POST HARVEST DISEASES IN SOME SELECTED ROOT AND TUBER CROPS AND IDENTIFICATION THEIR CAUSAL AGENTS

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JUNE, 2020

POST HARVEST DISEASES IN SOME SELECTED ROOT AND TUBER CROPS AND IDENTIFICATION THEIR CAUSAL AGENTS

BY

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Reg No: 18-09097

A Thesis

Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE

IN

PLANT PATHOLOGY

Semester: Jan-Jun, 2020

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This is to certify that the thesis entitled, " **POST HARVEST DISEASES IN SOME SELECTED ROOT AND TUBER CROPS AND IDENTIFICATION THEIR CAUSAL AGENTS**" submitted to **the Department of Plant Pathology**, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207 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 ISRAT JAHAN, Registration No. **18-09097,** under my direct 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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ACKNOWLEDGEMENT

Alhamdulillah, at first All the praises and gratitude goes to the omnipresent and omnipotent Allah, who has kindly enabled the author to complete the research work and complete this thesis successfully for achieving practical knowledge and wisdom.

The author honestly desires to express her deepest sense of gratitude, respect, deep appreciation and indebtedness to her research Supervisor, Dr. Nazneen Sultana, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her benevolent and scholastic guidance, endless effort, appreciated suggestions, inspiration, co-operation and constructive criticisms throughout the entire period of research work and the groundwork of the manuscript of this thesis.

The author expresses deepest gratitude and obligation to her Co-supervisor, Dr. M. Salahuddin M. Chowdhury Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his co-operation, criticisms on the manuscript and helpful suggestions for the successful accomplishment of the research work.

The author expresses her deepest veneration and boundless thankfulness to Prof. Dr. Fatema Begum, Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her sympathetic co-operation and inspiration throughout the research work.

Special thanks and obligation to all the respective teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their appreciated teaching, sympathetic co-operation and inspiration throughout the period of the study.

The author thankfully evokes the MS and PhD students of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their cooperation in the entire period of study.

The author also expands her thanks to all the staffs of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their assistance during the research work.

The author also likes to give appreciations to all of her friends for their support and inspiration throughout her study period in Sher-e-Bangla Agricultural University, Dhaka.

The author also expresses thanks to her senior companions Anannay Biswas Soma, Sifat Hosen shova, Sadia Sharman, Rakibul Hasan Nitol, Md Sajeduzzaman, Asif Noor, and her friends Tanvir ahmed, for their cordial support, cooperation and inspiration in preparing this thesis.

Finally, the author deeply grateful to her respected parents and her lovely sisters for their precious love, well wishes, sacrifice, never ending affection and endless efforts for bringing her dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of her studies.

The Author

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ABSTRACT

The experiment was conducted at MS Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of August 2019 to March 2020 to isolate, identify, and characterize the causal agents responsible for the post-harvest spoilage of some selected root and tuber crops. Potato, sweet potato, radish and carrot were used as samples. Infected samples were collected from different markets in Dhaka city. The post-harvest fungi were isolated on PDA medium following tissue planting method. Five different fungi genera were isolated and identified viz. *Aspergillus niger, Geotrichum candidum, Rhizopus stolonifer, Fusarium* spp. and *Penicillium digitatum*. Of these, *Fusarium* spp. has the highest percentage of frequency of occurrence in potato, carrot and radish. In carrot, *Aspergillus niger* showed more frequency of occurrence than *Geotrichum candidum* but in case of sweet potato, the occurrence of *Rhizopus stolonifer* was found to be more than *Penicillium digitatum*. Pathogenicity tests revealed that all the isolated fungi were pathogenic to their respective host as post-harvest pathogens. Effect of different media and temperature on growth of isolated fungi were studied. On PDA media, the highest radial mycelial growth (4.06 cm) at 8 DAI was recorded in *Aspergillus niger* (carrot) which was statistically similar with *Rhizopus stolonifer* (sweet potato) whereas, the lowest radial mycelial growth (2.54 cm) was recorded in *Fusarium oxysporum* (radish). The highest radial mycelial growth was observed in PDA followed by CDA media. Most of the isolated fungi showed the highest growth rate at 30°C temperature, whereas, the growth rate of *Rhizopus stolonifer* was found the highest at 25°C temperature.

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LIST OF SYMBOLS AND ABBREVIATIONS

No. = Number

% = Percentage

et al.= And others

°C = Degree Celsius

@ = At the rate

etc. = Etcetra

J. = Journal

Viz. = Namely

Cm = Centimetre

& = And

g = gram

ml = Millilitre

L = Litter

i.e., = That is

PDA = Potato Dextrose Agar

CDA = Carrot Dextrose Agar

DAI = Days After Incubation

SAU = Sher-e- Bangla Agricultural University

BBS = Bangladesh Bureau of Statistics

LSD = Least Significant Difference

ANOVA = Analysis of Variances

CV% = Percentage of Co-efficient of Variance

CHAPTER I

INTRODUCTION

Bangladesh is particularly an agrarian country. Agriculture sector play a part in 15% to the country`s Gross Domestic Product (GDP) and employs around 41% of the total labour force (BBS, 2018). Root and tuber crops are important for their energy sources after cereals, generally in tropical regions in the world. They include potato, cassava, sweet potato, carrot, radish, yams and aroids belonging to different botanical families but all types of root and tuber produce underground food (Okigbo,1989). Radish and carrot are rated as the low-calorie root vegetables. (Akhtari *et al*., 2016). Potato and sweet potato have a good source of food energy (Oyeyipo, 2012). In Bangladesh, 1174978 acre of land have been used for potato cultivation and in some root crops such as radish, carrot and sweet potatoes were cultivated in 65652 acres, 4533 acres, 63604 acres of land in Bangladesh, respectively (BBS, 2017). Total production of potato, radish, carrot and sweet potato was 10215957 metric tons, 280524 metric tons, 16306 metric tons and 262702 metric tons, respectively (BBS, 2017). In the year 2017, 494.6 million tons of roots and tubers (including potato) are produced worldwide (FAOSTAT, 2019). Root plants and vegetables are especially vulnerable to mechanical damage, both treatment and harvesting or technological processes because the tubers, roots, leaves and stems of these plants are exposed to mechanical damage such as bruises, abrasions, intersections and cracks, which in the vast majority of cases are the result of dynamic loads (Abraha *et al*., 2018; Zhang *et al*., 2018).

The production of root crops such as radish, carrot, potato and sweet potato has been decreased by postharvest handling, storage, marketing, some disease-causing organism, transportation and processing, thereby resulting in the reduction in quality, quantity and market value of agricultural goods (Kader *et al*., 2005 and Parfitt *et al*., 2010). Postharvest fungal disease is one of the significant factors that reduce the storage period and shelf life of radish, carrot,

potato and sweet potato and even result in serious economic losses worldwide. Storage losses are increased by biotic (insects, pests, rodents and fungi) and abiotic factors (temperature, humidity and precipitation). The moisture content and temperature are the most important factors affecting the storage duration. Most losses during storage increase rapidly at temperatures of 20-40℃ and relative humidity over 70% (Grudzińska and Barbaś, 2017; Abedin *et al*., 2012). Low relative humidity below 70% limits the growth of mold (Sawicka, 2019). Post-harvest diseases decrease 10-30% of the total yield of crops and in some perishable root vegetable crops especially in developing countries; they spoil more than 30% of the total yield (Kader, 2002; Agrios, 2005).

The microbial spoilage is mostly caused by fungi, bacteria, yeast and molds. However, a remarkable loss of vegetables during post-harvest period was attributed to diseases caused by fungi and bacteria (Elias *et al.,* 2010).

Several disease-causing fungi were identified which was responsible for storage rot of root and tuber crops such as *Aspergillus niger, Fusarium* sp*, Penicillium* sp*, Mucor* sp*, Geotrichum candidum, Aspergillus flavus, Rhizopus* sp*,* etc. were found to be associated with post-harvest storage rots of root crop (Ibrahim and Shehu, 2014). In most root vegetables, several mechanisms were involved in transforming a dormant infection into an active one that results in decay and cell damage. Wound infections cause the most severe damage in postharvest spoilage emphasised on harvested and bulk handling, and then wound infections were prominent (Benny, 1994). *Rhizopus* spp.*, Mucor* sp*, Curvularia* sp*, Aspergillus* sp*, Erwinia* sp and *Fusarium* sp resulted in rots and storage deterioration of yams, cassava and potato around the world (Mahovic and Bartz, 2019). *Rhizopus oryzae, Fusarium redolens, Butryodiplodia theobromae, Fusarium oxysporum, Aspergillus niger* and *Penicillium species* were responsible for postharvest dry rot of Irish potato tubers (Salami, 2007).

Fusarium rot is the most destructive for the postharvest root and tuber crop. *Fusarium* dry rot is identified by an internal light to dark brown or black rot of the potato tuber-and it is usually dry. Dry rot is characterized by several fungal

species in the genus *Fusarium,* thus the name *Fusarium* dry rot. The most important dry rot pathogen in the Northeast is *Fusarium sambucinum,* although *Fusarium solani is* also present. *Sclerotium rolfsii* and *Rhizoctonia solani*, can cause significant postharvest losses of vegetable crops such as carrot and potato while diseases caused by these pathogens are primarily field diseases, the development of symptoms often accelerates after harvest (Coates, 1995).

Spoilage mold fungi such as *Aspergillus niger, A. flavus, Rhizopus* sp, *Fusarium* sp, *Penicillium* sp were isolated from the infected vegetables. They were identified based on the morphological characteristics, conidial structure, arrangement of spore. This information will help us in removing these spoilage microorganisms so that it will be precautionary measure in order to increase its shelf life (Sulthana *et al.,* 2019).

The most significant losses in agricultural productions which involve the greatest costs on the farm economy occur by post-harvest diseases. It was recorded that 10 to 40% losses nation of agricultural produce occur due to post harvest diseases all over the world. Losses were more severe in developing country than developed country of the world (Enyiukwu1, 2014).

The effects of high RH and high temperature are closely interrelated for decay of many fruits and vegetables, relative humidity near saturation results in lower decay losses only if the temperature was near O°C. The relative humidity below 90% cannot permit micro-organisms to grow on the surface of fruits and vegetables (Danladi, 2000).

PDA is one of the most common culture mediums because of its simple formulation and ability to mycelial growth of a wide range of fungi. Several workers stated that PDA to be the best medium for mycelial growth and preservation for future use. (Xu *et al*., 1984; Maheshwari *et al*., 1999; Saha *et al.,* 2008). The colony character including diameter, culture characteristics

(texture, surface and reverse colouration, zonation) and sporulation of selected fungi were identified by the use of different growth media (Devi *et al*., 2018).

Objectives: Mentioned the facts of following specific objectives were as follows:

- To isolate, identify and characterize the causal agents of post-harvest fungal diseases of potato, sweet potato, carrot and radish.
- To determine the effect of different media and temperatures on growth of post-harvest fungal pathogens isolated from selected root and tuber crops.

CHAPTER II

REVIEW OF LITERATURE

Root and tuber crop (potato, sweet potato, radish and carrot) is one of the most cultivated crops of temperature and tropical regions of Bangladesh. There are several post-harvest diseases of root and tuber crop, among them *Fusarium* dry rot, *Aspergillus* rot, mold rot, sour rot and *Rhizopus* rot have been considered as the most important diseases of root and tuber crop in Bangladesh at different market in Dhaka city. Literature review represent on these diseases were enlisted here.

2.1. Importance of different root and tuber crops

Dias *et al.* (2019) stated that 17% of below ground vegetables were edible organs comprised and Asia produces and consumes more than 70% of the world's vegetables of root and tuber crops.

Dias *et al.* (2019) also reported that Potato was the world's most important tuber crop, nearly about 400 million t produced worldwide every year.

Hossain *et al.* (2018) reported that an excellent agriculture zone situated at Sylhet in Bangladesh which is covered 11% of hill areas. Among the cultivated lands 34.4% were high land and medium high land.

Department of Agriculture Extension (DAE) (2018) has set a target of yielding around 1.25 lakh tons' sweet potato from around 8,050 hectares of land in the country's northwest region during the current season. DAE and other government and non-government organizations concerned have taken necessary measures to make the sweet potato cultivation a success in the region. Additional Director of DAE Mustafizur Rahman said the sandy lands and the riverbeds (chars) are most effective for the sweet potato cultivation. So, people of the Ganges and Brahmaputra basins and the remote char areas are suitable in the farming widely besides consuming it.

Bisbis *et al*. (2018) reported that by increasing human population, vegetables have played an important role in our national economy. Maximum output came from vegetable and more national income per unit area of land to small-scale farmers, particularly when compared to cereals.

About 31768 tons of sweet potato was produced on 1452 ha area of land with an average yield of 21.88 t ha^{-1} in Bangladesh (DAE, 2017).

Total production of sweet potato at the Sylhet region in Bangladesh was comparatively higher than national average yield of 10.4 t ha^{-1} (MoA, 2017).

Bangladesh Bureau of Statistics (2016) reported that the cultivable land and yield rate of potato has increased, 7.83% total amount of production of potato has increased.

Bangladesh Bureau of Statistics (2016) also reported that total amount of potato has been estimated 20.443 metric tons per hectare area of land in Bangladesh.

Dhaliwal (2017) reported that maximum yield in radish varies depending on the variety and the crop season. Maximum root yield was obtained from the winter crop than the summer crop. The root yield varies from 150 quintals per hectare in summer to 600 quintals per hectare in winter season.

In Bangladesh, 70% vegetables could grow in winter seasons such as Cole crop, brinjal, carrot, radish different types of leafy vegetables (Akter *et al*., 2016). They take low temperature below than 20℃. Winter season called vegetables seasons in Bangladesh (BBS, 2016). All vegetables could not grow in all regions in our country it can depends on soil types such as potato can grow in northern region such as Thakurgon, Rangpur, Bogra, and south part Munsigonj. This soil and environment were good for better potato production (BBS, 2016).

BARI and other agricultural organization have developed a good number of HYV of different vegetable crops including improved production technology (Akter *et al*., 2016). If farmers use these technologies then all internal

requirement will be met up even, we can export some vegetables abroad and earn foreign currency.

In the year 2015-2016, the cultivable land of carrot was 1,768 hectares, total production of 15,679 metric tons in Bangladesh (BBS, 2016).

Siddique *et al.* (2015) stated that average yields of potato in Bangladesh with special preference to seed potato sources, formal and informal seed production, certification and actors involve in Bangladesh.

FAO (2015) reported that potato (*Solanum tuberosum* L.) is one of the most important tuber crops of the world and holds the fourth position in production after wheat *(Triticum aestivum*), rice *(Oryza sativa*) and maize (*Zea mays*).

Bangladesh Bureau of Statistics (2015) reported that vegetable crop contributes as much 55% of the total vegetable production in Bangladesh.

Siddique *et al.* (2015) informed that BARI developed 44 exotic potato varieties, exotic germplasm and germplasm developed in Bangladesh through crossing-breeding programme. The most suitable variety was selected as per producers and end user's requirement.

Area, production and yield of radish was increasing at a rate of 1.40 percent, 2.10 percent and 0.70 percent per annum, respectively. By using new varieties Productivity of radish increases like BARI Mula-1, pinky, durti and BARI mula-4 are high yielding variety of Bangladesh (Digital Herbarium, 2015) and improved technologies which helps there to get maximum return production.

Kopta and Pokluda (2013) reported that the production of radish was regulated to be about 7 mils. t year^{-1}, representing roughly 2% of all vegetables in the world. It was more suitable vegetable in both tropical and temperate regions of the world. It is consumed as a salad or cooked as a vegetable. There are several varieties of radish having varying length, size, colour, and taste, flavour, yield

potential and quality parameters. Chinese radish is more popular than any other country in the world.

BADC (2012) also reported that 12,000 metric tons of quality seed-potato among the farmers as a whole against the annual demand of 600000 metric tons at present which is only 5-6% of the total requirement of seed potato in Bangladesh.

BADC (2012) has undertaken a programme for producing 25,000 metric tons of seed-potato by applying tissue culture technology by 2012 in the country. Tissue culture laboratories would be set up soon as mentioned by BADC office for seed production.

Sweet potato has an excellent potential to be an effective and economic source of food energy (Oyeyipo, 2012).

In Bangladesh 22,052 hectares' area of land was cultivated for radish production in which 20,0840 metric tons of radishes were produced annually (Mondal *et al.*, 2011).
In Bangladesh potato was the third most important producer in Asia and standing sixth in the world (FAO, 2010).

Karim (2009) said that Both private and public sector supply only 5% quality seed of potato of the total requirement in Bangladesh.

Biswas (2009) said that Bangladeshi market provide seeds of both hybrid as well as open pollinated varieties were disposed of Bangladesh almost throughout the year. To fulfil this growing demand of radish seeds, BRAC also launched a variety development as well as seed production program of radish and has released 3 relatively short duration varieties (Sufola-40, China 35 and White Star) with adequate yield performance and quality through its own variety development as well as variety screening program.

Diversification into vegetables, crops and increasing commercialization can support the development of the agricultural sector in different ways. Vegetables contribute an important share in the total agricultural exports from Bangladesh. Vegetables and root crops sub-sector also share about 11.70% to the agricultural GDP (Bangladesh Economic Review, 2008).

Production of radish roots all over the world was estimated at 7 million t per year, about 2% of the total world production of vegetables (Schippers 2004). In China, Japan and Korea, as well as in Yemen, radish ranks high in importance (Schippers, 2004).

There were several foods, which was as flexible as potato. Potato is a popular food throughout the world, both in its fresh and processed forms. Potato is special in a sense that it can fit into any meal. In world, Bangladesh ranks 7th in potato production. The major potato growing districts in Bangladesh are; Rajshahi, Rangpur, Dinajpur and Munshigonj, however, the contribution of Munshigonj in potato production is prime (nearly 44%) in the country (Singh and Lal, 2003).

