POST HARVEST DISEASES OF SOME SELECTED FRUITS, THEIR CAUSES AND MANAGEMENT

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This is to certify that the thesis entitled, "**POST HARVEST DISEASES OF SOME SELECTED FRUITS, THEIR CAUSES AND MANAGEMENT**" submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e Bangla Nagar, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY** embodies the results of a laboratory research work carried out by **ANANNAY BISWAS SOMA** bearing **REGISTRATION NO. 18-09057** under my supervision and guidance. No part of the 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 this investigation has been duly acknowledged.

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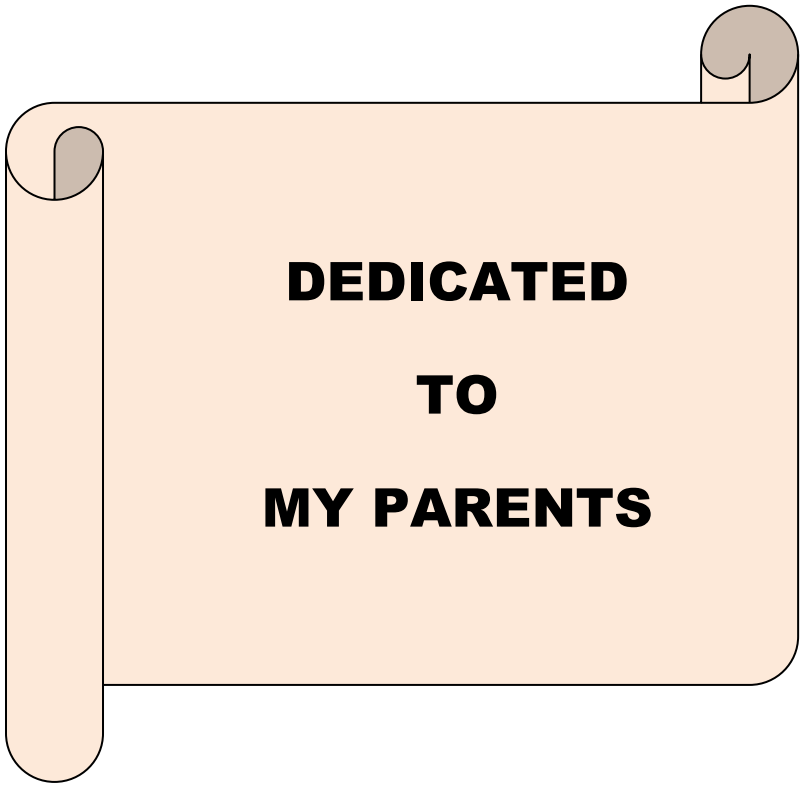
DEDICATED
TO
MY PARENTS

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ABSTRACT

A study was conducted at Plant Pathology Laborartory, Sher-e-Bangla Agricultural University, Dhaka-1207 during February 2019 to March 2020 to isolate, identify, and characterize the causal agents responsible for post-harvest diseases of fruits. Apple, pear, orange and papaya were used as samples and collected from different fruit markets in Dhaka city. Post-harvest fungi were isolated on PDA medium following blotter paper method. Four different genera of fungi were isolated and identified viz. *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Sclerotium* sp. Of these, *Aspergillus* spp. was common in apple, pear, orange and papaya. Pathogenicity test showed all isolated fungi pathogenic to their respective host. Effect of different media, temperature and packaging on growth and development of isolated fungi was studied. Study was carried out by completely randomized design with three replications. All isolates showed variation in myceial growth, surface colour, colony colour, colony shape and colony texture. On PDA media, the highest radial mycelial growth (88.19 *mm)* was recorded in *Sclerotium rolfsii* (Apple) and lowest (23.62 mm) in *Penicillium digitatum* (pear) at 10^{th} DAI. At 30℃ temperature, the highest radial mycelial growth (90.00mm) was recorded in *Sclerotium rolfsii* (Apple) and lowest (23.53 mm) *Penicillium digitatum* (orange) at 10^{th} DAI. In paper packaging, long days were required for deterioration in case of apple (60 days) and shortest days were required for deterioration in case of papaya (6days). *Sclerotium rolfsii* (Apple) showed best performance in radial mycelia growth observation (media and temperature) and apple showed best performance in all packaging observation. In this study, PDA medium, 30°C temperature showed best results for mycelial growth of isolated pathogen and paper bag showed best results for packaging of fruits at room temperature.

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et al.	=	And others
sp.	=	Species
J.	=	Journal
no.	=	Number
etc	=	Etcetera
˚C	=	Degree Celsius
ml	=	Milliliter
mm	=	Millimeter
µm	=	Micrometer
/	=	Per
kg	=	Kilogram
%	=	Percent
PDA	=	Potato Dextrose Agar
CDA	=	Carrot Dextrose Agar
PCDA	=	Potato Carrot Dextrose Agar
LSD	=	Least Significant Differences
CV%	=	Co-efficient of Variance
DAI	=	Days After Incubation/Inoculation

Chapter I

INTRODUCTION

A high incidence of post-harvest losses exacerbates markable complications of low agricultural productivity and food security in the world and the highest losses are found in developing countries. Postharvest losses cause the quality and quantity reduction of food severely, thereby affecting incomes and impacting on the rural poor society (FAO, 2018). The most common post harvest and storage fungi of fruits are *Aspergillus flavus, A. niger, Fusarium oxysporum, F. solani, Sclerotium* sp and *Penicillium* sp (Bhale, 2011). Lot of research has been done on fruit rot abroad but insufficient information is available in Bangladesh (Bashar *et al.*, 2012).

Apple (*Malus domestica*), pear (*Pyrus communis*), orange (*Citrus reticulata*) and papaya (*Carica papaya L.*) four economically important fruits which are widely consumed fruits, among which apple and pear are not yet cultivated in Bangladesh, but oranges are now cultivated in our country and papaya is native fruit of Bangladesh. Every year, consumption of fruits is increasing 5% (Daniel *et al.*, 2019). In FY 2018-19, total domestic fruits production stood 1.21 crore kg and public demand for foreign fruits resulted in expenses of Tk 20.3 billion on the import of different fruits where orange, tangerine, apple, pear and grape represented 85% of the fruit imports (FAO, 2018).

Post harvest diseases have not received the attention as a magnitude of the problem warrants which cause loss ranging from 20% to 50% between marketing and consumption (Kasso and Bekele, 2016). Fruits are inherently liable to deteriorate under different climatic conditions because they contain high moisture content (Kitinoja and Kader, 2012), remain biologically active and bring out respiration, transpiration and other biochemical activities, they become susceptible within time (Kasso and Bekele, 2016).

Postharvest diseases are caused primarily by microscopic fungi (Singh *et al.*, 2017) which can make infections through microscopic surface wounds created by mechanical or insect injury from growing season to consumption (Fallik *et al*., 2019). According to pathogenic signs and symptoms, diseases are primarily classified (Pierre *et al.*, 2011). Propagules of pathogenic fungi are abundant in the atmosphere (air, soil and water), on the surfaces of fruits (Baert *et al*., 2007) and containers used for storing and transporting fruit (Willson, 2013) produce extracellular enzymes to degenerate cells for nourishment (Simmonds *et al*., 1975).

Spores of *Penicillium* sp, *Fusarium* sp *and Aspergillus* sp are responsible for mould rot, a major postharvest disease of apple, pear, citrus and papaya worldwide (Schovánková and Opatová, 2011). *A. flavus* and *A. niger* are the most common and dominant species that affecting fruits (Abdullah *et al.*, 2016). Spores of *Penicillium* species are present in the soil, on the fruit surface, in the air of the store. *Fusarium* species is well represented among the communities of soil borne fungi, in every type of soil all over the world (Fravel *et al.*, 2003). Sclerotia of *Sclerotium rolfsii* easily spread with soil movement, infested plant materials, contaminated equipment, by splashing water to long-distance during shipment (Mullen, 2006). *Penicillium* sp, *Fusarium* sp *and Aspergillus* sp are not capable of penetrating the intact surfaces of the host, but they readily entry through injuries or natural openings and thus cause devastating rots of the mature produce (Snowden, l99l).

Preparation of suitable culture media for pathogenic growth is one of the prerequisites to study them. Different microorganisms nourish at different environments and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature (Magdalena, 2016). Media containing high carbohydrate source, nitrogen source are required for the growth of fungi at pH range of 5 to 6, and a temperature range from 15 to 37˚C, includes corn meal agar (CMA), potato dextrose agar (PDA), V-8 juice agar, and dung agar (Basu *et al*., 2015); carrot dextrose agar (CDA), potato-carrot dextrose agar (PCDA) (Aryal, 2015); soyabean dextrose, sawdust sucrose, sucrose and groundnut

dextrose broths (Ikechi *et al*., 2012); sabouraud agar (SA) Czapek's Dox Agar (CZA), nutrient agar (NA) etc (Hase and Nasreen, 2017).

Several factors contribute to post-harvest losses along the supply chain such as pre-harvest factors, environmental hazards (temperature, ventilation and relative humidity control, pests and diseases and senescence (FAO, 2018).

An appropriate disease management strategy firstly includes correct identification of the pathogen causing postharvest disease. Approximately 40% postharvest losses are estimated per year despite the use of modern storage facilities and techniques (Dubey, 2012). Harvested fruits require adequate and advanced postharvest processing for minimizing the losses after harvesting.

Management through physical ways can be observed as, temperature management, heat treatments, proper storage bag, packing sanitation, transportation etc rather than chemicals which can lengthen the storage life and maintain quality of horticultural crops (Kitinoja and Kader, 2012).

Temperature can be the main factor in controlling microbial growth in perishable foods after harvest, during storage and shipment (Silvestri *et al.*, 2018). Growth rate of fungi vary depending on temperature. The optimum growth temperatures for the majority of fungi studied was found to fall between 25˚C to 30˚C; was drastically reduced below 15˚C and above 35˚C and above 40˚C the growth was poor and in some cases mortality may occur (Sharma, 2015). Storage temperature of fresh produce is so critical to control postharvest diseases as moderately high temperatures cause fresh produce to decay (Choudhury, 2006) and excess low temperatures cause chilling injury (Sommer, 2009). Low temperature storage of fruit is used to delay ripening of disease, but temperatures are not lethal to the pathogen. For example, many temperate fruit (e.g. apples and pear) can be stored at 0ºC, whereas many tropical fruits cannot be stored below 10ºC as symptoms of chilling injury may arise (Kasso and Bekele, 2016).

Packaging is another important management phase to extend shelf life of fresh fruits. If both temperature and packaging are optimal, ageing of fruits can be slowed down significantly (Bigger *et al.*, 2003). The most common form of

commercial packaging is fibreboard carton, paper bags, tissue paper wraps, trays, net bags, cups or pads, moulded trays, waxed paper, polybags etc (Watson *et al*., 2016 and Scetar *et al.*, 2010).

Considering these factors, an intensive experiment was undertaken in Dr. M. A. Wazed Miah Research Centre, Sher-e-Bangla Agricultural University, Dhaka-1207; focusing on post-harvest fungal diseases of fruits (apple, pear, orange and papaya), their causes and the effect of different temperature, media and packaging on pathogenic growth to achieve the following objectives:

Objectives:

1. To isolate, identify and characterize causal agents of post harvest diseases of selected fruits.
2. To evaluate the effect of different cultural media, temperature and packaging on the growth and development of isolated fungi from selected fruits.

Chapter II

REVIEW OF LITERATURE

2.1.1 Post harvest diseases

Singh *et al.* (2018) reported that soil-infesting microorganisms like bacteria, fungi or nematodes that cause loss of fleshy tissues specially infect plants at the time of or just before harvesting. Infestation may occur during post harvest handling or storage as pathogenic propagules are abundant in atmosphere and on surfaces of fruits or inside the infected part.

Dauda and Adegoke (2014) mentioned that fruits and vegetables are among the most important foods of mankind because of their nutritive and indispensable effect in the management of health and fungi cause fruit and vegetables rot beacause they are fresh product containing high levels of moisture, sugars and nutrients element and their low pH values make them specifically desirable to fungal infection.

Visagie (2014) tested that approximately 25 species of fungi and bacteria are responsible for the major decays of plant products after harvest. Most of these microorganisms have the potentiality for attacking only a few different products from related species of plants. For example, *Penicillium digitatum* causes green mold on citrus fruits but does not cause disease in apples and pears. On the other hand, *P. expansum* causes blue mold on apples and pears but not on citrus fruits.

Dubey and Jalal (2013) studied that harvested products injured by physiological disorders such as excess cold, high heat, oxygen deficiency and other environmental agents push on the fresh agricultural products to post-harvest diseases. Alternaria black rot and bacterial soft rot of tomatoes have been reported to increase due to such storage conditions. Moderately high temperatures cause fresh produce to complete decay.

Bashar *et al.* (2012) found that diseases which are to develop on harvested parts of the plants like seeds, fruits and also on vegetables are particularly known as the post-harvested diseases. This can take place if the harvested products may get injured on the way of storage or to market or even just before their final consumption by human being. Considerably, the plant parts may also get infected in the field, but expression of symptoms can take place later, at any stage before final consumption.

Bhale (2011) reported that the most common post harvest and storage fungi of fruits are *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium oxysporum, Colletotrichum* spp., *Gloeosporioides* spp., *Geotrichum* sp, *Rhizopus nigricans, R. stolonifer and Penicillium* spp..

Moretti *et al*. (2010) found that postharvest losses occur by some common diseases like blue and green mould (caused by *Penicilium* spp.) and Fusarium rot (caused by *Fusarium* sp) resulted from wound infection.

2.1.2 Post harvest disease of fruits

Apple

Nabi *et al*. (2017) studied that latent infections are remarkable in postharvest wastage of many temperate fruits, such as apple, pear, apricot etc which is largely aided by mechanical injuries to the peel of produce or by direct penetration of the cuticle or entry through lenticels, stomata, wounds or scar tissue.

Ewekeye *et al.* (2016) observed an isolation practice which was carried out in Nigeria about various storage and shelf fungi of apple spolige ,where fungi were cultured on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The isolated fungi species were *Rhizopus* sp, *Aspergillus niger, Aspergillus terreus, Trichoderma* sp, and *Mucor* sp.

Oranusi and Wesley (2012) found that due to mechanical handling of apple fruits, its surface can harbor microorganisms which can attach to the surface or penetrate into fruits and redouble within its tissues and Dennis *et al.* (2005) revealed that the spores of pathogens take place on the surface of fruit at growing period when fruits remain naturally resistant, then fruit losses its resistancy due to gradual ripening and some mechanical damage which cause fruit decay.

Pierre *et al.* (2011) tested that pathogens like *Alternaria* sp, *Fusarium* sp, *Sclerotium* sp can destroy the nutritional value of apple and make the product damage to aesthetic value and harmful to human health.

Baert *et al.* (2007) studied that spores of *Penicillium* species are present in soil, on the surface of fruit, in air of store house etc. Pomiferous fruits like apple are prominent host of this pathogen. Blue mould rot, a major postharvest disease of apples worldwide that is caused by this species.

Valiuskaite *et al.* (2006) mentioned that most of the post harvest losses of apple can be caused by eminent postharvest fungi include *Penicillium expansum* (blue mould); *Monilinia fructigena* (brown rot), *G. album* and *G. fructigenum* (Gloeosporium rot) depending on fruit cultivar, growing season and production area among other factors.

Jijakli and Lepoivre (2004) reported that 5 to 25% losses of apples in storage room has been found even with modern storage facilities. Fungal pathogens like *Botrytis cinerea*, *Penicillium expansum* and *Gloeosporides* groups etc. are mainly responsible for massive economical diminution even physiological violation (bitter pit, water core and storage scald).

Papaya

Helal *et al.* (2018) experimented that 19 fungi were isolated from diseased yellow Shahi papaya collected from five different markets of Dhaka city in Bangladesh. Those were *Alternaria alternata*, *Aspergillus flavus, A. fumigatus,*

A. niger, *Colletotrichum dematium*, *C. gloeosporioides*, *Corynespora citricola*, *Curvularia lunata*, *Fusarium flocciferum*, *F. nivale*, *Fusarium* sp, *Lasiodiplodia theobromae*, *Monilia* sp, *Mucor* sp, *Penicillium* sp, *Pestalotiopsis guepinii*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Syncephalastrum* sp

Akinro *et al.* (2015) and Hamim *et al.* (2014) found that Anthracnose, Aspergillus rot, Fusarium rot, Penicillium rot, Alternaria rot, Rhizopus rot and stem end rot are recorded in Bangladesh as post-harvest diseases of papaya brings considerable deterioration in fruit quality and loss in fruit business.

Chowdhury *et al.* (2014) mentioned that post-harvest losses in papaya fruits are significantly high due to fungal infections. Various fungi cause rots at post harvest stage of papaya like *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Alternaria*, *Phomopsis, Fusarium, Aspergillus, Stemphylium, Pestalotiopsis etc.* and cause considerable damage to fruit quality and production.

Bhale (2011) observed that after harvesting, during storage and transportation fruits go through several biotic and abiotic stresses which plays a significant role in association of different pathogens and development of diseases over papaya fruits like Fusarium rot, Aspergillus rot etc.

Pear

Sutton *et al.* (2014) and Snowdon (1990) studied that pathogens as *Botrytis cinerea* (grey mold), *Penicillium expansum* (blue mold), *Mucor piriformis*, and *Monilinia fructigena* (brown rot) can cause decay of fruit in pre and postharvest ages.

Wenneker *et al*. (2014) experimented that postharvest diseases in long storage of apples and pears having major pathogens as *Neofabraea* spp. (apples and pears) and *Cadophora* spp. (pears) in Netherlands. At the same time *Botrytis*

spp., *Pencillium* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp. were also found at low frequencies and considered with minor importance.

Soto-Alvear *et al.* (2013), Spolti *et al.* (2012) and Weber (2009) found that postharvest rots results from infections at orchard remain quiescent during growing period and remain unnoticed at harvest. Development of symptom expression occurs at storage condition. Some common pathogens causing such late postharvest losses to pome fruits are able to infect fruits through lenticels, as *Colletotrichum acutatum species* (bitter rot), *Neofabraea alba* and *Neofabraea perennans* (Bull's eye rot).

Weber (2013), Sever *et al.* (2012) and Oranusi and Wesley (2012) identified that due to mechanical handling of pear fruits, its surface become perishable for microorganisms which can attach to the surface or invade into fruits and multiply within tissues such as *Aspergillus* sp, *Penicillium* sp, *Alternaria* spp. and *Fusarium* spp. etc.

Llorente and Montesinos (2006) reported that pear scab (*Venturia pirina*) and brown spot (*Stemphylium vesicarium*), stem-end rot (*Diplodia natalensis, Phomopsis citri*), gray mold (*Botrytis cineria),* sour rot *(Geotrichum candidum)* are the main diseases on pears.

Orange

Kahramanoglu *et al.* (2020) studied that because of highly juicy contents in citrus fruit, *Penicillium italicum* (blue mold) and *Penicillium digitatum* (green mold) make their entry to decay fruits through wounds by airborne spores which are greatly produced by rotten fruits and easily contaminate the surrounding fruits at temperature about 20–25°C.

Cerioni *et al.* (2017) found that green mold by *Penicillium digitatum* is the most considerable citrus decay and sour rot, although it is less common, causes significant losses in high rainfall areas.

Niem *et al.* (2007) mentioned that citrus fruits are severely affected by fungal pathogens as pre harvest infections caused by Brown rot (*Phytophthora* spp.*)*, Anthracnose (*Colletotrichum gloeosporioides*), Alternaria rot (*Alternaria* spp.), Grey mold (*Botrytis cineria*), Diplodia stem-end rot (Lasiodiplodia theobromae) etc. on the other hand post harvest infections caued by Green mold (*Penicillium digitatum* Sacc.), Blue mold (*P. italicum* Weh).

Villalta *et al*. (2004) experimented that green mold and blue molds represent the most serious diseases and cause remarkable economic losses at the period of fruit storage and marketing. *Penicillium digitatum* and *Penicillium italicum* specially infect blood oranges and infection starts through rind wounds the place where nutrients are available for spore germination. Particular warm and wet season may help in occurring incidence of other pathogen.

Dantas *et al.* (2003) conducted a survey in Northeastern Brazil of postharvest diseases of citrus and reported a 21.9% incidence of fungal rots. Several kinds of damaged tissue are prone to infection by pathogenic microorganisms.

2.1. 3 Isolation and identification of pathogen

Aspergillus niger

Lanette (2014) demonstrated that *Aspergillus niger* is not only a species of plant pathogen, but a group in the genus *Aspergillus* that is made up of 15 varieties, all with black conidia and generally recognized as safe by the USDA. In fact, many strains are used in commercial food production, including in fermentation processes and the production of citric and gluconic acid.

Sever *et al.* (2012) studied that *Aspergillus niger* typically reproduces in the asexual state, although sexual reproduction has been observed. In the typical asexual state, conidia (i.e. spores) are released and disseminated via wind. Conidia germinate into hyphae. Following hyphal colonization of the substrate, aerial hyphae will emerge, producing conidiophores (i.e. stalks) and conidial heads that form conidia.

Gautam and Bhadauria (2012) showed that morphological characters are not enough for fungal identification and it renders the need of microscopic features along with the morphological. Same colony colours of *A. niger* and *A. luchuensis*; *A. flavus* and *A. oryzae*; *A. anstelodani* and *A. parasiticus*, which supports the requirement of microscopic characters, along with the macroscopic for correct identification and classification.

Weber (2011) found that fungus can spread via the air, soil, and water. It is generally a saprophyte, living off dead and decaying matter. Therefore, it is commonly seen as a post-harvest disease. In the case of humans and animals, a compromised immune system is typically present when the disease manifests. In plants, irrigation practices such as drip irrigation lines buried in soil and hot, humid growth conditions are conducive to disease development.

Sharma and Sharma (2009) stated that the host range includes 37 genera of fruits and vegetables such as tomatoes, peanuts, grapes, onions, and mangoes. The fungus is commonly found in the soil, living as a saprophyte on decaying vegetation and leaves, compost piles, and stored grain. It is also found in indoor environments and as a contaminant of food. As the fungus is opportunistic, humans, birds, and animals can also be hosts, although this is extremely rare

Klich and Pitt (1988) experimented that *Aspergillus niger* is ubiquitous and commonly found in soils, seeds, plant litter, plant rhizospheres, dried fruit, and nuts. It is one of the most common fungi found on foods.

Aspergillus flavus

Shimwela (2014) identified that *Aspergillus flavus* is a fungal pathogen that causes Aspergillus ear and Kernel rot. The fungus is mostly found in soil as saprophytes, but it has a broad host range as an opportunistic pathogen. It causes significant losses in corn, peanuts, cottonseed, and tree nuts. *A. flavus* is also a pathogen of animals and insects. Contamination with aflatoxin, a toxic and carcinogenic compound, can occur when the fungus grows on food sources.

Klich and Xiao (2007) tested that powdery olive-green (yellow-green) mold grows on the ears of corn and then turns brown as the masses age. This pathogen is wide spread, particularly in tropical and subtropical soils.

