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MALAYSIA
KELANTAN

# Gray Mold *(Botrytis cinerea)* Disease Progress on Tomato Fruits in Different Packaging

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**F15A0098**

**A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Agrotechnology) with Honours**

**Faculty of Agro Based Industry**

**University Malaysia Kelantan**

**2019**

# DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled "Gray Mold (*Botrytis cinerea)* Disease Progress on Tomato Fruits in Different Packaging" by Nadirah Syamimi binti Yusof, matric number F15A0098 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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# ACKNOWLEDGEMENT

First of all, I thank the Almighty for getting me this far. Without His willing, I might not be able to finish up this thesis in a smooth manner.

I would like to thank all people who had lent me their support, encouragement, and guide during the time of doing this thesis on my Final Year Project.

I am grateful to Dr. Nurul Syaza binti Abdul Latif who is my supervisor for always guiding me in doing the experiment and thesis writing until I can finish it. I am thankful for her kindness and patience in supervisory and answering all my doubts all the time.

I would also like to express my thankful to my family and fellow friends for their continuous support, encouragement and commit their resources toward my education. Their sacrifices have helped me enable to becomes this far.

Last but not least, I appreciate all parties who involves directly or indirectly during my preparation of this Final Year Project.

**Gray Mold (*Botrytis cinerea*) Disease Progress on Tomato Fruits in different Packaging**


## ABSTRACT

This research is focusing on observing the growth progress of *Botrytis cinerea* on tomato fruits. Tomato fruits are one of the commercial fruits that often infected by *Botrytis cinerea* whether in the field or in the storage. Different packaging was used to find any differences that might occur during storage. The objectives of this research were to study the differences of spore spreading progress of *B. cinerea* on tomato fruits in different packaging storage and to analyze the disease incidence and severity during different packaging storage. The packaging used in this research were the cardboard box, plastic fruit container, and plastic zip lock bag. *B. cinerea* fungus was inoculated directly to the fresh tomatoes. After that, each packaging tomatoes was stored in the controlled environment and the progress was observed within a fixed time. Every three days, the inoculations of the *Botrytis cinerea* on the tomato were observed by using severity index scale. The disease incidence and severity were calculated and expressed in percentage. The percentage of incidence and severity were increased progressively and shows that the inoculated fruits were infected. Through this study, the progress of *B. cinerea* spore spread was observed and analyzed where most of the tomatoes in each packaging shows show symptoms as early as day three. The tomatoes in the packaging cardboard box and plastic fruit container were 100% severed until the day 30 but tomatoes in plastic zip lock bag were not fully severed until the last day of observation.



Keywords: Gray mold, *Botrytis cinerea*, disease progress, different packaging, tomato

**Perkembangan Penyakit Gray Mold (*Botrytis Cinerea*) Pada Buah Tomato dalam Simpanan Pembungkusan yang Berbeza**




## ABSTRAK

Kajian ini difokuskan untuk memerhatikan perkembangan pertumbuhan *Botrytis cinerea* pada buah tomato. Buah tomato adalah salah satu buah-buahan komersil yang sering dijangkiti oleh *Botrytis cinerea* sama ada di dalam ladang atau dalam penyimpanan. Pembungkusan yang berbeza digunakan untuk mencari sebarang perbezaan yang mungkin berlaku semasa penyimpanan. Objektif penyelidikan ini adalah untuk mengkaji perbezaan spora yang menyebarkan pertumbuhan *B. cinerea* pada buah tomato dalam simpanan pembungkusan yang berbeza, dan untuk menganalisis kejadian penyakit dan keparahan semasa penyimpanan bungkusan yang berbeza. Pembungkusan yang digunakan dalam penyelidikan ini ialah kotak kadbod, bekas buah plastik, dan beg kunci zip plastik. *B. cinerea* disuntik terus kepada tomato segar. Selepas itu, setiap tomato dalam simpanan pembungkusan yang berbeza disimpan dalam persekitaran terkawal dan pertumbuhan penyakit diperhatikan dalam masa yang telah ditetapkan. Setiap tiga hari, inokulasi *Botrytis cinerea* pada tomato diperhatikan dengan menggunakan skala indeks keparahan. Insiden dan keparahan penyakit dikira dan dinyatakan dalam peratusan. Peratusan kejadian dan keparahan buah meningkat secara progresif dan menunjukkan bahawa buah-buahan yang telah diinokulasi itu telah dijangkiti sepenuhnya. Melalui kajian ini, penyebaran spora *B. cinerea* dapat diperhatikan dan dianalisis di mana kebanyakan tomato dalam setiap pembungkusan menunjukkan tanda-tanda infeksi seawal hari ketiga. Tomato dalam kotak kadbod dan bekas buah plastik adalah 100% dijangkiti *B. cinerea* sehingga hari 30 tetapi tomato dalam beg kunci zip plastik tidak sepenuhnya dijangkiti sehingga hari terakhir pemerhatian.


Kata kunci: kulat, Botrytis cinerea, pertumbuhan penyakit, pembukusan berbeza, tomato

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## LIST OF ABBREVIATION AND SYMBOLS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| A.D | Anno Domini |
| Cm | Centimetre |
| G | Gram |
| PDA | Potato dextrose agar |
| ml | Millilitres |
| mm | Millimetre |
| CRD | Complete randomized design |
| $^oC$ | Degree(s) Celsius |
| % | Percentage |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

Fruits and vegetables are important in a human healthy diet because it supplies nutrients like vitamins, fibers, minerals, and antioxidants. The recommended green foods in a healthy diet are avocados, tomatoes, carrots and more. Regardless of the outstanding progress made in increasing food production at the global level, roughly half of the population in the Third World does not have access to sufficient food supplies (FAO, 1989). There are many reasons for this, which one of it is losing if food occurring during the postharvest and marketing period (FAO, 1989). The food supply could be enhanced by increasing food production and decrease the losses of postharvest.

