POST HARVEST DISEASES OF POTATO AT COLD STORAGE CONDITION IN NORTHERN REGION OF BANGLADESH

MD. SOURAV

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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BY
MD. SOURAV

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Approved by:

(Dr. M. Salahuddin M. Chowdhury)	**(Dr. Nazneen Sultana)**
Professor	Professor
Supervisor	**Co- Supervisor**

Prof. Dr. Khadija Akther
Chairman
Department of Plant Pathology
Examination Committee
Sher-e-Bangla Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

Sher-e-Bangla Agricultural University (SAU)
Sher-e-Bangla Nagar, Dhaka-1207
Bangladesh

Ref.: …… Date: ……

CERTIFICATE

This is to certify that the thesis entitled **"Post-harvest diseases of potato at cold storage condition in northern region of Bangladesh"** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in Plant Pathology**, embodies the result of a piece of *bona fide* research work carried out by Registration No. **12-04853** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated**:**

Place: Dhaka, Bangladesh

_____

(Prof. Dr. M. Salahuddin M. Chowdhury)
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

Dedicated to

my

Beloved Parents and Wife

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ABSTRACT

Cold storage environment was studied to assess its effect on post-harvest disease of potato in selected cold storage of Nilphamari district of Bangladesh. Total 360 samples of potato were collected from cold storages of Nilphamari districts viz. Shawon cold storage, Angkur seed & cold storage and Mukta cold storage during July, 2018 to December, 2018. Internal condition of cold-storages viz. temperature (^{0}C/^{0}F), Relative humidity (%), O_2 supply & CO_2 removal and NH_3 supply were recorded during the study period. The recorded parameters in the storage were; temperature ranged from 2.22^{0}C/36^{0}F to 4.44^{0}C/40^{0}F, relative humidity 85-90%, CO_2 removal and O_2 supply 48 minutes to 1 hr and NH_3 supply 9 to 13 hrs observed. Five significant pathogens were identified among them two fungi viz. *Fusarium oxysporum* (dry rot) and *Rhizopus stolonifera* (rhizopus rot) and three bacteria *Erwinia carotovora* (soft rot), *Streptomyces scabies* (scab) and *Rasltonia solanecearum* (brown rot). Storage disease incidence of potato was recorded and variations were observed which depended on internal conditions. The disease incidence of dry rot, rhizopus rot, soft rot, scab and brown rot was ranged from 0.85 to 0.30%, 0.40 to 0.0%, 0.50 to 0.10%, 0.50 to 0.19% and 0.62 to 0.11% respectively. Maximum disease incidence of storage tuber was recorded in July, 2018 while minimum loss was found in December, 2018.

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LIST OF SYMBOLS AND ABBREVIATIONS

% = Percentage

et al. = And others

^{0}C = Degree Celsius

^{0}F= Degree Fahrenheit

PDA = Potato dextrose agar

Ibs = Irritable bowel syndrome

Mins = Minutes

W = Weight

V = Volume

DMRT = Duncan's Multiple Range Test

Ha= Hectares

Th= Thousand

Kg = Kilogram

@ = At the rate

ISTA = International Seed Testing Association

M = Mega

Mt = Metric ton

etc. = Etcetra

J. = Journal

Viz. = Namely

Cm = Centimeter

Cfu = Colony forming unit

& = And

G = Gram

ml = Milliliter

hr = Hour (s)

i.e. = That is

mm = Millimeter

μm = Micrometer

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

FAO= The Food and Agriculture Organization

EPB = Export Promotion Bureau

SPP. = Species

ASAE = American Society of Agricultural Engineers

RH = Relative humidity

BoFED = Bureau of Finance and Economic Development

DAE = Department of Agriculture Extension

CIP = International Potato Centre

BBS = Bangladesh Bureau of Statistics

USA = United States of America

NA = Nutrient Agar (media)

TTC = Triphenyl Tetrazolium Chloride

ANOVA = Analysis of Variances

LSD = Least Significant Difference

CV% = Percentages of Co-efficient of Variance

CHAPTER I
INTRODUCTION

Agriculture is the main stay of majority of the people in Bangladesh and potato is one of the highly important crops in the state. Potato (*Solanum tuberosum L*.) is a tuber crop belonging to the family Solanaceae. It is an important food crop from the very beginning of human civilization and occupying its position just after wheat and rice both in respect of production and consumption (Thompson and Kelly, 1957). Potatoes are a versatile crop and are currently grown in 100 different countries and potatoes are a great source of Vitamin C and potassium, offering 45% and 18% of the recommended daily value respectively per 5.3-ounce potato with skin on (United States Potato Board 2015). Recent studies have shown potato mineral iron and zinc was heritable and therefore could be increased through breeding (Brown *et al.,* 2010 & Brown *et al*., 2011). Potato crops could yield 9.2M calories per acre, which was more than that of maize (7.5M), rice (7.4M), wheat (3M), and soybean (2.8M) (Ensminger *et al*., 1994). In Bangladesh, the cultivation of potato was started in the late 19th century (Siddique and Hossain, 1988). The major potato growing regions of Bangladesh were Dinajpur, Rangpur, Nilphamari, Joypurhat, Jamalpur, Sherpur, Bagura, Jashor, Shariatpur, Faridpur, Munshigang, Naranganj and Manikganj (Ahmed *et al.,* 2013). In Bangladesh, potato is a crop of great economic significance. Potato (*Solanum tuberosum* L.) popularly known as 'The king of vegetables', is the staple food for many countries of the world contributes alone as much as 54% of the total annual vegetable production of Bangladesh (Anonymous, 2006). Bangladesh was the third largest potato producer in Asia and standing sixth in the world (FAO, 2010). At present nearly 460 thousand hectares (ha) of cultivable land is under potato cultivation and the country produced 8,326 thousand tons potato in the year 2010-2011 (BBS, 2012). The average yield of potato per ha was 13.32 tha^{-1} which was very low in comparison to other potato producing countries like 43.2 tha^{-1} in France, 44.7 tha^{-1} in Netherlands and 44.6 tha-1 in the USA in 2007 (Anonymous, 2008). Hossain *et al.*, (2008) reported

that the national average yield of potato was very low (19.07 tha^{-1}) compare to its potential yield 30-40 tha^{-1}, due to lack of quality seed, cultivation of indigenous potato (yield 5-7 tha^{-1}) and high price of quality seed. Potato is grown in more than 100 countries in the world. According to FAO (2008), Potato was consumed by more than one billion people in the world. Potato was ranked as the fourth most important staple food crop, and the number one non-grain food commodity (Thomas and Sansonetti, 2009). Asia consumes almost half of the world's potato supply, but its huge population means that consumption per person was a modest 24 kg in 2005. Africa became first in consumption of potato as the quantity was 12571 Mt where Asia, Europe and Latin America consumed 94038 Mt, 64902 Mt and 11639 Mt respectively, (http://www.fao.org/potato-2008/en/world/).

The availability of the fresh potatoes is higher during the period of fresh consumption for 3-4 month. Hence, there should be proper storing facilities for potato in proper temperature and conditions to maintain its quality, morphological and physiological conditions. It is possible to store 25% of potato in cold-store condition. Rest of potatoes could be stored in farmers home. In room temperature, potato could be stored for 2 to 3 months. There are 34 model home storages were made at low cost to keep potato at normal condition for 2/3 months in Dhaka, Chittagong, Rajshahi, Khulna, Barisal and Rangpur. In the United Kingdom, approximately half of the total harvested tubers were stored for up to 11 months (Dale, 2014). Those potato tubers which were to be used for seed purpose are to be stored in 2^0C and those are used in processing and the consumption purposes these are stored at 8-12^0C. It helped to maintain the quality of the potatoes for processing (Mehta and Ezekiel, 2006). For storing of potatoes for longer period they were kept in cold storages until they were needed for the market. Different storage conditions and handling procedures affected the quality of the potato tubers (Bentini *et al*., 2009). Potatoes which are ultimately used for the seeding purposes were stored at lower temperature at about 4°C and those potatoes which were used for the processing purpose are stored at 8^0C (Van, 1987). Due to its perishable nature, certain quantity of produce was lost at

different levels of marketing as well as on the storage. To maintain potato quality during storage, the storage environment must be adjusted to minimize tuber deterioration. Temperature, humidity and air movement can always affect the keeping quality of stored potatoes. In most of the time, temperature is kept between 2-4^{0}C, relative humidity ranges from 80-95%, O_2 & CO_2 supply almost for 1 hour depending on weather condition and NH_3 supplied for 48 minutes to 1 hour during storage period of potato. Potatoes should always be kept in complete darkness to prevent greening. Potato is the crop which could be grown in only one season as field crop. It results in deficiency of the potato during other time period of the year. It is also important to store potato in such a way that, it would be less damaged by the climatic imbalance, pathogen and the quality of potatoes was also maintained (Kaur *et al*., 2009).

The growers have to sell major part of their produces immediately after harvesting at a very low price due to lack of storage facilities and cash needed of the growers. The trend to increase production of potato in Bangladesh is retarded mainly by post-harvest problems among which storage is an important one. According to Ahmad (1980) and Alauddin (1979), "potato production is directly proportional to its preservation space". Therefore, if storage facilities are not increased, the production of potato cannot be increased considerably.

In a preliminary survey of Khan *et al*. (2003) surveyed and found that the diseases of potatoes in cold storage in Bangladesh and it was also found that 2-9 percent of cold stored potatoes were lost in every year due to disease. Invasion of microorganisms into the tubers may cause considerable losses due to diseases. The diseases, which may develop during storage, are late blight, early blight, dry rot, pink rot, gangrene, skin spot, black dot, silver scurf, charcoal rot, soft rot, etc. High temperature and high relative are important factors responsible for disease development during cold storage. Normally infection takes place in field and in most of the occasions injuries of the tubers become the entry points to the microorganisms. Infection by some microorganisms may, under favorable storage conditions, cause rapid rotting of potatoes. As apparently, the affected

tubers also produce extra heat, temperature rises very rapidly and within a few days, the entire potato stack may have become rotten. To counteract the spread of diseases in the cold storage, care must first be taken at the beginning of storage period. Also, the potatoes must be maintained at desired low temperatures and kept dry throughout the storage period. Fakir (1979) stated that an amount of Taka 8 crores approximately was lost annually due to storage disease. Rahman (1969) and Kamaluddin (1970) reported that 2 to 9% losses of tubers take place every year in each storage due to these diseases in Bangladesh. In India post-harvest losses were 17% and in Pakistan losses ranged from 15-40% (Iqbal, 1996). Post-harvest losses of potato in different countries were reported as Colombia 25%, Costa Rica 24%, Dominican Republic 20% and United States 24% (Meyhuay, 2007). According to Miaruddun *et al.* (2009), average storage loss for cold storage was 3.82% for about nine months storage period. Dry rot of seed tubers can reduce crop establishment 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 (Wharton and Kirk, 2007). Rhizopus rot is often considered devastating post-harvest disease of potato, resulting in an estimated 2% loss in storage (Clark and Moyer, 1988). Holmes and Stange (2002) studied for couple of years and found that wounding and storage time can affect the potato's susceptibility to *Rhizopus stolonifer*. Potato tubers being nearly 80% water, they are especially susceptible to bacterial pathogens that cause soft rot, resulting to losses of up to 90% in the field and in storage (Czajkowski *et al*., 2011). In Zimbabwe, potato growers face the challenge of significant post-harvest losses of potato tubers (20% to 60%) due to soft rot (Manzira, 2010). According to Loria *et al.* (2006) and Wanner (2004) scab disease was indigenous in all potato growing areas in the world. About $9 million loss was made in Bangladesh in 2014 as *R. solanecearum* bacterium present in potato which retard export potato to Russia (EPB, 2014).

Considering the above facts, the present research program has been designed with following objectives:

1. To determine the incidence of post-harvest diseases of potato in selected cold storages of northern region of Bangladesh

2. To determine the storage condition that induce the diseases

3. To identify the causal agents of post-harvest potato diseases in cold storage condition

CHAPTER II
REVIEW OF LITERATURE

Storage diseases of potato constitutes a serious obstacle to store potato and becomes a serious threat during storage periods. The diseases are found in all storage areas of the world resulting severe yield losses both in terms of quality and quantity. The literature pertaining to the fungal and bacterial diseases of are reviewed here under.

2.1. Occurrence of diseases of potato in post-harvest period

Wijekoon *et al.* (2015) found that tubers dried quickly at high ventilation area and shrinkage at low ventilation.

According to the report of EPB (2014), Bangladesh faced a loss about 9 million dollars in 2014 as Russia stopped import potato due to the presence of a bacteria that causes brown rot in potato.

Nadia *et al*. (2013) studied on potato in some selected districts in Bangladesh to find the incidence and severity of bacterial wilt disease caused by *Ralstonia solanacearum*. The highest disease incidence and severity was recorded in Munshigonj (22.65% and3.80%) while lowest incidence and severity was recorded in Jamalpur (9.07% and 2.90%).

Czajkowski (2011) stated that soft rot bacterium was under the pectobacterium which caused damage to potato tuber and had great economic importance.

Czajkowski (2011) reported that potato tuber affected by soft rot bacteria after harvest and even during period of storage. The bacteria feed on parenchymatous tissues by maceration and infiltration through producing various cell-wall degrading enzymes in wide range of plants.

Ronald and Dennis (2011) found in their study that major storage diseases were dry rot, pink rot, sort rot, ring rot & late blight.

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

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

Pinhero *et al.* (2009) stated that supply of fresh air was critical for cooling and drying of tubers and to remove CO_2, volatiles and excessive heat & moisture as well.

Wanner (2009) and Loria *et al.* (2007) concluded that, total yield did not affect by common scab, since the economic losses are greatest when tubers infected for table stock and processing varieties, since appearance of potato is important for the market.

Microclimate like relative humidity (RH) above 80% & temperature 10-24^{0}C most favorable for appearance of the disease (Bhat and Singh, 2008).

Pathogens are both soilborne and seedborne, hence the diseases occur both in field and storage stages. Infection of plants in the field occurs on underground stems, stolons, or roots as tubers develop. Tubers can be infected by pathogenic fungi, oomycetes, and bacteria through lenticels, eyes and wounds inflicted during harvesting and loading into storage. The health of potato tubers in storage does not improve over time, but can be maintained by ensuring a proper storage environment. Once the tubers are harvested, they have to be cured at optimum temperature (10-13^o C), high humidity (95%), and good ventilation for two weeks. (Powelson and Rowe, 2008).

Hajhamed *et al* (2007) reported that, *Erwinia carotovora* sub sp. *Carotovora.* was the causal agent of bacterial soft rot. Either in the field or in storage, it was one of the most important and widespread bacterial disease of a variety of plants.

Some dry rot pathogens, Fusarium spp. have been reported to cause infection at 5^{o} C, while most of the species have their optimum temperatures ranging from 10-15^{o} C, and others range from 25-30^{o} C (Daami-Remadi *et al.*, 2006).

Elphinstone (2005) concluded that brown rot infected tubers and survived in tubers during storage and in the next season it could cause disease on tuber. The bacterium was spread through machinery and irrigation water. The disease was also spread through labor.

Mehedi (2004) worked on five different markets in Mymensingh on May and September 2013 taking Diamant as cultivar. Total 200kg potatoes were taken from five shops. Average soft rot incidence was 0.36% in May and 0.99% in September according to the survey result. According to the report, the average loss of potato tubers found 0.48% in May and 1.31% in September.

Under favourable conditions like high temperature (with an optimal of 25 °C), high humidity and poor ventilation, the bacteria-initiated lenticels or in wounds of tuber in a few days (Gardan *et al.*, 2003).

Banyal, (2002) mentioned that a survey of potato diseases was conducted during potato harvest in 1997-1998 in Lahaul Valley of Himachal Pradesh in India. Common scab caused by *Streptomyces scabies* was observed in all locations and the severity of these diseases ranged from 13 to 27% during 1997 and 1998.

