Effect of Betel leaf extract on the control of stem-end rot disease and overall quality retention of Papaya (*Carica papaya* L.) during cold storage

By

Fathumma Rizana Shiraz, BSc (Hons)

Thesis Submitted in Fulfillment of

the Requirement for the Degree of

Master of Philosophy

School of Biosciences, Faculty of Science

The University of Nottingham Malaysia Campus

March 2018

DEDICATION

To my family with my love

ABSTRACT

Effect of Betel leaf extract on the control of stem-end rot disease and overall quality retention of Papaya (*Carica papaya* L.) during cold storage

Postharvest losses of papaya due to fungal diseases is one of the major problem in fruit industry in Malaysia and worldwide. Stem-end rot an important postharvest fungal disease in papaya fruit is caused by *Lasiodiplodia theobromae* (synonym *Botryodiplodia theobromae*) This pathogen is a causative agent for the stem-end rot diseases in many other fruits such as Mango, Rambutan, Avocado, Annona and Citrus fruits. No specific control measures have been developed for this disease however, field sprays with systemic and contact fungicides could reduce field inoculum levels and reduce disease incidence. Moreover, due to developed resistance in pathogen and health concerns, there is a great demand to find alternatives to synthetic fungicides.

Betel leaves, *Piper betle* L., widely available in Malaysia and Asian region, has been known for its antifungal and antimicrobial activities due to its bioactive chemical constituents, such as hydoxychavicol, fatty acids (stearic acid and palmitic acid) and hydroxy fatty acid esters. However, for *Piper betel* L., most of the studies were carried out to find the antibacterial property of the plant, especially with human pathogens. The aim of this study was to evaluate the *in vitro* and *in vivo* efficacy of Betel Leaf Extract against *L. theobromae,* stem-end rot pathogen of Papaya and many other fruits.

Two crude extracts were used for this research, one was Betel leaf extract in propylene glycol (BLPG) and Betel leaf ethanol extract (BLET). 6%, 8%, 10%, and 12% concentrations of each type of Betel Leaf extracts, were tested for their antagonistic potential against *L. theobromae*. Maximum inhibition in radial mycelia growth 98.48% and 93.67% of *L. theobromae* was observed in 12% Betel leaf ethanol extract(BLET) and 12% Betel leaf propylene glycol(BLPG) extract respectively, as compared to the control. Maximum spore germination inhibition 93.2% was observed in 12% Betel leaf ethanol extract.

Scanning Electron Microscopy (SEM) images revealed that morphological changes in fungal hyphae and fungal conidial spores and complete cell lysis, with 10% and 12% of Betel leaf extract. *In vivo* studies show that, percentages of disease Incidence and disease severity of coated papaya during 28 days of cold storage ($10 \pm 2^{o}C$,

80%RH), is significantly low in coated fruits with Betel leaf extract as compared to the control.

Assessment of weight loss, colour development, firmness, soluble solids content (SSC), titratable acidity (TA) and antioxidant level (Ascorbic acid and Lycopene content) of the fruits during the cold storage at $10\pm2^{o}C$ (RH 80%) period was initially conducted at regular intervals for 28 days. Significant increase ($p < 0.05$) in the SSC, TA and Antioxidant amount of papaya was observed in response to the treatment with edible coating BLET and BLPG, along with delayed firmness loss. This provided physiological evidence of a relationship between treatment of Betel leaf extract edible coating and enhanced quality attributes in papaya 'Sekaki'.

In the present study, fruit treated with BLET and BLPG did not show the greatest changes in colour development and weight loss, despite better disease control provided by Betel leaf extracts, suggesting that fruit treated with BLET and BLPG delayed physiological changes in papaya fruit hence resulting in firmer fruit. Preliminary sensory results also confirmed the efficacy of using composite coatings based on Betel leaf extract. Delay ripening of fruit was obtained with the low ethylene production and low respiration rate in BLET coated papaya fruits.

The present results are the first to show that extracts from Betel leaf extract can act as antifungal agents against stem-end rot disease in papaya. It can be concluded from this study that 60% control of stem-end rot disease on papaya in the 28 days cold storage at $10\pm2^{0}C$ (RH 80%) achieved with 12% BLET extract combined with 1% Tween80. This could be considered as an effective and potential edible coating as an alternative to synthetic fungicides. This extract might be particularly suitable for the papaya growers and exporters as a postharvest biopesticide. In conclusion, Betel leaf ethanolic extract can be fruitfully utilized as an eco-friendly "green fungicide" to reduce stem-end rot disease at 12% concentration and extend quality and shelf-life of papaya in cold storage, thus minimize the postharvest economic loss.

ACKNOWLEDGEMENTS

Allah, the supreme ruler of the Universe, the Most Gracious and Most Merciful, has enabled me to complete the thesis for the degree of Master of Philosophy (MPhil.) in Bioscience from the School of Biosciences, the University of Nottingham Malaysia Campus (UNMC), Malaysia.

I would like to express my sincere gratitude and profound indebtedness to my honorable research Supervisor, Professor Asgar Ali Warsi, the Director of the Centre for Postharvest Biotechnology, UNMC for his valuable guidance, kind patient and generous help in carrying out of the research work and writing up the thesis. I thank you wholeheartedly, not only for your consistent encouragement and scholarly inputs but also for giving me constructive criticism and unconditional support.

I wish to convey my profound respect and heartfelt gratitude to Dr. Ajith Singh for his valuable guidance and teaching to learn statistics module that helped me to analyze my research data sets and moral support throughout the study. Statistical analysis of the experimental data would be difficult without your guidance and immense knowledge in statistics.

I will remain greatly indebted to honorable internal assessor, Dr. Soma Mitra for her constructive and brilliant suggestions and comments.

I am also hugely appreciative to my Co-supervisor Dr. Christina Vimala Supramanium for giving me an opportunity to learn Molecular plant pathology, which was helpful for me to understand and write my thesis in the area of fungal pathogens and diseases in my research and giving me encouragement throughout the study.

I am very thankful to Dr. Eunice Siew Ching Ngai, Postgraduate Advisor, for her support and guidance throughout my research study under School of Bio Sciences, UNMC.

I gratefully acknowledge Dr. Jean and Dr. Tissa for their valuable guidance and support through graduate programs and all the staff in the Graduate School for giving strength and support throughout my graduate study at UNMC.

I would like to express my gratitude and cordial thanks to Postgraduate administrator Mr. Marcus, staff in the administration

office and purchase officer Radha, Ms. Fifi, Ms. Carol and Vanitha for their quick help and support whenever I reached them.

I like to express my heartfelt gratitude to some faculty members of the institute who have been very kind enough to extend their help at various phases of this research, whenever I approached them, and I do hereby acknowledge all of them. I thank Ms. Siti Norazlin, Ms. Asma Musa, Ms. Shankari, Ms. Haslinda, Ms. Khatijah, Mr. Wong Siak Chung, Mr. Marcus and Mr. Wan Ghani for providing me the technical guidance and materials to complete my research.

I feel immense pleasure in expressing my gratitude to Dr. Paak W. Bordoh and Dr. Azhanee for heartily guiding me to use laboratory facilities and for the encouragement and best support extended to me during the study. I desire to express my cordial thanks to Mrs.Khaironi Jabbar at Scanning Electron Microscopy Lab, Faculty of Electric Engineering, for her valuable help to get SEM images. My deepest gratitude goes to my loving friends Ashrakath, Salwa, Nengi, Aloyce, Akesh, Yaseer, Eni, Asha and well-wishers for their good advices and motivational support, cheerfulness and inspiration during the study.

I like to express my special thanks and gratitude to my former lecturers during my bachelor's degree in the University of Colombo, Sri Lanka, especially Prof. Tara D. Silva, Prof. Shamala Tirimanne and Prof. Kshayanika Hirimburegama. Thank you for your continuous encouragement and support still with me.

I do not have words at command to express my gratitude and profound admiration to my husband Seyed Shiraz Mohamed Ameer without his love, encouragement and editing assistance, I would not have finished this thesis. I owe also my loving thanks to my loving daughters Faadhilah and Aanisha. They have missed and lost a lot due to my research. Above all, I must acknowledge my family for their loving support they provided me through my entire life and in especially my Mother, two Brothers and my Mother-in-law.

SUPERVISORY COMMITTEE

The thesis submitted to the School of Biosciences, Faculty of Science, The University of Nottingham Malaysia Campus has been accepted as fulfilment of the requirement for the degree of Master of Philosophy. The members of the Supervisory Committee are as follows:

Principal Supervisor:

Asgar Ali, PhD

Professor

Director of Centre of Excellence for Postharvest Biotechnology (CEPB)

School of Biosciences

The University of Nottingham Malaysia Campus

Co-supervisor:

Christina Vimala Supramaniam, PhD

Doctor

School of Biosciences

The University of Nottingham Malaysia Campus

DECLARATION

I hereby declare that he thesis is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at the University of Nottingham Malaysia Campus or other institutions.

…….

F. Rizana Shiraz

(Date : 29.05.2018)

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS vi

SUPERVISORY COMMITTEE viii

DECLARATION ix

TABLE OF CONTENTS x

LIST OF TABLES xiii

LIST OF FIGURES xiv

LIST OF ABBREVATIONS xviii

1. CHAPTER : INTRODUCTION 1

1.1. HYPOTHESIS 3

1.1. RESEARCH AIMS AND OBJECTIVES 4

2. CHAPTER: REVIEW OF LITERATURE 5

2.1. PAPAYA 5

2.1.1. Nutritional value 6

2.1.2. Papaya industry in Malaysia 8

2.1.3. Postharvest diseases in papaya 8

2.2. STEM-END ROT DISEASE 9

2.2.1. *Lasiodiplodia theobromae* 9

2.3. BETEL LEAF 10

2.3.1. The uses of betel leaf 11

2.3.2. Properties of Betel leaf 12

2.3.3. Chemical constituents of Betel leaf 12

2.3.4. Betel leaf extract against phytopathogens 15

3. CHAPTER : EFFECT OF BETEL LEAF EXTRACTS ON POSTHARVEST STEM-END ROT PATHOGEN 17

3.1. INTRODUCTION 17

3.2. MATERIALS AND METHODS 19

3.2.1. Isolation and identification of pathogen 19

3.2.2. Pathogenicity test 19

3.2.3. Preparation of Betel leaf extract 20

3.2.3.1. Betel leaf extract in Propylene glycol 20

3.2.3.2. Betel leaf extract using 80% ethanol 20

3.2.4. *In vitro* antifungal assay of Betel leaf extract 22

3.2.4.1. Dilution Method 22
3.2.4.2. Conidia Inhibition test 23
3.2.5. *In vivo* antifungal assay of Betel leaf extract 23
3.2.5.1. Disease Severity 25
3.2.5.2. Disease Incidence 25
3.2.6. Studies of Scanning Electron Microscopy (SEM) 26
3.2.7. Statistical analysis 26
3.3. RESULTS AND DISCUSSION 27
3.3.1. Isolation and identification of pathogen 27
3.3.2. Pathogenicity test 28
3.3.3. *In vitro* antifungal assay 28
3.3.3.1. Mycelial growth inhibition 28
3.3.3.2. Spore germination inhibition 31
3.3.4. *In vivo* antifungal assay 35
3.3.4.1. Disease Severity 35
3.3.4.2. Disease Incidence 37
3.3.5. Studies of Scanning Electron Microscopy (SEM) 40
4. CHAPTER: EFFICACY OF BETEL LEAF EXTRACT ON POSTHARVEST QUALITY OF FRUITS IN THE COLD STORAGE 44
4.1. INTRODUCTION 44
4.2. MATERIALS AND METHODS 46
4.2.1. Fruit samples 46
4.2.2. Coating solutions 46
4.2.3. Sample preparation 46
4.2.4. Determination of physical quality 47
4.2.4.1. Weight loss percentage 47
4.2.4.2. Fruit firmness 47
4.2.4.3. Peel colour 47
4.2.5 Determination of chemical quality 48
4.2.5.1. Soluble solids concentration (SSC) 48
4.2.5.2. Titratable acidity 48
4.2.6. Determination of antioxidants 49
4.2.6.1. Determination of Ascorbic acid content 49
4.2.6.2. Determination of total Lycopene 50
4.2.7. Sensory evaluation of ripe papaya 51

4.2.8. Gaseous exchange analysis 52
4.2.8.1. Determination of respiration rate 52
4.2.8.2. Determination of ethylene production 52
4.2.9. Experimental design 53
4.3. RESULTS AND DISCUSSION 54
4.3.1. Physical quality changes in papaya 54
4.3.1.1. Weight loss 54
4.3.1.2. Fruit firmness 55
4.3.1.3. Peel colour 57
4.3.2. Chemical quality changes in papaya 61
4.3.3. Nutritional quality changes in papaya 64
4.3.3.1. Changes in Ascorbic acid content 64
4.3.3.2. Changes in Lycopene content 65
4.3.4. Sensory evaluation of ripe papaya 67
4.3.5. Gaseous exchange analysis 70
5. CHAPTER: GENERAL CONCLUSION 73
REFERENCES 77
APPENDICES 87
BIODATA OF THE AUTHOR 100
LIST OF CONFERENCE ABSTRACTS 101
ORAL AND POSTER PRESENTATIONS 101
PUBLICATIONS 102

LIST OF TABLES

Table 1. Top papaya producing countries in the world..... 6

Table 2. Nutritive value of 100g of Papaya fruit..... 7

Table 3. Chemical compounds of *P. betle* and their uses..... 13

Table 4. Sensorial parameters of Betel leaf extract treated and negative control 'Sekaki' papaya after treated for 28 days under cold storage conditions (10±2^{0}C and 80 ± 5% RH). 68

LIST OF FIGURES

Figure 2.1. Papaya (Carica papaya, L.) fruit..... 5

Figure 2.2: stem-end rot on papaya (Carica papaya, L.)..... 9

Figure 2.3. (a) Betel vine plant, (b) Betel leaves and Betel leaf powder. 11

Figure 3.1. Preparation steps of Betel leaf ethanolic extract 21

Figure 3.2. Preparation of different concentrations of Betel leaf extract using Betel leaf ethanolic extract (BLET) and Betel leaf propylene glycol extract(BLPG) 24

Figure 3.3. Coated Papaya with Betel leaf extract were stored in the cold storage (10+2°C) for 28 days for in vivo studies. 25

Figure 3.4. (a)-Pure culture of Lasiodiplodia theobromae, and (b)-germination tube with conidial spore. 27

Figure 3.5. (a) & (b) Disease symptoms on inoculated papaya 28

Figure 3.6. Mycelial growth inhibition with Betel leaf ethanolic extract 29

Figure 3.7. Mycelial growth inhibition with Betel leaf propylene glycol extract 30

Figure 3.8. Effect of various concentrations of Betel leaf extracts on mycelial growth inhibition of L. theobromae. Values with different letters are significantly different according to ANOVA and Duncan's Multiple Range Test (DMRT) 30

Figure 3.9. Effect of concentrations 3%, 4%, 6%, 8%, 10% and 12% of Betel leaf ethanolic extracts on spore germination of L. theobromae, after 4 days incubation at room temperature (28+2°C). 31

Figure 3.10. Effect of concentrations 6%, 8%, 10% and 12% of Betel leaf propylene glycol extracts on spore germination of L. theobromae, after 4 days incubation at room temperature (28+2°C). 32

Figure 3.11: Effect of various concentrations of Betel leaf extracts on spore germination inhibition of L. theobromae. Values with different letters are significantly different according to ANOVA and Duncan's multiple range test (DMRT). 33

Figure 3.12. Spore germination inhibition test under light microscope; (a)-control, (b)-8% BLET, (c)-10% BLET, (d)-12% BLET, (e)-8% BLPG, (f)- 10% BLPG and (g)-12% BLPG. (BLET-Betel leaf ethanolic extract, BLPG- Betel leaf extract in propylene glycol). 34

Figure 3.13. Disease severity for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 35

Figure 3.14.Disease severity of inoculated papaya fruits after 2 weeks of cold storage at 10+2^{0}C treated with different concentrations of Betel leaf extract (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol). 36

Figure 3.15. Disease incidence (%) for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 37

Figure 3.16. Disease incidence and disease severity of inoculated papaya after 28 days cold storage at 10+2^{0}C with different concentrations of Betel leaf extract. 39

Figure 3.17. At the end of storage period, mycelial growth of pathogen inside the fruit, which was coated with 8% BLPG. 39

Figure 3.18. Images of Scanning Electron Microscope of mycelial growth on PDA amended with both types of Betel leaf extracts; (a)-Control, (b)-6% BLET, (c)-8% BLET, (d)-10% BLET, (e)-12% BLET, (f)-6% BLPG, (g)-8% BLPG, (h)-10% BLPG and (i)-12% BLPG. (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol). 41

Figure 3.19. Images of Scanning Electron Microscope of spore germination on PDA amended with both types of Betel leaf extracts; (a)-Negative, (b)-6% BLET, (c)-8% BLET, (d)-10% BLET, (e)-12% BLET, (f)-6% BLPG, (g)-8% BLPG, (h)-10% BLPG and (i)-12% BLPG. (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol). 42

Figure 4.1: Weight loss (%) of papaya for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates 54

Figure 4.2. Firmness of papaya for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 56

Figure 4.3. Lightness of papaya for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 58

Figure 4.4. Hue value of papaya for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 59

Figure 4.5. Chroma value (C) of papaya for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates 60

Figure 4.6. Soluble Solids Concentration (Brix %) of fruit pulp for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates. 61

Figure 4.7. Titratable acidity (%) of fruit pulp for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates 63

Figure 4.8. Ascorbic acid (mg) in 100g of "Sekaki" papaya fruit pulp for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P < 0.05$). 64

Figure 4.9. Lycopene content (mg) in 100g of "Sekaki" papaya fruit pulp for 28 days during cold storage at 10+2^{0}C with different concentrations of Betel leaf extract. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P < 0.05$). 66

Figure 4.10. Effect of coating with different concentrations of Betel leaf extracts on the sensory traits of "Sekaki" papaya fruits after four weeks of storage at 10+2^{o}C. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P < 0.05$). 69

Figure 4.11. Effect of different concentrations of Betel leaf extract on CO2 ($mlkg^{-1}hr^{-1}$) production of "Sekaki" papaya fruit during cold storage at 10+2^{0}C for 28 days. Each value is

the mean of four replicates; vertical bars indicate standard errors of the means; at (P < 0.05). 70

Figure 4.12. Effect of different concentrations of Betel leaf extract on C_2H_4($\mu lkg^{-1}hr^{-1}$) production of "Sekaki" papaya fruit during cold storage at 10+2^{0}C for 28 days. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at (P < 0.05). 71

LIST OF ABBREVATIONS

ANOVA	Analysis of Variance
BLET	Betel leaf ethanolic extract
BLPG	Betel leaf extract in propylene glycol
^{o}C	Celsius
CaCl2	Calcium Chloride
CRD	Completely randomized design
CO2	Carbon dioxide
DMRT	Duncan multiple range test
DNA	Deoxyribonucleic acid
DS	Disease Severity
DI	Disease Incidence
GC	Gas chromatograph
Fig	Figure
g	Gram
µg	microgram
Kg	Kilogram
$NaCO_3$	sodium carbonate
NaOH	sodium hydroxide
ml	milliliter
µl	microliter
ppm	parts per million
SE	standard error
SSC	soluble solid contents
TA	titratable acidity
TCD	Thermal Conductivity Detector
FID	Flame-Ionization Detector

1. CHAPTER : INTRODUCTION

Fruit industry is very important in Malaysia and worldwide because, it provides food for people and create jobs as well as generates revenues for economic development. Fruits provide essential vitamins, minerals and dietary fiber as well as reduce risk for cancers, obesity and chronic diseases. Papaya (*Carica papaya* Linn.) fruit in the family Caricaceae, is commonly known for its food and nutritional values throughout the world. It has a high demand due to its affordable price, easily available as well as its high nutrient value such as vitamin A, C, minerals, fibers and dietary antioxidants such as Beta-carotene, B-cryptoxanthin and Lycopene (Rivera-Pastrana, González-Aguilar, & Yahia, 2010).

Papaya is native to tropical America, and is widely cultivated as a commercial fruit crop in many tropical and sub-tropical regions around the world (Bautista-Baños et al., 2013). In Malaysia, papaya is one of the main fruit crop that gives high economic value for farmers and export market. Currently, 'sekaki' is the leading cultivar for domestic consumption and export market, where Hong Kong is the major export destination of Malaysian papaya followed by Singapore, United Arab Emirates and Brunei. In 2003, the export value of fresh papaya was estimated at RM 100.8 million, up from 23.6 million in 1992 (Rahman, 2008).

Postharvest losses are one of the main constraint in production of fresh fruits and vegetables. Papaya being a climacteric fruit, its rapid ripening and softening limits the storage, handling and transport potential (Ravneel Prasad, 2015). The postharvest losses reported for papaya are around 46 % (Jayathunge et al., 2014) and the losses are due to various factors such as mechanical damage, chilling injury, diseases and fruit overripens. Moreover, diseases caused by micro-organisms, specially fungi, add towards high percentage of postharvest losses. Although refrigeration is an effective method of storage of papaya in local market and during transportation in export market, the fungal diseases are still the major problem during cold storage. Bautista-Baños et al., (2013b) stated that papaya postharvest losses due to diseases ranged between one to 93% depending on postharvest handling and packing procedure. Generally, the diseases are characterized as; fruit surface rots, stem-end rots and internal fruit infections. According to previous findings 'sekaki' variety in Malaysia, is highly susceptible to anthracnose caused

by *Colletotrichum gloeosporioides*, followed by stem-end rot caused by *Botryodiplodia theobromae* (Rahman, 2008). The pathogen spores invade through crevices between the peduncle and the papaya flesh or invade through small wounds that occur at the harvest and cause stem-end-rot disease (Alvarez and Nishijima,1987).

Similarly, Netto et al.(2014) mentioned that disease incidence of stem-end rot caused by *Lasiodiplodia theobromae* is up to 70-80%, with a resulting reduction in commercial value of the fruit, worldwide. *L. theobromae* (synonym *Botryodiplodia*) species are common in tropical and subtropical regions and they cause a variety of diseases in up to 500 plant hosts where several economically important crops, especially avocado, banana, cashew, citrus, mango, rambutan, soursop, passion fruit, guava, water melon and coconut palm (Netto et al., 2014a).

Traditionally control of papaya postharvest fungal diseases depends on treatment with a series of chemical fungicides, namely, thiabendazole and benomyl. This treatment is reported to control the diseases up to 50% (Hewajulige & Wilson, 2010). However, prolonged application of fungicides on fresh fruits and vegetables may lead to development of resistant strains and reduce the effectiveness of fungicide against the target organisms. At the same time due to the carcinogenicity, long degradation time and high residual toxicity causes negative impact on human health and environment. Therefore, researchers are trying to find alternative treatment methods to synthetic fungicides for reducing postharvest diseases (A. Ali, Hei, & Keat, 2016a).

The utilization of natural products, especially the plant extracts, has been shown to be effective against many plant pathogens and considered to be safe for consumers and environments (Bussaman et al., 2012). *Piper betel*, a member of the Piperaceae, is also known "Sirih" in Malaysia and Indonesia, is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes (Guha, 2006). This plant is economically, medicinally and traditionally important in the whole world. It is originated from Malaysia but is distributed extensively in South and Southwest China. This crop is usually cultivated in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries.

P.betle, 'Betel' leaves contain a wide variety of biologically active compounds, which are responsible for its antioxidant, antifungal, antibacterial and chemo preventive activity (I. Ali et al., 2010a). The

leaves contain eugenol rich essential oil (1%–3%) which is the source for medicine, stimulant, antiseptic, tonic and other ayurvedic formulations. The essential oil also contains chavibetol, caryophyllene and methyl eugenol which are the potent source for preparation in ayurvedic medicine and herbal products.(Das et al., 2016).

In agreement with the current trend of giving value to the natural and renewable resources, the use of natural antimicrobial compounds to prevent or reduce postharvest diseases in fruits and vegetables, becomes very frequent. The direct addition of natural compounds to the fruit is the most common method of application, even if numerous efforts have been made to find alternative solutions to the aim of avoiding undesirable inactivation. This involves dipping, spraying, and coating treatment of fruits with active solutions prior to packaging. Numerous efforts were made to find alternative methods of treatment application with the aim of avoiding undesirable inactivation of bioactive compounds in natural products.

Piper betel is a medicinally important plant and well-studied for its antimicrobial activity but poorly explored to screen antifungal potency against fungal pathogens which cause various plant diseases. To our knowledge there are only few reports about the use of Betel leaf extract against plant pathogens (Jasrai & Patel, 2013; Kasiamdari & Sangadah, 2015; Srichana, Phumruang, & Chongkid, 2009).

Up to date, there are no reports on the antagonistic potential of Betel extract to suppress or inhibit the growth of postharvest diseases caused by fungi in papaya. Therefore, aim of the current work was to find out the effect of betel leaf extract as a coating of fruit to control the growth of stem-end rot pathogens in papaya and to apply it at a suitable concentration to inhibit the spore germination and mycelial growth of the pathogen. In addition, postharvest quality of the fruits during storage was also determined.

1.1. HYPOTHESIS

The application of Betel leaf extract will help to control *L. theobromae* and suppress disease of stem-end rot of papaya (*Carica papaya*, L.) due to its bio active components.

RESEARCH AIMS AND OBJECTIVES

The main objective is to develop antimicrobial coating out of Betel leaf extract and to study their efficacy on postharvest stem-end rot disease and the quality of papaya fruit to develop an alternative safe postharvest treatment method to control stem-end rot disease in Papaya.

1. To isolate and identify the causative fungal pathogens of stem-end rot disease in Malaysian papaya.

2. To determine the efficacy of Betel leaf extract concentrations formulations to control stem-end rot fungal pathogens and their spore germination *in vitro*.

3. To determine the efficacy of betel leaf extract incorporated with Tween-80 to control stem-end rot disease *in-vivo,* for 28 days cold storage at $10 \pm 2^{o}C$.

To determine the effect of betel leaf extract on postharvest physiochemical quality, nutritional quality, sensory quality and gaseous exchange of papaya fruits, during 28 days of cold storage at $10 \pm 2^{o}C$.

2. CHAPTER: REVIEW OF LITERATURE

2.1. PAPAYA



Figure 2.1. Papaya (*Carica papaya*, L.) fruit

Papayas are globular, pear shaped fruit which can grow up to 20 inches long. The skin of unripe fruit is green and turns to orange when ripe while the flesh inside is yellow, orange or red (Figure 2.1). Inside the center, there are many small black seeds, which are edible but bitter. The Papaya (*Carica papaya* L.) which is a climacteric fruit that belongs to the family Caricaceae is a commercial fruit crop in many tropical and sub-tropical regions around the world. Papaya is probably originated in Southern Mexico and Costa Rica, subsequently it was introduced as a plantation crop in Australia, Hawaii, Philippines, Malaysia, Srilanka, India, South Africa and in all tropical and sub-tropical regions (Krishna et al., 2008). India and Brazil are the leading production country of papaya industry in the world (Table 1).

Since green papaya contains an enzyme called papain, which can break down the tough protein chains found in muscle meat, people have used papaya to tenderize meat for thousands of years. Papaya is a common man's fruit due to availability and affordable price for everyone and its high nutritive value. Due to the fact it can be intensively cultivated, its rapid returns and the increased demand for the fresh fruits and its processed products, papaya is one of the major fruit product that gives high economic value for farmers and export market. Over the last two decades, papaya fruit ripening and handling research has taken on renewed importance, due to an increased worldwide consumer's

awareness of this fruit and the subsequent expansion in production and exports.

Table 1. Top papaya producing countries in the world.

Rank	Country	Production in millions of ton
1	India	5.5
2	Brazil	1.6
3	Indonesia	0.9
4	Nigeria	0.8
5	Mexico	0.8

(https://www.worldatlas.com/articles/top-papaya-producing-countries-in-the-world.html)

2.1.1. Nutritional value

The papaya is an incredibly healthy tropical fruit. Among the fruits Papaya leads first for its rich nutritive value and low in calories. It supplies natural vitamins and minerals; such as vitamins C, Vitamin A, riboflavin, folate, thiamine, niacin, iron, potassium, fibers and dietary antioxidants (Table 2). It is loaded with antioxidants that can reduce inflammation, fight diseases and help keep looking young. Papayas contain healthy antioxidants called carotenoids such as Beta-carotene, beta-cryptoxanthin and high amount of Lycopene (Table 2).

Schweiggert et al (2014) mentioned that even though carrot, tomato and papaya represent important dietary sources of β-carotene and lycopene, the bioavailability of β-carotene from papayas was approximately three times higher than that from carrots and tomatoes. Similarly, lycopene was approximately 2.6 times more bioavailable from papayas than from tomatoes. Furthermore, the bioavailability of β-cryptoxanthin from papayas was shown to be 2.9 and 2.3 times higher than that of the other papaya carotenoids β-carotene and lycopene, respectively. Particularly, the liquid-crystalline deposition of β-carotene and the storage of lycopene in very small crystalloids in papayas were found to be associated with their high bioavailability (Schweiggert et al., 2014).

Studies have found that fermented papaya can reduce oxidative stress in the elderly and people with prediabetes, mild hypothyroidism and liver disease (Marotta et al., 2006; Marotta et al., 2007; Prus & Fibach, 2012; Somanah et al., 2014; Tomella et al., 2014). Fermented papaya preparation (FPP) exhibited potent *in vitro* free radical scavenging activities attributed to residual phenolic or flavonoid compounds.

