ANTI-FUNGAL PACKAGING FORTIFIED WITH CLOVE ESSENTIAL OIL FOR PROLONGING SHELF LIFE OF 'BEAUTY SEEDLESS' GRAPES

SIRIPORN LUESUWAN

MASTER OF SCIENCE

IN

POSTHARVEST TECHNOLOGY AND INNOVATION

SCHOOL OF AGRO-INDUSTRY

MAE FAH LUANG UNIVERSITY

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Thesis Title	Anti-fungal Packaging Fortified with Clove Essential Oil for Prolonging Shelf Life of 'Beauty Seedless' Grapes
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ABSTRACT

Fungal growth in table grape (*Vitis vinifera* cv. Beauty seedless) triggered by *Botrytis cinerea*, *Penicillium* spp., *Aspergillus* spp., and *Rhizopus stolonifera*, deteriorates quality during postharvest storage. Sulfur dioxide fumigation or chemical- based antifungal pads that have been employed in grapes may affect consumer health. Considering the safety aspects, this research aimed to develop a hazard-free antifungal packaging embedded with essential oil (EO) to alleviate the fungal decay of table grapes. The various levels of EOs (0.5-5% v/v) from clove, cinnamon, thyme, peppermint, lemon, bergamot, ginger, spearmint, and lemongrass were tested against *Aspergillus* sp. The results attained in radial growth, disk diffusion method, minimal inhibitory concentration, and minimal fungicidal concentration revealed that 1% clove essential oil (CEO) showed higher efficacy against *Aspergillus* sp., compared to the untreated control and other treatments. CEO at 1% level exhibited pleasant odor intensity in table grapes than the other EOs. Active polyvinyl alcohol (7% PVA) film with 1% CEO resulted in lower weight loss, disease severity, and table grapes berry drop than the control and other treated samples. Additionally, the acceptance score in the table grapes sample wrapped with PVA film containing 1% CEO was augmented. Therefore, PVA film

with 1% CEO retarded the fungal growth and prolonged shelf-life of table grapes during storage of 21 days at 13 °C and 75% RH.

Keywords: Antifungal Packaging, Deterioration, Eco-friendly, Essential Oil, Pathogenic Fungi, Quality, Table Grape, Shelf-life

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background and Significance of the Research Problem

Table grape (*Vitis vinifera* L. cv. Beauty Seedless) is an important economic crop that suffers severe quality losses due to different spoilage and pathogenic microbial species during postharvest storage (Jayawardena et al., 2018). The deterioration of table grapes is catalyzed by the different types of microorganisms such as bacteria (*Gluconobacter* spp. and *Acetobacter* spp.), yeasts (*Zygosaccharomyces* spp.), and molds (*Botrytis cinerea, Penicillium* spp., *Aspergillus* spp., and *Rhizopus stolonifera*) (Gorrasi et al., 2020; Guerra et al., 2016a). Additionally, pathogenic fungi in grapes such as *Botrytis cinerea, Aspergillus* spp., and *Penicillium* spp. allow the development of aerial mycelium to spread rapidly to adjacent berries with severe economic repercussions (Xu et al., 2007). Besides microbial deterioration, grapes are perishable non- climacteric fresh food commodities with several other postharvest storage problems such as loss of firmness, berry drop, stem discoloration, and desiccation (Guerra et al., 2016b). Seedless grape cultivars may be more economical to the farmers, supermarkets, and export markets if wrapped with suitable bio-active packaging material (Youssef et al., 2015).

Over the past decades, several chemical methods have been used to preserve table grapes. Sulfur dioxide (SO_2) fumigation has been practiced commonly to prevent the microbial deterioration of table grapes. Although SO_2 fumes may retard the fungal growth, sulfite residues may be toxic to the consumer's health (Guerra et al., 2016a; Youssef et al., 2015). For ethical concerns, Food and Drug Administration (FDA) has set a maximum tolerance to sulfite residues at 10 ppm generated by SO_2 pads containing sodium metabisulfite ($Na_2S_2O_5$) enclosed in paper and plastic sheets (Ahmed et al., 2018; Sortino et al., 2017). Although commercial

SO2 pads may prevent fungal growth to some level, excess amounts may damage the postharvest quality of table grapes. Degradation of quality attributes by a high concentration of SO_2 may cause bleaching, shrinkage of grape berry, early browning of the rachis, cracking, fruit injury, unpleasant aftertaste, and food allergies (Ahmed et al., 2018).

Essential oils (EOs) are natural byproducts from plants Generally Recognized as Safe (GRAS) by the US FDA having antioxidant, antimicrobial, or antifungal properties (Atarés & Chiralt, 2016a; Ribeiro-Santos, Andrade, Melo, & Sanches-Silva, 2017). EOs have been extracted from different types of plants mainly from leaves, stems, flowers, seeds, branches, roots, buds, and bark of the plant (Ribeiro-Santos et al., 2017). EOs extracted from plants are free of toxicity and eco-friendly may be an efficient alternative to chemical fungicides (Aguilar-González, Palou, & López-Malo, 2015b). The active compounds of EOs are rich in volatile bioactive components mainly constituted of secondary metabolites such as aldehydes, fatty acids, phenols, ketones, esters, and alcohols and exhibit several nutraceutical properties (Abifarin, Otunola & Afolayan, 2020).

The use of EOs to extend the shelf-life of fruits has gained tremendous interest because of the promising health benefits (Echegoyen & Nerín, 2015). Application of biodegradable packaging films and edible coatings fortified with EOs have developed the concept of postharvest preservation of fresh produce (Aguilar-González, Palou & López-Malo, 2015a). Active packaging materials for grapes supplemented with EOs have been evidenced to develop a protective atmosphere rich in volatile antimicrobial or antioxidant components against microbial spoilage in table grapes (Mateo et al., 2017). Food packaging films from polyvinyl alcohol (PVA) and chitosan enriched with cinnamon EO were reported to have excellent antimicrobial properties (Ebrahimzadeh, Bari, Hamishehkar, Kafil & Lim, 2021). PVA loaded clove essential oil (CEO) nano- size capsules exhibited antifungal properties (Qing, Xiao, Ya, Cai-Ning, Guo-Yi & Hua-Bin, 2017). Nevertheless, there is no information about the preparation of PVA film loaded with EO on the retardation of fungal growth and quality changes of table grapes. Therefore, the current study was conducted to screen the desirable level of EO based on antimicrobial characterization under *in vitro* conditions. The impact of potential EO

fortified antifungal packaging material was also investigated on the fungal growth and postharvest quality changes of table grapes during storage of 30 days at 13 °C and 75% RH.