The origins of tomato were traced back to the early Aztecs around 700 A.D, and it is assumed that the tomato is native to the Americans (Veggie Cage, 2005). For many years before, tomatoes were considered venomous and were grown especially for their ornamental value. Then, tomatoes gradually spread through the central and South America where it was used as food. Tomatoes happen to be produced in Spanish fields, as early as the 1540s. The vegetables were used as a common food in the early 17th century but other European countries still not adapt to this vegetable straightaway (Vegetable Facts, 2018). In the 19th century, the tomatoes finally arrived in Asia. John Barker who is the British consul in Syria focussed the cultivation efforts first and the tomatoes gained much attention. After that, it started to being used widely in Syria, Iran, and China by the mid-19th century ("Vegetable Facts", 2018).

Tomato goes to family Solanaceae and genus of Lycopersicon. The size depends on cultivar because there were several hybrids available in the market. When it becomes to the Asian region, China is the prime producer of tomato followed by India. The tomato is the world demand vegetables and it often facing problems such as disease. The protected cultivation of tomato (*Solanum lycopersicum L.)* is common in an enormous number of countries. This crop is sheltered from rain excess and the temperature is more firm. Production yield and cycles are higher under protected cultivation but the condition is encouraging to pathogens, including *Botrytis cinerea* which causes gray mold or stem cancers (Dik and Wubben, 2004). In 2007, Elad, Williamson, Tudzinski, and Delen states that over 200 plant species, including tomato plant, are the host of *B. cinerea*, which infects the flowers fruits, leaves, and stems. The infections of fungus could lead to severe defoliation, flower death, reduce value in the market and stem lesion followed by plant death. *B. cinerea* causes postharvest decay that usually occurs at the field and can remain after storage.

The symptoms are variable depending on the variety and the sources of infection. The fungus can occur as both a parasite and in saprophyte on the same wide range of hosts. This disease is intriguing in where it can cause various disease including damping-off and blight of flowers, stems, fruits and foliage in vegetables and ornamental plants. *B. cinerea* could attack the healthy fruits besides it, causing extensive breakdown or entirely spoil (Zitter, 1986).

The inoculum sources of *B. cinerea* in the orchard are predominant and almost impossible to be destroyed. The spores usually grow during wet weather throughout the year and colonize dying flower parts during bloom. These infections either develop into visible rot in the orchard or remain latent infection and continue developed in store. The risk of this fungus infection can be assessed pre-harvest from previous orchard rot history and from raining seasons.

Tomato is the commercial fruits that are easy to be found in Malaysia. The purpose of using tomato fruits because of its availability in Kelantan area and it is easy to observe the progress of *B. cinerea* disease progress using tomato fruits. Tomato can easily be stored in different packaging. The packaging used is a plastic ziplock bag, plastic fruit container, and a cardboard box. The disease progress of B. cinerea is observed at different packaging to see any differences.

**1.2 Problem Statement**

One of the common diseases in tomatoes is *Botrytis cinerea*. When the stem end starts to turn gray or dark colour and leaves begin to drop off, it might indicate that the plant has been infected by *B. cinerea*. It often starts on the stems and eventually makes its way to where the fruit joins the stems. As result, the plant could not survive an eventually died. The typical symptoms lead to losses in economic due to the occurrence of spreading, fast-growing necrotic lesions bearing abundant pigmented and conidia.

Typically, *B. cinerea* causes losses in crops by damage to the harvestable part of the crop, flowers, fruits, or leaves, or by girdling stems (Shaw et al., 2016).

Tomato is one of the most commonly grown crops in the world. Mertely, and Peres, (2009) and Williamson, Tudzynski, Vankan, (2007) has stated that *Botrytis cinerea* is responsible for severe economic losses. The pathogen *B. cinerea* also causes significant losses during shipping and marketing which it makes it as the most economically important pathogen (Mertely and Peres, 2009).

Thus, there were many wastes of fruits and vegetables due to *B. cinerea* disease infection. Many wasted were comes from the wet market and supermarket due to the unsold product which cannot be used anymore. Due to the problems, pollution is also increasing. The wasted were dumped in the trash can and become smelly where it attracts other insects and pests.

Without proper storage, it will lead to the appearance of *B. cinerea* and spoil the fruits or vegetables. Tomatoes could only stand for one week on the counter without proper storage (Eatbydate, 2012). The shelf-life is really short and it is easy to be infected. *B. cinerea* could infect the tomatoes even when it still in the field and not harvested.

## 1.3 Objectives of the Study

The objectives of this study are

i. To study the differences of spore spreading progress on *Botrytis cinerea* on tomato fruits in different packaging storage.
ii. To analyse the disease incidence and severity during different packaging storage.

## 1.4 Scope of the Study

In this study, tomatoes fruit were inoculated to *Botrytis cinerea* to see the growth progress of the disease. Every three days, the observation was conducted to analyze the infected area. Different packaging storage was used which are plastic zip lock bag, plastic fruit container, and cardboard box. Disease incidence and disease severity were calculated and recorded.

## 1.5 Significant of the Study

*Botrytis cinerea* is the fundamental agent of gray mold. This pathogen is studied as the most crucial fungus for postharvest decay in fruits and vegetables. It has a wide host range with well over 200 reported plant host (Williamson et al., 2007). The affection of *B. cinerea* might occur at the field and remain until storage. This disease can appear on infected fruits as early as within three months in cold storage after harvest, but most decays that originate from infections of the stem and calyx of fruit develop after an extended period of time in storage. Research states that the shelf life of fruits and vegetables can be improved through modified atmosphere techniques using different types of packaging materials like cardboard boxes (Shahzad, Tahir, Jehan, & Luqman, 2015).