Table 2. Nutritive value of 100g of Papaya fruit

Constituents	**Ripe Papaya**	**Green Papaya**
Protein	0.6 g	0.7 g
Fat	0.1 g	0.2 g
Minerals -Potassium	0.5 g	0.5 g
Calcium & Magnesium	Trace amounts	
Fibre	0.8 g	0.9 g
Carbohydrates	7.2 g	5.7 g
Energy	32 kcal	27 kcal
Total Carotenoid	2,489 µg	0
Beta Carotene	228 µg	0
Cryptoxanthin	1483 µg	
Lycopene	2003 µg	
Vitamin C	157% of the RDI	
Vitamin A	33% of the RDI	

Vitamin B9 (Folate)	14% of the RDI
Vitamin B1, B3, B5, E & K	Trace amounts

[Krishna et al. (2008); Tee & Lim, (1991), www.healthline.com]

The many studies confirm that dietary supplementation with Lycopene reduces risk of cancers of many organs, and also retard the growth of the tumors. Lycopene has also chemo-preventive effects against other diseases such as cardiovascular disease, osteoporosis, male infertility and inhibits the toxic action of other agents. Numerous *in vitro* and animal studies showed that Lycopene may provide protection against damages induced by ionizing radiation. It suggests that supplementation of Lycopene might be useful in diminishing of negative effect of cancer radiotherapy or in mitigating the effects of possible radiation accidents on human health. (Gajowik & Dobrzyńska, 2014)

2.1.2. Papaya industry in Malaysia

Papaya industry eradicate poverty by generating income for farmers in rural, urban and peri-urban agriculture, due to its rapid return of profit. By increasing values of agricultural produce for export markets, papaya fruit industry contributes significantly, for economic development in Malaysia and many other countries. Azlina (2014), mentioned that fruit industry is one of the important sources in agriculture in Malaysia, with estimated market potential for RM 5,243.40 Million and fruits production is expected to grow annually by 3.8% in next decade.

2.1.3. Postharvest diseases in papaya

Postharvest losses are quite high in papaya fruit when compared with other tropical fruits. Short postharvest life and susceptibilities to physical damage, water loss, chilling injury, diseases and insect-pests are major postharvest constraints for papaya fruit. Amongst all postharvest losses due to the fungal diseases is a major constraint in papaya production and supply chain in Malaysia and worldwide. Postharvest fungal diseases of papaya can be categorized into three groups: fruit surface rots, stem-end rots and internal fruit infections.

2.2. STEM-END ROT DISEASE



Figure 2.2: stem-end rot on papaya (Carica papaya, L.)

Stem-end rot caused by *Lasiodiplodia theobromae* (Pat.) is an important postharvest disease of papaya crop in Malaysia and worldwide. Netto (2014a) mentioned that Disease incidence can be reached around 70–80%, and thereby reduction in the commercial value of the fruit. Stem-end rots of papaya occur when fungi invade the severed peduncle after harvest. Spores may also invade through crevices between the peduncle and the papaya flesh or invade through small wounds that occur at harvest (Alvarez and Nishijima, 1987). Symptoms of papaya stem rot begin at the stem and advance throughout the entire fruit. *Botryodiplodia theobromae* Pat. produced a wide margin of water-soaked tissues at the base of the fruit (Figure 2.2).

2.2.1. *Lasiodiplodia theobromae*

Lasiodiplodia theobromae is an economically important fungal pathogen with a worldwide distribution in tropical and subtropical regions. It has over 500 different kinds of host plants and can cause many diseases such as tree dieback, root rot, fruit rots and leaf spot (Masilamani & Muthumary, 1996). This fungus is a key pathogen that causes postharvest decay on many important horticultural fruits such as citrus, avocados, mangos, papayas, bananas, guava, Annona and rambutan in tropical and subtropical regions in the world (Kunz et al., 2004; Mohamed & Saad, 2009; Wijeratnam & Dharmatilaka, 2008; Sivakumar et al., 2002; Zhang, 2014). This postharvest decay can lead to

significant economic losses, and the fungus can also cause preharvest diseases on some horticultural crops, such as mango tree dieback disease (Khanzada et al., 2004).

Based on CABI databases for fungal classifications online (www.indexfung orum.org/), the current taxonomy status of *L. theobromae* is as follows: Kingdom: fungi; Phylum: Ascomycota; Class: Dothideomycetes; Order: Botryosphearíales; Family: Botryosphaeriaceae; Genus: *Lasiodiplodia*; Species: *theobromae* (Pat.) Griffon & Maubl. Synonyms: *Botryodiplodia theobromae* Pat.(Zhang, 2014).

The mycelial growth of *B. theobromae* isolates was classified as fluffy or depressed, uniform to irregular and cottony white turning to black. These isolates were fast to moderately fast growing and dark black pycnidia arranged either on the periphery, centered or scattered in the colonies (Shah, Verma, Singh, & Kaur, 2010). Colony of *Lasiodiplodia theobromae* produced very thin, uniform, thread like mycelia in all the media. Conidia are double layered, hyaline and unicellular at initial stage but on maturity it becomes light to dark-brown colour with typical striate formation, equally 2-celled, oblong, bilamilate (Chaudhuri, Panja, & Saha, 2017).

2.3. BETEL LEAF

Piper betel, a member of the Piperaceae, which is a large plant family, is also known "Sirih" in Malaysia and Indonesia, "Paan" in India and "Bulath" in Srilanka. The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves (Figure 2.3). It is a native of central and eastern Malaysia, which spread at a very early date throughout tropical Asia and later to Madagascar and East Africa. The betel plant is currently extensively cultivated in India, Bangladesh and Sri Lanka (Palaniappan, Sengottiyan, & Saravanan, 2012).

Palaniappan et al. (2012) mentioned that the betel farming activities can generate employment opportunities about 20 million people in India, derive their livelihoods directly or indirectly by producing, processing, handling, transporting and marketing. Consequently, leaves worth about Rs 30-40 million are exported to European countries. This

clearly indicates the profitability of the crop, which can be further exploited in the interest of the nation.



Figure 2.3. (a) Betel vine plant, (b) Betel leaves and Betel leaf powder.

2.3.1. The uses of betel leaf

In Malaysia, India, Srilanka and Pakistan the betel leaves play an important role in their culture, especially among Hindus and Buddhist. Many traditional ceremonies governing the lives of Hindus and Buddhist use betel leaves. The betel leaves and areca nuts are used ceremonially in traditional weddings (Palaniappan et al., 2012).

Piper betel is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes in traditional Ayurvedic medicine. The fresh leaves of betel leaves have been wrapped together with the areca nut, mineral slaked lime, catechu, flavoring substances and spices are chewed since the ancient time (Guha, 2006). The leaves contain eugenol rich essential oil (1%–3%) which is the source for medicine, stimulant, antiseptic, tonic and other ayurvedic formulations. As mentioned in the Table 3, the essential oil also contains chavibetol, caryophyllene and methyl eugenol which are the potent source for preparation in ayurvedic medicine and herbal products (Das et al., 2016).

Das et al. (2016) mentioned that, the betel leaves are mainly used as mouth freshener and is also well known for curing many communicable and non-communicable diseases like cold, cough, bronchial asthma, rheumatism, stomachalgia and used to treat other diseases like bad

breath, boils and abscesses, conjunctivitis, constipation, swelling of gums, cuts and injuries (Table 3).

2.3.2. Properties of Betel leaf

Investigations on the bioactivities of P. betle revealed the presence of antimicrobial, insecticidal, antioxidant, antinociceptive, antidiabetic and gastroprotective activities. In addition, P. betle was found to be safe in terms of hepatotoxicity, nephrotoxicity, haemotoxicity, gross morphology, weights of organs, stress or aversive behaviors in rats. The above findings indicate the vast potential of P. betle yet to be harnessed for the benefit of mankind and the betel industry. (Arawwawala et al., 2011).

The leaf juice is given systemically to treat cough and indigestion in children. It has anti-malarial activity, anti-diabetic activity, gastro protective activity, antinociceptive activity, cytotoxic activity and anti-platelet according to Baviskar et al. (2017).

2.3.3. Chemical constituents of Betel leaf

Piper betel contains a wide variety of biologically active compounds, which are responsible for its antioxidant, antifungal, antibacterial and chemo preventive activities and its concentration depends on the variety of the plant, season and climate. The aroma of betel leaf is due to the presence of essential oils, consisting of phenols and terpenes.

The various phytochemicals found in the betel plants are chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenol methyl ether, cadinene, γ-lactone, allyl catechol, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, p-cymene, terpinene, eucalyptol, carvacrol, sesquiterpenes, cadinene, caryophyllene, dotriacontanoic acid, hentriacontane, pentatriacontane, stearic acid, n-triacontanol, triotnacontane, piperlonguminine, allylpyrocatechol diacetate, isoeugenol, 1, 8-cineol, α-pinene, β-pinene, sitosterol, β-sitosteryl palmitate, γ-sitosterol, stigmasterol, ursolic acid, ursolic acid 3β-acetate (Sripradha, n.d.).

After analyzing the chemical components of the extract by Gas chromatography-mass spectrometry (GCMS), Nalina & Rahim, (2007)

found that leaves contains fatty acids (stearic acid and palmitic acid) and hydroxy fatty acid esters (hydroxy esters of stearic, palmitic and myristic acids) and hydroxychavicol, with the latter as main component.

Studies on the chemical constituents indicated that safrole is the major constituent, followed by chavibitol acetate, in the essential oil of common betel leaves of Sri Lanka (Arawwawala et al., 2011).

Table 3. Chemical compounds of P. betle and their uses

Compound	CAS no.	Molecular formula	Molecular weight	Uses
a-Pinene	80-56-8	C10H16	136.23	Anti-inflammatory and antibiotic
amphene	79-92-5	C10H16	136.24	Preparation of fragrances and food additive for flavouring
Sabinene	3387-41-5	C10H16	136.24	Antimicrobial activity
Myrcene	23-35-3	C10H16	136.24	Preparation of fragrances and Food additive
A-Terpinene	99-86-5	C10H16	136.23	Cosmetics and food industries
B-Phellandrene	555-10-2	C10H16	136.23	Cosmetics and personal care industries, cleaning products
B-Ocimene	13877-91-3	C10H16	136.23	Perfumery
Terpinolene	586-62-9	C10H16	136.26	Perfumery and food additive
Cis-sabinene	15826-82-1	C10H16	136.23	Anti-Infective Agents
Terpineol-4	8000-41-7	C10H18O	154.25	Fragrance compositions in disinfectants, polishes and household products
Safrole	94-59-7	C3H5C6H3O2CH2	162.19	Beverages and candy preparation
Eugenol	97-53-0	C10H12O2	164.22	Antiseptic and anesthetic, in dentistry
Iso-safrole	120-58-1	C3H5C6H3O2CH2	162.19	Fragrance industry
B-Bourbonene	5208-59-3	C15H24	204.3511	Flavour and fragrance agents

B-Elemene	515-13-9	C15H24	204.35628	Antiproliferative effect, used in chemotherapy for cancer treatment
Methyl Eugenol	93-15-2	C11H14O2	178.22	Fragrance ingredient in perfumes, toiletries and detergents
Caryophyllene	87-44-5	C15H24	204.35	Antioxidant, anti-inflammatory, anti-cancerous and local anesthetic.
Aromadendrene	489-39-4	C15H24	204.35	Antioxidants and anti-ageing
B-Farnesene	18794-84-8	C15H24	204.35	natural insect repellent
A-humulene	18794-84-8		204.39	Anti-inflammatory, effective in reducing platelet activating factor
Methyl isoeugenol	93-16-3	C11H14O2	178.23	Flavour and fragrance agents
Germacerene-D	23986-74-5	C15H24	204.4	Analgesic and anti-inflammatory properties
B-Selinene	17066-67-0	C15H24	204.35628	Antibacterial properties and used in aromatherapy
A-Selinene	473-13-2	C15H24	204.3511	Aroma active compound
A-Farnesene	502-61-4	C15H24	204.3511	Plant defense, biofuel precursor
Hydroxychavicol	1126-61-0	C9H10O2	150.1745	Antimutagenic effect
Eugenyl acetate	93-28-7	C12H14O3	206.24	Anti-virulence potential
A-Cadinene	24405-05-1	C15H24	204.36	Anticancer activity
Germacerene-B	15423-57-1	C15H24	204.356	Antimicrobial and insecticidal properties
E-Nerolidol	40716-66-3	C15H26O	222.37	Flavouring agent and in perfumery
Spathulenol	6750-60-3	C15H26O	220.4	Antibacterial activity
Globulol	489-41-8	C15H26O	222.37	Antimicrobial activity
Chavibetol	501-19-9	C10H12O2	164.2	Aromatic compound with a spicy odour
Allylpyrocatechol Diacetate	13620-82-1	C13H14O4	234.25 234.25	Antimicrobial activity against various obligate oral anaerobes

1,8-Cineol	470-82-6	C10H18O	154.28	Used in treatment of inflammatory diseases

(Das, S. et al., 2016)

2.3.4. Betel leaf extract against phytopathogens

There were few researches done to evaluate Betel leaf extract against phytopathogens. According to findings of Kasiamdari & Sangadah, (2015) in an *in vitro* test concentration of 100% aqueous betel leaf extract was the best concentration to inhibit the growth of *Colletotrichum gloeosporioides* (Penz.) colony by 70.26±0,61% and in an *in vivo* experiment, submersion of strawberry before pathogen infection (preventive) with 100% betel leaf extract decreased disease infection by 40% and disease severity by 16%, respectively. A research about use of an extraction of betel leaf with 95 % ethanol against *Aspergillus flavus* and *Fusarium verticillioides* which are contaminations in food grains, was reported by Srichana et al. (2009). They mentioned that 10,000 ppm concentration of the extract completely inhibited the growth of these fungi.

In a study by Jasrai & Patel (2013), the antifungal potency of *Piper betel* L leaf extracts prepared in various polarity solvents water, methanol, chloroform and petroleum were evaluated against the growth of *Alternaria alternata* (GUB01) isolated from apple fruit, *Aspergillus flavus* (GUB02) from peanuts, *Aspergillus Niger* (GUB03) from lemon, *Fusarium oxysporum* f.sp. *laginariae* (GUB04) from bottle gourd, *Fusarium solani* (GUB05) from potato tuber, *Fusarium solani* (GUB06) from tomato fruit and *Rhizoctonia solani* (GUB07) from potato tuber. A broad spectrum antifungal activity of *Piper betel* Chloroform extract found to inhibit all the selected fungal strains in the study. While *Piper betel* Methanol extract successfully inhibited some fungi. *Piper betel* Petroleum extract inhibited single fungi *Sarocladium oryzae*, while *Piper betel* aqueous extract found ineffective to inhibit the growth of selected fungal strains at a requisite concentration range.

3. CHAPTER : EFFECT OF BETEL LEAF EXTRACTS ON POSTHARVEST STEM-END ROT PATHOGEN

3.1. INTRODUCTION

As a tropical commodity papaya has a short storage life since the storage temperature should generally not be lower than 10°C. In addition, as papaya is a climacteric fruit with a high respiration activity, it is prone to ripen in a short period and as a result pathogen development occurs in short intervals of time. Fruit rots caused by fungi are the most common diseases found in the markets and stem-end rot caused by *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (syn. Botryodiplodia theobromae) is an important postharvest disease of this crop in worldwide. Bautista-Baños et al., (2013a) mentioned that anthracnose may be the leading postharvest pathogen but there are other microorganisms of great relevance since production may be completely lost by their infection as in the case of stem-end rots and *Fusarium oxysporum*. According to da Silva Pereira et al. (2012) the stem-end rot incidence at which it occurs is around 70–80%, with a resulting reduction in the commercial value of the fruit.

Fungicide use is one of the main disease management measures. Since fungicides are widely used for the control of papaya stem-end rot, and the amount of fungicide residue is one of the main limitations for fruit commercialization. However, several fungicides are not used for postharvest treatment or have been removed from the market due to possible toxicological risks (Meneses et al., 2009). Only thiabendazole (the methyl benzimidazole carbamates—MBC group) is registered for the control of *L. theobromae* in papaya (da Silva Pereira et al., 2012). However, one of the most troublesome factors is loss of efficacy due to fungicide resistance, which has become commonplace after the introduction of fungicides with a specific mode of action (SMA). Therefore, new and acceptable post- and pre-harvest disinfestation methods, such as hot air or hot water treatment, Ozone treatment, irradiation and controlled atmosphere technologies were developed.

Nishijima et al., (1992) reported that when hot-air treatment was combined with thiabendazole (TBZ) (4 g/liter) application or hot-water immersion (49° for 20 min), the incidence of most postharvest diseases was reduced, including those caused by *Phomopsis* sp., *Botryodiplodia theobromae* and *Mycosphaerella* sp. However, pitting and scalding

symptoms significantly ($P<0.01$) increased when hot-water preceded hot-air treatment, while hot-air treatment was associated with an increase in the incidence of internal lumpiness (hardened lumps of flesh in ripe fruit) compared with untreated fruit. however, this combination did not outperform the 96% control achieved with the application of the fungicide thiabendazole alone (Nishijima et al., 1992). Karaoglanidis & Bardas (2006), LaMondia & Douglas (1997) and Lennox & Spotts (2003) stated that due to the rapid development of fungicide resistance to MBCs, increasing fungicide use could be expected to result in increased resistance development.

Other studies carried out by Corra et al., (2011) found that ozone applications contributed to undesirable physiological changes occurring during ripening since papayas held for 24 h to an ozone continuous flow of 6 ppm ripen sooner than the untreated ones due to an early degradation of chlorophylls and the wax layer. On the other hand, due to the high start-up costs involved in irradiation and controlled atmosphere technologies, papaya suppliers and researchers try to find other profitable alternatives. The use of 'natural', biodegradable' or 'nontoxic' products to control postharvest diseases is a lively one, and hence, laboratory research has been aimed at identifying safe products in the laboratory.

Natural products may offer a new approach for control of postharvest diseases of horticultural products. It has long been known that plants produce antimicrobial compounds that may be present in different organs, i.e. seeds, leaves, flowers, etc. The constituents and secondary metabolites of from the seed and leaves of *Pithecellobium dulce* and *P. erosus* have demonstrated a remarkable control of various postharvest fungi of papaya, such as *Fusarium oxysporum, Rhizopus stolonifer* and *C. gloeosporioides* (Barrera-Necha et al., 2003). Experimental results of Meneses et al., (2009) indicated that mycelial growth of *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae* were reduced in culture media containing EPEM and dichloromethane fractions of Colombian propolis extracts due to their antifungal properties.

In this way, the use of natural products, either directly as crude preparations, or as pure compounds, can be a very attractive method for postharvest disease control of papaya fruits. Few number of earlier researchers has reported antifungal properties of Betel leaf extract against plant fungal pathogens (Jasrai & Patel, 2013; Kasiamdari &

Sangadah, 2015; Srichana et al., 2009). The aims of the present study were to evaluate the antifungal properties of Betel leaf extracts against *Botryodiplodia theobromae.*

3.2. MATERIALS AND METHODS

3.2.1. Isolation and identification of pathogen

Stem-end rot fungal pathogen was isolated from the upper surface of active margin of infected papaya. Symptomatic tissues (1 cm^2) from the skin of papaya were surface-sterilized using 1% sodium hypochlorite for 3 min, followed by three washes with purified water and drying on sterile paper. The edges of the surface-sterilized tissues were cut off with a sterile scalpel and the sterilized tissues were placed on Petri dishes containing solidified potato dextrose agar (PDA) and incubated at room temperature ($26\pm^{0}2$C) for one week for mycelial growth. Once mycelial growth was observed, the colonies were re-isolated on to fresh PDA dishes to obtain pure cultures. The colony morphology and pigmentation of the isolates were recorded before sub-culturing for purification and storage under refrigeration (approximately 5^{0}C).

Cultural and morphological characteristics were observed and considered for identification as earlier reported. Morphological structures of vegetative and reproductive structures were observed under compound light microscope, using lactophenol cotton blue dye. Pure culture mycelium of this isolate was maintained by sub-culturing on fresh PDA for every three weeks at 25°C to provide optimum conditions for growth of the fungus. Fungal pure culture slants were stored in the refrigerator (approximately 5^{o}C) until required. Two slants of inoculum were sent to CABI (Microbial Identification Service), UK for molecular identification by gene sequencing.

3.2.2. Pathogenicity test

Koch's postulate was carried out to test the pathogenicity of the selected isolates. The isolate was incubated for 10 days on PDA at 28 °C to induce sporulation. Healthy papaya fruits were surface sterilized using 0.5%

sodium hypochlorite solution followed by washing with purified water. To separate the spores from the 10 days old mycelium culture, the petri dish containing the pathogen was washed with 10 ml of sterilized water and two drops of Tween 80. Conidia were dislodged from mycelium by a sterile bent glass rod and were harvested by filtering through four layers of cheesecloth. Sterile purified water was used to adjust the conidial concentration to 1×10^6 conidia ml^{-1}, determined using a hemocytometer. A sterile sharp toothpick was used to make 2mm deep artificial wounds at the base of the fruit and each wound was inoculated with 20µl of conidial suspension using a sterile syringe needle. The fruits were placed in a cardboard box for incubation for 10 days with 90% relative humidity at 26 ± 2^0C. The fruits were observed daily for lesion development. Mock inoculations only with sterilized water were also be performed to serve as controls.

3.2.3. Preparation of Betel leaf extract

Two types of Betel leaf extract were used for all experiments all experiments in this study. One was Betel leaf ethanolic extract and other one was Betel leaf extract in propylene glycol.

3.2.3.1. Betel leaf extract in Propylene glycol

Betel leaf extract in propylene glycol was obtained from Indonesia (Haldin Pacific Semesta Company) and used as 100% original which contains 200 ppm total phenolic compounds.

3.2.3.2. Betel leaf extract using 80% ethanol

Betel leaf ethanolic extract was prepared locally following the method as (Bussaman et al., 2012). Fresh Betel leaves were bought from Semenyih, Selangor and washed thoroughly using tap water. Washed leaves were left to air dry. The air-dried, leaves were then dried in a hot-air oven at 45-50^{0}C for two days. Dried leaf samples were ground using a small commercial blender (WARING- model HGB2WTS3) and the leaf powder was placed in air tight glass bottles and stored at 4^{0}C until required.

The steps shown in the Figure 3.1 indicates 150g of leaf powder was added to 450ml of 80% ethanol which was obtained by adding purified water to 95% ethanol liquid (thus ratio between leaf powder and solvent was 1:3) and the mixture was agitated for 72h on a rotary shaker (120rpm).

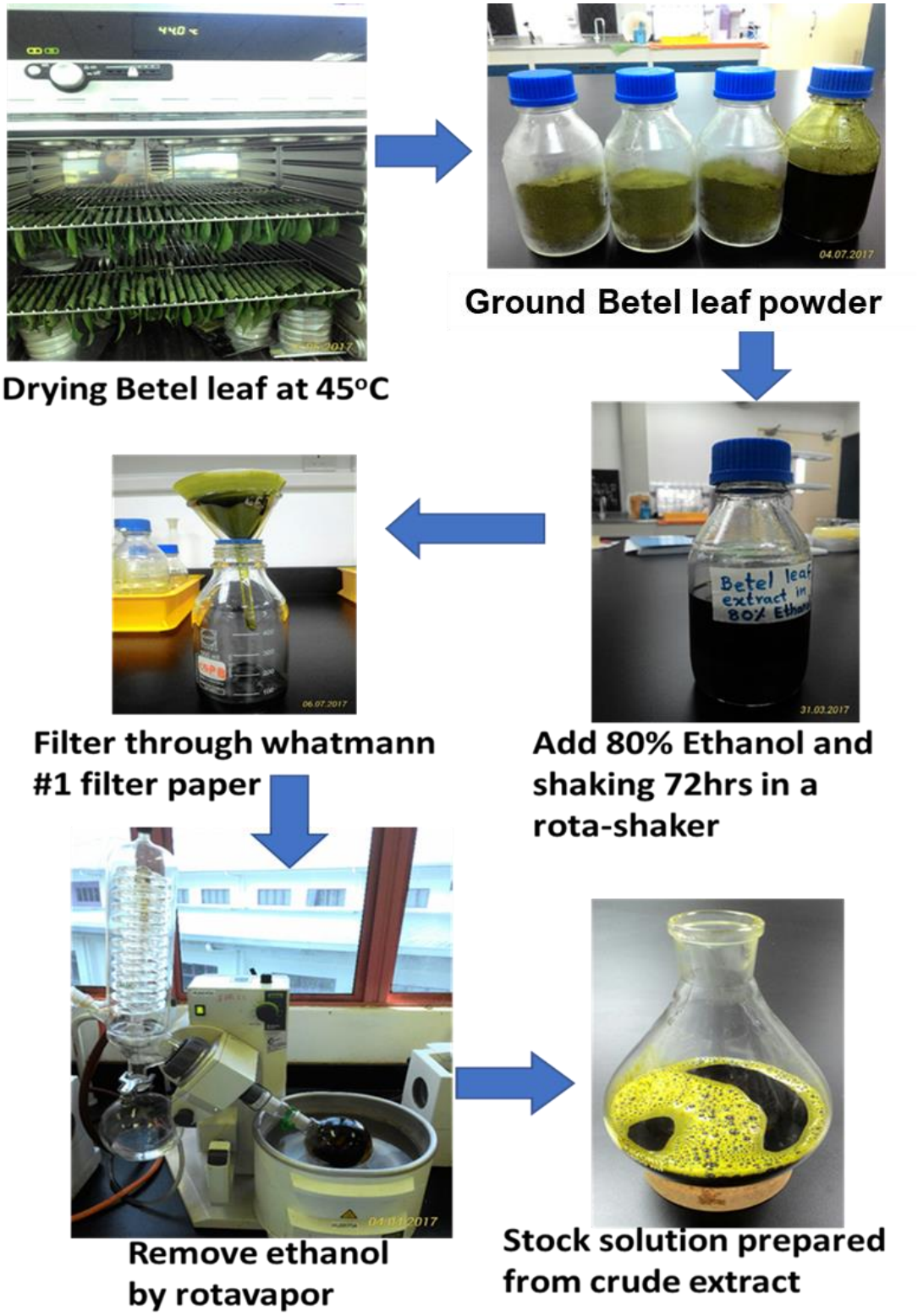


Figure 3.1. Preparation steps of Betel leaf ethanolic extract

The obtained extracts were centrifuged at 8,000 rpm for 10 minutes and filtered through Whatman filter paper no.1. The extracts were transferred to 500ml round-bottom flasks. Finally, the solvent ethanol was evaporated from extract using rotary evaporator (BUCHI Rotavapor R-200, HU00435) at 45^0C (Bussaman et al., 2012). The crude extract was then dissolved in 450ml of distilled water. This extract was used as the Betel leaf ethanolic extract 100% original solution (30mg/ml crude extract as 100%) for *in vitro* and *in vivo* studies.

3.2.4. *In vitro* antifungal assay of Betel leaf extract

Two types of original betel leaf extracts namely, Betel leaf ethanolic extract (BLET) and Betel leaf extract in propylene glycol (BLPG) were amended separately in a molten PDA to achieve 6%, 8%, 10% and 12% concentrations. Prepared concentrations were poured into Petri plates and allowed to solidify.

3.2.4.1. Dilution Method

The "poison food technique" (Sivakumar et al., 2002) was carried out to evaluate antifungal activity of Betel leaf extract on the inhibition in radial mycelial growth of *Lasiodiplodia theobromae* on PDA medium. 5mm diameter plugs were excised from actively growing pure culture of *L. theobromae* and placed in the center of each of the Betel leaf extracts amended PDA Petri dishes. Petri dishes containing only PDA was used as controls. All the inoculated petri dishes were incubated at room temperature (26 ± 2^0C). The radial mycelial growth was assessed every two days until the mycelium in control dishes reached the edge of the plate. The effect of the treatments was assessed by comparing growth (mm) of the fungal mycelium. The percentage inhibition in radial mycelial growth (PIRG) was calculated using formula described by Al-Hetar et al. (2011).

$$\% \text{ inhibition} = \frac{(X-Y)}{X} \times 100$$

X = diameter of fungal colony grown on the control plate
Y = diameter of fungal colony grown on plates containing crude leaf extracts.

3.2.4.2. Conidia Inhibition test

Spore suspension was prepared as described in section 3.2.2. Using a sterile, bent glass rod, 0.5ml of spore suspension of *L. theobromae* ($5x10^2$ spores/plate) were spread on the PDA plates containing different concentrations of betel leaf extract. The hemocytometer was used to determine the density of the spore suspension while sterile distilled water was added to adjust the desired concentration of the spore suspension. Control dishes contained PDA only. Plates were incubated at room temperature for 24 hours. After 24 hours, the number of germinated spores was counted in 10 microscopic fields of 100 spores in 20 replicated plates under a light microscope at 10× magnification. Percentage of inhibition in germination was calculated according to the method of Cronin *et al*. (1996).

$$\% \text{ inhibition of germination} = 1 - (G\infty/Gc) \times 100$$

$G\infty$ = Number of spore germination in the treatments

Gc = Number of spore germination in the control

3.2.5. *In vivo* antifungal assay of Betel leaf extract

For *in vivo* studies, papaya 'sekaki' variety was used to test the antagonistic effect of the Betel leaf extract against the stem-end rot disease on papaya. Healthy, matured, index-2 ripening stage (green with trace of yellow), uniform sized fruit without any detectible signs of infection were selected to evaluate the antifungal effects of Betel leaf extracts. Fruits were purchased from Exotic Star, Zone Perindustrian, Sungai Chua, Kajang, Selangor, Malaysia on the day of their harvesting. Both types of Betel leaf extracts used in *in vitro* studies, were used with 1% of tween-80 to coat the papayas. Tween-80, which is an emulsifier, helps to adhere Betel leaf extract well with the surface of the papaya. 8%, 10%, and 12% concentrations of Betel leaf extracts were prepared as shown in the figure 3.2, in separate containers (5L), amended with 1% Tween-80 emulsifier. Tween-80 has no antifungal or antimicrobial activity. Tween-80 is light yellow to amber oily viscose liquid, non-toxic. It is easily soluble in water and soluble in ethanol.

All the fruits were surface sterilized by rinsing with purified water, followed by washing with 1% Sodium hypochlorite and again washed with purified water.



Figure 3.2. Preparation of different concentrations of Betel leaf extract using Betel leaf ethanolic extract (BLET) and Betel leaf propylene glycol extract(BLPG)

After air drying for 30 minutes, a sterile sharp toothpick was used to make wounds approximately 2mm deep at stem end of each papaya. The wounded fruits were dipped in a spore suspension ($1x10^5$ spores/ml) containing 1% Tween 80 for 3 minutes. Inoculated fruit were air dried again for 30 minutes followed by dipping in each of the concentrations of Betel leaf extracts for 3 minutes. The treated fruits were kept for complete surface drying at ambient temperature for an hour. The treated fruits were packed in cardboard boxes and stored at 10 ± 2^oC, $80\pm5\%$ RH for 28 days (figure 3.3).