1.2 The Objectives of this Study

1.2.1 To investigate the *in vitro* anti-fungal properties of essential oils (EOs) from different herbs.

1.2.2 To evaluate the effect of packaging fortified with clove essential oil on the fungal growth and postharvest quality changes of table grape during cold storage

1.3 Scope of Research

1.3.1 Screening of the natural anti-fungal additive from different EOs at various percent levels could be used for the development of active antifungal packaging for table grape

1.3.2 Employing active packaging material (pads and films) supplemented with the optimum level of essential oil in table grape as a potential substitute to chemical fumigation in table grape

1.3.3 Application of active antifungal packaging material could preserve postharvest quality deterioration of table grape during cold storage

1.4 Expected Benefits

1.4.1 Research Outcome

The result of this research can summarize a novel way of developing an active packaging material fortified with essential oil against fungal growth and extending the overall shelf life of table grapes.

1.4.2 Research Output

The antifungal packaging material particularly an active PVA film supplemented with clove essential oil could be developed for grape storage and distribution under cold

1.5 List of Definitions of Technical Terms

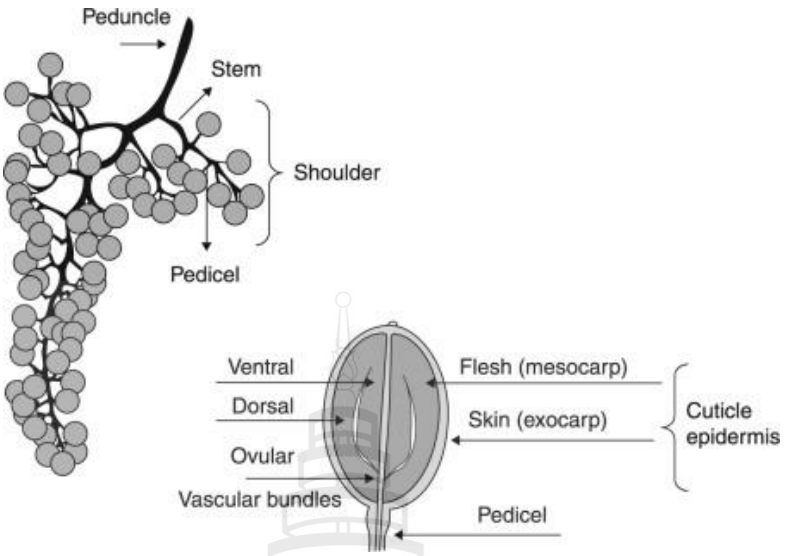
1. EO	essential oil
2. CEO	clove essential oil
3. PVA	polyvinyl alcohol
4. DMSO	Dimethyl sulfoxide
5. PDA	potato dextrose agar
6. TBRC	Thailand Bioresource Research Center
7. OPP	oriented polypropylene
8. MIC	Minimum inhibitory concentration
9. MFC	minimum fungicidal concentration
10. PDB	potato dextrose broth
11. GAP	Good Agricultural Practices
12. TSS	total soluble solids
13. CEO1-Pad	absorbent pad injected with 25 mL of
14. 1% CEO COM-SO2-Pad	commercial pad sodium metabisulfite 98%
15. CEO1-HC-Film	7% PVA film fortified with 1% CEO and 1% halloysite clay
16. CEO1-Film	7% PVA film fortified with 1% CEO

CHAPTER 2

LITERATURE REVIEW

2.1 Table Grapes

Table grapes are comprised of more than 50 cultivars and can be classified based on the color of the berry and the presence or absence of seeds inside the grape berries. The various cultivars grown worldwide include ‘Thomson Seedless’, ‘Red Globe’, and ‘Flame Seedless’ (Zoffoli & Latorre, 2011). Department of Agricultural Extension, Thailand (2017) reported that the main varieties of table grape grow in Thailand are white Malaga and Cardinal. The table grapes morphology and anatomy depict that the grape berries are composed of different layers such as exocarp, mesocarp, and endocarp that is skin, flesh, and tissue around seeds, respectively (Figure 2.1). The epidermal cells constitute the flavor and aromatic compounds (Zoffoli & Latorre, 2011).

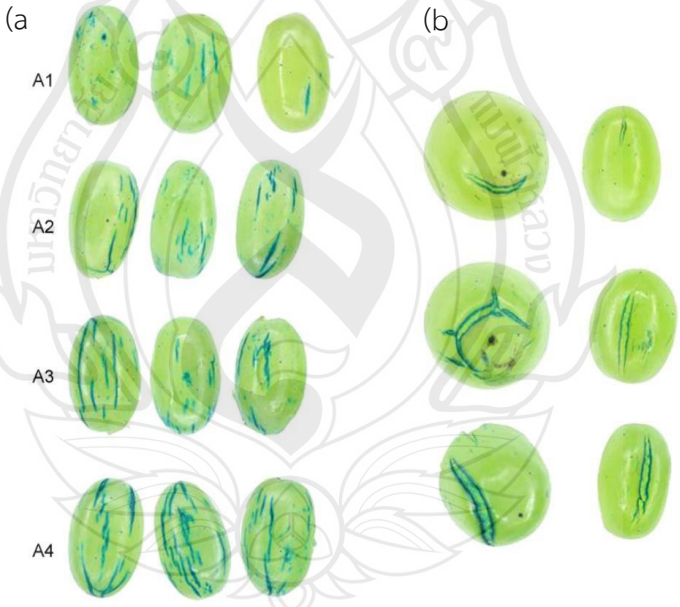


Source Zoffoli and Latorre (2011)

Figure 2.1 Schematic representation of table grape berry

2.2 Postharvest Storage Problems in Table Grapes

Table grape suffers severe quality problems during postharvest storage such as fruit wilting, fall grape, stem discoloration, and fungal rot leading to quality deterioration (Guerra et al., 2016b). The most visible quality defects during storage include fruit bleaching, water loss, hairline cracks, break apart, browning skin or flesh, berry splitting, and softness of berry (Figure 2.2) (Zoffoli & Latorre, 2011). The grape cultivar especially with thin skin more susceptible to cracking thereby resulting in short storage life and higher economic losses. The study of vineyard calcium spray reported that the presence of some elements such as iron (Fe), copper (Cu), zinc (Zn), and calcium (Ca) could increase the firmness in grape berry (Martins, Soares, Spormann, Fidalgo & Gerós, 2021).