Kozakiewicz *et al.* (1995) observed that hot, dry weather condition is favorable for *Aspergillus* infection and spread. *Aspergillus* fungal spores are produced on crop residue on the soil surface. The spores are disseminated by wind and insects to the silks of the maturing ears. During its growth, the fungus produces aflatoxin that is undesirable in the harvested produce.

***Penicillium* sp**

Canon (2016) and Samson (2004) mentioned that species of *Penicillium* species are ubiquitous soil fungi, saprophytic in nature, Commonly known as molds, and causes food spoilage. Some members of these ascomycetous fungi produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria.

Samson and Zoellner (2014) examined some species of *Penicillium* sp which have a blue color, commonly growing on old bread and giving it a blue fuzzy texture. The common apple rot fungus *P. expansum* was selected as the type species. Many species produce highly toxic mycotoxins.

Böhm *et al.* (2013) made a study that the thallus (mycelium) consists of highly branched networks of multinucleated cells located on a septum lacking hyphae that is often colorless. Conidiophores are at the end of each branch accompanied by green spherical constricted units called conidiospores. These individual units play a significant role in reproduction; conidiospores are the main dispersal route of the fungi.

Balgrie (2013) observed that some *Penicillium* species affect the fruits and bulbs of plants, including *P. expansum*, apples and pears; *P. digitatum*, citrus fruits and *P. allii*, garlic.

Thompson *et al.* (2010) reported that *Penicillium* species are present in the air and dust of indoor environments, such as homes and public buildings. The

fungus can be readily transported from the outdoors, and grow indoors using building material or accumulated soil to obtain nutrients for growth. *Penicillium* growth can still occur indoors even if the relative humidity is low, as long as there is sufficient moisture available on a given surface.

Leitao (2009) found that thallus referred to "pencil-like" (referring to a Camel's hair pencil brush. Three species *P. candidum*, *P. expansum*, and *P. glaucum* all of which produced a brush-like conidiophore (asexual fruiting structure).

Nicoletti *et al*. (2009) experimented that sexual reproduction involves the production of ascospores, commencing with the fusion of an archegonium and an antheridium, with sharing of nuclei. The irregularly distributed asci contain eight unicellular ascospores each.

Pitt *et al*. (2000) studied that the ability of these *Penicillium* species to grow on seeds and other stored foods depends on their propensity to thrive in low humidity and to colonize rapidly by aerial dispersion while the seeds are sufficiently moist.

da Costa *et al.* (1998) mentioned that some fungal species are pathogenic to animals; *P. corylophilum*, *P. fellutanum*, *P. implicatum*, *P. janthinellum*, *P. vir idicatum*, and *P. waksmanii* are potential pathogens of humans and other living animals..

Chang *et al.* (1995) found that *Aspergillus* and *Penicillium* type spores were the most prevalent in the indoor air of residential properties, and exceeded outdoor levels. Even ceiling tiles can support the growth of *Penicillium* if the relative humidity is 85% and the moisture content of the tiles is greater than 2.2%.

***Fusarium* sp**

Bakar *et al.* (2013) identified that *Fusarium* species is one of the common pathogens of post-harvest disease to cause rot perishable vegetable fruits. A total of 180 Fusarium isolates were obtained where *Fusarium solani* was most abundantly isolated (34%) followed by *F. semitectum* (31%) and *F. oxysporum*

(31%), *F. subglutinans* (3%) while the least was *F. equiseti* (1%). Pathogenicity test of isolates by injecting 1 mL of the conidial suspension onto healthy fruits showing majority of the isolated *Fusarium species* can potentially produce mycotoxins as their secondary metabolites. The potential production of mycotoxins by pathogenic isolates of *Fusarium* species in contaminated to fruits could pose health hazards when consumed.

Nurulhuda *et al.* (2013) reported that the disease causes the vegetable fruits unmarketable as consumer will only choose those that are fresh and healthy. Hence, *Fusarium* species have been isolated from decaying fruits. Fruits contaminated with *Fusarium* species are lethal for human and animals consumption since several species of them produce mycotoxins.

Burgess (1994) and Nelson *et al.* (1990) observed that *Fusarium* species can appear as saprophytes or pathogens on plants, animals as well as humans. They are well associated with a wide range of plants in their natural habitats such as tomato, banana, asparagus, barley, mango, pineapple, carnation, coffee, corn, grasses, legumes, oats, pine, rice, sorghum, sugarcane and wheat.

Oladiran & Iwu (1992) studied that Fusarium rot on papaya fruits are often caused by *Fusarium* species and the disease symptoms include rots softer and extend into the center of the fruit. The rotted tissue is often water-soaked and becomes covered by white, yellow or pinkish mycelium externally while the infected tissue is discolored and appears pale brown.

***Sclerotium* sp**

Billah *et al.* (2017) mentioned that *Sclerotium rolfsii* is found to be pathogenic on sunflower, moonbeam, betel vine, lentil, sugar beet, tomato, sweet pumpkin also attack the plants like maize, chick pea, apple, cotton, potato, soybean, oat and some ornamentals. During favorable weather conditions, sclerotia resume activity by either eruptive or hyphal germination.

Nandi *et al.* (2017) found that *S. rolfsii*, the polyphagous fungus has a wide host range of 500 species in about 100 families including groundnut, green bean, lima bean, onion, garden bean, pepper, potato, sweet potato, tomato and water melon worldwide causing huge losses. Though the fungus is seed and soil borne, soil borne inoculum is more important in causing infection and disease development. The fungus *S. rolfsii* produces abundant white fluffy, branched, septate mycelium with clamp connections only on the main hyphae, which spread like a fan. Small white tufts were formed on mycelium which later gives rise to smooth, hard and dark brown sclerotia.

Kator *et al.* (2015) examined that temperature and moisture are very important factors in the spread and development of this pathogen. Hyphal growth occurs over a temperature range of 8-40ºC / 46-104º F, but optimal growth and sclerotia production occurs between 27-35ºC / 81-95ºF. In addition to temperature effects, hyphal growth and sclerotia germination require a water-saturated soil. High humidity also favours fungal development. At 27ºC / 81ºF on Potato Dextrose Agar, the hyphal growth rate of S. rolfsii has been observed to be 0.8-0.9 mm per hour. Sclerotia form after 5-7 days. Host penetration and infection will proceed optimally at 27-30ºC / 81-86ºF, provided that moisture and high humidity are present.

Mishra and Khan (2015) surveyed on a global perspective and calculated losses of 10 to 20 million dollars associated with *Sclerotium rolfsii* have been recorded with yield depletion ranging from 1to 60% in fields. Sclerotia serve as primary inoculum for the pathogen and are spread to uninfected areas by wind, water, animals and soil. Sclerotia serve as the principle overwintering structures and primary inoculum for disease.

Oche *et al.* (2015) stated that *S. rolfsii* primarily attacks host stems, although it may infect any part of a plant under favourable environmental conditions including roots, fruits, petioles, leaves, and flowers. The fungus produces abundant white, fluffy mycelium on infected tissues. Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed.

Gupta and Pathak (2012) studied that control measures include excluding the pathogen from the area, plant removal, soil removal, soil treatment, heat, solarization, chemical soil treatment, cultural practices, resistance and transgenic plant resistance, plant treatment, crop rotation, amongst others. Despite considerable research on this pathogen, its control continues to be a problem.

Kalai-Grami *et al.* (2013) reported that *Sclerotium rolfsii* is a soil borne polyphagous phytopathogenic fungus infect any plant parts of apple including fruit, flower, pedicel, stem, root etc. characterized by prolific growth and ability to produce persistent sclerotia in soil and th infected plant part.

Fischer *et al.* (2007) and Browing *et al.* (1995) observed that *Sclerotium rolfsii* is a soil borne pathogen that causes stem rot disease on plants. It primarily attacks host stems including roots, fruits, petioles and leaves under favourable conditions. It commonly occurs in the tropics, subtropics and other warm temperate regions of the world. Common hosts are legumes, crucifers and cucurbits.

Corazza *et al*. (1999) reported that Apple collar rot was originally described as apple southern blight in the USA and has also been reported in India, China, Israel and Italy.

Dwivedi and Brown (1991) found that during the middle of the 20th century, *S. rolfsii* was controlled to some degree by fumigation or soil applied fungicides. These chemicals are often too expensive and too toxic for many situations, and future uses of fumigants are being restricted due to environmental concerns. Despite these efforts, *S. rolfsii*, like many other soil borne fungal disease agents, continues to be a difficult pathogen to control. The wide host range, prolific growth, and ability to produce persistent sclerotia contribute to the large economic losses associated with the pathogen. Sclerotia spread to uninfected areas by wind, water, animals, and soil. Mycelium is carried to new places by transplants and infected seeds.

2.1.4. Symptoms and characteristics

Mokobi *et al.* (2020) stated that *Aspergillus flavus* produces white soft velvety colonies that turn yellowish-green, a pigment of the conidial spores. They produce green conidia, with a dominated colony appearance. They are plain and flat at the edges and raised at the center and wrinkled cerebriform pattern. They also produce exudates that are colorless or brown. The sclerotia which are the compact mass of hardened fungal mycelia and deep brown in color. The colonies are encircled by a white border and a pale inner side.

Aycock (2017) mentioned that colonies of *S. rolfsii* are readily distinguished on plant material or artificial media by gross morphological characteristics. Rapidly growing, silky-white hyphae tend to aggregate into rhizomorphic cords. In culture, the whole area of a petriplate is rapidly covered with mycelium, including aerial hyphae which may cover the lid of the plate. Both in culture and in plant tissue, a fan-shaped mycelial expanse may be observed growing outward and branching acutely

Moslem *et al*. (2016) identified in microscope that the conidial heads are radiate with conidiogenous cells biseriate. Conidia is brown. Macroscopic observation of *Aspergillus niger* colonies on potato dextrose agar at 25°C is initially white, which quickly becomes black with conidial production. The reverse is pale yellow and growth may produce radial fissures in the agar.

Houbraken *et al.* (2014) observed that hyphae are septate and hyaline. Conidial heads are radiate to loosely columnar with age. Conidiophores are coarsely roughened, uncolored, up to 800 µm long x 15 – 20 µm wide, vesicles globose to subglobose (20 – 45 µm), metulae (8 – 10 x 5 – 7 µm) covering nearly the entire vesicle in biseriate species. Some isolates may remain uniseriate, producing only phialides (8 – 12 x 3 – 4 µm) covering the vesicle. Conidia are smooth to very finely roughened, globose to subglobose, 3 – 6 µm in diameter.

Houbraken *et al.* (2014) stated that *Penicillium* contain septate, hyaline hyphae. Conidiophores simple or branched. Phialides grouped in brush-like

clusters (penicilli) at the ends of the conidiophores; conidia unicellular, round to ovoid, hyaline or pigmented, rough walled or smooth, in chains.

Pitt (2014) reported that the genus *Penicillium* is a very important fungal genus because of its ubiquity and the role of many species in food spoilage and mycotoxin production. *Penicillium* is distinguished by its frequently greenish colonies and its branching or simple conidiophores supporting phialides in brush-like clusters known as penicillin. Texture is velvety to powdery; color green, blue-green, gray-green, pinkish and yellow on the culture surface.

Paolo (2013) studied that Sclerotia forming on a host tend to have a smooth texture, whereas those produced in culture may be pitted or folded. Serving as a protective structure, sclerotia contain viable hyphae and serve as primary inoculum for disease development.

William and Wambura (2011) found that *Aspergillus flavus* under the microscope, observe uncolored thick-walled conidiophores and rough or pitted vesicles. The vesicles are about 800-1200um in diameter, producing phialides. the phialides have uniseriate or biseriate or combined. The conidia are 250-450um with thin rough walls.

Ataga *et al.* (2010) mentioned that *Aspergillus niger* is a filamentous fungus, forming filamented hyphae that make them appear like small plants. Macroscopic observation of *Aspergillus niger* reveals that their growth is initially white but they change to black after a few days producing conidial spore. The edges of the colonies appear pale yellow producing radial fissures.
Elenwo *et al*. (2010) observed that microscopic view of *Aspergillus niger* reveals that it has smooth colored conidiophores and conidia. The conidiophores are protrusions from a septate and hyaline hyphae. The conidial heads appear radial and they split into columns (biseriate). The conidiophore vesicle produces sterile cells known as metulae which support the phialides on the conidiophores. Conidiophores are smooth and hyaline. The metulae and

phialides cover the vesicle. The phialides produce conidia that have a rough texture, are dark brown colored.

Moslem *et al.* (2010) found that the colonies of *Aspergillus niger* on PDA at 27°C are initially white, quickly becoming black with conidial production. Reverse is pale yellow and growth may generate radial fissures on the agar. Hyphae are septate and hyaline. Conidial heads are radiate initially, splitting into columns at maturity. They have a cottony appearance; initially white to yellow and then turning black made up of conidiophores. The reverse is white to yellow.

Rahman *et al.* (2008) examined that Fusarium rot caused by *Fusarium* sp appeared initially as a circular water-soaked lesion, which later became depressed. At advanced stage of disease development, the soft rotted area was covered with a white mycelial mat of the fungus. Conidia were hyaline, three to four-celled and crescent shaped with sharply pointed ends, which were produced from phialides.

Townsend and Willetts (2001) observed that Sclerotia (0.5-2.0mm diameter) begin to develop after 4-7 days of mycelial growth. Initially a white appearance, sclerotia quickly melanize to a dark brown coloration. Four zones in the mature sclerotium: i) thick skin, ii) rind of thickened cells, iii) cortex of thin walled cells, and iv) medulla containing filamentous hyphae.

Takahashi (1999) examined that at least two types of hyphae are produced. Coarse, straight, large cells (2-9um x 150-250um) have two clamp connections at each septation, but may exhibit branching in place of one of the clamps. Branching is common in the slender hyphae (1.5-2.5um in diameter) which tend to grow irregularly and lack clamp connections. Slender hyphae are often observed penetrating the substrate.

2.1.5 Pathogenecity test

Nandi *et al.* (2017) experimented that pathogenicity test using placing a parafilm-wrapped PDA plug bearing both mycelium and sclerotia near the

collar region of healthy ground cherry plants. After 7 days, yellowing of basal leaves, followed by drooping of leaves and wilting was observed on inoculated plants. The non inoculated control plants, on which only PDA plugs were deposited, remained healthy. The fungus was re-isolated from inoculated plants. Mycelial vegetative compatibility studies were done as standard method.

Rahman *et al*. (2008) studied on *Fusarium sp* and it showed small, round water-soaked lesions on wound inoculated papaya after three days of inoculation. As the infection advanced, lesions became circular and slightly sunken, and covered with dense whitish mycelial growth. In unwounded fruits, small water-soaked areas were observed on each inoculation site after five days of inoculation. At the advanced stage of disease development, a round sunken lesion with translucent, light brown margin was formed. After seven days of inoculation the fungus produced light orange spore masses in the central portion of the lesion.

2.2 Management of post harvest diseases

Media

Hase and Nasreen (2017) experimented that PDA is one of the most commonly used culture media because of its formulation and ability to support mycelia growth of wide range of fungi. Growth of some pathogenic fungi has been studied in various media viz. Potato Dextrose Agar (PDA); Czapek's Dox Agar (CZA); Corn Agar (CA); Nutrient Agar (NA); Sabouraud Agar (SA).

Nandi *et al.* (2017) experimented cultural characteristics by isolating fungus on Potato Dextrose Agar (PDA) directly from surface sterilized diseased tissue and maintained them on PDA plates (Potato-200 g; Dextrose-20 g; Agar-18 g; D/W 1000 ml) at 28±1°C.

Ewekeye *et al.* (2016) observed an isolation practice about various storage and shelf fungi of apple spolige ,where fungi were cultured on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The isolated fungi species were

Rhizopus sp, Aspergillus niger, Aspergillus terreus, Trichoderma sp, and *Mucor* sp.

Bakar *et al*. (2013) studied the phenotype-based identification of *Fusarium* sp pure isolates which were cultured on carnation leaf-piece agar (CLA) and incubated at room temperature (25±2°C) for 7 days. The isolates phenotype was examined for identification of shapes and sizes of macroconidia and microconidia; number of septa and shapes of the apical and basal cells of the macroconidia, conidiogenous cells, growth rate, presence of chlamydospore, colony colour, growth and pigmentation on PDA.

Sharma and Kaul (2012) identified that fungus is typically cultured on PDA (potato dextrose agar) media. After 3-4 days, *Aspergillus* sp form a compact layer of mycelia is covered by a thick layer of large (up to 3 µm x 15 -20 µm), dark-brown to black, conidial heads. Conidia are dark-brown to black.

Kumara and Rawal (2010) found that the fungus shows variation in growth, when grow on various nutrient media. The fungi shows the vegetative growth and colony morphology with pigmentation and sporulation depending on the composition of specific culture media, pH, temperature, light, humidity.

Temperature

Wenneker (2019) mentioned that fruits should be stored in regular atmosphere (RA) for short-term storage and in controlled atmosphere (CA) for long-term storage until packing. Pome fruit like apple and pear may remain for up to 12 months in storage, but during this time fruit rot diseases may also occur.

Shah *et. al* (2018) observed that at 25˚C and 30˚C the fungus attained maximum growth of 25mm and 35mm after 4 days of incubation. However, the growth of the fungus was drastically reduced below 15˚C and started to decline above 35˚C, as these temperatures did not favour the growth of the fungus.

Singh *et. al* (2017) experimented that temperature management is so critical to control postharvest diseases. At low temperatures the physiological and

biochemical changes of fruits become slow so the host can maintain high resistance to pathogens. But the incidence of Alternaria rot in pawpaw and apple is increased by exposure to excessive cold. The rate of deterioration increases by two- to three-fold for each 10 °C increase above optimum .

Parisa *et al.* (2017) observed that citrus postharvest diseases can be controlled by using cold rooms in storage and the use of refrigerated containers during transport of processed fruits delay the development of rots considerably which are important practices to reduce postharvest diseases.

Sui *et al.* (2016) reported that heat treatments can enhance resistance of fresh produce to environmental stress and help to preserve fruit and vegetable quality during prolonged storage and extended shelf-life, moreover these are residue-free and environment-friendly.

Giradu *et al*. (2012) reported that hot water postharvest treatment performs very effective not only against both *Neofabraea spp*., *Monilia fructigena* and *Phytophthora spp.* Adjunction of formulated clove oil enhances the efficacy. Sanitation is recommended to avoid infection by *Penicillium* during storage; furthermore, certain antagonistic microorganisms (such as *Candida spp*.) are effective against *P. expansum, Botrytis cinerea* and *M. fructigena.*

Sharma and Sharma (2009) found that *Penicillium* sp and *Trichoderma* sp exhibited maximum growth at a temperature range of 28-30˚C. The effect of temperature on growth of 17 strains of *Aspergillus* resulted that 28˚C and 31˚C temperatures to be the optimum temperature for growth of most of the test strains. Shine, *et al.* (2007) examined that proper management of temperature is so critical to postharvest disease control. Fruit rot fungi generally grow at 20 to 25ºC (68 to 77ºF). Fungi with a minimum growth temperature below -20°C (28.4ºF) cannot be completely stopped by refrigeration without freezing fruit.

Agostini *et al.* (2006) tested that waxing treatment is capable to reduce postharvest disease development on apple and citrus black spot, whereas the postharvest fungicides treatment had no effect on these.

Kim and Xiao (2005) made several studies and conducted the effect of environmental factors on the growth of the pathogen such as 24˚C to 36˚C temperature and relative humidity of 97% were reported to have a profound influence on infectiveness of a variety of fungi.

Zhang *et al.* (2004) demonstrated that postharvest infections can be reduced by sanitation of bins and packinghouse facilities (machines, chambers, floor tiles) with chlorine and quaternary ammonium containing products.

Malik and Singh (2004) examined that maximum growth of *Fusarium sp* at 30˚C. Farooq *et al.* (2005), the growth of *Fusarium oxysporium* was found to reach its maximum at 30˚C after 7 days of incubation which was drastically reduced below 15˚C and above 35˚C.

Packaging

Wohner *et al.* (2019) stated that packaging can be the major item of expense in fresh produce marketing, so the selection of suitable containers especially for commercial-scale marketing requires a careful consideration on understanding the need of the commodity, particularly in terms of physical protection.

Awoke *et al.* (2018) studied on three packaging materials (carton, jute sack and newspaper) and three post-harvest treatments (ginger, clove, hot water treatment with 55ºC for 40 seconds and ambient (control)) had been used to asses pH value, moisture content, physiological weight loss, peel to pulp ratio, decay percentage, color and shelf life for every four days.

Bhattarai and Shah (2017) experimented on fruits by five treatments of plastic wrapping without whole, plastic with 5 holes wrapping, plastic with 10 holes wrapping, newspaper wrapping, jute wrapping, no packaging materials

(control) with three replication. Physical attributes including shelf life, weight loss, color index, pathological disorder and marketability were observed in every three days.

Watson *et al*. (2016) and Scetar *et al*. (2010) mentioned that the most common form of commercial packaging is fibreboard carton, bulk bins, paper bags, tissue paper wraps, trays, net bags, cups or pads, moulded trays, waxed paper, polybags, reusable plastic containers, palletized containers, crates, baskets etc.

Scetar *et al*. (2010) observed that processing and packaging are the two important phases in food industry to extend shelf life of fresh fruit. The important parameters for shelf life extension are temperature, moisture and a modified atmosphere (oxygen, carbon dioxide and ethylene). If both temperature and packaging are optimal, ageing of fruit can be slowed down significantly.

Sandhu and Singh (2000) stated that the lower spoilage percentage in polyethylene flms lined boxes and crates might be due to retardation of enzymatic activity of post-harvest pathogens whereas an increase in spoilage of pear fruits with the advancement of storage period.

Gonzalez *et al.* (1997) reported that plastic covering plays an important role in preventing dehydration by creating a saturated micro-atmosphere around the fruit. Moreover, the polyethylene films have the characteristic feature of reducing the rate of transpiration by restricting the diffusion of gases and other mechanism.

Farooqi *et al.* (1975) reported that polyethylene and polyethylene green are impermeable to water; unipacking in such materials raises the humidity around the commodity and decrease moisture loss and results in decrease in weight loss.

Chapter III

MATERIALS AND METHODS

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

3.1 MATERIALS:

I. For Sample Collection:

- ✓ Sample collection bags
- ✓ Record keeping books.

II. For Laboratory Experiment:

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

3.2 Methods

3.2.1 Experimental site:

The experiment was conducted at the Plant Pathology Laboratory, Dr. M. A. Wazed Miah Research Centre, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.2.2 Experimental period:

The experiment was carried out during February 2019 to March 2020.

3.2.3 Collection of samples:

Fresh, matured, ripened and infected fruits sample of apple, pear, orange and papaya (5kg for each) were collected from different markets of Dhaka. Samples were stored at 4°C until the identification and isolation were made within 48 hours. Picking up the picture of the collected sample for further use.

3.2.4 Preservation of samples:

The collected samples were washed with running tap water to remove the sand and soils from the diseased sample and dried then kept in a poly bag and stored at 4ºC in refrigerator for future use.

