot diseases of some cultivated spices caused by postharvest pathogens :(Eds.) Fitz: Government of India Central Publication Branch.

Mohamed, H. Abd-Allaa, Shymaa, R. Bashandy, Stefan Ratering and Sylvia Schnell (2011). First report of soft rot of onion bulbs in storage caused by *Pseudomonas aeruginosa* in Egypt. *Journal of Plant Interactions*. **6** (4): 229-238.

Munoz, A. H. A. and Abdullah, A.K. (2014). Production of aflatoxins by *Aspergillus flavus* and *Aspergillus niger* strains isolated from onion in storage. *J. Food Agric. Environ*. **7:** 33-39.

Nadia, N. and Villamiel, M. (2013). Biological properties and postharvest diseases of onions and garlic. *Trends Food Sci. Tech*. **18**: 609-625.

Nirenberg, H.I., Feiler, U. and Hagedorn, G. (2002). Description of *Colletotrichum lupini* comb. nov. in modern terms. *Mycologia*. **94** (2): 307–320.

Noor, S., Khan, M. S., Shil, N. C. and Talukder, M. R. (2008). Integrated nutrient management for sustainable yield of major spice crops in Bangladesh. *Bangladesh Journal of Agricultural Environment.* **4**: 95-113.

Nurmalia, Purwanto, Y.A., Sobir, Sulassih and Naibaho, N. (2019). Effect of low temperature and period of storage on the quality of Garlic Seeds (*Allium sativum* L). IOP Conf. Series: Materials Science and Engineering 557.

Olivieri, F. P., Maldonado, S., Tonon, C. V. and Casalongue, C. A. (2004). Hydrolytic activities of *Fusarium solani* and *Fusarium solani f. speumartii* associated with the infection process of some tropical spices. *J. Phytopathol*. **152** (6): 337- 344.

Overy, D. P., Karlshøj, K. and Due, M. J. (2005). Low temperature growth and enzyme production in *Penicillium ser. corymbifera* species, casual agents of blue mold storage rot in bulbs. *J. Plant Pathol.* **87**: 57-63.

Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J. and Mongkolporn, O. (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in Capsicum. *Plant Breeding*. **124** (2): 206–208.

Prajapati, B.K. and Patil, R.K. (2015). Black mould rot: An important postharvest disease of onion and its management. *Popular Kheti.* **3** (1): 78.

Prusky, D. (2011). Reduction of the incidence of postharvest quality losses, and future Prospects. *Food Secur*. **3**: 463–474.

Prusky, D. and Reddy, B.N. (2005). The causes of poor yield of stored ginger (*Zingiber officinale*) in Queensland, Australia. *Australian Plant Pathology*. **33**: 203-210.

Prusky, D. (2005). Pathogen quiescence in postharvest diseases. *Phytopathol*. **34**: 413–434.

Prusky, D. and Plumbley, R.A. (1992). Quiescent Infections of *Colletotrichum* in Tropical and Subtropical spicess. In: Bailey JA, Jeger MJ, editors. Colletotrichum: Biology, Pathology, and Control. Wallingford: CAB International; 1992. pp. 289–307.

Pruthi, J.S. (1998). Spices and condiments. National Book Trust (5th Ed.) New Delhi, India. pp. 1-325.

PTRIC. (2005). Postharvest Technology Research Information Center. Department Plant Sciences. University of California-Davis.

Purseglove, J.W., Brown, E.G., Green, C.L. and Robbins, S.R.J. (1981). Ginger. In: Spices. Vol. II. Longman, New York. pp. 447-531.

Rafika, S. B., Mejda, D.R., Mohamed, B. K. and Hatem, C. (2006). Onion storage ability and an inventory of onion post-harvest fungi in Tunisia. *Trop. Sci*. **46**: 105-112.

Ramachandran, N. and Rathnamma, K. (2006). *Colletotrichum acutatum* a new addition to the species of chilli anthracnose pathogen in India, in Paper presented at the Annual Meeting and Symposium of Indian Phytopathological Society, Central Plantation Crops Research Institute (Kasarago:).

Ranathunge, N. P., Mongkolporn, O., Ford, R. and Taylor, P. W. J. (2012). *Colletotrichum truncatum* pathosystem on Capsicum spp.: infection, colonization and defence mechanisms. Australas. *Plant Path*. **41**: 463–473.