The farmers produced 95% quality potato seed themselves. Seeds of modern high yielding varieties were imported from the Netherlands around 1960 and continued there after (Kadian *et al*., 2000).

Yield of carrot depending on the production methods followed by the farmer and growing conditions. 25 to 30 tons' ha^{-1} of yield may be considered good for the small-rooted early market, but for processing more than 100 tons' ha-1 was often achieved. Fresh market bunched or topped pre-packed carrot yields range from 30 to 60 tons' ha^{-1} (Rubatzky *et al*., 1999).

Rashid and Sarker (1986) reported that total amount of yield of carrot was 35 tons per hectare in Bangladesh. This yield was comparatively low than other carrot producing countries like Belgium (47.64 t/ha), Netherlands (61.87 t/ha) and Sweden (43.6 t/ha) (FAO, 2012).

2.2. Post-harvest losses of root vegetable crop

Yahaya and Mardiyya (2019) stated that high temperature and high relative humidity increase the decaying and spoilage of vegetables, while decrease in temperature slow down the rate of microbial attack on different vegetable crops especially when it is below 5°C. Chilling injury was caused by low but not freezing temperature was mainly observed with tropical and subtropical vegetables. However, the symptoms were caused by chilling injury may not be showed while the vegetables were held at chilling temperature but may become visible only when the fruits and vegetables were transferred to room temperature at 37℃.

Fungi, bacteria and oomycetes were disease causing agents which create rots and deterioration of stored agro produce leading to postharvest losses of foods all over the world (Enyiukwu *et al*., 2014a; Kasso and Bekele, 2018).

Root crops and vegetables were particularly included to mechanical damage, both during treatments and during harvesting or technological processes, because the tubers, roots, leaves, and stems of these plants were resolved to mechanical damage, such as bruises, abrasions, intersections, and cracks, which in the vast majority of cases the result of dynamic consignments (Abraha *et al*., 2018; Zhang *et al*., 2018).

Post-harvest losses can be restricted as loss of weight due to deterioration, loss of quality, reduce nutritional value, loss of viability, and finally commercial loss (Boxall, 2001; FAO, 2017a).

In potatoes, mechanical injury and the formation of black spots in the tuber parenchyma cause significant economic losses and degradation of quality and market value (Grudzińska and Barbaś, 2017).

It was estimated that root crops and vegetables, these losses account for, respectively, approx. 19%, and 44% of losses (FAO, LEI 2015; Food and Agriculture Organization of the United Nations, 2016).

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

The most important losses in agricultural commodities which involve the greatest costs on the farm economy occur by post-harvest diseases. It was measured that 10 to 40% losses nation of agricultural produce occur due to post harvest diseases all over the world. Losses were more severe in developing than developed country of the world (Enyiukwu1, 2014).

Medagoda (2011) observed in a study that a very few percentages of total produce were taken as a food amounting 30 percent and greater percentages, consist of about 70% losses were observed in pre- and post-harvest stage. The major problem was observed in marketing where the marketing structures had not properly well, lack of processing plants and the less demand in local market for jack products. An integrated access would improve productivity, quality and income from jack cultivation contributing to poverty mitigation in the rural sector to a considerable extent.

Many microbes and disease-causing organisms could attack on vegetable. The microbial spoilage was caused by fungi, bacteria, yeast and molds. However, most of the losses of vegetables at the time of post-harvest period was attributed to diseases caused by fungi and bacteria. These organisms were easily invading of the root vegetables. (Elais *et al.*, 2010).

Muntad (2009) reported that both quantitative and qualitative losses of immensely variable magnitudes occurring at all stages in the post-harvest system from harvesting, through handling, storage, processing and marketing to final delivery to the consumer.

Miah *et al.* (2008) reported that the post-harvest practices of vegetables scheme were quite unacceptable and mostly constitute of traditional techniques practiced by the growers, traders and processors, owing to which considerable deterioration in physical and nutritional qualities of the harvested in Bangladesh. It was estimated that the post-harvest loss of vegetables in Bangladesh was about 25-35%.

Postharvest loss (20-25%) was one of the major challenges in the potato production. Another loss was sustained to the small holders that could have helped in nutrition, food security and wages of the producers (Bureau of Finance and Economic Development, 2007).

Hayatu and Sani (2000 and 2006) reported that the effect of post-harvest losses of vegetables was influenced by many factors. These factors involved losses due to physical, physiological, mechanical and hygienic conditions. Vegetables were mainly characterized by high level metabolic activities and known to posse's short shelf life. As a result of these factors, post-harvest losses of vegetables can occur harvesting to consumption of produce.

Valiuskaite *et al*. (2006) reported that fungi were the most essential post-harvest losses agent of different fruits and vegetables, based on cultivar, season and production area with other factors. Fungi were the most crucial and common pathogens that cause diseases. It affects a wide range of fruits and vegetables during storage and transportation (Sommer, 1985).

According to a report, around 1 billion people are facing by severe hunger in a number of nations of which 10% actually die from hunger-related complications. This problem arises due to inadequate agricultural storage, handling and produce preservation from microbes-induced spoilages.

post-harvest losses are caused by the disease which occurs on root vegetables. Post-harvest diseases spoiled 10-30% of the total yield of crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield all over the world (Kader, 2002; Agrios, 2005).

Wills *et al*. (2004) estimated that in order to tropical and subtropical climatic region, a variety of vegetables are grown in Bangladesh. Unfortunately, a considerable proportion of the harvested vegetables never reaches the consumers mainly because of postharvest losses. The estimated postharvest losses of vegetables are 20-40% in Bangladesh.

It was estimated that as much as 40% vegetables were wasted after harvest. Hence, the elimination of post-harvest losses of agricultural products is important to boost food security and availability in these countries (Mrema and Rolle, 2002).

Alao (2000) said that mechanical damage was caused by handling during harvesting, packing, transportation, storage, marketing etc. Some insects and birds were also responsible for the mechanical damage of vegetables. Mechanical injuries like bruising and cracking of vegetables render them more prone to attack by organisms and increase the rate of water loss and gaseous exchange. Several tines the damage cause by mechanical injury on vegetables as a result of pressure thrust during transportation and marketing though many times invisible, but causes damage of inner tissues and cells.

Root plants and vegetables were particularly vulnerable to mechanical damage, both during treatments and during harvesting or technological processes, because the tubers, roots, leaves, and stems of these plants are exposed to mechanical damage, such as bruises, abrasions, intersections, and cracks, which in the vast majority of cases were the result of dynamic loads (Abraha *et al*., 2018; Zhang *et al*., 2018).

The term “postharvest loss” - PHL refers to assessable quantitative and qualitative food loss in the postharvest system (de Lucia and Assennato, 1994). This system encompasses interconnected activities from the time of harvest through crop processing, marketing and food preparation, to the final decision by the consumer to eat or discard the food.

Wilson *et al.* (1991) reported that vegetables were highly perishable commodities, the quality of root vegetable was affected by post-harvest handling, transportation, storage and marketing. The inappropriate handling, packaging, storage and transportation may result in decay and production of microorganisms, which become activated because of the changing physiological conditions of the vegetables.

Postharvest losses of vegetables were 25-40% as reported by (Amiruzzaman, 1990), respectively. So, there is no dilemma that enormous amounts of vegetables are lost every year. Most of the data available are based mainly on experiments conducted at different research field and Universities. Most of the data on crop losses have been evolved from indirect sources.

FAO (1989) reported that estimation of the post-harvest losses of food grains and vegetables in the developing country from mishandling, spoilage and pest infestation are put at 25%, this means that one-quarter of what is produced never grasps the consumer for whom it was grown, and the effort and money required to produce it was lost-forever. Fruit, vegetables and root crops were much less hardy and were most promptly perishable, and if necessary, alarm was not taken in their harvesting, handling and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to court, but some authorities put losses of sweet potatoes, potatoes, carrot, tomatoes, bananas and citrus fruit sometimes as high as 50 percent, or half of what is grown. Particularly if it can economically be avoided, would be of great significance to growers and consumers alike.

Sharma (1987) reported that, post-harvest losses of vegetables in Bangladesh as tremendous as 43%. The average post-harvest loss rated by khan (1991) was 26%.

2.3. *Fusarium* dry rot of potato, radish and carrot

Association of yams, cassava, potato, wheat, cowpea, soybean, tomato and water melon for ground with *Rhizopus* spp., *Mucor* sp, *Curvularia* sp, *Aspergillus* sp,

Erwinia sp and *Fusarium* sp resulted in rots and storage deterioration of these crops around the world (Mahovic and Bartz, 2019; Mohammed, 2013; Enyiukwu *et al.*, 2014b).

Singha *et al.* (2016) reported that *Fusarium* dry rot (FDR) or potato dry rot was crucial potato disease worldwide and was caused by 13 species such as *Fusarium solani, F. sambucinum, F. avenaceum*, *F. graminearum, and F. oxysporum*, and causes post-harvest diseases of tuber rot and seed piece decay after planting. This disease can hamper crop establishment by affecting the developing potato sprouts and causing crop losses estimated to be up to 25%, while more than 60% of potato tubers can be infected during storage.

Charles *et al*. (2010) stated that *Aspergillus niger* and *Fusarium oxysporum*. *Fusarium* were found in cold storage along with *Aspergillus flavus* which was the most severe fungal species in cold storage.

Klosterman *et al.* (2009) reported that Possible causal agent of vascular wilt in red radish is *Verticillium dahliae*. Both of these fungi, *Fusarium* and *Verticillium*, cause wilts with identical symptoms and equal each other in isolate diversity and economic importance. *Verticillium dahliae*, like *F. oxysporum*, is a cosmopolitan, soilborne fungus infecting over 200 different species of plants, and has six known VCGs. Another parallel of *V. dahliae* to *F. oxysporum* as a wilt pathogen is that new wilt diseases are occurring on hosts that were once considered to not susceptible, probably when the plant was under stress (Bhat, 1999).

Fusarium dry rot of seed tubers can reduce the plant formation by killing the developing potato spouts. Also, it can enormously affect tuber quality and subsequently, can severely reduce its market value (Sadfi *et al.*, 2002; Peters *et al*., 2008).

Soil borne fungal diseases are still a major threat to potato cultivation in worldwide. Several important potato pathogens were isolated from soil borne inoculums. Potato plants were susceptible to destructive by various diseases such

as Black scurf caused by *Rhizoctonia solani* and dry rot caused by *Fusarium sambucinum* (Agrios, 1997; Yogen *et al*., 2006; Peters *et al*., 2008).

Wharton and Kirk (2007) reported that dry rot of seed tubers of potato can reduce crop enhancement by affecting the development of potato sprouts and causing crop losses up to 25%, while more than 60% of tubers can be infected during storage.

Salami (2007) identified *Rhizopus oryzae, Fusarium redolens, Butryodiplodia theobromae, Fusarium oxysporum, Aspergillus niger* and *Penicillium species* were responsible for postharvest dry rot of Irish potato tubers. This research was aimed at carrying out the mycological assessment of spoilt Irish potato tuber.

Owunbiko *et al.* (2005) reported that *Fusarium* dry rot of potatoes is one of the most economic problem in world wide. There are many species of *Fusarium* estimated to cause dry rot of potato Worldwide. The disease may cause significant losses of potatoes than any other-post harvest disease. Crop losses aspect to dry rot have been estimated to an average of 6 to 25%.

In 2004, *F. oxysporum* f. sp. *tuberosi* was also detected from potato plants as a serious disease wilting and tuber rotting agent (Daami-remadi and El Mahjoub 2004). It was revealed that dry rot caused by *F. oxysporum* f. sp. *tuberosi* is still common in Tunisia and that *F. graminearum* re-emerged as an increasingly important occurring dry rot pathogen in traditional as well as cold storages.

Yield losses indicated to dry rot in storage ranged from 6 to 25%, with up to 60% of tubers affected in some cases. Stevenson *et al.* (2001); Rahman (1969) and Kamal Uddin (1970) reported that 2 to 9% yield losses of potato tubers take place every year in each storage due to dry rot diseases. They also estimated that of all the diseases in cold storages, dry rot caused by *Fusarium caeruleum* (lib) sacc. is very common and causes most of the damage and spoilage, but no expanded work has been carried out to assess the loss due to dry rot in Bangladesh.

It was the first report of *Fusarium* wilt of radish caused by *F. oxysporum* f. sp. *raphani* in Washington, an important zone for radish seed production in the US (Schreiber *et al*., 1995). At the end of the growing season, the aggregate of *Fusarium* wilt and cabbage maggot injury resulted in a stand loss of about 90% in the stock seed crop, which was abandoned. Radish stock seed crops were measured to be three times the value of commercial radish seed crops

Hinman *et al*. (1997) given that *F. oxysporum* f. sp. *conglutinans* can be seedborne in Brassica spp. (Richardson,1990), and because of the potential for dissemination of seedborne pathogens, research is needed to determine whether *F. oxysporum* f. sp. *raphani* can be seedborne in radish.

Chelkowski (1989) and Secor and Salas (2001) estimated that dry rot of potato losses has been measured from 6% to 25%, and occasionally losses as high as 60% have been reported during long-term storage and transportation.

2.4. *Aspergillus* rot of carrot

Akhtari *et al.* (2016) reported that the pathogenicity test all the fungal isolates were pathogenic on radish roots. The percentage of pathogenicity was different from 35% to 87%. Among all the pathogenic fungi, *Aspergillus niger* was found to be comparatively more virulent than *Geotrichum candidum* and *Rhizopus oryzae*. The inoculated pathogens on pathogenicity test caused root rotting fungi. Their rotting percentage of root was 87%, 68% and 35%, respectively.

Samuel *et al.* (2016) reported that fungi were determined on the basis of their colonial and microscopic characteristics as *Penicillium digitatum, Rhizopus stolonifer, Aspergillus niger* and *Alternaria alternata. Escherichia coli* was widely isolated among the bacterial isolates (50%) while *Aspergillus niger* occurred more quickly than the other fungal species (40%). These organisms may have been identified to the carrots during growth, harvesting, handling, storage and distribution.

Hossain *et al.* (2014) reported that in case of seeds of root vegetables, six fungi were found namely *Fusarium* spp. (5.8%), *Alternaria* spp. (3.3%), *Penicillium* spp. (2%), *Aspergillus niger* (1.8%), *Aspergillus flavus* (1.7%) and *Curvularia* spp. (1.2%) Maximum incidence of *Alternaria* spp. was found in carrot (5.5%), but seeds of turnip were infected by *Alternaria* spp. In case of *Aspergillus niger*, the highest incidence was recorded in radish (3%), but no incidence in turnip. *Aspergillus flavus* (5%) and *Curvularia* spp. (3.5%) were shown only in radish. Maximum incidence of *Fusarium* spp. was recorded in radish (10.5%) and minimum in turnip (2.5%). The incidence of *Penicillium* spp. was high in turnip (3.5%), while low in carrot (1%). The highest total seed-borne fungal infection was recorded in radish (28%) followed by carrot (13.5%), while the lowest seed borne infections were recorded in seeds obtained from turnip seed (6%). In this case, most predominant fungus was *Fusarium* spp. (5.8%) followed by *Alternaria* spp. (3.3%), *Penicillum* spp. (2%), *Aspergillus niger* (1.8%), *Aspergillus flavus* (1.7%) and *Curvularia* spp. (1.2%).

Aspergillus sp were found to be the most common fungi which are responsible for post-harvest-loss of vegetable in South-Western Nigeria (Akintobi *et al.* 2011). Generally, pathogenic fungi are considered toxigenic or pathogenic (Al-Hindi *et al.*, 2011).

Al-Hindi *et al*. (2011) reported that *Aspergillus* spp. were found to be the most spoilage fungi that can be affected all types of fresh fruits and vegetables. And also found that *A. niger* was a commonly fungus on grapes (Chulze, 2006), apples (Oelofse, 2006) and orange (Gadgile and Chavan, 2010).

Sultana (2009) reported that *Aspergillus* spp., *Curvularia* spp., *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp. and *Botrytis* spp. found seed-borne fungi of vegetable.

2.5. Mold fungi of sweet potato

Penicillium sp*, Ceratocystis fimbriata, A. niger, A. fumigates, F. solani* and *Rhizopus stolonifer* were common pathogens aggregate with diseases in sweet potato (Agu *et al*., 2015).

Agu *et al.* (2015) reported that various types of fungi isolate increase different level of disease severity on sweet potato tuber. *Penicillium* sp*, Ceratocystis fimbriata, A. niger, A. fumigates*, *F. solani* and *Rhizopus stolonifer* were the most common pathogens related with diseases in sweet potato.

Olaitan (2012) found that *F. oxysporum, A. niger, Penicillum* sp*, R. stolonifer* and *Botryodiplodia theobroma* were pathogenic fungi of the sweet potato tuber.

Dov *et al.* (2010) found that *P. expansum* was the main causes of blue mold rot of vegetables and other fresh fruits, it was an example of the destructive post-harvest pathogens that cause a serious part of the economic losses during postharvest stage in which it leads pH to decreases from 3.95 to 4.31 in the healthy mesocarp to values ranging from 3.64 to 3.88 in the rotting tissue.

Ameinyo and Ataga (2006) reported that *F. oxysporum, R. stolonifer, Erwinia chrysentheli* and *C. fimbriata* are answerable for sweet potato diseases especially at the time of storage. Succeeding this, Olaitan (2012) found that *F. oxysporum, A. niger, Penicillum* sp*, R. stolonifer* and *Botryodiplodia theobroma* were pathogenic to the sweet potato tuber for disease development.

Several fungi have been involved in the spoilage of sweet potato. Onuegbu (2002) reported that *Penicillium* sp*. Ceratocystis fimbriata, Aspergillus niger, Diaporthe batatalis*, and *Aspergillus flavus* as fungi responsible for the post-harvest spoilage of sweet potato.