Nourian *et al*. (2002) worked on soft rot bacterium and reported that the bacterial soft rot caused by *E. carotovora* (Ecc) was a major disease potato during the period of storage.

According to Nora *et at.* (2001), seven diseases of potato were isolated in storage condition namely pink rot, pythium leak, late blight, early blight, dry rot, sort rot, black dot and silver scurf.

Secor and Gudmestad (1999) stated that diseases caused by fungal and fungal-like pathogens were the most detrimental to potato production. These diseases include late blight (*Phytophthora infestans*), dry rot (*Fusarium sambucinum* and

spp.), pink rot (*Phytophthora erythroseptica*), Pythium leak (*Pythium ultimum*), and silver scurf (*Helminthosporium solani*) in North America.

Singh (1998) reported that heavy sporulating fungus and spread of the disease takes place at 90% RH and temperature 16 - 22^0C.

Walker (1998) has summarized that a high humidity coupled with a temperature of 80^0F the bacteria was capable to cause the great injury.

Common scab is widely distributed in Bangladesh which gives ugly appearance to wear potatoes. Though the disease does not cause appreciable reduction in yield, it can cause great loss due to reduction of market value of tuber (Dutt, 1997).

According to ASAE (1991) relative humidity should be kept at least 90-95% unless there were diseased, or frozen potatoes, or wet potatoes which need to be dried.

Temperature, humidity, carbon dioxide and air movement are the most important factors during storage (Harbenburg *et al*., 1986; Maldegem, 1999).

Swanson and Van (1985) indicated that fungi grown well at temperatures ranged between 24 and 27°C with 80 to 90% relative humidity at storage.

Wong et al. (1984) found that temperature/moisture combinations of 10°C + 45% RH caused greatest pre-emergence of fungal pathogen of potato storage period.

Dry rot caused by *Fusarium caeruleum* (lib) sacc. was very common and causes most of the damage among all the disease in cold storage condition, but in Bangladesh, there were no extensive work has been carried out to assess the loss due to dry rot. (Rahman, 1969; Kamaluddin, 1970 and Stevenson *et al.*, 2001).

Nielsen (1968) found that the sensitivity of bacteria to tubers was increased at a combination of lower oxygen concentrations with high RH%.

Cairns (1939) stated that under high soil moisture in the field, or high humidity in storage, infection may occur directly through eyes as low moisture inhibits infection.

Cairns (1933) summarized that high humidity in storage with poor ventilation can cause heavy losses of affected tubers.

2.2. Post-harvest Loss of Potato

Rahman *et at.* (2017) in his study found that about 3 lakh tons of potato which was one third of the total annual production was wasted every year a mounting a loss of more than Taka 60 crores annually.

It has been estimated that losses of potato tubers due to improper storage conditions can reach even up to 40% of tuber weight, while losses caused by periderm injuries (resulting in excessive transpiration and respiration) can account for further 10% loss (Grudzińska *et al*., 2016; Zgórska and Grudzińska, 2012).

Farmers are bound to sale their perishable potatoes immediately after harvest at lower price or they store naturally in the field or in their houses; where, they can hardly store for 2 months, but a large amount of potato tubers are spoiled (Hoque, 2014).

The losses in potatoes during storage was an approximately 50% of the crop production which was due to the sprouting, loss in weight, rotting, greening and sweetening in the presence of inappropriate storage conditions (Rezaee *et al*., 2011; Karanja *et al*., 2013).

In another report of telegraphindia.com (September 23, 2013) about 0.38% potatoes were rotted in 2013.

Ayandiji *et al*. (2011) stated that, with the reduction in postharvest losses by 50%, food availability would be increased by 20% without cultivating an additional hectare of land for increasing crop yield of potato.

According to Hodges *et al.* (2011), postharvest losses of potato in developing countries can range from 15 to 50%.

The United Kingdom recorded overall losses of 17% (770,000 tons) in 2012, where premature sprouting and rotting during storage was the main cause of wastage (Terry *et al*., 2011; Pritchard *et al.*, 2012).

According to Terry *et al*. (2011) and Pritchard *et al.* (2012), The United Kingdom recorded overall losses of 17% (770,000 tons) in 2012 during storage.

In Zimbabwe, potato growers face the challenge of significant post-harvest losses of tubers ranging from 20 to 80% (Ngadze, 2010) leading to significant financial losses.

The potato tuber is also roughly made up of 75% water and 25% starch, and therefore is capable of losing the internal water if subjected to low external vapor pressure or relative humidity (CIP, 2009).

Babalola and Gbola (2008) stated that postharvest losses of potato have been highlighted as one of the determinants of the food problem.

In a report of the Daily start newspaper (April 23, 2008), around 0.74% loss was made at mushigang during cold storage.

Wale *et al.* (2008) reported the loss could be of up to 100% in storage both in developed and developing countries leading to insufficient planting material for the following season.

According to BoFED (Bureau of Finance and Economic Development), (2007) postharvest loss (20–25%) was one of the major problems in the potato production.

Dry rot of seed tubers can reduce crop establishment 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 (Wharton and Kirk, 2007).

According to DAE (2005), brown rot of potato was the most serious disease of potato worldwide and causes $950 million losses each year.

Bari (2004) found in his study that total postharvest losses of potato were 15% of the total production.

It is reported in different newspapers that 5 thousand of tons of potatoes was going to rot due to lack of adequate cold storage facility (Moazzem and Fujita, 2004).

U.S. potato losses during storage have averaged about 7.5% over the past several years, according to the National Potato Council 2004-2005 Potato Statistical Yearbook.

Risk of freezing or chilling injury, forming rots or decay and the high cost price are vital reasons to continuing search for alternative ways of mechanical refrigeration. (Chourasia and Goswami, 2001).

Stevenson *et al.* (2001); Rahman (1969) and Kamaluddin (1970) reported that 2 to 9% losses of tubers take place every year in each storage due to these diseases.

Potato should be stored in a suitable environment to prevent weight loss, rot, shrinkage, sweetening, discoloring and sprouting (Gottschalk and Christenbury, 1998).

In India post-harvest losses of potato are 17% and in Pakistan these losses ranged 15-40% (Iqbal, 1996).

Varns *et al.* (1985) investigated the potato losses during the first three months of storage for processing. Questionnaires indicated that 64 to 150 thousand metric tons were annually lost during early storage from the total crop stored for processing. This constitutes a range of 5.6- 13.2 million dollars lost in production costs.

2.3. Isolation and identification of the pathogen

Dariva (2011) studying characteristics of *Fusarium oxysporum* f.sp. *passiflorae* isolates, found an average mycelial growth of 6.36 cm in PDA after four days of incubation.

Dariva (2011) in her study, showed that *Fusarium solani* forms cylindrical macroconidia, with no convex curvature, as seen in *Fusarium oxysporum* f.sp. *passiflorae,* suggesting that the isolates of this study are *Fusarium oxysporum.*

Mahesh *et al.,* (2010) collected 41 *F. udum* isolates from different parts of India and studied with special reference to cultural characters on PDA medium. All the 41 *F. udum* isolates showed wide variations with respect to mycelial colour, pigmentation and colony characters.

Lesions of scab pathogen vary in size and colour but most probably these lesions were brown in color having a few millimetre diameter (Lerat *et al*., 2009).

One of the most important potato diseases was common scabe caused by *Streptomyces scabies*. It is indigenous in all potato growing areas in the world (Loria *et al*., 2006; Wanner, 2004).

Agrios (2004) found that when the epidermal cells were collapsed, the fungus emerges through the wounds and produces aerial sporangiophores, sporangia, stolons, and rhizoids, the latter capable of piercing the softened epidermis.

Potato-dextrose-agar is used as the most common, but malt-agar may not be the best medium to grow rhizopus fungus (Mari *et al*., 2002).

The most important characteristics to identify rhizopus morphologically was the spores (shape, size and colour) and fruiting bodies (Agrios, 2001).

S. *scabies* were identified by the following tests: Gram stain, catalase production, oxydase reaction (Gregerson, 1978), melanin production on peptone yeast extract-iron agar and tyrosine agar medium; starch hydrolysis; hydrogene sulfide(H_2S) production, and nitrate reduction; gelatin liquification (Schaad *et al.,* 2001).

Characterization and Identification of pectolytic erwinias were traditionally based on biochemical and phenotypic characteristics (De Boer and Kelman, 2000).

Bacterial organisms were isolated from different fruit samples by the "streak plate" technique as described by Mortensen (1997) and Kim *et al.* (2002).

All the strains of *E. carotovora* ssp. *carotovora* caused creamy white or light brown soft rot in potato slices (Obradovic and Arsenijevic, 1997).

A loop full culture was taken from the bottom of the beakers and streaked on to paradises which contained sterilized tetrazolium chloride (TZC) agar medium (Kelman, 1995).

Brown rot pathogen of potato was a gram negative, aerobic, motile and rod-shaped bacterium belonging to genus Rasltonia (Yabuuchi *et al.*, 1995).

Cultural, morphological microscopical and pathological properties were considered to identify the scab pathogens according to Burgess *et al*. (1994).

Erwinia carotovora was facultative anaerobe, nonspore-forming, Gram-negative enterobacteria that causes disease in a wide range of plants including many economically important crops (Schuerger and Batzer, 1993; Agrios, 2005).

Traditional identification and characterization of *Colletotrichum* species has relied primarily on differences in morphological features such as colony colour, size and shape of conidia and appressoria, growth rate, presence or absence of setae, and existence of the *Glomerella* teleomorph (Smith and Black, 1990).

In Europe, R. *solanacearum* was a quarantine pest (Council Directive, 1998-2000; Commission Directive, 2006) and in USA it was an important bioterrorism agent (Madden and Wheelis, 2003).

Champawat and Pathak (1989) observed the macroconidia as sickle shaped and that of microconidia as ovoid shaped in case of *F. oxysporum.*

Smith (1988) observed the aerial mycelium of *F. oxysporum* first appears white, then may change to a variety of colors, ranging from violet to dark purple, according to the strain (or special form).

Mycelial compatibility grouping was an important tool for identifying variability among isolates. Including *F. oxysporum* (Correll *et al.*, 1986), it occurred in many fungi.

R. stolonifer was one of the most common members of the Mucorales and has a worldwide distribution, although it was most commonly occurring in warmer areas (Domsch, 1980).

Isolates of brown rot pathogen were studied according to specific biochemical tests for R. solanacearun i.e., gram staining (Schaad, 1980), potassium hydroxide test (Suslow *et al*., 1982), catalase oxidase test (Schaad, 1980), kovacs oxidase test (Suslow *et al.*, (1982).

According to Gregerson (1978), scab pathogen was Gram positive, non-motile, utilized L-arabinose, D-fructose, D-glucose and rhamnose.

Microconidia varied in shape, from elliptical to cylindrical, with 0 to 2 septa which, according to Booth (1977) identifies the isolates as *Fusarium oxysporum*.

The *Fusarium* identification key proposed by Booth (1977) describes several characteristics, such as colony aspect, microconida formation structure, chlamydospore presence and characteristics, macro and microconidia presence and size.

A series of physiological and biochemical tests were performed for characterization of the isolated more pathogenic isolates. The physiological and biochemical tests were a. Potato soft rot test b. Fermentation of glucose (OF test) (Hugh and Leifson, 1953), c. Gram reaction (Suslow *et al*., 1982), d. Oxidase reaction (Kovacs, 1956), e. Catalase production (Hayward, 1992), f. Gelatin Liquefaction test (Schaad, 1988), g. Urease production (Schaad, 1988), h. Nitrate reduction test (Lelliott and Dickey, 1984).

CHAPTER III
MATERIALS AND METHODS

Three experiments were carried out throughout the research period in order to record the internal condition of cold storage, to observe disease incidence and to find out the causal organism of bacterial and fungal diseases of potato. The experiments were as follows:

I. Survey on the cold storages to observe the internal condition of storage room.

II. Isolation and identification of fungus and bacteria in the laboratory through cultural, morphological and biochemical tests.

III. Calculation of disease incidence of fungus and bacteria having visible and invisible symptoms of potato.

3.1. Experiment I. Survey on the cold storages to observe the internal condition of storage room

3.1.1 Survey on cold storages

Three cold storages of Nilphamari district were visited viz. Shawon cold storage, Angkur seed & cold storage and Mukta cold storage. The survey was done in each month from July, 2018 to Dec, 2018.

3.1.2. Observation of internal conditions

Different methods and equipment's were used to keep the potato at required environment inside the storage room. Temperature (c), Relative humidity (%), Ammonia supply (Hrs), Oxygen supply and Carbon di-oxide removal (Hrs) were observed for all three-cold storage in each month.

3.2 Experiment II. Isolation and identification of fungi and bacteria in the laboratory through cultural, morphological and biochemical tests.

3.2.1. Collection of potato tuber

Potatoes were collected from Shawon cold storage, Angkur seed & cold storage and Mukta cold storage. Potatoes were taken from the pile of rotten potato. For visual identification, 20 rotten potatoes were taken randomly to categorized while it was fungal or bacterial. Tubers were stored at 4^0C prior to experiment following by washing with water to clear dirt and mud.

3.2.2. Selection of area and period

Potatoes were collected from three different cold storages of Nilphamari district. No variety were taken under consideration while potatoes were collected. And the experiments were carried out in Plant Pathology Department, Faculty of Agriculture, Sher-e-Bangla Agriculture University during the period of July, 2018 to December, 2018.

3.2.3. Observation of the symptoms

According to Champoiseau *et al*. (2009), "Symptoms of the disease were studied by visual observation as per standard procedure. Samples were visually observed for the symptoms of bacterial and fungal diseases. Finally, the identification was done by isolation, microscopic view of fungal structures and different biochemical test.

3.2.4. Isolation and identification fungi in laboratory

3.2.4.1. Preparation of potato dextrose medium

Potato Dextrose Agar (PDA) medium was used to isolate fungus from diseased potato. To prepare potato infusion, boil 200 g sliced & unpeeled potatoes in 1liter distilled water for 30 min. Filtered through cheesecloth, saving effluent, which was potato infusion (or use commercial dehydrated form). Mixed with Dextrose, Agar and Water and boiled to dissolve. Autoclaved 15 min at 121^0C. Dispensed 20-25 ml portions into sterile petri dishes to make it ready for fungal growth.

3.2.4.2. Isolation of fungi

Infected potatoes were washed with tap water in order to remove all adhering soil particles. Potato pieces approximately 100mm x 25-50mm were cut with healthy portion, washed in sterile distilled water for 5 min, subjected to surface sterilization in 1% NaOCl for 30 sec. Then, they were transferred moist for incubation. After few days, fungus grew on rotted tissues were isolated aseptically and transferred to PDA media. Pure cultures were obtained by sub-culturing the fungal growth in separate petri-dishes containing the same medium. Then the *Fusarium oxysporum* were identified by following the key outline of Booth (1977) and *Rhizopus stolonifer* was identified by the key outline of Zheng *et al*., (2007).

3.2.5. Isolation and identification of bacteria

3.2.5.1 Preparation of Nutrient Agar (NA)

At first dehydrated Nutrient Agar (28g) was taken in an Erlenmeyer flask which contained 1000ml distilled water and shaken to mix them well for few mins. Then the mouth of the flask was covered with aluminum foil paper and tight with a thin rope. It was then autoclaved at 121^0C at 15 Ibs pressure for 15 mins. Then the media was taken to laminar air flow to cool down. When the media become near about 50-55^0C, then it was poured on sterilized glass petri-dish for making NA plates. Nutrient agar media was prepared by method of Schaad (1988).

3.2.5.2. Preparation of Triphenyl Tetrazolium Chloride (TTC) Solution

Triphenyl tetrazolium chloride was prepared according to method followed by Schaad (1988). For the preparation of 1% TTC medium at first 0.1 g 2,3,5 triphenyl tetrazolium chloride was taken in an Erlenmeyer flask containing 10ml distilled water. Then it was shaken thoroughly for few minutes. It was then autoclaved at 121^0C under 15 PSI pressure for 15 minutes.