Figure 3.3. Coated Papaya with Betel leaf extract were stored in the cold storage (10±2°C) for 28 days for in vivo studies.

3.2.5.1. Disease Severity

Disease severity (DS) was scored at interval of 7 days using the scale 1= 0% of fruit surface rotten, 2 = 1-25% rotten, 3 = 26-50% rotten, 4 = 51-75% rotten and 5 = 76-100% rotten (Sivakumar et al., 2002). Percentage of severity was calculated according to Kranz (1988).

$$\textbf{\%}\ DS = \sum \frac{\text{(number of symptomatic fruits x severity scale)}}{N \times Z} \times 100$$

N = Total number of sample fruits in each replicate

Z = highest rating scale

3.2.5.2. Disease Incidence

Disease incidence (DI) based on the stem-end rot symptoms on the fruit surface was recorded weekly for 28 days during cold storage. The data was pooled on weekly basis, analyzed and calculated in terms of

percentage of fungal disease incidence by the following formula (Singh et al., 2012).

$$\% \text{ DI} = \frac{\text{Number of diseased fruits affected by stem-end rot}}{\text{Total Number of fruits}} \times 100$$

3.2.6. Studies of Scanning Electron Microscopy (SEM)

PDA plates were amended with different concentrations (6%, 8%, 10% and 12%) of both types of Betel leaf extracts for inoculation with conidial spores and mycelium plugs separately. After inoculation, the plates with mycelium plugs were incubated for 6 days and plates with spores were incubated for 2 days at room temperature ($26 \pm 2^{o}C$). After incubation, a thin layer of $0.8cm^2$ squares of PDA with conidial spores and PDA with mycelium were cut off separately from each treatment plates and mounted on stubs to get SEM images, immediately. The SEM images were taken at ×500 and ×2500 magnification for conidia and ×1200 magnification for mycelia using FEI Quanta 400F, Field Emission Scanning Electron Microscope.

3.2.7. Statistical analysis

Four replicates were used for each treatment with five plates in each replicate for *in vitro* studies. Four replicates for each treatment and eight fruits for each replicate were used for *in vivo* studies. The experiments were carried out in a completely randomized design (CRD) throughout the experiment. All data were subjected to analysis of variance (ANOVA) using the general linear model procedure by GenStat Software 15^{th} Edition. Means were compared by Duncan's Multiple Range Test (DMRT) as $P < 0.05$.

3.3. RESULTS AND DISCUSSION

3.3.1. Isolation and identification of pathogen

Lasiodiplodia theobromae Pat. colonies grew rapidly on PDA plates, covering the entire surface of the petri dishes abundantly with fluffy mycelium within 5 days. The mycelium was white at the beginning later turn to dark greenish-gray (Figure 3.4a) and become black when mature. Under light microscope, the mycelium was very thin, hyaline, uniform, thread like structures. When matured the mycelium were filled with dark granular content. Initially the conidia were hyaline, unicellular, oblong in shape, thick-walled with granular content. When conidia matured they were two-celled, light brown in color (Figure 3.4b). According to CABI (Accession Number- IMI 506609), the ITS rDNA sequence obtained from the sample of pure culture of pathogen, produced top matches at 100% identity to sequence of *L. theobromae* (synonym *Botryodiploda theobromae*).

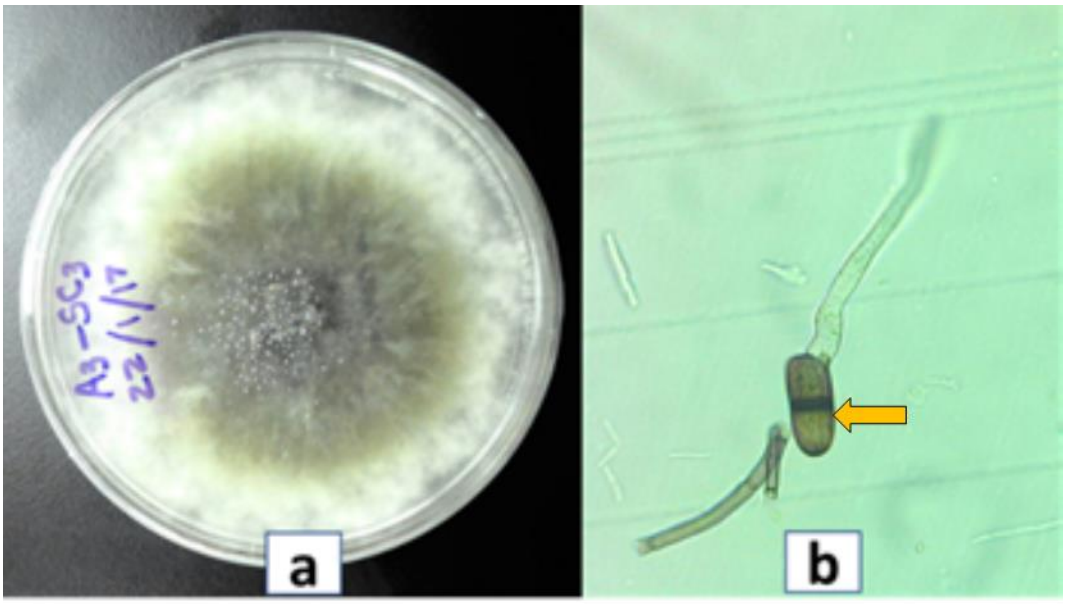


Figure 3.4. (a)-Pure culture of *Lasiodiplodia theobromae*, and (b)-germination tube with conidial spore.

3.3.2. Pathogenicity test

Initially, water soaked wide margins were produced at the base of the papaya fruit by the pathogen. With the progression of the infection, the lesion margin remains translucent as the rest of the infected tissues wrinkled, turned black and became dry (Figure 3.5a). At an advanced stage of infection white mycelium was observed at the stem end on the affected area (Figure 3.5b). Similar results were reported by Rahman *et al*. (2008) and Netto et al., (2014).

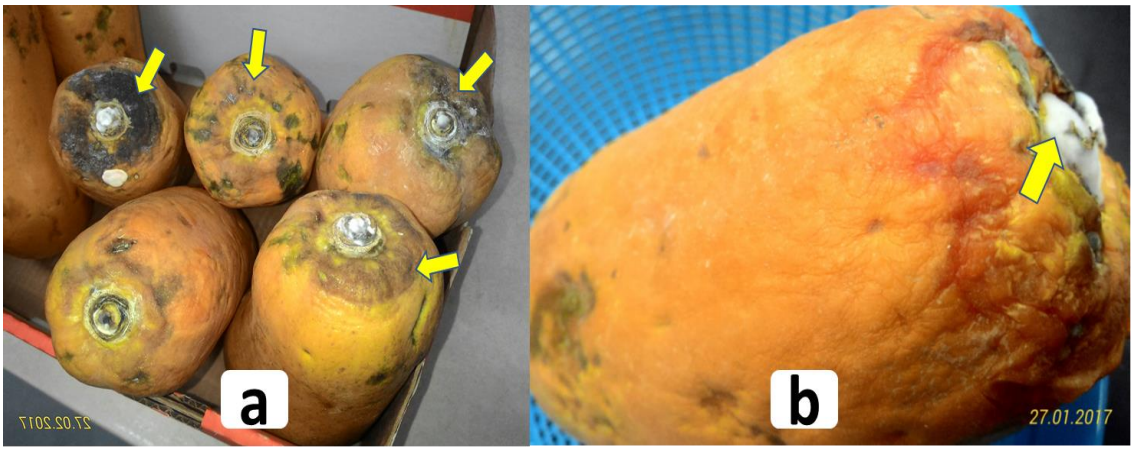


Figure 3.5. (a) & (b) Disease symptoms on inoculated papaya

3.3.3. *In vitro* antifungal assay

3.3.3.1. Mycelial growth inhibition

Inhibition of radial mycelial growth of *L. theobromae* was observed after 5 days of incubation at 26 ± 2^0C using two types of Betel leaf extracts (locally prepared BLET extract and BLPG which was bought). The growth was decreased significantly ($P<0.05$) by the different types of Betel leaf extract at all concentrations tested as compared to the control (Figure 3.6 and Figure 3.7).

The maximum percentage of inhibition in mycelial growth was observed as 98.48% with 12% locally prepared Betel leaf ethanolic extract where

as 93.64% was observed with 10% extract (Figure 3.6 and Figure 3.8), followed by 93.67% with 12% BLPG extract (Figure 3.7 and Figure 3.8).

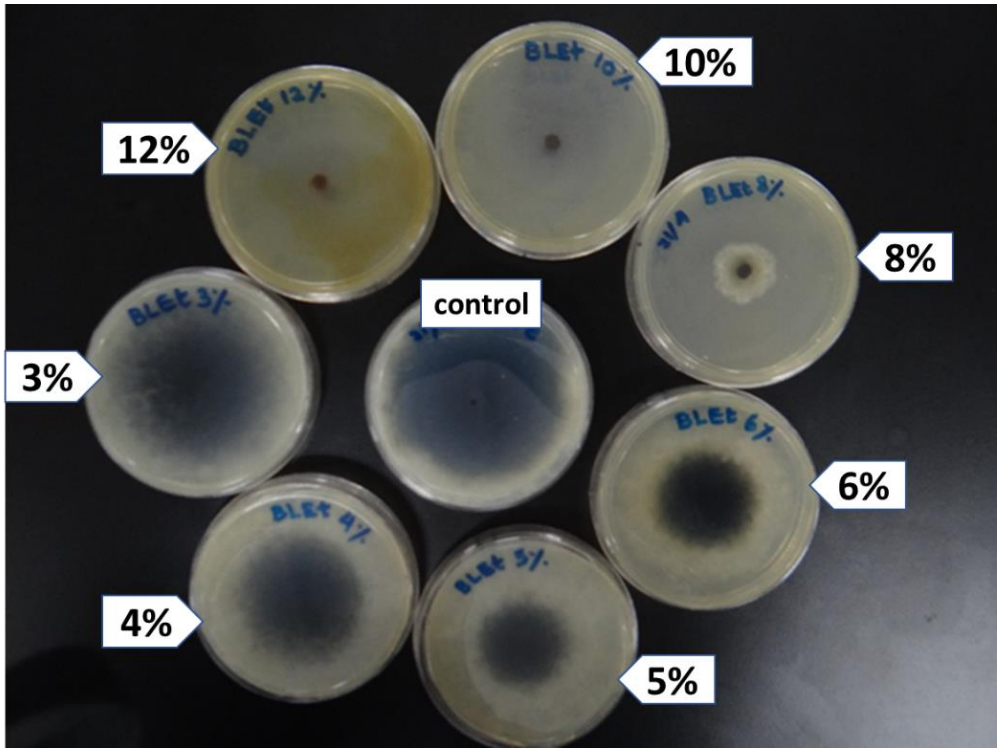


Figure 3.6. Mycelial growth inhibition with Betel leaf ethanolic extract

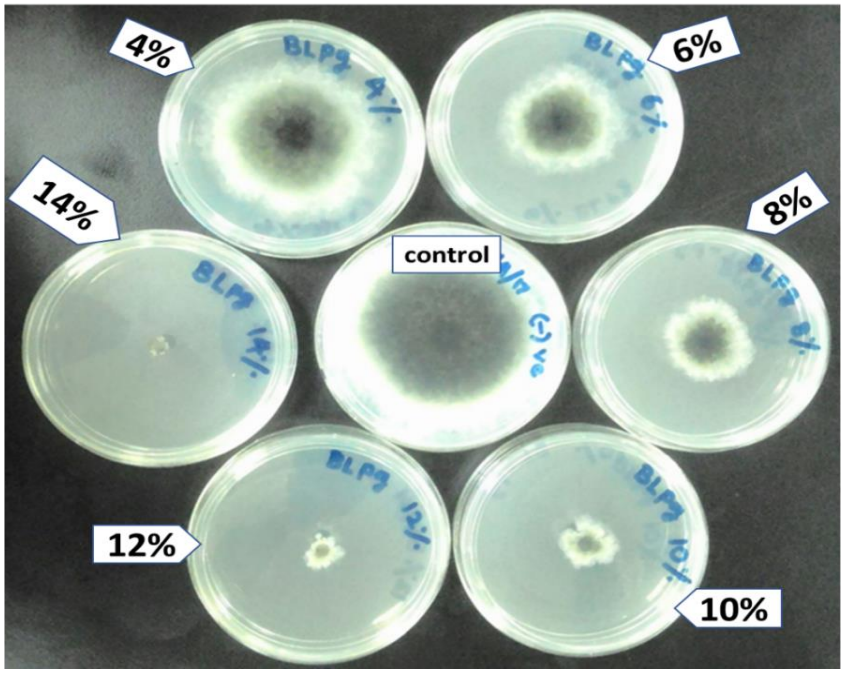


Figure 3.7. Mycelial growth inhibition with Betel leaf propylene glycol extract

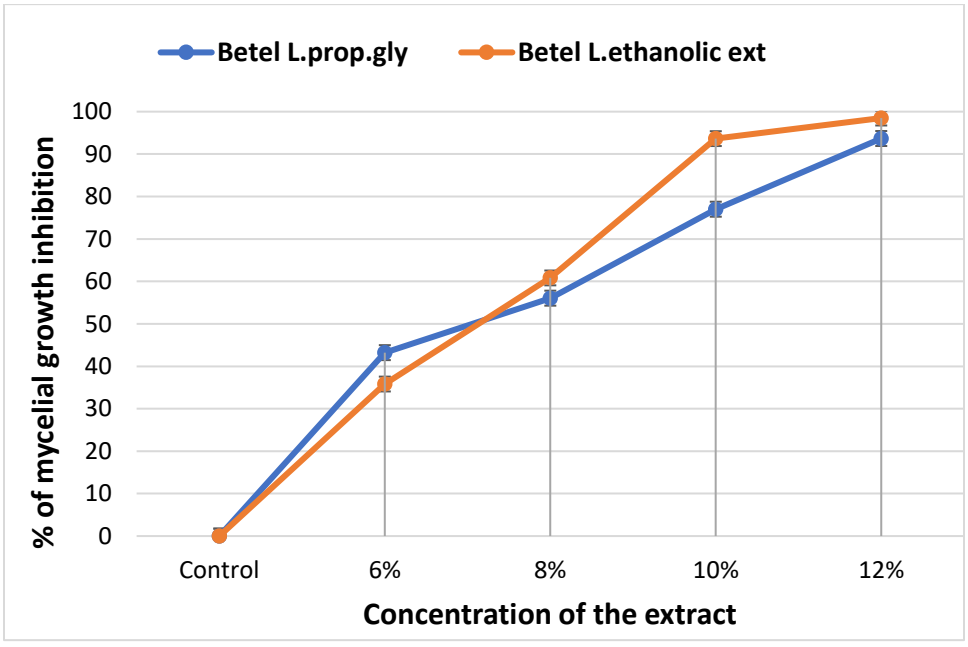


Figure 3.8. Effect of various concentrations of Betel leaf extracts on mycelial growth inhibition of *L. theobromae*. Vertical bars indicate standard errors; means of four replicates.

3.3.3.2. Spore germination inhibition

The germination of *L. theobromae* conidia was significantly ($P<0.05$) inhibited by both types of Betel leaf extracts tested at all concentrations compared to the control. The graph in Figure 3.11 shows that, the maximum percentage inhibition in conidial germination 93.91% and 90.26% of *L. theobromae* was observed in 12% Betel leaf ethanol extract and Betel leaf propylene glycol extract respectively. Figure 3.9 and Figure 3.10 shows that high sensitivity of conidia of *L. theobromae* to Betel leaf ethanolic extract was observed with completely inhibition in spore germination in most number of plates at 10% and 12% concentrations, while with 12% of Betel leaf extract in propylene glycol. In addition, complete inhibition of mycelial growth was observed with most number of plates, in each replicate of 12% and 10% BLET added PDA media as well as in 12% BLPG added PDA media. According to the all above results we can observe that Betel leaf ethanolic extract is most effective in inhibition of both, mycelial growth and spore germination, when compared with Betel leaf propylene glycol extract.

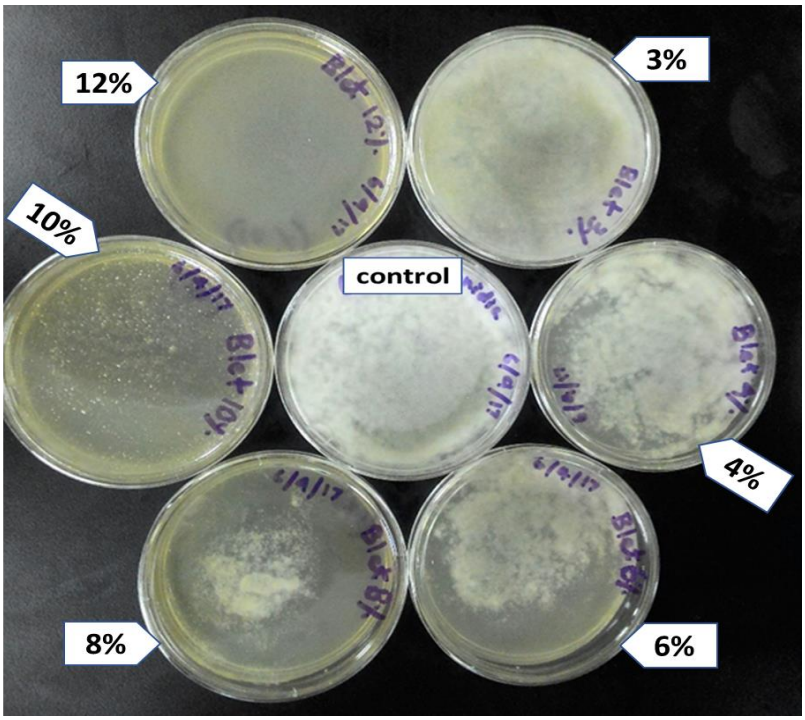


Figure 3.9. Effect of concentrations 3%, 4%, 6%, 8%, 10% and 12% of Betel leaf ethanolic extracts on spore germination of *L. theobromae*, after 4 days incubation at room temperature (28±2°C).

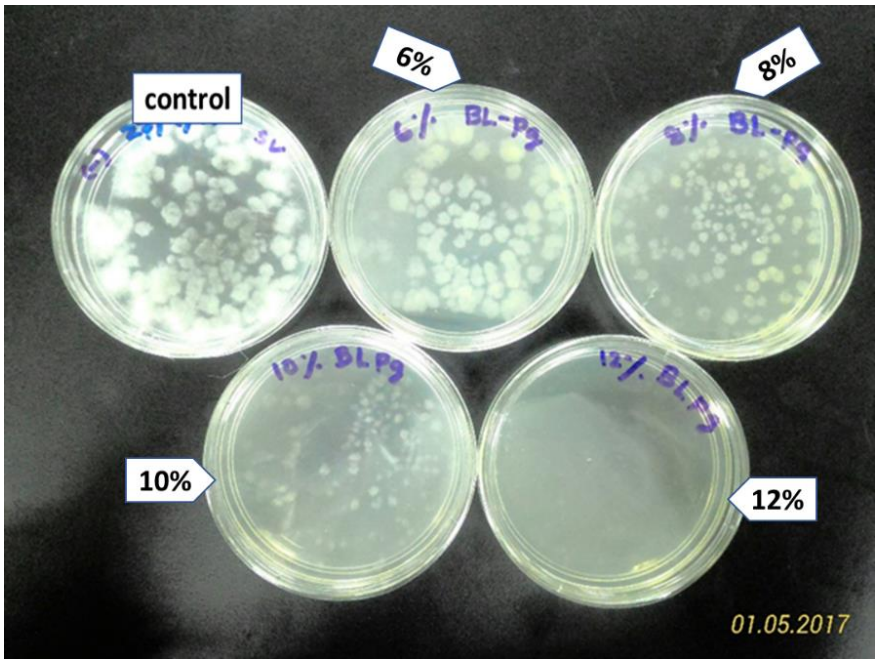


Figure 3.10. Effect of concentrations 6%, 8%, 10% and 12% of Betel leaf propylene glycol extracts on spore germination of L. theobromae*, after 4 days incubation at room temperature (28±2°C).*

The findings agree with Tripathi et al. (2008), where antifungal activity of secondary metabolites depends on the method and solvent used for extraction, its concentration and composition.

Even though, the germ tube of few conidia grew (which could be seen under light microscope) for 24 hours in 10% Betel leaf ethanolic extract and 12% Betel leaf propylene glycol extract, but they ruptured after two days, thereby no mycelium colonies could be seen in these plates, after incubation for 4 days (Figure 3.12). Antimicrobial activity of Betel leaf extract has been detected towards many bacteria and fungi. (Hoque et al., 2012, Sugumaran et al., 2011 and I. Ali et al., 2010b).

The active ingredient of piper betle oil which is obtained from the leaves are primary a class of allyl benzene compounds, chavibetol, Chavicol, Estragole, Eugenol, methyl Eugenol and hydroxycatechol (Sugumaran et al., 2011). The ethanolic extract turned out to be most effective for its antifungal activity against *L. theobromae* by penetrating active ingredients into the fungal cell wall and causing leakages from fungal cells and spores.

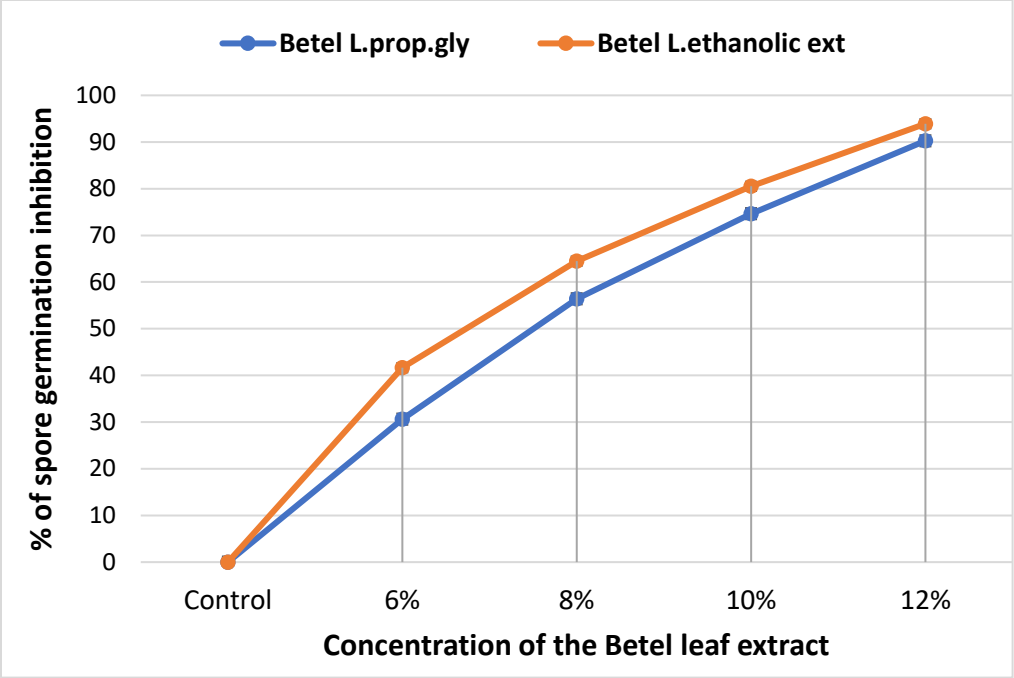


Figure 3.11: Effect of various concentrations of Betel leaf extracts on spore germination inhibition of *L. theobromae*. Vertical bars indicate standard errors; means of four replicates.

In addition. these antifungal compounds may involve with inhibiting or downregulating of fungal protein production or DNA replication. Jasrai & Patel (2013) also stated that the fungal inhibition can be due to the limitation of the fungal growth by interfering with the fungal protein production, DNA replication, interference with cellular metabolism, damage to the membrane, following death of the fungal cells.

Inthzar Ali (2010) mentioned that, *Piper betle* exhibited more effective anti-bacterial and antifungal properties and Hydroxychavicol is one of the major constituents of *P. betle*. Kasiamdari & Sangadah (2015) reported that the common terpene compounds such as carvacrol, linalool and eugenol have been known to exhibit antifungal activity towards several strains of microorganisms and there are many secondary metabolites and chemical constituents produced in *P. betle* extracts that may contribute to the observed positive antifungal effects.

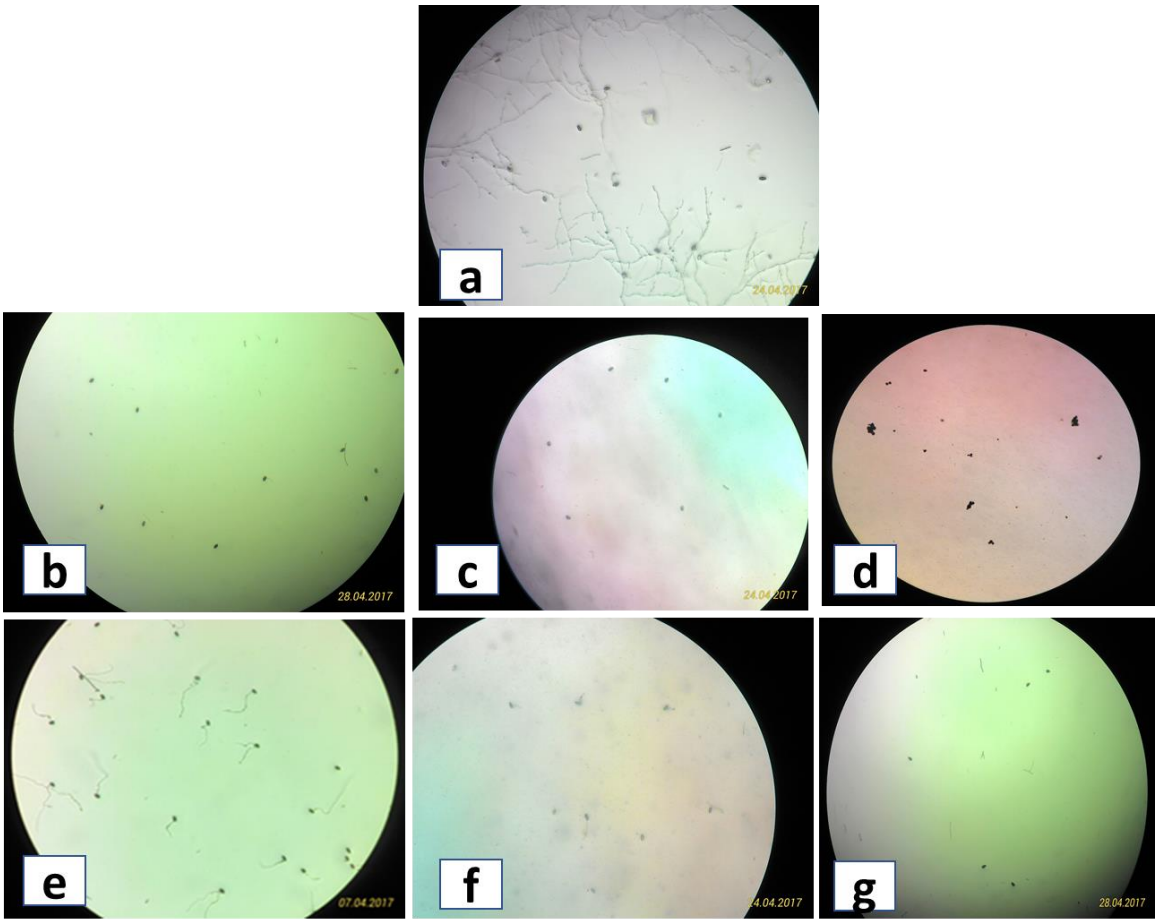


Figure 3.12. Spore germination inhibition test under light microscope; (a)-control, (b)-8% BLET, (c)-10% BLET, (d)-12% BLET, (e)-8% BLPG, (f)- 10% BLPG and (g)-12% BLPG. (BLET- Betel leaf ethanolic extract, BLPG- Betel leaf extract in propylene glycol).

3.3.4. *In vivo* antifungal assay

3.3.4.1. Disease Severity

During the 28 days of cold storage, disease severity (DS) was significantly low ($P < 0.05$) by 12%, 10% and 8% BLET (Betel leaf ethanolic extract) as well as 10% and 12% concentrations of BLPG (Betel leaf extract in propylene glycol), compared with the control. The percentage of disease severity in coated papaya with 8% BLPG, was as same as **control** after two weeks (Figure 3.13).