Onuegbu (2002) reported that *Penicillium* sp, *Ceratocystis fimbriata, Aspergillus niger, Diaporthe batatalis*, and *Aspergillus flavus* as fungi accountable for the post-harvest spoilage of sweet potato.

2.6. Sour rot of carrot

Bartz (2007); Bartz *et al. (*2012) indicated that *G. candidum* infections were most severe after wet harvest conditions, short drops in temperature due to rainfall, and improper post-harvest handling and storage procedures.

Yaghmour *et al. (*2012) reported that Sour rot occurs in a variety of geographic area and environments, making it difficult to know the exact in-field and postharvest conditions that influence disease incidence. Recently, high incidence of sour rot of fruit and vegetables and nectarine has increased in California, which were produced in semi-arid conditions and environmental moisture was not found to play a role in infection rate.

Sour rot of carrot caused by *G. candidum* has been estimated previously in Korea (Kim *et al*., 2011).

Fatima *et al*. (2009) and Saude *et al*. (2005) reported that sour rot on vegetables like oriental melon, tomato, cherry tomato, cucumber, potato, pumpkin and carrot caused by *G. candidum. G. candidum were* identified wide host range and strong pathogenicity against carrot, cucumber, tomato and pumpkin. Occurrence in this disease on several root vegetables indicate that *G. candidum* can be a serious threat to stable production of fresh vegetable. Postharvest decay of lime fruits caused by *Geotrichum candidum* (sour rot) *Penicillium digitatum* (Green mold) and *P. italicum* (blue mold) were the most important factors affecting harvested lime fruits during handling, transportation, exportation and storage (Morris,1982).

Postharvest decay of vegetables caused by *Geotrichum candidum* (sour rot); *Penicillium digitatum* (Green mold) and *P. italicum* (blue mold) were the most important factors affecting root vegetables during handling, transportation, exportation and storage (Morris, 1982; Brown and Eckert, 1988 and Morsy and Abdel-Kader, 1994).

It was also believed that sour rot infections were more important during the growing season, thus growers have converted their cultivar selection, planting date, and harvest time based on this (personal observation). In certain years, *G. candidum* can reach "epidemic" status and result in measured 25% loss of packaged fruit (Richard Davis, Lipman Produce, personal comm.). Infected vegetables may remain asymptomatic for a number of days during processing and de-greening, presenting symptoms during storage and throughout distribution (Bartz, 2007; Coates and Johnson, 1997).

A survey revealed that 12.5% of asymptomatic vegetables in grocery stores were infected by *Geotrichum* species (Tournas, 2005) and another study found 1 out of 6 vegetable infected with *G. candidum* also contained Salmonella typhimurium (Wells and Butterfield, 1999).

Mishra and Rath (1986) reported that *Geotrichum candidum* storage rot was a minor disease was occurred on injured or physiologically week roots, actually these were found during late winter or autumn.

2.7. *Rhizopus* rot of sweet potato

Agu *et al*. (2015) stated that fungi initiate a menace in storage rots of many agricultural commodities. It was noticed that *Aspergillus niger* and *Rhizopus stolonifer* were the most often isolated fungus from spoilt sweet potato tubers in Anmabra State. The results of pathogenicity test designated that fungi induce different level of decay with *Aspergillus niger* the most virulent fungus.

According to a report of Charles Tortoe *et al.* (2010), *Aspergillus flavus* was the most devastating fungal species during postharvest storage condition of sweet potato followed by *Aspergillus niger, Rhizopus stolonifer, Trichoderma viride, Fusarium oxysporum, Penicillium digitatum*, *Cladosporium herbarum*, and *Aspergillus ochraceus*

Ray *et al.* (2010) reported that *Rhizopus* spp. Because soft rot is wide spread all over the countries where Sweet potato are grown. Different species of Rhizopus have been reported to cause rotting i.e. *R. stolonifera, R. oryzae*, and *R. nigricans*. diseased roots are usually decayed totally by a rapidly developing soft and watery rot. Under humid conditions, the root of sweet potatoes is shriveled, at places where the skin ruptures there is a copious development of coarse white mold bearing characteristics globular spore head (sporangia). At first the sporangia are black then turn black as they mature and entire mycelium remain grey.

Amienyo (2007) reported that *Rhizopus stolonifer* was the most regularly isolated fungus from spoilt sweet potato tubers in South western, Nigeria.

Salami (2007) reported that *Rhizopus oryzae* as the most virulent among the fungi correlated with storage rots of sweet potato tubers in South western, Nigeria

Oyewale (2006) reported fungi that were related with post-harvest fungal rot of sweet potato and they include *Motierella ramanniana, Rhizopus stolonifer, Mucor pusiluss, Botrytis cinerea, Erysiphe polygoni* and *Aspergillus flavus*. During the post-harvest storage of sweet potato, *Aspergillus flavus* was the most prominent fungal species followed by *Aspergillus niger*, *Rhizopus stolonifer*, *Trichoderma viride, Fusarium oxysporum, Penicillium digitatum*, *Cladosporium herbarum* and *Aspergillus ochraceu*

Agrios (1997) reported that *Rhizopus* soft rot has been affected fruits and vegetables throughout the world on harvested fleshy organs of vegetables, fruits and flower crops during preservation, transit, and marketing of these products all over the world.

Snowdor (1991) and Oyewale (2006) reported that fungi related with postharvest fungal rot to include *Motierella ramanniana, Rhizopus stolonifer, Muco pusiluss, Botrytis cinerea, Erysiphe polygoni* and *A. flavus*. These fungi create local discoloration of the surrounding tissues of infected tubers resulting in changes in

appearance, deterioration of texture and possibly flavour or taste. Postharvest losses due to fungi, reduction in the market value and misfortune to farmers. Fungicides such as Dichloronitroanline are used to safe tubers against *Rhizopus* wet rot (Clark and Moyer, 1988).

Clark and Hoy (1994) Onuegbu (2002) reported that the fungi identify with rotting of sweet potato include*, Fusarium oxysporum, Ceretocysts fimbriata, Fusarim solani, Monilochaetes infuscans*, *Macrophomina phaseolina* and *Botryodiplodia theobromae* implicated *Penicillium* sp, *Cerocystis fimbriata, Diaporthe batatalis, Aspergillus flavus* and *Aspergillus niger,* as fungi responsible for decay of sweet potato tuber.

Ceponis *et al*. (1974) estimated that 2% of sweet potatoes were reduce to *Rhizopus* soft rot by the time they reach the retail market, but losses can be spread since only a few rotted roots can cause package to be rejected. *R. stolonifer* requires a wound for infection and to cause disease. Spores of *R. stolonifer* are familiar in the environment, and in the presence of a suitable wound, they germinate to produce mycelia and pectolytic enzymes which cause a soft, watery rot. Whiskery, white mycelium that becomes covered with powdery, black sporangiospores is a characteristic sign of *R. stolonifer*. An entire root can become fully rotted in 3 to 4 days after roots leave the packinghouse in good condition to arrive at their destination completely decayed.

2.8. Culture and preservation

Skovgaard *et al.* (2003) and Leslie *et al.* (2006) reported that *Fusarium* isolates were grown on PDA and SNA in Petri plates at 22-24°C with 12 h light/12 h darkness for 10 days. Species of the *Fusarium* isolates were identified based on their colour character and growth pattern of colonies on PDA and morphology of macro and micro-conidia and chlamydospores on SNA.

Babadoost *et al.* (2004) reported that *Fusarium* inoculum, a conidial suspension was prepared from 7- to 10-day-old Fusarium cultures on PDA plates by adding 10 ml SDW to each plate and dislodging the conidia using a soft brush. The concentration of conidia (micro- and 25 macro-conidia) were made to 105 conidia per 1 ml using a haemocytometer. Sticky inoculum suspension was prepared for Fusarium inoculum was prepared in a sterilized 0.5% agar solution (Atibalentja and Eastburn, 1998; Babadoost *et al*., 2004).

PDA is one of the most common and valuable used culture media because of its simple formulation and its ability to mycelial growth of a wide range of fungi. Several workers stated that PDA to be the best media for mycelial growth and preservation for future use. (Xu *et al*., 1984; Maheshwari *et al*., 1999; Saha *et al.*, 2008)

2.9. Effect of temperature on isolated pathogen growth

Knowles and Plissey (2008) reported that 90-95% relative humidity has been maintained throughout the storage period. Proper air circulation within the potato pile should be influenced to avoid build-up of carbon dioxide, which has been connected with development of soft rots. In the cases of tubers approaching to storage having 1-2% of disease symptoms, then curing temperatures call to be lowered below 10℃ as soon as the tubers are put in preserve.

Danladi (2000) reported that during post-harvest environment, Relative Humidity (RH) is important as like as temperature. The effects of temperature and relative humidity both were interrelated because the capacity of air to hold moisture varies with the temperature fluctuation. The aeration in the storage containers has its bearing on RH and hence indirectly on disease development. The effects of high RH and high temperature are closely interrelated for decay of many fruits and vegetables, relative humidity near saturation results in lower decay losses only if the temperature was near O°C. The relative humidity below

90% cannot permit micro-organisms to grow on the surface of fruits and vegetables.

Daami-Remadi (1996) found that growth of mycelium different fungi such as: *F. culmorum, F. graminearum* and *F. sambucinum* was optimum at 20-25°C whereas the highest sporulation occurred at temperatures between 25 and 30°C. On the other hand, for *F. solani* var. *coeruleum*, the optimum growth and sporulation were 25-30 and 20-25°C, respectively.

Daami-Remadi *et al.* (2006e) reported that the offensive of *Fusarium* spp. was significantly influenced by storage temperature. *Fusarium species* possessed two thermal peaks of hostility on potato cv. 'Spunta' tubers: the first one situated at 10-15°C and the second at higher temperatures (30-35°C). At temperatures Below 25°C temperature, *F. sambucinum* and *F. graminearum* increased the severest dry rot symptoms while *F. solani* was the most aggressive at temperatures above 30°C.

Sharma and Razak (2003) reported that optimum growth temperatures for most of the fungi was found to fall between 25˚C to 30˚C and above 40˚C the growth was poor and in some cases mortality may occur.

CHAPTER III

MATERIALS AND METHODS

MATERIALS:

A. For sample collection:

- ✓ Sample collection bags (shopping bag, polythene, net bag and paper bag etc.)
- ✓ Voucher
- ✓ Data recording books.

B. For lab experiment:

- ✓ **Media preparation:** Potato, carrot, dextrose powder, agar powder, lactic acid, Tissue paper, conical flask, measuring cylinder, test tubes and cotton plug etc.
- ✓ **Machinery tools:** Laminar Air Flow cabinet**,** autoclave, oven, balance, stove, microscope and camera or digital mobile phone etc.
- ✓ **Sterilizer:** Alcohol or Hexi sol, tissue and mask etc.
- ✓ **Slide preparation:** Slide, cover slip, cotton blue, glycerin and nail polish etc.
- ✓ **Pathogen transfer:** Block cutter, forceps and needle.
- ✓ **Others:** Water, boiling pot, Bunsen burner, gas light, knife, filler, bowl, marker pen, cellophane, parafilm/aluminum foil, glass rod, test tube holder, Skoch tape, forceps, needle, blade scissor and record book etc.

METHODS

3.1. Experimental site

The experimental samples were collected from different markets in Dhaka city and experiment was conducted in Plant Pathology Laboratory, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh.

3.2. Experimental period

The experiment was carried out during the period August 2019 to March 2020.

3.3. Collection and preservation of samples

Root and tuber like, potato, sweet potato, carrot, and radish which showing the deterioration and rotting like samples were collected from different markets/ shops of Dhaka city. Samples were stored at 4°C until the identification and isolation were made within 48 hours.

3.4. Isolation, purification and preservation of pathogens

Blotter paper method and PDA media were followed for isolation of fungi from infected potato, carrot, sweet potato, radish. Infected part of root samples was first washed in tap water to make free from sand and soils. The infected part along with healthy part of the root sample were cut into small pieces 0.5-1.0 (cm) and then surface sterilized with 70% ethanol 2-3 minutes. Then the root sample pieces 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 (Plate 1) via standard blotter protocol (ISTA, 2003). The plates were incubated for 7 days at 25±1℃ and under 12h/light: 12h dark. After incubation, fungi developed on each root and tuber samples, were isolated as pure cultures on PDA media. A bit of mycelia was taken with the help of sterile needle the mycelia grew over the moisten filter paper and transferred to the media on PDA (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 ± 1^0 C. After

incubation the fungi that grew on PDA were identified following the key outlined by (Leslie and Summerell, 2006, Kibria *et al*., 2016, Gautam *et al.,* 2012). 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 4^0C in the refrigerator as stock culture for future study.

3.4.1. Identification of causal organism

After incubation the pathogen was examined under compound microscope for identification of the pathogen. Temporary slides of diseased tissues were prepared and observed under light microscope. Fungi were identified according to reference of Barnett and Hunter (1998), Dix & Webster (1995), Domsch *et al*. (1980), Dugon (2006), Erwin & Ribeiro, (1996) and Nelson *et al*. (1983). 10X-40X magnification were used for the examination of pathogenic isolates under microscope.

3.4.2. Slants preservation of isolates

PDA media was poured in test tubes and autoclaved at 121℃ for 15 min leads 15psi. After autoclaved media was permitted to solidify in test tubes at an angel of 65℃. A loopful inoculums of each isolates were transferred to PDA slants, keep the tubes in an incubator at 25ºC±1 for 7 days and stored at 4ºC as pure culture in refrigerator for future use. (Iqbal *et al*., 2017).

Figure 1. Laboratory work

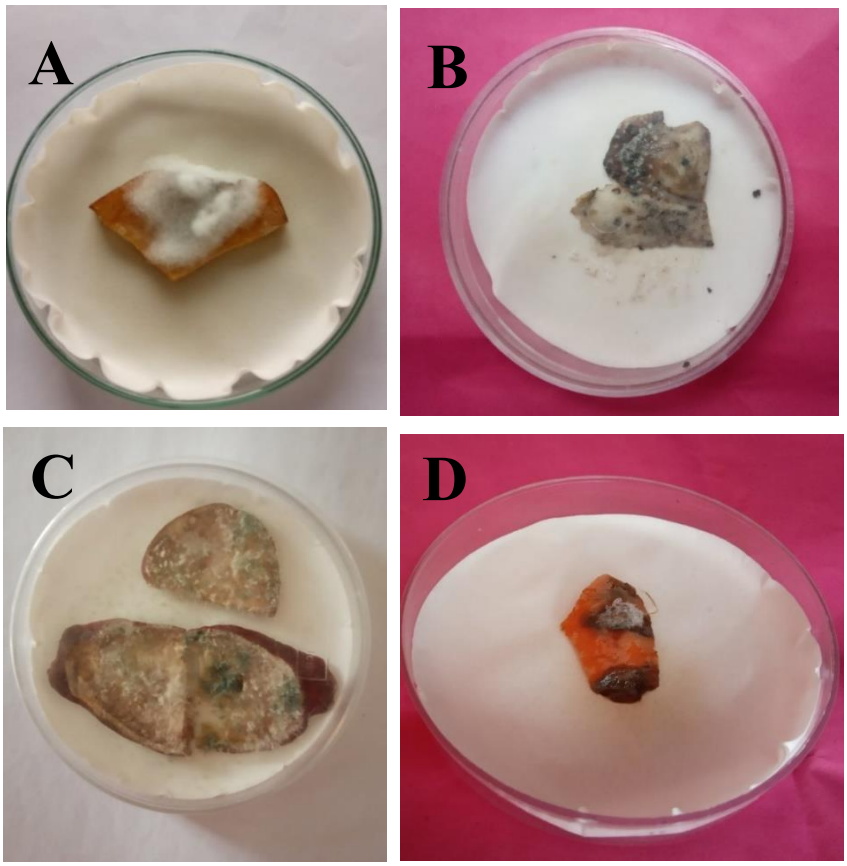

Plate 1. Growth of fungi on moisten filter paper (moist chamber showing the fungal growth over the slice of the infected roots and tubers).

A. Infected potato slice

B. Infected radish slice

C. Infected sweet potato slice

D. Infected carrot slice

3.5. Pathogenicity test

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

3.6. Effect of culture media and temperature on growth of fungi

Under aseptic condition, each pure isolate was transferred to PDA and CDA media 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 day up to seven days after inoculation.

3.6.1. Preparation of culture media

3.6.1. 1. Preparation of PDA medium

Potato dextrose agar (PDA) medium was prepared as described by Sagar *et al*. (2018). 200 g of sliced peeled potatoes extract, 20 g agar and 20 g dextrose, 1000 ml distilled water were taken in a conical flask and mix well it properly for the preparation of 1L PDA medium. Prepared media were Sterilized by autoclaving at 20 min 121ºC under 15 PSI pressure. At the same time Petri dishes were sterilized in oven for 90 minutes unto 55/60℃. After conclusion of autoclaving and oven sterilization the media, Petri dishes and other necessaries were transferred in Laminar 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 Petri dishes aseptically and left in laminar air flow for solidifying properly.

Potato Dextrose Agar (PDA), 1000ml	
Component	Amount
Potato Extract	200 g
Dextrose	20.0 g
Agar	20.0 g
dH_2O	1500 ml

3.6.1.2. Preparation of CDA medium

Carrot dextrose agar (CDA) was prepared as described by Sagar *et al*. (2018). 200g peeled carrot extract, 1000 ml distilled water, 20 g agar, 20 g 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℃. After conclusion 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 in laminar air flow for solidifying properly.