3.2.5.3. Preparation of Triphenyl Tetrazolium Chloride (TTC) media

At first Casamino Acid (1g), Peptone (9g), Glucose (5g) and Agar (17g) were taken in Erlenmeyer flask containing 1000ml of water to make CPG media. Then they were mix thoroughly and cover the mouth of flask with aluminum foil paper following by tightening with thin rope. Then flask then autoclaved at 121^0C for 15 mins at 15 Ibs pressure. Then media was then taken to laminar air flow and mixed 1% 2,3,5 triphenyl tetrazolium chloride solution which was prepared previously by dissolving 1g 2,3,5 triphenyl tetrazolium chloride powder in 100ml distilled water. The 1% TZC solution was also autoclaved before adding with CPZ media. TZC media was prepared by method of Schaad (1988).

3.2.5.4. Isolation of *Ralstonia solanecearum*

The bacterium was isolated by dilution plate method from collected potato samples. According to Goszczynska and Serfontein (1998), diseased potato tubers were first washed with tap water then surface sterilized with 95% ethanol for 3 min then rinsed thoroughly 3 times with sterilized distilled water to remove sterilant. The rotted tissues of tuber were macerated in sterile water to make a bacterial suspension. Tenfold serial dilution was made from the stock solution. 0.1 ml of each dilution was placed on the surface of a nutrient agar plate and distributed using a sterile, L- shaped glass rod at three replications. The inoculated NA plates were incubated at 30^0C and observed after 24 hrs and 48 hrs. A part of a well isolated typical colony was taken using a sterile wire loop and streaked on fresh NA plate to get pure culture.

3.2.5.5. Preservation of bacteria

A slant culture of purified bacteria was done on NA media in small screw-cap test tubes in order to preserve the bacteria for future use and kept it in refrigerator at 4^0C.

3.2.5.6. Identification *Rasltonia solanecearum*

Rasltonia solanecearum was identified based on morphological, cultural (size of colony, form, pigment and elevation) and biochemical features as per standard microbiological procedures and growth of bacteria over semi-selective media.

3.2.5.6.1. Growth on 2,3,5 triphenyl tetrazolium chloride (TTC) media

Ralstonia solanecearum was transferred to TTC (2,3,5 triphenyl tetra zolium chloride) media from NA plates with the help of sterile loop through streaking method. *Ralstonia solanecearum* was identified following the key feature by Schaad (1988).

3.2.5.6.2. Morphological and Biochemical character *Ralstonia solanecearum*

3.2.5.6.2.a. Gram staining

A clean microscope slide was taken and a small drop of distilled water was placed on the slide. A small part of young (18-24hrs old) colony was taken with the help of sterile loop from NA medium and then the bacterial smeared was prepated on the slide. The smear then air dried and heat fixed by passing it through the Bunsen flame. Then the slide was flooded with crystal violet solution for 1 minute followed by rinsing under with tap water for a few seconds and excess water was drained off. Then the slide was flooded with Lugol's iodine solution for 1 minute followed by rinsing under with tap water for a few seconds and excess water was drained off. After that decolorization was done with 95% ethanol for 30 seconds and again rinsed with water and air dried. Then counter-stain with safranine for 10 seconds was done. It was then rinsed with water and dried. Then the glass slide was observed at 100x magnification using oil immersion. The bacterium was identified on the basis of color crystal violet color retaining (Pastor *et al.*,2010).

3.2.5.6.2.b. KOH solubility test

At first 3% KOH solution was made. Then one drop of 3% KOH was taken on a sterilized and clear slide. Then a loopful of bacteria was removed from young colony i.e. 24 hrs old and mixed with 3% KOH for 10 seconds. After a few minutes the loop was lifted from the mixture and observed whether it formed threat of not (Suslow *et al*.,1982).

3.2.5.6.2.c. Catalase test

A few drops of freshly prepared 3% H_2O_2 (Hydrogen peroxide) was placed at the center of a clean glass slide. 48 hours old pure culture of bacterium grown on NA plate was added with it and observed whether the bacteria formed bubbles within a few seconds or not (Schaad, 1988).

3.2.5.6.2.d. Oxidase test

1% aqueous (w/v) solution of NNN'Ntetramethyl-p-phenylene-diamine-dihydrochloride solution was used as test reagent. Whatman filter paper was soaked with reagent and the paper was placed on a petri dish. Then young colony of bacteria was picked up with a sterile tooth pick and rubbed onto the moistened (oxidase reagent) filter paper and observed up to 10 seconds whether it changed color to dark purple or not (Kovacs, 1956).

3.2.5.6.3. Other potato disease such as soft rot and scab

Potato soft rot and scab disease was identified by visual observation of their typical symptoms. Diseased potato samples were selected based on visible symptoms of soft rot and characteristic odor as described by Agrios (1997) and Singh (2001) and scab was identified on the basis of key characteristics by Phillip (2015).

3.3 Experiment III Calculation of disease incidence of fungus and bacteria having visible and invisible symptoms of potato

3.3.1. Disease incidence of potato

The total number of potato and the number of infected potato tuber were counted to calculate the disease incidence (Islam, 1995).

% Potato infection/ Disease incidence = (Number of infected potato X 100) / Total number of potatoes

The diseased tubers from these bags were weighed and these amounts was divided by the total amount observed potatoes and multiplied by 100 so as to find out the parentage of loss due to diseases (Islam, 1995).

Few things were brought under consideration to calculate the disease incidence in cold storage conditions such as-

a) Total number of bags of potato
b) Amount of potato in each bag
c) Amount of rotten potato separated at the time of pre-cooling
d) Average amount of rotten potato from each bag
e) Types of rot or symptom

Then percentage of fungal (Black dot, Dry rot, Leak and Rhizopus rot) disease and bacterial (Soft rot, Scab and Brown rot) disease were calculated in cold storage and in laboratory as well.

3.3.2. Data analysis

Data on disease incidence was recorded from time to time (at one-month intervals) during the tenure of the work for requisite parameter. Data was statistically analyzed to obtain the level of significance using the MSTAT-C package program (Russell, 1986). Duncan's multiple range test (DMRT) was used to compare the treatment means.

CHAPTER IV

RESULTS

4.1 Variation of internal condition of different cold-storages during data collection time.

Acute variation among the internal conditions in different cold-storage and six-month data recording time were observed.

The internal condition of cold-storages were almost same as conditions were kept similar throughout the storage period but a very little variation was observed from the data recording (table 1). The maximum temperature was observed 4.44^0C or 40^0F in the month of November and December at Angkur seed and cold-storage and Mukta cold-storage while minimum temperature was 2.22^0C or 36^0F from July to October at Shawon cold-storage and in September at Angkur seed and cold-storage. Maximum relative humidity (RH%) was recorded 90% in July at Shawon cold-storage, in July, August, November & December in Angkur seed and cold-storage and in July and August at Mukta cold-storage while minimum was 85% from August to December at Shawon cold-storage and September to October at both Angkur and Mukta cold storage. Maximum O_2 supply and CO_2 removal (Hr) was mentioned 13 hours in July at all three cold-storage while minimum was 9 hours from October to December at Mukta cold-storage. Maximum NH_3 supply was mentioned 1 hour in July and October to December at Shawon cold-storage, from July to December except September and July & December at Mukta cold-storage while minimum was found 0.75 hour in August at Shawon cold-storage.

Table 1. Internal conditions of cold-storage during data recording from July 2018 to December 2018.

Name of Cold storage	Month	Temperature (^{0}C/^{0}F)	Relative Humidity (%)	O_2 supply and CO_2 removal (Hr)	NH_3 supply (Hr)
Shawon Cold Storage	July	2.22/36	90	13	1
	August	2.22/36	85	12	0.75
	September	2.22/36	85	12	0.8
	October	2.22/36	85	10	1
	November	3.33/38	85	10	1
	December	3.33/38	85	10	1
Angkur Seed and Cold Storage	July	2.78/37	90	13	1
	August	2.78/37	90	12	1
	September	2.22/36	85	12	0.8
	October	2.78/37	85	10	1
	November	4.44/40	90	10	1
	December	4.44/40	90	10	1
Mukta Cold Storage	July	2.78/37	90	13	1
	August	2.78/37	90	12	0.8
	September	2.78/37	85	12	0.8
	October	2.78/37	85	9	0.8
	November	4.44/40	90	9	0.8
	December	4.44/40	90	9	1

Almost similar temperature (^{0}C/^{0}F) was found in term of monthly data collection of cold-storages. Maximum temperature was 4.44^{0}C or 40^{0}F in the month of November and December in both Angkur and Mukta cold storages where minimum temperature was 2.22^{0}C of 36^{0}F at Shawon cold-storage from July to October.

Table 2. Distribution of temperature among all three cold-storages during July 2018 to December 2018.

Month	Temperature (^{0}C/^{0}F)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	2.22/36	2.78/37	2.78/37
August	2.22/36	2.78/37	2.78/37
September	2.22/36	2.22/36	2.78/37
October	2.22/36	2.78/37	2.78/37
November	3.33/38	4.44/40	4.44/40
December	3.33/38	4.44/40	4.44/40

Almost similar relative humidity (RH%) was found in term of monthly data collection of cold-storages. Maximum RH was 90% in July and in July, August, November and December at both Angkur and Mukta cold storages where minimum RH was 85% at Shawon cold-storage from July to December and in September and October at rest two cold-storages.

Table 3. Distribution of relative humidity (RH%) among all three cold-storages during July 2018 to December 2018.

Month	Relative humidity (%)		
	Shawon cold-storage	Angkur seed and cold-storage	Mukta cold-storage
July	90	90	90
August	85	90	90
September	85	85	85
October	85	85	85
November	85	90	90
December	85	90	90

Almost similar O_2 supply and CO_2 removal were found in term of monthly data collection of cold-storages. Maximum O_2 supply and CO_2 removal was 1 hour while minimum was 0.75 hour.

Table 4. Distribution of O_2 supply and CO_2 removal (Hr) among all three cold-storages during July 2018 to December 2018.

Month	O_2 supply and CO_2 removal (Hr)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	1	1	1
August	0.75	1	0.8
September	0.8	0.8	0.8
October	1	1	0.8
November	1	1	0.8
December	1	1	1

Almost similar NH_3 supply was found in term of monthly data collection of cold-storages. Maximum NH_3 supply was 13 hours in July at all three cold-storages where minimum NH_3 supply was 9 hours at Mukta cold-storage from October to December.

Table 5. Distribution of NH_3 supply (Hr) among all three cold-storages during July 2018 to December 2018.

Month	NH_3 supply (Hr)		
	Shawon cold-storage	Angkur seed and cold-storage	Mukta cold-storage
July	13	13	13
August	12	12	12
September	12	12	12
October	10	10	9
November	10	10	9
December	10	10	9

4.2 Identifying the causal agents of post-harvest diseases of potato

4.2.1. Identification of fungus

Fungus were grown on PDA media to identify them by their colony and morphological characteristics. Colony characters were observed on due time and morphological characters were observed under motic microscope through preparing slide using either glycerin or lactophenol cotton blue.

4.2.1.1. Identification of *Fusarium oxysporum* by cultural and morphological characteristics

After 8 days growth on PDA media, it was seen that, the culture was white in color and growing rapidly. Mycelia was also white and hyaline. Some time it showed pink color pigmentation at the bottom of the petri-dish. Under microscope, it was observed that, microconidia were abandoned but macroconidia were less. Conidiophores are short and single. The fungi were

identified on the basis of macro conidia characteristics which were sickle shaped, blunt ends, thin walled generally 3 septate, fusiform macro conidia with hyaline color.

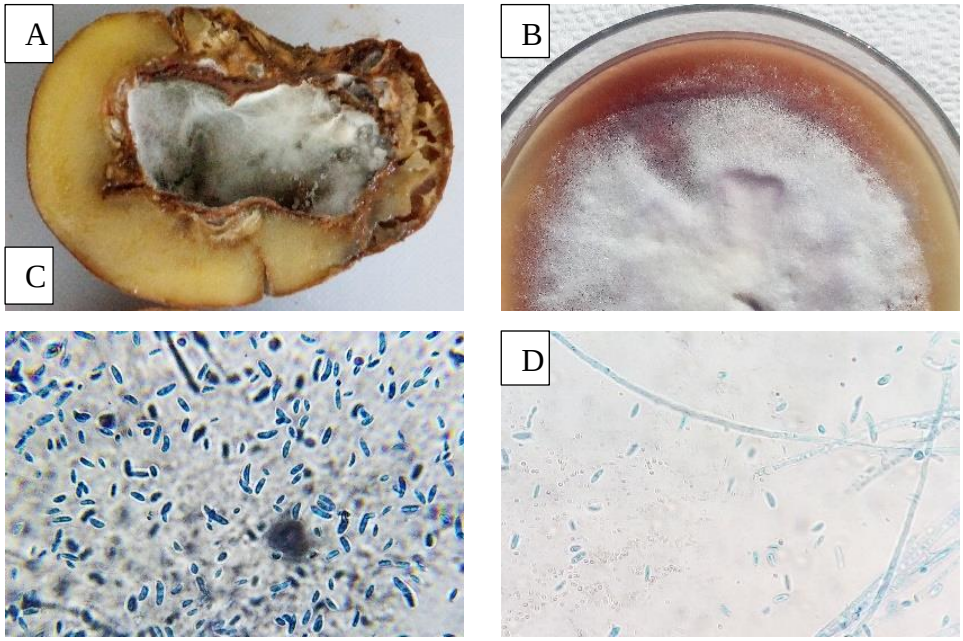

Plate: 1. Cultural and morphological view of F. *oxysporum.* A - showing the diseased potato. B- Culture of *F. oxysporum* on PDA media. C- a number of macro-conidia. D- Hyaline mycelia.

4.2.1.2. Identification of *Rhizopus stolonifer* by cultural and morphological characteristics

The colonies, grown on potato dextrose agar at 25ºC, were white cottony. Then the colony became heavy and brownish black. *Rhizopus stolonifera* produced coenocytic hyphae which were two types: rhizoid and stolon. Sporangiophores were pale to dark brown, usually straight, light brown, smooth-walled, simple, long, and arising in groups. Rhizoids and stolons were dark brown, hyaline abundantly branched rhizoids.

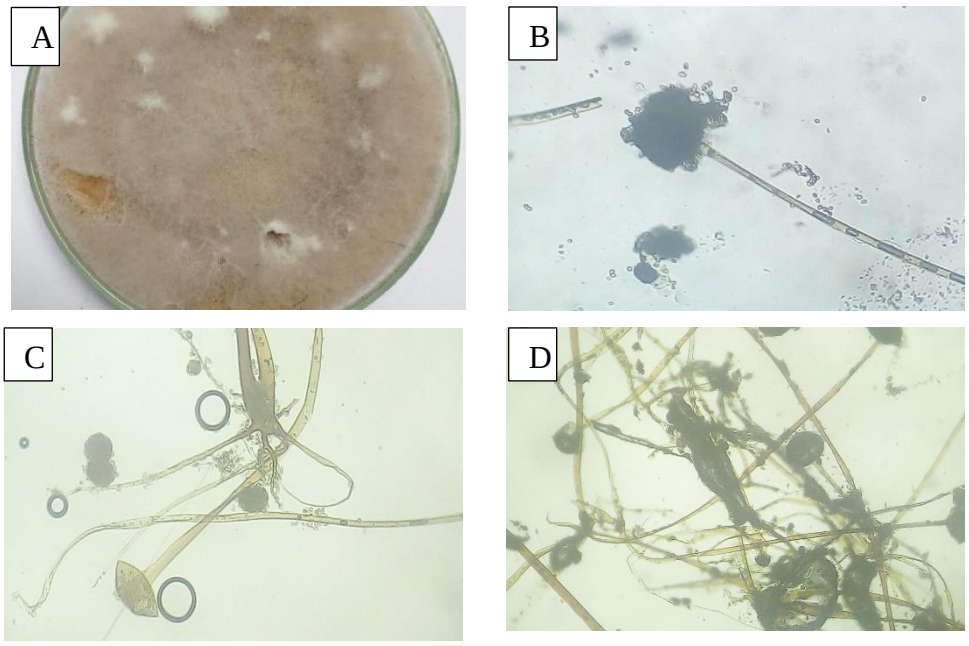

Plate: 2. Cultural and morphological view of *R. stolonifer* A - Culture of *R. stolonifier* on PDA media. B- Sporangia & Conidiophore. C- Rhizoid. D- Compact structure.