*B. cinerea* exists in all vineyards in the world. This disease seriously reduces the quality and quantity of the crop. It is a wide range of major and minor host plant. Through this study, the growth progress of *B. cinerea* could be observed and analyzed. There are still no major resistance genes that could be used against the fungus infection in tomatoes and other fruits.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 *Botrytis cinerea*

*Botrytis cinerea* is a necrotrophic fungal pathogen which also known as gray mold. The fungus able to cause gray mold rot in more than 200 host plant species where is especially destructive on fruits and vegetables causing serious economic losses (Williamson et al., 2007).

*Botrytis cinerea* is a soft rot that collapsed and has a water-soaked appearance on soft fruits and leaves. Some brown lesion will slowly appear on the fruits and the symptoms are visible at wound sites where the fungus begins to rot the plants. Gray masses with a smooth appearance like velvet the conidia on the plant tissues indicate the sign of pathogen infection (Coley-Smith, Verhoeff, & Jarvis, 1980). Then, conidia will continue to affect the surrounding plants and hosts through the growing seasons. Figure 2.1 shows *Botrytis cinerea* fungus on tomato.

Figure 2.1: Gray mold on tomato fruit

(Source: Victoria State Government, 2017)

*Botrytis cinerea* appears as white cottony, which then turns into light grey with age on the potato dextrose agar (PDA). The young hyphae are thin, hyaline, and 8-16 μm wide, and become brown and septate with age (Xiao, 2006). Conidiophores are light brown, septate with slightly enlarged tips bearing small pointed sterigmata bearing 1 to 2 celled, hyaline, oval conidia forming clusters. Characteristics and 3 sporulation of *B. cinerea* depend on and vary with, nutrient medium, temperature, and ecological factors (Xiao, 2006).

*Botrytis cinerea* is in the kingdom of Mycota where the phylum is Ascomycota. This fungus was in class Leotiomycetes and family Sclerotiniaceae. It is from genus *Botrytinia*. Conidia are the one-celled spores that borne on branched conidiophores, where the arrangement of the fungus is the grape cluster arrangement spores. In 1986, Zitter T. A. has noted that the spores give the fungus its name, from the Greek botrys which means a bunch of grapes. When conidiophores dry out, the spores will gently move and release out. Usually, the air movements are sufficient to get the spores flying.

The fungus often landed on the injured tissues to infect and continues as saprophyte for long periods.

*B. cinerea* is an airborne fungus. It keeps well inside the soil in the form of small hard nodules, dark in colour and made of intertwined hyphae. These nodules are known as sclerotia or resting bodies. The life functions of this fungus are in slow motion where it can wait until years for a favourable condition for it to return. This fungus can tolerate low temperature from -2$^{o}$ C to high temperature, 33$^{o}$ C. It fits well in dead organic materials, as saprophyte and able to sustain a long continuous development on the dead, and rot materials. The sclerotium might spawn into mycelium which produces conidia by simple budding-off from the specialized parts. The spores are the agent for germination of disease (Cyclamen.com, 2015).

The fungus could live through the winter as mycelium or sclerotia in plant debris while, as for the seed borne, it tends to become as spores or mycelium. Other crops are also becoming the source of the pathogen and have the chance to cross-infect. Conidia are carried by wind and the surface of splashing raindrops. High relative humidity is required for abundant spore production. When in the field, germination and infection of the disease often arise when the spores land on the tomato plant when there is favourable condition for it to grow.

*B. cinerea* is host to over 200 plants species including tomato plant. The fungus contaminates the flowers, fruits, leaves as well as the stem of the plant (Elad, Williamson, Tudzinski, and Delen, 2004). The infection of the fungus leads to severe defoliation, flower death, falling of market values of the fruits, the stem lesions, and plant death. Stem breakage may occur when the severity of the gray mold is high. Tomato stem lesions can result from infections in wounds caused by the defoliation or pruning of plants as well as fungal colonization from the petiole (Dik, Koning, and Kohl, 1999). The disease can cause severe losses in greenhouses, leading to the early death of more than 70% of tomato plants and, thus, reducing yields.

## 2.2 Sign and Symptoms of Infection of *Botrytis cinerea*

The common sign and symptoms of gray mold on tomato plants are leaves have irregular to V-shaped brown blotches, frequently starting at the edge of the leaves. The dieback symptoms appear as infection progresses from leaves (figure 2.2), through petioles, toward the main stem. Then, the stem is brown in colour, and have oval bruise girdle on the stem. Pale, soft and watery rot symptoms show up at the infected fruits as shown in figure 2.3. It often happens on green and red fruit which on the plant or during postharvest. Failed fruit infection results in white rings or halos on the fruits, which are called ghost spots and the infected flowers turn brown and die. Fluffy gray spores cover the infected part and have spores that are light brown-gray on black stalks.

Figure 2.2: Plant dieback

(Source: Dr Pam Robert, no date)

Figure 2.3: Infected tomato fruit

(Source: Dr Pam Robert, no date)

As Gonzalez-Dominguez, Caffi, Ciliberti, Rossi, (2015), concluded in their research that *B. cinerea* infection pathways differ for conidia and mycelium. The conidia infect inflorescences young clusters and ripening berries. For mycelium, it is responsible for the berry to berry infection. They state that for grapes, on inflorescences and young grape clusters, the strength of infection increases with the hour of wetness and the optimal temperature is about $20^{o}$ C. At ripening, the fungus could infect the whole cluster of grape and damage the great loss in the crop. Infection incidence in mature fruits is higher at temperatures between $15^{o}$ C to $25^{o}$ C. Moreover, disease infection is higher on wounded fruits with favourable conditions.