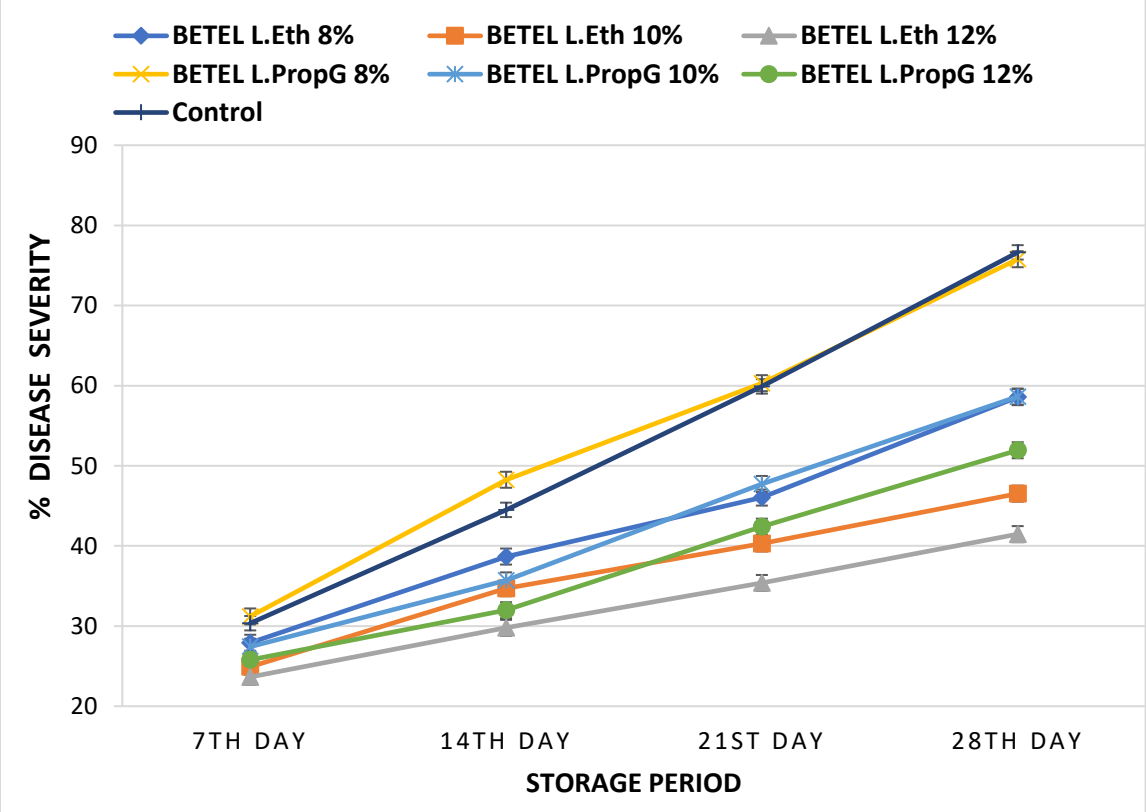


Figure 3.13. Disease severity for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

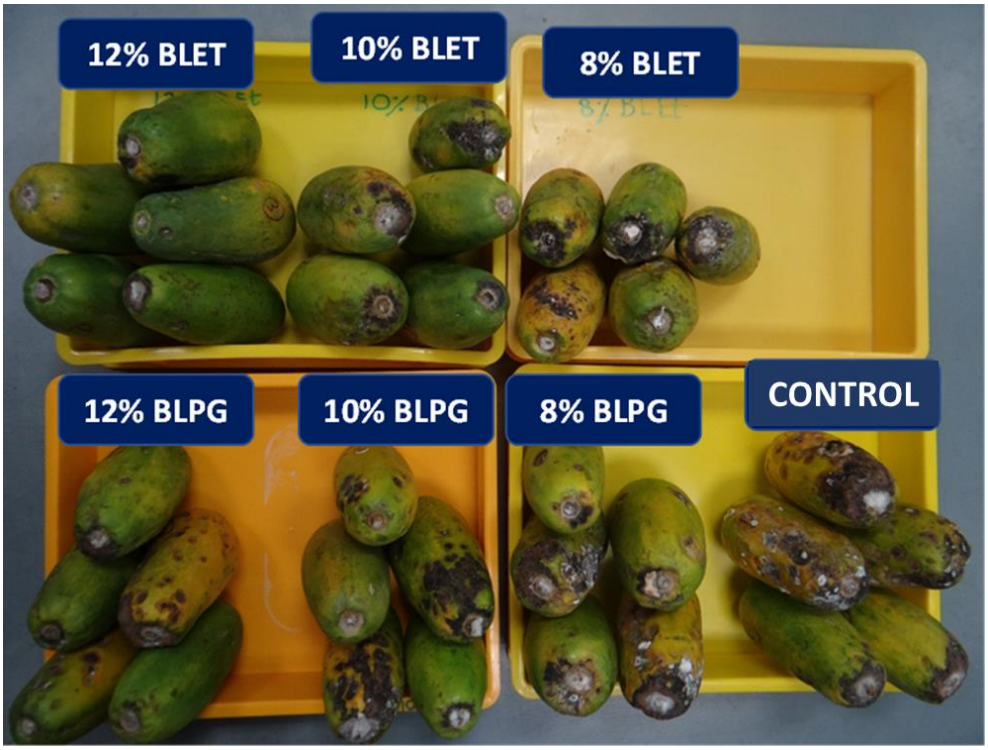


Figure 3.14.Disease severity of inoculated papaya fruits after 2 weeks of cold storage at 10 ± 2^0C treated with different concentrations of Betel leaf extract (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol).

The Betel leaf ethanolic extract (BLET) was consistently more effective than Betel leaf extract in propylene glycol (BLPG) in minimizing the disease severity and 12% Betel leaf ethanolic extract was the best treatment (Figure 3.13). In control fruit, disease severity reached 76%, for 28 days cold storage, while minimum disease severity 42% was recorded with 12% Betel leaf ethanolic extract. Differences in disease severity among all treatments were significant ($P< 0.05$) after 28 days of storage, while with 10% BLET treatment and 12% BLPG treatment showed same percentage of disease severity at the end of storage period (Figure 3.13 and Figure 3.14).

The essential oil of betel leaves possesses anti-bacterial, anti-protozoan and anti-fungal properties (Punuri et al., 2012). Arawwawala et al. (2011) stated that antifungal activity of betel oil was investigated against *Colletotrichum sp., Fusarium oxysporium sp., Corynospora cassicola, Rigidoporous sp*. and *Phytophthora sp*., using the disk method. All fungi species except *Phytophthora sp*. showed significant growth inhibition in betel oil.

3.3.4.2. Disease Incidence

During the 28 days of cold storage, disease severity (DS) was significantly low ($P < 0.05$) by 12%, 10% and 8% BLET (Betel leaf ethanolic extract) as well as 10% and 12% concentrations of BLPG (Betel leaf extract in propylene glycol (Figure 3.17), when compared with the control (Figure 3.15). The Betel leaf ethanolic extract (BLET) was consistently more effective than Betel leaf extract in propylene glycol (BLPG) at reducing disease incidence and 12% Betel leaf ethanolic extract was the best treatment (Figure 3.16). It agrees with the statement by Tripathi et al. (2008). He reported that antifungal activity of secondary metabolites depends on the method and solvent used for extraction, its concentration and composition.

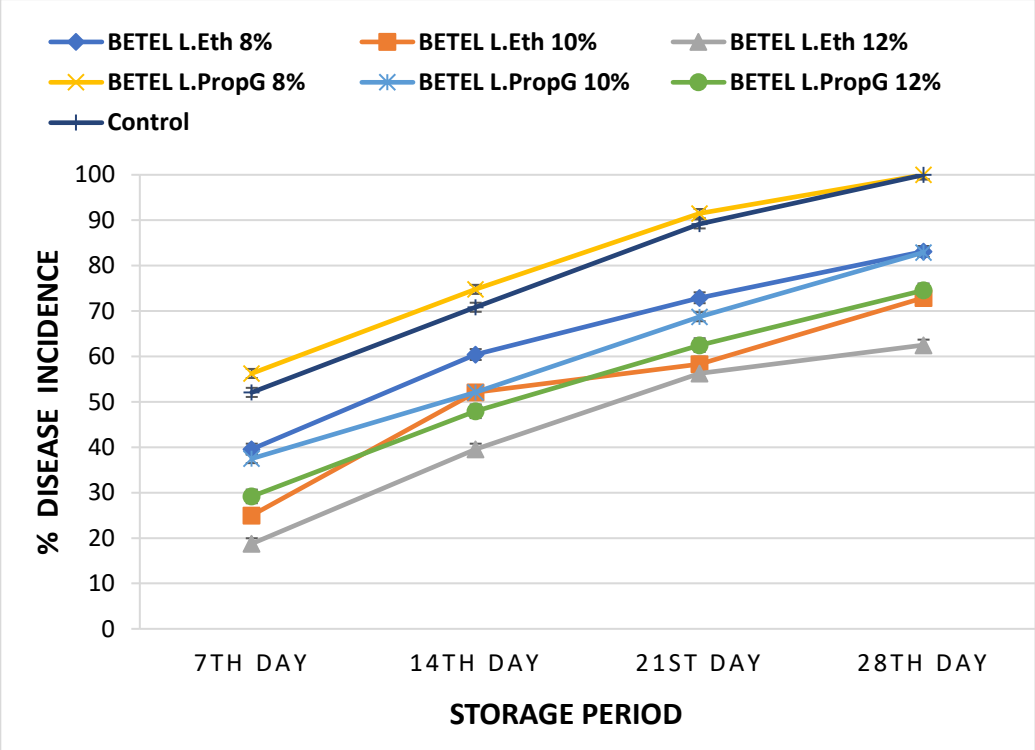


Figure 3.15. Disease incidence (%) for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

Ethanol extract of betel leaf might have a potential application as natural food preservative to improve the microbiological safety of the foods due to its bioactive antifungal compounds. It agrees with the statement of Xu & Li (2011), where a number of compounds from Piper species have

been reported to possess significant antifungal activities, in recent years. Srichana et al. (2009) suggested in her report about the high potential inhibition effect of the betel leaf ethanolic extract on the fungi *Aspergillus flavus* and *Fusarium verticillioides* which are contaminations in food grains. She found that Ethanol extracts of betel leaf at 10,000 ppm completely inhibited growth of *A. flavus* TISTR 3366 and *F. verticillioides* TISTR 3175.

Pyrethroids and neem products are well established commercially as botanical pesticides and recently some essential oils of higher plants have also been used as antimicrobials against storage pests because of their relatively safe status and wide acceptance by the consumers. Some of the volatile oils, which often contain the principal aromatic and flavouring components of herbs and spices, have been recommended as plant based antimicrobials to retard microbial contamination and reduction in spoilage of food commodities (Tripathi et al., 2008).

A research by Mohamed et al. (1996) reported that two varieties of *Piper betle*, showed strong activity against all the pathogens tested (*Colletotrichum capsici*, *Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Alternaria alternata*, *Penicillium citrinum*, *Phomopsis caricae-papayae* and *Aspergillus niger*), with inhibition diameters significantly (P<0·01) bigger than 2·5 mg ml^{-1} prochloraz or 10 mg ml^{-1} clotrimazole.



Figure 3.16. Disease incidence and disease severity of inoculated papaya after 28 days cold storage at 10±2^{0}C with different concentrations of Betel leaf extract.

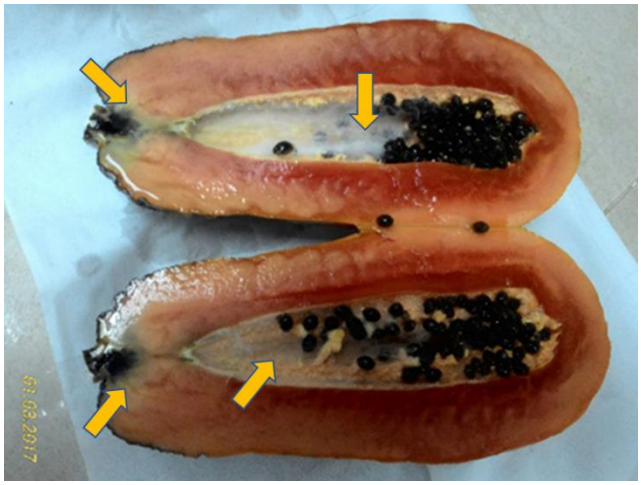


Figure 3.17. At the end of storage period, mycelial growth of pathogen inside the fruit, which was coated with 8% BLPG.

3.3.5. Studies of Scanning Electron Microscopy (SEM)

The results of Scanning Electron Microscopy (SEM) images in Figure 3.18 and Figure 3.19 shows disruption and distortion of fungal mycelium and fungal conidial spores grown in 8%, 10% and 12% of Betel leaf ethanolic (BLET) extract and Betel leaf extract in propylene glycol (BLPG).

In the control (Figure 3.18a), 6% BLET (Figure 3.18b) and 6% BLPG (Figure 3.18f) the mycelium growth was abundant and the mycelium hyphae was thinner when compared with all other concentrations of BLET and BLPG. In 8%, 10%, 12% BLET (Figure 3.18c) and 8%, 10%, 12% BLPG (Figure 3.18g), mycelium hyphae became thicker in size when compare with control. This might be due to the alteration of membrane due to the presence of antifungal bioactive compounds in Betel leaf extract.

At the same time, the density of the mycelial growth was low in all concentrations of BLET as well as all concentrations of BLPG when compared with control. The mycelial growth was reduced remarkably in 10%, 12% BLET (Figure 3.18 d & e) and 10%, 12% BLPG (Figure 3.18 h & i) and the hyphae were more branched near the old mycelium plug, shortened, and got thickened, thereby by the growth of mycelium was restricted near to the old mycelium plug 1-2mm around the plug (Figure 3.6 and Figure 3.7). Then they were distorted, deformed and destroyed, thus inhibited the mycelium growth in 10% and 12% both types of Betel leaf extracts.

In the control treatments the conidia were well germinated, and the conidial structure was defined well in the SEM image (Figure 3.19a). Ruptured and deformed conidia with sunken walls were observed with both types of Betel leaf extract, BLET and BLPG in all the concentrations **(**Figure 3.19). Even though, with 6% BLET (Figure 3.19b) and 6% BLPG (Figure 3.19f), germination of conidia was observed, but the germination tube did not produce long mycelium and growth were stopped with a short hypae. At the same time many conidia remained ungerminated. With 8% , 10% and 12% both BLET (Figure 3.19 c, d & e) and BLPG (Figure 3.19 g, h & i), the conidia were damaged, ruptured and sunken cell walls were observed with all conidia spores. This may due to leakage of cell wall material and cell membrane. It was reported that hydroxychavicol in Betel leaf extracts alters the cell membrane

structure, resulting in the disruption of the permeability barrier of microbial membrane structures (Ali et al., 2010b).

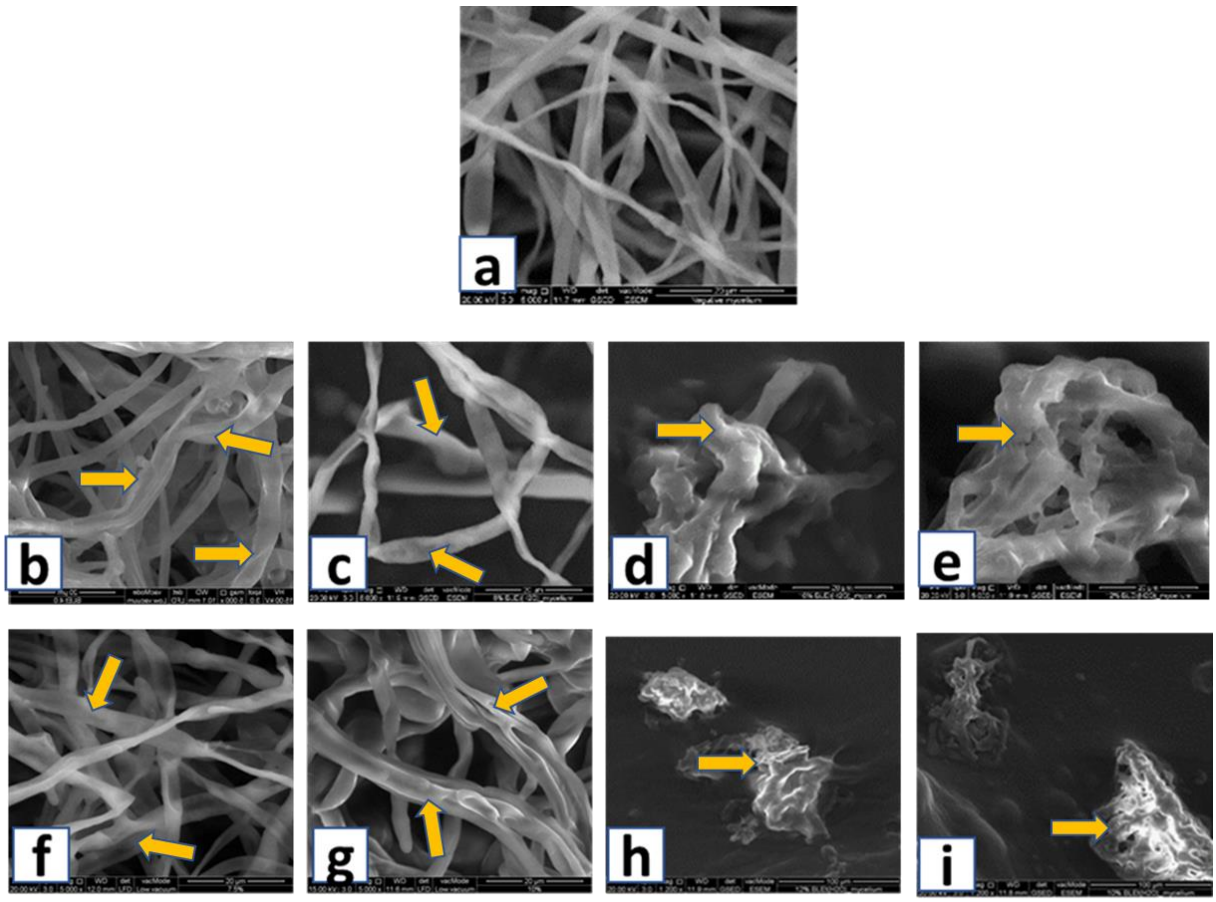


Figure 3.18. Images of Scanning Electron Microscope of mycelial growth on PDA amended with both types of Betel leaf extracts; (a)-Control, (b)-6% BLET, (c)-8% BLET, (d)-10% BLET, (e)-12% BLET, (f)-6% BLPG, (g)-8% BLPG, (h)-10% BLPG and (i)-12% BLPG. (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol).

Piper betel extract contains large quantity of sterols which is the bioactive molecule responsible for antimicrobial activity. Sterols present in the extract interact with the cell wall and membrane of the pathogen altering the primary structure of cell wall and membrane which ultimately lead to pore formation and degradation of the fungal cell components. For example, the betel leaves extract possesses antimicrobial activity against several pathogenic bacteria. The bioactive compound against bacteria were identified as sterol and their mode of

action was the disruption of the permeability barrier of the microbial membrane structure (Singburaudom, 2015).

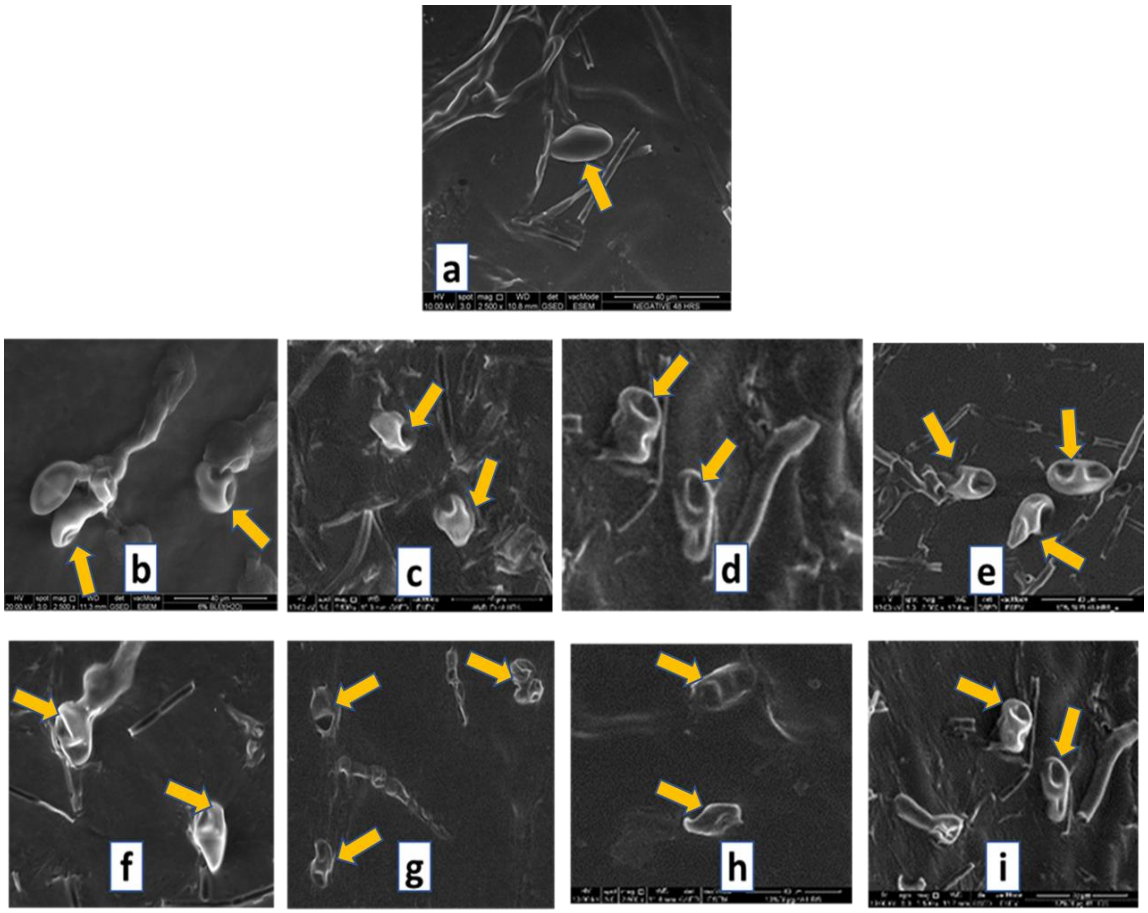


Figure 3.19. Images of Scanning Electron Microscope of spore germination on PDA amended with both types of Betel leaf extracts; (a)-Negative, (b)-6% BLET, (c)-8% BLET, (d)-10% BLET, (e)-12% BLET, (f)-6% BLPG, (g)-8% BLPG, (h)-10% BLPG and (i)-12% BLPG. (BLET-Betel leaf ethanolic extract, BLPG-Betel leaf extract in propylene glycol).

Moreover, *Piper betle* extracts containing high concentration of fatty acids like palmitic acid, stearic acid and hydroxy fatty acid esters exhibits potent antimicrobial activity against diverse pathogenic microorganisms. From the present investigation, it was observed that Piper betel leaf extracts successfully inhibited the growth of *L. theobromae*.

It has been reported that the major constituents of Piper betel leaf are phenolic in nature that may be responsible for inhibiting fungal mycelium growth and conidial spore germination. The fungal inhibition

can be due to the limitation of the fungal growth by interfering with the fungal protein production, DNA replication, interference with cellular metabolism, damage to the membrane, following death of the fungal cells (Jasrai & Patel, 2013).

Piper betle leaf also possesses some volatile oils. The major constituents of Piper betel leaf are phenolic groups which are slightly acidic in nature and possess slight polarity. Ethanol is slightly polar organic solvent that sufficiently dissolve slightly polar bio active organic compounds in the Betel leaf. Therefore, high activity was observed for ethanolic extracts.

The reported isolation and extraction techniques were employed using solvent-ethanol, water, acetone and methanol. According to Sharma et al. (2009), the trend of results with respect to solvents reported was in relation that the antimicrobial activity is more in organic solvent extract than in aqueous solvent extract. This agree with our positive results of ethanolic extract of Betel leaf against fungal pathogen.

Spore germination kinetic study done by Basak & Guha (2017) revealed that the efficacy of Betel leaf essential oil on inhibiting the germination of *Penicillium expansum* spores at much lower concentration as compared to other essential oils reported. It was also found that increase in Betel leaf essential oil concentration delayed the germination time of spore up to a point, beyond that no germination occurred. Paridhi et al. (2015) mentioned that high concentrations of flavonoids and polyphenols especially hydroxychavicol present in *Piper betle* oil exhibits potent antibacterial activity against oral cavity pathogens.

4. CHAPTER: EFFICACY OF BETEL LEAF EXTRACT ON POSTHARVEST QUALITY OF FRUITS IN THE COLD STORAGE

4.1. INTRODUCTION

Quality retention with postharvest storage life of papaya is the major constraints on marketing of this fruit in local and export market. Due to numerous changes in biochemical and physiological attributes with rapid ripening of papaya, which is a climacteric fruit, the quality of the fruit decrease with the postharvest storage time thus reduce the shelf life. Ripening-related cell wall changes and the enzymes possibly involve in changes with physiochemical properties, nutrient quality and sensory quality of the fruit.

Therefore, to delay the ripening process and senescence, the respiratory and metabolic activities of fruit must be controlled at a lower rate after harvesting. Coating the fruits with preservative compounds reduces the rate of respiration by creating a modified atmosphere around the fruits, thereby retarding ripening and senescence Ali, et al., 2011). The modified atmosphere created by edible coating around the fruit provide a semi-permeable barrier to O_2 and CO_2, thereby reducing respiration and oxidation reaction rates (Maqbool et al., 2010). Alginate, Chitosan, gum Arabic and many other natural product as edible coating maintains fruit quality during postharvest storage Ali, et al., 2010; Ali et al., 2011; Takma & Korel, 2017; Valero et al., 2013).

The effects of two types of Betel leaf extracts as an antimicrobial coating at different concentration (8, 10 and 12%) were studied on physiochemical attributes, nutrient quality and sensory quality of the fruit during cold storage for 28 days at 10 ± 2 ^{o}C (RH 80%). Texture or firmness is a good indicator for fruit quality and maturity as it is directly relating to postharvest shelf life, susceptibility to mechanical damage and disease. Firmness of a fruit can be measured as force (Newton) per surface area, to penetrate the fruit. Fruit colour depends on the pigmentation of fruit skin. The pigment components occurred in fruits includes chlorophyll, anthocyanins, carotenoids and lycopene. The colour can be measured by ***L*** (lightness), ***a*** (red/green values) and ***b*** (yellow/blue values), colour-opponent dimensions.

Total soluble solids (TSS) are total sugar, organic acid and soluble mineral content in the fruit. About 80 to 90% of the soluble solids content consists of sugars. Soluble solids concentration, particularly

when expressed relative to titratable acidity, often determines consumer perception of fruit flavor. Acidity in fruit is an important factor in determining maturity. Acids determine fruit pH, contribute to color stability, and inhibit enzyme activity. Acidity (titratable acidity) can be determined for a sample of extracted juice by titrating it with an alkaline solution (normally 0.1N NaOH) to a colour change of a pH indicator (e.g. phenolphthalein).

Lycopene amount and Vitamin C (Ascorbic acid) are major antioxidant types in papaya which is contributing to the nutrient quality of the fruit. As an antioxidant, ascorbic acid and Lycopene help in preventing diseases such as heart disease and cancer. Thus, it is vital to preserve these antioxidants in the fruit, without deteriorate with storage period. The effect of Betel leaf extract on preservation of antioxidants in coated fruit during storage is one of major factor that must be evaluated.

Respiration and ethylene biosynthesis are the primary physiological processes, which influence the postharvest deterioration rate of the commodity as well as storage life. Therefore, the effects of coating with Betel leaf extract on the respiration and ethylene evolution of "Sekaki" papaya for 28 days cold storage at $10 \pm 2^{o}C$ (RH 80%) were studied each week, comparing with control.

Sensory quality is the key factor in the consumer acceptance in the market. The demand in the fruit industry is depend on the sensory quality of the fruit. Thus, preservation of the sensory quality of the fruit with storage time is vital to supply quality product in export market as well as local market. Therefore, the sensory evaluation with the different treatment at the end of the storage period is very important to observe.

Therefore, the main objectives of the present study were:

1. To evaluate the effect of Betel leaf extracts on physiochemical quality attributes of papaya "Sekaki", such as weight loss, peel colour, firmness, total soluble solid (TSS) concentrations and titratable acidity.
2. To observe the effect of Betel leaf extract on preservation of antioxidants in coated fruit during cold storage.
3. To determine the effect of Betel leaf extract on sensory quality of the fruits at the end of storage period.

4.2. MATERIALS AND METHODS

4.2.1. Fruit samples

Healthy, matured, 'Sekaki' papaya fruits of colour index 2 (green with trace of yellow), uniform sized (800g-900g) without any detectible signs of infection were selected to evaluate the effects of Betel leaf extracts on physiological response and quality in the postharvest storage period. Fruit were obtained immediately after harvest from a local exporter (Exotic Star, Zone Perindustrian, Sungai Chua, Kajang, Selangor, Malaysia) during July-September 2017. The experiments were conducted in the months of July and September 2017.

4.2.2. Coating solutions

Both types of Betel leaf extracts prepared as mentioned in *in vitro* studies section 3.2.3., have been used with 1% of tween-80 to coat the papaya fruits. Tween-80, which is an emulsifier, helps to adhere Betel leaf extract well with the surface of the papaya fruits. 8%, 10%, and 12% concentrations of Betel leaf extracts were prepared in separate containers (5L). Treatments for the fruit consisted of control (distilled water with 1% Tween-80), Betel leaf ethanolic extract 8%, 10% and 12% and Betel leaf in propylene glycol 10% and 12% concentrations. Each treatment solution contains 1% Tween-80 emulsifier.

4.2.3. Sample preparation

The fruit were rinsed well with purified water, then washed with 1% Sodium hypochlorite and again washed with purified water. Fruit were air dried for 30 minutes and dipped in Betel leaf extract for 3 minutes to apply the solution uniformly over the whole fruit surface. The fruit in the control treatment were dipped in distilled water plus 1% Tween-80 solution. Then fruit were allowed for complete surface drying at ambient temperature for 30 minutes and then packed in cardboard boxes and stored at $10 \pm 2^{o}C$, $80 \pm 5\%$ RH for 28 days. The data were measured and recorded before treatment (Day 0) and each week.

4.2.4. Determination of physical quality

4.2.4.1. Weight loss percentage

Three fruits in each replication were marked and weighed using a digital balance, (EK-600H, Japan) before storage for weight loss determination. Same fruits were then weighed at 7-day intervals over the 28-storage period. The results were expressed as percentage weight loss relative to the initial weight.

$$\% \text{ Weight loss} = \frac{(W1 - W2)}{W1} \times 100$$

where W1 = initial weight of the fruit at Day 0,

while W2 = weight of fruit after 7 days storage.

4.2.4.2. Fruit firmness

Texture is a critical fruit quality attribute to measure fruit firmness which relates to customer acceptance. Fruit firmness was determined using an Instron texture analyser (Instron 2519-104, MA), equipped with an eight mm plunger tip. Three fruits in each replication were penetrated, at a speed of 50 mm min−1 on 3 random positions and the average of these results were taken as the firmness of each fruit. The compression force measured at the maximum peak of the recorded force on the chart was expressed in Newtons (N).

After performing the firmness assay, the same fruits were used for determination of chemical quality and antioxidant quality.