4.2.2. Isolation and identification of different bacteria

Bacteria were grown on NA and TZC media to identify them by their colony color, colony size and morphological characteristics.

4.2.2.1. Identification of *Ralstonia solanecearum*

1a. Colony morphology

Bacterium was isolated and cultural & morphological characteristics were observed (Plate:3)**.**

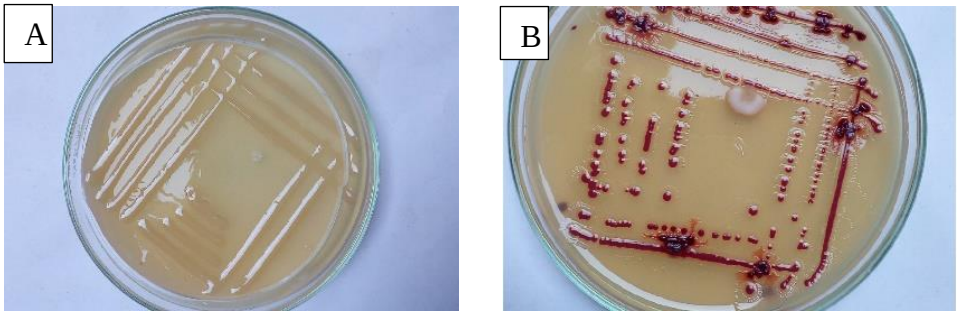

Plate :3. Growth of *R. solanecearum* on nutrient agar and TZC media. A- Culture on NA media. B- Streaking on TZC media showing red color.

Table 6. Cultural characterization of *Ralstonia solanecearum* on NA plates

Name of cultural character	Observation
Colony size	Moderate
Form	Circular
Pigmentation	Creamy white
Elevation	Convex

1b. Biochemical tests of *Ralstonia solanecearum*

The isolated bacteria, *Ralstonia solanecearum*, was confirmed by different biochemical tests. After a series of reaction test a red color was developed, under microscope, which indicated as it was a Gram-negative bacterium. In KOH solubility test, a mucoid thread was produced when lifted with the help of toothpick. Bubbles formed by bacteria resulting positive catalase test when a few

drops of freshly prepared 3% H_2O_2 added with pure culture of bacterium grown on NA plate. The bacteria didn't form dark purple color on oxidase test.

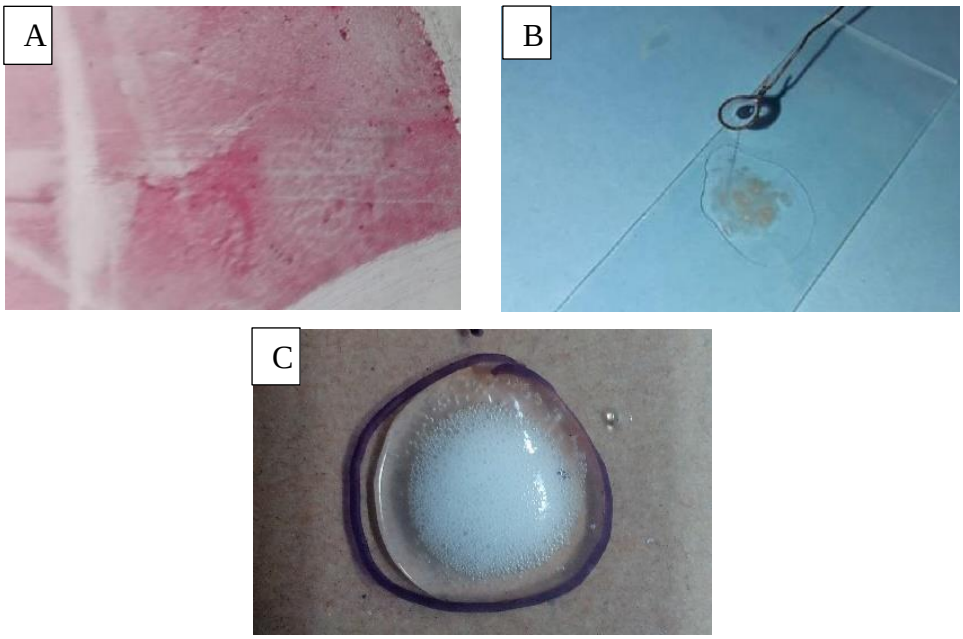

Plate: 4. Biochemical test of *R. solanecearum*. A- Gram staining test. B- KOH test. And C- Catalase test

Table 7. Characteristics of isolated *R. solanecearum* to different tests are listed below.

Name of tests	Reaction
Gram staining	-
KOH solubility test	+
Catalase test	+
Oxidase test	-

4.2.2.2. Identification of other disease such as soft rot and scab of potato

The soft rot disease was identified easily as rotten potato produced a bad odor. The rotten potato became watery and very soft. A black border developed and it separated the diseased area and healthy area. A hole is formed while pressing the diseased infected potato with finger.

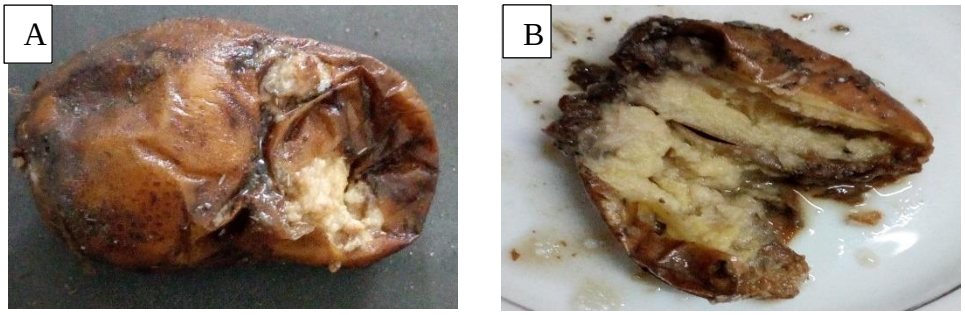

Plate: 5. Symptoms of soft rot. A- Potato become soft. B- Potato become watery.

The scab of potato developed cork like lesion. Mostly the lesions were circular. A shallow pitted lesion was observed. A characteristic raised corky area also found. Sometime lesion covered the whole surface of the potato.

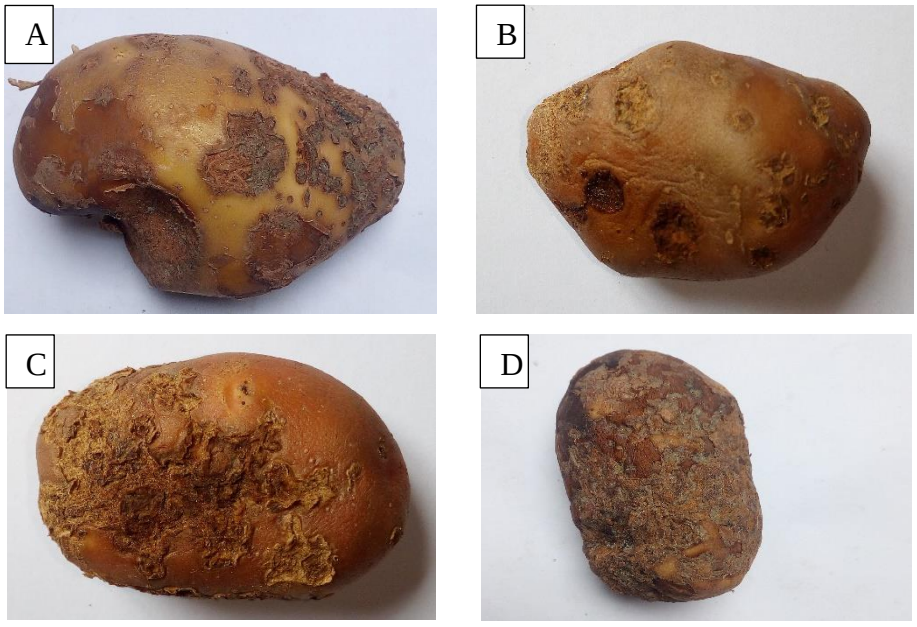

Plate: 6. Symptoms of scab. A- Normal corkey lesion. B- Pitted lesion. C- Raised corkey lesion and D- Severe infection.

4.3 Effect of different internal condition of cold-storages on the incidence of different diseases of potato.

Effect of different intenal condition (temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply) on incidence of different disease at different cold-storages were observed.

Table 8. Incidence of dry rot of potato among three cold-storages during the period of July 2018 to December 2018.

Month	Incidence of dry rot of potato (%)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	0.85a	0.50a	0.54a
August	0.60b	0.42b	0.30b
September	0.55b	0.30d	0.30b
October	0.40c	0.40c	0.30b
November	0.40c	0.30d	0.30b
December	0.30d	0.20e	0.30b
LSD (0.05)	0.10	0.02	0.06
CV (%)	11.25	5.78	9.76

Significant variations of the incidence of dry rot disease of potato was found due to variation to temperature, RH%, O_2 & CO_2 supply and N_2 supply. In case of dry rot disease of potato, maximum incidence was found 0.85% at Shawon cold-storage in July at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand, minimum incidence was 0.20% at Angkur seed and cold-storage in September at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 2.22^0C or 36^0F, 85%, 0.80 hour and 12 hours (Table 1) respectively.

4.3.1a Incidence of dry rot of potato at Shawon cold-storages during July 2018 to December 2018.

The incidence of dry rot disease varied significantly from month to month at Shawon cold-storage which ranges 0.85% to 0.30% (Table 12). Maximum disease incidence recorded in July 0.85% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^{0}C or 36^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.30% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 3.33^{0}C or 38^{0}F, 85%, 1 hour and 9 hours (Table 1) respectively.

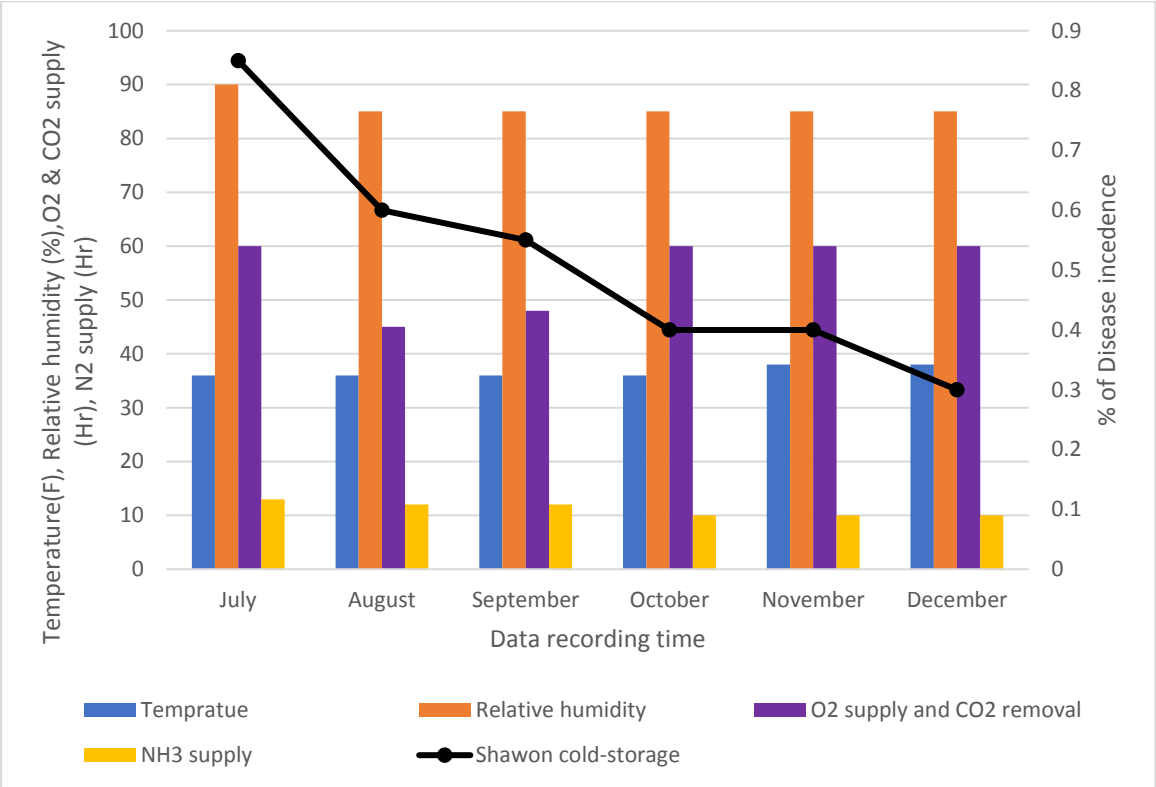

Figure: 1. Effect of internal condition at Shawon cold-storage on incidence of dry rot disease of potato during storage period July 2018 to December 2018.

4.3.1b Incidence of dry rot of potato at Angkur seed and cold-storages during July 2018 to December 2018.

The incidence of dry rot disease varied significantly from month to month at Angkur seed & cold-storage which ranges 0.50% to 0.20% (Table 12). Maximum disease incidence recorded in July 0.85% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.20% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 2.22^{0}C or 36^{0}F, 85%, 0.80 hour and 12 hours (Table 1) respectively.

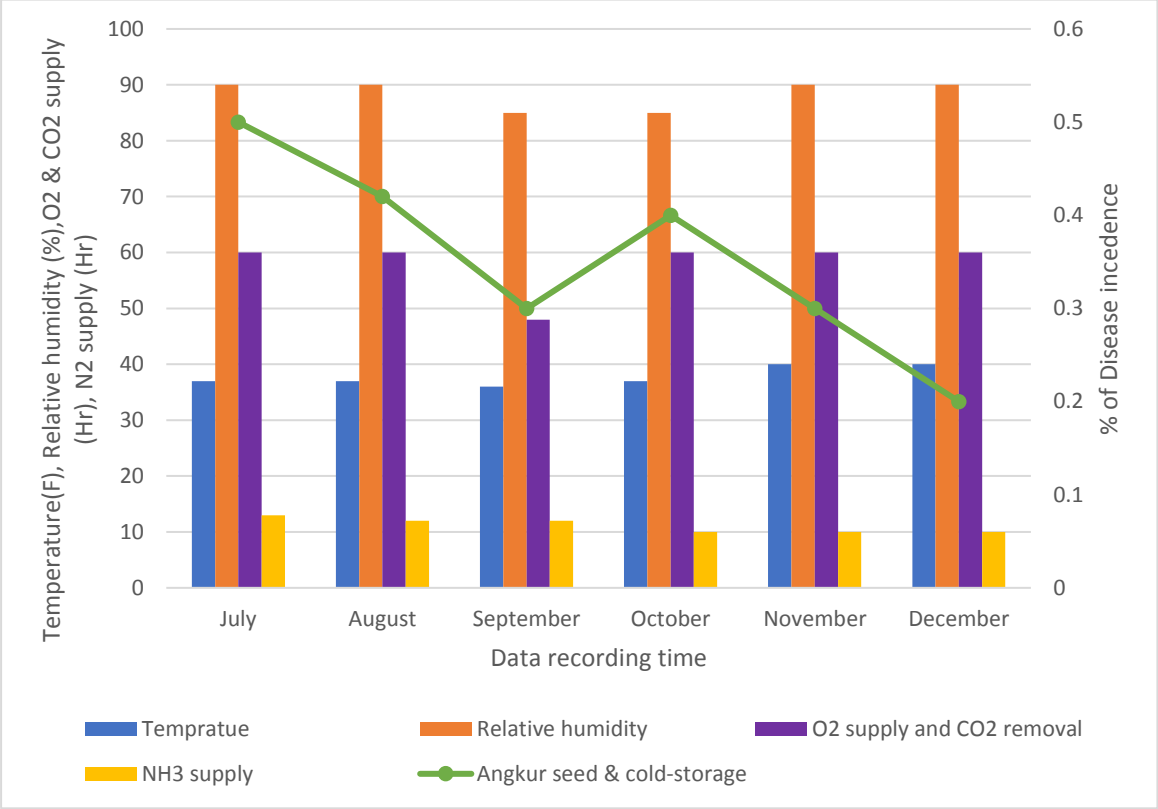

Figure: 2. Effect of internal condition at Angkur seed and cold-storage on incidence of dry rot disease of potato during storage period July 2018 to December 2018.