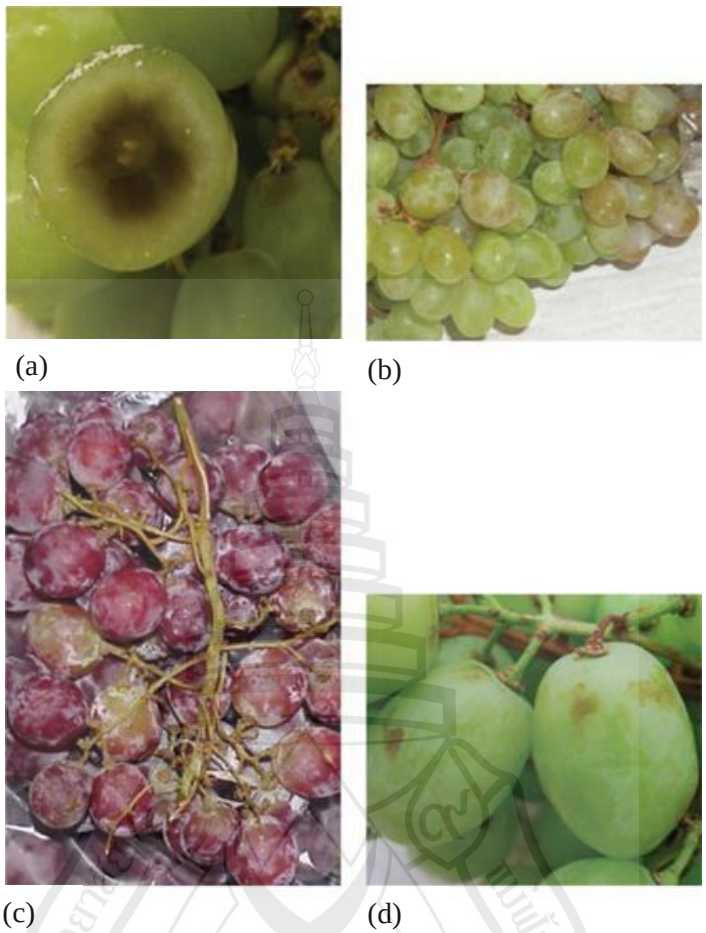


Source Zoffoli and Latorre (2011)

Figure 2.2 Hairline cracks (a) and berry splitting (b) symptoms on table grapes stained by methylene blue

At present, the consumer demand for fresh and healthy may be hinder the economic growth of the farmers due to inadequate methods that may shorten the shelf life of fresh fruits by tissue bruising leading to microbial deterioration (Martins et al., 2021).

The abscission zones developed on the grape were documented as a result of postharvest hormonal changes when stored in different environmental conditions (Wu, Xin, Xu, Chu, Du, Tian & Zhu, 2021). During the extended storage time, a process of aging was observed known as senescence visualized by weight loss, berry softening, berry bruising, and stalk browning in grapes (Zhang et al., 2019). Moreover, the quality deterioration leading to the softness of tissue may accelerate the abscission and the stem becomes brown of grapes (Wu et al., 2021). Postharvest quality defects may be further triggered by chilling injuries, internal tissue, and seed browning leading to stem discoloration and abrasion damage (Figure 2.3) (Zoffoli & Latorre, 2011).



Source Zoffoli and Latorre (2011)

Figure 2.3 Internal tissue browning (a), chilling injuries (b), stem browning (c), and abrasion damage (d) during postharvest storage of different grape cultivars

2.3 Microbial Deterioration of Grapes During Storage

One of the major postharvest problems of table grapes is fungal decay which comes with a big cause of rejection in marketability. The main mold infection including *Botrytis cinerea*, *Penicillium expansum*, *Aspergillus niger*, and *Rhizopus stolonifera* is the main cause of postharvest diseases of grape (Guerra et al., 2016b). *Botrytis cinerea* and *Aspergillus* spp. are usually known as the main postharvest decay microorganisms of table grapes from the field during harvesting of fruits,

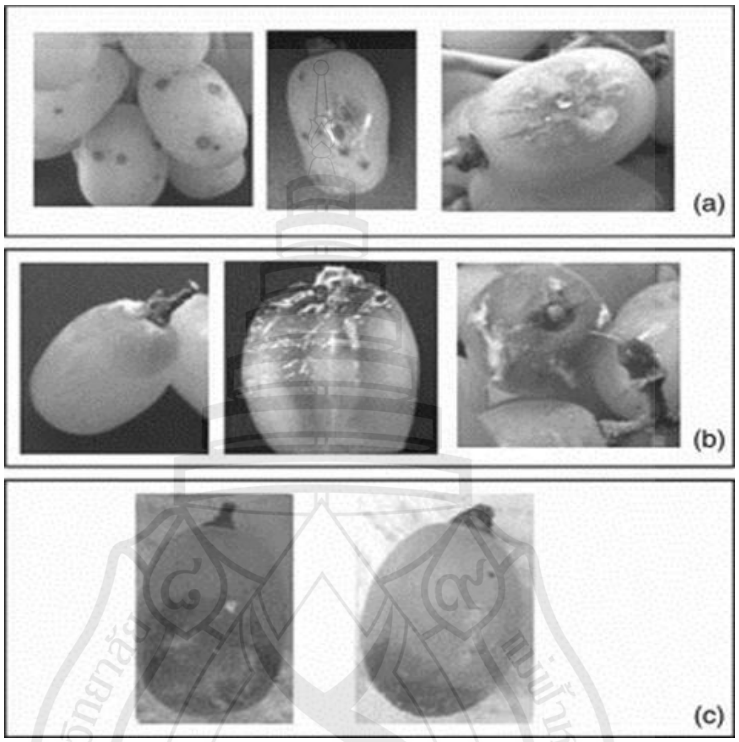
distribution, for shelf-life assessment under low- temperature storage (Feliziani, Romanazzi & Smilanick, 2014; Guerra et al., 2016b; Xu et al., 2007).

After harvest, about 10-40% of grapes are wasted due to fungal growth (Solairaj et al., 2020). Even though grapes are highly consumed and demanded by consumers, but the problem of fungal decay reduces its marketability as a result of decreased shelf- life. The problem such as stem change in color, decrease in firmness, desiccation, berry drop from the stem, the fungal infection makes it less economical to be sold in the market (Solairaj et al., 2020). The most important postharvest pathogenic fungi in grapes are *Aspergillus carbonarius*, *Botrytis cinerea*, *Penicillium expansum*, *Rhizopus stolonifer*, and *Alternaria alternate*. Moreover, *P. expansum* and some *Aspergillus* spp. can produce mycotoxins such as patulin, aflatoxin, and ochratoxin A (OTA) (Solairaj et al., 2020). OTA was classified as a human carcinogen (group 2B) by the International Agency for Research on Cancer (Jiang, Shi, Lui & Zhu, 2014). The mycotoxin production and other plant pathogenic microorganisms associated with fungal growth vary on climatic conditions and environmental factors. The fungus responsible for inducing potential infections in grapes may depend on the specific climatic conditions might be regarded as less virulent in a different set of other climatic factors (Solairaj et al., 2020). Apart from the classic method of morphological approach of classification of fungi and bacteria, novel methods have been developed using DNA sequencing methods for the detection of diverse pathogenic fungi (Zabel & Morrell, 2020).

2.3.1 Gray mold

Gray mold caused by *Botrytis cinerea* is a major postharvest disease of grapes. *B. cinerea* is a broad-spectrum host-range fungal pathogen that triggers the infection of gray mold also correlated with the bunch rot caused by *Vitis vinifera*. Being a necrotrophic fungus, the host tissue is attacked for its sustenance to grab the nutrients for its growth. Such fungal infection may be involved in the early-stage invasion at the flowering time on receptacles during the blooming of grape buds and may be inherited to berry until it ripens (Bhatia, Upadhyay & Singh, 2020). Gray mold is a major threat during the pre-harvest and postharvest periods of table grapes. It makes a soft and watery berry. Gray mold was infected in the field, during storage, and wounded infection (Figure 2.4). Conidia are dispense by wind

(Zoffoli & Latorre, 2011). This fungal rot disease can be evidenced in more than 200 plant species that may accelerate the global economic losses (Zambounis, Ganopoulos, Valasiadis, Karapetsi & Madesis, 2020).