3.2.5 Isolation, identification, purification and preservation of pathogen:

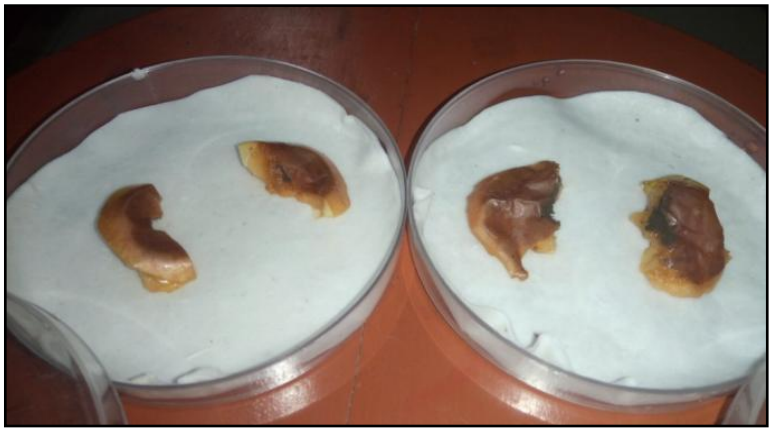
3.2.5.1 Isolation of pathogen

Blotter paper method and growth media (PDA) were followed for isolation of fungi from infected fruit sample (Apple, pear, orange and papaya). At first infected part of fruit samples were washed in tap water to make free from sand and soils. The infected parts along with healthy part of the sample were cut into small pieces (0.5-1.0cm) and then surface sterilized with 70% ethanol 2-3 minutes. Then the pieces of sample were washed with sterilized water thrice and placed on filter paper to remove excess water adhering to the pieces and plated onto wet blotter disc via standard blotter protocol (ISTA, 2003) (Plate

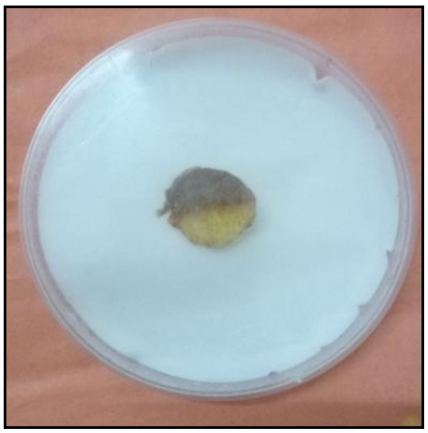
1). The plates were incubated for 7 days at 22℃ and under 12h / light: 12h dark. After incubation, fungi developed on each samples, were isolated as pure cultures on PDA plates. A bit of mycelia that grew over the moisten filter paper was taken with the help of sterile needle and transferred to the media on Potato Dextrose Agar medium (6.5 pH) plates in sterile conditions (Rahman *et al*., 2008, Bhale, 2011 and Gadgile, 2017) from moisten blotter paper. The plates were incubated for 7 days at 25± 1^0C. After incubation the fungi that grew on PDA were identified following the key outlined by Booth (1971) and Agrios (2005). The isolated fungal pathogen was purified by using PDA (Begum *et al.*, 1998). The pure culture of all isolates was preserved in PDA slants at 4^0 C in the refrigerator as stock culture for future study.

3.2.5.2. Identification of causal organism:

After incubation the fungus mycelia were examined under compound microscope for identification of the pathogen. Temporary slides of diseased tissues were prepared and observed under light microscope (Onyemata and Ibrahim, 2018). Fungi were identified according to reference of Collins *et al*., (2004); William and Wambura, (2011); Domsch *et al*., (2007); Barnett and Hunter (1998); Klich and Xiao, (2007); Samson, (2004); Agrios, (2005). 10X-40X magnification were used for the examination of pathogenic isolates under microscope (Leslie *et al.*, 2006; Gautam and Bhadauria, 2012 and Iqbal *et al*., 2017). Microscopic view has been taken for future use.



a. Apple



b. Pear

c. Papaya

d. Orange

Plate 1. Surface sterilized diseased samples placed on moisted blotter paper (a. Apple; b. Pear; c. Papaya; d. Orange)

3.2.5.3. Slants preservation of isolates:

PDA media was poured in test tubes and autoclaved at 121°C for 15min at 15psi. After autoclaved media was permitted to solidify in test tubes at an angel of 65º. A loopful inoculum of each isolates was transferred to Potato Dextrose Agar (PDA) slants, then kept the tubes in an incubator at 25ºC±1 for 7 days for fungal growth and preserved the pure slant culture in refrigerator managing at 4ºC as for future use. (Wilson, 2013; Ikechi–Nwogu and Elenwo, 2012 and Iqbal *et al.*, 2017).

3.2.6 Pathogenicity Test:

Healthy fruits (Apple, pear, orange and papaya) were collected from market places. The LAF (Laminar Air Flow) and surface of the fruits were sterilized by 70% ethanol. Firstly, the cork borer was flamed red hot with a spirit lamp and confessed to cool before use. A hole was prepared by pressing with the sterilize cork borer. Around 4 mm mycelial discs of 5 days old cultures of the isolates was cut by cork borer and inserted into the holes created in the fresh fruits consequently. The discs of fresh fruit in the cork borer was restored and then covered with vaseline jelly to make it air tight. Then they were placed in incubation chamber for future growth of the pathogen. The samples were examined every day to determine the effect of the pathogens on them. Re-isolation of fungi and record of fungi characteristics were done and compared with the first one and then confirmed (Sharma, 2015; Ewekeye *et al*, 2016 and Ivana *et al.*, 2019). After inoculation, pictures of pathogenic symptoms on inoculated fruits were taken.

3.2.7 Effect of culture media and temperature on growth of pathogen

Under aseptic condition in LAF, each pure isolate was transferred to different growth media (PDA, CDA and PCDA) on the same date and kept in incubation chamber at different temperature (20ºC, 25ºC, 30ºC) respectively, at each temperature for 7 days. Data were recorded on radial mycelial growth in each two days interval up to 10^{th} day after inoculation.

3.2.7.1. Preparation of culture media

3.2.7.1.1 Preparation of Potato dextrose agar (PDA) media:

Potato dextrose agar (PDA) medium was prepared as described by Sagar *et al*. (2018). 200 g of sliced peeled potatoes extract, 20g agar and 20g dextrose, 1000ml distilled water were taken in a conical flask and mix well it properly for the preparation of 1000ml PDA medium. Prepared media were sterilized by autoclaving at 121ºC, 15 PSI pressure for 20 min. At the same time petri dishes were sterilized in oven for 90 minutes up to 55/60℃. After completion of autoclaving and oven sterilization the media, petri dishes and other necessaries were transferred in Laminar Air Flow (LAF) for few minutes to cool, 40-50 drops of lactic acid were added and shacked carefully. At last, media was poured into sterilized petri dishes aseptically and left under Laminar Air Flow for drying properly.

3.2.7.1.2 Preparation of Carrot dextrose agar (CDA) media:

Carrot dextrose agar (CDA) was prepared as described by Sagar *et al*. (2018). 200g peeled carrot extract, 1000ml distilled water, 20 g agar, 20g dextrose in a conical flask and autoclaved at 121℃ under 15 PSI for 20 minutes. At the same time petri dishes were sterilized in oven for 90 minutes unto 55/60 ºc. After completion of autoclaving and oven sterilization the media, petri dishes and other necessaries were transferred in Lamina Air Flow (LAF) for few minutes for cool, 40-50 drops of lactic acid were added and shacked carefully. At last, media was poured into sterilize0d Petri dishes aseptically and left under Laminar Air Flow for drying properly.

3.2.7.1. 3. Preparation of Potato carrot dextrose agar (PCDA) media:

Potato carrot dextrose agar (PCDA) was prepared as described by Sagar *et al*. (2018). 200g peeled potato-carrot extract(100g peeled potato extract and 100g peeled carrot extract), 1000ml distilled water, 20 g agar, 20g dextrose in a conical flask and autoclaved at 121℃ under 15 PSI for 20 minutes. At the same

time petri dishes were sterilized in oven for 90 minutes unto 55/60 ºc. After completion of autoclaving and oven sterilization the media, petri dishes and other necessaries were transferred in lamina air flow (LAF) for few minutes for cool, 40-50 drops of lactic acid were added and shacked carefully. At last, media was poured into sterilized petridish aseptically and left under Laminar Air Flow for drying properly.

3.2.8. Radial growth assay:

A wire loop was used to take inoculums from the rotten fruit samples and inoculated in already prepared Potato-dextrose Agar (PDA) media, Carrot-dextrose Agar (CDA) media and Potato-carrot dextrose Agar (PCDA) media using different petri dishes for each of the fruit sample and left to grow at room temperature (25±1℃). Then the pure cultures were collected from the different petri dishes and stored in PDA slants. Fungal isolates from petri dishes were prepared into mounts on microscopic slides and examined under the compound microscope for comparison of fungal morphology with description given by Sutton *et al.* (2014); Ellis *et al.* (2007); Agrios, (2005); Barnett and Hunter (1998) and Booth (1971). Then they were studied for the radial growth assay. The assay was carried out at 20℃, 25℃ and 30℃ at every 12h interval (twice daily) for ten days. Measurement of growth was done in millimeter by using a centi meter-rule (that is from the center of the petri- dish to the circumference of the isolated pathogen (Cerioni, *et al*., 2017).

3.2.9 Study on cultural characteristics of different isolates

20 isolates were isolated from four different fruit samples (Apple, pear, orange and papaya) and cultured on PDA media at 25ºC and colony characters such as surface color, subsurface color, colony shape, colony structure and texture were studied carefully (Ivana *et al.*, 2019).

3.2.10 Packaging of fruits at room temperature

Healthy fresh fruits (apple, pear, orange and papaya) were collected from market. Washing with running tap water and surface sterilization of fruits with 70% ethanol were done and stored at room temperature and 90% relative humidity in various ways as in paper bags, net bags, polythene and shopping bags. The initiation of pathogenic growth on fruit was observed at every 4 days interval of store. The asymptomatic fruits were stored for 14 days before discharged (Cerioni *et al*., 2017).

3.2.11 Data analysis:

Data were arranged on Microsoft office excel worksheet and analysed by using the analysis of variance following Completely Randomized Design (CRD) with Statistix-10.0 software at 5% significant level and LSD (Least Significant Differences) test.

Chapter IV

RESULTS

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

Experiment 1 (4.1.1-4.1.4): Isolation, identification and characterization of causal agents of post harvest diseases of apple, pear, orange and papaya fruits.

4.1.1 Visual symptoms of collected samples

According to chapter III, section 3.2.3, post harvest fruit diseases were identified from collected sample by visual symptoms and compared with secondary data. The Various disease symptoms are shown in plate 2 (a-i) below with recorded pictures:

I. ***Aspergillus flavus:*** Fruits appeared with olivacious or greenish colored pathogenic growth, developing soft rot and water soaked tissue (Plate 2: f and g).

II. ***Aspergillus niger****:* Fruits appeared with black colored pathogenic growth resulting water soaked decayed tissue (Plate 2: a, c and g).

III. ***Fusarium oxysporum (*white*)***: Affected fruits had an earthy, musty odor, caused soft rot in infected area with white cottony pathogenic growth (Plate 2: i)

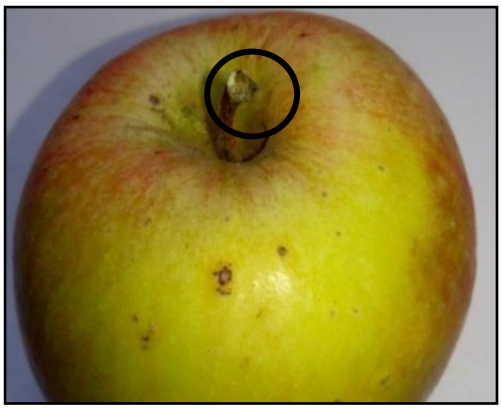
IV. ***Fusarium sp (*pink*):*** Affected fruits had soft rot in infected area with white to gray colored pathogenic growth (Plate 2: i).

V. ***Penicillium italicum* and *P. expansum* (Blue mold):** Caused distension of the fruit surface, infected area covered with blue colored pathogenic growth, spread through the surface having inward progress of infection, infected tissue were shrinkled (Plate 2: e and g).

VI. ***Penicillium digitatum* (Green mold):** Infected area covered with green colored pathogenic growth, soft decayed tissue can be easily scooped out of the surrounding healthy tissue (Plate 2: d).

VII. ***Sclerotium rolfsii*:** Fruit stem appeared with white mycelial mats, symptoms started with shrinkage of stem. The heavily infected fruits tissue became watery and rotted (Plate 2: b).

***a. Aspergillus niger* on Apple**



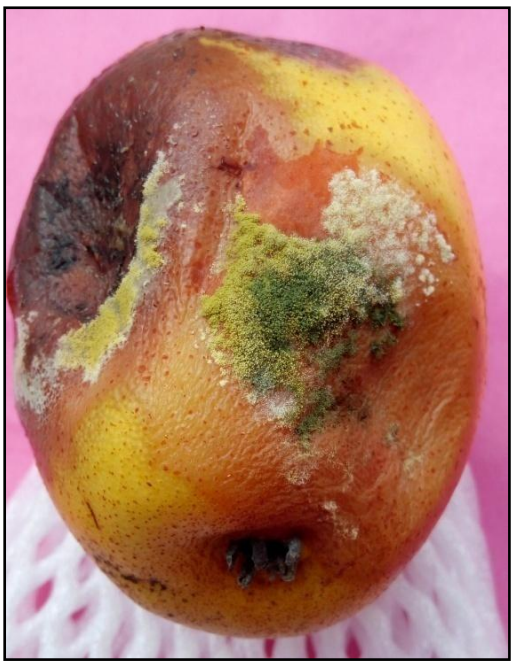
***b. Sclerotium rolfsii* on Apple**

***c. Aspergillus niger* on Orange**

***d. Penicillium digitatum* on Orange (Green Mold)**

***e. Penicillium italicum* on Orange (Blue Mold)**

Cont.



***f. Aspergillus flavus* on Pear**

***g. Penicillium expansum* (Blue Mold) and Aspergillus niger on Pear**

***h.Aspergillus flavus* and *Fusarium sp* (pink) on Papaya**

***i.Fusarium oxysporum* on Papaya**

Plate 2. Identification of fruit diseases (a-i) by visual symptoms on collected fruit samples

4.1.2 Isolation of post harvest fruit pathogens from collected sample:

According to chapter III, section 3.2.5, the pathogens were isolated from collected fruit samples.

Table 1. Name of isolated causal organisms from selected fruits and their disease name:

Fruits	**Disease**	**Causal organism**
Apple	Aspergillus rot	*a.* *Aspergillus flavus*
		b. *Aspergillus niger*
	Sclerotium rot	*c.* *Sclerotium sp*
Pear	Blue mold	*a.* *Penicillium expansum*
	Aspergillus rot	*b.* *Aspergillus flavus*
		c. *Aspergillus niger*
Orange	Green mold	*a.* *Penicillium digitatum*
	Blue mold	b. *Penicillium italicum*
	Aspergillus rot	*c.* *Aspergillus niger*
Papaya	Fusarium rot	*a.* *Fusarium sp (white)*
		b. *Fusarium sp (pink)*
	Aspergillus rot	c. *Aspergillus flavus*

4.1.3 Identification of isolates on PDA media and under microscope

According to chapter III, section 3.2.5.1 and 3.2.9, the isolates were identified by cultural and microscopic view, which are presented below (Plate 3)

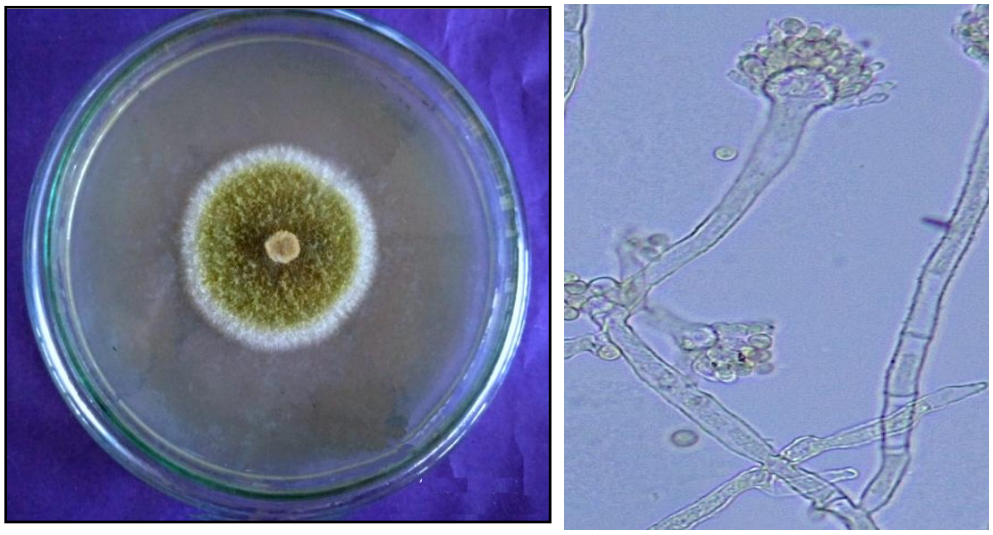
a. *Aspergillus flavus* (Apple, Pear and Papaya): (Plate 3)

A. flavus was isolated from three individual fruits as apple, pear and papaya.

Cultural view: The cultured isolate colony was characteristically olivacious green in color with a dominated circular appearance. Colony was plain and flat at the edges and raised at the center. The colony was encircled by a white border and a pale inner side. Colony texture was velvety.

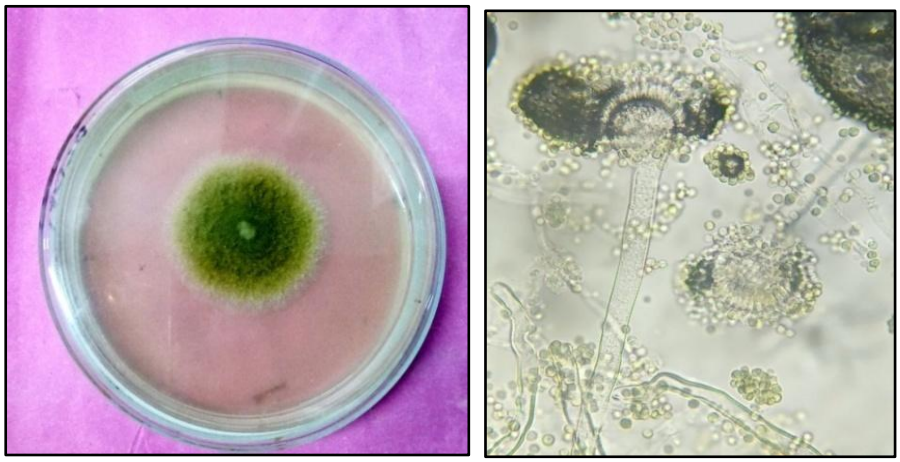
Microscopic view: Hyphae were septate and hyaline. Conidial heads are radiate to loosely columnar. Conidiophores are coarsely roughened, uncolored, vesicles globose to subglobose, metulae covering nearly the entire vesicle.

a.Aspergillus flavus

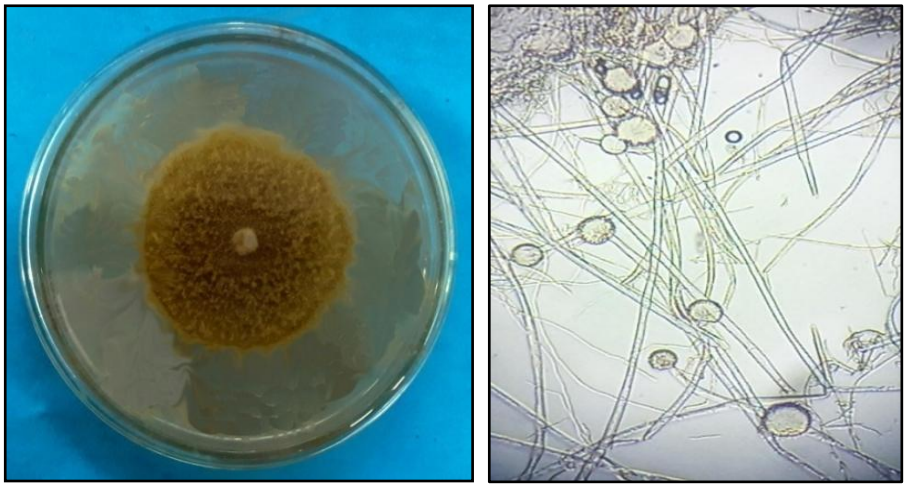


(a.1) *A. flavus* isolated from Apple (Cultural and Microscopic view (40X))

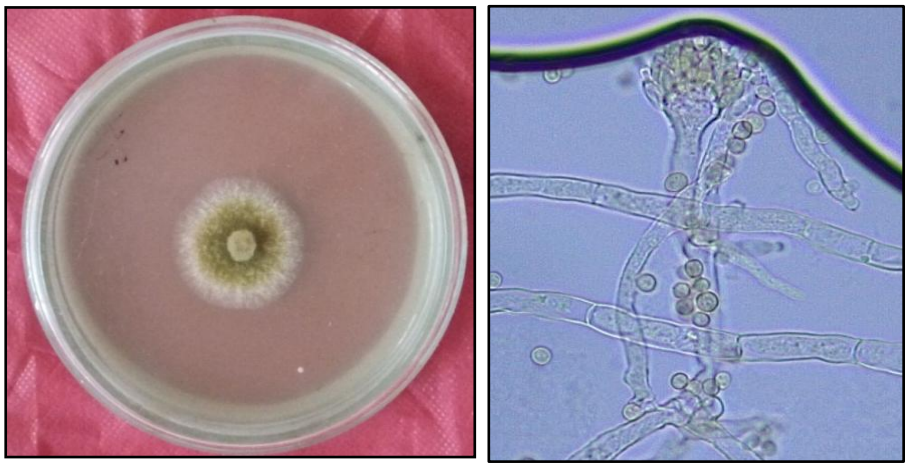
Cont.