4.3.1c Incidence of dry rot of potato at Mukta cold-storages during July 2018 to December 2018.

The incidence of dry rot disease varied significantly from month to month at Mukta cold-storage which ranges 0.54% to 0.30% (Table 12). Maximum disease incidence recorded in July 0.54% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.30% from August to December.

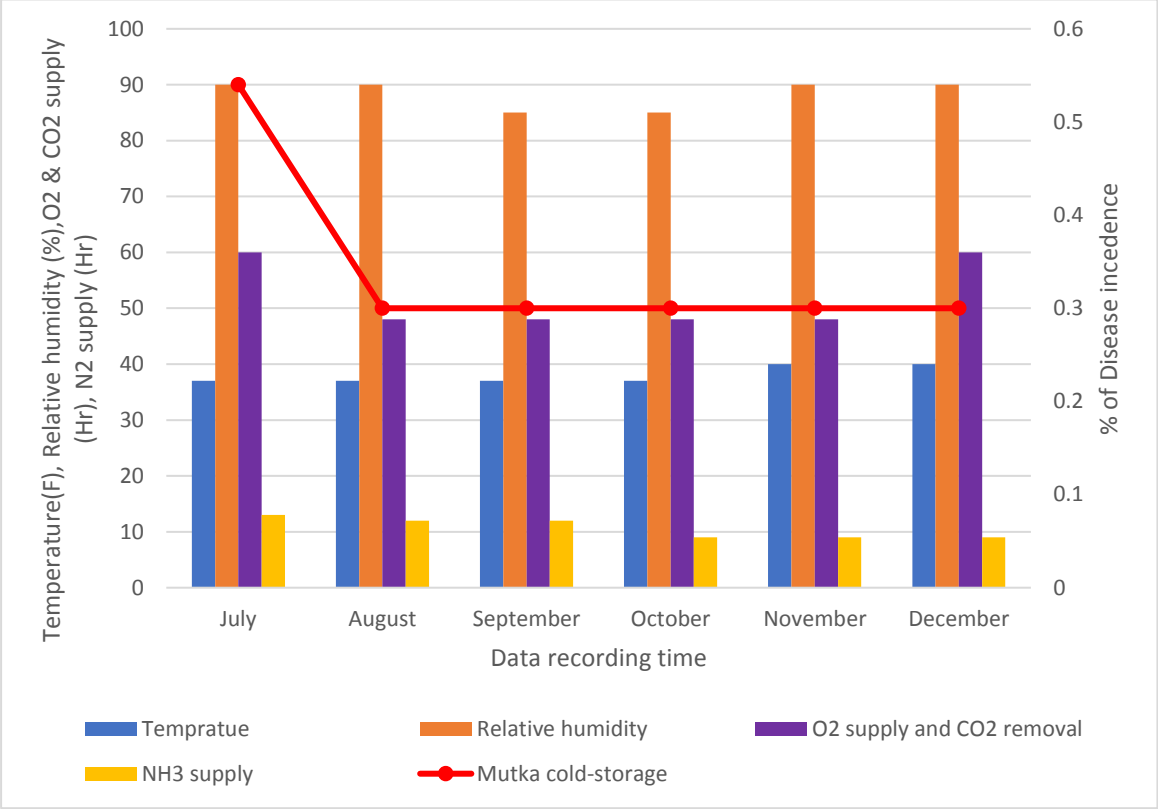

Figure: 3. Effect of internal condition at Mukta cold-storage on incidence of dry rot disease of potato during storage period July 2018 to December 2018.

Table 9. Incidence of Rhizopus rot of potato among three cold-storages during the period of July 2018 to December 2018.

Month	Incidence of Rhizopus rot of potato (%)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	0.40a	0.19a	0.19a
August	0.15b	0.19a	0.10b
September	0.15b	0.10c	0.00c
October	0.10b	0.00c	0.10b
November	0.00c	0.00c	0.10b
December	0.00c	0.00b	0.00c
LSD (0.05)	0.06	0.02	0.02
CV (%)	16.90	14.90	14.45

Significant variations of the incidence of rhizopus rot disease of potato was found on different data recording months. In case of rhizopus rot disease of potato, maximum incidence was found 0.40% at Shawon cold-storage in July at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand, minimum incidence was 0.00% at Shawon cold-storage in November and December, at Angkur seed and cold-storage from September to November and at Mukta cold storage in September and December.

4.3.2a Incidence of Rhizopus rot of potato at Shawon cold-storage during July 2018 to December 2018.

The incidence of rhizopus rot disease varied significantly from month to month at Shawon cold-storage which ranges 0.85% to 0.30% (Table 14). Maximum disease incidence recorded in July 0.33% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.20% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 3.33^0C or 38^0F, 85%, 1 hour and 9 hours (Table 1) respectively.

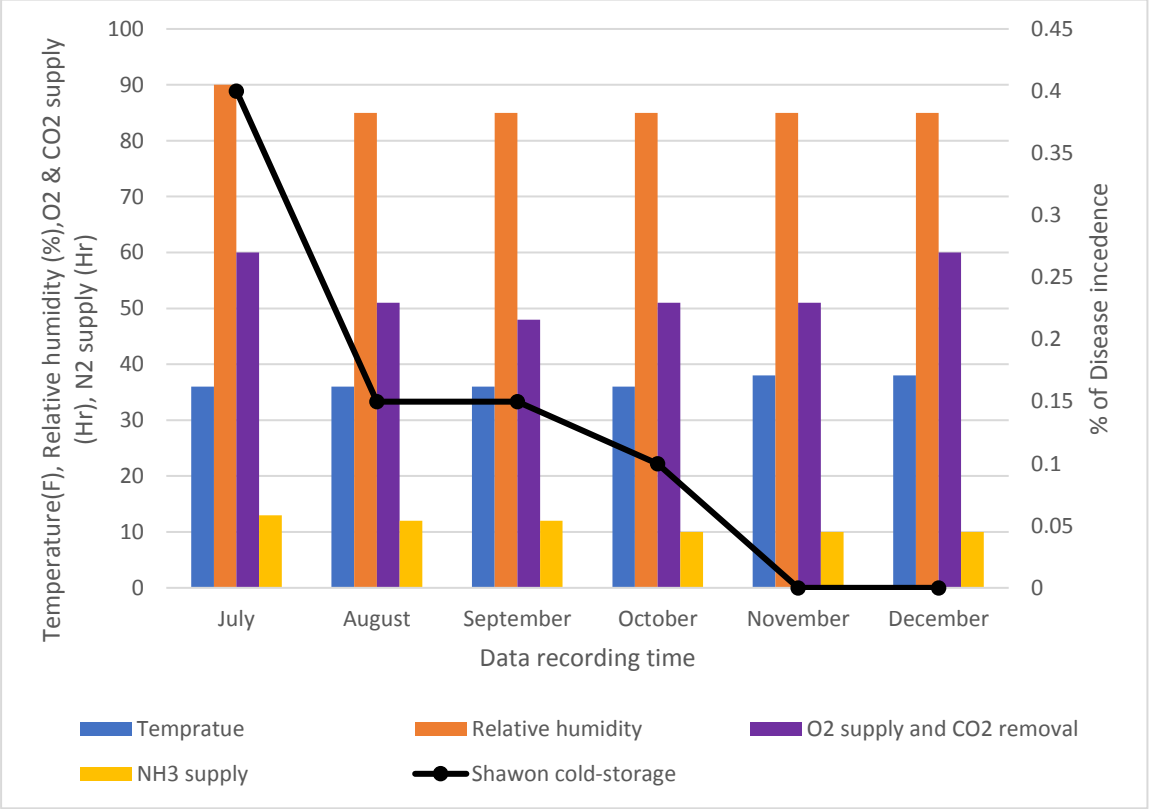

Figure: 4. Effect of internal condition at Shawon cold-storage on incidence of rhizopus rot disease of potato during storage period July 2018 to December 2018.

4.3.2b Incidence of Rhizopus rot of potato at Angkur seed and cold-storage during July 2018 to December 2018.

The incidence of rhizopus rot disease varied significantly from month to month at Angkur seed & cold-storage which ranges 0.19% to 0.00% (Table 14). Maximum disease incidence recorded in July 0.19% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^0C or 37^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.00% from October to December.

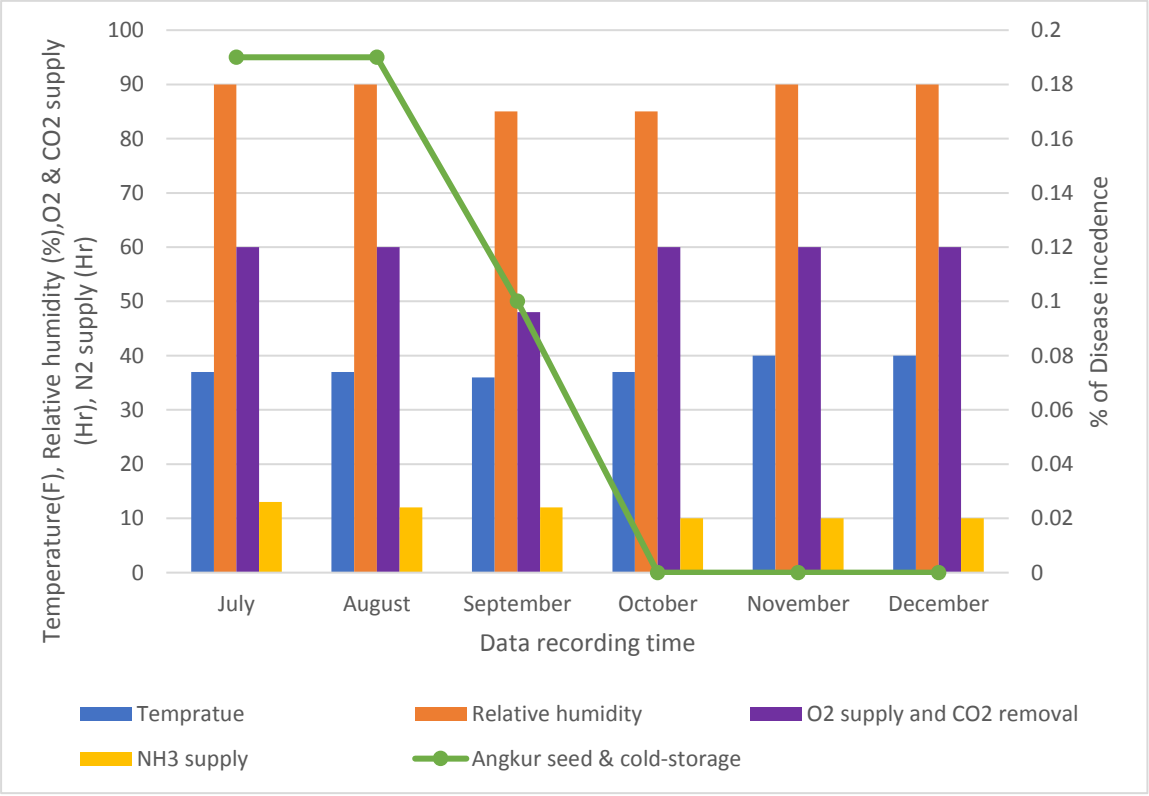

Figure: 5. Effect of internal condition at Angkur seed & cold-storage on incidence of Rhizopus rot disease of potato during storage period July 2018 to December 2018.

4.3.2c Incidence of Rhizopus rot of potato at Mukta cold-storages during July 2018 to December 2018.

The incidence of rhizopus rot disease varied significantly from month to month at Mukta cold-storage which ranges 0.19% to 0.00% (Table 14). Maximum disease incidence recorded in July 0.19% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.0% in November and December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 4.44^{0}C or 40^{0}F, 90%, 1 hour and 9 hours (Table 1) respectively.

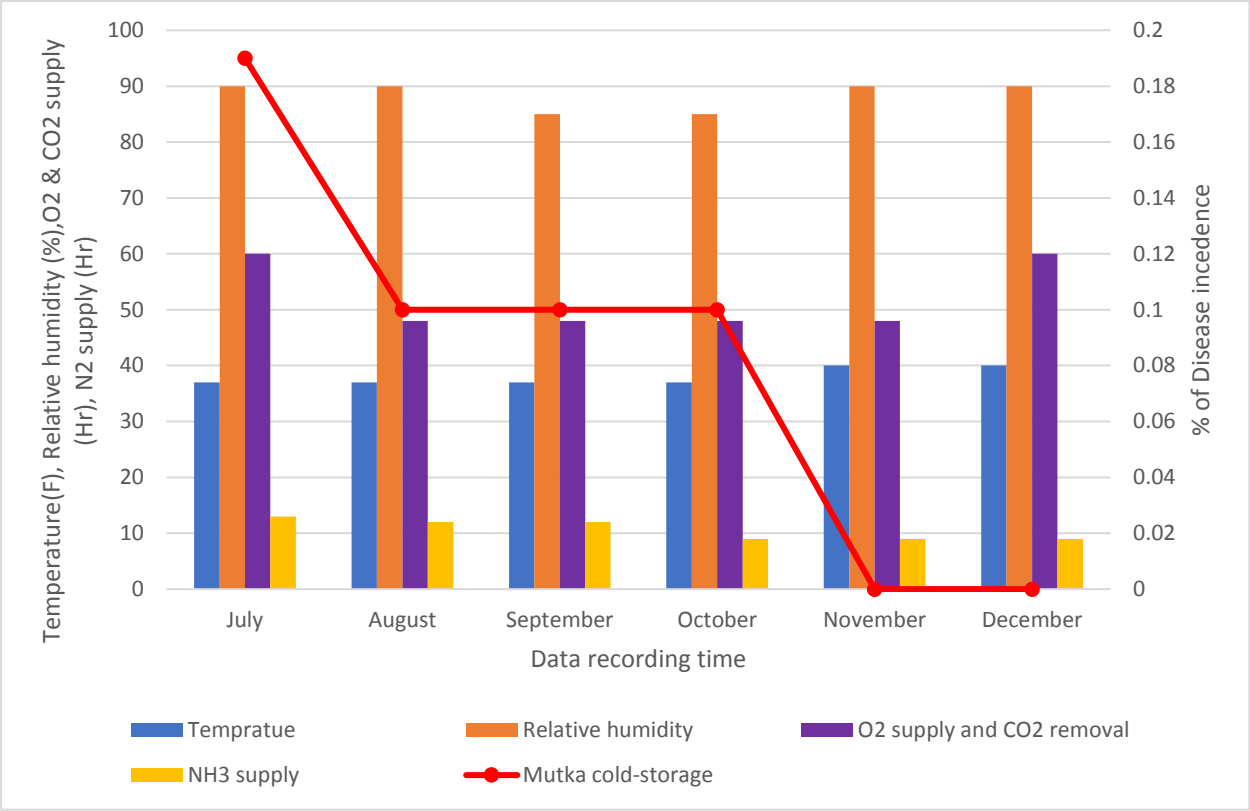

Figure: 6. Effect of internal condition at Mukta cold-storage on incidence of rhizopus rot disease of potato during storage period July 2018 to December 2018.