Source Zoffoli and Latorre (2011)

Figure 2.4 Small spots on fruits (a), brown discoloration (b), and brown discoloration (c) are the symptoms of gray mold on table grapes

Fresh fruits and vegetables are susceptible to the fungal attack by *Penicillium* species during postharvest handling and storage. Most of the species responsible for blue mold have been reported to be psychotropic thereby causing spoilage at cold storage temperature in table grapes (Rodriguez Assaf, Pedrozo, Nally, Pesce, Toro, Castellanos de Figueroa & Vazquez, 2020). Blue mold caused by *P. expansum* has been identified to develop a soft rot in fresh fruits especially in grapes during cold storage (Zoffoli & Latorre, 2011). The disease symptoms appear on the grape berries such as softness, watery lesions that are light brown in color, and lesion edge, the

non-motile fungal spore of conidia have been observed to appear as blue-green color in spoiled grapes (Errampalli, 2014).

2.3.2 *Aspergillus* rot

The genus *Aspergillus* is comprised of several species that produce mycotoxins, which may impact the large quantity of agricultural produce may cause epidemiological and economic concerns in grape cultivators and consumers. It is one of the main mycotoxin- producing microbe associated with agricultural contamination and environmental pollution (Plascencia-Jatomea, Susana, Gomez & Velez-Haro, 2014). The black mold causes decay in grapes with serious health and economic repercussions due to black spore on table grapes worldwide (Li et al., 2017). *Aspergillus* rot creates brown lesions on bruised grape berries followed by dark-black conidia. The growth of *Aspergillus* spp. is favored in warm temperatures in the fields and may be inhibited under refrigerated conditions. *Aspergillus* rot is often regarded as sour rot in grapes (Zoffoli & Latorre, 2011). *Aspergillus niger* has been reported to cause black mold disease in during postharvest storage of grapes and during transportation under to markets may be associated with the secretion of mycotoxin (ochratoxin A) that can be pathogenic or carcinogenic to human health (An et al., 2019).

2.3.3 Rhizopus rot

Rhizopus stolonifer is the fastest-growing species responsible for triggering Rhizopus rot in grapes. Disease caused by this fungus is detected apparently as soft rot, black mold, or *Rhizopus* rot. It often grows in a short period under moist and humid conditions in stored grapes (Bautista-Baños, Bosquez-Molina & Barrera-Necha, 2014). *Rhizopus* rot is infected causes softness of mature grape berries indicated by the off-flavor or watery berry texture (Zoffoli & Latorre, 2011).

2.3.4 Other fungi

Therefore, several other fungi such as *Alternaria alternate, Botryodiplodia theobromae* may also contribute to the fungal spoilage of mature table grape berries during postharvest storage (Zoffoli & Latorre, 2011).

2.4 Application of Techniques and Treatments During the Distribution of Grapes

The various techniques have been employed in the supply chain management of grapes from the harvest site to the final packaging along with treatments for safeguarding the quality of grapes. Sulfur dioxide sheets that release SO_2 fumes have been used in the boxes ready for import and export of grape packaged in boxes. An estimated shelf-life of 2 months has been typically associated with the antimicrobial effect of SO_2 producing pads. However, SO_2 can cause several quality constraints such as bleaching, injuries to berries, changes in flavor, and maybe allergic to humans (Gabler, Smilanick, Mansour & Karaca, 2010). The method, technique, and treatment used for the inhibition of microorganisms were shown in Table 2.1.

Table 2.1 The method, technique, and treatment used for inhibition of microorganism

Method	Technique	Treatment	Inhibition microorganism	Reference
Chemical control	Fumigation	Sulfur dioxide	*Penicillium expansum*	(Franck, Latorre, Torres & Zoffoli, 2005)
	Dipping	Ethanol	*Botrytis cinerea*	(Lichter et al., 2002)
	Vapor	Acetic acid 20 $\mu L.L^{-1}$	*Botrytis cinerea*	(Venditti, Ladu, Cubaiu, Myronycheva & D'hallewin, 2017)
	Spraying (Pre- and Postharvest	44% Calcium chloride	*Botrytis cinerea*	(Ciccarese, Stellacci, Gentilesco & Rubino, 2013)
Biological control	Fumigation	*Muscodor albus*	*Botrytis cinerea*	(Gabler, Mercier, Jiménez & Smilanick, 2010)
	Coating	Chitosan	*Botrytis cinerea*	(Xu et al., 2007)
	Essential oil	Thyme	*Botrytis cinerea* *Rhizopus stolonifer*	(Bhaskara Reddy, Angers, Gosselin & Arul 1998)
	Coating	Lemongrass oil 0.5-4.0 g/100g	*Salmonella typhimurium* *Escherichia coli*	(Kim, Oh, Lee, Song & Min, 2014)
	Coating	Lemongrass oil+chitosan 1g/100g	*Salmonella typhimurium*	(Oh et al., 2017)
	Fumigation	Ozone 0.100µl/L (day) and 0.300µl/L (night)	*Botrytis cinerea*	(Feliziani et al., 2014)
	Hot water treatment	Dipping in 45°C water, 8 min	Postharvest loss and chilling damage	(Wu et al., 2015)
	Fumigation	Ozone 200-350 µl.L-1, 1 h at 35, 75, and 95% RH, 25°C	*Penicillium digitatum* *Penicillium italicum* *Botrytis cinerea*	(Ozkan, Smilanick & Karabulut, 2011)

Table 2.1 (continued)

Method	Technique	Treatment	Inhibition microorganism	Reference
	Biofumigation	*Mucodor albus*+ozone 5,000 µl.L-1	*Botrytis cinerea*	(Gabler, Smilanick, Mansour & Karaca, 2010)
	UV-C+Chitosan	UV-C light+Chitosan coating	*Botrytis cinerea*	(Freitas, López-Gálvez, Tudela, Gil & Allende, 2015)