(a.2) *A. flavus* isolated from Pear (Cultural and Microscopic view (40X))



(a.3) *A. flavus* isolated from Papaya (Cultural and Microscopic view (10X))



(a.4) *A. flavus* isolated from Papaya (Cultural and Microscopic view (40X))

Plate 3. (a.1- a.4) *Aspergillus flavus* isolated from different fruit sample on culture media and under microscope

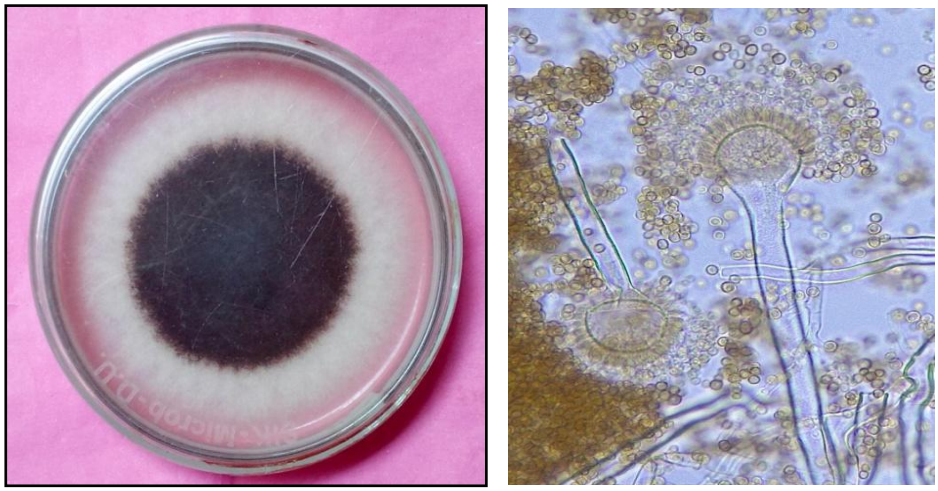
b. Aspergillus niger: **(Apple, Pear and Orange):** (Plate 4)

A. niger was isolated from three individual fruits (apple, pear and orange).

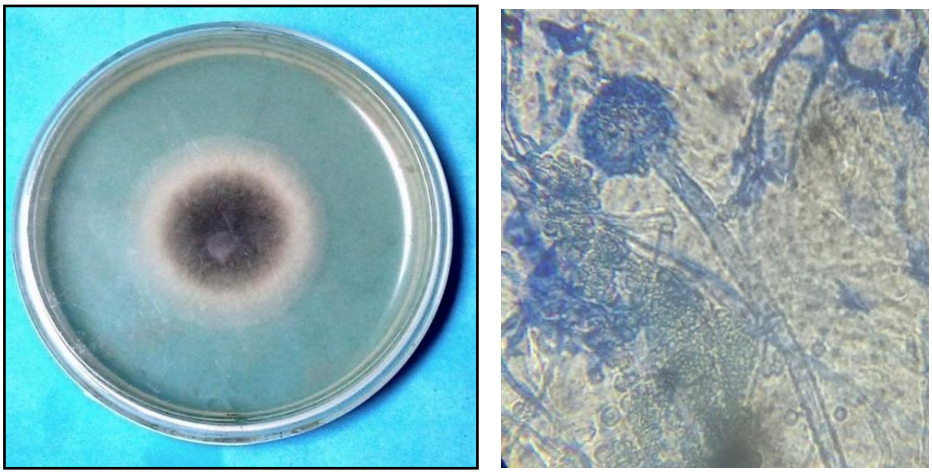
Cultural view: The cultured mycelial colony was visually black in color with a perfect circular shape plain and slopy from centre to edges, encircled by a wide white border attatched with a pale inner side. Texture was cottony.

Microscopic view: Conidiophores were long, smooth and hyaline with a foot cell, becoming darker at the apex and terminating in a globose vesicle. Conidia were brown to black, very rough and globose.

b. *Aspergillus niger (A. niger)*

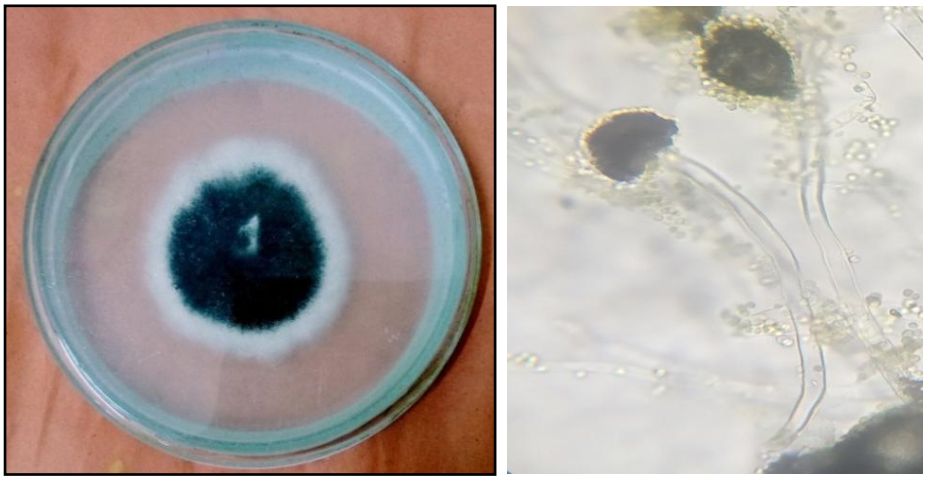


(b.1) *A. niger* isolated from Apple (Cultural and Microscopic view (40X))

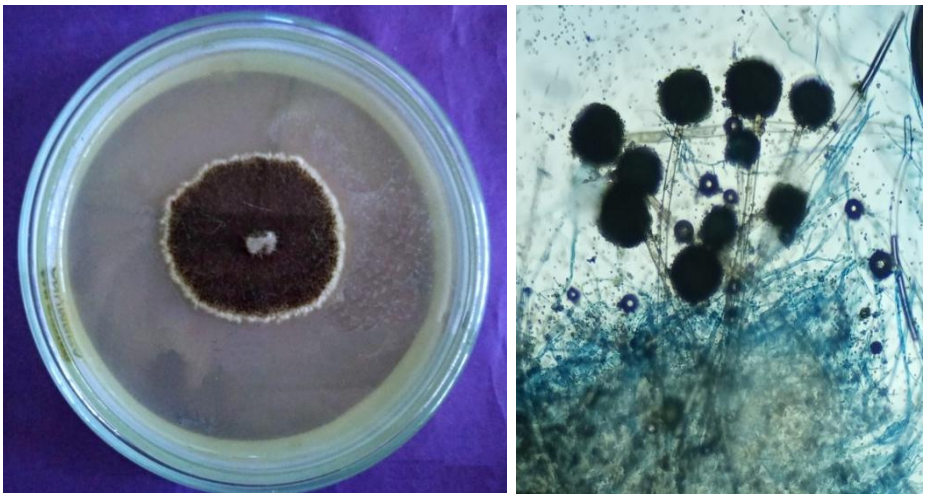


(b.2) *A. niger* isolated from Apple (Cultural and Microscopic view (40X))

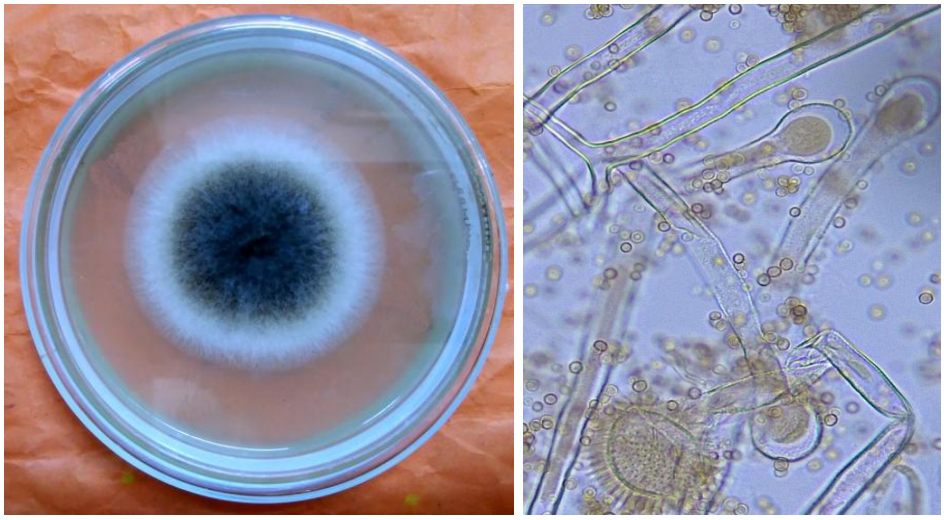
Cont.



(b.3) *A. niger* isolated from Orange (Cultural and Microscopic view (40X))

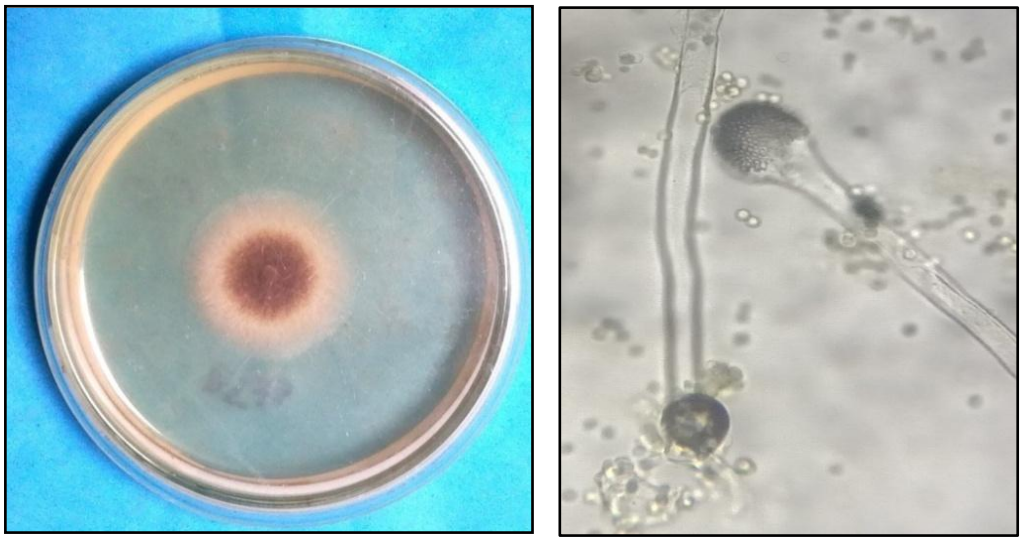


(b.4) *A. niger* isolated from Orange (Cultural and Microscopic view (10X))

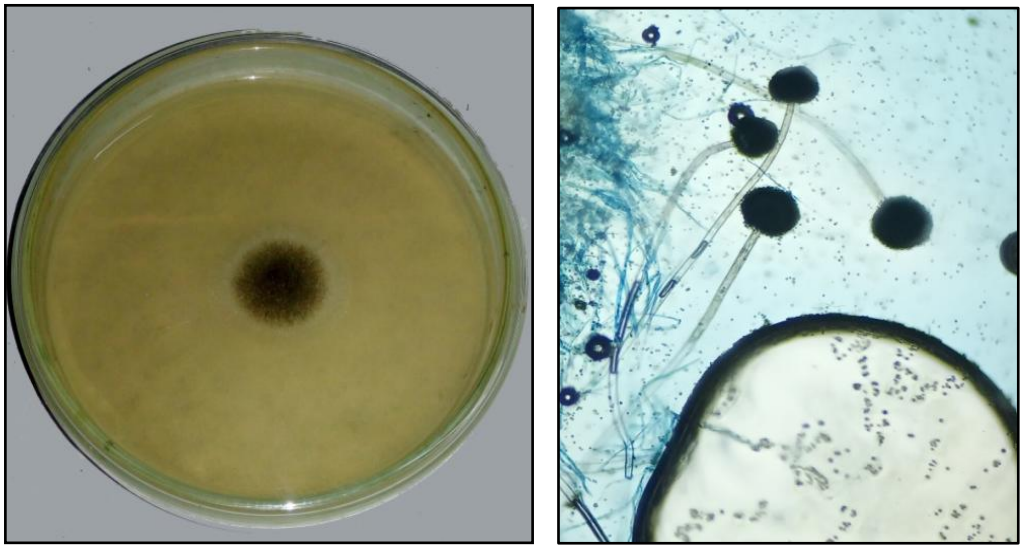


(b.5) *A. niger* isolated from Pear (Cultural and Microscopic view (40X))

Cont.



(b.6) *A. niger* isolated from Pear (Cultural and Microscopic view (10X))



(b.7) *A. niger* isolated from Apple (Cultural and Microscopic view (10X))

Plate 4. (b.1-b.7) *Aspergillus niger* isolated from different fruit sample on culture media and under microscope

c. Fusarium sp (Papaya): (Plate 5, 6)

Fusarium spp. with two different appearances was isolated from one individual fruit as papaya.

Cultural view: One of the cultured mycelial colony was light pinkish from both sides (pink *Fusarium oxysporum)* and another one was pure white in color (white *Fusarium solani*). Both of them were cottony in texture and circular in shape.

Microscopic view: Species of *Fusarium* typically produce both macro- and microconidia. Microconidia were small, one or two-celled, hyaline, pyriform, fusiform to ovoid, straight or curved.

d. Penicillium spp.*:* (Pear and Orange): (Plate 7)

Penicillium spp. with three different appearances was isolated from two individual fruits (orange and pear).

Cultural view: Colonies were usually fast growing. Isolated pathogen that caused blue mold appeared with a blue color colonial growth on media.

And another isolate that caused green mold appeared with a greenish color colonial growth on media. In both cases, colony margin was entire and narrow. The cultures had a characteristic musty, earthy odor.

Microscopic view: Hyphae hyaline. Conidiophores arised from mycelia were hyaline, simple or branched. Phialides grouped in brush-like clusters (penicilli) at the ends of the conidiophores. Conidia were hyaline, arranged in long chains or scattered, round to ovoid.

c.*Fusarium* sp

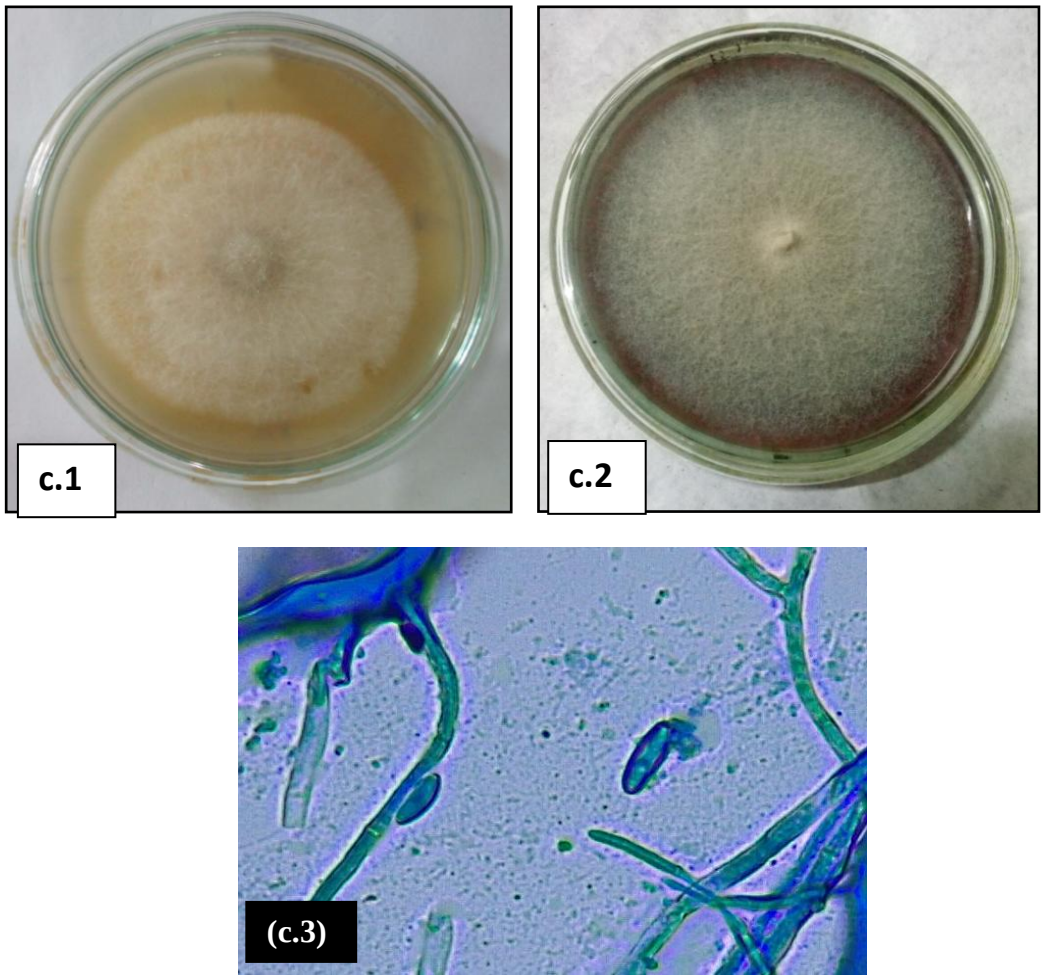


Plate 5. *Fusarium solani* (white) isolated from Papaya; (c.1)Young Culture; (c.2) Matured Culture; (c.3) Microscopic View (40X)

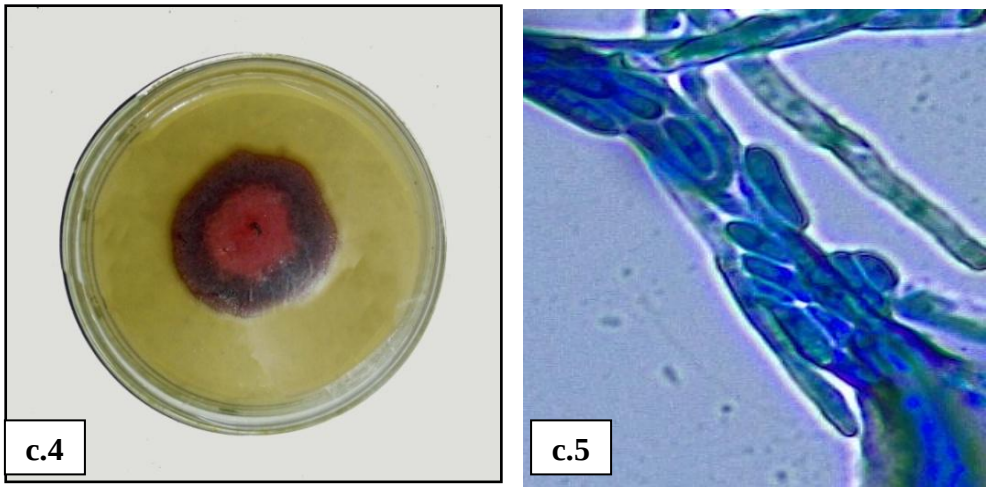
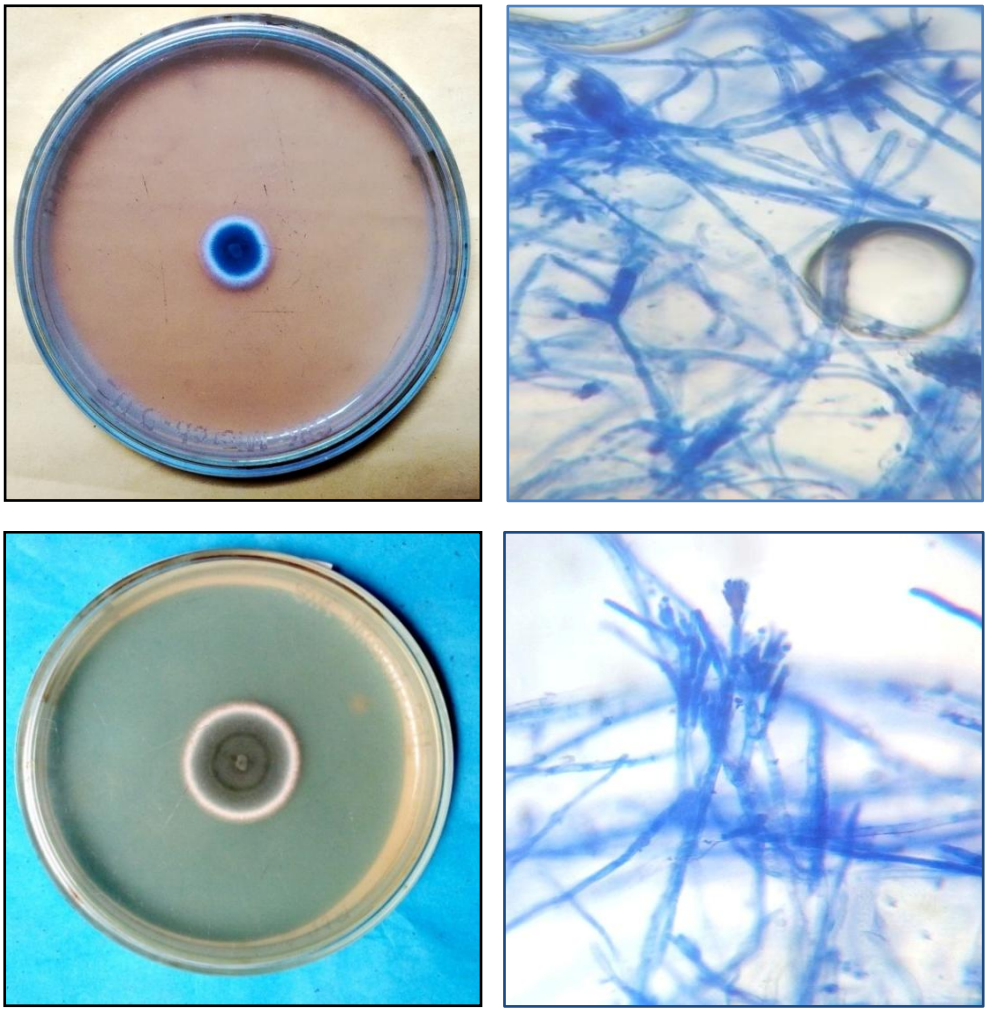
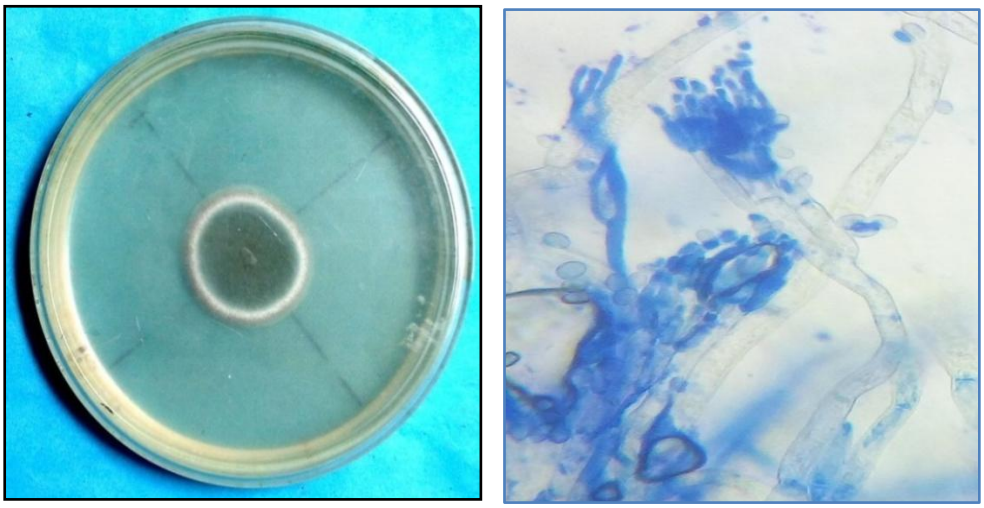