Carrot Dextrose Agar (CDA), 1000ml	
Component	Amount
Carrot Extract	200 g
Dextrose	20.0 g
Agar	20.0 g
dH_2O	1500 ml

3.7. Radial mycelial growth assay

A wire loop was used to take inoculums from the rotten roots and tubers vegetable samples and inoculated in already prepared PDA and CDA media, using different Petri dishes for each of the 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 Robert *et al.* (1988). Then transferred the pure cultures onto petri-dishes for the radial growth assay. The assay was carried out at 20℃, 25℃ and 30℃ at every 12 h interval (twice daily) for eight days. Measurement of growth was done using a centi meter-scale (that is from the center of the Petri-dish to the circumference of the isolated pathogen (Palacios-Cabrera *et al*., 2005).

3.8. Study on cultural characteristics of different isolated post-harvest pathogens

All isolates were segregated from four different roots and tubers (carrot, radish, potato and sweet potato) were cultured on PDA and CDA media at 25ºC and colony characters such as surface color, subsurface color, colony shape, colony structure and texture were studied carefully.

3.9. 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 AND DISCUSSION

4.1. Visual symptoms of collected samples

4.1.1. *Fusarium* dry rot of potato

Infection started at the infected side. Once infection occurred, it slowly enlarges in all directions. wrinkled and shrink aged area were found over the infected area. The skin sometimes in concentric ring also found due to the fungus drying out the flesh of the tuber. Internally, infected area was light brown to whitish as the fungus kills the cells of the tuber. Internal cavities created by dry rot infections generally contain fungal mycelium of various colours (Plate 2: A, B, C, D and E).

4.1.2. Identification of *Fusarium* sp isolated from potato

Characteristics of *Fusarium* sp on PDA and CDA medium

Cultures of *Fusarium* sp on both PDA and CDA medium were white in colour. Mycelium of this species were sparse densely, soft and fluffy. No pigmentation was found on agar medium. (Plate 3: A, B, C and D)

Characteristics of macro conidia (Plate 3: E)

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

Characteristics of micro conidia (plate 3: F)

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

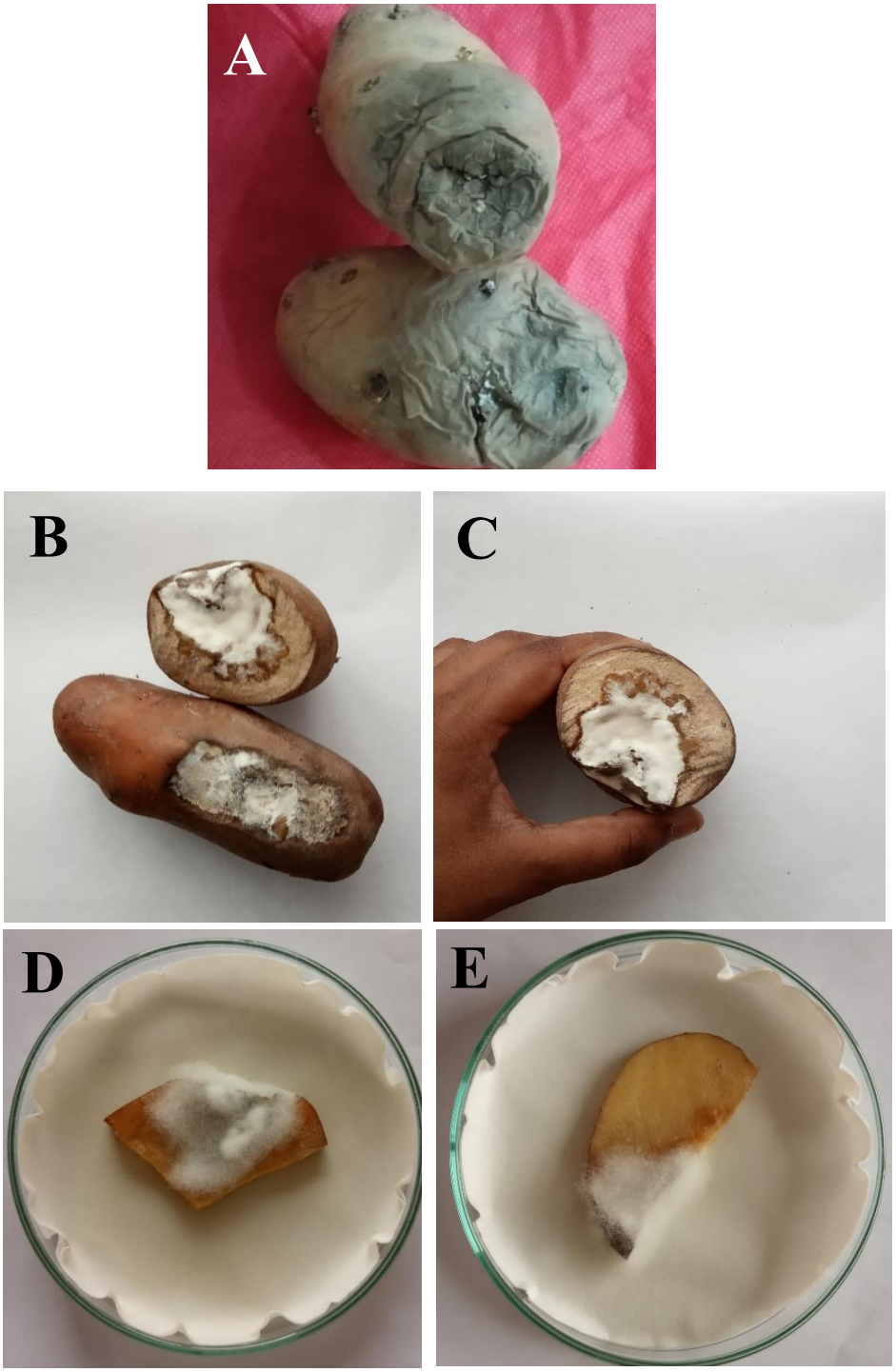

Plate 2. ***Fusarium*** **dry rot of potato on infected potato tuber (A, B and C). Infected potato flesh over the moisten filter paper (D and E) in moist chamber showing the fungal growth over the slice of the infected tuber.**

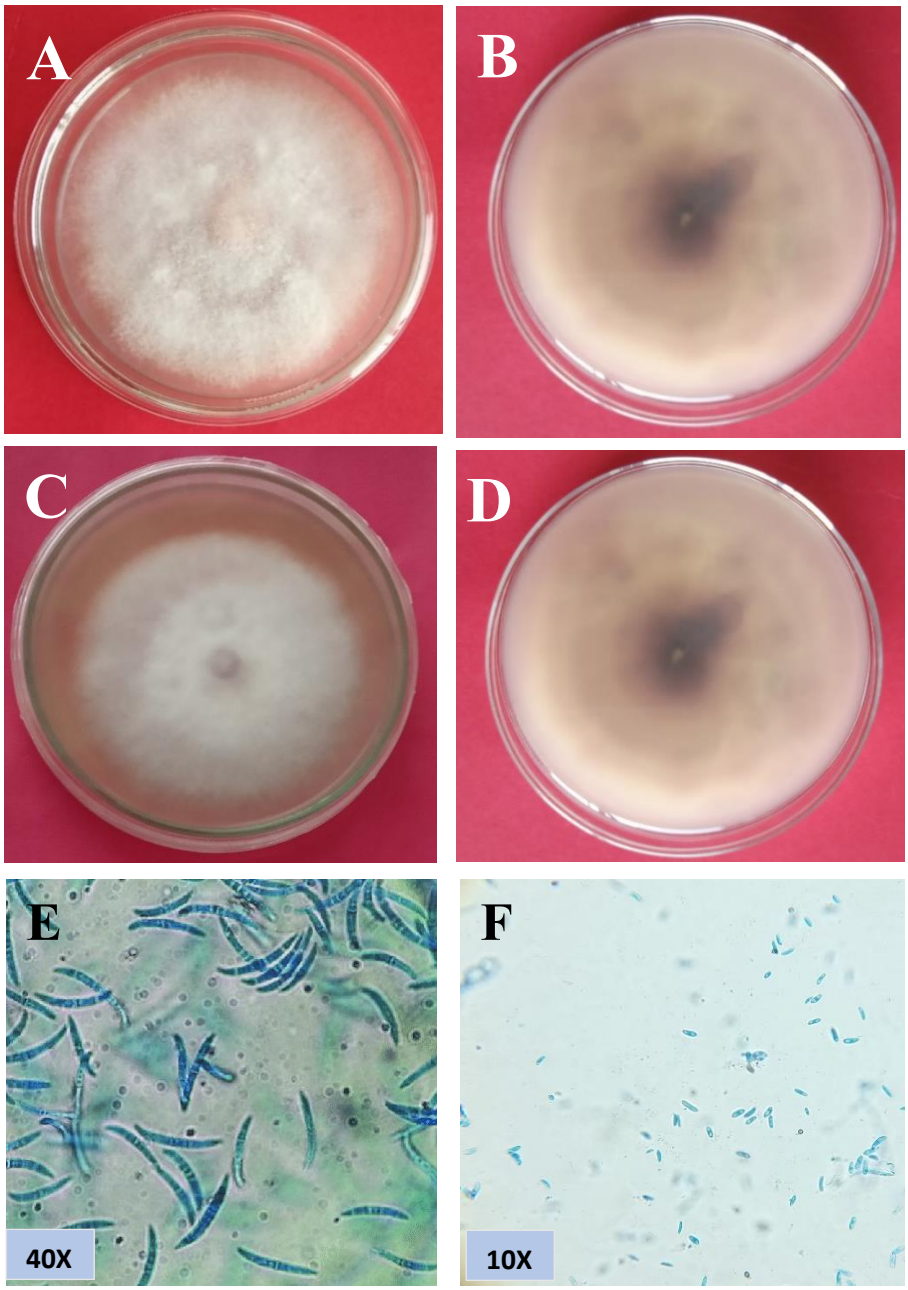

Plate 3. Cultural and microscopic view (at 10x and 40X) of *Fusarium* sp

A. **Surface side on PDA**
B. **Subsurface side on PDA**
C. **Surface side on CDA**
D. **Subsurface side on CDA**
E. **Macroconidia of *Fusarium* sp**
F. **Microconidia of *Fusarium* sp**

4.1.3. Sooty rot/ black mold of carrot

On the root, superficial, irregular black lesions were developed. The discoloration on the root, caused by masses of dark brown to chlamydospore, was limited the skin. Soft and watery rot with black spore masses were found over the infected root. (Plate 4: A, B)

4.1.4. Identification of *Aspergillus niger* isolated from black mold infected carrot

Characteristics of *Aspergillus niger* on PDA and CDA medium

Aspergillus niger on PDA were initially white, quickly becoming black with conidial production. Reverse was pale yellow and white in colour. Spores were gradually black in colour and the media was covered by this spore. (Plate 5: A, B, C and D)

Characteristics of *Aspergillus niger* under microscope

Cultural morphology (Plate 5: A, B, C and D)

At first surface was white than turn brown or black. Texture velvety and cottony were present. Reverse was white, golden or brown.

Microscopic morphology (Plate 5: E, F)

Hyphae were septate. Unbranched conidiophore arises from foot cells. Colour less conidiophore and spores were present. Conidiophores was long and produce sporangiospores with large, round head.

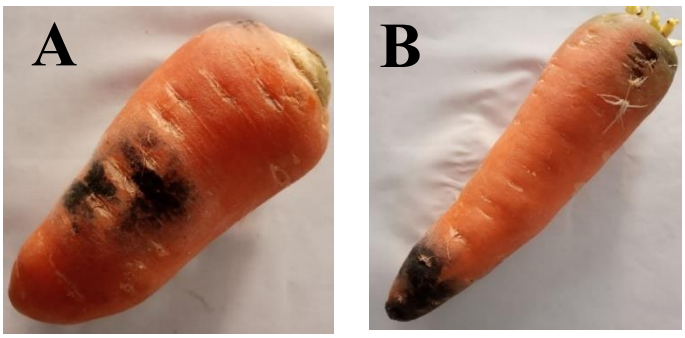

Plate 4. Sooty rot/ Black rot (A and B) of carrot

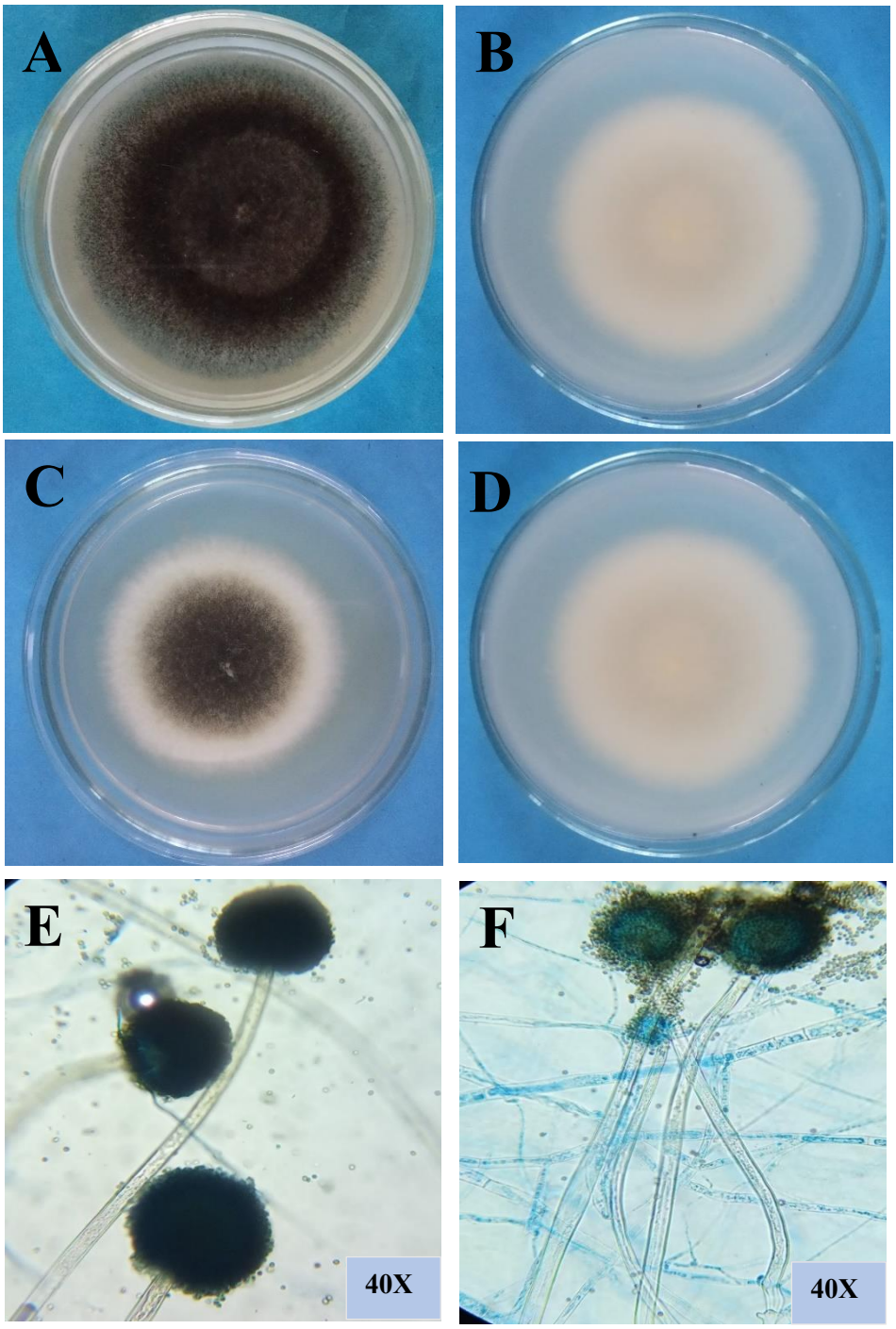

Plate 5. Cultural and microscopic view (40X) of *Aspergillus niger*

A. **Surface side on PDA**
B. **Subsurface side on PDA**
C. **Surface side on CDA**
D. **Subsurface side on CDA**
E. **Microscopic view of *Aspergillus niger***
F. **Microscopic view of *Aspergillus niger***

4.1.5. Sour rot of carrot

Soft, watery, colourless decay were found on carrot roots. Decay area covered with dull, white spores of pathogen. Vinegar like odor was developed on the infected part of carrot root and the tissue of the root became dull. (Plate 6: A, B)

4.1.6. Identification of *Geotrichum candidum* isolated from sour rot disease infected carrot

Characteristics of *Geotrichum candidum* on PDA and CDA medium

Colonies were fast growing, flat, white to cream, dry and finely leather like whose surface has been made slightly rough and soft but not shiny. (Plate 7: A, B, C and D)

Characteristics of *Geotrichum candidum* under microscope

Hyphae were hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled. Conidia were cylindrical shape or barrel shape. Conidiophore were not produced. (Plate 7: E, F)