In 2008, Xiao and Kim analyze that gray mold on Gala apple fruits infected at the wound such as cracks on stem bowl area and punctures as well as bruises that are created during postharvest handling. According to Xiao and Kim, (2008), gray mold can also derive from a fruit-to-fruit spread of disease in field bins during storage. The decayed area of the infected apples is depending on apple cultivar and storage conditions. When the entire fruits are decaying, it may appear like a baked apple. The infected area is spongy and the diseased tissue attached to healthy tissues.

The immature green fruits might turn light brown or white, starting at the point where they touch other infected plant parts. The soft rot might develop with skin that remains perfect, but inside the fruits, the tissues are mushy and watery. Later, fuzzy mold can begin to grow on the tomatoes and sclerotia may appear, under dead calyx or sepal. If these stages occur in the greenhouse, the fruit will fall off the plant and litter the floor of the house. In the field, the alleyways will be filled with discarded fruits (figure 2.4).


Figure 2.4: Discarded fruits in alleyways

(Source: Zitter T. A., 1986)

Green fruits also have the possibility to get infected directly by the airborne spores. The white circular (halo) spots appear on the fruit and have been termed "ghost spots". It can appear on green, breaker or mature fruits where the colour of the halos will change. The halos colour change from white to yellow as the fruits ripen.

High relative humidity which is about 90% humidity combined with mild temperature, less than 20%, favours the development of gray mold (Jarvis, 1989). The effect of temperature on the infection is similar to what has been reported for other plant organs. It shows the optimum range for infection is between 10$^{o}$ C and 20$^{o}$ C, even though the infection may occur between 5$^{o}$ C to 25$^{o}$ C. However, other factors such as plant age and the infection site may affect the host susceptibility, which decreases with the time following injury. The data were obtained from studies conducted in tempered regions.

## 2.3 Disease Cycle of *Botrytis cinerea*

The life cycle of *Botrytis cinerea* was illustrated in figure 2.5 below. The sources of inoculum in *Botrytis cinerea* starts in the orchard itself. That is where the fungus lives which are on the plant materials. Usually where there is wound or injuries are the infection starts to occur. The fungus starts to develop faster in cold storage temperature where states by Beattie, et al, 1989. The spores of *B. cinerea* are stored in orchard soil and produced on the decaying plant materials, where then it was brought into storage by bins or the containers. Additional inoculum is provided by the fruits that decay during storage. The infection spreads to adjacent fruit in cartons or bins causing nests or pockets of infection (Beattie *et al.*, 1989).

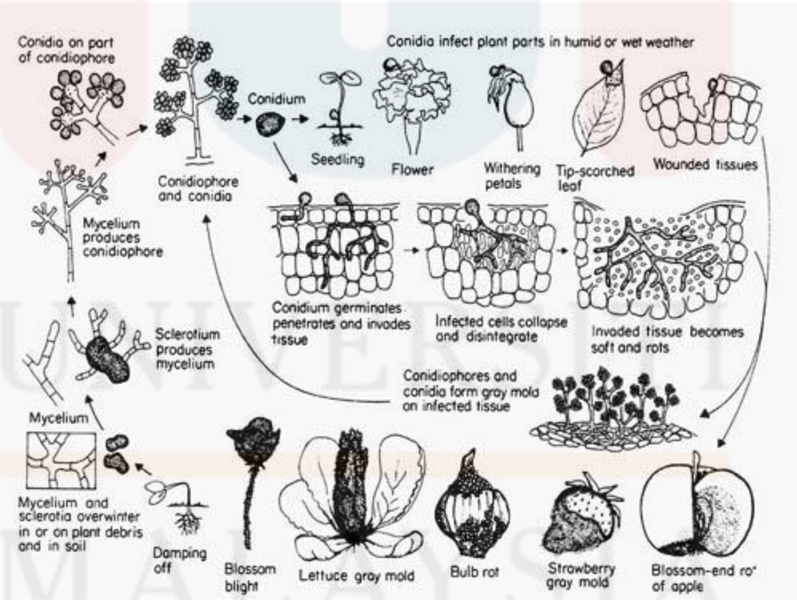


Figure 2.5: *Botrytis cinerea* life cycle

(Source: Cornell University, 2018)

## 2.4 *Solanum Lycopersicum* (Tomato)

The along with the paper, eggplant, and potato are belong to the Solanaceae family. They are known as nightshades and economically important. Tomatoes have several scientific names includes *Solanum lycopersicum* and *Solanum esculentum*. These plants are in kingdom Plantae and order Solanales. The genus of tomato is Solanum and species *S. lycopersicum*. Tomato plants are vines and typically grow about 180 cm or more on the ground if supported. The leaves are less hairy, have strongly odorous and the five-petaled flowers are yellow. The fruit berries are usually red, scarlet or yellow and vary in diameter from 1.5 to 7.5 cm. The shape is spherical to oval and elongate to pear-shaped (Encyclopaedia Britannica, 2018). Tomato plants are dicots and it grows branching series of a stem with terminal buds at the tip. Nowadays, due to the advance of genomics, scientist found out more about the gene that responds to certain characteristics. Through genetic engineering, the scientist manages to create many new varieties of tomatoes (Tomatosphere, 2018).

Tomato commonly cultivated extensively for its edible fruits. It labelled as vegetables for its nutritional purpose, tomatoes are best sources of vitamin C and phytochemical lycopene. Usually, the fruits were eaten raw where it was served in salad, also as cooked vegetables. It also used for the ingredient in various prepared dishes and pickles. In the commercial market, tomatoes were used for processing products which include canned tomatoes, juices, ketchup, puree, paste, and “sun-dried” tomatoes or dehydrated pulp (Encyclopaedia Britannica, 2018).