2.4.1 Conventional or Chemical Methods

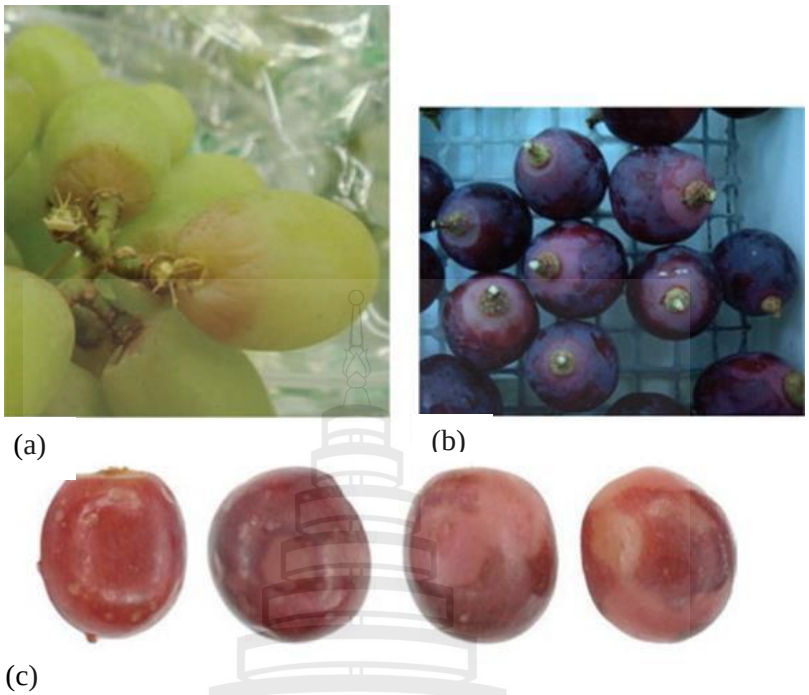
2.4.1.1 Sulfur dioxide treatments

The control of mold infection by chemical fungicides as SO_2 during pre and postharvest time has been documented in grapes (Guerra et al., 2016a; Youssef et al., 2015). The structure of fumigation SO_2-releasing pads used inside grape boxes or in cold storage rooms is presented in Figure 2.5 (Lichter et al., 2002).

Source Postharvest (2018)

Figure 2.5 SO_2- releasing pad

SO_2 pad can control fungal decay caused by *B. cinerea* (Zoffoli & Latorre, 2011). But its use has become limited in many countries because the residue of SO_2 is hazardous to human health (Guerra et al., 2016a; Youssef et al., 2015). Moreover, SO_2 is not considered as GRAS and named a pesticide, and is not allowed to apply on organic grape berries (Romanazzi, Smilanick, Feliziani & Droby, 2016; Xu et al., 2007). Moreover, The sunken syndrome occurs when SO_2 transfer inside of berry by injuries and weak of fruits (Figure 2.6) (Zoffoli & Latorre, 2011).



Source Zoffoli and Latorre (2011)

Figure 2.6 Berry bleaching symptom produced by SO_2 (a) Thompson Seedless (b) Red Globe (c) Red Glob

2.4.1.2 Ethanol dipping

Lichter et al. (2002) reported that dipping grapes in 70% ethanol can eliminate the fungal and bacterial load on the surface, but not for yeast. But *in vitro*, the ethanol concentration of more than 20% can inhibit the growth of *B. cinerea*, which shows more effective role in 40% ethanol.

2.4.2 Biological Methods

Over the past decade, laboratory- scale experiments have been shown the success of biocontrol agents to control the gray mold of table grapes (Romanazzi et al., 2016). *Bacillus subtilis* can inhibit several fungi such as *Aspergillus, Penicillium,* and *B. cinerea* (Jiang et al., 2014). Gabler et al. (2010) reported the synergistic effect of bio-fumigation using *Muscodor albus* and pre- treated ozone or SO_2 potentially inactivated the gray mold in table grapes. They found that the combination of ozone and *M. albus* could control the decay of gray mold significantly than the less effective sulfur dioxide releasing treatments and could be alternatives to organic production. Cordero-Bueso et al. (2017) studied on Wild Grape-associated yeasts as biocontrol agents to control fungi in grape they found that the effective yeasts against *B. cinerea* was isolated from infected grape.

2.4.3 Controlled and Modified Atmosphere Packaging Method

2.4.3.1Controlled and modified atmosphere packaging of grapes

Feliziani et al. (2014) compared the atmosphere of ozone in the day and night-time and analyzed that 0.100 µL/L of ozone in the day and 0.300 µL/L at night reduced gray mold growth during storage. The effectiveness of high CO_2 levels in a controlled atmosphere (CA) can prolong the shelf life of fresh products. Deng, Nu and Li (2006) studied the physiological responses and quality attributes of Kyoho grapes (*Vitis vinifera* x *V. labrusca*) and found that grape storage in 4% O_2, 9% CO_2, and 80% O_2 can extend 60 days shelf-life of grapes under CA storage.

Table 2.2 The study of modified atmosphere and packaging (MAP) with table grape

Grape variety	Composition and packaging	Result	Reference
Organic Scarlotta seedless grape	polyamide and polyethylene, pre- treatments of O_3 (5, 10, 20 μL L^{-1}) or CO_2 (50%, 70%) + MAP 2% O_2-5%CO_2	The O_3 at 20 μL L^{-1} maintain rachis chlorophyll content and skin colour. CO_2 caused stem browning and off-flavours.	(Admane et al., 2018)
Michele Palieri	oriented polypropylene +gas O_2:CO_2:N_2 ratio of 5:3:92, 10:3:87 and 15:3:82	The varies concentration of gas in active MAPs were not significant in prolong shelf-life, because of product	(Costa et al., 2011)
Red Globe	perforated polyethylene (PPE), add or not add SO_2 pads or ethanol vapor sachets	Ethanol vapor enhanced berry color, but occur stem browning and did not reduce fungal decay.	(Candir et al., 2012)
'Red Globe' table grapes	perforated plastic bags+ 7 mm holes every 15 mm of area of bag	MAP storage help to increases rachis postharvest quality by reduce green color loss.	(Silva-Sanzana et al., 2016)
'Superior seedless' table grapes	micro-perforated PP-30 and an oriented PP (OPP)	MAP storage show the overall quality of clusters nearby at harvest time.	(Artés-Hernández, Tomás-Barberán & Artés, 2006)
Flame Seedless, Crimson Seedless	Perforated film, macro-perforated (5-mm hole) or micro-perforated with 1 or 3, 100 μm diameter holes.	Monoterpenes can promote berry Flavor during storage.	(Tyagi et al., 2020)

2.4.3.2 Coating

Edible coating containing chitosan and essential oils was preferred by the researcher to the use of synthetic fungicides. That is environmentally friendly control pathogenic fungi and maintains the quality of grape during storage (Guerra et al., 2016a). (Chen et al., 2019) report that two edible coatings of 1.5% chitosan and 1.0% poly-ε- lysine were SO_2-induced abscission, reduce weight loss, reduce cell-wall-degrading enzyme activities, maintain the fruit quality in 'Kyoho' table grapes during storage. While Castelo Branco Melo et al. (2018) report that the coating of chitosan nanoparticles was reduced the ripening process, weight loss, soluble solids, and reducing sugar and sensory test. So, chitosan nanoparticles can be maintaining the quality of grapes after storage. The gelatin hydrogels were added with curcumin-loaded zein nanoparticles or calcium propionate and applied to Benitaka grapes. The result shows that the edible coating did not affect the texture, but color increased when curcumin was applied (Lemes et al., 2017).