Plate 6. Sour rot (A and B) of carrot

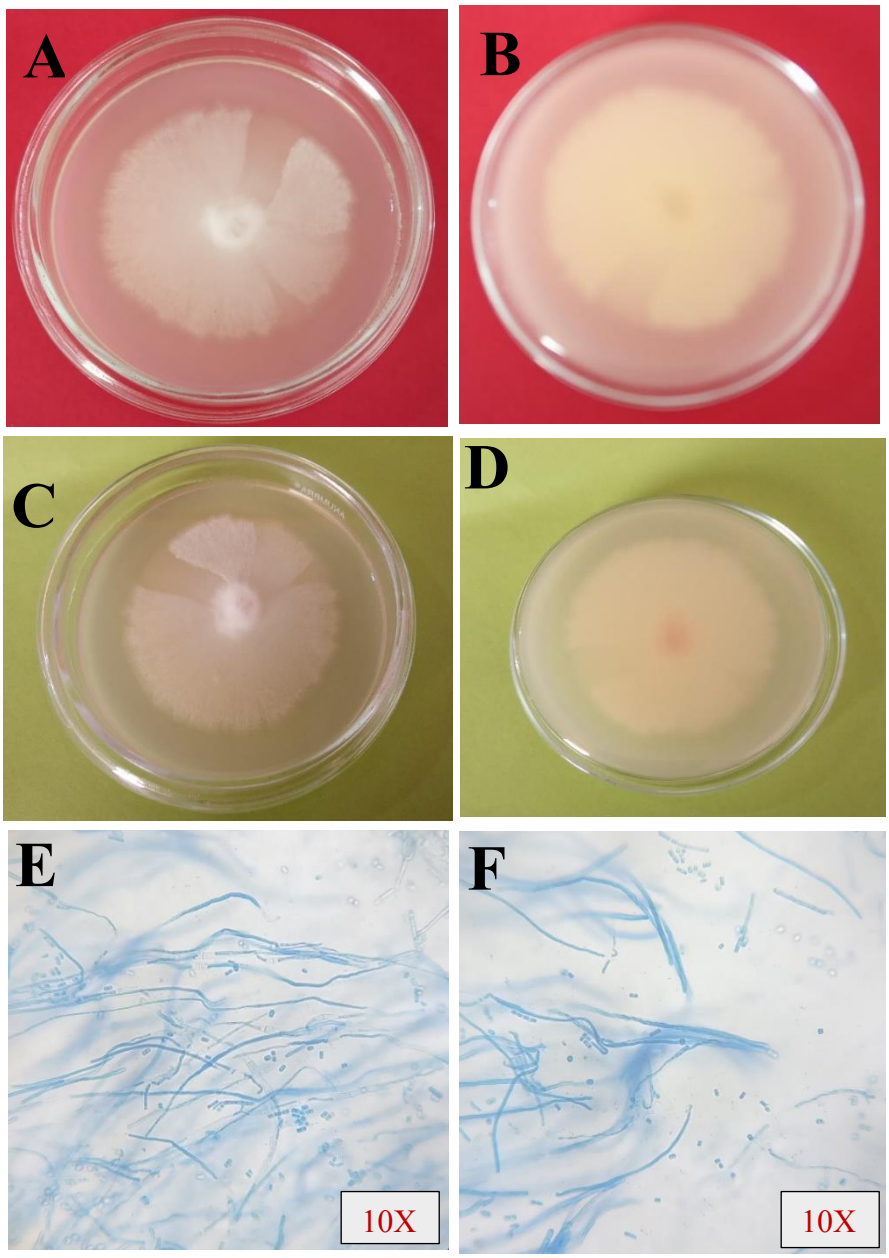

Plate 7. Cultural and microscopic view (10X) of *Geotrichum candidum*

A. Surface side on PDA

B. Subsurface side on PDA

C. Surface side on CDA

D. Subsurface side on CDA

E. Microscopic view of *Geotrichum candidum*

F. Microscopic view of *Geotrichum candidum*

4.1.7. *Fusarium* dry rot of carrot

Symptoms included dry rot of the root as well as large, brown to dark brown, circular, sunken lesions on the stored roots. Regularly, abundant whitish mycelium was observed covering the surface of the colonized roots. Cottony aerial mycelium covered the infected root. (Plate 8: A, B)

4.1.8. Identification of *Fusarium solani* isolated from *Fusarium* rot disease infected carrot

Characteristics of *Fusarium solani* on PDA and CDA medium

Mycelia were floccose and abundant. Both PDA and CDA media colony formed with white aerial mycelia. No pigmentation found on the media. (Plate 9: A, B C and D)

Characteristics of macro and micro conidia (Plate 9: E)

General morphology under microscope were relatively wide, wide straight to slightly curved. Apical cell morphology was found blunt and rounded. Basal cell morphology was found straight to almost cylindrical. 3 to 5 septation with rounded ends was found. Size of conidia was larger. The size of conidia was smaller. 1 septation was present which was oval, ellipsoid and fusiform. Micro conidia were formed in round false headed with aerial mycelium.

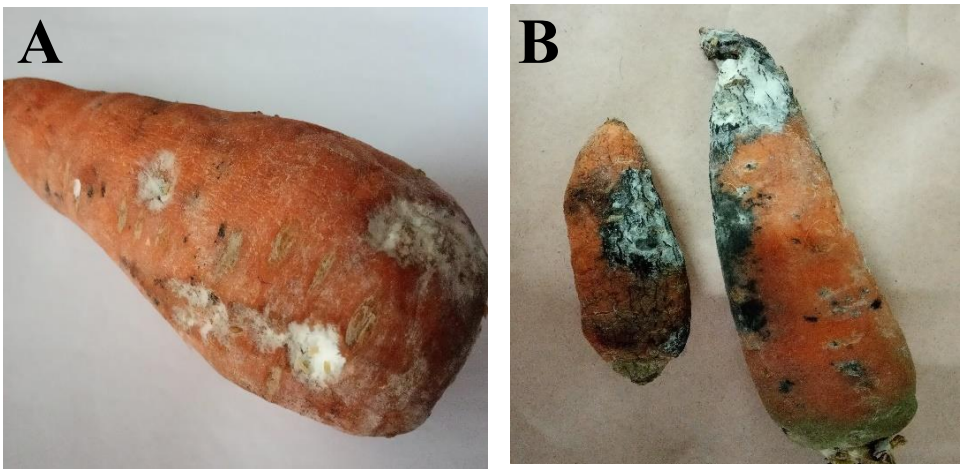

Plate 8. *Fusarium* dry rot (A and B) of carrot

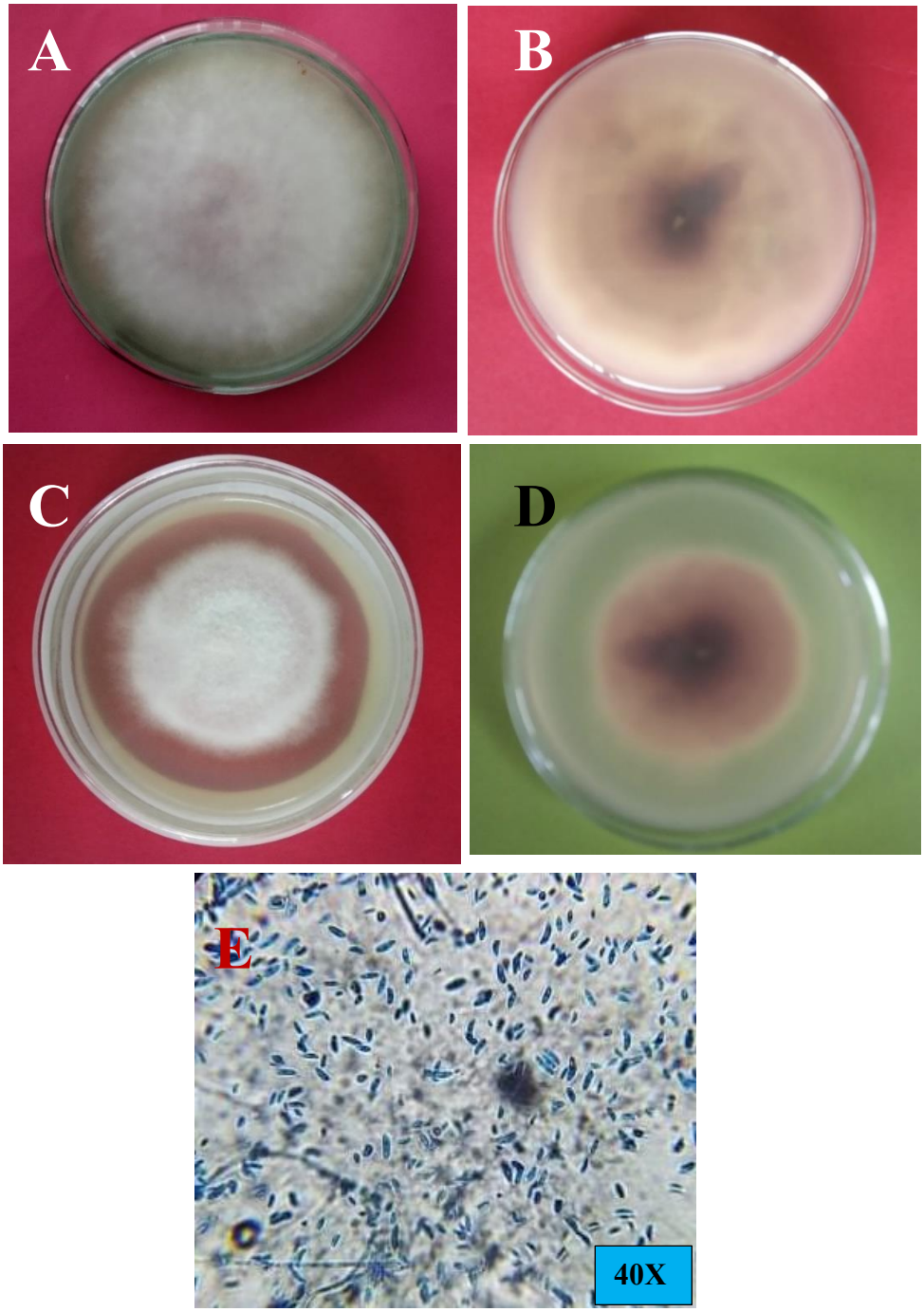

Plate 9. Cultural and microscopic view (40X) of *Fusarium solani* on PDA and CDA medium

A. **Surface side on PDA**
B. **Subsurface side on PDA**
C. **Surface side on CDA**
D. **Subsurface side on CDA**
E. **Macro and microconidia of *Fusarium solani***

4.1.9. *Fusarium* dry rot of radish

The rotted areas of radish were brown to black. Rot depressions was started in the surface of the infected portion on the radish root. (Plate 10: A, B)

4.1.10. Identification of *Fusarium oxysporum* isolated from *Fusarium* rot disease infected radish

Characteristics of *Fusarium oxysporum* on PDA and CDA medium

Cultures of *Fusarium oxysporum* on both PDA and CDA medium were pink in colour. Mycelium of this species was sparse densely. White colour mycelium slightly covered on the pink colour mycelium. Pinkish pigmentation was found on agar medium. (Plate 11: A, B, C and D)

Characteristics of macro and micro conidia (Plate 11: E)

Macro conidia observed under microscope were relatively wide, wide straight to slightly curved. Apical cell morphology was found blunt and thin. Basal cell morphology was observed straight to almost cylindrical. 3 septation with thin ended. Large sized conidia present. The size of conidia was smaller. 0 to 1 septation present, oval, ellipsoid and fusiform. Micro conidia are formed in round false heads.

Plate 10. *Fusarium* dry rot of radish (A). Infected radish flesh over the moisten filter paper (B) moist chamber showing the fungal growth over the slice of the infected root.

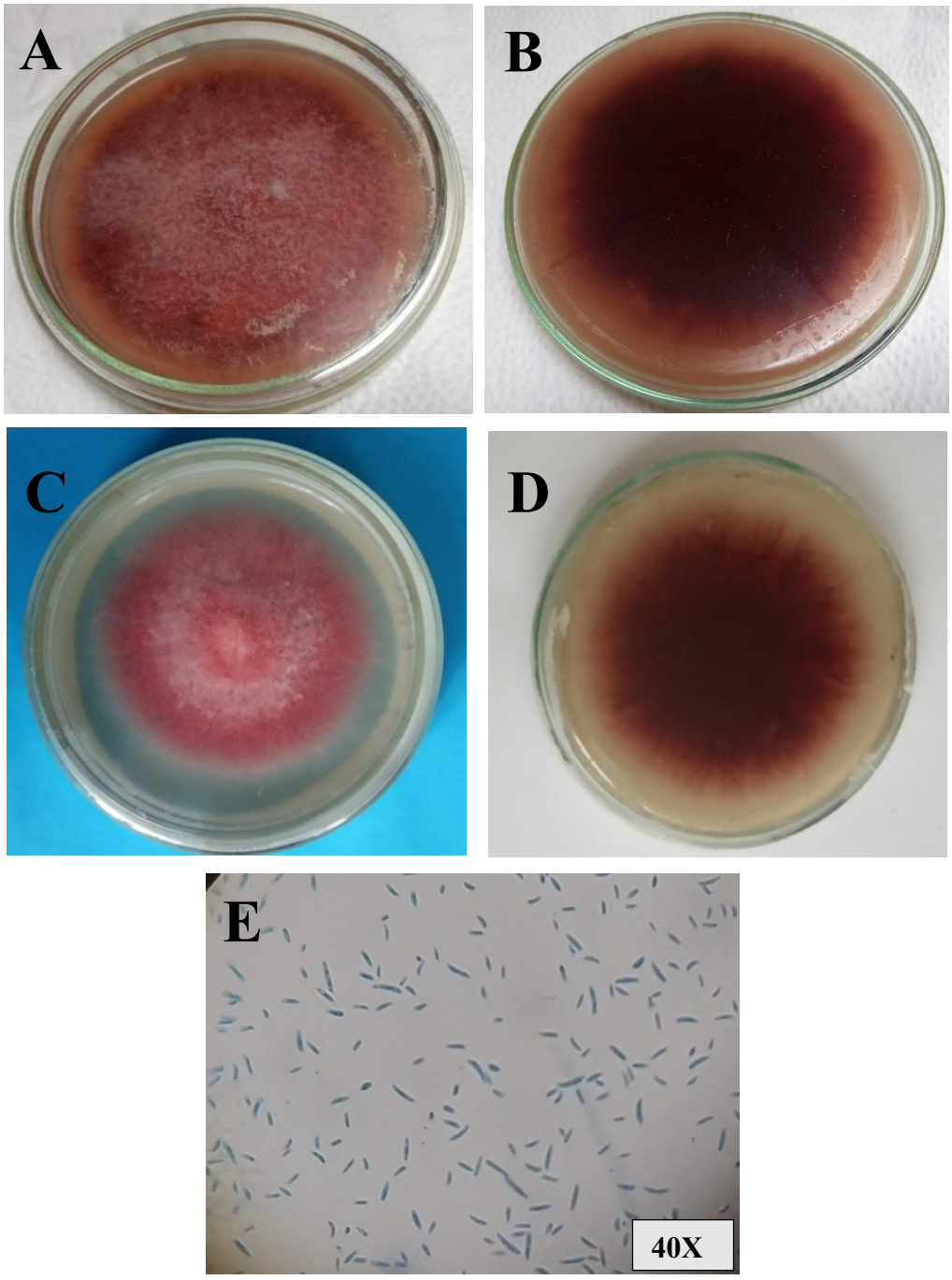

Plate 11. Cultural and microscopic view (40X) of *Fusarium oxysporum*

A. Surface side on PDA
B. Subsurface side on PDA
C. Surface side on CDA
D. Subsurface side on CDA
E. Macro and microconidia of *Fusarium oxysporum*

4.1.11. *Rhizopus* rot of sweet potato

Symptoms originate at the wounded area in the sweet potato and consist of a soft, watery rot that progresses quickly under favourable conditions and can result in full decay of an infected root within three days. White to grey fungal mycelium produce black sporangia (Plate 12: A, B).

4.1.12. Identification of *Rhizopus stolonifer* isolated from *Rhizopus* rot disease infected sweet potato

Characteristics of *Rhizopus stolonifer* on PDA and CDA medium

The texture was typically cotton- candy like both PDA and CDA media. From the front, the colour of the colony was white initially and then turns grey to yellowish brown in the time. After some days the colour of the media turns white to pale. (Plate 13: A, B)

Characteristics of *Rhizopus stolonifer* under microscope (Plate 13: C)

Rhizopus fungi were identified by a body of branching mycelium composed of three types of hyphae: stolon, rhizoids, unbranching sporangiophores. The black sporangia produced multinucleate spores for asexual reproduction. Hyphae broad, not scarcely septate. Rhizoids and stolon were present. Sporangiophores were brown in colour. Sporangia was round that were black colour spore. Apophysis was absent or scarcely apparent, sporangiophores ovoid.

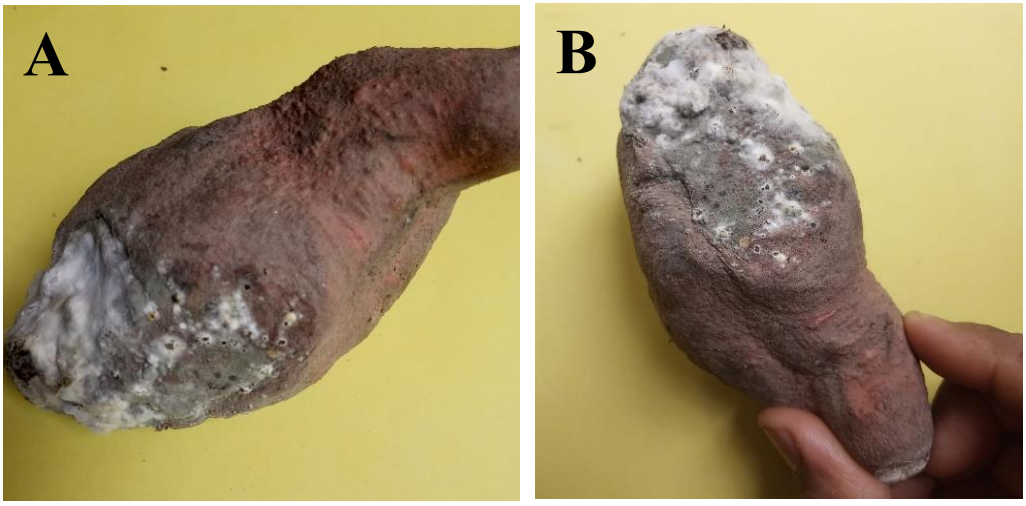

Plate 12. *Rhizopus* rot (A and B) of sweet potato.