## 2.5 Packaging in Tomato Fruits

There are several types of packaging materials used in storage of tomatoes. For examples, open market bag, cardboard boxes, wooden boxes, sealed boxes, and plastics. Packaging system was used to extend the storage life of many fresh fruits and vegetables. The efficiency of packaging materials determines the shelf life of the food materials. Packaging could give modified gas atmospheres around the product which can slow down the respiratory activity of fruits including tomatoes. Both packaging and storage temperature could affect the quality of tomatoes. The type of packaging materials significantly influences the firmness and sensory quality of tomato fruits. Disease of tomatoes also could be controlled with proper packaging materials. Among the packaging materials, it states that sealed packaging seems to be better packaging material for storing tomatoes (Mekonnen, 2017).

Agreeing to the statement from Shahnawaz, Sheikh, Soomro, Panhwar, Khaskheli in 2012, the quality and type of packaging materials also influence the quality of product stored. The different packaging materials are used in the wholesale and retail market for packing the fresh produce like tomatoes.



## 2.6 Economic Importance

Gray mold is a disease of mature fruit which is of minor importance in well-managed plantations. *Botrytis* diseases are probably the most common and widely distributed diseases of vegetables, ornamentals, fruit, and even field crops throughout the world (Agrios, 1997). According to Rosslenbroich et al. (1998), the economic importance is higher when considering the fact that these cash crops is not only endangered in the field but also during transport and storage. *Botrytis* diseases commonly appear as blossom blights and fruit rots. Gonsalves and Ferreira (1994) had mentioned that fungus *B. cinerea* could cause some disease. The example of the disease is damping off, leaf spot, rots, and stem cankers.

# CHAPTER 3

# METHODOLOGY

In order to determine the parameter values of this study, proper experimental data is required. To observe the growth this fungus, laboratory work had been carried out. After that, the data was analysed which consist of numerical computation.

## 3.1 Laboratory Work

### 3.1.1 Fruit disinfection

About 90 tomatoes were used during the experiment. The tomatoes were collected from farmers in Pasar Borong Tanah Merah. Those tomatoes were transported to University Malaysia Kelantan Jeli Campus for further action. Uniform fruit and without physical injuries will be used as experimental materials. The tomatoes were selected specifically according to the size, weight, colour and maturity stages.

The tomatoes used were index 2 and 3 of maturity and size medium (M). Index 2 of maturity is breaking or turning where the colour other than green appears on tomato surface. About 10% to 30% of the tomato surface is pale yellow. Index 3 is pink where there is 30% to 60% of the tomato surface show pink or light red colour. The diameter of the tomatoes was around 41 mm to 50 mm (FAMA, 2018). This is to standardize the tomatoes for the experiment.

The fruits were rinsed with sterile tap water and let air-dried on a clean bench about 2 hours. After dried, the fruits were wounded at the equator with a sterile nail. The wounded were about 2 mm to 3 mm depth. Then, the wounded area was inoculated with the *Botrytis cinerea* (Zhang, Qin, Li, & Tian, 2014[a]).

### 3.1.2 Culture

The spore of *Botrytis cinerea* was bought from the spore bank. The spores then were cultured on Potato Dextrose Agar (PDA) media about two to three weeks. The characteristic of PDA is a universal media which suitable to grow most of the fungus.

The PDA was prepared in the laboratory by using PDA powder and double distilled water. 18.5 g of PDA powder was mixed into 500 ml distilled water. The mixture was mixed gently and the solutions were sterilised in the pressure cooker. After the pressure cooker cool, the PDA solution was taken out and poured the media into the petri dish. Using aseptic condition, the media were allowed to solidify to produce the agar plate and sealed with parafilm to avoid external contamination.

Once PDA media was ready, the spores of *Botrytis cinerea* were cultured on the media agar. The colony of *B. cinerea* began to grow from time to time until it fully grown on the agar plate.

### 3.1.3 Inoculation of *Botrytis cinerea*

*Botrytis cinerea* was obtained from University Malaysia Kelantan. As Zhang et al. (2014[a]) mentioned the tomato fruits were inoculated with 10µl spore suspension in each wounded site using a pipette. The spore suspension shows 1 X $10^5$ cfu after checked using haemocytometer. Ten inoculation fruits will be stored in different packaging respectively. The packaging system was used in this experiment was cardboard box, plastic fruit container and plastic zip lock bag.

### 3.1.4 Packaging System Used to Store Tomatoes

The tomatoes were divided into three different packaging at a fixed temperature. The packaging that was used is plastic zip lock bag, plastic fruit container, and cardboard box. In the ambient temperature, the fruits were stored in different packaging system respectively. The tomatoes were put into the cardboard boxes (figure 3.1), inside the plastic fruit containers (figure 3.2), and inside the plastic ziplock bags (figure 3.3). Afterward, each packaging was left at the ambient temperature ($21^o$ C to $25^o$ C). The experiment then was done in a controlled and closed environment to prevent the spread of disease. The inoculation was observed for the spore spreading progress for a fixed time. For every three days, the fruits were check and observed. The data were recorded for every growth of the spore on the tomatoes for every three days.