Xiao et al. (2020) reported a novel nanocomposite poly (ethylene- *co*-vinyl acetate) (EVA) film with controlled release of iprodione (ID) under *in vitro* conditions. Chitosan (CS) used was reinforced onto EVA to improve the water and oxygen permeability of films. ID loaded poly (ethylene glycol) - poly (ε- caprolactone) (PEG-PCL) (IPP) micelles were used as the drug carriers to exhibit antifungal and controlled release properties. After loading of IPP micelles on films improved antifungal ability and temperature- sensitive drug release behavior in grapes during pre-harvest spraying.

2.4.3.3 Essential oils

Essential oils are biologically active solution (Servili, Feliziani & Romanazzi, 2017). The application of essential oils was a new way to control the postharvest pathology of fruits. It is consumer prefer, organic solution, environment protection (Vitoratos, Bilalis, Karkanis & Efthimiadou, 2013) and are natural substances classified as GRAS by the US food and Drug Administration and some of them shown antioxidant/antifungal properties that makes them interested for application in the food industry (Atarés & Chiralt, 2016b; Mateo et al., 2017).

2.5 Applications of Essential Oil as Inhibited Diseases on Grape

Essential oil (EOs) and their components are becoming extensively applied due to the presence of volatile natural bioactive compounds and safe for consumption and are considered GRAS. EOs are eco- friendly and are nontoxic, may act as biopesticides to control pests and disease for alleviating food- borne pathogenicity. The volatility of EOs allows their application as biofumigant to protect stored fruits during postharvest storage (Nguyen, Joly & Dantigny, 2016). EOs are natural products obtained from plant materials such as flowers buds, leaves, stems, bark, and seeds (Ribeiro-Santos et al., 2017).

Several researchers reported that SO_2 application on table grapes and fruits is not allowed because it is harmful to human health. So, biological control could be used as an alternative to control postharvest diseases fungi in table grapes (Meng, Qin & Tian, 2010). Essential oil- incorporating coating or films has been seen to reduce microbial diversity in fresh fruits (Oh et al., 2017). However, consumer interest in organic food and biologically control methods are interest in ensuring health benefits and ecofriendly decomposition of waste packages (Godana et al., 2020). The different uses of aromatic plant- based EOs have been applied in fruit coatings and packaging are presented in Table 2.3 and 2.4.

Table 2.3 Application of different essential oils against fungal growth in fruits

Essential oils	Oil concentration	Fruit	Fungal pathogen	Reference
Thyme	50-200 ppm	Strawberry	*Botrytis cinerea* *Rhizopus stolonifer*	(Bhaskara Reddy et al., 1998)
Thyme	0.2 µl/ml	Guava	*Botrytis cinerea*	(Abdel-Rahim & Abo- Elyousr, 2017)
Clove Mustard	92.56 µL/L_{air} 15.42 µL/L_{air}	Strawberry	*Botrytis cinerea*	(Aguilar-González et al., 2015b)
Peppermint	5 µL/mL	Cherry tomato	*Aspergillus niger* *Botrytis cinerea* *Penicillium expansum* *Rhizopus stolonifer*	(Guerra et al., 2016b)
Rosemary Peppermint	Pure essential oil, 24 hr exposure	Table grapes	*Botrytis cinerea*	(Servili et al., 2017)
Tea tree	0.9 g/L	Strawberry	*Botrytis cinerea* *Rhizopus stolonifer*	(Shao, Wang, Xu & Cheng, 2013)
Thyme Cumin	200 µL/L High concentration	Strawberry	*Botrytis cinerea*	(Zamani-Zadeh, Soleimanian-Zad, Sheikh-Zeinoddin & Goli, 2014)
Origanum Lavender Rosemary	0.2 µg/mL	Tomato	*Botrytis cinerea*	(Soylu, Kurt & Soylu, 2010)
Thyme (4 species)	500-2000 µl L^{-1}	Citrus	*Penicillium digitatum* *Penicillium italicum,Geotrichum citri-aurantii*	(Boubaker et al., 2016)
Clove	0.25-0.50 µl/ml	Navel oranges	*Penicillium digitatum*	(He et al., 2016)

Table 2.3 (continued)

Essential oils	Oil concentration	Fruit	Fungal pathogen	Reference
Cinnamon	Pure essential oil	Citrus	Blue mold Green mold	(Kouassi, Bajji & Jijakli, 2012)
Carvacrol Thymol	Pure essential oil	Lemon	*Penicillium digitatum* *Penicillium italicum*	(Pérez-Alfonso et al., 2012)
Thyme Mexican lime	0.1% 0.5%	Papaya	*Colletotrichum gloeosporioides* *Rhizopus stolonifer*	(Bosquez-Molina, Jesús, Bautista-Baños, Verde-Calvo & Morales-López, 2010)
Citrus	>2%	Date	*Aspergillus flavus*	(Aloui et al., 2014)
Summer savory Satureja hortensis L.	25, 12.5, and 6.25 µl/ml	Lemon	*Aspergillus flavus*	(Dikbas, Kotan, Dadasoglu & Sahin, 2008)
Perillaldehyde	0.25-1 µl/ml	Grape	*Aspergillus niger*	(Tian et al., 2015)

Table 2.4 Inhibition effect of essential oils in combination with coating materials on the microbial load of diverse fruits

Edible coating	Essential oil	Fruit	icMicrobial growth inhibition	Reference
5%Cassava Starch .5% chitosan	1.25% Myria ovate Cambessedes	angaba fruits	*B. cinerea* *B. subtilis* *Seratia marcescens*	(Frazão, Blank & de Aquino Santana, 2017)
cyclodextrin	Cinnamon Oregano	-	*Botrytis* sp.	(Munhuweyi, Caleb, van Reenen & Opara, 2018)
ginate-based	0.3-0.5%w/v Lemongrass	Fresh-cut pineapple	Bacteria	(Azarakhsh, Osman, Ghazali, Tan & Mohd Adzahan, 2014)
protein lignin blend film	3% w/w Citronella	Banana	*Fusarium oxysporum*	(Arancibia, López-Caballero, Gómez-Guillén & Montero, 2014)
llulose acetate	Oregano +oreganophillic montmorillonite clay	-	*Alternaria alternata* *Geotrichum condidum* *Rhizopus stolonifer*	(Pola et al., 2016)
in film	10% *Zataria multiflora* Boiss.	-	*Listeria monocytogenes* *Escherichia coli*	(Kashiri et al., 2017)
tosan- carboxymethyl cellulose films	25,50,75 µl cinnamon and ginger	-	*Aspergillus niger*	(Noshirvani et al., 2017)
itosan film	Basil Thyme	-	*Aspergillus niger* *Botrytis cinerea* *Rhizopus stolonifer*	(Perdones, Chiralt & Vargas, 2016)

2.6 Modified Atmosphere Packaging (MAP) of Grapes

Modified atmosphere packaging (MAP) has been widely used to control decay and quality changes in table grapes. MAP uses a semi-permeable film to manage the atmosphere inside the package. It was reported that MAP filled with 15% O_2 and 10%

CO_2 was a potential alternative to sulfide fumigation in table grapes for quality preservation (Admane et al., 2018). Water vapor, oxygen, and carbon dioxide can be exchanged between internal or external environments through the polymer film. The suitable barrier properties of the film were important to maintain the quality of table grapes. Polyethylene and polypropylene film with or without perforated surfaces were used to maintain passive modified atmosphere conditions (Costa et al., 2011). The recent findings from the literature related to the application of MAP with different gas composition use for prolonging the shelf life of table grapes have been given in Table 2.5.