4.2.4.3. Peel colour

Fruit peel colour was determined using a Colorimeter (Hunter Lab), measures fruit colour accurately based on *L**(lightness), *a** and *b** parameters. The values of lightness (L*) form the vertical axis with values ranging from 0 = black to 100 = white, a* (red-green axis) and b*(yellow-blue axis) which represent coordinates in the colour chart indirectly reflecting chroma (C*) and hue angle (h◦). The C*(colour saturation) which refers to the vividness of colour was computed from values of a* and b*,

i.e. $C^* = (a^{*2} + b^{*2})½$, which represents the hypotenuse of a right angle triangle with values ranging from 0 = least intense to 60 = most intense (A. Ali et al., 2014). The h◦ was referred to as the perceived colour and was the angle of the tangent^{-1} b*/a*, where 0◦ =red purple, 90◦= yellow, 180◦ = bluish-green and 270◦ = blue.

4.2.5 Determination of chemical quality

4.2.5.1. Soluble solids concentration (SSC)

The total soluble solids can be measured by chemical means. However, it is much easier and just as useful to measure the concentration of soluble solids in extracted juice with a refractometer. Refractometer operates based on reflection of light which relates to density of fruit juice and expressed in Brix◦. SSC of papaya fruit pulp was measured using a Palette Digital Refractometer (Model: PR-32a, Atago Co. Ltd, Japan). The refractometer was calibrated with purified water to give 0% reading before sample analysis.

Fruit pulp (10g) was sampled from a mixture of 3 fruits in each treatment homogenized in a kitchen blender with 40ml of purified water. The mixture was centrifuged at 5000 x*g* for 5 minutes and then filtered through cotton wool. A drop of the filtrate was placed on the prism glass of the refractometer to obtain the %SSC reading. The readings were multiplied by the dilution factor to obtain the SSC of the papaya fruit pulp.

4.2.5.2. Titratable acidity

The titratable acidity (TA) was determined using the titration method described by Ranganna (1999). Ten gram of pulp tissue was homogenized with 40 ml of distilled water using the kitchen blender for 2 min. The mixture was then filtered through muslin cloth. 10ml of filtrate with one to two drops of 0.1% phenolphthalein as a pH indicator was titrated against 0.1 N sodium hydroxide (NaOH) to a pink endpoint (at least for 10 s). Titratable acidity was expressed as percentage of citric acid equivalents.

$$\% \text{ Titratable acidity} = \frac{N \times V_{NaOH} \times V \times \text{eq. Wtacid} \times 100}{W \times V_u \times 1000}$$

N = Normality of Titrant (0.1N)

V_{NaOH} = Volume of NaOH used

V = Volume of fruit juice made for analysis (50ml)

$Eq.Wt_{acid}$ = Equivalent weight of predominant acid (Citric acid = 64)

W = Weight of sample blended (10g)

V_u = Volume of sample used for titrate (10ml)

$$\% \text{ Titratable acidity} = V_{NaOH} \times 3.2$$

4.2.6. Determination of antioxidants

4.2.6.1. Determination of Ascorbic acid content

Ascorbic acid was determined by using the 2,6-dichlorophenolindophenol (DCPIP) dye titration method (Nielsen, 2010) with some modifications. DCPIP dye (Fisher Chemicals, USA) was prepared by dissolving 42 mg ± 0.1 of sodium carbonate in 150 ml ± 0.1 of hot distilled water followed by adding 50 mg ± 0.1 of DCPIP sodium salt. The solution was then cooled, and the volume made up to 200 ml by adding distilled water and refrigerated until used.

Samples were prepared with 20g of fruit pulp tissue from each fruit, blended in 80 ml of 3.0% metaphosphoric acid (HPO3) solution. From the filtrate, 2ml aliquot of the sample was titrated against 2,6-dichlorophenolindophenol dye until a pink colour persisted for 15 s. The ascorbic acid content was expressed as mg per 100 g of fresh fruit.

The dye factor was determined by standardizing the DCPIP dye. A 2ml aliquot of freshly prepared standard L-ascorbic acid solution (1 mg/ml in metaphosphoric acid) was mixed with 5 ml metaphosphoric acid and titrated against DCPIP dye.

For the blank titration, 7ml of metaphosphoric acid was added to a volume of distilled water approximately equal to the volume of dye used for the standard sample. This mixture was titrated against DCPIP dye and recorded the data as B.

The "Titer" (F) was then calculated using the following equation;

$$\text{Titer} = F = \frac{\text{mg } ascorbic\ acid \text{ in volume of standard solution titrated}}{(\text{average ml dye used to titrate standards-average ml dye used to titrate blank})}$$

Ascorbic acid content was calculated using the following equation and expressed in mg of ascorbic acid in ml of fruit sample.

mg Ascorbic acid / ml = [(X – B) x (F / E) x (V / Y)] mg/ml

where;

X = average ml for sample titration

B = average ml for sample blank titration

F = Titer of dye (= mg ascorbic acid equivalent to1ml Indophenol standard solution)

E = ml assayed (2 ml)

V = volume of initial assay solution (= 7ml)

Y = volume of sample aliquot titrated (= 7ml)

Ascorbic acid content (mg) in 100g, FW was calculated using the following equation and expressed in mg of ascorbic acid in 100g of fruit sample.

Ascorbic Acid (mg/100g, FW) = [(X – B) x (F / E) x (V / Y)] x (100/20) x 100

(FW =Fruit Weight)

4.2.6.2. Determination of total Lycopene

Following the spectrophotometric assay developed by Fish et al. (2002), Lycopene was extracted from papaya pulp and amount of Lycopene was found out using absorbance given by the spectrophotometer (Variskon

Flash Multimode Reader, Thermo Fisher Scientific, USA) at 503 nm. To each amber vial 5 mL of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 mL of 95% ethanol, and 10.0 mL of hexane were added at room temperature.

A given volume of sample was removed from the stirred puree and pipetted into its corresponding assay vial whose tare weight had been determined. The weight of sample was determined to the nearest 0.01 g or 0.001 g. The amount of sample used in the reduced volume assay can range from 0.4 to 0.6 g. Vials were laid on their sides in a rectangular container, covered with a second container that contained ice and placed on an orbital shaker to mix at 180 rpm for 15 min. After 15 min of shaking, 3 mL of deionized ice-cold water were added to each vial, and the samples shaken for another 5 min. Shaking was stopped, and vials were left at room temperature for 5 min to allow for phase separation. The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm versus a blank of hexane solvent. The lycopene content of tissue was then estimated by using the formula of;

$$\text{total Lycopene (mg / 100g FW)} = \frac{(A_{503} \times 3.12)}{\text{grams of sample}}$$

4.2.7. Sensory evaluation of ripe papaya

At the end of the storage period (after 28 days from harvest), the sensory evaluation of all fruits, which were treated with different treatments, was performed for taste, peel colour, pulp colour, texture and flavor using the scale-5 Hedonic scale Ali et al., 2011). Based on their consistency and reliability of judgement, a panel of 15 participants were selected. The panelists were asked to score the differences between the samples according to their judgement following the scale 0-5. (0= very poor, 1= poor, 2= fair, 3= good, 4= very good, 5= excellent).

4.2.8. Gaseous exchange analysis

The respiration rate and ethylene production of papaya were carried out at the harvest and each week for 28 days, as described by (Ong et al., 2013) with some modifications. Each fruit was placed in 3.6 L air tight containers. The lid of the containers was modified and equipped with a rubber septum. After incubated at room temperature for 1 h, 1 ml of headspace sample was withdrawn with a gas tight syringe through the septum and analyzed for CO_2 and ethylene in a gas chromatograph (GC) (Claru-500, Perkin-Elmer, USA).

4.2.8.1. Determination of respiration rate

CO_2 analysis was carried out on the gas chromatography equipped with a thermal conductivity detector (TCD) using a 1.2 m long stainless steel packed column (Porapak R, mesh size 80/100). Helium was used as a carrier gas at the flow rate of 20 ml min-1. Temperature for packed injector, oven and detector was set at 150, 60 and 200 ^{o}C, respectively. 1 ml of 1% CO_2 in nitrogen was used as the external standard for calibration. The production rate of CO_2 was expressed as ml kg-1 h-1.

$$K = \frac{\text{volume of jar (ml)} - \text{weight of sample (g)}}{\text{Weight of sample (kg)} \times \text{hours} \times 100}$$

$$\text{ml } CO_2/\text{kghr}^{-1} = \frac{K \times (\text{volume of gas injected}) \times (\text{Peak area of sample})}{(\text{Peak area of standard})}$$

4.2.8.2. Determination of ethylene production

For ethylene analysis, 1 ml of gas was withdrawn from the bottle headspace and quantified in the gas chromatograph), equipped with a flame ionization detector (FID). Nitrogen, hydrogen and air flow rates were 20 ml min−1. Nitrogen served as the carrier gas. Temperatures were 150, 200 and 200°C for the oven, injector and FID, respectively.

One milliliter of ethylene gas (Scotty Gases) used as external gas standard, was injected for calibration. Independent ethylene samples were taken from each replication and the results expressed as means (µl kg−1 h−1).

$$K = \frac{\text{volume of jar (ml)} - \text{weight of sample (g)}}{\text{Weight of sample (kg)} \times \text{hours} \times 1000}$$

$$\mu l\ C_2H_4/kghr^{-1} = \frac{K \times (\text{volume of gas injected}) \times (\text{Peak area of sample})}{(\text{Peak area of standard})}$$

4.2.9. Experimental design

Fruit were evaluated 0, 7, 14, 21 and 28 days after harvest. The experiments were conducted in a completely randomized design (CRD) with three replications. Each replicate consisted of 15 fruits. In each replicate, three fruits were randomly selected for physicochemical analysis at each evaluation time. The analyses were conducted at the postharvest laboratory, School of Biosciences, University of Nottingham Malaysia Campus. The data was subjected to analysis of variance (ANOVA) using GenStat software, while least Duncan's Multiple Range Test (DMRT) was used to compare differences among treatments at 95% confidence level for each variable. The whole experiment was repeated twice, and data were pooled before analyses.

4.3. RESULTS AND DISCUSSION

4.3.1. Physical quality changes in papaya

4.3.1.1. Weight loss

After harvesting, all fruits and vegetables undergo weight loss, which is a common and unavoidable phenomenon due to series of metabolic processes and physiological changes during ripening. Loss of water from harvested fruits and vegetables is a major cause of postharvest deterioration. Loss of substantial amounts of water may result in important quality and economic losses, as well as deteriorate the visual, compositional and eating quality of the produce.

According to our results percentages of fruit weight loss increased during cold storage in both control and treated fruit with time.

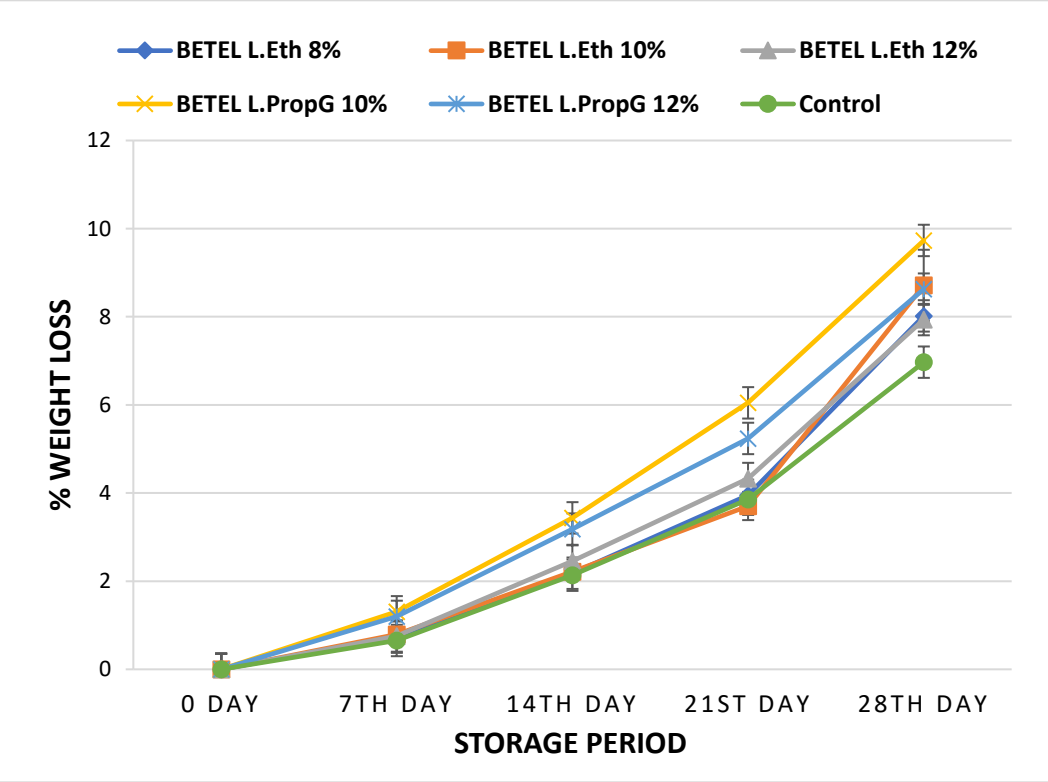


Figure 4.1: Weight loss (%) of papaya for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates

Coating with Betel leaf propylene glycol (BLPG) extract had a significant effect on fruit weight loss during the 28 days of cold storage compared to the control and BLET (Figure 4.1). At the 14th day and onwards, there were significant increment ($p < 0.05$) in the weight loss of fruits could be observed with BLPG when compared with control and other treatments (Figure 4.1). The weight loss reached a maximum of 9.732% with 10% BLPG while it was in the range between 7% and 8.7% with other all treatments and control.

The increased percentage of weight loss with BLPG extract may be due to the damage or disruption of the cuticle and/or epidermal tissues of the fruits by solvents in the extract. Our findings are well corroborated with Paull & Chen (1989) who report that, when cuticle was disrupted with solvent there was six folds of weight loss in solo papaya. It agrees with weight loss percentage was increased when exposed to higher concentrations of ozone which damage cuticle and epidermal cells of the fruit (Ali et al., 2014).

Some edible coatings can provide selective barriers against respiration and moisture loss (Maqbool et al., 2011a). Different kinds of coatings such as gum Arabic, Chitosan and some plant oils maintain the quality of fruit by maintaining the turgidity through water retention in the fruit (Chien et al., 2007) and by creating additional barrier against diffusion through stomata Ali et al., 2016a, 2010, Maqbool et al., 2011a, 2010). However, the application of Betel leaf extracts may not support as an additional barrier for reduce the weight loss. Our results are in agreement with the findings of Duan et al. (2011) and (Guerreiro et al.(2015) who reported that alginate-based and polysaccharide-based edible coatings may not have the ability to reduce water loss, needing additional lipid incorporation for water loss reduction improvement.

Differences of water loss when compared with Betel leaf ethanolic extract (BLET) and control were not significant until the third week during storage. From third week onwards, weight loss increased in BLET than in control. However, overall water loss in papaya with the storage time in this experiment was very low due to the greater thickness of the papaya peel and the greater fruit size when compare with other fruits.

4.3.1.2. Fruit firmness

Firmness is one of the major criteria by which consumer judge the quality and freshness of the fruits. Firmness depends on water content or turgor, cell wall components and cell membrane integrity. In our

results, fruit firmness decreased for 28 days cold storage in both control and treated fruit with time.

From second week onward, a highly significant (P > 0.05) retention of firmness in fruits were observed with all other treatments except 8% Betel leaf ethanolic extract when compared with the negative control. 8% Betel leaf ethanolic extract showed a similar decrease in firmness as the control during the 28 days of cold storage (Figure 4.2). In the control fruit and fruit treated with 8% Betel leaf ethanolic, firmness declined rapidly to 18.7N and 22.44N respectively, at the end of storage period. They were the maximum firmness reduction in the fruit at 28th day of storage.

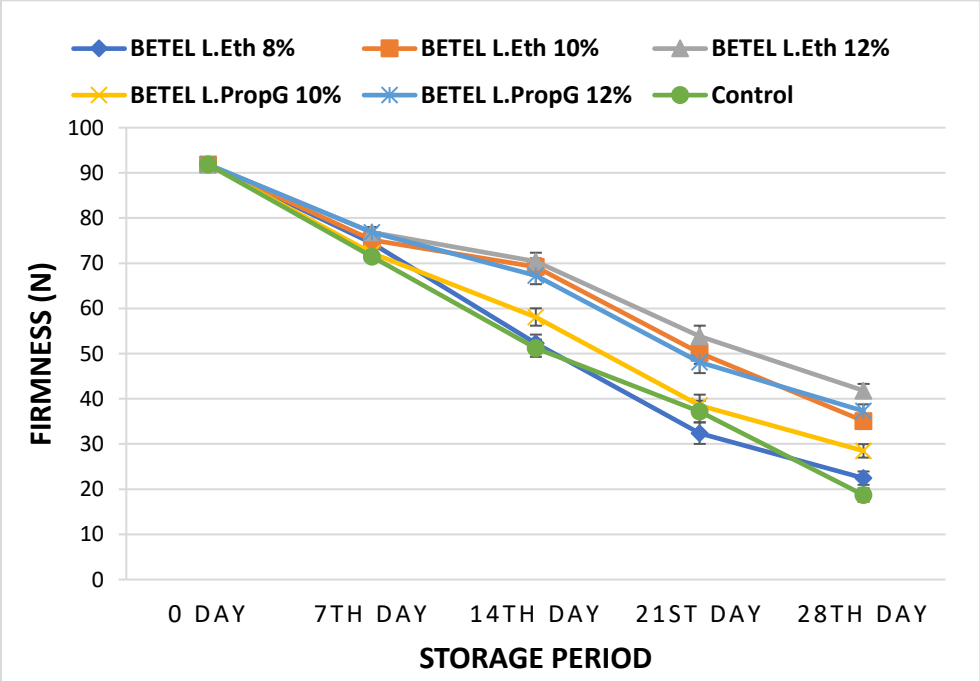


Figure 4.2. Firmness of papaya for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

Maximum retention of firmness was observed with 12% Betel leaf ethanolic (BLET) extract and it was 41.81N at the end of the storage period. The firmness results with 10% BLET extract and 12% BLPG extract showed same firmness trend along with time period but with 10% BLPG it was significantly lower than 10% BLET, 12% BLET and 12% BLPG (Figure 4.2). This may due to 10% BLET, 12% BLET and 12% BLPG better maintained the fruit quality without deterioration by the microorganisms, thus maintained the fruit firmness. Earlier reports

support this evident where fruit retained their firmness in the storage with different coatings such as Gum Arabic (Ali et al., 2010; Maqbool et al., 2011b), Chitosan (Ali et al., 2011) and Hydroxypropyl methylcellulose (Klangmuang & Sothornvit, 2018).

After harvest fruits and vegetables continue most biochemical and physiological processes during storage (Valero, 2010). Fruit softening could arise due to one of the three mechanisms: loss of turgor; degradation of starch; or breakdown of fruit cell walls. Pectin are the common and major components of primary cell wall and middle lamella, contributing to the texture and quality of fruits. Fruit softening is triggered by biochemical processes involving the hydrolysis of pectin and starch in the cell wall by Pectin degrading enzymes such as polygalacturonase, pectin methyl esterase, lyase, and rhamnogalacturonase. (Prasanna, Prabha, & Tharanathan, 2007). Zhou et al. (2011) reported that softening of Huanghua pears during storage could be partly attributed to an increase in depolymerization and degradation of the cell-wall polysaccharides containing pectin, hemicellulose, and cellulose.

Betel leaf extract may reduce the rate of hydrolysis process of pectin and starch in the cell wall by inhibiting the pectin-degrading enzymes and thereby retention of fruit firmness. Zhou et al., (2008) mentioned that coating may also inhibit the activities of pectin-degrading enzymes closely related to fruit softening by reducing the rate of metabolic processes during senescence resulting in maintaining firmness in the fruit. Kim et al. (2013) and Maqbool et al. (2011a) reported that Edible coatings incorporated with essential oil (lemon grass oil and cinnamon oil with gum arabic) delayed textural changes, thus reducing firmness loss in papaya and banana.

4.3.1.3. Peel colour

Quality and consumer acceptability depend mostly on peel colour of papaya. Alteration in skin color and texture is common with ripening process of papaya. Towards the final stages of papaya fruit ripening, pigmentation changes often involve with peel colour change from green to yellowish-orange and red. The skin color changes of the papaya is caused by the chlorophyll degradation and also biosynthesis of lycopene and carotenoid development which later will turns the skin color from green into red (Wills & Widjanarko, 1995).

In our results, the trend for the color values of Lightness (L*) and Chroma shows similar sigmoid curve in which the value increase throughout the ripening period (Figure 4.3 & Figure 4.5). Only the h value shows opposite trend with negative sigmoid as the value decrease along the ripening period. The results of peel colour of papaya showed that the lightness value (L*) increased from 41.077 on the day zero, with all the treatments and negative control through storage time, as brightness of fruit increased with ripening. The increase in L* agrees with the findings by (Ruslan & Roslan, 2016) who studied skin color changes of papaya during ripening.

the color *L** value did not show statistically significant differences until 21 days when compared to control (Figure 4.3). However, after 28 days storage, 10% and 12% BLET (Betel leaf ethanol extract) showed significantly lower *L** value than control and other treatments.

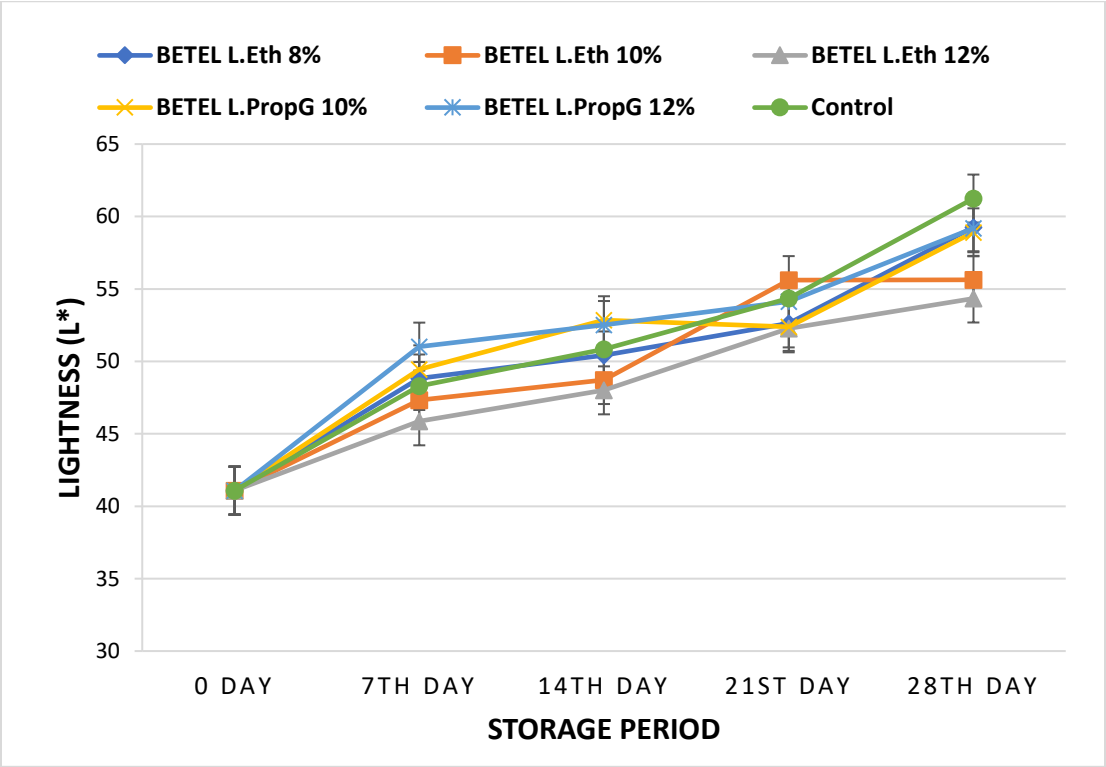


Figure 4.3. Lightness of papaya for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

Overall, it shows some reduce rate of lightness value changes with 10% and 12% BLET extract when compared with all others. This illustrated that fruit treated with 10% and 12% BLET extract have delayed ripening

and underwent slower changes in peel colour, as indicated by the slower increase in L* value. This may due to some effect on reduction of ripening process by 10% and 12% BLET on the fruit.

By the end of the storage period, the control fruit had changed from green to yellow with L* = 61.2, chroma =59.91 and hue angle =87.5. The hue angle measured for papaya was reduced from 121 to 87.5. At the end of storage period, the highest hue value, 104.25 was observed in 12% BLET treated papaya followed by 101.75 with 10% BLET treated papaya compared to control (Figure 4.4).

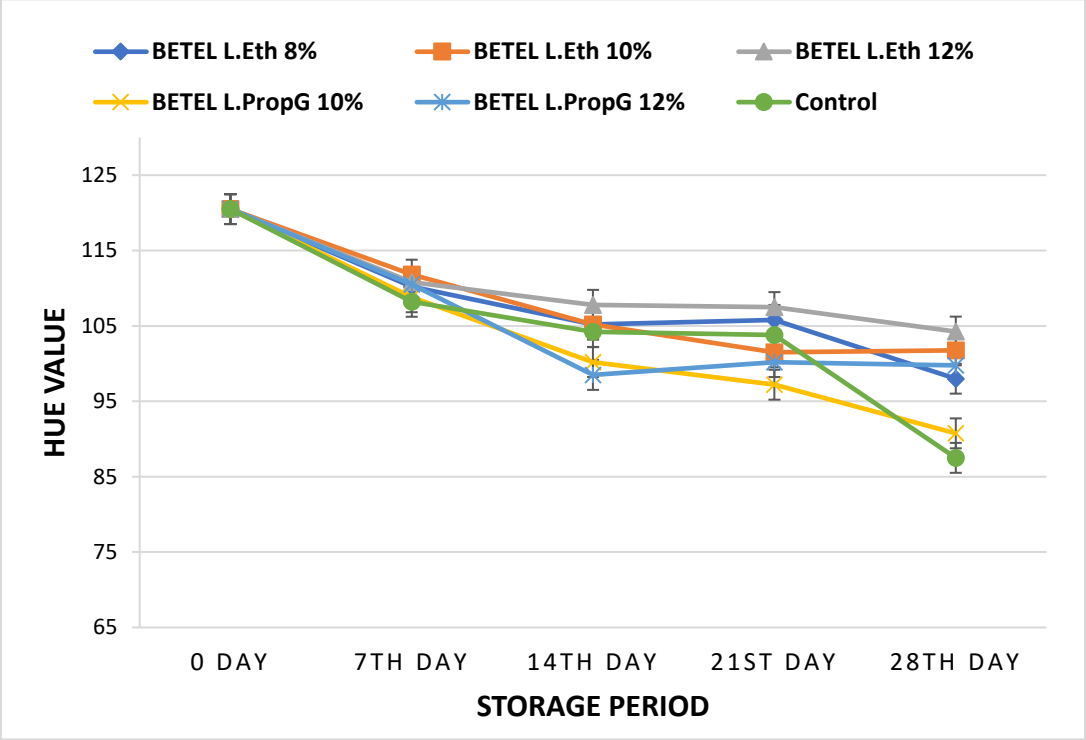


Figure 4.4. Hue value of papaya for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

Reduction of fruit firmness is corelated with reduction in hue value, due to ripened fruit which is less turgid has less hue value (Adolfo González-Aguilar et al. 2010). It agrees with the higher firmness values and hue values indicated by 10% and 12% BLET treated fruits, in the results of this study when compared with control and others. Guerreiro et al. (2015) also mentioned that, the °hue decreased significantly through 28 days storage time throughout the ripening period.

There was not much significant difference in Chroma value between the peel of fruit treated with Betel leaf extract and control (Figure 4.5). But, control and BLPG (Betel leaf propylene glycol) extract revealed some amount of faster change in color compared to other treatments as shown by L* and C* values through storage period. In contrast, the peel color of fruit coated with 10% and 12% Betel leaf ethanolic extract changed more slowly, as indicated by the slower increase in L*and C* values. The skin color shift obviously to more vivid color where the skin color of papaya changes to a brighter yellow color which indicates higher Chroma value.

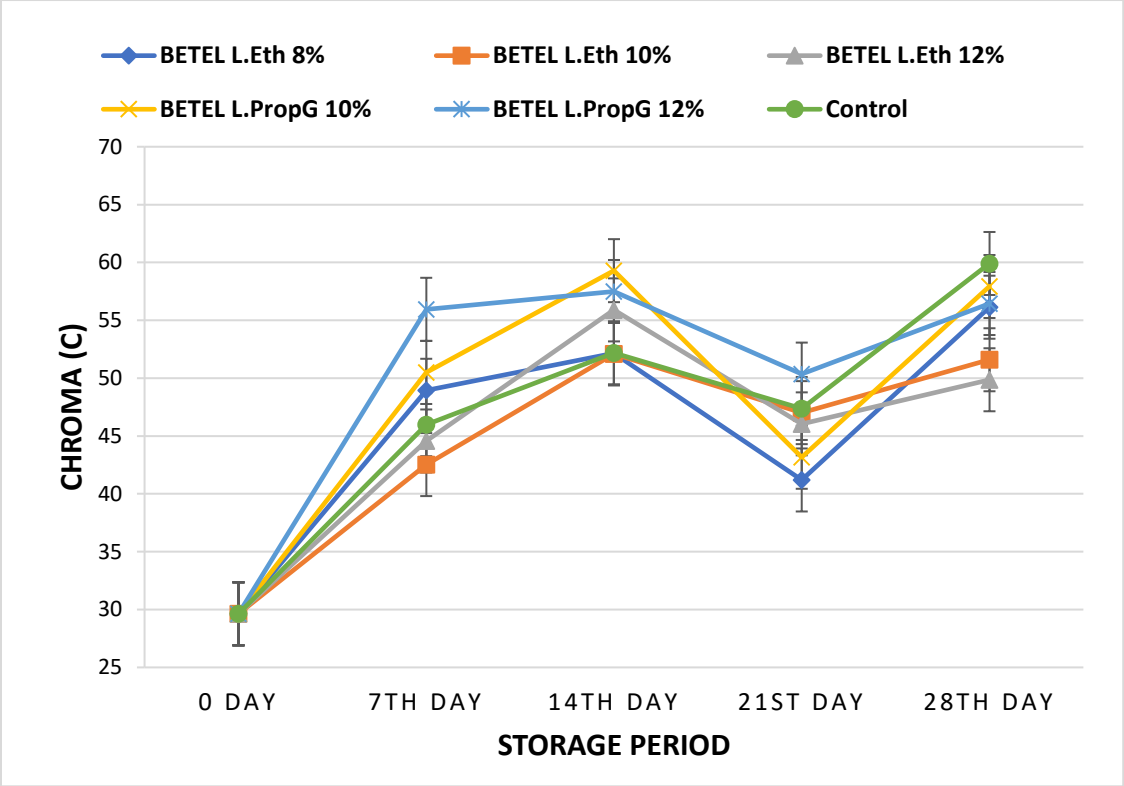


Figure 4.5. Chroma value (C) of papaya for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

The quality of Malaysian local papaya variety, Sekaki can best be described by the skin color changes and L*C*h is best to describe the quality of papaya through the skin color changes. Ruslan & Roslan (2016) found that the hue values can be used to indicate early and late ripening stage while the L* color values can best indicate middle ripening stage.