Rangaswami and Mahadevan (2004). Postharvest management of black mould rot of onion. *Indian Phytopathology*. **59** (3): 333-339.

Raju, K. and Naik, M. K. (2006). Effect of pre-harvest spray of fungicides and botanicals on storage diseases of onion. *Indian Phytopath*. **59**: 133-141.

Ravindran, P.N. and Nirmal, B.K. (2005). Introduction. In: Ginger: the genus *Zingiber*. (Eds.): Ravindran, P.K. and Nirmal, B.K. CRC Press, New York. pp 1-14.

Razi, S. J. (2012). Biology and epidemiology of bacterial soft rot caused by *Pseudomonas spp.*: Annual review. *Phytopathology*. **11**: 1-20.

Reddy, K. R. N., Raghavender, C. R., Salleh, B., Reddy, C. S. and Reddy, B. N. (2011). Potential of aflatoxin B1 production by *Aspergillus flavus* strains on commercially important spices. *Int. J. Food Sci. Tech.* **24**: 161–165.

Richard, L. and Kristan, H. (2017). Diagnosis and control of onion disease. *Plant Pathology*. **5** (1):18.

Ross, F.M. (2008). Pathogenic fungi in spices from the United State and China and efficacy of fungicides against pathogens in spice germplasm in Washington State. *J. Phytopathol*. **155**: 437 -445.

Sadeghi-Seraji, J., Gholam Khodakaramian and Kiomars Rouhrazi (2018). First report of garlic soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* in Iran. *Journal of Plant Pathology*. **100**: 125.

Sang, M. K., Han, G. D., Oh, J. Y., Chun, S.C. and Kim, K. D. (2018). *Penicillium brasilianum* as a novel pathogen of onion (*Allium cepa* L.) and other fungi predominant on market onion in Korea. *Crop Prot.* **65**: 138-142.

Saxena, A., Raghuvanshi, R. and Singh, H. B. (2014). Molecular, phenotypic and pathogenic variability in *Colletotrichum* isolates of subtropical region in North Eastern India, causing fruit rot of chillies. *J. Appl. Microbiol*. **117**: 1422–1434.

Schwartz, H.F. and M. Krishna (2006). Compendium of onion and garlic diseases and pests, 2nd Ed. APS Press.

Schwartz, H.F., and Mohan, S.K. (1995). Compendium of Onion and Garlic Diseases. St. Paul, MN: APS Press.

Selvaraj, S. (1976). Onion: Queen of the kitchen. *Kisan World*. **3** (12): 32-34.

Sharma, N.D. and Jain, A.C. (2017). Report submitted to Indian Council of Agricultural Research on Ginger postharvest diseases and its control. Report Department of Plant Pathology, Jawahar Lal Nehru Krishi VishvaVidyalaya, Jabalpur, Indian: 30pp.

Sharma, R. R., Singh, D. and Singh, R. (2009). Biological control of postharvest diseases of spices by microbial antagonists: a review. *Biol. Control*. **50:** 205–221.

Sharma, P.N., Kaur, M., Sharma, O.P., Sharma, P. and Pathania, A. (2005). Morphological, pathological and molecular variability in *Colletotrichum capsici*, the cause of fruit rot of chillies in the subtropical region of north-western India. *Journal of Phytopathology*. **153** (4): 232–237.

Sharma, N.D. and Jain, A.C. (1977). A checklist and selected bibliography of ginger diseases of the world. *PANS*. **23**: 474-481.

Shehu, K. and Muhammad, S. (2011). Fungi associated with storage rots of onion bulbs in Sokoto, Nigeria. *Int. J. Mod. Bot*. **1**: 1-3.

Shelly, T.E., Mcinnis, D.O. and Pahio, E. (2004). Aromatherapy in the Mediterranean fruit fly (diptera: Tephritidae): Sterile males exposed to ginger root oil in prerelease storage boxes display increased mating competitiveness in field-cage trials. *J. Economic Entomology*. **97** (3): 846-853.

Sherf, A.F. and. Macnab, A.A (1986). Vegetable diseases and their control, 2nd Ed., A Wiley Interscience Publication, John Wiley and Sons, Inc. New York.

Shing, R.S. (1985). Diseases of Vegetable Crops, Oxford and IBA Publishing Company Pvt. Ltd., New Delhi, p. 331.