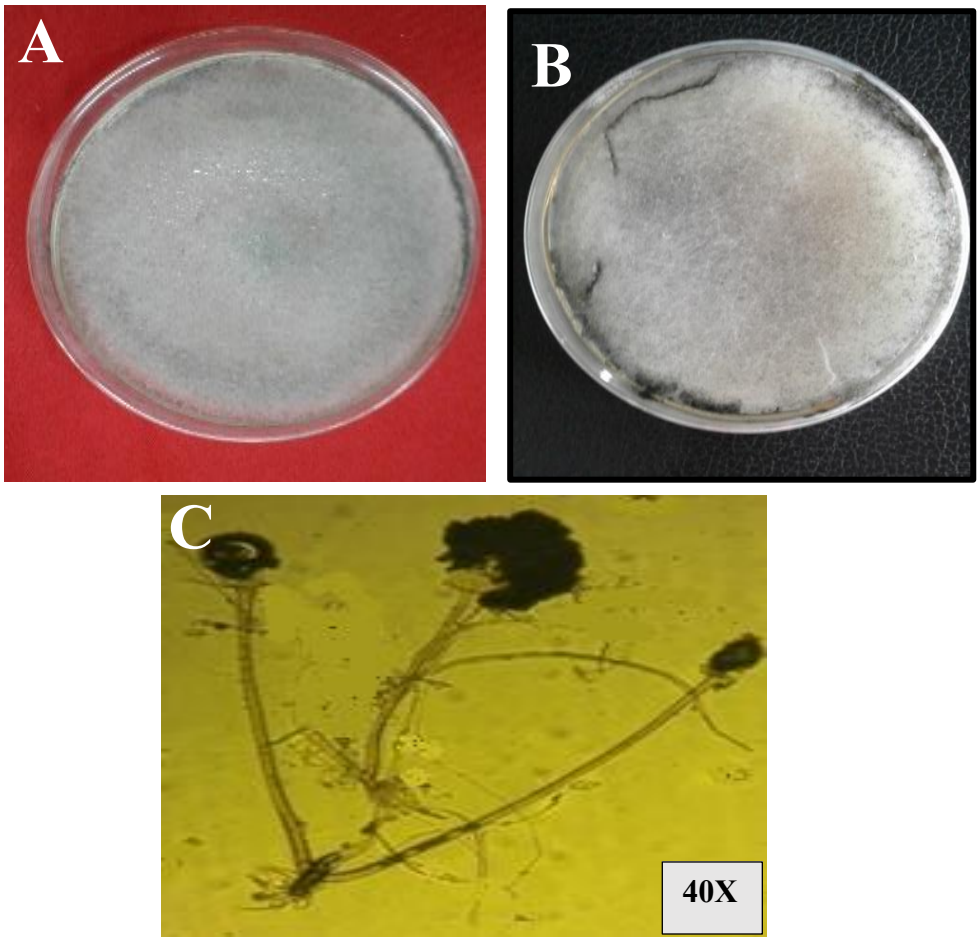

Plate 13. Cultural and microscopic view (40X) of *Rhizopus stolonifer* on PDA medium (A) *Rhizopus stolonifer* on CDA medium (B). Microscopic view of *Rhizopus stolonifer* (C).

4.1.13. Green mold of sweet potato

The root of the sweet potato appeared green colour. White colour mold forming in cavities within the areas of rot. The mold become whitish green flappy within few days. (Plate 14: A, B, C)

4.1.14. Identification of *Penicillium digitatum* isolated from green mold infected sweet potato

Characteristics of *Penicillium digitatum* on PDA and CDA medium

Fine white with green spore on the surface of PDA media. On the reverse side there was no colour formation. Concentric zone with heavy spore masses had seen on the PDA media. (Plate 15: A, B, C and D)

Characteristics of *Penicillium digitatum* under microscope (Plate 15: E)

Simple or branching structures that are slightly elongated and in clusters of flask-shapes known as phialides and are called conidiophores. Hyphae were septate, hyaline. Conidiophores were simple or branched. Phialides grouped in brush like clusters (penicillin) at the ends of the conidiophores. Conidia were unicellular, round to ovoid, hyaline or pigmented, rough walled or smooth in chains.

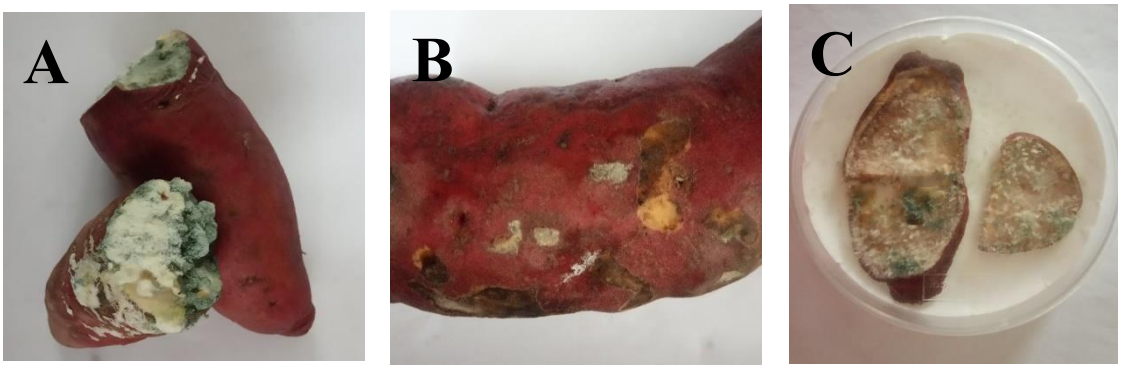

Plate 14. *Penicillium* rot of sweet potato in infected sweet potato tuber (A and B). Infected sweet potato flesh over the moisten filter paper (C) moist chamber showing the fungal growth over the slice of the infected tuber.

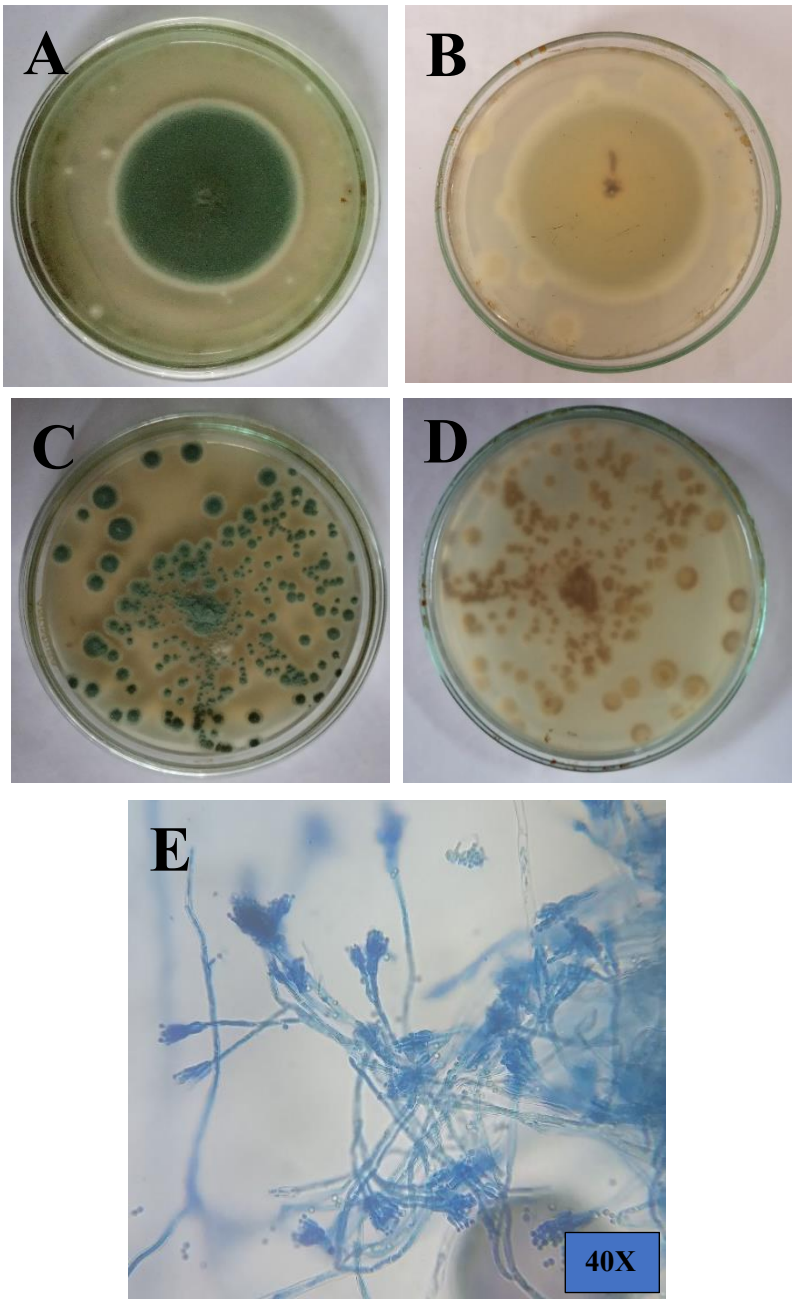

Plate 15. Cultural and microscopic view (40X) of *Penicillium digitatum*

A. Surface side on PDA

B. Subsurface side on PDA

C. Surface side on CDA

D. Subsurface side on CDA

E. Microscopic view of *Penicillium digitatum*

4.2. Pathogenicity test

The pathogen isolated from different roots and tubers samples was confirmed by the inoculation of fresh roots and tubers. After inoculation all of the isolates successfully showed typical symptoms in roots and tubers collected diseased samples. After completion of inoculation pathogenic growth were developed on fresh root and tuber crops within 7 days post inoculation at 25±1℃. Re-isolated pathogen showed similar morphological and microscopic character. When the morphological character was similar to the isolated pathogen it was confirmed the pathogenicity test successfully completed.

4.2.a. *Fusarium* dry rot of potato

Infected area found at the inoculation side. Once infection occurs, it slowly enlarges in all directions. Wrinkled and shrink aged area were found over the infected area. The skin sometimes in concentric ring also found due to the fungus drying out the flesh of the tuber this visual symptom closely related to the isolated tuber as clearly shown in figure 2.

4.2.b. Sooty rot of carrot

On the root, superficial, irregular black lesions were developed at the inoculation side then black spore mass covered on the whole root. Soft, watery rot with black spore masses were found over the infected root. It was similar to the isolated root as clearly shown in figure 3.

4.2.c. Sour rot of carrot

Soft, watery, colourless decay were found on inoculation side of the carrot. Decay area covered with dull, white spores of pathogen. Vinegar like odour may developed on the infected part of carrot root and the tissue of the root became dull which was similar to the isolates as shown in figure 4.

4.2.d. *Fusarium* dry rot of carrot

Symptoms included dry rot of the root as well as large, brown to dark brown, circular, sunken lesions on the stored roots. Whitish mycelium was observed covering the surface of the roots. Cottony with fluffy mycelium covered the infected root as found in figure 5.

4.2.e. *Fusarium* dry rot of radish

The rotted areas of radish were whitish to black at the inoculation side. Rot depressions in the surface of the infected portion on the radish root as shown in figure 6.

4.2.f. *Rhizopus* rot of sweet potato

Symptoms originate at an inoculation area in the sweet potato and consist of a soft, black spore mass that progresses quickly under favourable conditions and can result in full decay of an infected root within three days. White to grey fungal mycelium produce black sporangia similar to the isolates as shown in figure 7.

4.2.g. Green mold of sweet potato

The root of the sweet potato appeared green colour. White colour mold forming in cavities within the areas of rot. The mold become whitish blue fluffy within few days as clearly appears in figure 8.

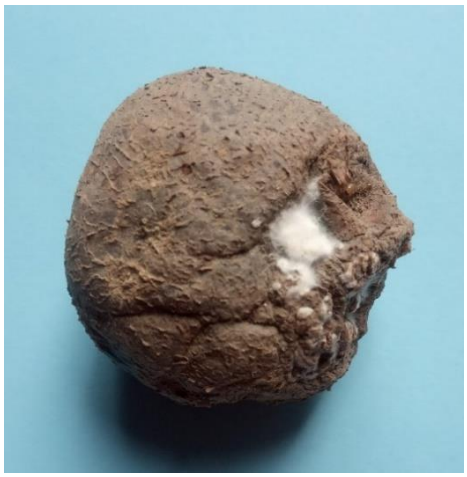

Figure 2. *Fusarium* dry rot of potato

Figure 3. Sooty rot/ Black rot of

Figure 4. Sour rot of carrot

Figure 5. *Fusarium* dry rot of carrot

Figure 6. *Fusarium* dry rot of radish

Figure 7. *Rhizopus* rot of sweet potato

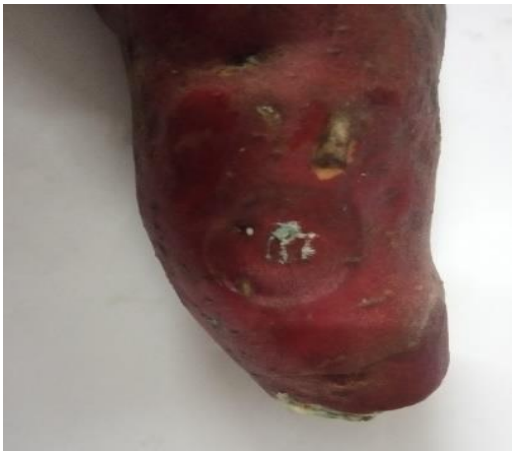

Figure 8. Green mold of sweet potato

4.3. Effect of different media on radial mycelial growth of different pathogens isolated from root and tuber crops

4.3.1. Radial mycelial growth of isolated pathogen on PDA media

The effect of different media on radial mycelial growth of different isolates of root crop was recorded at 2 DAI, 4 DAI, 6 DAI, 8 DAI respectively. Radial mycelial growth of different isolates of different species of fungi remarkably varied on PDA.

After two DAI, the maximum radial mycelial growth was noticed in CaAn (2.64 cm), followed by CaFs (2.58 cm) which was statistically similar. The minimum radial mycelial growth was RFs (1.56 cm). After four DAI and six DAI, of inoculation the maximum radial mycelial growth of were recorded in CaAn 3.02 cm, 3.43 cm, respectively and the minimum radial mycelial growth were in RFs 1.88 cm, 2.22 cm, respectively. After eight DAI days of inoculation the maximum radial mycelial growth of was measured in CaAn (4.06 cm), followed by CaFs (3.81 cm) whereas the minimum radial mycelial growth was recorded in RFs (2.54 cm) which was statistically different to CaGec (2.73 cm). Results are presented in table 1.

4.3.2. Radial mycelial growth of isolated pathogens on CDA medium

After two DAI, the maximum radial mycelial growth on CDA media was noticed in CaAn (2.40 cm), followed by CaFs (2.40 cm) which was statistically similar. The minimum radial mycelial growth was RFs (1.16 cm). After 4 DAI and 6 DAI, 6th day, of inoculation the maximum radial mycelial growth of were recorded in 2.67 cm, 2.96 cm, respectively and the minimum radial mycelial growth were 1.37 cm, 1.61 cm, respectively. After 8 DAI, the maximum radial mycelial growth of was measured in CaAn (3.34 cm), followed by CaFs (3.33 cm) which was statistically similar whereas the minimum radial mycelial growth was recorded in SpPs (1.82 cm) which was statistically similar to RFs (2.12 cm). Results are presented in table 2.

Table.1 Radial mycelial growth (cm) of different isolates of root and tuber crops at different days after incubation (DAI) on PDA medium.

Isolates	**2DAI**	**4DAI**	**6DAI**	**8DAI**
CaFs	**2.58 a**	2.96 ab	**3.36 a**	3.81 b
CaAn	**2.64 a**	**3.02 a**	**3.43 a**	**4.06 a**
CaGec	1.89 c	2.19 d	2.36 d	2.73 d
SpPs	1.79 c	2.12 d	2.32 d	2.80 d
SpRs	2.22 b	2.65 c	3.02 c	4.06 a
PFs	2.28 b	2.83 b	3.19 b	3.56 c
RFs	1.56 d	1.88 e	2.22 d	2.54 e
CV%	6.71	6.87	5.16	5.55

Ca = Carrot

P = Potato

R = Radish

Sp = Sweet potato

Fs= *Fusarium* sp

An= *Aspergillus* niger

Gec= *Geotrichum candidum*

Ps= *Penicillium digitatum*

Rs= *Rhizopus stolonifer*

PDA= Potato Dextrose Agar

2 DAI= Two days after incubation

4 DAI= Four days after incubation

6 DAI= Six days after incubation

8 DAI= Eight days after incubation

Table.2 Radial mycelial growth (cm) of different isolates of roots and tuber crops at different days after incubation (DAI) on CDA medium.

Isolates	2DAI	4DAI	6DAI	8DAI
CaFs	**2.40 a**	**2.62 a**	**2.96 a**	3.33 b
CaAn	**2.40 a**	**2.67 a**	**2.89 a**	3.34 b
CaGec	1.54 c	1.67 c	1.81 d	2.16 d
SpPs	1.19 d	1.42 d	1.61 e	1.82 e
SpRs	1.84 b	2.24 b	2.72 b	3.58 a
PFs	1.80 b	2.23 b	2.47 c	2.72 c
RFs	1.16 d	1.37 d	1.63 e	2.12 d
CV%	7.06	6.95	6.85	6.07

Ca = Carrot

P = Potato

R = Radish

Sp = Sweet potato

Fs= *Fusarium* sp

An= *Aspergillus niger*

Gec= *Geotrichum candidum*

Ps= *Penicillium digitatum*

Rs= *Rhizopus stolonifer*

CDA= Carrot Dextrose Agar

2 DAI= Two days after incubation

4 DAI= Four days after incubation

6 DAI= Six days after incubation

8 DAI= Eight days after incubation

4.4. Effect of different temperature on radial mycelial growth of different pathogens isolated from different root and tuber crops.

In case of radial mycelial growth significant variations were observed on different temperature (Figure 9). All isolates produced higher mycelial growth at 30˚C and lower mycelial growth was observed on 20℃ but 2 isolates of *Fusarium solani* from carrot and *Rhizopus stolonifer* from sweet potato were observed the highest radial mycelial growth at 25℃.