Figure 3.1: Packaging system used to store tomato (Cardboard boxes)

Figure 3.2: Packaging system used to store tomato (Plastic fruit basket)

Figure 3.3: Packaging system used to store the tomato (Zip lock bag)

**3.2 Disease Measurement and Observation**

The observation was based on how fast the spore spread to appear on the inoculated tomatoes and the time taken for the fungus to infect the whole fruits. After three days of inoculation, the fruits were observed for any infection or symptoms appear. The time for fruits to be infected was recorded. Disease incidence was measure along the appearance of any symptoms and severity on the fruits. A direct quantitative method was used. The formula used to calculate disease incidence and disease severity are as below:

$$\text{Disease Incidence} = \left(\frac{Number\ of\ infected\ plant\ units}{Total\ number\ of\ plant\ unit\ assessed}\right) \times 100 \qquad (3.1)$$

$$\text{Disease Severity} = \left(\frac{(Number\ of\ units \times Disease\ grade)}{(Total\ plants\ observed\ in\ set \times Maximum\ disease\ grade)}\right) \times 100 \qquad (3.2)$$

## 3.3 Data Analysis

There were three replication used in this study and was displayed in a complete randomized design (CRD). Each replication contains ten tomatoes. The replication of the treatment used labelled as Control, A and B. The control of the treatment was not given any inoculation of the fungus *Botrytis cinerea*. One-way analysis of variance (ANOVA) was used in analysing the results. When the p-value is same or less than 0.05 ($P \leq 0.05$), significant value due to the differences was obtained. But when p-value is more than 0.05 ($P > 0.05$), this indicated that the significant value is not significant to the level 0.05.

## 3.4 Experimental Design

Botrytis cinerea spores were cultured on PDA media

↓

Prepared the tomatoes by disinfection them from any dirt

↓

Tomatoes were rinsed with sterile tap water and let dried

↓

The tomatoes were wounded around 2 mm to 3 mm with sterile nail to inoculate the pathogen

↓

10µl spore suspension with 1 x $10^5$ cfu were inoculated on the tomatoes using pipette

↓

The fruits were divided to three different packaging

↓

The inoculated fruits were observed every three days for 30 days

Figure 3.4: The flow process of the experiment

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Disease Incidence of Tomatoes in Different Packaging

Disease incidence is defined as the relative number of plants that are affected by the disease. It often expressed as a percentage of the total number of plants. Disease incidence is about the disease occurrence in a population. In this study, disease incidence was observed from the appearance of any symptoms such as a white spot on the tomatoes in every packaging, after few days of inoculation the *Botrytis cinerea* into the tomatoes. The *B. cinerea* fungus inoculated on the tomatoes was 1 x $10^5$ cfu.

Based on the experimental data, *Botrytis cinerea* started to appear as early as day 3. The packaging cardboard boxes and zip lock bag has the peak value of disease incidence which is 70%. This is means that 7 tomatoes in each packaging were infected. Fruit basket has the least on day 3 which is only 45% of disease incidence. The experiments have two replication for each packaging which is labelled as A and B. Both replications were taken the average value and recorded in the data.

From the data tabularized, it is assumed that the actual time for the appearance of the symptoms was between day 1 and day 3. The experiment was observed until day 30 and the disease incidence was increased gradually day by day. On day 12 almost all tomatoes in every packaging were infected by the *Botrytis cinerea*. The percentage of disease incidence in fruit basket is 95% while both cardboard boxes, and zip lock bags were 100%. The packaging itself might be the factor that the *B. cinerea* are easy to spread. As for the cardboard boxes it gives the tomatoes minimum light and high humidity. Same goes for the zip lock bag which contains more humidity.

All tomatoes in every packaging show the signs of infection on day 15. This means that the percentage of disease incidence become 100% on day 15. It acquired about 9 to 12 days for the tomatoes to fully infect once the first symptoms appeared.

For control tomatoes in each packaging, they remain uninfected and have no sign of disease by the pathogen of *Botrytis cinerea*. The controls began damage in each packaging after a week. They turn out too mushy and watery, smell awful and rot. They show the symptoms of rotting and damages.

As claimed by Zhang, Qin, Li, and Tian (2014[b]), after two days of inoculation pathogen *Botrytis cinerea* the lesion on tomatoes and apples were measured. On tomato fruit, the lesion expansion rate was lower than the apple but the strain was also developed more mycelia and sporulated abundantly to the wounded site. After four days of inoculation, slight lesions were detected in the mutant-inoculated apples fruit, which the lesion size was about 30 mm. Based on Tanovic, Delibasic, Milivojevic, and Nikolic, (2009) after 7 days inoculation of pathogen, thick and woolly mycelium was developed on raspberry fruit of their research. It was confirmed that the pathogen was successfully inoculated the fruits. Based on the previous study, this experiment was conducted. This shows that the experiment obtains the same outcome as mention from the previous study.