Table 2.5 Impact and application of packaging materials and the employed technologies for prolonging the shelf life of table grape

Packaging	Method	Treatment	Reference
Perforated polyethylene (PPE)	Modified atmosphere packaging	Ethanol vapor generating (Sachets and pad)	(Candir et al., 2012)
Oriented polypropylene	Passive and active modified atmosphere packaging	difference thickness bag (20, 40, and 80 µm)	(Costa et al., 2011)
Perforated polyethylene (PPE)	Modified atmosphere packaging	Ethanol vapor generating (Sachets and pad)	(Ustun et al., 2012)
Conventional polyethylene (PE)	Modified atmosphere packaging	Active slow-release pasteurizing packaging	(Zhang, Li, Xu & Fu, 2014)

2.7 The Antimicrobial Packaging

Fruits and vegetables vary in their respiration rates, ethylene production and sensitivity to the release of ethylene, storage temperature, and relative humidity that may require a potential packaging material for the shelf- life extension. Apart from MAP, active packaging technologies such as ethylene scavengers/ absorbers, antimicrobial packaging, and moisture absorbers along with cold storage can be applied to fruits and vegetables to increase their shelf life (Ayhan, 2019). Antimicrobial packaging materials of different compositions containing bioactive compounds have been studied extensively and applied commercially. The various forms of antimicrobial sachets that have been tested in different materials show controlled release of bioactive compounds employed as edible coatings, and in sachet form placed in MAP of fruits and vegetables (Jung & Zhao, 2016).

Durability and long- lasting effect of novel packaging are important aspects for preserving the quality of fruits and vegetables. Traditional packaging is

ineffective and not sufficient in comparison with the novel packaging material. The concept of active packaging involves scavenging or absorption of undesirable compounds such as oxygen, carbon dioxide, ethylene, flavor/ odor, excessive water to prevent fungal or bacterial growth and to ensure the safety and biochemical quality of packaged fruits and vegetables for a longer period in the supermarkets (Bodbodak & Rafiee, 2016). The active packaging use for ready-to-eat food if directly placed on the food may contaminate the food surface, subsequently affecting the sensorial quality of the product such as color, taste, and texture. Antimicrobial packaging with no direct contact with the food surface has been developed to prevent the microbial deterioration of fresh fruits during postharvest storage (da Costa et al., 2020). Antimicrobial packaging material incorporated with organic, inorganic, and other antimicrobial agents has been proposed. However, the antimicrobial active packaging fortified with natural and non- toxic additives has been postulated to be potential antifungal materials and to ensure the safety of fruits during postharvest cold storage (Yang, Zheng, Liu, Kong & Wang, 2020). Therefore, antimicrobial packaging from the natural source has been implemented in different wrappings to control the growth of pathogenic fungi in fruits (Silveira et al., 2020).

Several studies have reported the application of metal nanoparticles and edible coatings in biodegradable packaging for promising results in fresh produce. It was noted that the application of bio- nanocomposite films and edible coatings supplemented with metal nanoparticles exhibited a notable effect on the keeping quality of tropical fruits and vegetables, classified as climacteric or non-climacteric commodities. Moreover, it was also reported that eco- friendly edible coatings and bio- nanocomposite film packaging employed in fruits and vegetables decelerated the changes in color, rate of respiration, weight loss, and delaying ripening thereby extended the postharvest shelf- life of fruits and vegetables including grapes (Jafarzadeh et al., 2021).

Table 2.6 Active packaging fortified with essential oils for delaying quality changes or inhibition of microbial growth in fruits and vegetables

Packaging	Medium+ Essential oil	Crop Produce	Delay/ Inhibition	Reference
Active papers	solid and emulsion paraffin with cinnamon essential oil	mushroom	Browning	(Echegoyen & Nerín, 2015)
Active paper	*Zataria multiflora* + *Cuminum cyminum European cumin*		*Staphylococcus aureua Listeria innocua Pseudomonas* sp. *Salmonella enterica* subsp. *enterica Escherichia coli*	(Akrami et al., 2015)
Paper coating	4 and 6% w/w Zataria		*Staphylococcus aureus Listeria innocua Pseudomonas sp. Salmonella enterica* subsp. *enterica*	(Akrami et al., 2015)
Active paper	Paraffin coating+clove, cinnamon, and oregano		Candida albicans Aspergillus flavus Eurotium repens Pennicilium nalgiovense Pennicilium roqueforti Salmonella cholerasius Escherichia coli	(Rodríguez, Batlle & Nerín, 2007)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials and Chemicals

Chemicals and microbial media used in the experiments were of analytical grade. Dimethyl sulfoxide (DMSO) (RCI Labscan Limited, Thailand), Tween-80, potato dextrose agar (PDA), and halloysite nano clay were purchased from Sigma Chemical Co., St. Louis, USA. All the commercial-grade essential oils (EOs) including lemongrass, spearmint, ginger, bergamot, lemon, peppermint, thyme, cinnamon, and clove were obtained from JAGAT Aroma oils distillery, Namsiang company Ltd. Bangkok, Thailand. Polyvinyl alcohol (PVA) and oriented polypropylene (OPP) films were bought from Loba Chemie PVT. Ltd, India. Sterile absorbent food-grade pads (25 mL capacity) were purchased from Dry Square Co., Ltd, Thailand. The fungal culture typically *Aspergillus* sp. was identified species of *Aspergillus* sp. by the Thailand Bioresource Research Center (TBRC).

3.2 Effect of Different Levels of EOs on *Aspergillus* sp.

The different kinds of EOs from clove, cinnamon, thyme, peppermint, lemon, bergamot, ginger, spearmint, and lemongrass were screened against *Aspergillus* sp. All the EOs were preprepared in dimethyl sulfoxide (DMSO) at various levels of 0.5, 1, 2, and 5% (v/v), respectively. The various levels of EOs were subjected to *in vitro* microbial analyses.

3.2.1 Radial Growth and Disk Diffusion Method

Different levels of EOs (0.5, 1, 2, and 5; v/v) were prepared and 100 µL of each level of EOs were mixed with 20 mL of sterile potato dextrose agar (PDA). The mixtures of EOs and PDA were allowed to solidify on the Petri dishes. A hole of 3 mm in diameter was made using a sterile cork-borer at the center of each petri dish. The plate of *Aspergillus* sp. of 14 days' mycelium was used for suspension preparation. Ten microliters of *Aspergillus* sp. spore suspension (10^6 spores/mL) were dropped on the circular spot of Petri dishes with different levels of EOs. Control was prepared by the addition of 10 µL of sterile distilled water instead of EOs on the spot of the inoculated Petri dish. Inoculated Petri dishes were sealed using paraffin film to avoid contamination. The plates were incubated for 7 days at 25±2 °C and the radial growth was measured from the developed mycelium on the plate (Vitoratos et al., 2013). Disk diffusion was analyzed by placing a 6 mm sterile paper disk at the center of the inoculated plate with *Aspergillus* sp. the suspension (100 µL). Diluted concentrations of EOs (10 µL) were dropped on paper disks and incubated. The inhibition zones of different levels of EOs were measured based on the growth of mycelium (Jakowienko, WÓJcik- StopczyŃSka & Jadczak, 2011).