Plate 6. (c.4-c.5) *Fusarium oxysporum* (Pink) isolated from Papaya (Cultural and Microscopic View (40X))

d. *Penicillium* sp

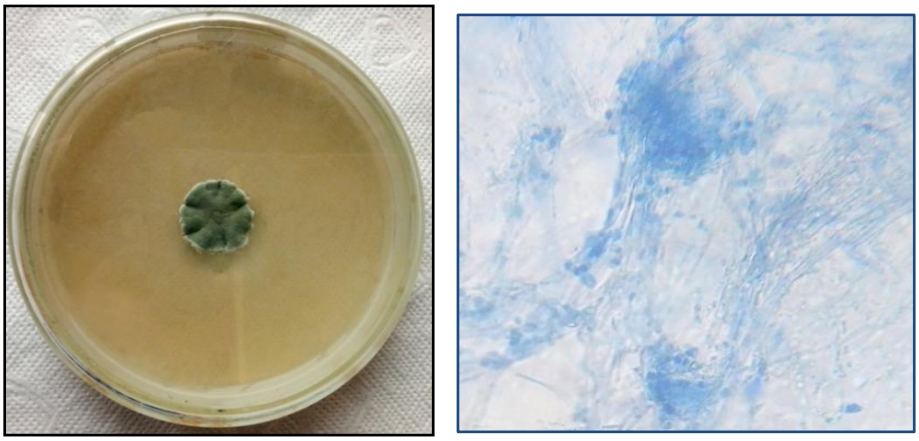


(d.1) *Penicillium italicum* isolated from Orange (Blue Mold) (Cultural and Microscopic view (40X))

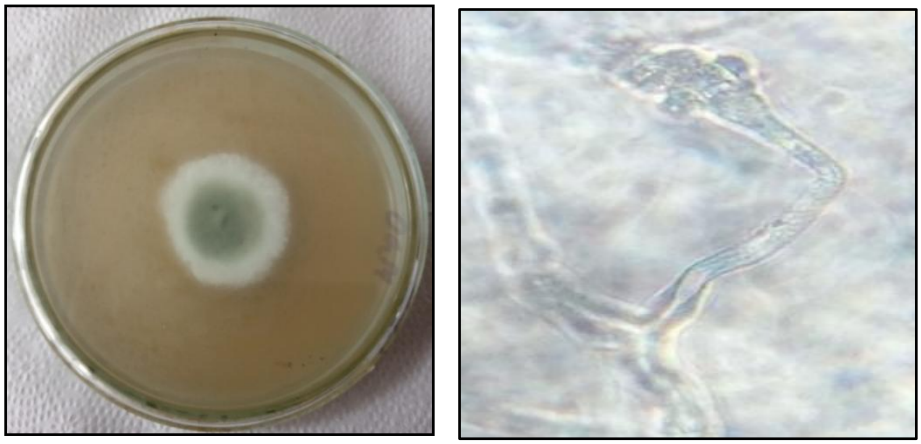


(d.2) *Penicillium italicum* isolated from Pear (Blue Mold) (Cultural and Microscopic view (40X))

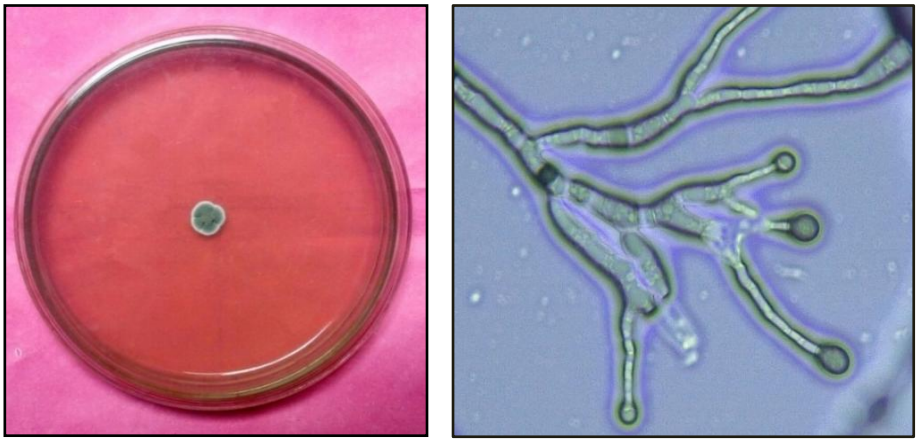
Cont.



(d.3) *Penicillium digitatum* of Orange(Green Mold) (Cultural and Microscopic view (10X))



(d.4) *Penicillium digitatum* of Orange (Green Mold) (Cultural and Microscopic view (10X))



(d.5) *Penicillium expansum* of Pear (Green Mold) (Cultural and Microscopic view (40X))

Plate 7 (d.1-d.5): *Penicillium* spp. isolated from Orange and Pear Fruits

***e. Sclerotium rolfsii* (Apple)**: (Plate 8)

Cultural view: Colony of *Sclerotium sp* isolate was white to light brown in color with fluffy texture. The colony appeared with white cottony mycelium with ropy strands. The colony produced sclerotia at the edges of the petri plates. The colour of the sclerotia was mostly light brown to reddish brown at maturity.

Microscopic view: Only thread like structures were found under microscope.

e.Sclerotium rolfsii.

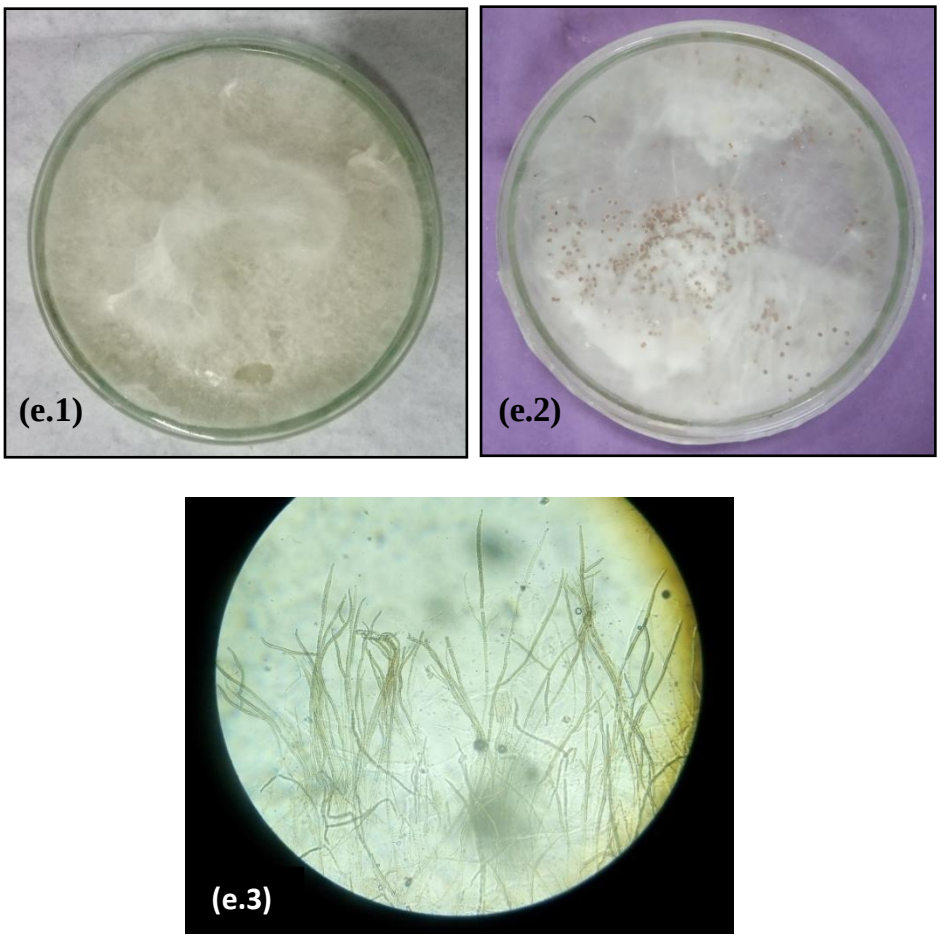


Plate 8. *Sclerotium rolfsii* isolated from Apple : Cultural and Microscopic View (e.1) Young Culture, (e.2) Matured Culture with sclerotia (e.3) Microscopic View (10X)

4.1.4. Pathogenecity test

According to Materials Methods (chapter:III, section: 3.2.6), pathogenecity test was done on apparently healthy mature fruits (apple, pear, orange and papaya). In this test, all the fungal isolates were observed positive for causing spoilage in fruits. All of the isolated organisms were found to be pathogenic as they successfully performed the ability to cause decay in healthy fruits. In most cases pathogenic growth was developed on inoculated fruits 7 days post inoculation at 25°C and 30°C. Characteristics of each isolates were present at the individual inoculation sites of every selected fruits. On Visual inspection, a sharp margin between infected and healthy tissue could easily be seen and the decayed tissue felt out leaving a proof of pathogenecity. But no symptoms were observed on the control fruits. The symptoms on inoculated fruits were very similar to those of natural infection. Re-isolated fungi showed the same morphological characteristics as original isolates, thus fulfilling pathogenecity test. The Various disease symptoms caused by each of the isolates were observed and recorded as follows:

A. ***Aspergillus niger*****:** Fruits appeared with water soaked and soft decayed area with black powdery colonial growth. Developed rapidly through fruit tissue resulting in total rot and exudation of liquids (Plate 9).

B. ***Aspergillus flavus*****:** Soft rot spoilage, the infected area turned brown and water soaked with olivacious colored colony growth, colony texture was powdery, scattered or velvety (Plate 10).

C. ***Sclerotium rolfsii*****:** Fruit surface appeared with white mycelial mats, symptoms started with water soaking lesion and progressed into the surface of the fruit. The heavily infected fruits became watery and eventually rotted (Figure 1).

D. ***Fusarium solani*** **(white)**: Cause soft rot in the affected area with white cottony pathogenic growth and covered the surface having inward progress of infection (Plate 11.a).

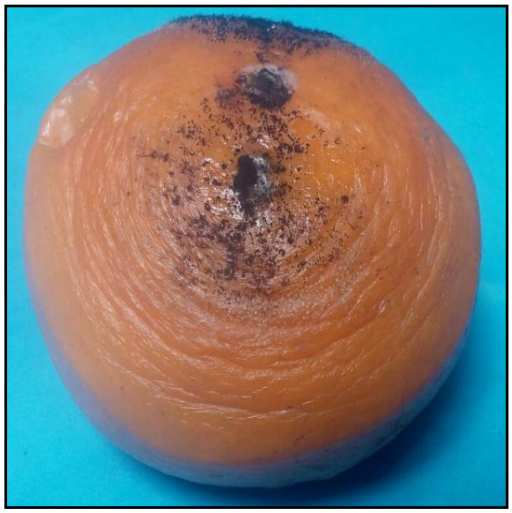
E. ***Fusarium oxysporum*** **(pink)**: Caused soft rot, infected area covered with white to browny cottony pathogenic growth (Plate 11.b).

F. ***Penicillium expansum*** **and** ***P. italicum*** **(Blue mold):** Fruits decayed gradually with lesions having blue colored mats structure of pathogen around inoculated area and rotten tissue was soft and watery (Plate 12).

G. ***Penicillium digitatum*** **(Green mold):** Infected areas were clearly delineated and developed lesions having green colored mats structure of pathogen around inoculated area (Plate 13).

A. niger **on Pear**

A. niger **on Apple**

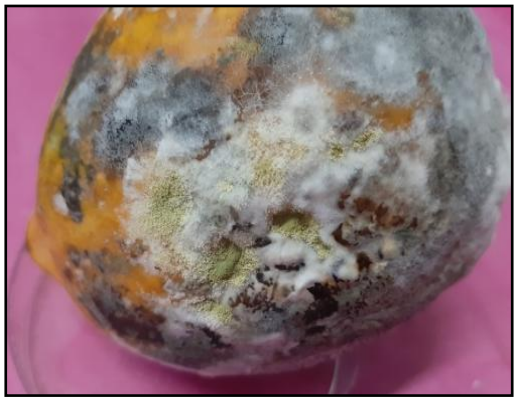


A. niger **on Orange**

Plate 9. Inoculated fruits with symptoms for pathogenecity test of *A. niger* (Pear, Apple and Orange)

***A. flavus* on Pear**

***A. flavus* on Apple**



***A. flavus* on Papaya**

Plate 10. Inoculated fruits with symptoms for pathogenecity test of *Aspergillus flavus* (Papaya, Pear and Apple)

Figure 1. Inoculated fruits with symptoms for pathogenecity test of *Sclerotium rolfsii* (Apple)

***a. Fusarium solani* on Papaya (white)**

***b. Fusarium oxysporum* on Papaya**

Plate 11. Inoculated fruits with symptoms for Pathogenecity test of *Fusarium* spp. (Papaya)

***P. expansum* on Pear**

***P. italicum* on Orange**

Plate 12. Inoculated fruits with symptoms for pathogenecity test of *Penicillium* spp. (Blue Mold) (Pear and Orange)

Plate 13. Inoculated fruits with symptoms for pathogenecity test of *P.digitatum* of Orange (Green Mold)

Experiment 2 (4.2.1-4.2.3): Effect of different media, temperature and packaging on the growth and development of isolated fungi

4.2.1 Effect of different media (PDA, CDA and PCDA) on radial mycelial growth (mm) of isolates at different days after inoculation

4.2.1.1 Radial mycelial growth (mm) of isolates on Potato Dextrose Agar (PDA) media at different days after inoculation (DAI)

Radial mycelial growth of individual isolates from different fruits significantly not varied on PDA (Table 2).

At 2nd day after inoculation (2DAI) the maximum radial mycelial growth of *isolated* species was observed in APSC (23.27 mm) followed by APANc (14.11 mm), PRANa (14.11 mm) and ORANa (14.11 mm). The minimum radial mycelial growth was identified in ORGMa (4.38 mm) which was statistically close to PRBM (4.7 mm).

At 4th day, 6th day and 8th day after inoculation (4DAI, 6DAI, 8DAI and 10DAI) the maximum radial mycelial growth were recorded in APSC which were 46.57 a mm, 59.79 mm and 76.44 mm respectively and the minimum mycelial growth were found in ORGMa (9.72mm, 15.64 mm and 19.87 mm) and PRGM (9.72mm, 15.64 mm and 19.76 mm) in respective days after inoculation.

At 10th day after inoculation (10DAI) the maximum radial mycelial growth were recorded in APSC (88.19mm), PRANa (88.19 mm) which was statistically similar to APANc (88.07 mm) and those were followed by APANa (86.22mm) and PRANb (85.94 mm) whereas the minimum mycelial growth were founnd in ORGMa (23.84mm) and PRGM (23.62) which was statistically close to PRBM (27.20mm).

Table 2: Radial mycelial growth (mm) of isolates on PDA media at different days after incubation (DAI)

Isolates	Isolates Full Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	13.47 cde	26.22 c	43.57 d	57.79 c	68.71 e
APANa	Apple *Aspergillus niger* (a)	14.00 bc	34.27 b	55.83 b	71.86 b	86.22 b
APANb	Apple *Aspergillus niger* (b)	14.00 bc	34.17 b	55.83 b	71.52 b	85.44 bc
APANc	Apple *Aspergillus niger* (c)	14.11 b	33.78 b	54.22 c	72.47 b	**88.07 a**
APSC	Apple *Sclerotium rolfsii*	**23.27 a**	**46.57 a**	**59.79 a**	**76.44 a**	**88. 19 a**
PRBM	Pear *Penicillium expansum*	4.7 ij	10.56 g	17.06 hi	22.72 g	27.20 h
PRGM	Pear *Penicillium digitatum*	4.28 J	9.72 g	15.64 i	19.76h	23.62 i
PRANa	Pear *Aspergillus niger* (a)	14.11 b	33.67 b	54.22 c	72.69 b	**88.19 a**
PRANb	Pear *Aspergillus niger* (b)	13.92 bcd	33.56 b	53.67 c	72.36 b	85. 94 b
PRAF	Pear *Aspergillus flavus*	13.39 de	24.11 d	40.56 e	53.50 d	68.42 e
ORGMa	Orange *Penicillium digitatum* (a)	4.38 j	9.72 g	15.64 i	19.87 h	23.84 i
ORGMb	Orange *Penicillium digitatum* (b)	7.77 h	16.03 f	24.33 g	31.82 f	39.51 g
ORBMa	Orange *Penicillium italicum* (a)	7.83 h	16.03 f	24.44 g	31.93 f	39.40 g
ORBMb	Orange *Penicillium italicum* (b)	5.17 i	10.66 g	17.36 h	22.83 g	27.31 h
ORANa	Orange *Aspergillus niger* (a)	14.11 b	33.78 b	54.56 bc	72.47 b	84.20 d
ORANb	Orange *Aspergillus niger* (b)	13.80 bcde	33.78 b	54.56 bc	72.47 b	84.42 cd
PFW	Papaya *Fusarium solani* (White)	12.44 f	23.22 d	37.00 f	51.78 de	67.11 f
PAFa	Papaya *Aspergillus flavus* (a)	10.58 g	21.22 e	39.44 e	51.67 e	66.39 f
PAFb	Papaya *Aspergillus flavus* (b)	13.98 bc	33.33 b	54.22 c	72.24 b	84.33 cd
PFP	Papaya *Fusarium oxysporum* (Pink)	13.22 e	26.61 c	39.33 e	50.22 e	67.06 f
CV%		**5.39**	**4.83**	**4.12**	**3.59**	**1.88**

4.2.1.2 Radial mycelial growth (mm) of isolates on Carrot Dextrose Agar (CDA) media at different days after inoculation (DAI)

Radial mycelial growth of individual isolates from different fruits significantly not varied on CDA media (Table 3).

At 2nd day after inoculation (2DAI) the maximum radial mycelial growth of isolated species was observed in APSC (20.98 mm) followed by ORANa (11.94 mm). The minimum radial mycelial growth was detected in PRGM (3.43 mm) which was statistically similar to PRBM (3.60 mm) and ORGM (3.60 mm).

At 4th day, 6th day and 8th day after inoculation (4DAI, 6DAI and 8DAI) the maximum radial mycelial growth were recorded in APSC which were 42.22 a mm, 54.82 mm and 69.07 mm respectively and the minimum mycelial growth were founnd in ORGMa 7.79mm, 12.86 mm and 17.46 mm in respective days of inoculation.

At 10th day after inoculation (10DAI) the maximum radial mycelial growth were recorded in APANc (84.11mm) and PRANa (84.11 mm) which was statistically similar to APANa (83.56 mm) and APSC (83.47mm) and those were followed by APANb (76.33mm), ORANa (76.80mm) and ORANb (76.94mm) whereas the minimum mycelial growth were founnd in PRGM (21.04mm) and ORGMa (21.19mm)

Table 3: Radial mycelial growth (mm) of isolates on CDA media at different days after incubation (DAI)

Isolates	Isolates Full Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	11.50 bcd	23.94 d	41.00 c	53.02 c	63.167 c
APANa	Apple *Aspergillus niger* (a)	11.68 bc	29.98 b	48.10 b	66.83 b	**83.56 a**
APANb	Apple *Aspergillus niger* (b)	11.39 cd	29.53 bc	47.58 b	66.09 b	76.33 b
APANc	Apple *Aspergillus niger* (c)	11.86 bc	30.00 b	47.89 b	67.28 b	**84.11 a**
APSC	Apple *Sclerotium rolfsii*	**20.98 a**	**42.22 a**	**54.82 a**	**69.07 a**	**83.47 a**
PRBM	Pear *Penicillium expansum*	3.60 h	9.44 i	15.55 i	20.94 i	25.88 h
PRGM	Pear *Penicillium digitatum*	3.43 h	7.52 j	12.69 j	17.11j	21.04 i
PRANa	Pear *Aspergillus niger* (a)	11.86bc	29.44 bc	47.89 b	66.44 b	**84.11 a**
PRANb	Pear *Aspergillus niger* (b)	11.76 bc	28.56 c	47.56 b	66.22 b	**84.00 a**
PRAF	Pear *Aspergillus flavus*	11.67 bc	24.33 d	37.87 d	50.66 d	62.61 cd
ORGMa	Orange *Penicillium digitatum* (a)	3.60 h	7.79 j	12.86 j	17.46 j	21.19 i
ORGMb	Orange *Penicillium digitatum* (b)	6.77 g	14.09 h	19.99 h	26.88 h	34.13 g
ORBMa	Orange *Penicillium italicum* (a)	6.76 g	14.19 h	21.04 h	28.73 g	34.83 g
ORBMb	Orange *Penicillium italicum* (b)	3.61 h	9.33 i	15.44 i	20.78 i	25.39 h
ORANa	Orange *Aspergillus niger* (a)	11.94 b	30.00 b	47.94 b	67 28 b	76.80 b
ORANb	Orange *Aspergillus niger* (b)	11.90 bc	29.78 b	47.72 b	67.17 b	76.94 b
PFW	Papaya *Fusarium solani* (White)	9.82 f	20.66 f	33.00 f	46.89 e	61.89 d
PAFa	Papaya *Aspergillus flavus* (a)	10.98 de	19.24 g	30. 39 g	45.00 f	58.67 f
PAFb	Papaya *Aspergillus flavus* (b)	11.90 bc	30.06 b	48.00 b	67.11 b	76.80 b
PFP	Papaya *Fusarium oxysporum* (Pink)	10.63 e	22.67 e	34.67 e	45.22 f	59.89 e
CV%		**5.67**	**5.73**	**3.72**	**3.51**	**2.02**

4.2.1.3 Radial mycelial growth (mm) of isolates on Potato-Carrot Dextrose Agar (PCDA) media at different days after inoculation (DAI)

Radial mycelial growth of individual isolates from different fruits significantly not varied on PCDA (Table 4).

At 2nd day after inoculation (2DAI) the maximum radial mycelial growth of *isolated* species was observed in APSC (21.60 mm). The minimum radial mycelial growth was identified in PRBM (3.49 mm) which was statistically similar to PRGM (3.52mm), ORGMa (3.54 mm) and ORBMb (3.97mm).

At 4th day and 6th day after inoculation (4DAI and 6DAI) the maximum radial mycelial growth were recorded in APSC which were 43.18 mm and 52.87 mm respectively and the minimum mycelial growth were found in PRGM (7.18mm and 11.28mm) and ORGMa (7.42 mm and 11.68 mm) in respective days after inoculation.

At 8th day after inoculation (8DAI) the maximum radial mycelial growth were recorded in APSC (67.40mm) and APANb (67.39 mm) and followed by ORANb (66.42mm) whereas the minimum mycelial growth were found in PRGM (16.60mm) and ORGMa (16.84mm)

At 10th day after inoculation (10DAI) the maximum radial mycelial growth were recorded in APANb (83.81mm), APSC (83.81 mm),statistically similar to APANc (83.77mm), PRANa (83.77mm) and PRANb (83.63mm) whereas the minimum mycelial growth were found in PRGM (20.33mm) statistically similar to ORGMa (20.43mm).