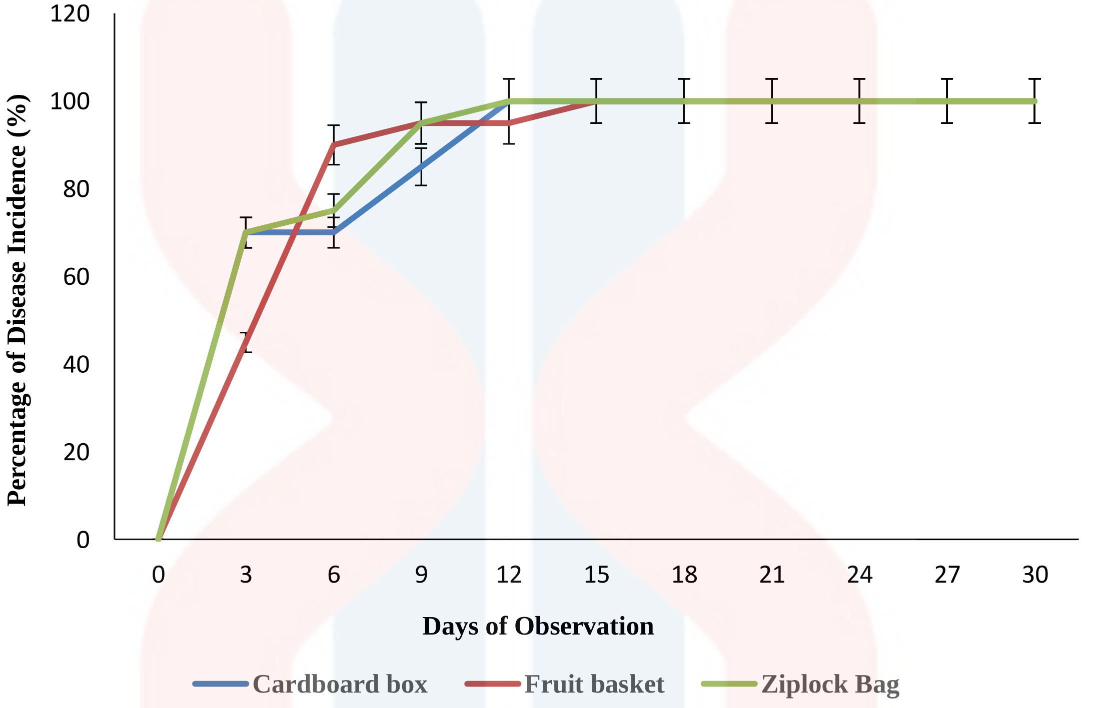


Figure 4.1: The graph of disease incidence for each packaging

The graph above shows the percentage of disease incidence for every treatment used in this experiment. The average value from both replications was taken in plotting the graph. The graph indicates that disease incidence is increasing from day 0 until day 30.

## 4.2 Disease Severity of Tomatoes in Different Packaging

Disease severity is used to express the amount of disease on individual fruits assessed. It is the percentage of relevant host tissues or organ covered by symptoms, lesion or damaged by the disease. The severity results from the size and number of lesions. It is stated that disease severity is the extent of damage caused by the disease (Sharma, n.d).

The severity of gray mold was rated on the scale of zero to four. The scale 0 is no disease symptom, scale 1 is 0.1 to 5% disease symptoms, scale 2 is 5.1 to 20% disease symptoms, scale 3 is 20.1 to 40% disease symptoms while 4 is 40.1 to 100% disease symptoms. The index of disease severity was described by Lee, Lee, Kim, Son, Lee, et al (2006).

For every 3 days, the tomatoes were observed. The tomatoes were determined if it has the disease or not. This is by determining the relative amount of area that showing any symptoms which are expressed as a percentage. The symptoms shown are the area covered by any lesion, or extent of damage that caused by the disease. A lightly infected plant may only have 5% of the infected area, while the bigger infected area may have about 50% of its infected area.

Three days after inoculation of *Botrytis cinerea*, all packaging showed the symptoms of the disease. The percentage of disease severity in the cardboard box was 8.75% on day 3 which were the highest among the three packagings as shown in the Appendix Table A.2. The other two packagings which were plastic fruit container and plastic zip lock bag showed 3.75%. The *Botrytis cinerea* infects and damages the fruit within three days. It shows that the symptoms able to form as early as day 3.

The percentage of severity on the fruits began to increase until day 30. The increasing of disease severity percentage was constant and uniform. Until day 24, both cardboard boxes and plastic fruit basket reach 100% of disease severity. The severity on the fruit had fully infected all ten tomatoes available in the packaging. Surprisingly, the plastic zip lock bag only reaches 93.75% of disease severity.

The tomatoes in cardboard boxes and plastic fruit container were infected 100% until day 30. Some of the tomatoes in the packaging fully damage and dried. Meanwhile, the tomatoes in the plastic zip lock bags have 96.25% of the percentage of disease severity on day 30. The tomatoes are not fully infected by *Botrytis cinerea*.

The tomatoes in each packaging show an early sign of disease on day 3. The packaging with tomatoes is infected in day 24 which designate that the disease severity was 100%. Unfortunately, the plastic zip lock bags are not fully infected until day 30. This might indicate that the tomatoes in packaging plastic zip lock bags need more time to fully infect by *Botrytis cinerea*. That means, this treatment performed better than other treatment.

For control, the tomatoes are not having severity because of the pathogen *Botrytis cinerea*. The tomatoes in every packaging began to rot and become watery. The tomatoes are slightly damage and have the lesion on the fruits but it was not caused by the disease.

According to Gao, Qin, Li, and Zhou, in 2018, the value of disease severity was calculated using formula as in equation (3.2). In the research, the result of the inoculation assays on tomato fruits showed that B. cinerea causes a necrotic lesion on all tomato fruits in every treatment.