Table 10. Incidence of Soft rot of potato among three cold-storages during the period of July 2018 to December 2018

Month	Incidence of soft rot of potato (%)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	0.50a	0.43a	0.35b
August	0.40b	0.30b	0.40a
September	0.40b	0.18c	0.18d
October	0.18d	0.18c	0.28c
November	0.12d	0.18c	0.18d
December	0.12d	0.11d	0.10e
LSD (0.05)	0.06	0.02	0.02
CV (%)	9.01	9.20	7.20

Significant variations of the incidence of soft rot disease of potato was found due to on different data recording months. In case of dry rot disease of potato, maximum incidence was found 0.43% at Angkur seed and cold-storage in July at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand, minimum incidence was 0.10% at Mukta cold-storage in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply supply 4.44^0C or 40^0F, 90%, 1 hour and 9 hours (Table 1) respectively.

4.3.3a Incidence of soft rot of potato at Shawon cold-storages during July 2018 to December 2018.

The incidence of soft rot disease varied significantly from month to month at Shawon cold-storage which ranges 0.50% to 0.12% (Table 16). Maximum disease incidence recorded in July 0.50% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^{0}C or 36^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.12% in November & December.

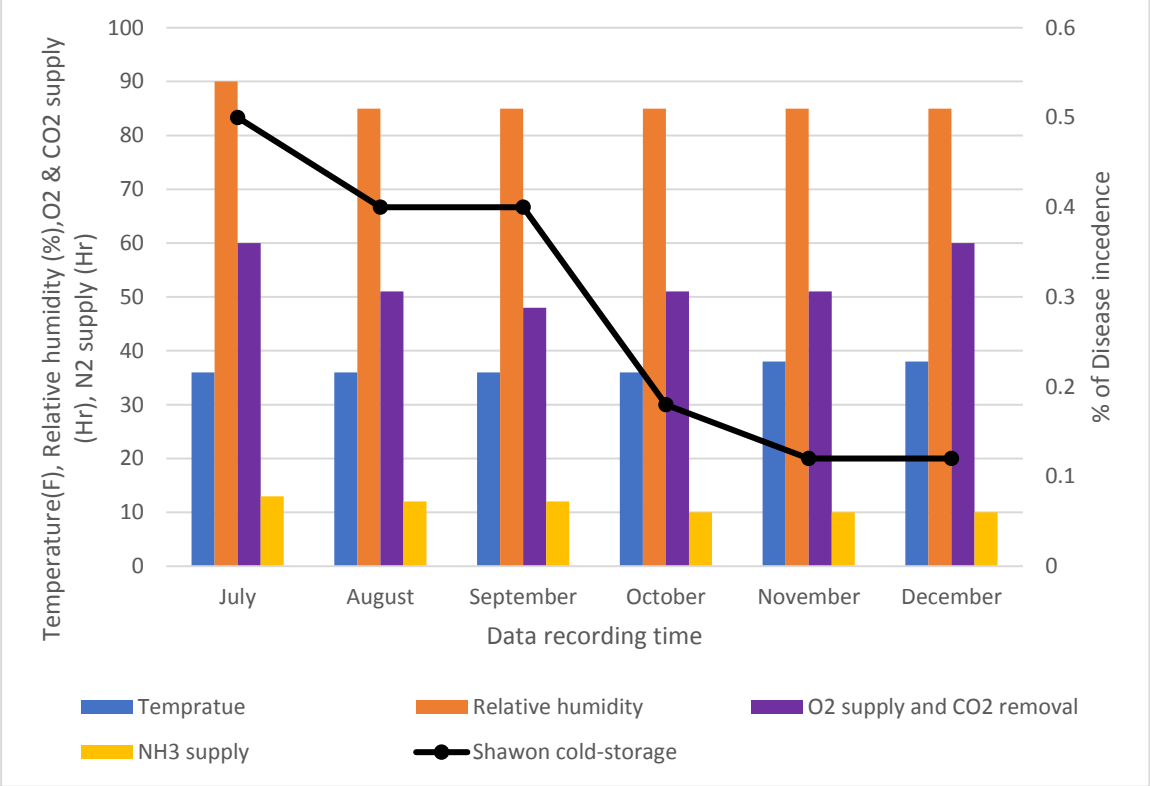

Figure: 7. Effect of internal condition at Shawon cold-storage on incidence of soft rot disease of potato during storage period July 2018 to December 2018.

4.3.3b Incidence of soft rot of potato at Angkur seed and cold-storages during July 2018 to December 2018.

The incidence of soft rot disease varied significantly from month to month at Angkur seed & cold-storage which ranges 0.43% to 0.11% (Table 16). Maximum disease incidence recorded in July 0.43% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.11% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 2.78^{0}C or 37^{0}F, 85%, 1 hour and 10 hours (Table 1) respectively.

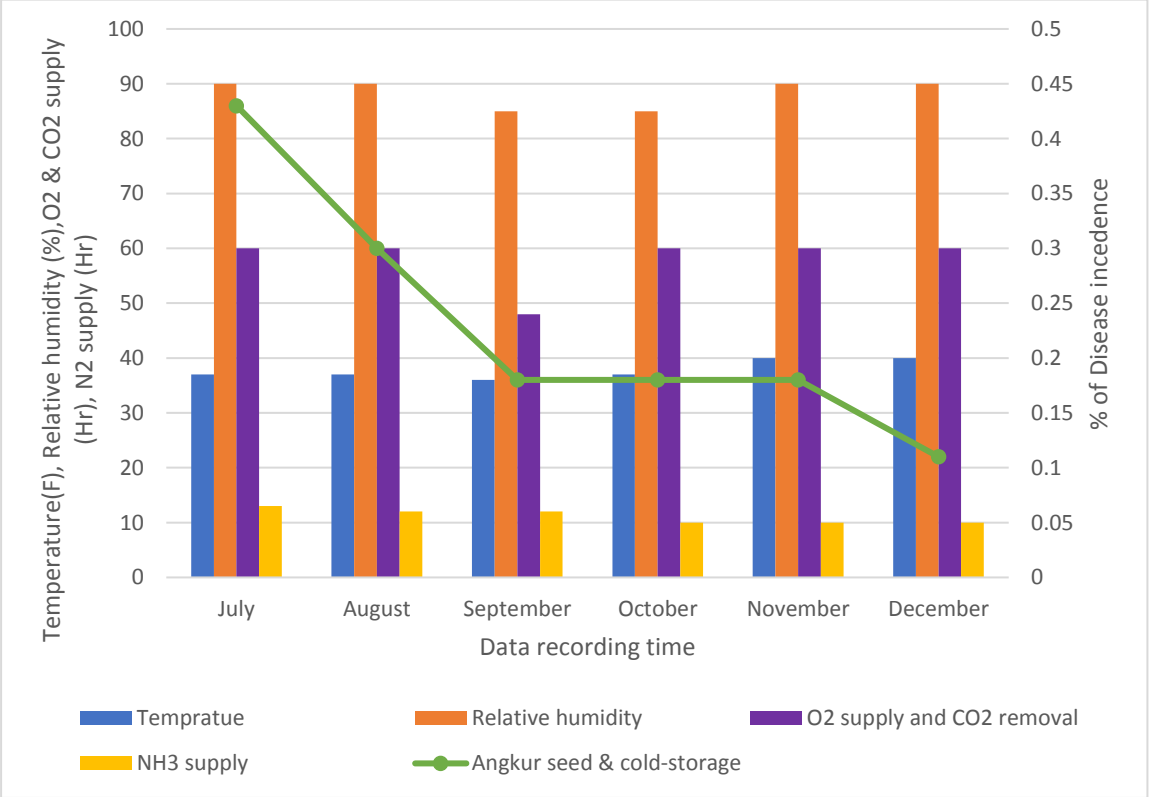

Figure: 8. Effect of internal condition at Angkur seed & cold-storage on incidence of soft rot disease of potato during storage period July 2018 to December 2018.

4.3.3c Incidence of soft rot of potato Mukta cold-storages during July 2018 to December 2018.

The incidence of soft rot disease varied significantly from month to month at Mukta cold-storage which ranges 0.40% to 0.10% (Table 16). Maximum disease incidence recorded in July 0.19% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 0.8 hour and 12 hours (Table 1) respectively. On the other hand minimum incidence was 0.10% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 4.44^{0}C or 40^{0}F, 90%, 1 hour and 9 hours (Table 1) respectively.

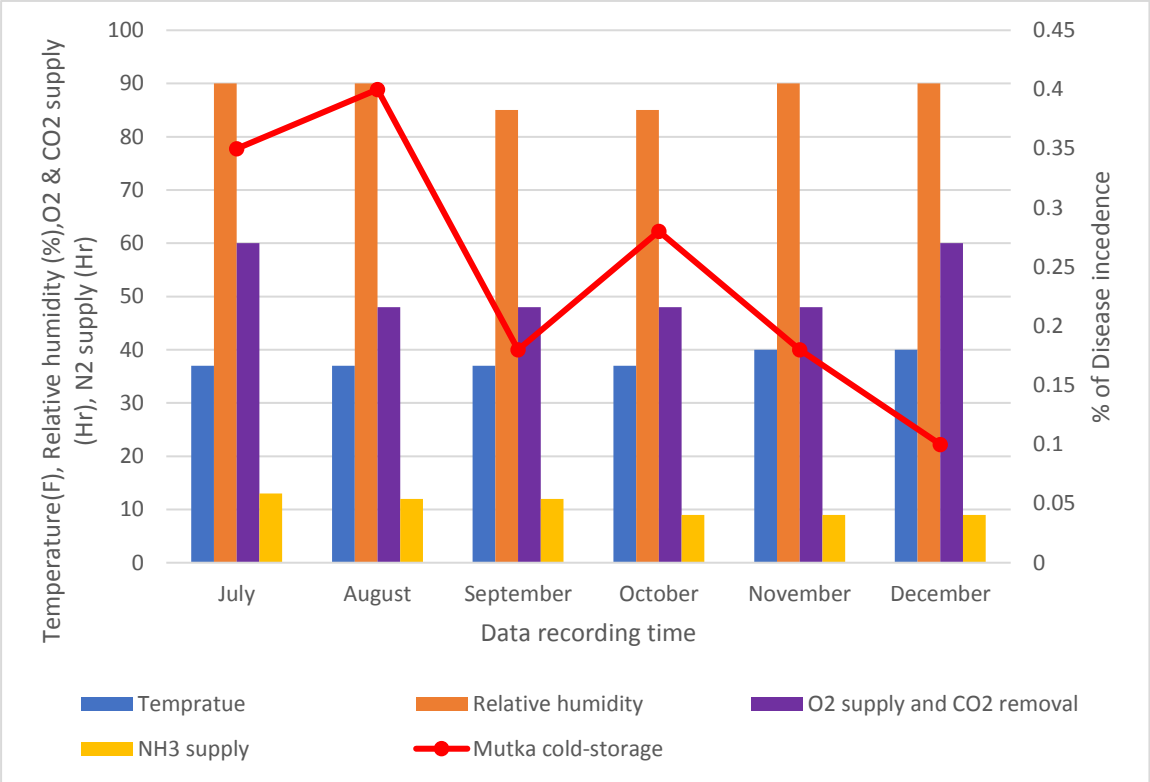

Figure: 9. Effect of internal condition at Mukta cold-storage on incidence of Soft rot disease of potato during storage period July 2018 to December 2018.

Table 11. Incidence of scab of potato among three cold-storages during the period of July 2018 to December 2018.

Month	Incidence of scab of potato (%)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	0.50a	0.32a	0.40a
August	0.34b	0.29b	0.20b
September	0.30b	0.20b	0.20b
October	0.30 b	0.29a	0.19b
November	0.30b	0.30a	0.19b
December	0.30b	0.20a	0.19b
LSD (0.05)	0.06	0.06	0.02
CV (%)	9.45	8.31	9.60

Significant variations of the incidence of scab disease of potato was found on different data recording months. In case of dry rot disease of potato, maximum incidence was found 0.50% at Shawon cold-storage in July at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand, minimum incidence was 0.19% at Mukta cold-storage from October to December.

4.3.4a Incidence of scab disease of potato at Shawon cold-storage during July 2018 to December 2018.

The incidence of scab disease varied significantly from month to month at Shawon cold-storage which ranges 0.50% to 0.30% (Table 17). Maximum disease incidence recorded in July 0.50% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^{0}C or 36^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.30% from September to December.

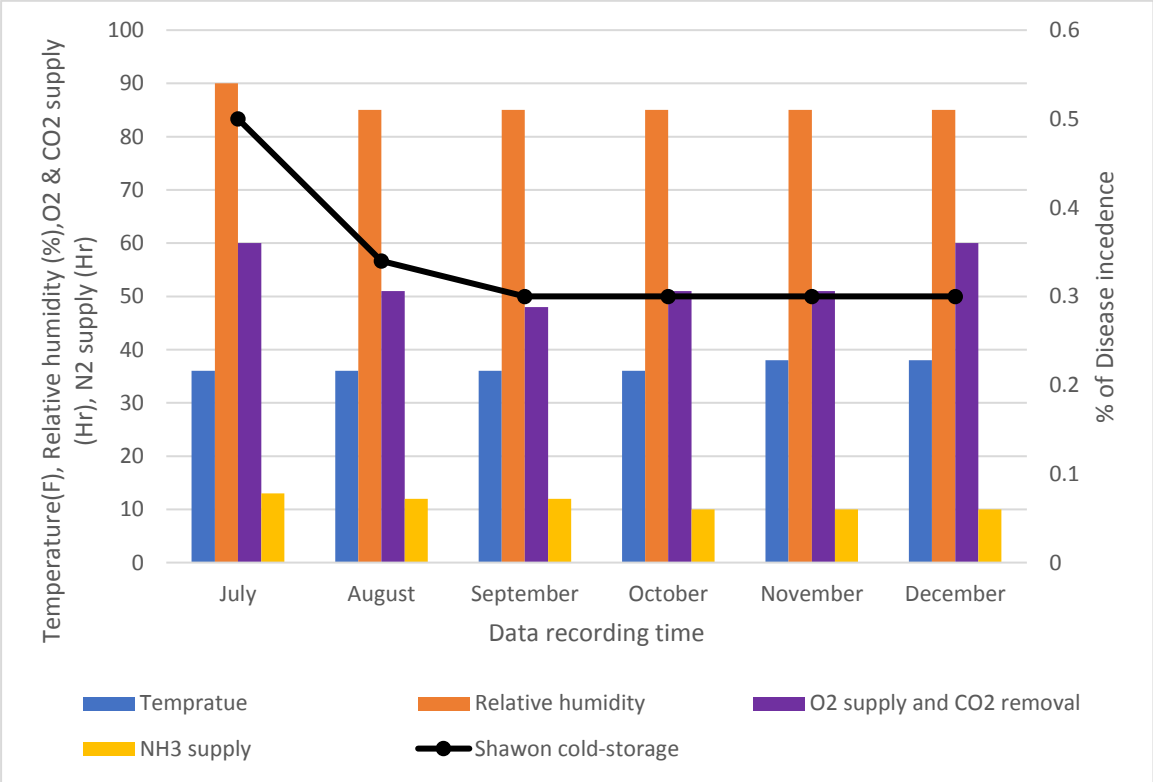

Figure: 10. Effect of internal condition at Shawon cold-storage on incidence of scab disease of potato during storage period July 2018 to December 2018.

4.3.4b Incidence of scab of potato at Angkur seed and cold-storage during July 2018 to December 2018.

The incidence of scab disease varied significantly from month to month at Angkur seed & cold-storage which ranges 0.32% to 0.20% (Table 17). Maximum disease incidence recorded in July 0.32% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.20% in September & December.