Table 4: Radial mycelial growth (mm) of isolates on PCDA media at different days after incubation (DAI)

Isolates	Isolates Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	11.69 c	22.56 c	38.73 d	52.21 d	65.00 c
APANa	Apple *Aspergillus niger* (a)	12.61 b	30.56 b	48.86 b	65.03 c	79.68 b
APANb	Apple *Aspergillus niger* (b)	12.61 b	30.67 b	48.63 b	**67.39 a**	**82.81 a**
APANc	Apple *Aspergillus niger* (c)	12.61 b	30.13 b	46.74 c	65.98 bc	**83.77 a**
APSC	Apple *Sclerotium rolfsii*	**21.60 a**	**43.18 a**	**52.87 a**	**67.40 a**	**83.81a**
PRBM	Pear *Penicillium expansum*	3.49 i	8.28 gh	15.13 i	21.36 i	25.38 h
PRGM	Pear *Penicillium digitatum*	3.52 i	7.18 h	11.278 j	16.60 j	20.33 i
PRANa	Pear *Aspergillus niger* (a)	12.61 b	29.80 b	46.74 c	65.94 bc	**83.77 a**
PRANb	Pear *Aspergillus niger* (b)	12.71 b	29.81 b	47.06 c	66.06 b	**83.63 a**
PRAF	Pear *Aspergillus flavus*	10.92 d	22.19 c	33.53 e	46.94 e	59.39 d
ORGMa	Orange *Penicillium digitatum* (a)	3.54 i	7.42 h	11.68 j	16.84 j	20.43 i
ORGMb	Orange *Penicillium digitatum* (b)	7.06 h	13.71 f	20.97 h	29.00 h	34.41 g
ORBMa	Orange *Penicillium italicum* (a)	7.00 h	13.66 f	20.86 h	29.00 h	34.81 g
ORBMb	Orange *Penicillium italicum* (b)	3.97 i	8.59 g	15.13 i	21.13 i	25. 38 h
ORANa	Orange *Aspergillus niger* (a)	12.57 b	30.13 b	46.93 c	65.98 bc	78.64 b
ORANb	Orange *Aspergillus niger* (b)	12.68 b	30.13 b	47.04 c	66.42 ab	79.33 b
PFW	Papaya *Fusarium solani* (White)	9.07 f	19.58 d	32.89 e	46.56 e	59.89 d
PAFa	Papaya *Aspergillus flavus* (a)	7.61 g	16.33 e	29.56 g	41.78 g	55.44 f
PAFb	Papaya *Aspergillus flavus* (b)	12.60 b	30.33 b	46.71 c	65.87 bc	79.44 b
PFP	Papaya *Fusarium oxysporum* (Pink)	9.83 e	18.50 d	30.78 f	44.78 f	56.78 e
CV%		**5.88**	**5.47**	**3.16**	**2.24**	**2.23**

4.2.2 Effect of different temperature (20°C, 25°C and 30°C) on radial mycelial growth (mm) of isolates at different days after incubation

4.2.2.1 Radial mycelial growth (mm) of isolates at 30°C temperature at different days after incubation (DAI)

Radial mycelial growth of individual isolates from different fruits was significant at 30°C (Table 5).

At 2nd day after incubation (2DAI) the maximum radial mycelial growth of isolated species was observed in APSC (27.52mm) followed by APAF (16.78mm) and PRAF (16.76 mm). The minimum radial mycelial growth was identified in ORGMa (4.83mm) and PRBM (4.83mm).

At 4th day, 6th day and 8th day after incubation (4DAI, 6DAI and 8DAI) the maximum radial mycelial growth were recorded in APSC which were 52.83 mm, 62.19 mm and 76.44 mm respectively and the minimum mycelial growth were founnd in PRGM (9.97 mm) at 4DAI, PRGM (14.64mm) and ORGMa (14.52 mm) at 6DAI and PRGM (19.24mm) and ORGMa (19.24mm) at 8DAI.

At 10th day after inoculation (10DAI) the maximum radial mycelial growth were recorded in APSC (90.00 mm) followed by APANc (89.63mm) and PRANb (89.50 mm)whereas the minimum mycelial growth were founnd in ORGMa (23.53 mm).

Table 5: Radial mycelial growth (mm) of isolates on 30°C temperature at different days after incubation (DAI)

Isolates	Isolates Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	16.78 b	34.44 de	55.39 bc	68.40 d	79.79 d
APANa	Apple *Aspergillus niger* (a)	14.78 cd	34.78 c	56.21 b	74.64 b	88.66 b
APANb	Apple *Aspergillus niger* (b)	14.78 cd	35.00 b	56.21 b	74.64 b	86.31 c
APANc	Apple *Aspergillus niger* (c)	14.44 d	34.56 d	55.11 c	73.47 c	**89.63 a**
APSC	Apple *Sclerotium rolfsii*	**27.52 a**	**52.83 a**	**62.19 a**	**76.44 a**	**90.00 a**
PRBM	Pear *Penicillium expansum*	4.83 j	12.04 hi	18.61 h	25.08 i	29.31 h
PRGM	Pear *Penicillium digitatum*	4.94 i	9.97 j	14.64 i	19.24 j	23.77 i
PRANa	Pear *Aspergillus niger* (a)	14.44 d	34.44 de	55.11 c	73.47 c	87.11 bc
PRANb	Pear *Aspergillus niger* (b)	14.77 cd	33.89 e	54.31 c	73.02 cd	**89.50 a**
PRAF	Pear *Aspergillus flavus*	16.76 b	31.78 f	50.78 d	66.19 d	76.73 d
ORGMa	Orange *Penicillium digitatum* (a)	4.83 j	10.01 ij	14.52 i	19.24 j	40.03g
ORGMb	Orange *Penicillium digitatum* (b)	8.59 f	16.16 h	23.59 g	32.64 h	23.53 i
ORBMa	Orange *Penicillium italicum* (a)	8.42 g	16.10 h	23.53 g	32.76 h	39.89 gh
ORBMb	Orange *Penicillium italicum* (b)	5.50 h	12.47 hi	18.78 h	25.33 i	29.20 h
ORANa	Orange *Aspergillus niger* (a)	14.28 e	34.56 d	55.30 c	73.47 c	85.06 c
ORANn	Orange *Aspergillus niger* (b)	14.39 de	34.67 c	55.52 bc	73.47 c	86.17 c
PFW	Papaya *Fusarium solani* (White)	15.81 c	30.11 g	45.67 f	61.11 f	76.44 d
PAFa	Papaya *Aspergillus flavus* (a)	15.89 c	29.56 gh	47.78 e	62.67 e	75.67 e
PAFb	Papaya *Aspergillus flavus* (b)	14.36 de	35.11 b	55.41 bc	73.24 cd	86.06 c
PFP	Papaya *Fusarium oxysporum* (Pink)	16.34 bc	29.89 gh	45.22 f	59.89 g	74.22 f
CV%		6.89	5.95	3.96	2.33	1.93

4.2.2.2 Radial mycelial growth (mm) of isolates at 25°C temperature at different days after incubation (DAI)

Radial mycelial growth of individual isolates from different fruits was significant at 25°C (Table 6).

At 2nd day after incubation (2DAI) the maximum radial mycelial growth of isolated species was observed in APSC (20.66 mm) followed by ORANa (13.11 mm). The minimum radial mycelial growth was identified in PRGM (4.06 mm) which was statistically similar to ORGMa (4.18 mm).

At 4th day, 6th day and 8th day after incubation (4DAI, 6DAI and 8DAI) the maximum radial mycelial growth were recorded in APSC which were 41.90 mm, 55.49 mm and 71.34 mm respectively and the minimum mycelial growth were founnd in PRGM 8.27 mm, 14.52 mm and 18.79 mm in respective days after incubation.

At 10th day after inoculation (10DAI) the maximum radial mycelial growth were recorded in APANa (87.78 mm) which is statistically similar to APSC (87.62 mm) and APANc (87.52 mm) whereas the minimum mycelial growth were found in PRGM (22.80 mm) and ORGNa (22.80mm).

Table 6: Radial mycelial growth (mm) of isolates on 25°C temperature at different days after incubation (DAI)

Isolates	Isolates Full Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	12.43 d	23.67 f	40.75 f	57.77 d	72.98 f
APANa	Apple *Aspergillus niger* (a)	12.83 c	31.80 c	50.22 c	69.50 bcd	**87.78 a**
APANb	Apple *Aspergillus niger* (b)	12.83 c	31.59 c	50.22 c	69.50 bcd	82.33 d
APANc	Apple *Aspergillus niger* (c)	12.72 cd	32.00 b	50.22 c	69.92 b	**87.52 a**
APSC	Apple *Sclerotium rolfsii*	**20.66 a**	**41.90 a**	**55.49 a**	**71.34 a**	**87.62 a**
PRBM	Pear *Penicillium expansum*	4.41 i	9.78 i	18.67 ij	25.39 i	30.67 i
PRGM	Pear *Penicillium digitatum*	4.06j	8.27j	14.52 j	18.79 j	22.80 j
PRANa	Pear *Aspergillus niger* (a)	12.72 cd	31.22 d	50.22 c	69.61 b	84.32 cd
PRANb	Pear *Aspergillus niger* (b)	12.73 cd	31.22 d	50.56b	69.50 bcd	86.53 b
PRAF	Pear *Aspergillus flavus*	11.83 e	31.22 d	36.67 g	51.00 f	69.44 g
ORGMa	Orange *Penicillium digitatum* (a)	4.18 j	8.87 j	14.64 ij	18.83 j	22.80 j
ORGMb	Orange *Penicillium digitatum* (b)	7.40 h	14.86 h	22.62h	30.00 h	36.50 h
ORBMa	Orange *Penicillium italicum* (a)	7.40 h	14.86 h	22.51 h	30.56 h	37.23 h
ORBMb	Orange *Penicillium italicum* (b)	4.69i	9.44 i	18.61 ij	24.81 i	30.67 i
ORANa	Orange *Aspergillus niger* (a)	13.11 b	32.00 b	50.83 b	69.92 b	82.28 d
ORANb	Orange *Aspergillus niger* (b)	13.08 bc	32.00 b	50.61 b	70.26 ab	82.64 d
PFW	Papaya *Fusarium solani* (White)	10.82 f	26.78 e	41.67 e	56.33 e	72.11 f
PAFa	Papaya *Aspergillus flavus* (a)	8.06 g	19.17 g	32.61 gh	45.67 g	63.33 gh
PAFb	Papaya *Aspergillus flavus* (b)	12.90 c	31.56c	50.11cd	69.76 b	81.97 e
PFP	Papaya *Fusarium oxysporum* (Pink)	12.02 de	26.00 e	39.44 g	50.78 fg	68.78 g
CV%		**8.05**	**4.35**	**4.72**	**2.48**	**4.8**

4.2.2.3 Radial mycelial growth (mm) of isolates at 20°C temperature at different days after incubation (DAI)

Radial mycelial growth of individual isolates from different fruits was significant at 20°C (Table 7).

At 2nd day and 4th day after incubation (2DAI and 4DAI) the maximum radial mycelial growth of isolated species was observed in APSC (17.67 mm and 37.23 mm). The minimum radial mycelial growth was identified in PRGM (2.13mm and 5.59 mm).

Similarly at 6th day after incubation (6DAI) the maximum radial mycelial growth was recorded in APSC (49.80 mm) followed by APANa (46.35 mm). The minimum radial mycelial growth was identified in PRGM (10.44 mm) and ORBMb (10.54 mm)

As following at 8th day after incubation (8DAI) the maximum mycelial growth was found in APSC (62.76 mm). The minimum mycelial growth at was recorded for PRBM (14.56 mm) and ORBMb (14.60 mm)

At 10th day after inoculation (10DAI) the maximum radial mycelial growth was recorded in APSC (78.96 mm) and APANc (78.54 mm) whereas the minimum mycelial growth were found in ORBMb (18.11 mm).

Table 7: Radial mycelial growth (mm) of isolates on 20°C temperature at different days after incubation (DAI)

Isolates	Isolates Full Name	2DAI	4DAI	6DAI	8DAI	10DAI
APAF	Apple *Aspergillus flavus*	7.44 d	14.61 ef	27.15 f	36.85 d	44.11 e
APANa	Apple *Aspergillus niger* (a)	10.68 c	28.22 b	46.35 b	61.93 bc	77.17 ab
APANb	Apple *Aspergillus niger* (b)	10.39 c	27.78 b	45.61c	60.86 c	75.94 bc
APANc	Apple *Aspergillus niger* (c)	11.41 b	27.36 cd	43.52 d	62.33 b	**78.54 a**
APSC	Apple *Sclerotium rolfsii*	**17.67 a**	**37.23 a**	**49.80 a**	**62.76 a**	**78.96 a**
PRBM	Pear *Penicillium expansum*	2.54 g	6.46 i	10.47 j	14.56 j	18.48 ij
PRGM	Pear *Penicillium digitatum*	2.13 h	5.59 j	10.44 j	15.43 i	18.43 ij
PRANa	Pear *Aspergillus niger* (a)	11.41 b	27.24 cd	43.52d	62.00 b	76.78 bc
PRANb	Pear *Aspergillus niger* (b)	10.89 c	26.80 d	43.41d	62.11 b	77.54 ab
PRAF	Pear *Aspergillus flavus*	7.39 d	15.63 e	24.51 g	33.91 e	43.64 e
ORGMa	Orange *Penicillium digitatum* (a)	2.51 g	6.06 ij	11.01 ij	16.09 hi	19.13 i
ORGMb	Orange *Penicillium digitatum* (b)	5.60 e	12.82 f	19.08 hi	25.06 h	31.52 h
ORBMa	Orange *Penicillium italicum* (a)	5.77 e	12.92 f	20.30 h	26.36 gh	31.92 h
ORBMb	Orange *Penicillium italicum* (b)	2.56 g	6.67 i	10.54 j	14.60j	18.11 j
ORANa	Orange *Aspergillus niger* (a)	11.23 b	27.36 cd	43.52 d	62.33b	72.31 c
ORANn	Orange *Aspergillus niger* (b)	10.91 c	27.02 cd	42.97e	62.33 b	71.89 cd
PFW	Papaya *Fusarium solani* (White)	4.70 f	6.57 i	15.56 i	27.78 g	40.33 g
PAFa	Papaya *Aspergillus flavus* (a)	5.22 e	8.08 h	19.00 hi	30.11 f	41.50 fg
PAFb	Papaya *Aspergillus flavus* (b)	11.22 b	27.06 cd	43.41d	62.22 b	72.56 c
PFP	Papaya *Fusarium oxysporum* (Pink)	5.32 e	11.89 g	20.11 h	29.56 fg	40.72 g
CV%		**8.84**	**7.43**	**4.18**	**3.78**	**2.36**

4.2.2.4 Comparison of radial mycelial growth (mm) of isolates on different media at 10th day after inoculation (10th DAI)

Significant variations were observed on pathogenic radial mycelia growth and PCDA on different media (Figure 2). Radial mycelia growth of isolates were higher on PDA media, on the other hand moderate to lower radial mycelia growth of isolates were observed on CDA and PCDA media.

4.2.2.5 Comparison of radial mycelial growth (mm) of isolates at different temperature at 10th day after incubation (10th DAI)

Significant variations were observed on pathogenic radial mycelia growth at different temperature (Figure 3). Radial mycelia growth of all isolates were higher at 30°C temperature except ORBMb (Orange *Penicillium italicum*, 23.53 mm). At 25°C temperature, moderate radial mycelia growth of all isolates were observed and at 20°C, lower radial mycelia growth of all isolates were observed .

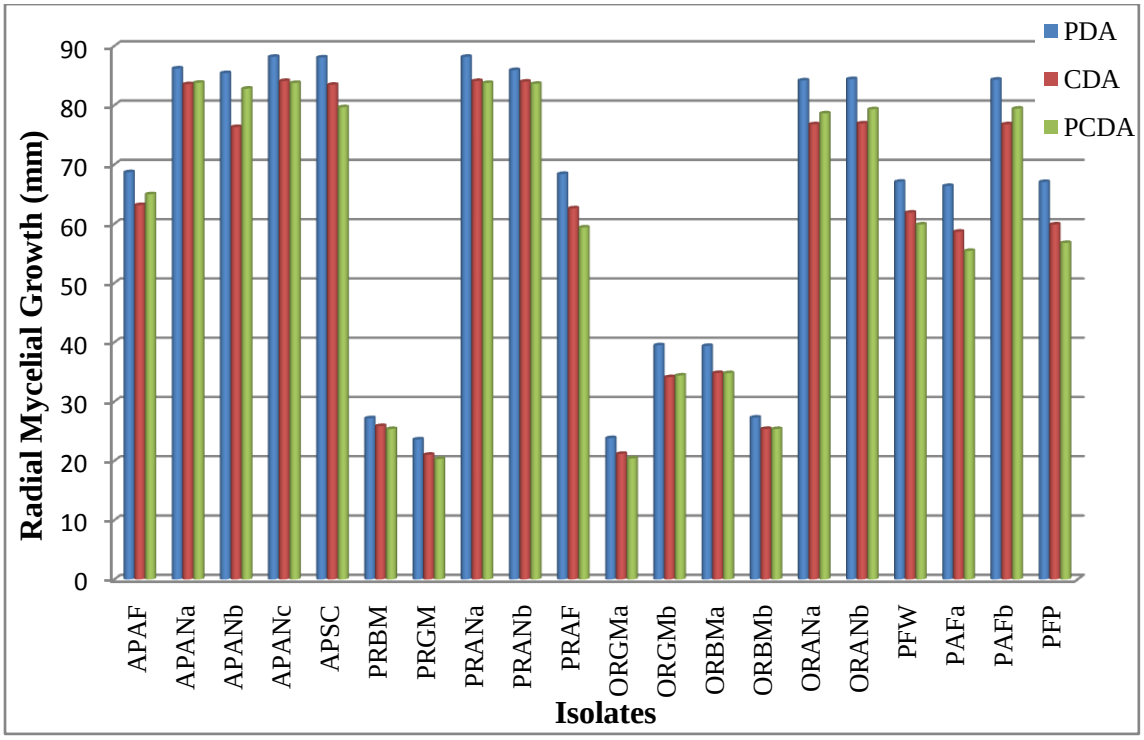


Figure 2. Comparison of radial mycelial growth (mm) of isolates on different media (PDA, CDA and PCDA) at 10th day after inoculation (10th DAI)

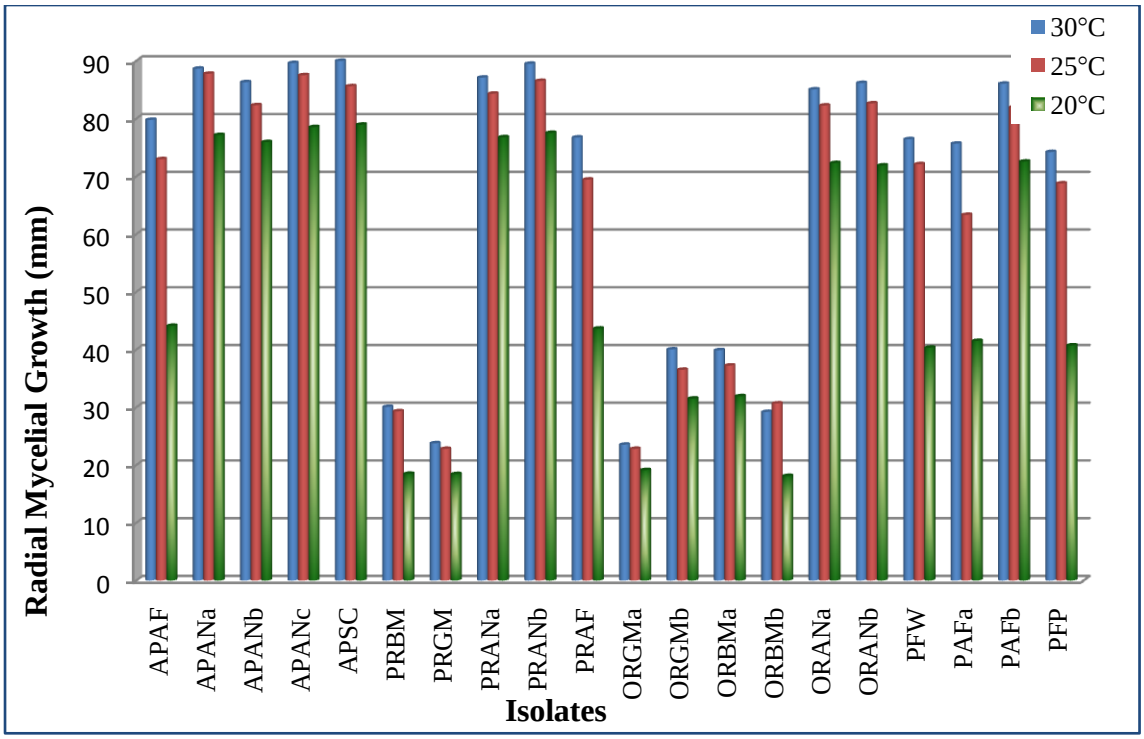


Figure 3. Comparison of radial mycelial growth (mm) of isolates at different temperatures (20°C, 25°C and 30°C) at 10th day after incubation (10th DAI)

4.2.3 Effect of different packaging on post harvest deterioration of fruits at room temperature (23°±1)

Effect of different packaging on post harvest deterioration of fruits at room temperature (23°±1) were recorded for three months and presented in figure: 4 and plate: 14-17. Among the packaging used variation were observed in post harvest deterioration of different fruits.

In case of net bags deterioration varied from 4 to 50 days, where the higher value counted from apple and the lowest value from papaya.

In case of polythene bags deterioration varied from 2 days to 40 days when the highest and the lowest value recorded from apple and papaya.

In case of paper bags deterioration varied from 6 days to 60 days when the highest and the lowest value recorded from apple and papaya.

In case of cloth bags deterioration varied from 2 days to 40 days when the highest and the lowest value recorded from apple and papaya.

So, among the fruits used the highest shelf life (60days) recorded in apple and the shortest shelf life recorded in papaya in all packaging methods. And among the packaging used the highest shelf life (60days) recorded in paper bag and the shortest shelf life recorded in polythene bag followed by cloth bag.