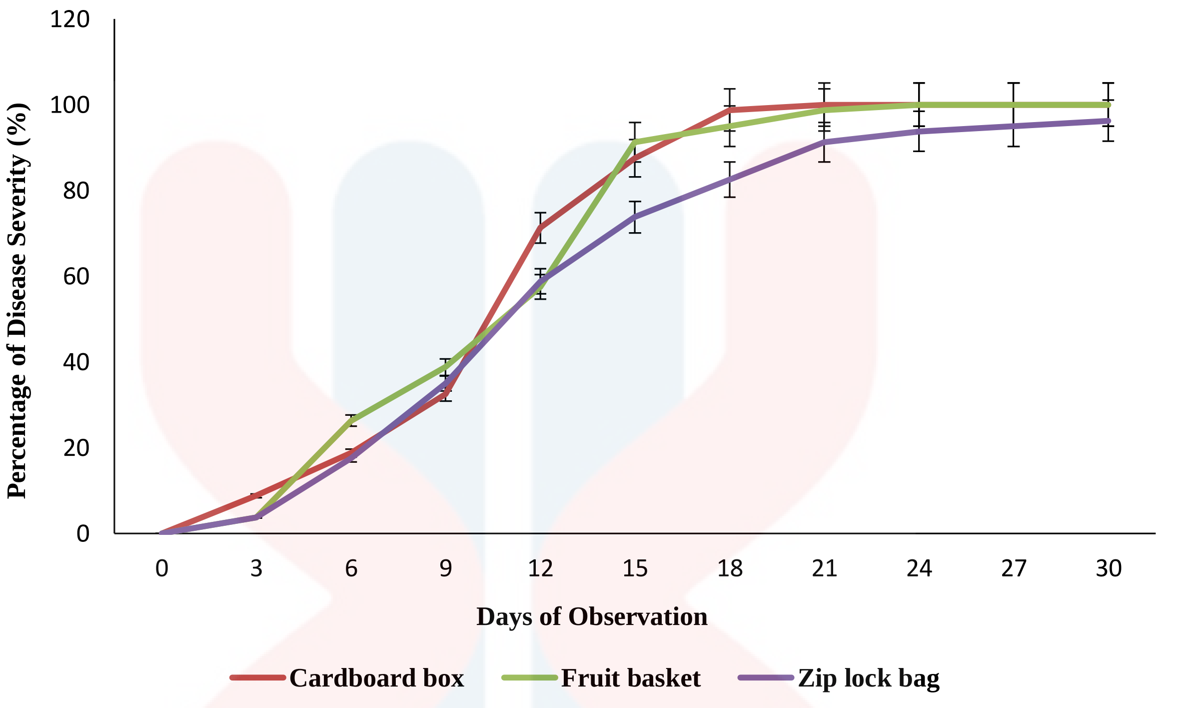


Figure 4.2: The graph of disease severity in each packaging

From the figure 4.2, the graph displays the percentage of disease severity for each packaging system of tomatoes used in 30 days of observation. It shows that the percentage of disease severity was increasing progressively. The data was plot using the average of two replication of each packaging, A and B.

From the tabularized result, the data were analysed using One-Way ANOVA. When the p-value is same or less than 0.05 ($p \leq 0.05$), significant value due to the differences was obtained. When the p-value is more than 0.05 ($p > 0.05$), the significant value due to the differences was not obtained. This means that the significant value of each packaging is different. Due to that, the hypothesis was rejected.

For the treatment used, the ANOVA values are 0.904 which are more than 0.05. This indicates the ANOVA for each treatment is not significant to the level 0.05. The hypothesis made was rejected. The mean for each packaging is different for all packaging or at least one of the mean was different.

This ANOVA result can be supported by the graph shown in Figure 4.2, where on day 15 of the observation, the tomatoes in the fruit basket were highly susceptible to pathogen *Botrytis cinerea* (almost 100% severed). On day 21 tomatoes in cardboard boxes were 100% severed while in the fruit basket were fully severed (100% disease severity) on day 24. Day 30 shows that the tomatoes in zip lock bag were not 100% severed.

According to the experimented data, it shows that the percentage of incidence and severity were increased progressively. The infected fruits at 100% were eventually rotted, damaged and died caused by the infection. It shows that no fruits were recovered and could be safe.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

From the study, the packaging used to store the fruits may give different impact on the fruits. The type of packaging that had been used might extend the storage life of many fruits and vegetables. The effectiveness of the packaging also determined the shelf life of the fruits and vegetables store whether it spoil them or increase the freshness of the fruits and vegetables. The storage temperature and humidity also could affect the quality of the food materials. The packaging used and time for storing the foods influences the firmness, freshness, and quality of the tomatoes. From the result obtained, it states that the sealed packaging seems better choice materials that could be used to store the fruits and vegetables.

## 5.2 Recommendation for Further Study

The tomato fruit is a climacteric fruit that necessitates suitable packaging and control of temperature to extend the shelf life. The packaging material to stores the tomatoes really influences quality of the fruits. Besides, the temperature of storage also plays the roles in maintaining the quality. The recommended packaging to store the fruits is Xtend bag as well as the sealed box for better performances. The suitable temperature to store the fruits is around 4$^{o}$C, according to Mekonnen, Z. T., (2017).

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# APPENDIX

Table A.1: The percentage of disease incidence

| Days/Packaging | Percentage of Disease Incidence (%) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | **27** | **30** |
| Cardboard box | 0 | 70 | 70 | 85 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Fruit basket | 0 | 45 | 90 | 95 | 95 | 100 | 100 | 100 | 100 | 100 | 100 |
| Zip lock Bag | 0 | 70 | 75 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table A.2: The percentage of disease severity

| Days/Packaging | Percentage of Disease Severity | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | **27** | **30** |
| Cardboard box | 0 | 8.75 | 18.75 | 32.5 | 71.25 | 87.5 | 98.75 | 100 | 100 | 100 | 100 |
| Fruit basket | 0 | 3.75 | 26.25 | 38.75 | 57.5 | 91.25 | 95 | 98.75 | 100 | 100 | 100 |
| Zip lock bag | 0 | 3.75 | 17.5 | 35 | 58.75 | 73.75 | 82.5 | 91.25 | 93.75 | 95 | 96.25 |

Table A.3: One-Way ANOVA of disease severity

| | Sum of Squares | Df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 203.535 | 2 | 101.768 | 0.101 | 0.904 |
| Within Groups | 30133.712 | 30 | 1004.457 | | |
| Total | 30337.247 | 32 | | | |

Figure A.1: Tomato used in the experiment

Figure A.2: Inocultion of *Botrytis cinerea* on tomato (Day 0)

Figure A.3: Day 3 of inoculation *Botrytis cinerea*

Figure A.4: Day 30 of observation (Cardboard box)

Figure A.5: Day 30 of observation (Plastic fruit basket)

Figure A.5: Day 30 of observation (Zip lock bag)