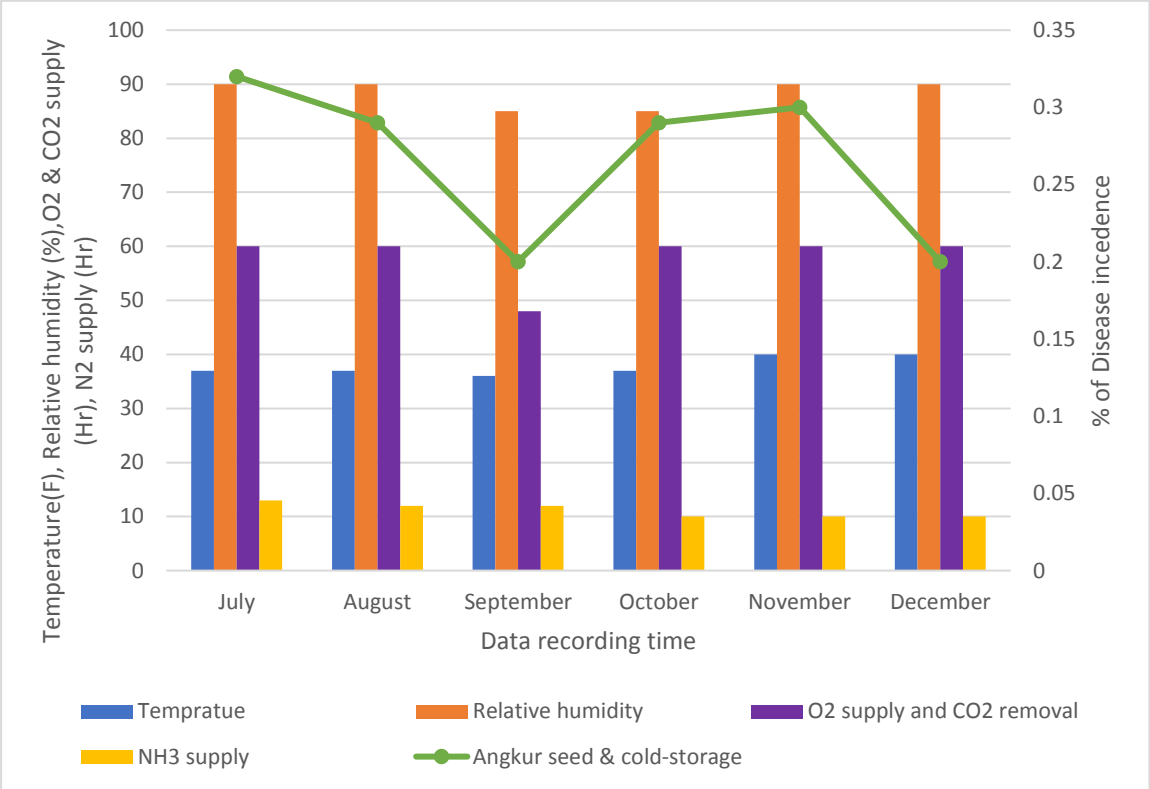

Figure: 11. Effect of internal condition at Angkur seed & cold-storage on incidence of scab disease of potato during storage period July 2018 to December 2018.

4.3.4c Incidence of scab of potato at Mukta cold-storage during July 2018 to December 2018.

The incidence of scab disease varied significantly from month to month at Mukta cold-storage which ranges 0.40% to 0.19% (Table 17). Maximum disease incidence recorded in July 0.40% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^0C or 37^0F, 90%, 0.8 hour and 12 hours (Table 1) respectively. On the other hand minimum incidence was 0.19% from October to December at temperature.

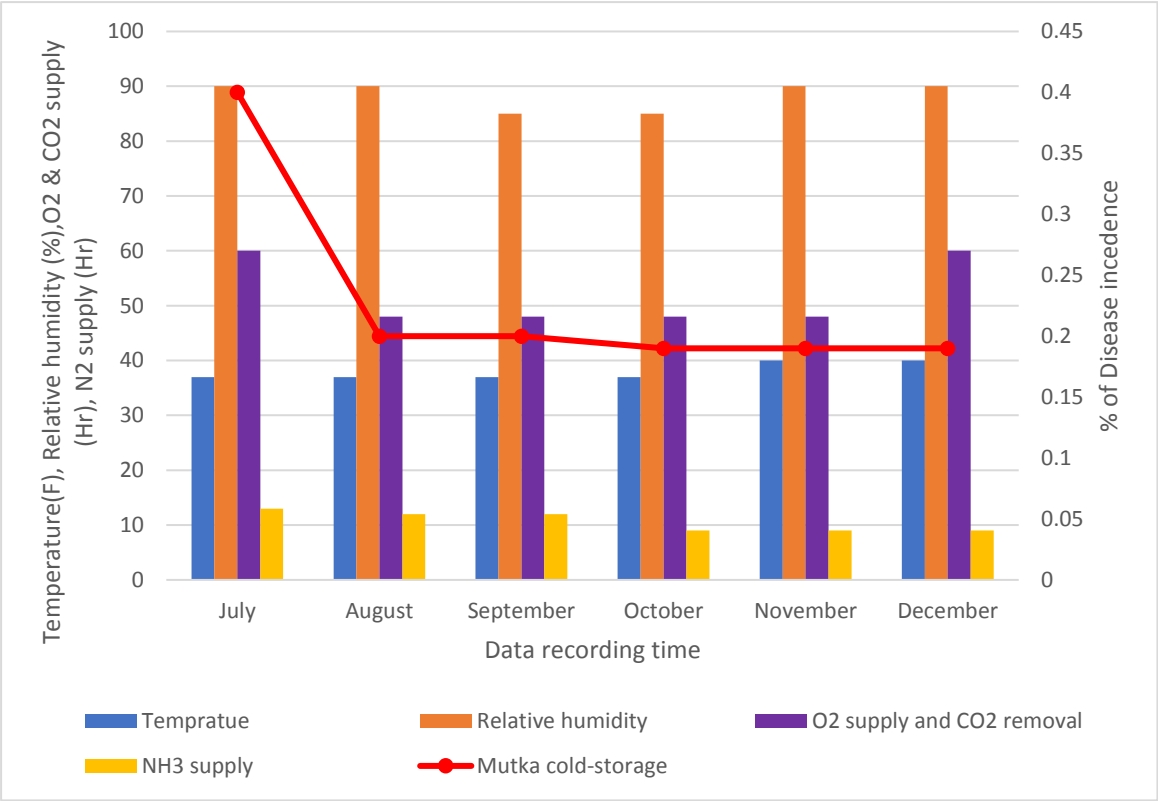

Figure: 12. Effect of internal condition at Mukta cold-storage on incidence of scab disease of potato during storage period July 2018 to December 2018

Table 12. Incidence of Brown rot of potato among three cold-storages during the period of July 2018 to December 2018.

Month	Incidence of brown rot of potato (%)		
	Shawon cold-storage	Angkur seed & cold-storage	Mukta cold-storage
July	0.62a	0.30a	0.30a
August	0.20c	0.12c	0.20b
September	0.30 b	0.12c	0.18c
October	0.18d	0.18b	0.12d
November	0.18d	0.12c	0.12d
December	0.12e	0.11c	0.12d
LSD (0.05)	0.02	0.02	0.02
CV (%)	6.60	9.76	9.69

Significant variations of the incidence of brown rot disease of potato was found on different data recording months. In case of dry rot disease of potato, maximum incidence was found 0.62% at Shawon cold-storage in July at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand, minimum incidence was 0.11% at Angkur seed and cold-storage in August at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 2.78^0C or 37^0F, 90%, 1 hour and 12 hours (Table 1) respectively.

4.3.5a Incidence of Brown rot of potato at Shawon cold-storage during July 2018 to December 2018.

The incidence of brown rot disease varied significantly from month to month at Shawon cold-storage which ranges 0.62% to 0.12% (Table 18). Maximum disease incidence recorded in July 0.62% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.22^0C or 36^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.12% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 3.33^0C or 36^0F, 85%, 1 hour and 10 hours (Table 1) respectively.

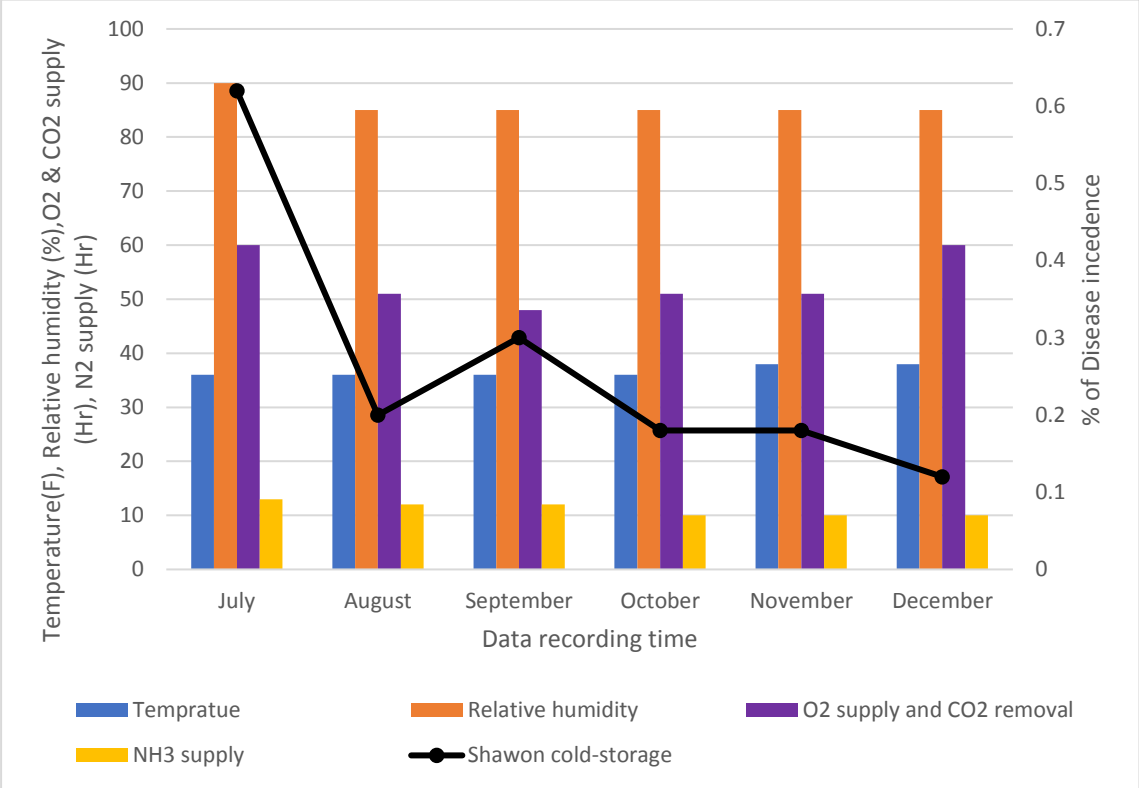

Figure: 13. Effect of internal condition at Shawon cold-storage on incidence of brown rot disease of potato during storage period July 2018 to December 2018.

4.3.5b Incidence of Brown rot of potato Angkur seed and cold-storages during July 2018 to December 2018.

The incidence of brown rot disease varied significantly from month to month at Angkur seed & cold-storage which ranges 0.30% to 0.11% (Table 18). Maximum disease incidence recorded in July 0.43% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^0C or 37^0F, 90%, 1 hour and 13 hours (Table 1) respectively. On the other hand minimum incidence was 0.11% in December at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply 2.78^0C or 37^0F, 90%, 1 hour and 12 hours (Table 1) respectively.

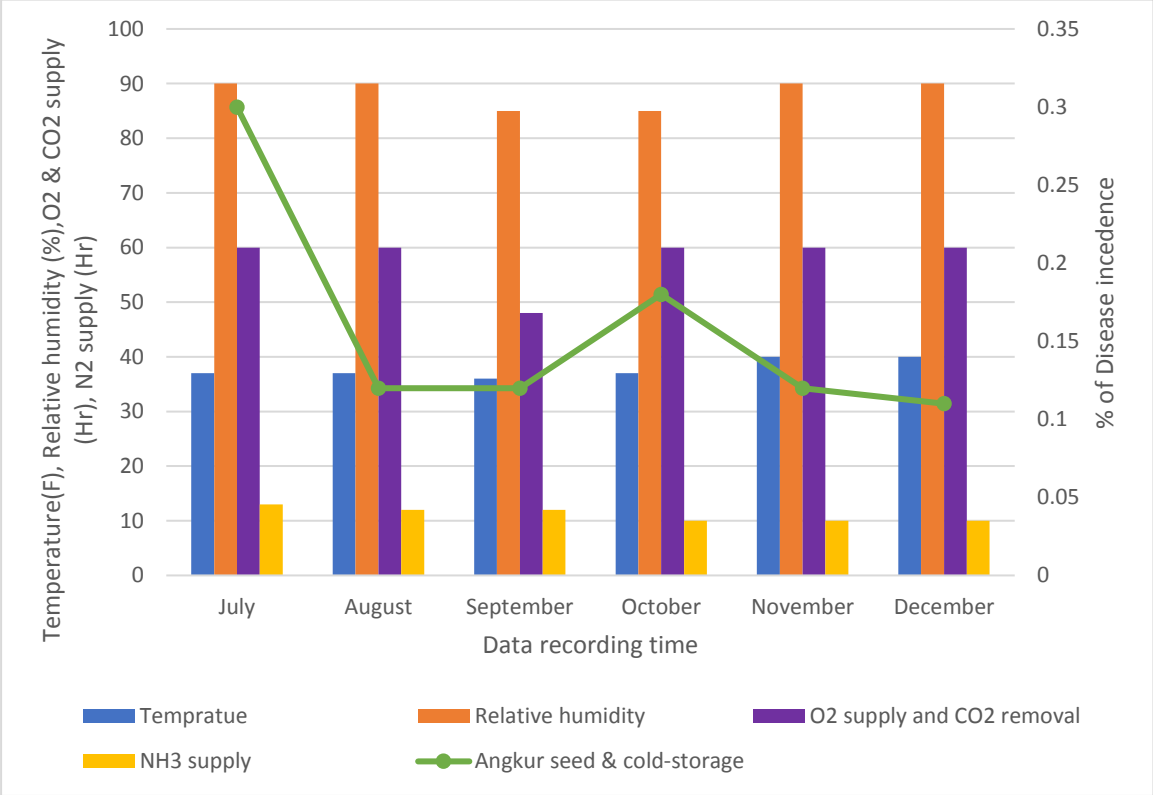

Figure: 14. Effect of internal condition at Angkur seed & cold-storage on incidence of brown rot disease of potato during storage period July 2018 to December 2018.

4.3.5c Incidence of Brown rot of potato at Mukta cold-storage during July 2018 to December 2018.

The incidence of brown rot disease varied significantly from month to month at Mukta cold-storage which ranges 0.30% to 0.12% (Table 18). Maximum disease incidence recorded in July 0.30% at temperature, RH%, O_2 supply & CO_2 removal and NH_3 supply was 2.78^{0}C or 37^{0}F, 90%, 0.8 hour and 12 hours (Table 1) respectively. On the other hand minimum incidence was 0.12% from October to December.