4.3.2. Chemical quality changes in papaya

The soluble solids content of fruit is composed of sugars, acids, and other substances dissolved in the cell sap. During ripening, organic acid such as malic acid is converted to sugar (fructose). This contributes to the sweet taste of ripened fruit. As the sugar content increased, Brix◦ value increases. Fruit with high SSC are generally acceptable to consumers. To assess the metabolic status of the fruit, the Soluble solids content (SSC) can be used as an indicator. According to the results, there was an increment in SSC content in all the treatments with storage time (Figure 4.6).

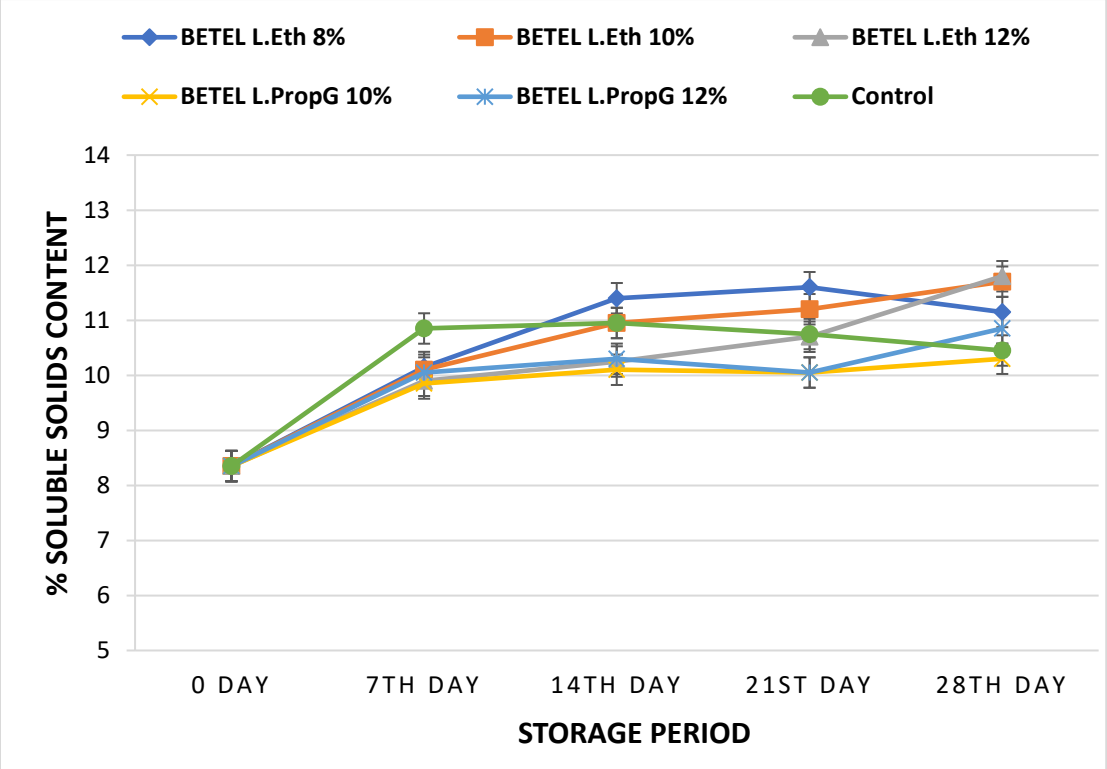


Figure 4.6. Soluble Solids Concentration (Brix %) of fruit pulp for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

González-Aguilar et al. (2010) mentioned that, in general, a series of events and reactions occur during the ripeness process, where molecules with high molecular weight (such as starch) are degraded, resulting in molecules with low molecular weight (such as sugars and organic acids, among others). The total soluble solids (TSS) of the papaya fruit increased continuously with fruit ripening advancing and it

may due to the hydrolysis of starch to sugars as fruit advances in maturation (Zuhair et al., 2013).

The negative control the SSC Brix value was highest in the 7th day and declined slowly after 14th day (Figure 4.6). The SSC was increased on 7th day due to ripening and then it decreased due to the overripe and senescence in the negative treated fruit. The delayed peak in SSC in the Betel leaf extract treated papaya at day 14 was associated with the reduced metabolic actions in the fruit and delayed ripening compared to the negative.

At the end of storage period the highest SSC obtained by 10% and 12% BLET extract while the Lowest SSC was with negative control and 10% BLPG (Betel leaf propylene glycol extract). That means at the end of storage period the sweetness or SSC significantly ($p<0.05$) well maintained at a higher level by 10% and 12% BLET extract. The effect of antimicrobial activity of 12% and 10% BLET maintained high SSC level with no or very less infection on 28th day. This is supported by the findings of A. Ali et al. (2016b) , Maqbool et al. (2011b) and Velickova et al. (2013) reported that slower sugar metabolism through storage in coated papaya, banana and strawberries associated with the slowing down of respiration and metabolic activity, hence retarding the ripening process. In addition, the lower level of SSC in negative and 10% BLPG treated fruit was associated with the amount of microbial infection on the fruit and senescence as well as consumption by respiration. Soluble solids and organic acids of fruit are substrates of energy reserve during ripening (Yaman & Bayoındırlı, 2002).

The titratable acidity (TA) values decreased with storage time in all the treated fruits, but there was no significance difference ($p <0.05$) between all treatments including negative control until 14th day. But a faster decrease was observed with the negative control throughout the storage period (Figure 4.7). Corral et al. (2010) mentioned that TA value of whole papaya tended to decline with maturation. The titratable acidity reflects the organic acid content of the fruit and Citric acid is the major organic acid in ripe papaya fruit. Generally the level of acid decreased during ripening due to the consumption of organic acid in respiration, as organic acid provide most of the hydrogen ion (A. Ali et al., 2014). Due to high respiration level in our negative control fruit, the TA level decreased faster when compared with other treatments. Ali et al. (2011)

mentioned that in control fruit, faster reduction in TA resulted in earlier senescence.

Even though the lower rate of decline in the TA level was maintained in the cold storage with all the treated fruit with both types of Betel leaf extract, but on 28th day the TA level was retained significantly higher ($P < 0.05$) with 12% and 10% BLET extract coated fruit (Figure 4.7), when compared with all other treatments. This may due to respiration rate and physiological function was maintained at lower level with 10% and 12% BLET extracts.

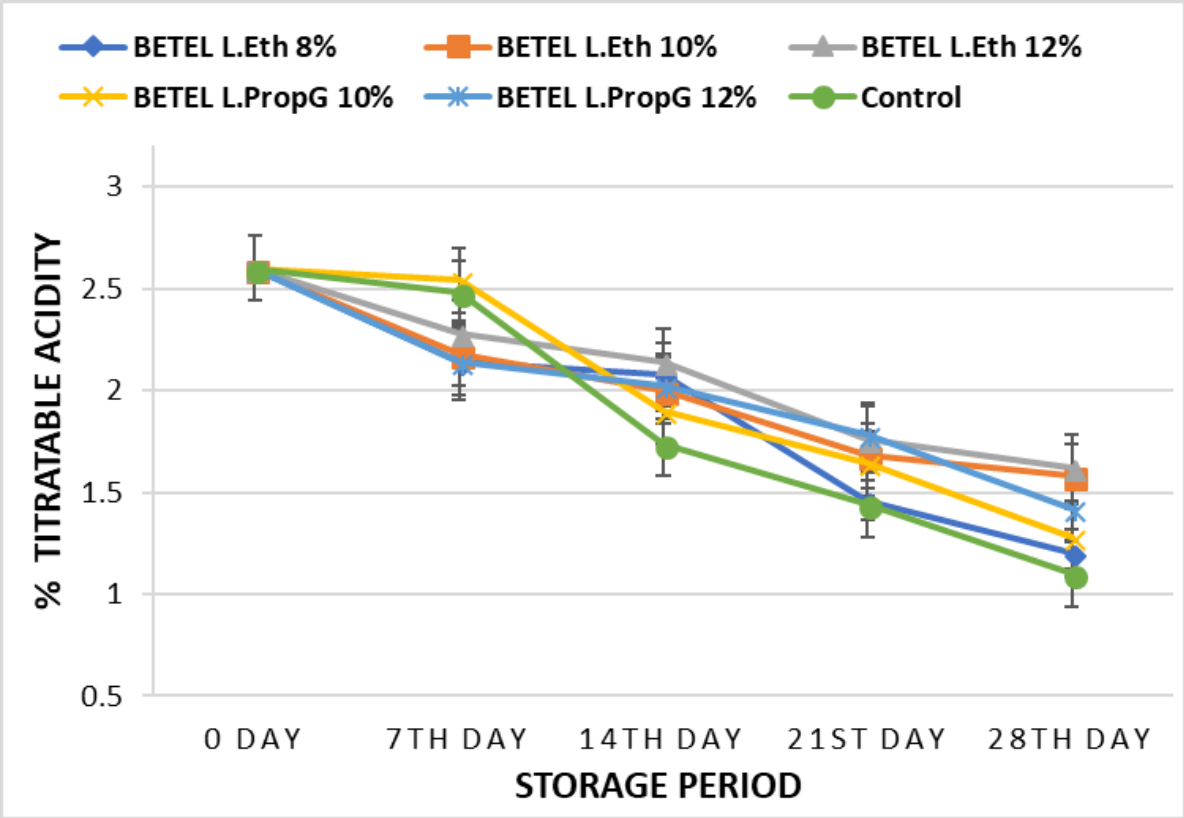


Figure 4.7. Titratable acidity (%) of fruit pulp for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract; vertical bars indicate standard errors; means of four replicates.

In addition to that, the antimicrobial activity of 10% and 12% BLET extract were higher than all other treatments as mentioned earlier findings in our *in vitro* and *in vivo* studies. Thus, maintained the minimal level of fungal infection resulting the low respiration and physiological activity in these coated papayas, thereby preserved the SSC and TA

levels. Martínez-Romero et al. (2006) also reported that, the higher antifungal activity present in coated fruit can reduce infection by covering the cuticle and lenticels in fruit skin, hence minimising respiration and other ripening processes.

4.3.3. Nutritional quality changes in papaya

4.3.3.1. Changes in Ascorbic acid content

Generally, in our results the Ascorbic acid content of all the papaya increased as the fruit ripens within 28 days cold storage. The Ascorbic acid content was significantly better maintained ($P < 0.05$) with all the Betel leaf extract coatings when compared with the control throughout the storage period (Figure 4.8). Also, Gol et al. (2013) and Zhou et al. (2008) found better preservation of ascorbic acid with chitosan and Shellac edible coatings than control.

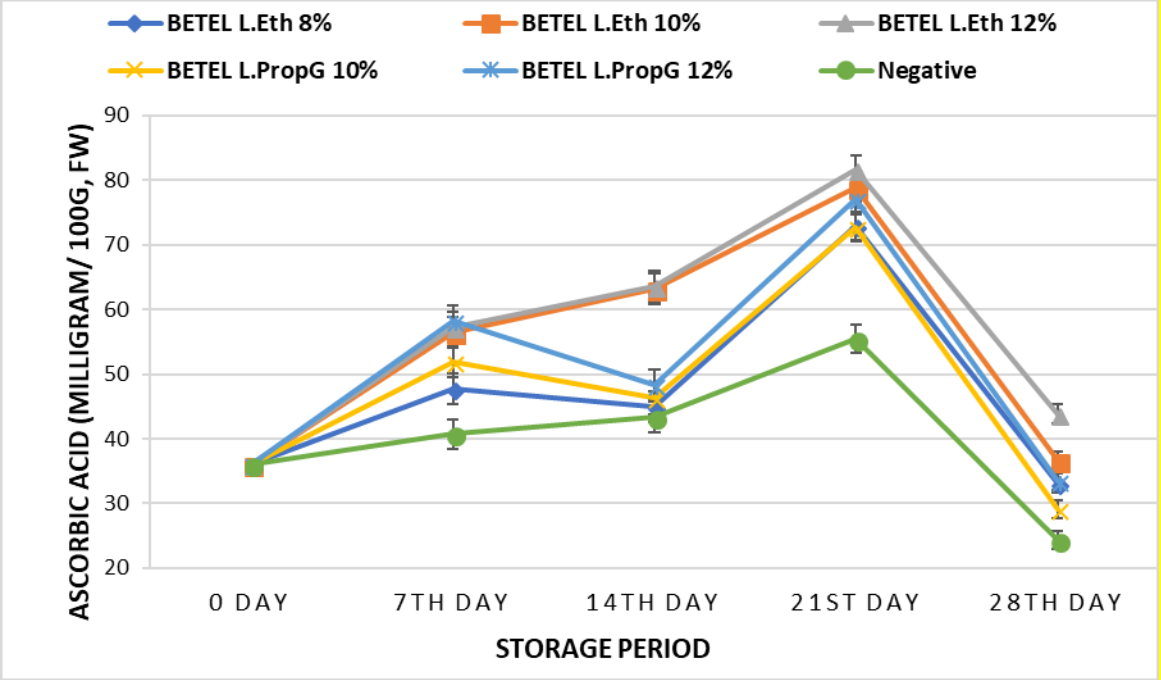


Figure 4.8. Ascorbic acid (mg) in 100g of "Sekaki" papaya fruit pulp for 28 days during cold storage at 10 ± 2^0C with different concentrations of Betel leaf extract. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P \leq 0.05$).

According to our results, until third week the Ascorbic acid content increased with time from 36mg /100g (FW) on the day zero to range of 73-83mg /g (FW) on the 21st day, with all treated fruits with Betel leaf extract. The highest content of Ascorbic acid 83mg / 100g (FW) was observed with 12% BLET treatment on the 21st Day, while the negative treatment showed 55mg /g (FW) Ascorbic acid content only.

However, on the 28th day there were slight decrease of Ascorbic acid amount in all the treated fruits. This may due to the degradation of Ascorbic acid in the long storage with ripening and the growth of pathogens. Ascorbic acid is primarily regulated by ascorbic acid oxidase and phenoloxidase, whose activities are influenced by the oxygen contents in the storage condition. Yaman & Bayoındırlı (2002).

Throughout the storage, the higher retention of Ascorbic acid content in the fruits treated with Betel leaf extract may be due to the lower gas permeability of the coating and antioxidant activity of the Betel leaf extract as well as less fungal attack on the fruit as higher antifungal activity of the Betel leaf extracts coatings. Rathee et al. (2006) reported that 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay of the ethanol extracts of three varieties (Bangla, sweet, and Mysore) of Piper betel revealed the Bangla variety to possess the best antioxidant activity that can be correlated with the total phenolic content and reducing powers of the respective Extracts. Dwivedi & Tripathi (2014) mentioned that, the Betel leaf has the great potency to act as natural antioxidant.

4.3.3.2. Changes in Lycopene content

Lycopene is a vibrant red carotenoid that serves as an intermediate for the biosynthesis of other carotenoids (Fish et al., 2002). In our results, with all treatments the lycopene level increased with time until 2nd week then it decreased with storage period. According to our results, the Lycopene amount was significantly higher with the 10% and 12% BLET treated fruits than other treatments and negative control (Figure 4.9). The highest content of Lycopene 2mg / 100g (FW) was observed with 12% BLET treatment on the 14th Day while the negative treatment showed 1.35mg /g (FW) Lycopene content only on the same day. However, from 21st day there were slight decrease of Lycopene amount in all the treated fruits. This may due to the degradation of Lycopene in the long storage with the high respiration rate at the end of the storage and pathogen development.

However, throughout the storage period we observed overall Lycopene content was better maintained with all the fruits with Betel leaf extract coatings than the negative control. This may due to high reducing power of antioxidant compounds in the Betel leaf extract.

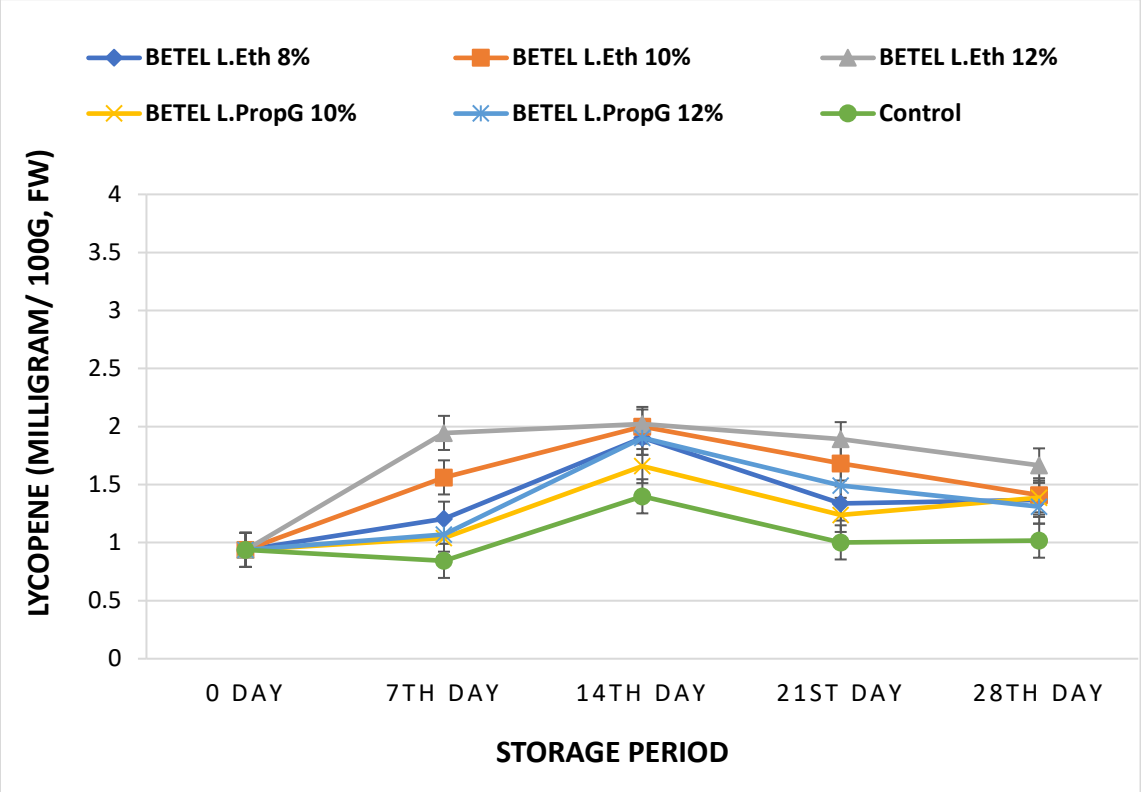


Figure 4.9. Lycopene content (mg) in 100g of "Sekaki" papaya fruit pulp for 28 days during cold storage at 10±2^{0}C with different concentrations of Betel leaf extract. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P \leq 0.05$).

Punuri et al. (2012) mentioned that, P. betel leaves contain a significant number of antioxidants such as hydroxychavicol, eugenol, ascorbic acid, and β-carotene and they have successfully synthesized AuNPs by hypothesizing that the presence of strong antioxidants and flavonoids would assist in the reduction of gold ions to AuNPs. Additionally, Sharma et al. (2009) found that hydroxychavicol in Betel leaf extract showed significant antioxidant activity, measured in terms of the inhibition of lipid peroxidation.

In addition, Lycopene content maintained at higher level in the 12% and 10% BLET at the end of the storage period, as in our case probably due

to the reduced microbial growth caused by the protective effect of antifungal and antimicrobial edible coating. There have been reports on the antifungal activities of *P. betle*. Hydroxychavicol in *P. betle* exhibited a broad range of antifungal activity against clinically significant human fungal species (I. Ali et al., 2010). Experimental findings by Hoque et al., (2012) revealed that the ethanol extract of betel leaves potentially inhibit the growth of foodborne pathogens and ethanol extract of betel leaf might have a potential application as natural food preservative to improve the microbiological safety of the foods.

Nowadays, the worldwide increase in diseases has motivated consumers to increase the intake of fruits and vegetables, in response to various research reports indicating that fruits and vegetables can help prevent certain types of illnesses, due to their potentially high antioxidant properties. Lycopene is an efficacious free radical scavenger (Di Mascio et al., 1989) and its presence in the diet positively correlates with reduced cancer incidence. We evaluated the effect of the 12% and 10% BLET extract coatings of papaya fruit (*Carica papaya* L.) on the contents of antioxidant capacity, Lycopene and Ascorbic acid were significantly high. So BLET extract could maintain high nutritious value of the fruit in the cold storage.

4.3.4. Sensory evaluation of ripe papaya

Since edible coatings can change sensory properties of the fruit, the acceptance by consumers of fruit after treated with edible coatings is a major issue in the consumer market. The sensory evaluation is of great importance because treatments and storage time can change the edible quality of fruit (Guerreiro et al., 2015). A complete sensory evaluation was done after 28 days cold storage for visual appearance (peel colour and pulp colour), taste, texture and flavor for 'Sekaki' papaya. Interestingly all treatments, including negative control gave a good appearance (peel colour and pulp colour), which did not significantly change through 28 days storage time (Table 4).

In our survey, the highest value of 3.667 was obtained with 12% BLET for pulp colour, while 3.467 was obtained with 10% and 12% BLPG for peel colour. That was an average acceptable value in the Hedonic scale range of 0-5. At the same time, the lowest value 2.933 was obtained

with negative control for pulp colour and while it was 2.867 with 8% BLET for peel colour.

Taste was the most affected by Betel leaf extract edible coatings, mostly at higher concentrations 12% BLET and 12% BLPG coatings gave best taste 3.733 and 3.6 respectively, when compared with control (Table 4 and Figure 4.10). Zuhair et al. (2013) reported that the sweetness and flavor produced from full-ripe papaya fruits were found to be the most preferred in comparison to those from the half-ripe and unripe papaya.

Table 4. Sensorial parameters of Betel leaf extract treated and negative control 'Sekaki' papaya after treated for 28 days under cold storage conditions (10±2^{0}C and 80 ± 5% RH).

Extract			Sensory traits		
Treatments	**Taste(0-5)**	**Peel Colour(0-5)**	**Pulp Colour(0-5)**	**Texture(0-5)**	**Flavour(0-5)**
BETEL L.Eth 8%	3.133ab	2.867a	3.4a	3.667ab	3ab
BETEL L.Eth 10%	3.333ab	3.133a	3.2a	2.8c	3.333ab
BETEL L.Eth 12%	3.733a	3.333a	3.667a	3.667ab	3.6a
BETEL L.PropG 10%	2.667b	3.467a	2.933a	3.133bc	2.933ab
BETEL L.PropG 12%	3.6a	3.467a	3.4a	4a	3.533a
Control	1.667c	3.2a	2.933a	3.467abc	2.467b

The high acceptable score for taste and flavor with 10% and 12% BLET might be with high sugar amount and high SSC achieved by the coated papaya with these treatments at 28th day. Zuhair et al. (2013) also mentioned that, as starch in fruits is changed into sugars during ripening process, the taste changes to sweet as a result of increased sugar composition contributing to the taste of the fruit.

The negative control gave the poor taste at the end of 28 cold storage period. It was 1.667 in the scale range of 0-5. The negative control had a significant difference ($P < 0.05$) score value for the taste and flavor (aroma) which were in lower quality when compare to other all treatments. This lower value of taste in the fruit of negative control due

to the intense change caused by microbial growth on the fruit, and due to a faster advance in senescence.

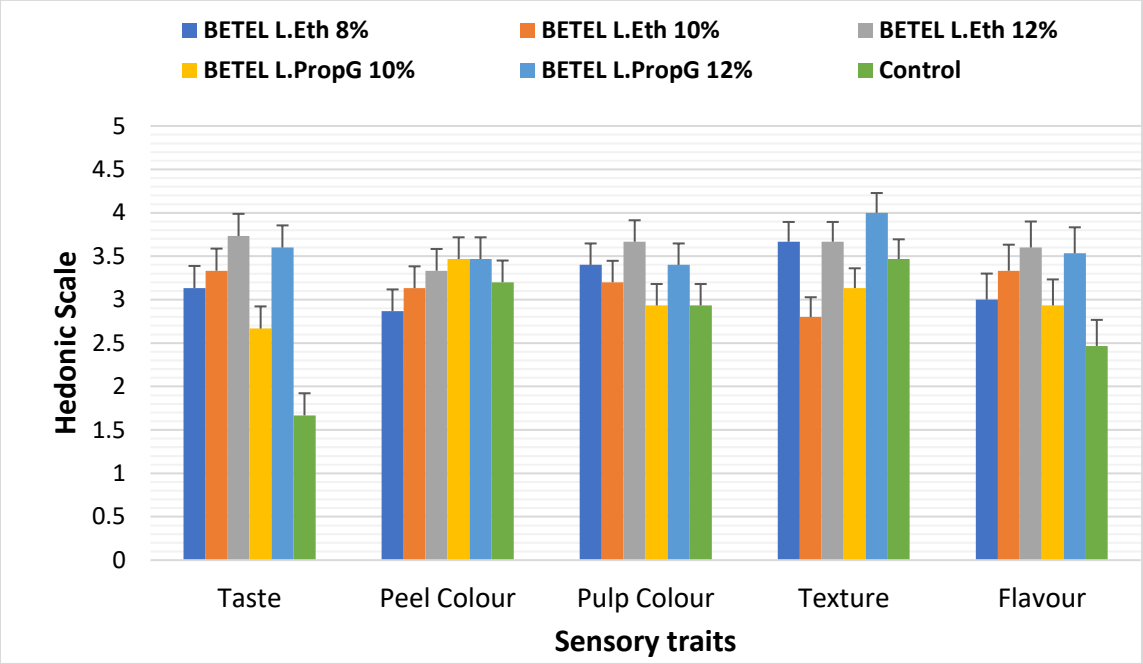


Figure 4.10. Effect of coating with different concentrations of Betel leaf extracts on the sensory traits of "Sekaki" papaya fruits after four weeks of storage at 10±2°C. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P \leq 0.05$).

Ali et al. (2014) reported that, sensory evaluation of ozone treated papaya fruit after storage for 12 days at ambient temperature revealed significant differences in sweetness and texture which gave higher scores when compared with negative control (Figure 4.10). In this manner, after 28 days storage treatments with 12% BLPG had the highest texture preference values followed by and 12% and 8% BLET (Figure 4.10). Edible coatings incorporated with essential oil delayed textural changes, as reported with lemongrass oil nanoemulsions for plums (Kim et al., 2013) and gum Arabic/cinnamon oil for papaya and bananas (Maqbool et al., 2011c). The sensory analyses revealed beneficial effects of coating with Betel leaf extract, in terms of maintenance of fruit visual aspect without any detrimental effect on taste, texture or flavour.

4.3.5. Gaseous exchange analysis

The results of respiration rate recorded in the fruit for 28 days storage, showed that the respiration rate was higher in negative control and BLPG coated fruits when compared with BLET coated fruits until 14th day. Then it dropped on 3rd week and then the maximum respiration rate was recoded with negative control at the end of 28 days storage (Figure 4.11). The early peak of respiration with negative control and BLPG coated fruits, were due to early ripening of the fruit with these treatments while at the end of 28 days storage, the maximum respiration rate on control fruit due to the growth of high number of pathogens and microbes.

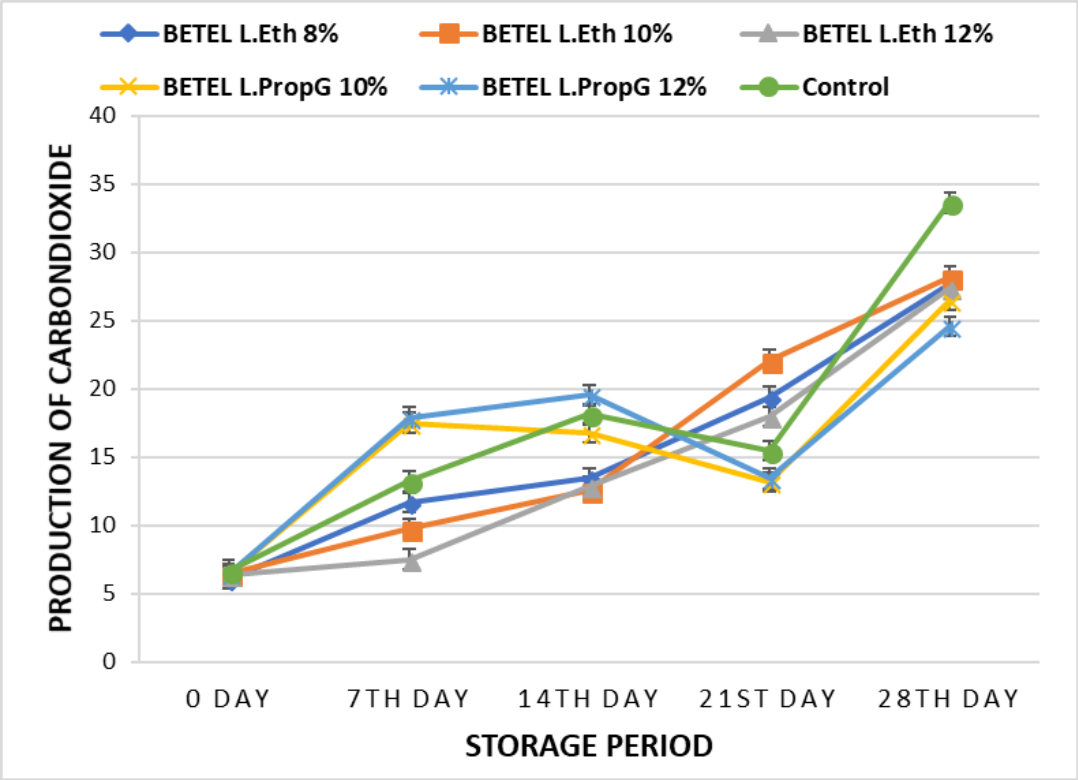


Figure 4.11. Effect of different concentrations of Betel leaf extract on CO2 (mlkg^{-1}hr^{-1}) production of "Sekaki" papaya fruit during cold storage at 10±2^{0}C for 28 days. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P \leq 0.05$).