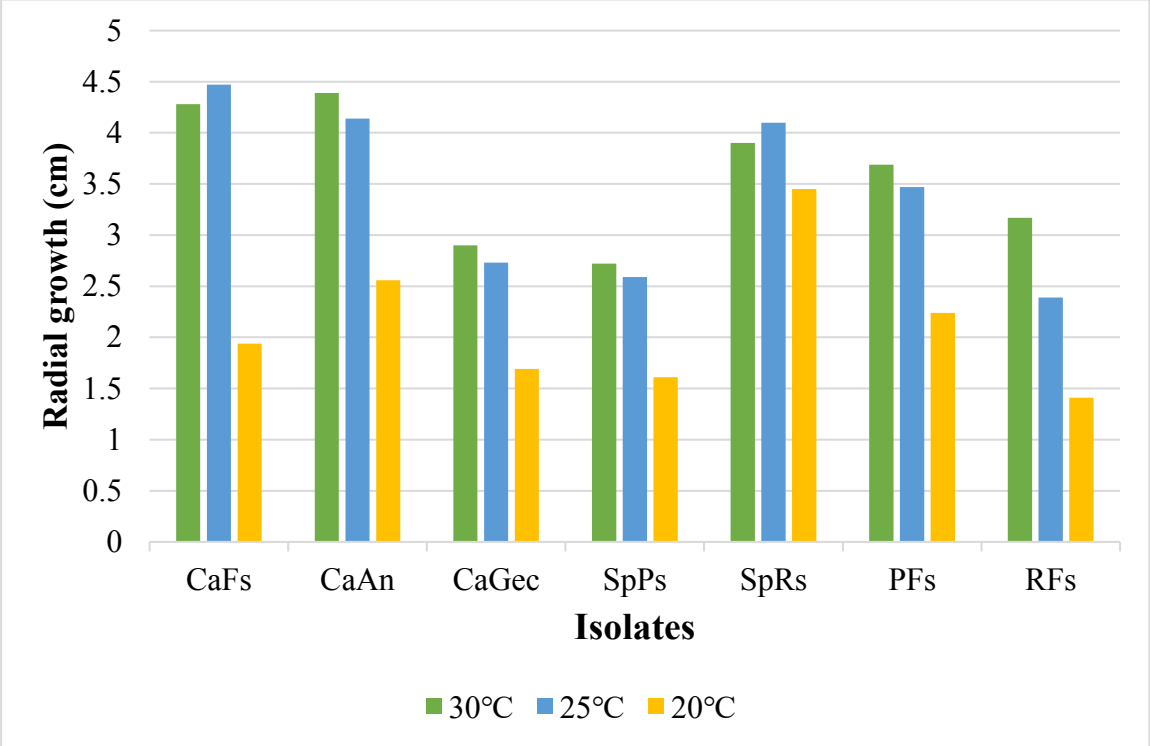

Figure 9. Effect of different temperature on radial mycelial growth of different isolates at 8 DAI

Ca=Carrot, **Fs**=*Fusarium* sp, **An**= *Aspergillus niger*, **Gec**=*Geotrichum candidum*, **Sp**=Sweet potato, **Ps**= *Penicillium digitatum*,

Rs= *Rhizopus stolonifer*, **P**=Potato**, R**=Radish

4.4.1. Effect of different temperature and media on radial growth (cm) of *Fusarium solani* isolated from carrot at 8 days after incubation (DAI).

No Significant variations were observed on radial mycelial growth on different media and temperature at 25℃ and 30℃ but the significant variation was observed at 20℃ (Figure 10). *Fusarium solani* from carrot was higher radial mycelial growth on PDA media than CDA media.

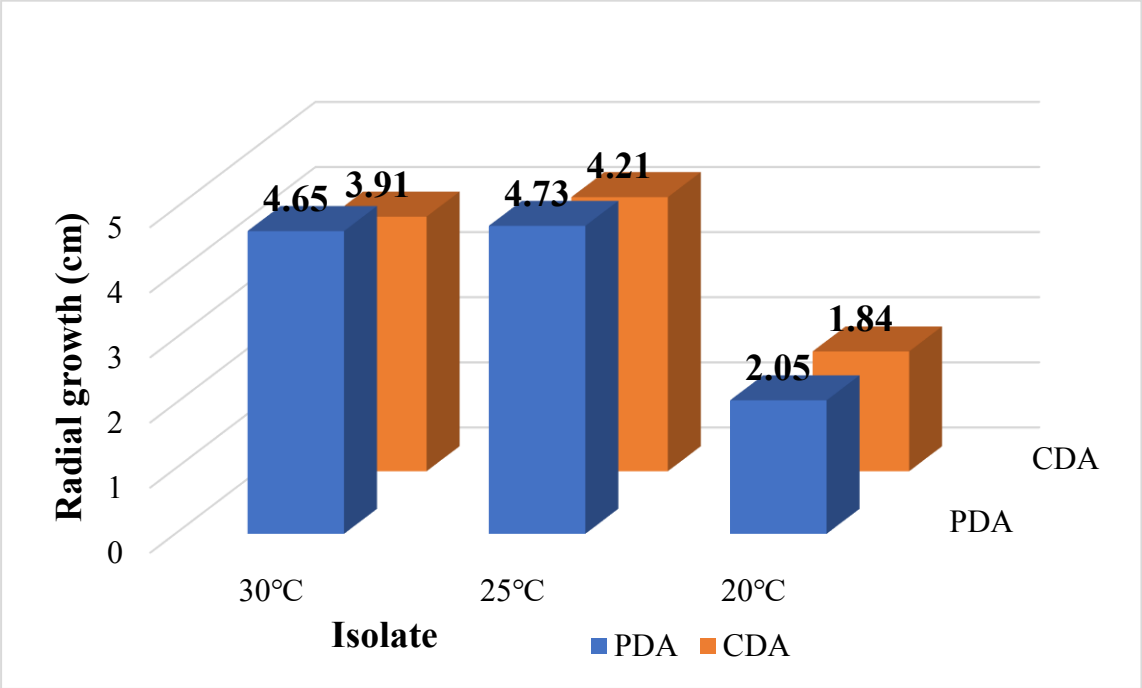

Figure 10. Effect of different temperature and media on radial mycelial growth (cm) of *Fusarium solani* isolated from carrot at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.2. Effect of different temperature and media on radial mycelial growth (cm) of *Aspergillus niger* isolated from carrot at 8 days after incubation (DAI).

Significant variations were observed on *Aspergillus niger* of carrot on different media and temperature (Figure 11). This isolate produced higher mycelial growth on PDA 30℃ media than CDA 30℃ media.

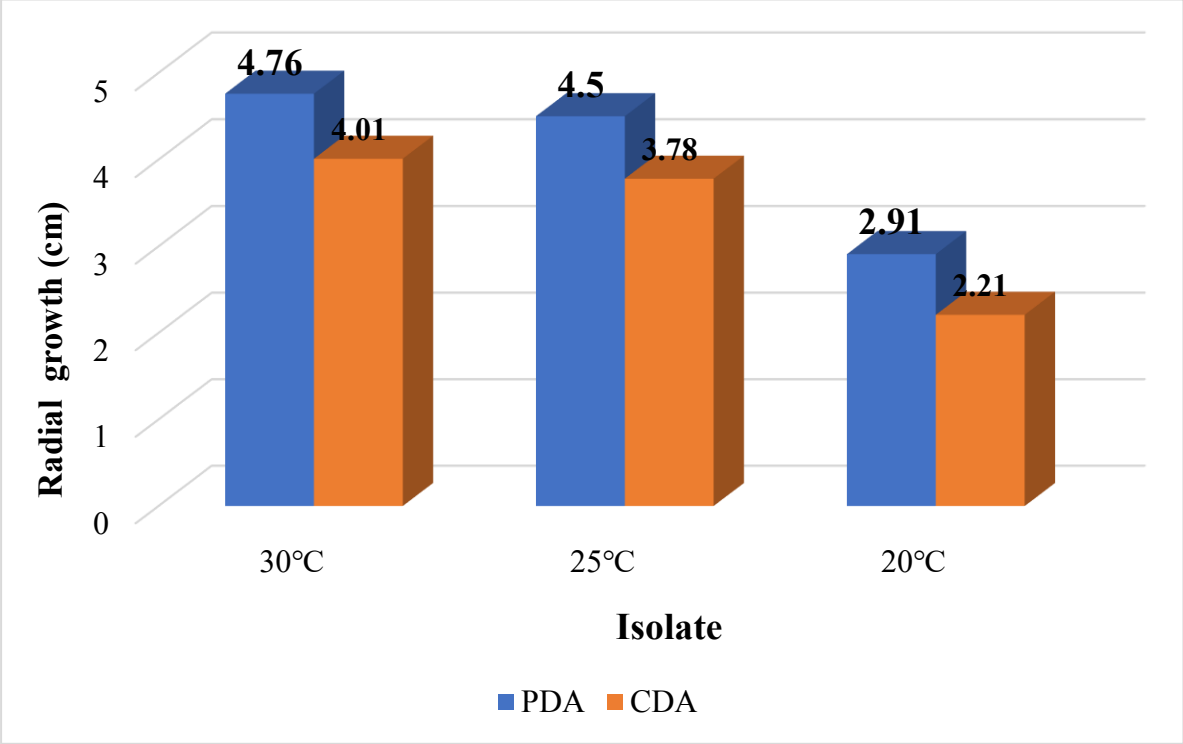

Figure 11. Effect of different temperature and media on radial mycelial growth (cm) of *Aspergillus niger* isolated from carrot at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.3. Effect of different temperature and media on radial mycelial growth (cm) of *Geotrichum candidum* isolated from carrot at 8 days after incubation (DAI).

Significant variations were observed on *Geotrichum candidum* of carrot on different media temperature (Figure 12). This isolate produced higher mycelial growth on PDA 30℃ media while on CDA at 25℃.

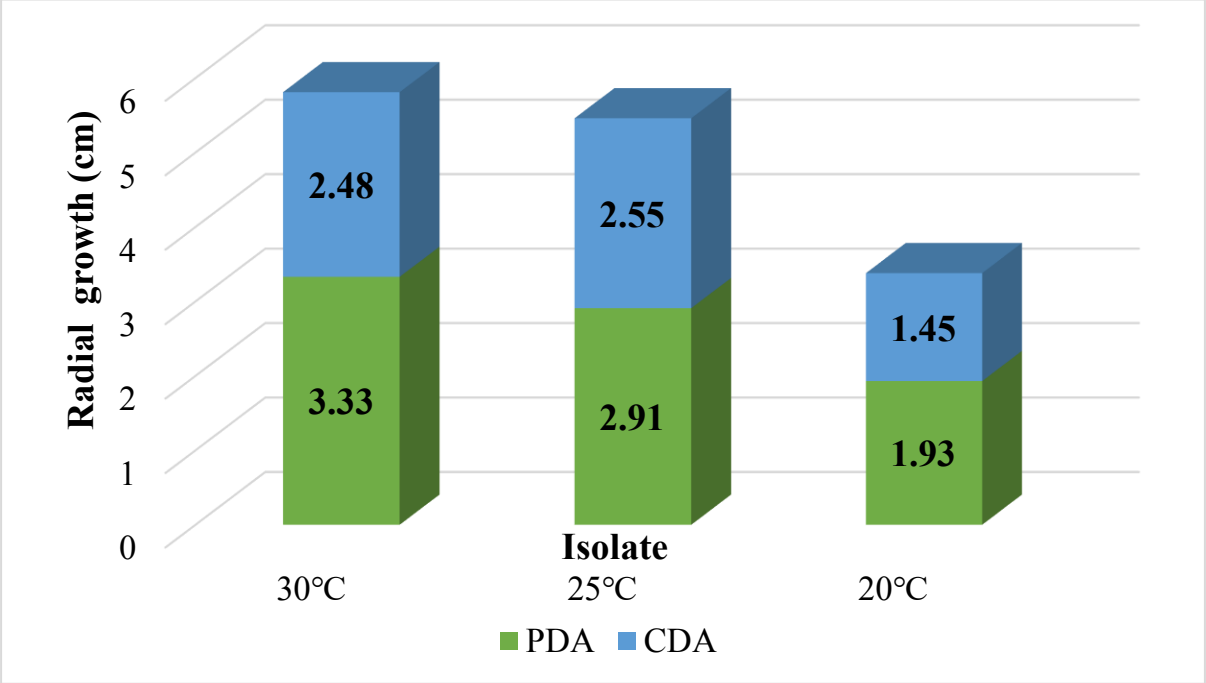

Figure 12. Effect of different temperature and media on radial mycelial growth (cm) of *Geotrichum candidum* isolated from carrot at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.4. Effect of different temperature and media on radial growth (cm) of *Penicillium digitatum* isolated from sweet potato at 8 days after incubation (DAI).

No Significant variation were observed on *Penicillium digitatum* of sweet potato on different media and temperature (Figure 13). This isolate produced higher mycelial growth on PDA and CDA media at 30℃.

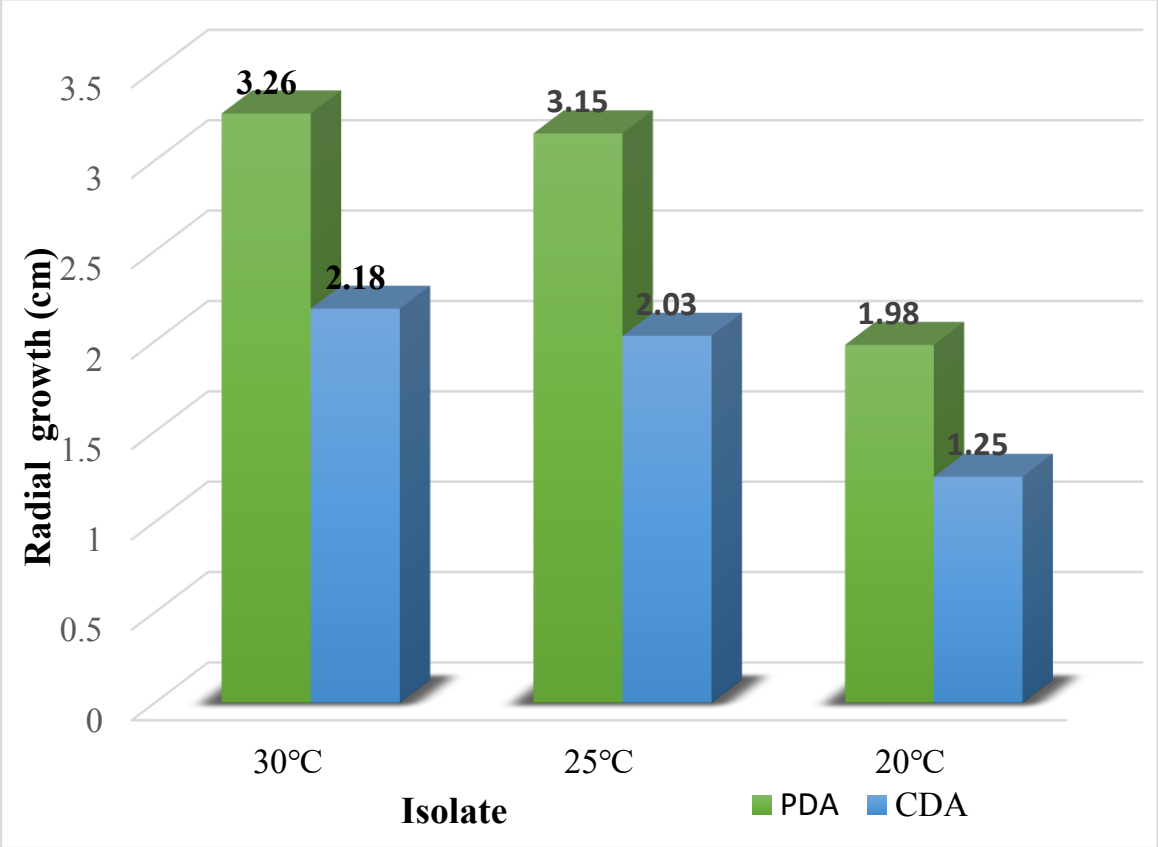

Figure 13. Effect of different temperature and media on radial mycelial growth (cm) of *Penicillium digitatum* isolated from sweet potato at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.5. Effect of different temperature and media on radial mycelial growth (cm) of *Rhizopus stolonifer* isolated from sweet potato at 8 days after incubation (DAI)

No Significant variation was observed on *Rhizopus stolonifer* of sweet potato on different media and temperature. This isolate produced higher mycelial growth on PDA 25℃ and CDA media at 25℃ media (Figure 14).