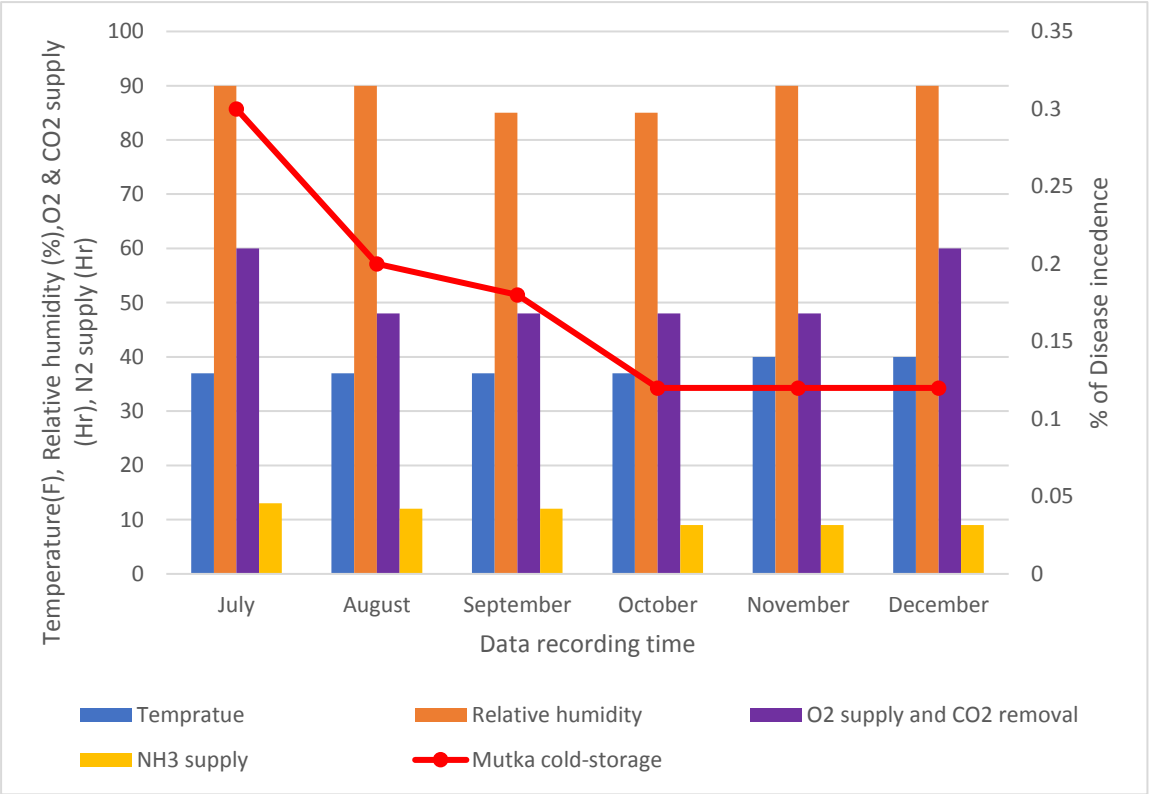

Figure: 15. Effect of internal condition at Mukta cold-storage on incidence of brown rot disease of potato during storage period July 2018 to December 2018.

CHAPTER V

DISCUSSION

Potato samples were collected from three different cold storages of Nilphamari district viz. Shawon cold storage, Angkur seed and cold storage and mukta cold storage during the period of July 2018 to December 2018. About 10 samples were collected from each cold storage as working sample. Before collecting samples, internal conditions of cold storages viz. temperature (^{0}C/^{0}F), relative humidity (RH%), O_2 supply & CO_2 removal (hr) and NH_3 supply(hr) were recorded. The internal conditions of cold storage play important role to keep potato healthy and fresh and scientists researched on suitable environment of cold storage to prevent weight loss, rot, shrinkage, sweetening, discolour and sprouting around the world (Gottschalk and Christenbury, 1998; Harbenburg *et al.*, 1986; Maldegem, 1999; Moazzem and Fujita, 2004). Gottschalk and Christenbury (1998) stated that potato should be stored at suitable environment to prevent weight loss, rot, shrinkage, discolor and sprouting. According to Harbenburg *et al.* (1986) and Maldegem (1999), temperature, humidity, carbon dioxide and air movement are the most important factors during storage. According to the report of Moazzem and Fujita (2004), five thousand tons of potato were rotted due to inadequate cold-storage facilities. Temperature recorded in the studied cold storage were ranged from 2.22^{0}C/36^{0}F to 4.44^{0}C/40^{0}F and it differs from month to month. The optimum range of temperature inside cold stores were 2-4 ^{0}C for long term storage (Van't, 1999). Seed potatoes were stored at 2^{0}C and potato for consumption were stored at 8-12^{0}C (Mehta and Ezekiel, 2006). Van (1987) reported that potatoes used for the seeding purposes were stored at about 4°C and those potatoes used for the processing purpose are stored at 8°C. Potatoes stored at higher temperature has higher swelling power than the potato stored at lower temperature (Kaur *et al.*, 2009). With the decrease in storage temperature there was increase in the content of the amylose content (Singh *et al*., 2008). For long-term storage temperature for processing potatoes was approximately 45^0 F. Seed potatoes could be stored at slightly lower temperatures (38° to 40° F.) for better weight loss and sprout

control (Nourian *et al*., 2003). In present study the relative humidity was recorded 85% to 90% in different months. Van't (1999) also suggested that the optimum range relative humidity of the air inside cold stores should be 85-90% for long term storage. Relative humidity should be kept at least 90-95% (ASAE 1991) unless there were diseased, or frozen potatoes, or wet potatoes which need to be dried. Hunter (1985) concluded that at 97.8% relative humidity, the rate of water loss would not be affected by airflow velocity. Nourian *et al*. (2003) suggested to maintain a 95% relative humidity at all times at entire storage period because it would help to minimize tuber weight loss as weight loss increased below 90% RH. In a study Kader, (2006) showed that tubers stored at 60-65% and 90-95% RH, the weight loss was more at 60-65% RH. Burton (1989) reported that under drier conditions, the rate of evaporation from tubers increases. In the present study, it was recorded that cold storages supplied O_2 and CO_2 removal for around 48 minutes to 1 hours simultaneously at dawn time. Concentrations of 0.2 to 0.3% CO_2 were common when potatoes were ventilated (ASAE 1991). Mazza and Siemens (1990) found that CO_2 could accumulate to 3.2% in less than 48 h in a sealed Manitoba commercial storage and above normal raised in CO_2 were correlated to increased reducing sugar concentrations in potatoes. In ASAE (1991) standard, the level of 1% CO_2 should be considered as the upper allowable threshold. Both reduced O_2 and elevated CO_2 concentrations stimulate sprouting (Burton, 1958; and Rastovski *et al.*, 1981), and caused disease incidence (Lund and Wyatt, 1972). In the present study it was recorded that liquid N_2 were supplied around 9 hours to 13 hours to cool the storage room. Temperature was maintained at desired level through supplying gasious NH_3 at storage room and it was varied from month to month. At higher levels of nitrogen supply, the vitamin C concentration has been shown to decrease in potatoes (Augustin, 1975). There was chance of losing the internal water if subjected to low external vapor pressure or relative humidity (CIP, 2009).

During the survey from July 2018 to December 2018, 2 fungi and 3 bacteria were isolated and recorded. The significant fungi were *Fusarium oxysporum* and *Rhizopus stolonifera* while significant bacteria of *Erwinia carotovora, Streptomyces scabies and Ralstonia solanecearum* were identified. This finding coroborates with the findings of scientists worded in the similar study (Secor and Gudmestad, 1999; Nora *et at.*, 2001; Ronald and Dennis, 2011). Secor and Gudmestad (1999) stated that diseases caused by fungal and fungal-like pathogens were late blight, dry rot, pink rot, Pythium leak, and silver scurf. Nora *et at.* (2001) recorded seven diseases of potato in storage condition namely pink rot, pythium leak, late blight, early blight, dry rot, sort rot, black dot and silver scurf. Ronald and Dennis (2011) studied and recorded the major storage diseases those were dry rot, pink rot, soft rot, ring rot and late blight. In the present study, *Fusarium oxysporum* causing dry rot of potato was identified by observing presence of macro and micro-conidia under microscope following by preparing the slide along with mycelia. Booth (1977) suggested the identification keys to identify *F. oxysporum*. Dravia (2011) confirmed *F. oxysporum* by observing macro-codinia. Sickle shape macro-conidia and ovoid shaped micro-conidia were seen as per the confirmation key of *F. oxysporum* according to Champawat and Pathak (1989). In the present study, rhizopus rot was confirmed as globose sporangia, sporangiophore, stolon and rhizoids were observed under microscope. Agrios (2004) stated that rhizopus produced aerial sporangiophores, sporangia, stolons, and rhizoids, the latter capable of piercing the softened epidermis. The most important characteristics to identify rhizopus morphologically are the spores (shape, size and colour) and fruiting bodies (Agrios, 2001). In the present study, soft rot of potato by *Erwinia carotovora,* brown rot potato by *Ralstonia solanecearum* and scab of potato by *Streptomyces scabies* were identified by observing morphological feature and biochemical test. *Ralstonia solanecerum* showed morphological feature described by Schaad (1992) and Gerhardt (1981) as colony size, form, pigmentation and elevation were found small to moderate, circular, creamy white and convex respectively. In the present study, the pathogen bacteria were found gram negative as red color developed (Pastor *et*

al.,2010), showed positive result on KOH test as mucoid thread produced by (Suslow *et al.*,1982), on catalase test as bubbles formed by (Schaad 1988), on oxidase test as formed dark purple color by (Kovacs, 1956). In the present study, soft rot and scab was identified by observing the key visual symptom. It was observed that the soft rot makes the potato soft and watery and creates bad odor (Agrios, 1997 and Singh, 2001) and scab creates different types corky lesion (Phillip, 2015).

The disease incidence of identified diseases was recorded during the period of July 2018 to December 2018 at three cold storages and effect of internal conditions of cold storages on studied diseases were also observed. All the diseases showed maximum incidence in July, 2018 viz. dry rot 0.85%, Rhizopus rot 0.40%, Soft rot 0.50%, Scab 0.50% and Brown rot 0.62%. The temperature, relative humidity, O_2 supply & CO_2 removal and NH_3 supply was 36^0F to 37^0F, 90%, 13 hours and 1 hour respectively in July. Minimum disease incidence was recorded for dry rot 0.20%, Rhizopus rot 0.0%, soft rot 0.10%, Scab 0.19% and brown rot 0.11% in December. The temperature, relative humidity, O_2 supply and CO_2 removal and NH_3 was 38^0 to 40^0F, 85% to 90%, 9 to10 hours and 1 hour respectively in December. Bari (2004) found that total postharvest losses of potato were 15% of the total production. Stevenson *et al.,* (2001); Rahman (1969) and Kamaluddin (1970) reported that 2 to 9% losses of tubers take place every year in each storage due to these diseases. According to Terry *et al*. (2011) and Pritchard *et al.* (2012), the overall losses of 17% (770,000 tons) were recorded during storage in United Kindom. In a report of the Daily star (April 23, 2008) around 0.74% loss was observed at Munshigang cold storage in 2008. In another report of telegraphindia.com (September 3, 2013) about 0.38% potatoes were rotted in 2013. Singh (1998) reported that heavy sporulating fungus and spread of the disease takes place at 90% RH and temperature 16 - 22^0C. Microclimate like relative humidity (RH) above 80% & temperature 10-24^0C most favorable for appearance of the disease (Bhat and Singh, 2008). Cairns (1939) stated that under high soil moisture in the field, or high humidity in storage, infection may occur directly through eyes as low moisture inhibits

infection. Cairns (1933) summarized that high humidity in storage with poor ventilation can cause heavy losses of affected tubers. Swanson and Van Gundy (1985) indicated that fungi grown well at temperatures ranged between 24 and 27°C with 80 to 90% relative humidity at storage. Wong *et al.* (1984) found that temperature/moisture combinations of 10°C + 45% RH caused greatest pre-emergence of fungal pathogen of potato storage period. The sensitivity of bacteria to tubers was increased at a combination of lower oxygen concentrations with high RH (Nielsen, 1968). Under favourable conditions like high temperature (with an optimal of 25 °C), high humidity and poor ventilation, the bacteria-initiated lenticels or in wounds of tuber in a few days (Gardan *et al.*, 2003). Walker (1998) has summarized that a high humidity coupled with a temperature of 80^0F the bacteria was capable to cause the great injury. Pinhero *et al.* (2009) stated that supply of fresh air was critical for cooling and drying of tubers and to remove CO_2, volatiles and excessive heat and moisture as well. Drying of tubers with high ventilation capacity is much quicker and with less overall shrinkage (Wijekoon *et al.*, 2015). Few things could be the reason for variation of incidence among the studied disease; poor handling, wet sacks in some cases, insufficient O_2 supply, improper sorting, delayed removal of CO_2.

CHAPTER VI
SUMMARY AND CONCLUSION

Potatoes are attacked by a number of pathogens at cold storage. Internal condition of cold storage plays an important role to keep potato disease free during storage period. Disease incidence vary significantly from month to month during storage of potato. Considering the above points, the present research work has been designed with the following objects- to determine the incidence of post-harvest diseases of potato in selected cold storages of northern region of Bangladesh, to determine the storage condition that induce the diseases, to identify the causal agents of post-harvest potato disease in cold storage condition.

Three sets of experiments were carried out namely i) Survey on the cold storages to observe the internal condition of storage room ii) Isolation and identification of fungus and bacteria in the laboratory through cultural, morphological and biochemical tests iii) Calculation of disease incidence of fungi and bacteria having visible and invisible symptoms of potato.

A survey was carried out on three cold storages of potato in Nilphamari district of Bangladesh during July, 2018 to December, 2018. An acute variation of internal condition viz. temperature, relative humidity, O_2 supply & CO_2 removal and NH_3 supply were observed from month to month. Temperature, relative humidity, O_2 supply & CO_2 removal and NH_3 supply ranged from $2.22^0C/36^0F$ to $4.44^0C/40^0F$, 85-90%, 1 hr to 48 minutes and 9 hrs to 13 hrs respectively.

The fungi were isolated on PDA media followed by blotter paper method. The pure cultures were observed under microscope to identify the fungi. *Fusarium oxysporum* showed sickle shaped, blunt ends and fusiform macroconidia. *Rhizopus stolonifer* showed rhizoid, stolon, sporangia. The bacteria were isolated on NA media through dilution plate method. *Ralstonia solanecearum* produced creamy white, small to moderate, circular and convex colony on NA media. On TZC media, *Ralstonia solanecearum* produced red color surrounding with

creamy white colony. *R. solanecearum* showed Gram negative in Gram staining test and oxidase test, Positive in KOH solubility test, catalase test. Soft rot produces soft and watery tissue and scab produce corky tissue.

Disease incidence for fungal and bacterial pathogen were recorded for six months at three cold storage. In July the, the disease incidence was maximum for both fungal and bacterial pathogen while minimum incidence was found in December. Dry rot caused maximum loss 0.85% to 0.20% where minimum loss recorded from 0.40% to 0.0% by rhizopus rot.

The present study clearly showed that fungal and bacterial diseases of potato were responsible for cold storage loss. Internal condition influenced the disease incidence. Significant cultural, morphological and biochemical feature were observed during isolation and identification of fungi and bacteria. In July disease incidence was maximum although moderate incidence found in between July and December. Minimum disease incidence found in December. Maximum disease incidence found 0.85% for dry rot of potato and minimum was 0.0% for rhizopus rot. Relative humidity put a significant role in disease development at cold storages.

CHAPTER VI

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APPENDICS

Appendix I: Preparation of culture media

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

PDA media

Potato infusion : 200.00 g
Dextrose : 20.00 g
Agar agar : 20.00 g
Distilled water : 1000.0 ml

NA media

Nutrient agar : 28.00 g

Distilled water : 1000.0 ml

Triphenyl Tetrazolium Chloride (TTC)

2,3,5 triphenyl tetrazolium chloride (Soluble) : 10.0 g

Distilled water : 1000 ml

Appendix II: Preparation of biochemical reagent

Gram's staining reagents

Gram's Crystal violet (Hucker's modification)

Solution A: Crystal violet (90% dye content) = 2.0 g

Ethyl alcohol = 20.0 ml

Solution B: Ammonium oxalate = 0.8 g

Distilled water = 80.0 ml

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

Gram’s Iodine (Gram’s modification of Lugol’s solution)

Iodine : 1.0 g

Potassium iodide (KI) : 2.0 g

Distilled water : 300.0 ml

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

Gram’s alcohol (decolorizing agent)

Ethyl alcohol (95%) : 98 ml

Acetone : 2 ml

Safranin (counter stain)

Safranin (2.5% solution in 95% ethanol) : 10 ml

Distilled water : 100 ml

KOH solubility reagent

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

Catalase reagent

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

Oxidase reagent

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