Effect of different packaging on post harvest deterioration of fruits at room temperature (23°±1)

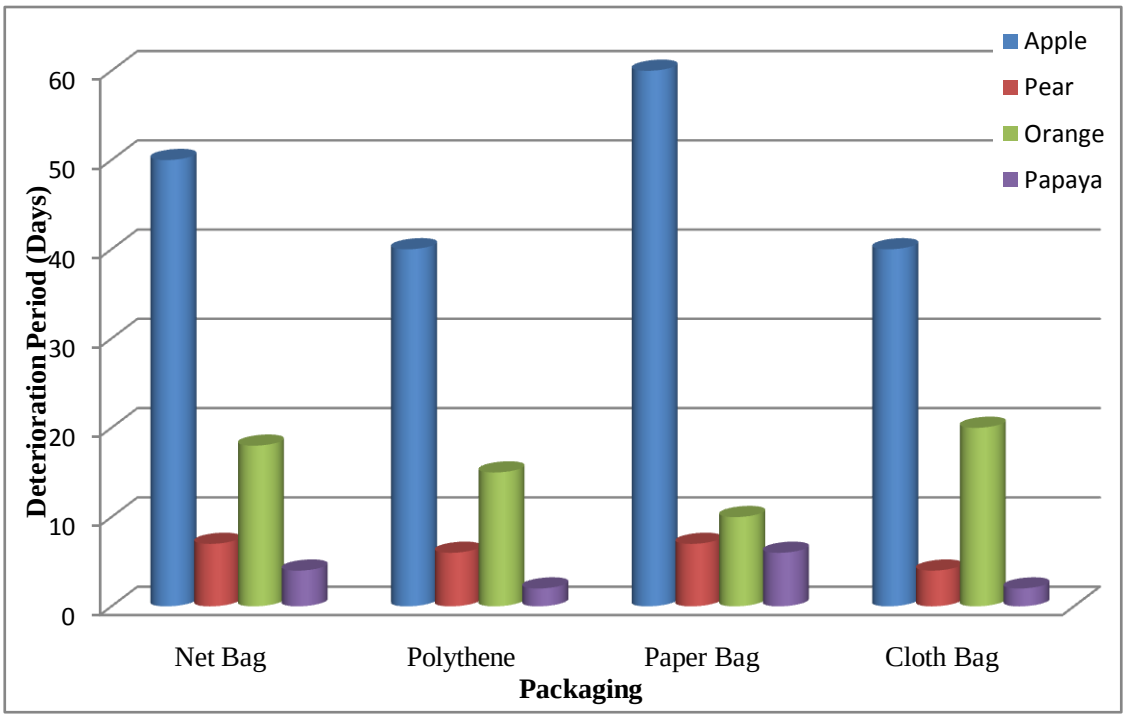


Figure 4. Effect of different packaging on post harvest deterioration of fruits at room temperature (23°±1)

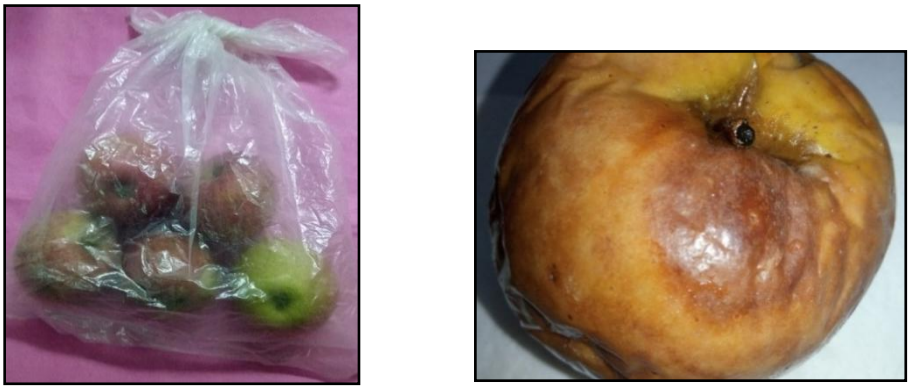

Apples


Pears

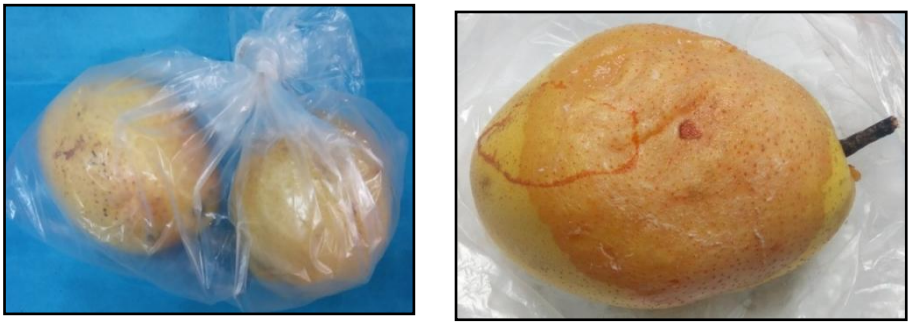

Oranges


Oranges

Plate 14. Effect of net packaging on post harvest deterioration of fruits at room temperature (23°±1)



Apples



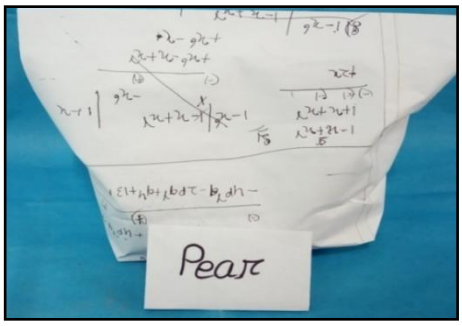
Pears

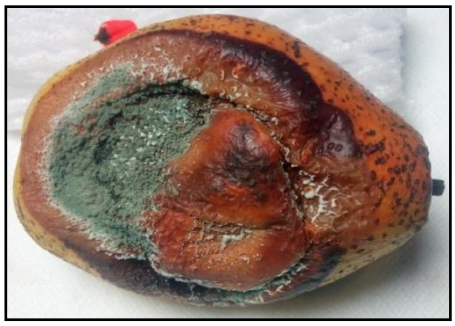
Oranges

Papaya

Plate 15. Effect of polythene packaging on post harvest deterioration of fruits at room temperature (23°±1)

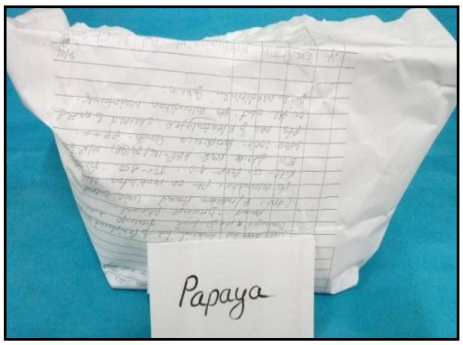
Apples





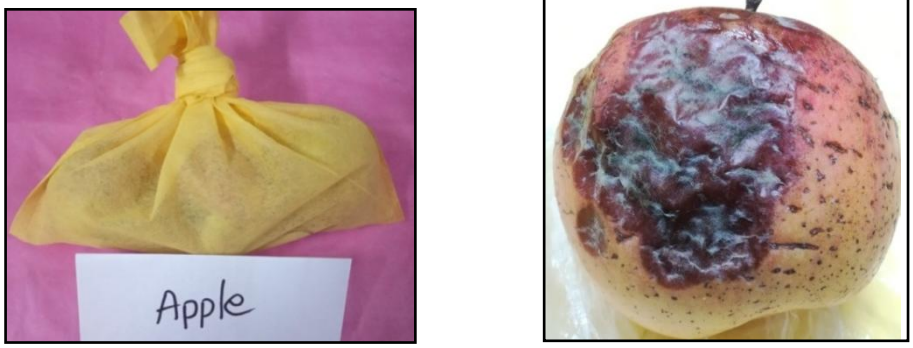
Pears

Oranges

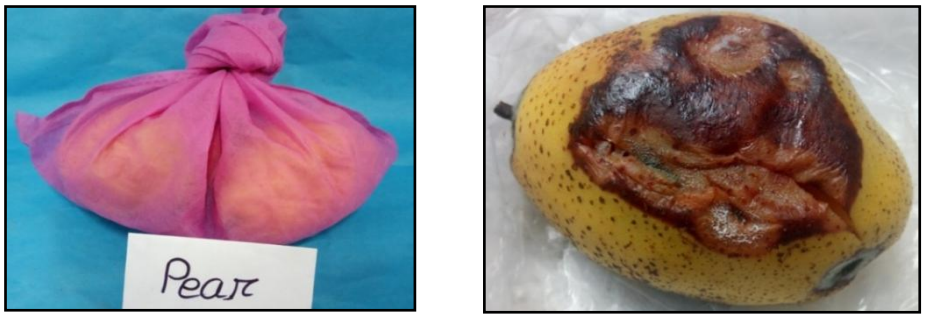


Papaya

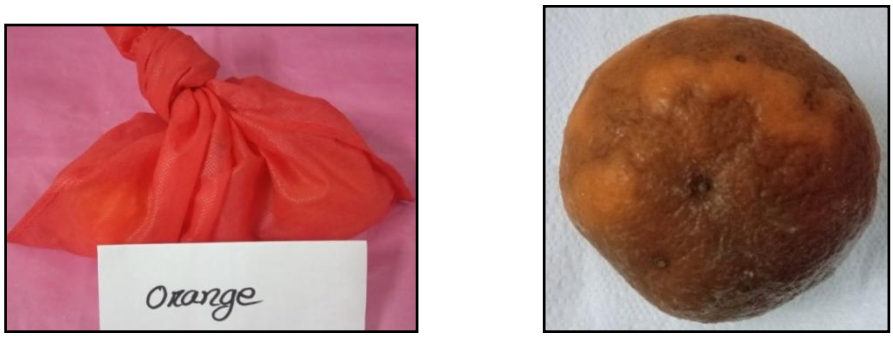
Plate 16. Effect of paper packaging on post harvest deterioration of fruits at room temperature (23°±1)



Apples



Pears



Oranges

Papaya

Plate 17. Effect of cloth bag packaging on post harvest deterioration of fruits at room temperature (23°±1)

Chapter V

DISCUSSION

A study was conducted at Plant Pathology Laborartory, Dr. M. A. Wazed Miah Research Centre, Sher-e-Bangla Agricultural University, Dhaka-1207 to study on different post harvest fungal diseases of some selected fruits (Apple, pear ornge and papaya*) and* the effect of different media and temperature on the radial mycelial growth. In this study, fruit samples having typical symptoms were collected from fruit market in Dhaka and the causal organism were isolated on PDA media. After isolating pure culture of fungi, pathogenicity test was done for each pathogen. The effects of media and temperature on mycelial growth isolated fungal pathogens were investigated. Pathogens were identified on the basis of morphological, cultural and microscopic studies. The radial mycelia growth (mm/day) of fungi was observed on three culture media: potato dextrose agar (PDA), carrot dextrose agar (CDA) and potato-carrot dextrose agar (PCDA). All isolates showed significant variations in respect of their cultural and morphological characteristics on different media. In respect of cultural characteristics, all isolates showed variation in mycelial growth, colony colour, shape, textures, surface colour.

Four genera of fungi had been identified from four different fruits (apple, pear, orange and papaya) as *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Sclerotium* sp. These pathogens were also identified by Samuel *et al*. (2017). They also isolated *A. niger, P. digitatum, A. flavus,* and *F. solani.* Iniekong *et al.*, (2015) and Bashar *et al*., (2012) detected that *Aspergillus niger, Fusarium oxysporum* and *Sclerotium* sp play dominant role in the spoilage of fruits.

Aspergillus spp. were common in all fruit specimen, they were fast growing and easily detectable which can be supported by Onyemata and Ibrahim (2018) who showed that *Aspergillus* sp has the highest occurrence in fruits. Abdullah *et al.,* (2016) observed that *A. flavus* and *A. niger* are the most common and dominant species that affecting fruits (apple, orange, and pear fruits).

Penicillium spp. produced green and blue color colony on media and hyphae, conidiophores under microscope etc. This was supported by Abdullah *et al*., (2016). They found that *Penicillium* sp was the second most common pathogenic genus for fruits like apple, grape and orange. Pitt (2014) reported that, *P. italicum, P. digitatum, P. expansum, P. chrysogenum* etc. cause decay of various fruits.

Sclerotium rolfsii of apple were white to gray colored on culture media can be compared by Billah *et al*.,(2017). They reported that *S. rolfsii* is pathogenic on apple, soybean, maize, potato, chick pea, cotton and ornamental plants. Kator *et al*., (2015) reported about *S. rolfsii* that it attacks host stems including other parts like fruits, leaves, roots and petioles. Kalai-Grami *et al*., (2013) and Corazza *et al*. (1999) described apple collar rot as apple southern blight caused by *S. rolfsii* is a phytopathogenic fungus born in soil and can infect any plant parts of apple including fruit, flower, stem, root, pedicel etc. and able to produce persistent sclerotia in soil and at the infected parts of the plant.

PDA media was most prominent in mycelial growth of fungi (88.19mm) followed by CDA (84.11mm) and PCDA(83.81mm) media at 10^{th} day after inoculation. It is partially supported by Hase and Nasreen (2017) who found that PDA has easy formulation, capability to support a wide range of fungal mycelial growth and useful for fungal identification. Sharma and Pandey (2010) stated that type of culture media affect mycelia growth and conidial production of different fungi. Saha *et al.,* (2008) stated that PDA encourages mycelial growth with various colour of spores through which fungi are recognized and identified.

In case of temperature. maximum growth of fungus were observed at 30˚C (90.00 mm) followed by 25˚C (87.78 mm) and 20˚C (78.96 mm) at 10^{th} day after incubation. This result can be partially supported by Ibrahim *et al*., (2011), they reported that maximum fungi grow between 25˚C-30˚C and above 40˚C growth is poor. Sharma and Sharma (2009) and Farooq *et al.* (2005) obtained maximum fungus growth at 28˚C-30˚C temperatures and reduced

growth below 15˚C and above 35˚C temperature. Kator *et al.* (2015) identified that hyphal growth occurs between 8-40ºC but optimum growth and sclerotia production occurs between 27-35ºC on Potato Dextrose Agar Media (PDA) after 20 days.

In all containers, long days were required for deterioration in case of apple and longest shelf life of fruits was observed in case of paper bag. In all cases apple showed the longest shelf life. It was supported by Bhattarai and Shah (2017) that transpiration and respiration are important to extend shelf life of fresh fruit. Newspaper wrapping can absorb moisture during transpiration and reduce pathogenic susceptibility but plastic bag wrapping arrests evaporation within the package and creates a saturated micro-atmosphere around the fruit that increases fruit deterioration rate. Bigger *et al*. (2013) observed that if both temperature and packaging are optimal, ageing of fruit can be slowed down significantly. Rahman *et al*. (2008) stated that apple is less prone to damage at normal to heavy condition because of its waxy layer on surface and Agostini *et al.* (2006) tested that waxing treatment is capable to reduce postharvest disease development on apple and other perishable fruits.

The result of present study confirmed that these fungi are prominent post harvest diseases of fruits. Potato Dextrose Agar (PDA) and 30°C temperature were the best combination to enhance growth of fungi. Paper bag showed long shelf life of these fruits at room temperature. Findings of the present study will be helpful for regular users. For further information more research work can be taken in hand.

Chapter VI

SUMMARY AND CONCLUSION

Apple (*Malus domestica*), pear (*Pyrus communis*), orange (*Citrus reticulata*) and papaya (*Carica papaya L.*) are worldwide consumed fruits because of their nutritive effect on health. Spoilage of fruits is occurred by various diseases in Bangladesh making it toxic and less consumable and the major losses occur after harvest due to post harvest decay, handling, storage, transfer and disease-causing organism. The present study was conducted to study on different post harvest fungal diseases of these fruits, their causal agents and their physical treatments. Effect of different cultural media, temperature and storage bags on fungal growth was studied. Infected and diseased fruit samples were collected from different market in Dhaka city. The experiment was carried out following the completely randomized design with three replications. Three different temperature (20°C, 25°C and 30°C) and three different media (PDA, CDA and PCDA) were used to measure growth and development of isolated pathogen from collected samples. Pathogenicity test was done by fresh fruits inoculation which was compared to the isolated pathogen. Four types of storage bags (polythene, net, shopping, paper) were used to study the storage longevity of fruits at room temperature.

Twenty isolates belonging to 4 fungal genera from the collected samples of the infected fruits were isolated as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp. and *Sclerotium rolfsii.*

All isolates showed variation in the terms of cultural and morphological character. All isolates developed higher radial mycelial growth at 30˚C followed by 25°C and 20°C. But one isolate of orange blue mold showed higher growth at 25°C. PDA media showed the best performance on radial mycelial growth followed by PCDA and CDA media. *Aspergillus niger* and *Sclerotium* sp showed highest growth on every media.

All isolates produced circular colony but colour and texture of the mycelia on cultural media was different among all post harvest fungal isolates from collected samples. *Aspergillus flavus* showed olivacious green colored, flat and velvety mycelia on media. *Asperguillus niger* showed black cottony mycelia. *Fusarium* sp of papaya showed fluffy white mycelia and pinkish fluffy mycelia. *Penicillium* sp showed slow growth, blue color mycelia (blue mold) and greenish color mycelia (green mold). *Aspergillus niger* showed initially white colony then gradually turned black. *Sclerotium* sp isolated from apple showed white cottony mycelium with ropy strands. Different isolates produce different colour on surface and the subsurface colour ranges from whitish to brown colour.

Packaging at room temperature showed that paper bag was more or less slower in pathogenic growth than polythene, net bag and shopping bag. Among selected fruits, apple was less prone to damage but pear and papaya was susceptible to pathogenic infection. Orange and papaya were slightly prone to pathogenic attack. But over ripened papaya was observed damaged within overnight.

On the basis of the above results and discussion it can be summarized that- Potato dextrose agar medium and 30˚C temperature were seems to be the best medium for mycelial growth of isolated pathogen.

The present study was based on post-harvest fungal diseases of fruits, their causal organisms, effect of different media and temperature on radial growth of isolated fungi and physical treatment of selected fruits. Further study is needed to increase storage period and post-harvest disease management of these types of valuable fruits.

Chapter VII

REFERENCES

Abdullah, Q., Mahmoud, A. and Al-harethi, A. (2016). Isolation and Identification of Fungal Post-harvest Rot of Some Fruits in Yemen. *PSM Microbiol*. **1**(1): 36-44.

Agostini, J. P., Mackenzie, S. J., Timmer, L. W. and Adaskaveg, J. E. (2006). Effect of Fungicides and storage conditions on postharvest development of citrus black spot and survival of *Guignardia citricarpa* in Fruit Tissues. *Plant Disease*. **90**:1419-1424.

Agrios, G. N. (2005). Introduction to plant pathology. Elsevier Academic Press Publication. pp.703-922.

Akinro, E. B., Adetuberu, I. A., Efunwole, O. O. and Olakunle, T. P. (2015). Isolation and identification of fungal species associated with the spoilage of some selected edible fruits in Iree town of Boripe Local Government, Osun State. *Nigeria. J. Res. Pharm. Sci*. **2**(7): 07-10.

Aryal, N. (2015). Potato Dextrose Agar (PDA): Principle, Composition and Colony Characteristics . *J. Agric. Bio*. **7**(2): 275- 277.

Ataga, A. E., Elenwo, E. N. and Nwachukwu, E. O. (2010). Laboratory Exercises and Series in Mycology. *ACOTEC Technology, PH, Nigeria*. pp. 327-333.

Awoke, M., Abiso, E., Alemnew, A. and Eshetu, S. (2018). Effect of packaging materials and postharvest treatments on postharvest quality and shelf life of banana fruits (*Musa* spp.). *Annals. Food Science and Technology*. **19:**2.

Aycock, R. (2017). Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. *Agr. Expt. St. Tech. Bul.* p.174.

Baert, K., Devlieghere, F., Flyps, H., Oosterlinck, M., Ahmed, M.M., Rajkovi, A., Verlinden B, Nicola B, Debevere J and De Meulenaer B (2007). Influence of storage conditions of apples on growth and patulin production by *Penicillium expansum*. *International Journal of Food Microbiology*. **119**: 170-181.

Bakar, Brown, G. E., Eckert, J. W. (2013). Diplodia stem-end rot. **In:** Timmer, L. W., Garnsey, S. M., Graham, J. H. (Ed.2) Compendium of citrus diseases. Saint Paul: APS Press. pp. 43-44.

Balgrie, B. (2013). Taints and Off-flavours in Food and evolution of bacterial and fungal growth media. *J. Bioinformation.* **11(**4): 182–184.

Barnett, H. L. and Hanter, B. B. (1998). Illustrated Genera of Imperfect Fungi. Burgess Publishing Co, Minneopolis Minnesota. p.15.

Bashar, M. A., Shamsi, S. and Hossain, M. (2012). Fungi associated with rotten fruits in Dhaka metropolis. Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh. *Bangladesh. J. Bot.* **41**(1): 115-117.

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M. and Khurana, S. (2015). Evolution of bacterial and fungal growth media. *Bioinformation*. **11**(4): 182–184.

Begum, H. A., Umesha and Momin, A. (1998). Comparison between two detection techniques of seed-borne pathogens in cucurbits in Bangladesh. *Pakistan Journal of Scientific and Industrial Research.* **43**(4):244-248.

Bhale, U. N. (2011). Survey of market storage diseases of some important fruits of Osmannabad District (M.S) India. *Science Research Reporter*. **1**(2): 88-91.

Bhale, U. N. (2011). Survey of market storage diseases of some important fruits of Osmannabad District (M.S) India. *Science Research Reporter*. **1**(2): 88-91.

Bhattarai, B.P. and Shah, R. (2017). Effect of Different Packaging Materials on Post-Harvest Status of Mandarin (*Citrus reticuleta* Blanco). *J Hortic*. **4**: 218.

Bigger, S. W., Suppakul, P., Miltz, J. and Sonneveld, K. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*. **68**(2):408-420.

Billah, K. M., Hossain, M., Prince, M., Masud, M., Sumon, P. and Parvez, J. (2017). Pathogenicity of *Sclerotium Rolfsii* on Different Host, and Its over Wintering Survival; A Mini Review. *International Journal of Advances in Agriculture Sciences.* **2**: 2456-75152.

Böhm, J., Hoff, B., O'Gorman, C. M., Wolfers, S., Klix, V., Binger, D., Zadra, I., Kürnsteiner, H., Pöggeler, S., Dyer, P.S. and Kück, U. (2013). Sexual reproduction and mating-type-mediated strain development in the penicillin-producing fungus *Penicillium chrysogenum*. *Proc. Natl. Acad. Sci. U.S.A.* **110** (4): 1476–81.

Booth, C. (1971). The Genus Fusarium. CMI, Kew, Surrey, England. pp. 237.

Burgess, L. W. (1981). General Ecology of the Fusarium. **In:** Nelson, T.A., Toussoun, R. J., Cook (eds.) *Fusarium:* disease, biology, and taxonomy. University Park, PA, USA: *The Pennsylvania State University Press*. pp. 225 – 235.

Canon (2016). Fast and Accurate Microplate Method (Biolog MT2) for Detection of *Fusarium* sp Fungicides Resistance/Sensitivity. *Front Microbiol*. **7**: 489.

Cerioni, L., Paula B. Bennasar, B., Lazarteb, D., Sepulvedab, M., Joseph L., Smilanick, C., Ramallob, J., Viviana, A. and Rapisarda,A. (2017). Conventional and reduced-risk fungicides to control postharvest Diplodia and Phomopsis stem-end rot on lemons. *Scientia Horticulturae*. **225**: 783-787.

Chang, J. C. S., Foarde, K. K. and Vanosdell, D. W. *(1995).* "Growth evaluation of fungi (*Penicillium* and *Aspergillus* spp.) on ceiling tiles". *Atmospheric Environment.* **29** (17): 2331-37.