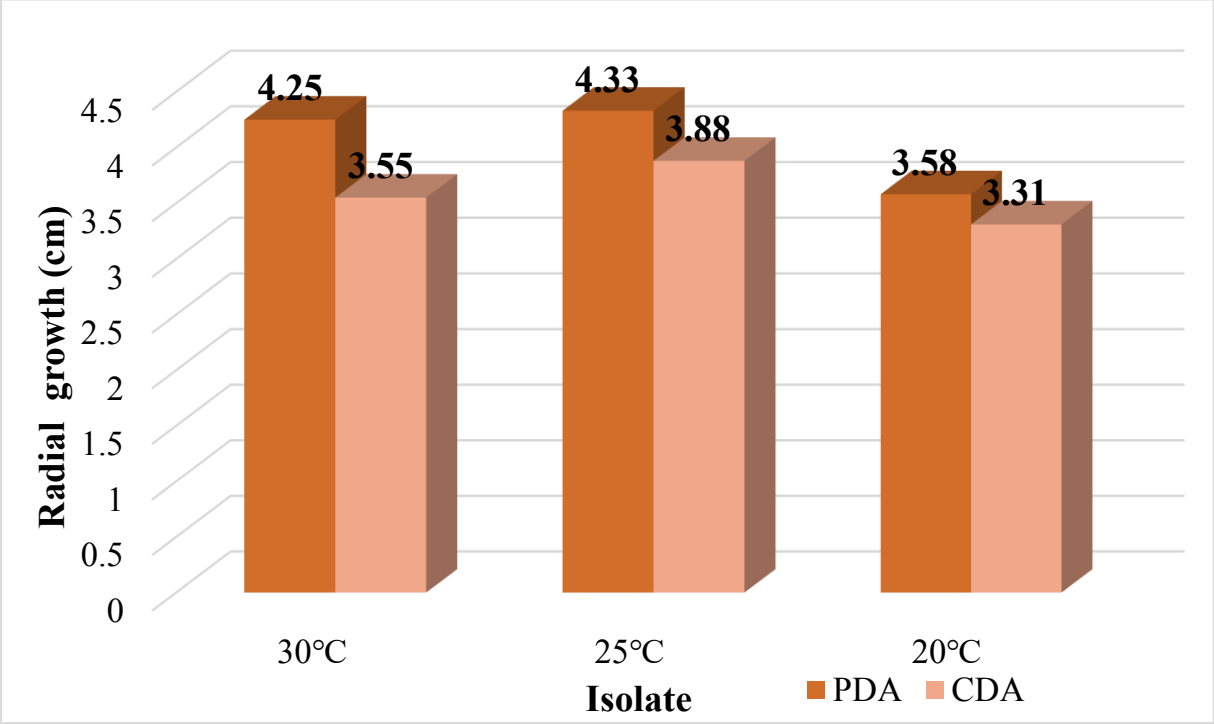

Figure 14. Effect of different temperature and media on radial mycelial growth (cm) of *Rhizopus stolonifer* isolated from sweet potato at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.6. Effect of different temperature and media on radial mycelial growth (cm) of *Fusarium* sp isolated from potato at 8 days after incubation (DAI)

No Significant variation was observed on *Fusarium* sp of potato on different media and temperature. This isolate produced higher mycelial growth on PDA and CDA media at 30℃ (Figure 15).

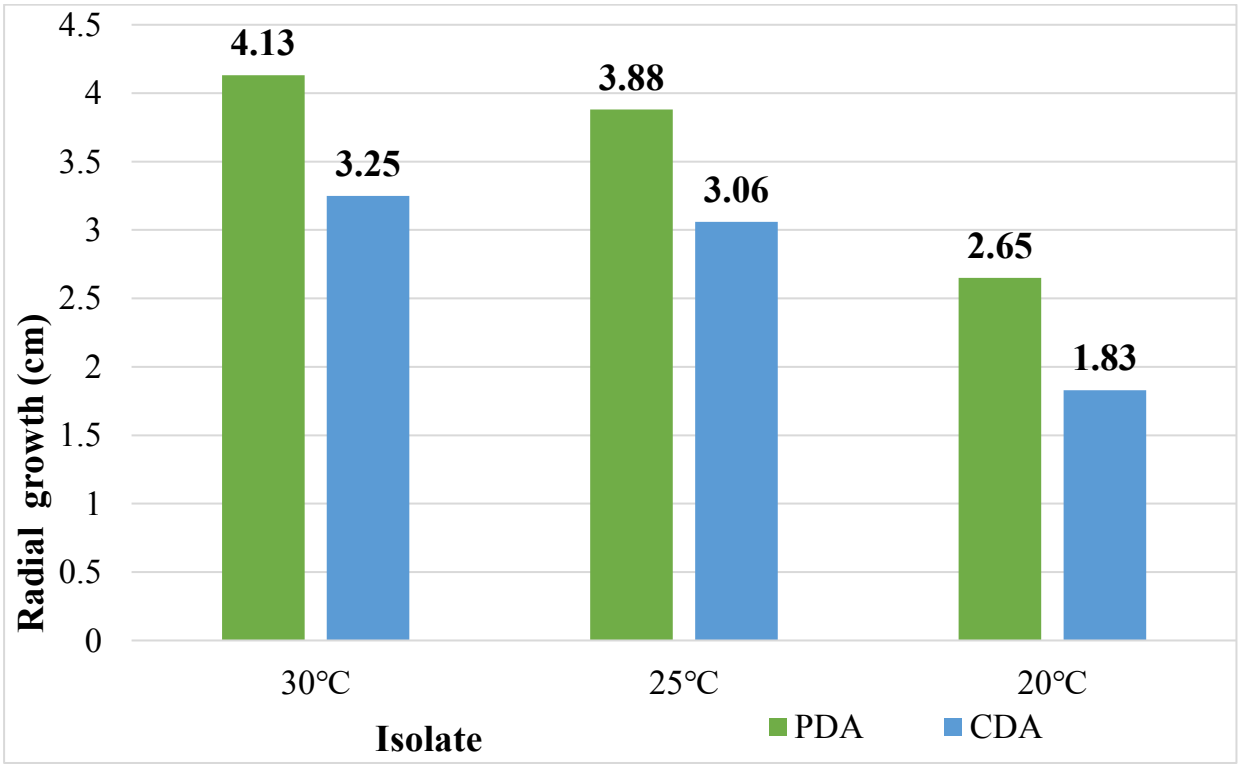

Figure 15. Effect of different temperature and media on radial mycelial growth (cm) of *Fusarium* sp isolated from potato at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

4.4.7. Effect of different temperature and media on radial mycelial growth (cm) of *Fusarium oxysporum* isolated from radish at 8 days after incubation (DAI)

Significant variation was observed on *Fusarium oxysporum* from radish on different media and temperature. This isolate produced higher mycelial growth on PDA and CDA media at 30℃ (Figure 16).

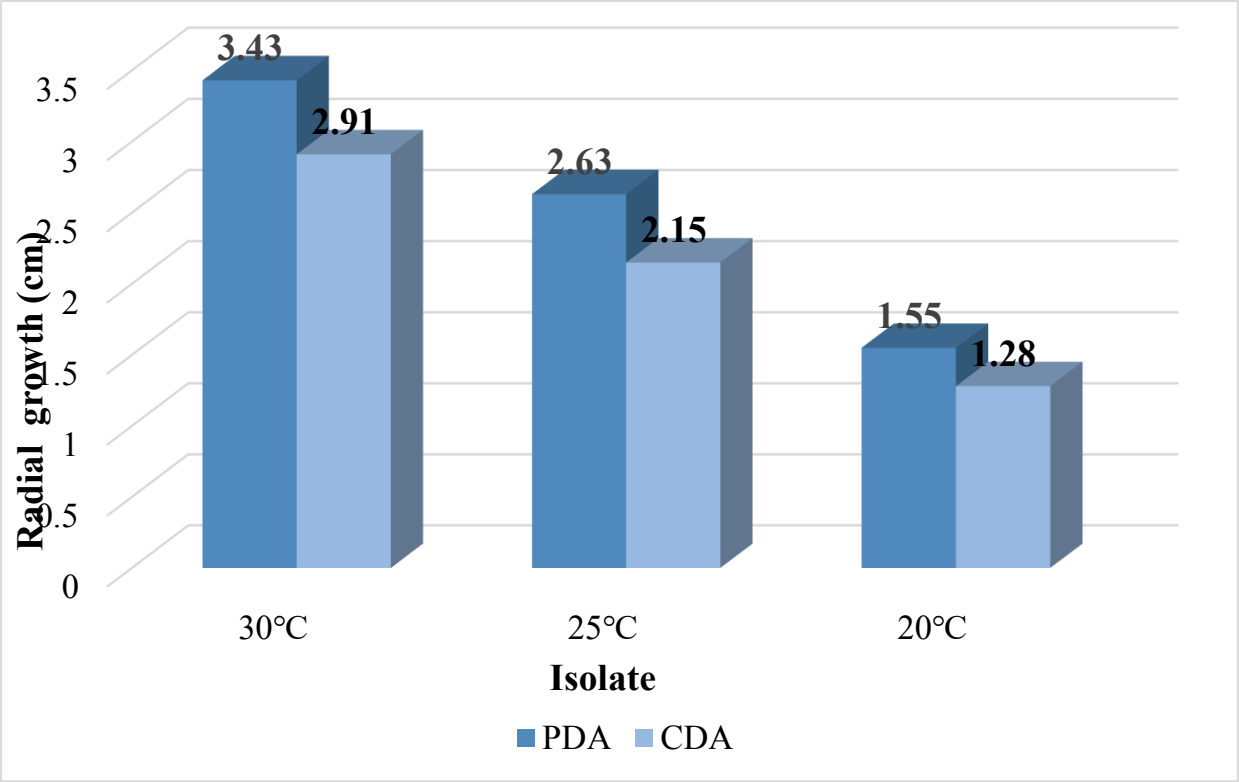

Figure 16. Effect of different temperature and media on radial growth (cm) of *Fusarium oxysporum* isolated from radish at 8 DAI

PDA= Potato Dextrose Agar

CDA= Carrot Dextrose Agar

DISCUSSION

An experiment was carried out in Plant Pathology Laboratory of Sher-e- Bangla Agricultural University to find the possibility of postharvest fungal diseases in root and tuber crops (potato, sweet potato, carrot, radish). In this study, the root and tuber crops having typical symptoms were collected from vegetable market in Dhaka city and the causal organism were isolated on PDA media. After getting pure culture of associated fungi then, pathogenicity test was done for each pathogen.

The effects of media and temperature on mycelial growth and sporulation of isolated fungal pathogens were investigated. The pathogens were identified on the basis of morphological, cultural and microscopic studies. The radial growth (cm/day) of the fungi was assessed on two artificial culture media viz. potato dextrose agar (PDA) and carrot dextrose agar (CDA).

Five fungi genera were isolated and identified as post-harvest pathogens in collected samples (carrot, potato, sweet potato and radish). These were *Fusarium, Aspergillus, Geotrichum, Penicillium* and *Rhizopus.* This finding was found to be similar with the finding of Salami (2007), who determined *Fusarium roselens, Rhizopus stolonifer, Aspergillus fumigatus, penicillium, Aspergillus niger* species were responsible for post-harvest rot of sweet potato tubers, carrot, Amienyo (2007) reported that *Rhizopus stolonifer* is the most generally isolated fungus from spoilt sweet potato tubers in South western, Nigeria. The results of this study are in arrangement with the findings of other researchers (Agu *et al*., 2014) that fungi establish a menace in storage rots of many agricultural goods. Species of *Alternaria, Fusarium, Penicillium, Aspergillus, Geotrichum* as well as to *Botrytis* have been recorded as prevalent post-harvest fungi (Splittstoesser, 1987; Adaskaveg *et al*., 2002).

In the present study *Fusarium* dry rot of potato disease have been recorded in the present of external evidence of the following description of (Turkensteen 2005; Wale, 2008). However, when a wide-ranging of the tuber surface were affected, symptoms progress externally into typical wrinkling recognize in concentric

rings. The internal spoilage is expressed as brown streak of rotten tissue indicating hollows filled with mass of fungal spore and mycelia. Highly infected tubers will ultimately shrivel and become mummified.

All isolates showed remarkable variations in respect of their visual symptoms and morphological characteristics in different media. In respect of cultural characteristics, all isolates of causal agent showed variation in mycelial growth, colony colour, shape, textures, subsurface colour, zonation (Leslie and Summerell, 2006).

In the present study both PDA and CDA media were shown little difference for the radial mycelial growth of *Fusarium* sp and the colour of the colony was white cottony that was similar to both media. It was confirmed to the findings of Gupta *et al*. (2010) they were found that PDA was best for the growth of the *Fusarium* spp. isolates. *F. oxysporum* f. sp *psidii* is rapid growing than *F. solani*. Colour of the colony ranged from cottony white to varying colony character, and metabolite colour from yellowish to no colour for both *F. oxysporum* f. sp *psidii* and *F. solani*. An absolute colony growth pattern (i.e., cottony white) of *F. oxysporum* f. sp *psidii* isolates was listed when they were grown on CDA. When *F. solani* isolates grown on CRBA media had a pinkish cottony type growth. Both *F. oxysporum* f. sp *psidii* and *F. solani* isolates did not outcome a metabolite colour when grown on CMA media.

In radish, *Fusarium* sp colour of the mycelium in PDA and CDA was whitish pink at first then turn dark pink and the radial growth of the pathogen was higher in PDA media against CDA media Other studies are similar of *F. oxysporum* f. sp *dianthi* on solid media designated that, on potato dextrose agar radial growth was maximum which was significantly remarkable over all other media viz. Czapek's Dox Agar, Malt Extract Agar, Oatmeal Agar and Rose Bengal Agar. Bountiful sporulation was seen in the case of Oatmeal Agar (Chittem and Kukarni, 2008).

In the present study radial mycelial growth of the isolated pathogens were developed at different temperature. The growth of mycelium is higher at the

temperature (25°C-30℃) similar result found by Daami-Remadi (1996). In this study, all isolated pathogen from root and tuber crops maximum radial mycelial growth were recorded in PDA at 30℃ without *Fusarium* rot of carrot and *Rhizopus* rot of sweet potato these two pathogens were showed the highest mycelial growth on PDA at 25℃ preceded by CDA media. This result is similar to the Ahamad *et al*. (2002), Sharma *et al*. (2005) they also observed that excellent growth and sporulation took place at 30°C followed by 25°, 20° and 30°C. Moderate sporulation was listed at 25℃. In present study the highest mycelial growth on PDA media at 8 DAI was recorded *Aspergillus niger* on carrot (4.06 cm) preceded by *Fusarium* rot of carrot (3.81 cm) and the lowest mycelial growth recorded at *Fusarium* rot of radish (2.54 cm) and the highest mycelial growth on CDA media at 8 DAI was recorded (3.58 cm) in case of *Rhizopus* rot of sweet potato preceded by *Aspergillus niger* (3.34 cm) isolated from carrot. and the lowest mycelial growth (1.82 cm) recorded at *Penicilliun digitatum* from sweet potato.

The present study revealed that five fungi viz. *Fusarium* spp., *Aspergillus niger*, *Geotrichum candidum*, *Penicillium digitatum*, *Rhizopus stolonifer* have been found to cause storage rot of potato, sweet potato, carrot and radish roots during storage condition. These pathogens lead to enormous loss of these tubers not only in terms of quantity but also reduce its economic and nutritive value. Some of these fungi are capable of producing mycotoxins which are hazardous to the health of consumers. Therefore, attention is required for the disease, thereby increasing the economic yield of the produce. This will ensure substantial contribution of the crop to food supply and national economy.

CHAPTER V

SUMMARY AND CONCLUSION

Root and tuber crops (potato, sweet potato, radish and carrot) are valuable for their energy source which is grown all over the world. It belongs to the different botanical families. The production of the root crop is not satisfactory because of the land crises and by various diseases in Bangladesh and the major losses occur after harvest due to post harvest decay, handling, storage, and disease-causing organism. This study was conducted to find out the post-harvest fungal diseases of selected root and tuber crops in which *Fusarium* dry rot, sooty rot, mold fungi, sour rot, *Rhizopus* rot are very common post-harvest fungal disease. The present study was performed to study on different fungal diseases of selected root and tuber crops and to identify the causal agents of isolated pathogen. Infected and diseased sample were collected from different markets in Dhaka city. The experiment was carried out in the completely randomized design with three replications. Three different temperature and two different media (PDA and CDA) were used to measure growth and development of isolated pathogens from different root and tuber crops. Pathogenicity test was done by using fresh root and tuber inoculation which was similar to the isolated pathogens.

The causal agent of post-harvest fungal diseases was isolated from infected root and tuber selected vegetable transferred to the moist chamber. After isolation of pathogens, they were inoculated on PDA medium. After completion of inoculation on PDA media then radial mycelial growth were measured by using different media PDA and CDA media with different temperature in the incubation chamber.

All the isolates showed variation in the terms of cultural and morphological character. All isolates produced higher mycelial growth at 30˚C and lower mycelial growth was observed on 20℃ but 2 isolates of *Fusarium solani* from carrot and *Rhizopus stolonifer* from sweet potato were observed the highest radial mycelial growth at 25℃.

Colour of the mycelia were different among all isolated pathogens isolates from different crops. *Fusarium* dry rot of potato showed fluffy white mycelia on PDA medium. All isolates produce circular colony and the texture and colour of all isolated pathogen were from one another. *Fusarium* sp showed white fluffy on PDA medium, *Geotrichum candidum* showed flat, white to cream colour on PDA medium, *Rhizopus stolonifer* showed initially white colour colony then turns grey to yellowish, *Penicillium digitatum* were slow growing on PDA medium and the colour of the species white with green surface on PDA medium, *Aspergillus niger* showed initially white colony then turns black and another *Fusarium* sp isolated from radish pink colour mycelium grown on PDA media. Variation also observed between surface and sub-surface colour. Different isolates produce different colour on surface and the subsurface colour whitish to brown colour. The highest mycelial growth on PDA media at 8 DAI was recorded in *Aspergillus niger* on carrot (4.06 cm) preceded by *Fusarium* rot of carrot (3.81 cm) and the lowest mycelial growth recorded at *Fusarium* rot of radish (2.54 cm) and the highest mycelial growth on CDA media at 8 DAI was recorded (3.58 cm) in case of *Rhizopus* rot of sweet potato preceded by *Aspergillus niger* (3.33 cm) isolated from carrot and the lowest mycelial growth (1.82 cm) recorded at *Penicillium digitatum* isolated from sweet potato.

On the basis of the above results, it can be summarized that- Potato dextrose agar medium and 30˚C temperature was seeming to be the best medium and temperature, respectively for the radial mycelial growth of this fungal pathogens.

The present study was based on post-harvest fungal diseases of root and tuber crops and their causal organisms isolated from selected root and tuber crops. Further studies needed to increase storage period and post-harvest disease management of different root and tuber crops.

CHAPTER VI

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