BLET treated fruits showed a delay ripening and slower metabolism by no peak during first two weeks period. At the end of 28 days storage, the fruit coated with BLET achieved maximum CO_2 emission or respiration rate, which indicates maximum ripening and metabolism activity of fruits with all BLET concentration at the 4th week. This shows

BLET extract helps to elongate shelf life of papaya by reducing metabolism and respiration rate for first three weeks.

Ethylene is involved in the initiation and coordination of ripening related process such as internal and skin color development, softening and flavor and aroma development. An increase in ethylene production was observed in all treatments until 21 days and it was reduced with negative and 10% BLPG coated fruits at the end of 28 days storage (Figure 4.12). Throughout the storage period of four weeks, the ethylene production of BLET coated fruits were significantly lower than the control and BLPG treated fruits. This explained that BLET treatment delay ripening of papaya.

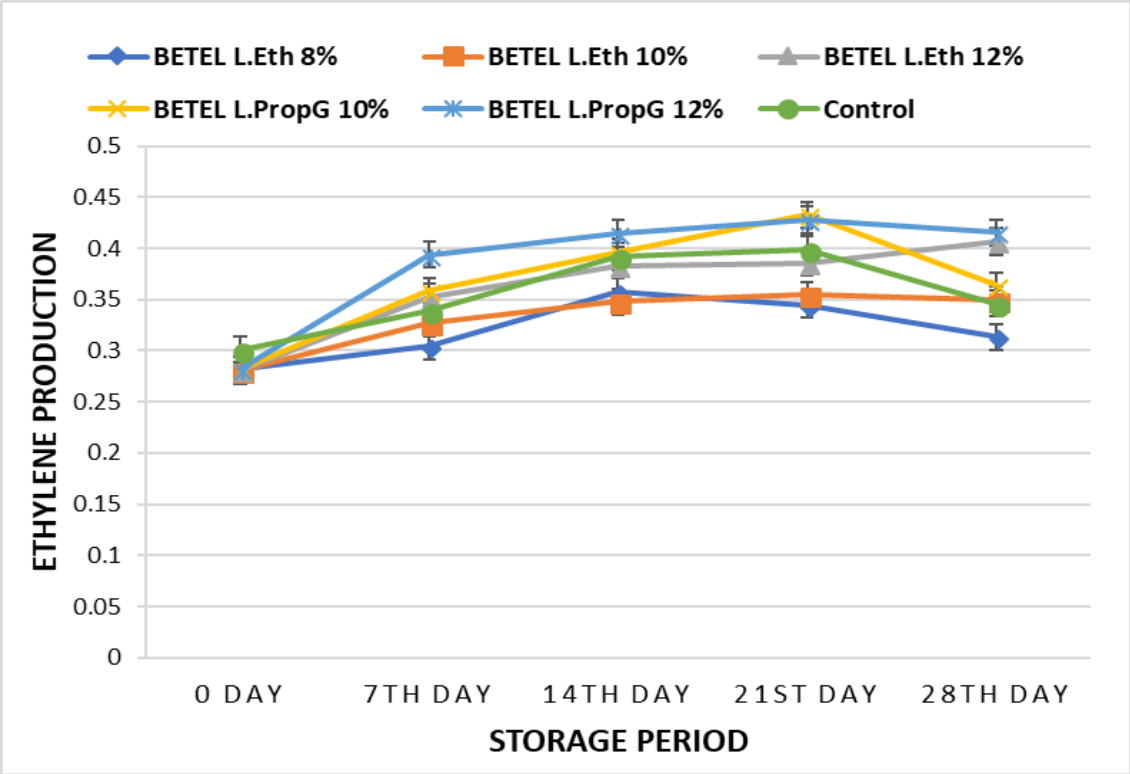


Figure 4.12. Effect of different concentrations of Betel leaf extract on C_2H_4($\mu l kg^{-1} hr^{-1}$) production of "Sekaki" papaya fruit during cold storage at 10 ± 2^0C for 28 days. Each value is the mean of four replicates; vertical bars indicate standard errors of the means; at ($P \leq 0.05$).

5. CHAPTER: GENERAL CONCLUSION

Papaya is an economically important fruit that is extensively cultivated and marketed worldwide. Papaya, being a nutritious fruit, is relished for its pleasant flavor, mouthfeel and texture. In general, postharvest life of fresh papaya varies from 3 to 6 days under tropical climatic conditions, due to enhanced physiological activities such as respiration and other metabolic processors that are associated with deterioration of products.

According to our research study papaya coated with Betel leaf extract, will ensure the extending shelf-life of this fruit in the cold storage up to one month. Commercial viability of extending shelf-life of papaya with Betel leaf extract will help for farmers and sellers to prevent or reduce the amount of profit loss due to the decay and damage of the product in the local and export market by fungal stem-end rot disease. It improves food safety as Betel leaf extract is a safe green fungicide which will make a positive impact on the consumers and the environment.

To our knowledge, this is the first time that Betel leaf ethanolic extract (BLET) and Betel leaf extract in Propylene glycol (BLPG) combined with Tween-80 based edible coatings showed beneficial effects in controlling *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*) in papaya "Sekaki". Tween-80 which has no antifungal or antimicrobial activity, acts as an emulsifier.

Maximum inhibition in radial mycelia growth 98.48% and 93.67% of *L. theobromae* was observed in 12% Betel leaf ethanol extract and 12% Betel leaf propylene glycol extract respectively. In addition, complete inhibition of mycelial growth and spore germination were observed with the greatest number of plates, in each replicate of 12% and 10% BLET added PDA media as well as in 12% BLPG added PDA media.

In vivo studies showed the percentages of Disease severity of coated papaya with 12% and 10% of BLET were significantly low 41.67% and 46.6% respectively and with 12% BLPG extract was 52.13%, while control was 76.67%, during 28 days of cold storage at $10 \pm 2^{o}C$.

The results suggest that betel leaf extract possess bioactive compounds containing antifungal properties that could be key components in controlling *L. theobromae* spore germination and mycelial growth. The ethanolic extract was most effective for its antifungal activity against *L.*

theobromae by penetrating active ingredients into the fungal cell wall and causing leakages of cell wall material and cell membrane from fungal cells and spores as seen in SEM studies. As the results of leakage, deformed conidia with sunken cell walls or ruptured conidia were formed, thereby complete cell lysis of fungal pathogen were occurred with Betel leaf extract

Piper betel extracts containing high concentration of fatty acids like palmitic acid, stearic acid and hydroxy fatty acid esters exhibits potent antifungal activity against pathogenic microorganism. Other possibilities of the fungal inhibition could be due to the limitation of the fungal growth by damage to the membrane, following death of the fungal cells. Antifungal properties of betel leaf ethanolic extract and betel leaf propylene glycol extract could be fruitfully utilized in postharvest stem-end rot disease, thus to extend shelf-life of papaya.

In this study, a novel edible coating based on Piper betel extract has been used as postharvest treatment to maintain papaya 'Sekaki' quality and safety. During 28 days cold storage, there were no significant effect on colour development and weight loss by Betel leaf extract coatings when compared with negative control. Negative control fruit showed increased microbial populations which associated with rapid SSC loss and TA changes, accelerated softening and nutrient depletion. The changes in fruit quality parameters related to papaya postharvest ripening, such as soluble solid content and acidity losses, softening and nutrient losses, were significantly delayed by both Betel leaf extract edible coatings (BLET and BLPG). Betel leaf ethanolic extract as edible coating maintains papaya nutrient quality during postharvest storage by maintaining high level of Lycopene and Ascorbic acid content. Antioxidant activity of Betel leaf extract may be associated with retention of high level of Lycopene and Ascorbic acid in the papaya during cold storage.

When consider the overall liking of papaya 'Sekaki' treated with 12% BLET and 12% BLPG edible coatings, the sensory panel gave a good overall acceptance after 28 days cold storage. On day 28, there was a large increment of SSC in fruit treated with 12% and 10% BLET extract, which indicated that the fruit can be preserved until day 28 due to the increment in their sweetness. The sensory analyses revealed beneficial effects in terms of maintenance of fruit visual aspect without any

detrimental effect on taste, texture or flavour. As far as we are aware, this is the first time *Piper betel* extract is used as an edible coating in fruit, which would be an innovative and interesting means for commercial application and as alternative of the use of postharvest chemical treatments.

Betel leaf ethanol (BLET) extract helped to elongate shelf life of papaya by reducing metabolism and respiration rate for first three weeks. Throughout the storage period of four weeks, the ethylene production of BLET coated fruits were significantly lower than the control and BLPG treated fruits. This explained that BLET treatment delay ripening of papaya fruit.

The findings from all the experiments in this research showed that locally prepared, Betel leaf ethanol (BLET) extract at 12% concentration could be used commercially as an eco-friendly "green fungicide", in local and export market during postharvest storage and transportation of papaya "sekaki", as this concentration shows the maximum effect with the fungal pathogen *in vitro*, *in vivo* as well as maintain the quality attributes in the cold storage up to 28 days with high consumer overall acceptance. Therefore, it can be suggested to be used as an edible coating for extending shelf-life by delay in ripening and improving quality of papaya during storage. Ethanol extract of betel leaf might have a potential application as natural food preservative to improve the microbiological safety of the foods.

Being non-toxic and biodegradable, betel leaf extract with fungi-static or fungi-toxic activity could be considered as suitable alternative to synthetic fungicides in fruit industry to reduce postharvest economic losses due to the stem-end rot disease in papaya and other fruits, thus assisting sustainable agriculture.

However, this investigation has opened new areas and given rise to new ideas on use of antifungal ingredients in *Piper betel* leaves against postharvest fungal diseases of papaya and their non-chemical management of stem-end rot disease in papaya. Hence, the following future lines of work are being suggested.

1. There is a need to undertake an experiment to find out the effect of Betel leaf extract on inhibition of other postharvest fungal

diseases in papaya and other fruits to develop it as broad-spectrum coating treatments.

2. Detailed studies on active ingredients, quantification and the optimal concentration of Betel leaf extract on inhibition of different plant fungal pathogens is required.

3. Studies on gene expression and of pathogenesis related proteins in response to different concentrations of Betel leaf extract is necessary to understand the mode of action.

4. We have used the crude extract of the Betel leaf ethanolic extract which gave the best control with 12% to stop the growth of mycelium and germination of the pathogen spores. Crude extract means all the compounds in the Betel leaf which can be dissolved in the ethanol, what we extracted in our original solution. Thus, it makes the weight percentage as high as 12%. The most effective concentration could be reduced if isolate the bioactive compounds and separate and do the experiment with the pure compounds. So there is a need to do a study with pure bio active compounds of Betel leaf extract which has antimicrobial and antifungal activity.

REFERENCES

Alvarez, A.M., Nishijima, W.T., 1987. Postharvest diseases of papaya. Plant Dis.71, 681-686.

Adolfo González-Aguilar, G., Gayosso-García Sancho, L. E., Yahia, E. M., & Angel Martínez-Téllez, M. (2010). Effect of Maturity Stage of Papaya Maradol on Physiological and Biochemical Parameters. *American Journal of Agricultural and Biological Sciences*, *5*(2), 194–203. Retrieved from http://thescipub.com/pdf/10.3844/ajabssp.2010.194.203

Al-Hetar, M. Y., Zainal Abidin, M. A., Sariah, M., & Wong, M. Y. (2011). Antifungal activity of chitosan against Fusarium oxysporum f. sp. cubense. *Journal of Applied Polymer Science*, *120*(4), 2434–2439. https://doi.org/10.1002/app.33455

Ali, A., Hei, G. K., & Keat, Y. W. (2016a). Efficacy of ginger oil and extract combined with gum arabic on anthracnose and quality of papaya fruit during cold storage. *Journal of Food Science and Technology*, *53*(3), 1435–1444. https://doi.org/10.1007/s13197-015-2124-5

Ali, A., Maqbool, M., Ramachandran, S., & Alderson, P. G. (2010). Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (Solanum lycopersicum L.) fruit. *Postharvest Biology and Technology*, *58*, 42–47. https://doi.org/10.1016/j.postharvbio.2010.05.005

Ali, A., Muhammad, M. T. M., Sijam, K., & Siddiqui, Y. (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (Carica papaya L.) fruit during cold storage. *Food Chemistry*, *124*(2), 620–626. https://doi.org/10.1016/j.foodchem.2010.06.085

Ali, A., Ong, M. K., & Forney, C. F. (2014). Effect of ozone pre-conditioning on quality and antioxidant capacity of papaya fruit during ambient storage. *Food Chemistry*, *142*, 19–26. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.07.039

Ali, I., Khan, F. G., Suri, K. A., Gupta, B. D., Satti, N. K., Dutt, P., … Khan, I. A. (2010a). In vitro antifungal activity of hydroxychavicol isolated from Piper betle L. https://doi.org/10.1186/1476-0711-9-7

Arawwawala, L. D. A. ., Dissanayake, D., Kumarasingha, S.,

Arambewela, L. S. ., Kumaratunga, K., & Ratnasooriya, W. (2011). Investigations on Piper betle grown in Sri Lanka. *Pharmacognosy Reviews*, *5*(10), 159. https://doi.org/10.4103/0973-7847.91111

Azlina, N., Abdul, Z., & Abdul, R. (2014). An Overview of Fruit Supply Chain in Malaysia. *Jurnal Mekanikal*, *37*(June), 36–46. https://doi.org/10.4028/www.scientific.net/AMM.695.792

Bautista-Baños, S., Sivakumar, D., Bello-Pérez, A., Villanueva-Arce, R., & Hernández-López, M. (2013). A review of the management alternatives for controlling fungi on papaya fruit during the postharvest supply chain. *Crop Protection*, *49*, 8–20. https://doi.org/10.1016/j.cropro.2013.02.011

Baviskar, H. P., Dhake, G. T., Kasai, M. A., Chaudhari, N. B., & Deshmukh, T. A. (2017). Review of Piper Betle. *Research Journal of Pharmacognosy and Phytochemistry*, *9*(2), 128. https://doi.org/10.5958/0975-4385.2017.00024.3

Bussaman, P., Namsena, P., Rattanasena, P., & Chandrapatya, A. (2012). Effect of Crude Leaf Extracts on *Colletotrichum gloeosporioides* (Penz.) Sacc. *Psyche: A Journal of Entomology*, *2012*, 1–6. https://doi.org/10.1155/2012/309046

Chien, P.-J., Sheu, F., & Yang, F.-H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering*, *78*(1), 225–229. https://doi.org/10.1016/J.JFOODENG.2005.09.022

Corral, R. D., Carrillo, A., Yahia, E. M., & González, G. (2010). The correlation between some nutritional components with total antioxidant capacity (measured with six different assays) in eight horticultural commodities. *Acta Horticulturae*, *877*, 1267–1274. https://doi.org/10.1021/jf801983r

Das, S., Parida, R., Sriram Sandeep, I., Nayak, S., & Mohanty, S. (2016). Biotechnological intervention in betelvine (Piper betle L.): A review on recent advances and future prospects. *Asian Pacific Journal of Tropical Medicine*, *9*(10), 938–946. https://doi.org/10.1016/j.apjtm.2016.07.029

Di Mascio, P., Kaiser, S., & Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics*, *274*(2), 532–538. https://doi.org/10.1016/0003-9861(89)90467-0

Duan, J., Wu, R., Strik, B. C., & Zhao, Y. (2011). Effect of edible

coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. *Postharvest Biology and Technology*, *59*(1), 71–79. https://doi.org/10.1016/J.POSTHARVBIO.2010.08.006

Evans, P. H., Bowers, W. S., & Funk, E. J. (1984). Identification of fungicidal and nematocidal components in the leaves of Piper betle (Piperaceae). *Journal of Agricultural and Food Chemistry*, *32*(6), 1254–1256. https://doi.org/10.1021/jf00126a011

Fish, W. W., Perkins-Veazie, P., & Collins, J. K. (2002). A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, *15*(3), 309–317. https://doi.org/10.1006/jfca.2002.1069

Gajowik, A., & Dobrzyńska, M. M. (2014). Lycopene - antioxidant with radioprotective and anticancer properties. A review. *Roczniki Panstwowego Zakladu Higieny*, *65*(4), 263–71. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25526570

Guerreiro, A. C., Gago, C. M. L., Faleiro, M. L., Miguel, M. G. C., & Antunes, M. D. C. (2015). The effect of alginate-based edible coatings enriched with essential oils constituents on Arbutus unedo L. fresh fruit storage. *Postharvest Biology and Technology*, *100*, 226–233. https://doi.org/10.1016/J.POSTHARVBIO.2014.09.002

Guha, P. (2006). Betel Leaf : The Neglected Green Gold of India. *Journal of Human Ecology*, *19*(2), 87–93.

Gundala, S. R., Yang, C., Mukkavilli, R., Paranjpe, R., Brahmbhatt, M., Pannu, V., … Aneja, R. (2014). Hydroxychavicol, a betel leaf component, inhibits prostate cancer through ROS-driven DNA damage and apoptosis. *Toxicology and Applied Pharmacology*, *280*(1), 86–96. https://doi.org/10.1016/j.taap.2014.07.012

Hewajulige, I. G. N., & Wilson, S. (2010). Alternative Postharvest Treatments to Control Anthracnose Disease in Papaya during Storage.

Hoque, M. M., Rattila, S., Shishir, M. A., Bari, M. L., Inatsu, Y., & Kawamoto, S. (2012). Antibacterial Activity of Ethanol Extract of Betel Leaf (Piper betle L.) Against Some Food Borne Pathogens. *Bangladesh Journal of Microbiology*, *28*(2), 58–63. https://doi.org/10.3329/bjm.v28i2.11817

Jayathunge, K. G. L. R., Gunawardhana, D. K. S. N., Illeperuma, D. C. K., Chandrajith, U. G., Thilakarathne, B. M. K. S., Fernando, M. D.,

& Palipane, K. B. (2014). Physico-chemical and sensory quality of fresh cut papaya (Carica papaya) packaged in micro-perforated polyvinyl chloride containers. *Journal of Food Science and Technology*, *51*(12), 3918–3925. https://doi.org/10.1007/s13197-013-0952-8

Khanzada, M. A., Lodhi, A. M., & Shahzad, S. (2004). Mango Dieback and Gummosis in Sindh, Pakistan Caused by Lasiodiplodia theobromae. *Plant Health Progress*. https://doi.org/10.1094/PHP-2004-0302-01-DG

Kim, I.-H., Lee, H., Kim, J. E., Song, K. Bin, Lee, Y. S., Chung, D. S., & Min, S. C. (2013). Plum Coatings of Lemongrass Oil-incorporating Carnauba Wax-based Nanoemulsion. *Journal of Food Science*, *78*(10), E1551–E1559. https://doi.org/10.1111/1750-3841.12244

Klangmuang, P., & Sothornvit, R. (2018). Active coating from hydroxypropyl methylcellulose-based nanocomposite incorporated with Thai essential oils on mango (cv. Namdokmai Sithong). *Food Bioscience*. https://doi.org/10.1016/j.fbio.2018.02.012

Konuk Takma, D., & Korel, F. (2017). Impact of preharvest and postharvest alginate treatments enriched with vanillin on postharvest decay, biochemical properties, quality and sensory attributes of table grapes. *Food Chemistry*, *221*, 187–195. https://doi.org/10.1016/j.foodchem.2016.09.195

Krishna, K. L., Paridhavi, M., & Patel, J. A. (2008). Review on nutritional, medicinal and pharmacological properties of papaya (Carica papaya linn.). *Indian Journal of Natural Products and Resources*, *7*(4), 364–373. https://doi.org/0975-6299

Kunz, R., Wijeratnam, S. W., Jaenicke, H., & Heller, J. (2004). Control of Post-Harvest Disease (Stem End Rot) of Rambutan and Annona Species by Using a Bio-Control Agent (Trichoderma spp .).

Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., & Zahid, N. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology*, *62*(1), 71–76. https://doi.org/10.1016/j.postharvbio.2011.04.002

Maqbool, M., Ali, A., Ramachandran, S., Smith, D. R., & Alderson, P. G. (2010). Control of postharvest anthracnose of banana using a new edible composite coating. *Crop Protection*, *29*(10), 1136–1141. https://doi.org/10.1016/j.cropro.2010.06.005

Marotta, F., Pavasuthipaisit, K., Yoshida, C., Albergati, F., & Marandola, P. (2006). Relationship Between Aging and Susceptibility of Erythrocytes to Oxidative Damage: In View of Nutraceutical Interventions. *Rejuvenation Research*, *9*(2), 227–230. https://doi.org/10.1089/rej.2006.9.227

Marotta, F., Yoshida, C., Barreto, R., Naito, Y., & Packer, L. (2007). Oxidative-inflammatory damage in cirrhosis: Effect of vitamin E and a fermented papaya preparation. *Journal of Gastroenterology and Hepatology*, *22*(5), 697–703. https://doi.org/10.1111/j.1440-1746.2007.04937.x

Martínez-Romero, D., Alburquerque, N., Valverde, J. M., Guillén, F., Castillo, S., Valero, D., & Serrano, M. (2006). Postharvest sweet cherry quality and safety maintenance by Aloe vera treatment: A new edible coating. *Postharvest Biology and Technology*, *39*(1), 93–100. https://doi.org/10.1016/j.postharvbio.2005.09.006

Masilamani A N, S., & Muthumary, D. J. (1996). Development of conidiomata in Botryodiplodia theobromae. *Mycological Research*, *100*(11), 1383–1387. https://doi.org/doi:10.1016/S0953-7562(96)80067-0

Mohamed, H., & Saad, A. (2009). The biocontrol of postharvest disease (Botryodiplodia theobromae) of guava (Psidium guajava L.) by the application of yeast strains. *Postharvest Biology and Technology*. https://doi.org/10.1016/j.postharvbio.2009.04.001

Nalina, T., & Rahim, Z. H. A. (2007). The crude aqueous extract of Piper betle L. and its antibacterial effect towards Streptococcus mutans. *American Journal of Biochemistry and Biotechnology*, *3*(1), 10–15. https://doi.org/10.3844/ajbbsp.2007.10.15

Netto, M. S. B., Assunção, I. P., Lima, G. S. A., Marques, M. W., Lima, W. G., Monteiro, J. H. A., … Câmara, M. P. S. (2014a). Species of Lasiodiplodia associated with papaya stem-end rot in Brazil. *Fungal Diversity*, *67*(1), 127–141. https://doi.org/10.1007/s13225-014-0279-4

Nielsen, S. S. (Ed.). (2010). *Food Analysis*. Boston, MA: Springer US. https://doi.org/10.1007/978-1-4419-1478-1

Ong, M. K., Forney, C. F., Alderson, P. G., & Ali, A. (2013). Postharvest profile of a Solo variety "Frangi" during ripening at ambient temperature. *Scientia Horticulturae*, *160*(160), 12–19. https://doi.org/10.1016/j.scienta.2013.05.026

Palaniappan, G., Sengottiyan, A., & Saravanan, T. (2012). BETEL LEAF: THE GREEN GOLD OF INDIA. Retrieved from http://www.efymag.com/admin/issuepdf/Betel Leaf_April-12.pdf

Paridhi, B., Ashita, U. S., Swati, P., & Dilip, N. G. (2015). An Invitro Study Of Determination Of Anti-Bacterial , Antioxidant , Anti-Inflammatory Potential Of Piper Betel Essential Oil, *6*(2), 37–44.

Paull, R. E., & Chen, N. J. (1989). Waxing and plastic wraps influence water loss from papaya fruit during storage and ripening. *Journal of American Society for HorticulturAL Sciences*, *114*(May), 937–942.

Prasanna, V., Prabha, T. N., & Tharanathan, R. N. (2007). Fruit Ripening Phenomena–An Overview. *Critical Reviews in Food Science and Nutrition*, *47*(1), 1–19. https://doi.org/10.1080/10408390600976841

Prus, E., & Fibach, E. (2012). The antioxidant effect of fermented papaya preparation involves iron chelation. *Journal of Biological Regulators and Homeostatic Agents*, *26*(2), 203–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22824747

Punuri, J., Sharma, P., Sibyala, S., Tamuli, R., & Bora, U. (2012). Piper betle-mediated green synthesis of biocompatible gold nanoparticles. *International Nano Letters*, *2*(1), 1–9. https://doi.org/10.1186/2228-5326-2-18

Rahman, A. (2008). Major Postharvest Fungal Diseases of Papaya cv. "Sekaki" in Selangor, Malaysia. *Agric. Sci*, *31*(1), 27–34. Retrieved from http://pertanika.upm.edu.my/Pertanika PAPERS/JTAS Vol. 31 (1) Feb. 2008/07 PAGE 27-34.pdf

Rathee, J. S., Patro, B. S., Mula, S., Gamre, S., & Chattopadhyay, S. (2006). Antioxidant Activity of *Piper betel* Leaf Extract and Its Constituents. *Journal of Agricultural and Food Chemistry*, *54*(24), 9046–9054. https://doi.org/10.1021/jf061679e

Ravneel Prasad, R. (2015). Effects of Plant Leaf Extract against Colletotrichum gloeosporioides (Penz) Sac. Causing Post-Harvest Disease of Papaya. *Journal of Agricultural Science*, *7*(5). https://doi.org/10.5539/jas.v7n5p195

Rivera-Pastrana, D. M., González-Aguilar, G. A., & Yahia, E. M. (2010). Identification of phenolic and carotenoid compounds in Carica papaya L. "Maradol" using liquid chromatography - Mass spectrometry. *Acta Horticulturae*, *877*, 1197–1204.

Ruslan, R., & Roslan, N. (2016). Assessment on the skin color changes of Carica papaya L. cv. Sekaki based on CIE L*a*b* and CIE L*C*h color space. *International Food Research Journal*, *23*(December), S173–S178.

Schweiggert, R. M., Kopec, R. E., Villalobos-Gutierrez, M. G., Högel, J., Quesada, S., Esquivel, P., … Carle, R. (2014). Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised cross-over study. *The British Journal of Nutrition*, *111*(3), 490–8. https://doi.org/10.1017/S0007114513002596

Shanthi Wilson Wijeratnam, yasodha Dharmatilaka, D. W. (2008). Host Specificity of Colletotrichum gloeosporioides and Botryodiplodia theobromae Isolates from mango, papaya and Rambutan and their Response to Trichoderma harzianum. *International Research on Food Security*, (1997), 1–4.

Sharma, S., Khan, I. A., Ali, I., Ali, F., Kumar, M., Kumar, A., … Qazi, G. N. (2009). Evaluation of the Antimicrobial, Antioxidant, and Anti-Inflammatory Activities of Hydroxychavicol for Its Potential Use as an Oral Care Agent. *Antimicrobial Agents and Chemotherapy*, *53*(1), 216–222. https://doi.org/10.1128/AAC.00045-08

Singburaudom, N. (2015). Hydroxychavicol from piper betel leave is an antifungal activity against plant pathogenic fungi. *Journal of Biopesticides*.

Singh, P., Mishra, A. K., & Tripathi, N. N. (2012). Assessment of mycoflora associated with postharvest losses of Papaya Friuts. *Journal of Agricultural Technology*, *8*(3), 961–968.

Sivakumar, D., Hewarathgamagae, N. K., Wilson Wijeratnam, R. S., & Wijesundera, R. L. C. (2002). Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. *Phytoparasitica*, *30*(5), 486–492. https://doi.org/10.1007/BF02979753

Sivakumar, D., Wilson Wijeratnam, R. S., Wijesundera, R. L. C., & Abeyesekere, M. (2002). Control of postharvest diseases of rambutan using controlled atmosphere storage and potassium metabisulphite or Trichoderma harzianum. *Phytoparasitica*, *30*(4), 403–409. Retrieved from https://www.scopus.com/inward/record.uri?eid=2-s2.0-0036037982&partnerID=40&md5=f6d4552143cce30814751ea04d57b07c

Somanah, J., Bourdon, E., Rondeau, P., Bahorun, T., & Aruoma, O. I. (2014). Relationship between fermented papaya preparation supplementation, erythrocyte integrity and antioxidant status in pre-diabetics. *Food and Chemical Toxicology*, *65*, 12–17. https://doi.org/10.1016/j.fct.2013.11.050

Sripradha, S. (n.d.). Betel Leaf – The Green Gold. Retrieved from http://www.jpsr.pharmainfo.in/Documents/Volumes/vol6issue01/jpsr06011409.pdf

Sugumaran, M., Suresh Gandhi, M., Sankarnarayanan, K., Yokesh, M., Poornima, M., & Rajasekhar, S. R. (2011). Chemical composition and antimicrobial activity of vellaikodi variety of Piper betle Linn leaf oil against dental pathogens. *International Journal of PharmTech Research*, *3*(4), 2135–2139.

Taukoorah, U., Lall, N., & Mahomoodally, F. (2016). Piper betle L. (betel quid) shows bacteriostatic, additive, and synergistic antimicrobial action when combined with conventional antibiotics. *South African Journal of Botany*, *105*, 133–140. https://doi.org/10.1016/j.sajb.2016.01.006

Tee, E., & Lim, C.-L. (1991). Carotenoid Composition and Content of Malaysian Vegetables and Fruits by the AOA < : and HPLC Methods. *Food Chemistry*, *41*, 309–339.

Tomella, C., Catanzaro, R., Illuzzi, N., Cabeca, A., Zerbinati, N., Celep, G., … Marotta, F. (2014). The Hidden Phenomenon of Oxidative Stress During Treatment of Subclinical-Mild Hypothyroidism: A Protective Nutraceutical Intervention. *Rejuvenation Research*, *17*(2), 180–183. https://doi.org/10.1089/rej.2013.1495

Valero, D. (2010). *Postharvest biology and technology for preserving fruit quality / Daniel Valero and Maria Serrano.* Boca Raton, Florida.