Silvino, I.M., Deiziane da C.D., Augusto C.R., José R.O., Onkar D.D. and Olinto L.P. (2013). Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. *Trop. plant pathol*. **38**: 3.

Simmonds, J.H. (1965). A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. *Queensland Journal Agriculture and Animal Science*. **22**: 437–459.

Sinclair, P. J. and Letham, D. B. (2006). Incidence and sites of visible infection of *Aspergillus niger* on bulbs of two onion (*Allium cepa*) cultivars. *Australasian Plant Pathol.* **25**: 8-11.

Singh, S., Singh A.P. and Sinha, S.B. (1994). Effect of spacing and various levels of nitrogen on seed crops of kharif onion. *Veg. Sci.* **21**: 1-6.

Sloand, E.D. and Vessey, J.A. (2001). Self-medication with common household medicines by young adolescents. *Compr Pediatr Nurs*. **24** (1): 57-67.

Spices Research Centre, (2005). Production technology of spice crops, Bklt-07/2005. Bangladesh Agricultural Research Institute, Shibgonj, Bogra.

Srivastava, K.C. and Mustafa, T. (1989). Ginger (*Zingiber officinale*) and rheumatic disorders. *Med Hypotheses*. **29** (1): 25-28.

Stevenson, W. R., Curtis, R.W. and Tuite, J. (2011). Losses in quality and quantity of *Aspergillus niger* infected onion bulbs (*Allium cepa*). *Appl. Microbial.* **28**: 362-365.

Stirling, A.M. (2004). *Erwinia chrysanthemi* the cause of soft rot in ginger (*Zingiber officinale*) in Australia. *Australasian Plant Pathology*. **31**: 419-420.

Sumner, D. R. (2015). Disease of bulbs caused by fungi-black mould, P. 26-27.

Sutton, E. F. (2006). Evaluation of an avirulent strain of *Pseudomonas sp.* for biological control of bacterial soft rot of garlic. *American Spice Journal*. **65**: 255-268.

Tan, B.K.H. and Vanitha, J. (2004). Immunomodulatory and antibacterial effects of some traditional Chinese medicinal herbs: a review. *Curr Med Chem.* **11** (11): 1423-1430.

Tanaka, K. (2011). Studies on the black mould disease of onion bulbs caused by *Aspergillus niger* van Tieghem. Bulletin of Faculty of Agriculture. Saga University. **70**: 1-54.

Taskeen-un-Nisa, (2010). Pathological studies on fungal rots of some vegetables in Kashmir valley. Ph.D Thesis, Department of Botany, University of Kashmir, Srinagar, Kashmir, 271 pp.

Than, P. P., Jeewon, R., Hyde, K. D., Pongsupasamit, S., Mongkolporn. O. and Taylor P. W. J. (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathol*. **57**: 562–572.

Trujilo, E.E. (1964). *Fusarium* yellows and rhizome rot of common ginger. *Phytopathology*. **53:** 1370-1371.

UMM.University of Maryland Medical Center.2004. Garlic.

Varga, J., Kocsubé, S., Szigeti, G., Man, V., Tóth, B., Vágvölgyi, C. and Bartók, T. (2012). Black *Aspergilli* and fumonisin con-tamination in onions purchased in Hungary. *Acta Aliment. Hung*. **41**: 414-423.

Varga, J., Houbraken, J., Samson, R. A. and Frisvad, J. C. (2008). Molecular diversity of *Aspergillus* and *Penicillium* species on spicess and vegetables. In: Mycotoxins in spicess and Vegetables, Ed. by R. Barkai-Golan and N. Paster, pp. 205-234.

Velez, G. (2004) Management of the Black Mould Disease of Onion. *Journal of Plant Patho Microbes*. **5** (3): 312-315.

Voorrips, R. E., Finkers, R., Sanjaya, L. and Groenwold, R. (2004). QTL mapping of anthracnose (*Colletotrichum spp.)* resistance in a cross between *Capsicum annuum* and *Capsicum chinense*. *Theor. Appl. Genet*. **109**: 1275–1282.

Walker, J. C. and Lindegren, C. C. (1924). Further studies on the relation of onion scale pigmentation to disease resistance. *J. Agric. Res*. **29**: 507-514.