Choudhury, M. L. (2006). Recent developments in reducing post-harvest losses in the Asia–Pacific Region. **In**: Rolle, R.S. (ed.), Postharvest Management of Fruit and Vegetables in the Asia-Pacific Region. Asian Productivity Organization, and Food and Agriculture Organization of the United Nations, Tokyo. p. 312.

Chowdhury, S. M., Sultana, N., Mostofa, G., Kundu, B. and Rashid, M. (2014). Post harvest diseases of selected fruits in the whole sale market of Dhaka. *Bangladesh J. Plant Pathol.* **30**(1&2): 13-16.

Collins, C. H., Lyne, P. M., Grange, J. M. and Falkinham, J. O. (2004). Collins and Lyne Microbiological methods, 8th Edition. By Arnold, a member of the Hodder Headline Group, 338. Euston Road, London NW1 3BH. pp. 1-97.

Corazza L., Belisario A., Forti E., (1999). First report of root and collar rot by *Sclerotium rolfsii* on apple trees in Italy. *Plant Disease.* **83**: 695.

da Costa, G. L., de Moraes, A. M., and de Oliveira, P. C. (1998). Pathogenic action of *Penicillium* species on mosquito vectors of human tropical diseases. *Journal of Basic Microbiology.* **38**(5–6): 337–41.

Daniel, M. D., Bogard, J. R., Sulser, T. B., Nicola, C., Dunston, S., Herrero, M. and Wiebe, K. (2019). Gaps between fruit and vegetable production,

demand, and recommended consumption at global and national levels: an integrated modelling study. **3**(7): 318-329.

Dantas, S. A. F., Oliveira, S. M. A. and Michereff, S. J. (2003). Doenças fúngicas pós-colheita em mamões e laranjas comercializados na Central de Abastecimento do Recife. *Fitopatologia Brasileira*. **28**:528-533.

Dauda and Adegoke, G. (2014). Preservation of Some Physico-Chemical Properties of Soymilk-Based Juice with Aframomum Danielli Spice Powder. *American Journal of Food Science and Technology*. **2**: 116-121.

Domsch, K. H., Gams, W. and Anderson, T. H. (2007). Post-harvest fungal spoilage in sweet orange (*Citrus sinensis*) and acid lime (*Citrus aurentifolia* Swingla) at different stages of marketing. *Agric. Sci. Digest*. **28**:265-267.

Dubey, S. R. and Jalal, A. S. (2013). Species and Variety Detection of Fruits and Vegetables from Images. *International Journal of Applied Pattern Recognition.* **1**(1): 108-126.

Dubey, S. R.(2012). Automatic Recognition of Fruits and Vegetables and Detection of Fruit Diseases. Master's theses, GLA University Mathura, India. **21**(1): 26.

Dwivedi, L. and Brown, G. E. (1991). Decay control/fungicides applications. **In:** BROW, G. E. (Ed.) Fresh citrus quality short course proceedings. *Fort Pierce: AREC*. pp.1-24.

Elenwo, Y., Fairs, A., Wardlaw, A. J., Thompson, J. R. and Pashley, C. H. (2010). Guidelines on ambient intramural airborne fungal spores. *Journal of Investigational Allergology and Clinical Immunology.* **20**(6): 490-98.

Ellis, D., Davis, S., Alexiou, H., Handke, R. and Bartley, R. (2007). Descriptions of medical fungi. 2nd ed. Mycology Unit, Women's and Children's Hospital, North Adelaide, Australia. pp. 1–198.

Ewekeye, Eckert, T. and Tolulope, p. (2016). Studies on post harvest rot of apple (*Malus domestica Borkh*). *Indian Journal of Plant science*. **5**(1): 36-41.

Fallik, Elazar & Ilic, Zoran. (2019). Control of Postharvest Decay of Fresh Produce by Heat Treatments; the Risks and the Benefits. **In**: Postharvest Pathology of Fresh Horticultural Produce, Ministry of Science and Technological Development of the Republic of Serbia. pp. 521-538.

Food and Agricultural Organization (FAO) (2018). Prevention of Postharvest Food Losses: Fruits, Vegetables and Root Crops. A Training Manual. Food and Agricultural Organizations of the United Nations, Rome. p.157.

Farooq, S., Iqbal, S. M. and Abdulrauf, C. (2005). Physiological studies of Fusarium oxysporum. Inter. *J. Agric. Bio*. **7**(2): 275- 277.

Farooqi, W. A., Ahmad, M., Hussain, A. M. and Qureshi, M. J. (1975). Effects of thiabendazol and kininig materials on kinnow mandrins during storage. *The Nucleus*. **12**:25-29.

Fravel, D., Olivain, C., and Alabouvette, C. (2003). *Fusarium oxysporum* and its biocontrol. *Soil microbes and plant production.* **157**(3): 493-502.

Gadgile, D. P. (2017). Post-harvest fungal diseases of papaya. *Bioinfolet*. **14**(4): 367-368.

Gautam, A. K. and Bhadauria, R. (2012). Characterization of *Aspergillus* species associated with commercially stored triphala powder. *African Journal of Biotechnology*. **11**(104): 16814-16823.

Giradu, M., Bompeix, G., Gupta. M., Kumari, M. and Grover T. (2012). Effect of various media types on the rate of growth of *Aspergillus niger*. *Plant Protection Science*. **2**(2):141-144.

Gonzalez, A. G., Vasquez, C. and Felix, R. (1997). Low oxygen treatment before storage in normal or modifed packaging of mango. *Food Science Technology*. **34**: 339-404.

Gupta, A. K. and Pathak, V. N. (2012). A survey of fruit market for papaya fruit rot by fungal pathogens. *Indian J. Mycol*. **10**: 152-154.

Hase, V. and Nasreen, S. (2017). Influence of different culture media on growth of plant pathogenic fungi. Department of Botany, Post graduate and Research Center, Government Institute of Science, Aurangabad, Maharashtra, India. *International Journal of Multidisciplinary Research and Development Online.* **4**(1):67-70.

Helal, B. R., Sarowar, H. and Shamim, S. (2018). Mycoflora associated with post-harvest disease of papaya (*Carica papaya L*.) and their pathogenic potentiality. Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh. *Bangladesh J. Bot.* **47(**3): 389-395.

Houbraken, J. Hosen, S. and Samson, R. A. (2014). Modern Taxonomy of Biotechnologically Important *Aspergillus* and *Penicillium* Species in Advances in Applied Microbiology**.** *J. Mycol. Plant Pathol*. p.123.

Ikechi–Nwogu C. G. and Elenwo, E. N. (2012) Comparative Evaluation of Growth Media for the Cultivation of Fungal Cultures. *J. Plant Pathol. Microb.* **3**:139.

International Seed Testing Association (ISTA) (2003). International Rules for Seed Testing , 7-002a: Alternaria radicina on Daucus carota (Blotter). ISTA Handbook of Seed Health Testing. **5**(3).

Iqbal, S., Muhammad, A., Malik, A. H., Inam-ulhaq, Khan, K. S. and Mathews, P. (2017). Isolation, preservation and revival of *Trichoderma Viride* in culture media. *J. of Entom. and Zoo. St.* **5**(3): 1640-1646.

Ivana-Vico, Nataša, D., Miljan, V. and Milica, N. (2019). Identification of *Penicillium expansum* causing postharvest blue mold decay of apple fruit. *Pestic. Phytomed.* (Belgrade), **29**(4): 257–266.

Jijakli, M. H. and Lepoivre, P. (2004). State of the Art and Challenges of Post-harvest Disease Management in Apples. **In**: Mukerji, K. G. (eds) Fruit and Vegetable Diseases. *Disease Management of Fruits and Vegetables*. **1**:59-94.

Kahramanoglu, I., Nisar, M. F., Chen, C., Usanmaz, S., Chen, J. and Wan, C. (2020). Light: An Alternative Method for Physical Control of Postharvest Rotting Caused by Fungi of Citrus Fruit. *Journal of Food Quality*. **20**(20): 12.

Kalai-Grami, L., Mnari-Hattab, M., Terres, R., Dridi, M. and Hajlaoui, M.R. (2013). First report of apple collar rot incited by *Sclerotium rolfsii* in Tunisia. *Journal of Plant Pathology*. **95**(4): 69-77.

Kasso, M. and Bekele, A. (2016). Post-harvest loss and quality deterioration of horticultural crops in Dire Dawa Region, Ethiopia. *J. of the Saudi Society of Agric. Sci.* **7**:577-581.

Kator, L., Zakki, Y. H. and Daniel, O. O. (2015). *Sclerotium rolfsii*; Causative organism of southern blight, stem rot, white mold and sclerotia rot disease. *Scholars Research Library Annals of Biological Research.* **6** (11):78-89.

Kim, V. K. and Xiao, C. L. (2005). Influence of culture media and environmental factors on mycelial growth and picnidial production of Sphaeropsis pyriputrescens. *Mycologia*. **97(**1): 25-32.

Kitinoja, L. and Kader, A. A. (2012). Small-Scale Postharvest Handling Practices: A Manual for Horticultural Crops (fourth ed.), University of California, Postharvest Technology Research and Information Center, Davis.

Klich, M. A. and Pitt, J. I. (1988). A laboratory guide to common *Aspergillus* species and their teleomorphs. Commonwealth Scientific and Industrial Research Organisation, Division of Food Processing, Australia. pp. 474-478.

Klich, M.A. and Xiao, C. L. (2007). *Aspergillus flavus*: the major producer of aflatoxin. *Molecular Plant Pathology*. **8**:713-722.

Kozakiewicz, Z., Wang, H., Cao, G. and Prior, R.L. (1995). *Aspergillus flavus*. IMI Descriptions of Fungi and Bacteria. Surrey, UK: *CABI Bioscience.***126:**1251.

Kumara, K. L. W. and Rawal, R. (2010). Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (*Carrica papaya*). *Trop Agric. Res. Ext*. **11**:7-12.

Lanette, S. (2014). Diagnostic Image Series Development for Supporting IPM in the Southern Region, *Aspergillus niger*. National Institute of Food and Agriculture, U.S. Department of Agriculture. p. 818.

Leitão, A. L. (2009). Potential of *Penicillium* species in the bioremediation field. *International Journal of Environmental Research and Public Health.* **6** (4): 1393–417.

Leslie, J. F., Summerell, B. A. and Bullock, S. (2006). The Fusarium Laboratory Manual. Blackwell Publishing, Ames, IA. p.388.

Llorente, I. and Montesinos, E. (2006). Brown spot of pear: an emerging disease of economic importance in Europe. *Plant Disease*. **90**: 1368-1375.

Magdalena, F., Gryta, A., Oszust, K. and Kotowicz, N. (2016). Fast and Accurate Microplate Method (Biolog MT2) for Detection of *Fusarium* sp Fungicides Resistance/Sensitivity. *Front Microbiol*. **7**: 489.

Malik, V. K. and Singh, S. (2004). Effect of temperature and relative humidity on teliospore germination in Ustilago hordei. *J. Mycol. Plant Pathol*. **34**: 410-411.

Mishra, P. R. and Khan, F. N. (2015). Effect of different growth media and physical factors on Biomass production of *Trichoderma viride*. *People's Journal of Scientific Research*. **8**(2):11-16.

Mokobi, Gandarilla, Fatima, Torres-Caraballo, De Luna-Santillana, Erick, Quintero-Zapata and Nancy. (2020). Inhibitory effect of essential oils on the mycelial growth of *Penicillium digitatum* (pers.) sacc. isolated from sweet orange (*Citrus sinensis osbeck*). *Agrociencia*. **54**: 209-225.

Moretti, C. L., Mattos, L. M., Calbo, A. G. and Sargent, S. A. (2010). Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. *Food ReS. Int.* **43**:1824–1832.

Moslem, Leggo, D., Kiely, T. B. and Ghosoph, J. M. (2010). Effect of skin coatings on the development of black spot in stored Valencia oranges. *Australian Journal of Experimental Agriculture and Animal Husbandry*.**7**:593-600.

Moslem, Mohamed & Mashraqi, Abdullah & Elsalam, A., Kamel & Bahkali, Ali & Elnagaer, M.A.. (2016). Molecular detection of ochratoxigenic *Aspergillus species* isolated from coffee beans in Saudi Arabia. Genetics and Molecular Research. **9**: 2292-9.

Mullen, J. (2006). Southern blight, Southern stem blight, White mold. *The Plant Health Instructor.* **10**:101-104.

Nabi, U. N., Sajad, R., Wasim, K., Javid, Sharma, O. C. and Sheikh, M. (2017). Post Harvest Diseases of Temperate Fruits and their Management Strategies-A Review. *International Journal of Pure and Applied Bioscience*. **5**:885-898.

Nandi, S., Hembaram, S., Adhikari, A., Tiwari, B. K. and Dutta, S. (2017). Host Infection beyond the Traditional Range of *Sclerotium (Athelia) rolfsii* with *Physalis minima. Bioinformation*, **13**(10): 333-338.

Nicoletti, R., Buommino, E., De Filippis, A., Lopez-Gresa, M., Manzo, E., Carella, A., Petrazzuolo, M. and Tufano, M. A. (2009). Bioprospecting for antagonistic *Penicillium* strains as a resource of new antitumor compounds. *World Journal of Microbiology.* **24** (2): 185–95.

Niem, J., Miyara, I., Ettedgui, Y., Reuveni, M., Flaishman, M. and Prusky, D. (2007). Core rot development in red delicious apples is affected by susceptibility of the seed locule to *Alternaria alternata* colonization. *Phytopathology.* **97:** 1415–1421.

Nurulhuda, Weber, R. W. S. and Dralle, N. (2013). Fungi associated with blossom-end rot of apples in Germany. *European Journal of Horticultural Science.* **78**: 97-105.

Oche, Köhl and Jürgen. (2015). Postharvest decay of apples and pears in the Netherlands. *Acta Horticulturae*. **1053**: 107-112.

Oladiran, W. and Iwu, B. L. (1992) The effects of waxing on citrus fruit. *Rural Newsletter.* **79**:14-19.

Onyemata, E. K and Ibrahim, R. O. (2018). Isolation and identificationof fungi and pathogenicity assessment of somespoilt fruits sold in Wuse Market, Abuja, Nigeria. *International Journal of Current Research.* **10**(12):76256-76259.

Oranusi, U. S. and Wesley, B. (2012). Microbiological Safety Assessment of Apple Fruits (*Malus domestica Borkh*) Sold in Owerri Imo State Nigeria. *Advance Journal of Food Science and Technology*. **4**(2): 97-102.

Paolo, M. A. (2013). A sclerotium seed rot and seedling stem rot of mango. *Philippine Journal of Science.* **52**:237-261.

Parisa, Rathod, G. and Chavan, A. M. (2017). Status of biochemical content in papaya (Carica papaya L.) after post-harvest pathogenesis by fungi. *Current Botany*. **3**(3): 28-33.

Pierre, G., Edmond J. B., Gabriela, A., Matthew, C. F., Joseph, H. and Tatiana,G. (2011). Epidemiology and Evolution of Fungal Pathogens in Plants and Animals. *Genetics and Evolution of Infectious Disease.* pp. 59-132.

Pitt, J. I., Basílico, J. C., Abarca, M. L. and López, C. (2000). Mycotoxins and toxigenic fungi. *Medical Mycology.* **38** (1): 41-46.

Pitt, J. I. (2014). PENICILLIUM | Penicillium and Talaromyces. *Encyclopedia of Food Microbiology.* **1**(2).

Rahman, M. A., Mahmud, T. M. M., Kadir, J., Rahman, A. R. and Begum, M. M. (2008) Major Postharvest Fungal Diseases of Papaya cv. 'Sekaki' in Selangor, Malaysia. *Pertanika J. Trop. Agric. Sci*. **31**(1): 27-34.

Sagar, A., Tuset, P. and Eckert, L. (2018). Potato Dextrose Agar (PDA)-Principle, Uses, Composition, Procedure and Colony Characteristics. *International Journal of Microbiology*. **5**(1):43

Samson, R. A. (2004). Phylogenetic analysix of *Penicillium* subgenus : *Pencillium* using partial beta-tubulin sequences (PDF). *Studies in Mycology*. **49**: 175–200.

Samson, R. A. and Zoellner, J. J. (2014). Sensitivity of tomatoes at mature: green and breaker ripeness stages to internal bruising. *Journal of the American Society for Horticultural Science*. **117**:119-123.

Sandhu, S. S. and Singh, A. P. (2000). Effects of harvesting dates and individual seal packaging on pear fruits Le Conte during cold storage. Haryana. *Horticu. Sci*. **29**: 48-52.

Scetar, M., Kurek, M. and Galic, K. (2010). Trends in Fruit and Vegetable Packaging – a Review. *Croatian Journal of Food Technology, Biotechnology and Nutrition*. **5** (3-4): 69-86.

Schovánková, J., & Opatová, H. (2011). Changes in phenols composition and activity of phenylalanine-ammonia lyase in apples after fungal infections. *Horticultural Science* (Prague). **38**: 1–10.

Sever, Z., Ivić, D., Kos, T. and Miličević, T. (2012). Identification of Fusarium species isolated from stored apple fruit in Croatia. *Archives of Industrial Hygiene and Toxicology*. **63**: 463-470.

Shah, Souza, J. R. B., Kupper, K.C. and Augusto, F. (2018). *In vivo* investigation of the volatile metabolome of antiphytopathogenic yeast strains active against *Penicillium digitatum* using comprehensive two-dimensional gas chromatography and multivariate data analysis. *Microchem. J*. **141**:204–209.

Sharma, R. L. and Sharma J. B. (2009), Horticulture: mechanical injury. *South Australian Research And Development Institute – Sardi*. **16**(4): 121-130.

Sharma, R. L. and Kaul, J. L. (2012). Susceptibility of apples to brown rot in relation to quantitative characters. *Indian Phytopathology*. **43**:113-115.

Sharma, V. (2015). Evaluation on incidence and alternative management of postharvest fungal diseases of papaya fruit (*Carica papaya L*.) in Western U.P. *International Journal of Theoretical & Applied Sciences.* **7**(1): 6-12.

Shimwela, M. (2014). Diagnostic Image Series Development for Supporting IPM in the Southern Region, *Aspergillus flaavus*. National Institute of Food and Agriculture, U.S. Department of Agriculture. p.708.

Shine, Skaria, M., Eayre, C. G., Miao, H., Solis-gracia, N. and Mackey, B. (2007). Effect of packing on rot and fruit damage in Rio Red Grapefruit. *Subtropical Plant Science*. **55**:75-78.

Silvestri, D. A., Ferrari, E., Gozzi, S., Marchi, F. and Roberto, F. (2018). Determination of Temperature Dependent Growth Parameters in Psychrotrophic Pathogen Bacteria and Tentative Use of Mean Kinetic Temperature for the Microbiological Control of Food. *Frontiers in Microbiology*. **9**: 302-3**.**

Simmonds, Granato, L. M. and Brown, G. E. (1975). Factors affecting postharvest development of *Colletotrichum gloeosporioides* in citrus fruits. *Phytopathology*. **65**:404-409.

Singh, B. K., Singh, Y. K. and Verma, A. (2017). Impact of postharvest diseases and their management in fruit crops: An Overview, Department of Horticulture, Institute of Agricultural Science, Banaras Hindu University Varanasi. p. 221.

Singh, E., Ab-Rahman, S. F. S., Pieterse, C. M. and Schenk, P. M., (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **267**:102–111.

Snowden, A. L. (l99l). A colour at Las of post-harvest diseases and disorders of fruits and vegetables. *Wolfe Scientific Inndon*. **2.**

Sommer, N. F. (2009). Strategies for control of post-harvest disease of selected commodities. In: Post-harvest Technology of Horticultural Crops. University of California Press. pp. 83-98.

Soto-Alvear, S., Lolas, M., Rosales, I. M., Chávez, E. R., Latorre, B. A., (2013). Characterization of the bull's eye rot of apple in Chile. *Plant Disease*. **97**: 485-490.

Sui, Y., Wisniewski, M., Droby, S., Norelli, J. and Liu, J. (2016). Recent advances and current status of the use of heat treatments in postharvest disease management systems: Is it time to turn up the heat? *Trends in Food Science and Technology*. **51**: 34–40.

Sutton, T. B., Aldwinckle, H. S., Agnello, A. M. and Walgenbach, J. F. (2014). Compendium of apple and pear diseases and pests. APS Press. pp.28-33.

Takahashi, T. (1999). A sclerotium disease of larkspur. *Phytopathology*. **17:**239-245.

Thompson, J. R., Fairs, A., Wardlaw, A. J. and Pashley, C. H. (2010). Guidelines on ambient intramural airborne fungal spores. *Journal of Investigational Allergology and Clinical Immunology*. **20** (6): 490–98.

Townsend, B. B. and Willetts H. J. (2001). The development of sclerotia of certain fungi. *Ann. Bot.* **21**:153-166.

Valiuskaite, Alma & Survilienė, Elena & Lugauskas, Albinas & Levinskaitë, Loreta. (2006). Ecological aspects of distribution of potential toxin-producing micromycetes on stored apple fruit. *Lietuvos mokslų akademija*. pp. 60-63.

Villalta, O. N., Washington, W. S. and McGregor, G. (2004). Susceptibility of Asian pears to pear scab. *Plant Protection Quarterly*. **19**: 2-4.

Visagie, C. M. (2014). Identification and nomenclature of the genus *Penicillium. Studies in Mycology*. **78**: 343-371.

Watson, J. A., Danielle, T., Steven A. S., Jeffrey K. B. and William, P. (2016). Postharvest Storage, Packaging and Handling of Specialty Crops: A Guide for Florida Small Farm Producers. pp.4-5.

Weber, R.W. S. (2011). Phacidiopycnis washingtonensis, cause of a new storage rot of apples in Northern Europe. *Journal of Phytopathology*. **159**: 682-686.

Weber, R.W.S. (2013). Fungi associated with blossom-end rot of apples in Germany. *European Journal of Horticultural Science*. **78**: 97-105.

Wenneker, M. (2019). Fungal pathogens in pome fruit orchards and causal agents of postharvest decay. PhD Thesis, Wageningen University, Wageningen, The Netherlands. p.317.

Wenneker, Marcel & Köhl, Jürgen. (2014). Postharvest decay of apples and pears in the Netherlands. *Acta Horticulturae*. **1053**: 107-112.

William, O. N. and Wambura M. (2011). Isolation and characterization of pathogenic bacteria and fungi from *Leptoptilos crumeniferus* (Marabou stork) droppings. Indonesian Society of Sanitary and Environmental Engineers, Jakarta. **1**(1): 93-106.

Willson, C. (2013). FGSC culture preservation methods. Fungal Genetic Newsletter. **33**:47-48.

Wohner, B., Pauer, E., Heinrich, V. and Tacker, M. (2019). Packaging-related food losses and waste: an overview of drivers and issues. *Journal of Horticulture, Forestry and Biotechnology.* **11**(1): 264

Zhang, J. X. (2004). The potential of a new fungicide fludioxonil for stem-end rot and green mold control on Florida citrus fruit. *Postharvest Biology and Technology*. **46**: 262-270.