Valero, D., Díaz-Mula, H. M., Zapata, P. J., Guillén, F., Martínez-Romero, D., Castillo, S., & Serrano, M. (2013). Effects of alginate edible coating on preserving fruit quality in four plum cultivars during postharvest storage. *Postharvest Biology and Technology*, *77*, 1–6. https://doi.org/10.1016/J.POSTHARVBIO.2012.10.011

Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V. D., & Moldão-Martins, M. (2013). Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (Fragaria ananassa cv Camarosa) under commercial storage conditions. *LWT - Food Science and Technology*, *52*(2), 80–92.

https://doi.org/10.1016/j.lwt.2013.02.004

Wills, R. B. H., & Widjanarko, S. (1995). Changes in physiology, composition and sensory characteristics of Australian papaya during ripening. *Australian Journal of Experimental Agriculture - AUST J EXP AGR*, *35*.

Xu, W., & Li, X. (2011). Antifungal Compounds from Piper Species. *Current Bioactive Compounds*, *7*(4), 262–267. https://doi.org/10.2174/157340711798375822

Yaman, Ö., & Bayoındırlı, L. (2002). Effects of an Edible Coating and Cold Storage on Shelf-life and Quality of Cherries. *LWT - Food Science and Technology*, *35*(2), 146–150. https://doi.org/10.1006/FSTL.2001.0827

Zhang, J. (2014). Chapter 10 – Lasiodiplodia theobromae in Citrus Fruit (Diplodia Stem-End Rot). *Postharvest Decay: Control Strategies*, 309–335. https://doi.org/10.1016/B978-0-12-411552-1.00010-7

Zhou, R., Li, Y., Yan, L., & Xie, J. (2011). Effect of edible coatings on enzymes, cell-membrane integrity, and cell-wall constituents in relation to brittleness and firmness of Huanghua pears (Pyrus pyrifolia Nakai, cv. Huanghua) during storage. *Food Chemistry*, *124*(2), 569–575. https://doi.org/10.1016/J.FOODCHEM.2010.06.075

Zhou, R., Mo, Y., Li, Y., Zhao, Y., Zhang, G., & Hu, Y. (2008). Quality and internal characteristics of Huanghua pears (Pyrus pyrifolia Nakai, cv. Huanghua) treated with different kinds of coatings during storage. *Postharvest Biology and Technology*, *49*(1), 171–179. https://doi.org/10.1016/J.POSTHARVBIO.2007.12.004

Zuhair, R. A., Aminah, A., Sahilah, A. M., & Eqbal, D. (2013). Antioxidant activity and physicochemical properties changes of papaya (Carica papaya L. cv. Hongkong) during different ripening stage. *International Food Research Journal*, *20*(4), 1653–1659.

https://www.worldatlas.com/articles/top-papaya-producing-countries-in-the-world.html

APPENDICES

Appendix - A

Enquiry YM6/17/H49 Final Identification Report

Date received: 11/05/2017 Date started: 11/05/2017 Date completed: 30/05/2017

Methods:

The accredited service covers molecular identification of bacteria and fungi. Fungi processed under the accredited service are members of the Kingdom Fungi (including filamentous fungi and yeasts) and members of the Oomycota, Chromista and Stramenopiles.

All procedures are validated and processing undertaken in accordance with CABI's in-house methods as documented in TPs 61-68 and TP70 for bacteria and TPs 72-82 for filamentous fungi and yeasts.

Procedures involve the following steps:

All original samples are subjected to a purity check.

Molecular assays are carried out on each sample using nucleic acid as a template. A proprietary formulation [microLYSIS®-PLUS (MLP), Microzone, UK] is subjected to the rapid heating and cooling of a thermal cycler, to lyse cells and release deoxyribonucleic acid (DNA).

Following DNA extraction, Polymerase Chain Reaction (PCR) is employed to amplify copies of the rDNA in vitro.

The quality of the PCR product is assessed by undertaking gel electrophoresis.

PCR purification step is carried out to remove unutilised dNTPs, primers, polymerase and other PCR mixture compounds and obtain a highly purified DNA template for sequencing. This procedure also allows concentration of low yield amplicons.

Sequencing reactions are undertaken using BigDye® Terminator v3.1 kit from Applied Biosystems (Life Technologies, UK) which utilises fluorescent labelling of the chain terminator ddNTPs, to permit sequencing.

Removal of excess unincorporated dye terminators is carried out to ensure a problem-free electrophoresis of fluorescently labelled sequencing reaction products on the capillary array AB 3130 Genetic Analyzer (DS1) DyeEx™ 2.0 (Qiagen, UK).

Modules containing prehydrated gel-filtration resin are optimized for clean-up of sequencing reactions containing BigDye® terminators. Dye removal is followed by suspension of the purified products in highly deionised formamide Hi-Di™ (Life Technologies, UK) to prevent rapid sample evaporation and secondary structure formation. Samples are loaded onto the AB 3130 Genetic

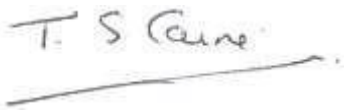
IMI Number	Identification and comments
506609	**Identified as: Lasiodiplodia sp.** **Process:** This sample was identified by ITS rDNA sequence analysis using the FASTA algorithm with the Fungus database from EBI and the BLAST algorithm with the NCBI database, limited to sequences from type material. **Result:** The ITS sequence obtained from this sample produced top matches at 100% identity to sequences from members of the genus *Lasiodiplodia*. Top matches included multiple sequences of *L. thoebromae* (synonym *Botryodiplodia theobromae*) including the type strain of this species (CBS 164.96). However there were also 100% matches to numerous other species in this genus e.g. sequence EF445362 from type strain of *L. plurivora* (STE-U 5803) published in Damm U. *et al.* (2007) Botryosphaeriaceae as potential pathogens of prunus species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* **99** (5) 664-80. Type strains with matches at 100% identity also included *L. euphorbicola* (sequence KF234543 from type strain CMM 3609) and *L. marypalmiae* (sequence KC484843 from type strain CMM 2275). Since there was no clear distinction between species from the ITS sequence result, identification is given to genus level only.

Analyzer and sequencing undertaken to determine the order of the nucleotide bases, adenine, guanine, cytosine, and thymine in the DNA oligonucleotide.

Also, for both **bacteria and fungi**, identification is given to genus level where matches of 97-100% identity are obtained to more than one species within a genus and there is no clear sequence distinction between taxa.

I certify that this report has been checked and approved.

Signed:



Miss T.S. Caine

ID Operations Manager

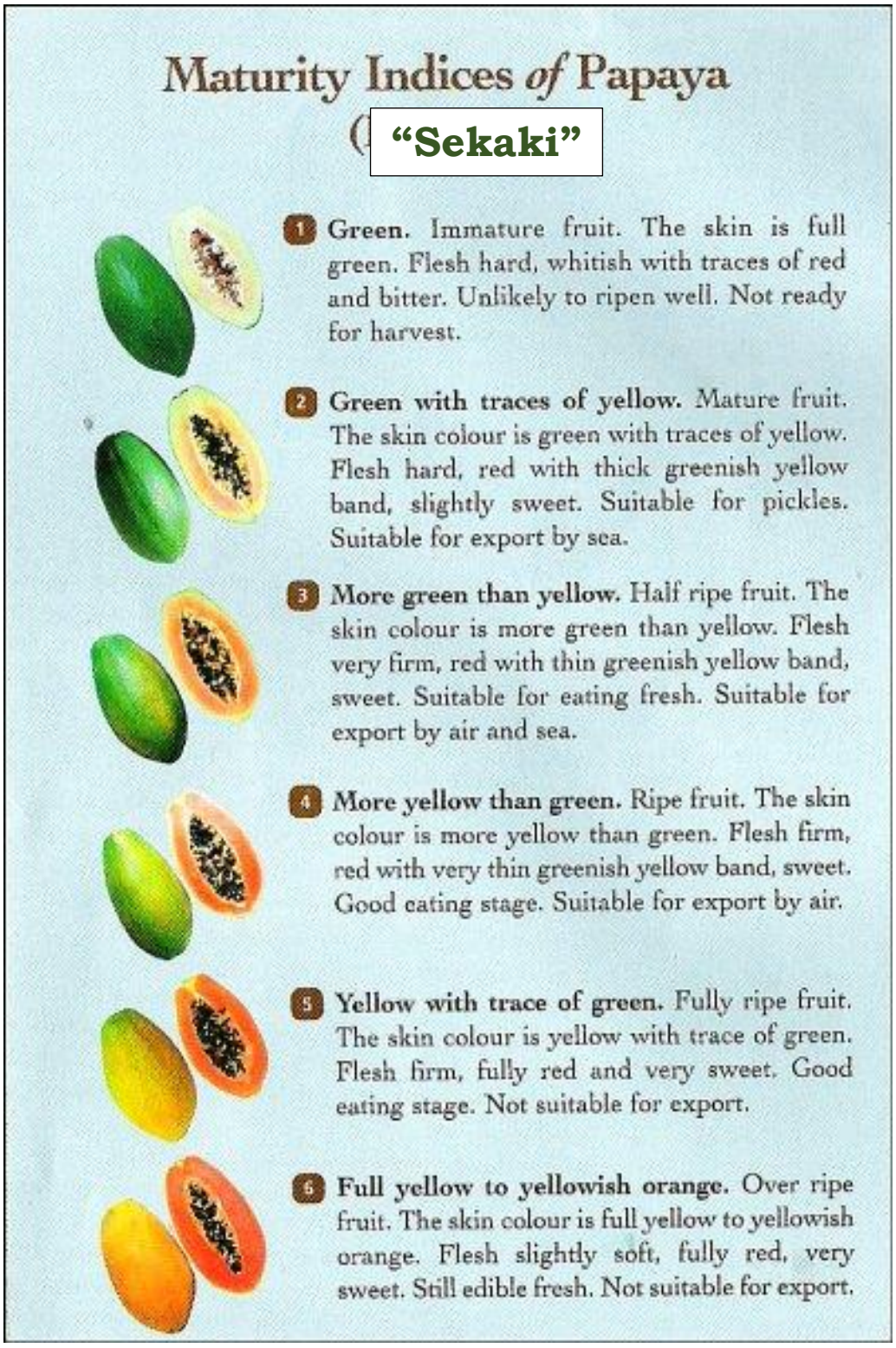
Microbial Identification Service

CABI is a not for profit organisation

CABI improves people's lives worldwide by providing

CABI UK Centre (Egham),

Appendix – B



(http://yumyumbites.blogspot.com/2006/12/fruits-maturity-indices.html)

Appendix – C

haldin

PRODUCT SPECIFICATION

Betel Pepper Liquid Extract

Product Code | **10000014**

PRODUCT DESCRIPTION

Type	: Paraben free, natural glycolic extract produced from selected leaves of *Piper betle*
Common Name	: Sirih (Indonesian), Betel Pepper (English)
Botanical Name	: *Piper betle*
Part Used	: Leaves
INCI Name	: *Piper betle* (leaf) extract, Propylene Glycol , Aqua

PRODUCT CHARACTERISTIC

Appearance	: Liquid
Color	: Clear colorless
Odor	: Characteristic of Piper betle
pH at 25^{o}C	: 3.0 – 3.9
Specific Gravity at 25^{0} C	: 0.990 - 1.055
Refractive Index	: 1.3216 - 1.3475
Solubility	: Soluble in water
Total Phenol	: NLT 200 ppm

MICROBIOLOGICAL DATA

Total Plate Count	: NMT 100 cfu/ml
Yeast and Mold	: Not detectable
E. Coli	: Not detectable / g
Salmonella	: Not detectable / 25 g

APPLICATIONS AND RECOMMENDED USE LEVELS

Application	: Suitable for feminine hygiene, mouth wash, soap, antibacterial wipes, and hand sanitizer.
Use Level	: 1% - 2%

SHELF LIFE AND STORAGE RECOMMENDATION

Storage	: Store in a tight container in a cool, area away from direct heat or light. Best when store at temperature 20 ^{o}C – 25 ^{o}C
Self Life	: One year when stored properly

SFG/TSD/264-07

IMPORTANT NOTICE

The information herein is reliable to the best of our knowledge. However, the recommendations or suggestions herein shall not be construed as a warranty or representation as to the results, safety and efficacy. Users should make their own evaluations and tests suitable for their particular need. We cannot be held liable for any loss or damage arising from the use of the information herein.

Copyright ©2010 by Haldin Pacific Semesta, PT

ingredients... partnership... shared success

Haldin Pacific Semesta, PT
Jl. Jababeka IV Blok C No. 3A
Cikarang Industrial Estate
Bekasi 17530, Indonesia

Ph. +62 21 893 4452
Fx. +62 21 893 4056
source@haldin-natural.com
www.haldin-natural.com

Appendix – D

Analysis of variance of % of Mycelial growth inhibition

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	2	35039.3	17519.66	1407.72	<.001
concentration	4	31644	7911	635.65	<.001
Extract.concentratior	2	1155.11	577.55	46.41	<.001
Residual	63	784.06	12.45		
Total	71	68622.5			

Analysis of variance of % of Spore germination Inhibition

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Extract	2	82281	41140	2243.7	<.001
concentration	4	69422	17356	946.51	<.001
Extract.concentration	2	297.5	148.75	8.11	<.001
Residual	171	3135.5	18.34		
Total	179	155136			

Analysis of variance of Disease Severity (DS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treat	6	47.87578	7.9793	95.24	<.001
days	3	63.84509	21.2817	254.01	<.001
Treat.days	18	19.71815	1.09545	13.07	<.001
Residual	84	7.03777	0.08378		
Total	111	138.4768			

Duncan's multiple range test - Mean comparisons DS (Treat.days)

(Treat x Day)	Mean (sqrt)	Mean Comparision	Mean DS	SEM
control day 28	8.754	a	76.63252	0.1442
BLPG 8% day 28	8.705	a	75.77703	0.1442
BLPG 8% day 21	7.766	b	60.31076	0.1442
control day 21	7.74	b	59.9076	0.1442
BLPG 10% day 28	7.658	b	58.64496	0.1442
BLET 8% day 28	7.653	b	58.56841	0.1442
BLPG 12% day 28	7.207	c	51.94085	0.1442
BLPG 8% day 14	6.947	cd	48.26081	0.1442
BLPG 10% day 21	6.909	cd	47.73428	0.1442
BLET 10% day 28	6.821	cde	46.52604	0.1442
BLET 8% day 21	6.785	cdef	46.03623	0.1442
control day 14	6.671	defg	44.50224	0.1442
BLPG 12% day 21	6.512	defg	42.40614	0.1442
BLET 12% day 28	6.44	efg	41.4736	0.1442
BLET 10% day 21	6.349	fgh	40.3098	0.1442
BLET 8% day 14	6.219	gh	38.67596	0.1442
BLPG 10% day 14	5.975	hi	35.70063	0.1442
BLET 12% day 21	5.948	hij	35.3787	0.1442
BLET 10% day 14	5.893	hijk	34.72745	0.1442
BLPG 12% day 14	5.655	ijkl	31.97903	0.1442
BLPG 8% day 7	5.586	ijkl	31.2034	0.1442
control day 7	5.51	jklm	30.3601	0.1442
BLET 12% day 14	5.457	klm	29.77885	0.1442
BLET 8% day 7	5.283	lmn	27.91009	0.1442
BLPG 10% day 7	5.237	lmn	27.42617	0.1442
BLPG 12% day 7	5.078	mn	25.78608	0.1442
BLET 10% day 7	4.992	n	24.92006	0.1442
BLET 12% day 7	4.863	n	23.64877	0.1442

Analysis of variance of Disease Incidence (DI)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treat	6	172.977	28.8294	29.77	<.001
days	3	340.085	113.3615	117.06	<.001
Treat.days	18	15.1427	0.8413	0.87	0.617
Residual	196	189.809	0.9684		
Total	223	718.013			

Duncan's multiple range test - Mean comparisons DI (Treat.days)

(Treat x Day)	Mean (sqrt)	Mean Comparision	Mean DI	SEM
BLPG 8% day 28	10	a	100	0.3479
control day 28	10	a	100	0.3479
BLPG 8% day 21	9.564	ab	91.470096	0.3479
control day 21	9.444	abc	89.189136	0.3479
BLET 8% day 28	9.117	abcd	83.119689	0.3479
BLPG 10% day 28	9.106	abcd	82.919236	0.3479
BLPG 8% day 14	8.647	bcde	74.770609	0.3479
BLPG 12% day 28	8.635	bcde	74.563225	0.3479
BLET 10% day 28	8.526	bcdef	72.692676	0.3479
BLET 8% day 21	8.51	bcdef	72.4201	0.3479
control day 14	8.39	cdef	70.3921	0.3479
BLPG 10% day 21	8.269	defg	68.376361	0.3479
BLET 12% day 28	7.875	efgh	62.015625	0.3479
BLPG 12% day 21	7.875	efgh	62.015625	0.3479
BLET 8% day 14	7.755	efgh	60.140025	0.3479
BLET 10% day 21	7.618	efgh	58.033924	0.3479
BLET 12% day 21	7.481	fgh	55.965361	0.3479
BLPG 8% day 7	7.456	fgh	55.591936	0.3479
BLPG 10% day 14	7.182	ghi	51.581124	0.3479
control day 7	7.157	ghi	51.222649	0.3479
BLET 10% day 14	7.108	hi	50.523664	0.3479
BLPG 12% day 14	6.883	hij	47.375689	0.3479
BLET 8% day 7	6.211	ijk	38.576521	0.3479
BLET 12% day 14	6.185	ijk	38.254225	0.3479
BLPG 10% day 7	6	jk	36	0.3479
BLPG 12% day 7	5.302	kl	28.111204	0.3479
BLET 10% day 7	5.09607692	lm	25.97	0.3479
BLET 12% day 7	3.647	m	13.300609	0.3479

Analysis of variance of Weight loss (%)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	25.7916	5.1583	10.16	<.001
days	4	1054.17	263.5425	519.08	<.001
Treatments.days	20	16.5625	0.8281	1.63	0.062
Residual	90	45.6937	0.5077		
Total	119	1142.2178			

Analysis of variance of Firmness (N)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	3038.05	607.61	50.53	<.001
days	4	56748.06	14187.02	1179.86	<.001
Treatments.days	20	1608.36	80.42	6.69	<.001
Residual	90	1082.19	12.02		
Total	119	62476.66			

Analysis of variance of Lightness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	1073.83	214.77	19.58	<.001
days	4	2165.73	541.43	49.36	<.001
Treatments.days	20	4197.66	209.88	19.13	<.001
Residual	90	987.24	10.97		
Total	119	8424.45			

Analysis of variance of Hue

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	597.1	119.42	7.59	<.001
days	4	7726.25	1931.56	122.73	<.001
Treatments.days	20	812.65	40.63	2.58	0.001
Residual	90	1416.5	15.74		
Total	119	10552.5			

Analysis of variance of Chroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	414.34	82.87	2.8	0.022
days	4	10429.13	2607.28	87.94	<.001
Treatments.days	20	754.15	37.71	1.27	0.219
Residual	90	2668.35	29.65		
Total	119	14265.98			

Analysis of variance of SSC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	8.1227	1.6245	5.32	<.001
days	4	117.1447	29.2862	95.85	<.001
Treatments.days	20	13.1473	0.6574	2.15	0.008
Residual	90	27.5	0.3056		
Total	119	165.9147			

Analysis of variance of TA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	0.5905	0.1181	1.16	0.335
days	4	23.6011	5.9003	58	<.001
Treatments.days	20	1.7423	0.0871	0.86	0.64
Residual	90	9.1552	0.1017		
Total	119	35.0891			

Analysis of variance of Ascorbic acid (mg in 100g, FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	3472.53	694.51	37.91	<.001
days	4	24164.63	6041.16	329.78	<.001
Treatments.days	20	1769.5	88.48	4.83	<.001
Residual	90	1648.71	18.32		
Total	119	31055.37			

Analysis of variance of Lycopene content (mg in 100g, FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	5.12979	1.02596	11.89	<.001
days	4	8.28724	2.07181	24	<.001
Treatments.days	20	2.40646	0.12032	1.39	0.146
Residual	90	7.76864	0.08632		
Total	119	23.59212			

Analysis of variance of Taste

Source of variat	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	3	40.2143	13.4048	13.74	<.001
Extract	2	4.0111	2.0056	2.06	0.133
Concentration.E	1	1.0889	1.0889	1.12	0.293
Residual	98	95.6	0.9755		
Total	104	140.9143			

Analysis of variance of Peel colour

Source of variat	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	3	0.9238	0.3079	0.33	0.806
Extract	2	2.8444	1.4222	1.51	0.226
Concentration.E	1	0.8222	0.8222	0.87	0.353
Residual	98	92.4	0.9429		
Total	104	96.9905			

Analysis of variance of Pulp colour

Source of variat	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	3	4.9571	1.6524	1.8	0.151
Extract	2	2.5	1.25	1.37	0.26
Concentration.E	1	0.2	0.2	0.22	0.641
Residual	98	89.7333	0.9156		
Total	104	97.3905			

Analysis of variance of Texture

Source of variat	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	3	11.3238	3.7746	4.85	0.003
Extract	2	0.0444	0.0222	0.03	0.972
Concentration.E	1	3.7556	3.7556	4.83	0.03
Residual	98	76.2667	0.7782		
Total	104	91.3905			

Analysis of variance of Flavour

Source of variat	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	3	13.095	4.365	3.23	0.026
Extract	2	0.711	0.356	0.26	0.769
Concentration.E	1	0.556	0.556	0.41	0.523
Residual	98	132.267	1.35		
Total	104	146.629			

Analysis of variance of CO2 ($mlkg^{-1}hr^{-1}$) production (Respiration rate)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	151.808	30.362	7.13	<.001
days	4	11821.44	2955.36	693.8	<.001
Treatments.days	20	1717.526	85.876	20.16	<.001
Residual	210	894.532	4.26		
Total	239	14585.305			

Duncan's multiple range test - Mean comparisons of CO2 ($mlkg^{-1}hr^{-1}$) production

(Treat x Day)	Mean (CO2)	Mean Comparision	SEM
control day 28	33.64	a	0.754
BLET 10% day 28	28.2	b	0.754
BLET 8% day 28	27.64	b	0.754
BLET 12% day 28	27.38	b	0.754
BLPG 10% day 28	26.47	bc	0.754
BLPG 12% day 28	24.55	c	0.754
BLET 10% day 21	22.1	d	0.754
BLPG 12% day 14	19.53	e	0.754
BLET 8% day 21	19.41	e	0.754
control day 14	18.12	ef	0.754
BLET 12% day 21	17.96	ef	0.754
BLPG 12% day 7	17.9	ef	0.754
BLPG 10% day 7	17.49	efg	0.754
BLPG 10% day 14	16.76	fg	0.754
control day 21	15.45	gh	0.754
BLET 8% day 14	13.45	hi	0.754
BLPG 12% day 21	13.44	hi	0.754
control day 7	13.23	hi	0.754
BLPG 10% day 21	13.15	hi	0.754
BLET 12% day 14	12.88	i	0.754
BLET 10% day 14	12.58	i	0.754
BLET 8% day 7	11.66	ij	0.754
BLET 10% day 7	9.75	j	0.754
BLET 12% day 7	7.49	k	0.754
control day 0	6.71	k	0.754
BLPG 12% day 0	6.47	k	0.754
BLPG 10% day 0	6.44	k	0.754
BLET 10% day 0	6.42	k	0.754
BLET 12% day 0	6.38	k	0.754
BLET 8% day 0	6.11	k	0.754

Analysis of variance of C_2H_4 ($ppmkg^{-1}hr^{-1}$) production

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	5	0.115512	0.023102	17.74	<.001
days	4	0.352696	0.088174	67.7	<.001
Treatments.days	20	0.06049	0.003024	2.32	0.002
Residual	210	0.273516	0.001302		
Total	239	0.802214			

Duncan's multiple range test - Mean comparisons of C_2H_4 ($ppmkg^{-1}hr^{-1}$) production

(Treat x Day)	Mean (C2H4)	Mean Comparision	SEM
BLPG 10% day 21	0.4329	a	0.01276
BLPG 12% day 21	0.4279	a	0.01276
BLPG 12% day 28	0.4153	ab	0.01276
BLPG 12% day 14	0.4147	ab	0.01276
BLET 12% day 28	0.4066	ab	0.01276
control day 21	0.3986	abc	0.01276
BLPG 10% day 14	0.3964	abc	0.01276
BLPG 12% day 7	0.3937	abcd	0.01276
control day 14	0.3922	abcd	0.01276
BLET 12% day 21	0.3858	bcde	0.01276
BLET 12% day 14	0.3832	bcde	0.01276
BLPG 10% day 28	0.3636	cdef	0.01276
BLPG 10% day 7	0.3587	cdef	0.01276
BLET 8% day 14	0.3577	cdef	0.01276
BLET 10% day 21	0.3544	defg	0.01276
BLET 12% day 7	0.3523	defg	0.01276
BLET 10% day 28	0.3501	efg	0.01276
BLET 10% day 14	0.3478	efg	0.01276
control day 28	0.3461	efg	0.01276
BLET 8% day 21	0.3448	efg	0.01276
control day 7	0.3388	fgh	0.01276
BLET 10% day 7	0.3271	fghi	0.01276
BLET 8% day 28	0.3136	ghij	0.01276
BLET 8% day 7	0.3045	hij	0.01276
control day 0	0.2924	ij	0.01276
BLET 8% day 0	0.2817	j	0.01276
BLPG 10% day 0	0.2817	j	0.01276
BLPG 12% day 0	0.2817	j	0.01276
BLET 10% day 0	0.2813	j	0.01276
BLET 12% day 0	0.2806	j	0.01276

BIODATA OF THE AUTHOR

Fathumma Rizana Shiraz was born in Colombo, Sri Lanka on 2nd June 1974. She had her primary education at St Anthony's Girls School, Modera, Colombo and continued her secondary education in Science Stream at Sirimavo Bandaranayaka Girl's School, Colombo. After completing her secondary education, she joined Faculty of Science, University of Colombo in 1997 and completed her B.Sc. (Hons.) Bio Sciences with specialization in Plant Sciences.

After completion of her studies at University of Colombo, she joined the Department of Plant Sciences, Faculty of Science at University of Colombo in 2001 as a Demonstrator/Tutor. She gained lot of Teaching knowledge working with Undergraduate students.

After her Marriage to Seyed Shiraz in August,2003, she left from her job and joined her husband in Brunei Darussalam. She is blessed with two beautiful daughters.

In Brunei Darussalam, she was involved in teaching Science at Secondary Schools.

In October 2016 she joined School of Biosciences, Faculty of Science, The University of Nottingham, Malaysia Campus to continue her postgraduate studies. Her MPhil Research Studies was done under the supervision of Professor Asghar Ali Warsi at the Post-Harvest Bio Technology Unit. During her research period she has joined in many conferences, seminars and symposiums in Malaysia as an Oral / Poster Presenter and Proceedings.

LIST OF CONFERENCE ABSTRACTS

1. Fathumma Rizana Shiraz & Asgar Ali. (2017). "Anti-fungal activity of Betel leaf extract against stem-end rot disease of papaya during cold storage". 27th Malaysian Society of Plant Physiology Conference (MSPPC-2017), organized by Malaysian Society of Plant Physiology, at the Pulai Springs Resort, Johor Bahru, 21st – 23rd August 2017.

2. Fathumma Rizana Shiraz & Asgar Ali. (2017). "Anti-fungal assay of Betel leaf extract against stem-end rot disease of papaya during cold storage". 3rd Annual Biomedical Sciences Postgraduate Research Symposium, 21st July 2017, organized by Department of Biomedical Science, The University of Nottingham Malaysia Campus, Malaysia.

3. Fathumma Rizana Shiraz & Asgar Ali. (2017). "Novel biodegradable green fungicide to combat stem-end rot in papaya and to improve postharvest shelf-life in the cold storage". International Congress of Malaysian Society for Microbiology (ICMSM), 4th-7th December 2017, Puthrajaya, Malaysia.

ORAL AND POSTER PRESENTATIONS

Fathumma Rizana Shiraz and Asgar Ali. (2017). "Novel biodegradable green fungicide to combat stem-end rot in papaya and to improve postharvest shelf-life in the cold storage". the International Congress of the Malaysian Society for Microbiology 2017 (ICMSM-2017), Puthrajaya, Malaysia, 4th-7th December 2017.

Fathumma Rizana Shiraz and Asgar Ali. (2017). "Novel biodegradable green fungicide to combat stem-end rot in papaya and to improve postharvest shelf-life in the cold storage". 3rd International Conference for Crop Improvement, organized by University of Reading Malaysia, Plymouth University and Universiti Putra Malaysia (UPM), at the University of Reading Malaysia, Johor Bahru, 8-10th November 2017.

Fathumma Rizana Shiraz and Asgar Ali. (2017). "Anti-fungal activity of Betel leaf extract against stem-end rot disease of papaya during cold storage". 27th Malaysian Society of Plant Physiology Conference (MSPPC-2017), organized by Malaysian Society of Plant Physiology, at the Pulai Springs Resort, Johor Bahru, 21st – 23rd August 2017.

Fathumma Rizana Shiraz and Asgar Ali. (2017). "Anti-fungal assay of Betel leaf extract against stem-end rot disease of papaya during cold storage". 3rd Annual Biomedical Sciences Postgraduate Research Symposium, 21st July 2017, organized by Department of Biomedical Science, The University of Nottingham Malaysia Campus, Malaysia.

Fathumma Rizana Shiraz and Asgar Ali. (2017). "Green-fungicide to combat fruit decay". Postgraduate Research Showcase, 27th April 2017, organized by The Graduate School, The University of Nottingham Malaysia Campus, Malaysia.

PUBLICATIONS

Fathumma Rizana Shiraz & Asgar Ali. (2017). "Anti-fungal activity of Betel leaf extract against stem-end rot disease of papaya during cold storage". The research was published in the journal of "Transactions of the Malaysian Society of Plant Physiology" (Volume 25, pg. 176-182) in June 2018.