Wright, P.J., Clark, R.G. and Hale, C.N. (1993). A storage soft rot of New Zealand onions caused by *Pseudomonas gladioli* pv. *alliicola*. *New Zealand J. Crop and Hort. Sci*. **21**: 225-227.

APPENDICES

Preparation of culture media

The compositions of the media which were used in this thesis work are given below: Unless otherwise mentioned all media were autoclaved at 121^0C for 20 minutes at 15 lb. pressure.

PDA Medium

Peeled Potato	200 g
Agar Powder	20 g
Dextrose	20 g
Distilled Water	1000 ml

NA Medium

Dehydrated Medium	28 g
Distilled Water	1000 ml

Specific Medium for *Bacillus spp.*

Dehydrated Medium	20.5 g
Distilled Water	475 ml

Specific Medium for *Pseudomonas spp.*

Dehydrated Medium	46.7 g
Distilled Water	1000 ml

KOH Solubility Test Reagent

3% aqueous solution of KOH was prepared from the KOH granules.

Catalase Test Reagent

3% aqueous solution of H_2O_2 was prepared from the H_2O_2 absolute solution.

Oxidase Test Reagent

1% aqueous solution of NNN'N-tetramethyl-p-phenylene-diamine dihydrochloride was prepared from the absolute solution.

Motility Test Reagent

SIM Medium

Pancreatic Digest of Casein	20 g
Peptic Digest of Animal Tissue	6.1 g
Ferrous Ammonium Sulfate	0.2 g
Sodium Thiosulfate	0.2 g
Agar Powder	3.5 g
Distilled Water	1000 ml

Casein Test Reagent

Skim Milk Agar Medium

Dry Milk, Instant Nonfat	50 g
Pancreatic Digest of Casein	5 g
Yeast Extract	2.5 g
Glucose	1 g
Agar	12.5 g
Distilled Water	1000 ml

Citrate Test Reagent

Simmons Citrate Agar Medium

Sodium Chloride (NaCl)	5 g
Sodium Citrate (Dehydrated)	2 g
Ammonium Dihydrogen Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate (Heptahydrate)	0.2 g
Bromothymol Blue	0.08 g
Agar	15 g
Distilled Water	1000 ml

Lactose and Sucrose Test Reagent

Phenol Red Sucrose Broth Medium

Proteose Peptone	10000 g
Beef Extract	1000 g
Sodium Chloride (NaCl)	5000 g
Sucrose	5000 g
Phenol Red	0.018 g
Distilled Water	1000 ml

Gram Staining Reagents

Gram's Crystal Violet (Hucker's modification)	
Solution A: Crystal Violet (90% dye content)	2 g
Ethyl Alcohol	20 ml
Solution B: Ammonium Oxalate	0.8 g
Distilled Water	80 ml

Solution A and B in equal volume to prepare crystal violate solution.

Gram's Iodine (Gram's Modification of Lugol's Solution)	
Iodine	1 g
Potassium Iodide (KI)	2 g
Distilled Water	300 ml

Add iodine after KI is dissolved in water to prepare Gram's Iodine solution.

Gram's Alcohol (Decolorizing Agent)

Ethyl Alcohol (95%)	98 ml
Acetone	2 ml

Safranin (Counter Stain)

Safranin (2.5% Solution in 95% Ethanol)	10 ml
Distilled Water	100 ml

Reagents used for DNA sequencing

DNA Isolation:

i) Maxwell Blood DNA Kit, Model: AS1010, Origin: Promega, USA.

PCR:

i) Hot Start Green Master Mix (dNTPs, Buffer, $MgCl_2$, Taq Pol), Cat: M7432, Origin: Promega, USA.

ii) Primer 27F: AGA GTT TGA TCM TGG CTC AG

iii) Primer 1492 R: CGG TTA CCT TGT TAC GAC TT

Gel:

i) Agarose, Cat: V3125, Origin: Promega, USA.

ii) 100 bp DNA Ladder, Cat: G2101, Origin: Promega, USA.

iii) 1kb DNA Ladder, Cat: G5711, Origin: Promega, USA.

iv) Diamond™ Nucleic Acid Dye, Cat: H1181, Origin: Promega, USA. (A non-carcinogenic DNA binding dye).

v) TAE Buffer: Cat: V4251, Origin: Promega, USA.

PCR Clean Up:

i) SV Gel and PCR Clean Up System, Cat: A9281, Origin: Promega, USA.