3.2.2 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The plate of *Aspergillus* sp. of 14 days' mycelium was used for suspension preparation. Spore suspension of *Aspergillus* sp. was prepared in potato dextrose broth (PDB) by serial dilution (10^5 spores/mL). The 10 µL was added from all the EO samples in the wells of a sterile microplate. Microplates were incubated at 25±2 °C for 24 h. Absorbance was taken using a microplate reader for the determination of MIC of EOs (Tayel Moussa, Salem, Mazrou & El-Tras, 2016). MFC was analyzed by spreading 100 µL from the selected wells of MIC microplate on the PDA plate. All the plates were incubated at 25±2 °C for 48 h to determine MFC. The MFC spreads all of the incubated fungi in the MIC part on PDA plates and incubated at 25±2 °C for 72 hr before checking the growth of fungi. (Tayel et al., 2016).

3.2.3 Procurement and Preparation of Table Grapes Samples

Fully ripened table grapes (Vitis vinifera cv. Beauty seedless) were harvested from PB Valley Chiang Rai orchard located in the north of Thailand. Good Agricultural Practices (GAP) were followed until the harvesting time. Freshly harvested grapes ranged 14-18 ^{o}Brix in total soluble solids (TSS), certified by the Department of Agriculture, Thailand. Fresh table grapes were transported to the Postharvest Technology and Packaging Laboratory, Mae Fah Luang University, Chiang Rai, Thailand. Grapes were selected based on uniform size, color, appearance, and absence of mechanical bruises (Xu et al., 2007). After sampling, grapes were immersed in 200 ppm sodium hypochlorite for 2 minutes to remove the extraneous material and microbial contamination. Washed table grapes were dried in a biosafety cabinet to avoid cross-contamination until used.

EOs at 0.1% level were analyzed for order intensity in which 10 trained panelists, aged between 25-32 years were recruited from the Department of Postharvest Technology, Mae Fah Luang University, Chiang Rai, Thailand. EOs (1 mL) at 0.1% level were poured in all the sealable cups followed by the addition of a table grapes sample (10 berries, 50 g). All the cups were sealed airtight and incubated at 13 °C for 24 h. After incubation, all the sample cups were kept at 25±2 °C for 2 h before presenting to the panelists. The panelists were asked to open the sealed cups and sniff the headspace to determine the odor intensity of grape berries (Fan, 2004). All the grape berry samples treated with different EOs were scored as level 0-4; 0 = none, 1 = mild odor/ highly acceptable, 2 = moderated odor/ high acceptable, 3 = strong odor/ unacceptable, and 4 = extremely strong odor/ extremely unacceptable (Sae-Leaw, Benjakul & O'Brien, 2016).

3.3 Preparation of Antifungal Active Packaging for Quality Preservation of Table Grapes

Clove essential oil (1% CEO) was selected and supplemented as an active antifungal ingredient into different packaging materials: (1) 25 mL of 1% CEO injected into sterile absorbent pads, (2) 1% CEO fortified into PVA film. (3) PVA film with

1% CEO added with 1% halloysite nanoclay. For PVA film preparation, polyvinyl alcohol (PVA) 7% w/v) was dissolved in distilled water and heated on a magnetic stirrer set at 98±2 °C to attain a clear solution and cooled down at room temperature. Then, 20% tween 40 and 1% CEO with and without 1% halloysite nanoclay were mixed into the PVA solution. PVA film-forming solutions were cast on the orientated polypropylene film (OPP) and then dried at room temperature 25 °C for 24 h.

Washed table grapes samples (300±20 g/treatment) free of contamination were packed in polypropylene bags (PP bag) with 6-8 holes of 1 cm diameter and placed in a corrugated box. Different treatments of table grapes were prepared i.e., control (without any treatment), CEO1-Pad (absorbent pad injected with 25 mL of 1% CEO), COM-SO_2-Pad (commercial pad sodium metabisulfite 98%), CEO1-HC-Film (7% PVA film fortified with 1% CEO and 1% halloysite clay), and CEO1-Film (7% PVA film fortified with 1% CEO). All the pad and film treatments were placed on the grape samples packaged in PP bags and sealed in the corrugated boxes and stored at 13 °C until analysis. The treatments were prepared 6 replications per treatment (n=6) up to 30 days of storage.

3.4 Effect of Antifungal Active Packaging on Postharvest Quality Losses of Table Grapes

3.4.1 Disease Severity and Grape Berry Drop

Disease severity was analyzed following the 6-point empirical scale (0 = 0% fruit surface infected; 1 = 1-20% fruit surface infected; 2 = 21-40% fruit surface infected; 3 = 41-60% fruit surface infected; 4 = 61-80% fruit surface infected; 5 = 81% or more surface of fruit infected and showing sporulation) (El-Garhy et al., 2020; Santos et al., 2017). The fruit drop of table grapes was checked and recorded every 3 days of storage for 30 days (Santos et al., 2017).

3.4.2 Weight Loss (%)

The weight loss during postharvest storage was determined in various replications (n=6) every 3 days interval up to 30 days of storage (Youssef et al., 2015).

$$\textit{Weight loss } (\%) = \left[\frac{(Mi - Ms)}{Mi}\right] \times 100$$

Where *Mi* and *Ms* are the initial and final weight of the samples, respectively.

3.4.3 Acceptance Score

Fifty untrained panelists comprising of 27 males and 23 females aged 25–32 years were recruited from the School of Agro- Industry, Mae Fah Luang University, Chiang Rai, Thailand. All the panelists were asked to evaluate the appearance, firmness, odor, and overall acceptability of table grapes samples without and with active antifungal packaging material, using a 9- point hedonic scale at day 0 and day 21 of storage at 13 °C (Amaral et al., 2018; Shiekh, Zhou & Benjakul, 2021).

3.4.4 Statistical Analysis

Analysis of variance (ANOVA) and Duncan's multiple range test was performed using a statistical program, SPSS v. 10.0. Samples at a level of significance ($p<0.05$) for all the parameters were analyzed.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Impact of EOs at Different Levels on the Radial Growth, Disk Diffusion Method, MIC, and MFC of *Aspergillus* sp.

The efficacy of different levels of EOs in the inhibition of fungal growth displayed on the PDA plates inoculated with *Aspergillus* sp. is shown presented in Table 1. Radial growth in the control (without any treatment) was higher than in the plates treated with different levels of EOs ($p<0.05$). Inoculated plates treated with EOs at various levels of 0.5, 1, 2, and 5% (v/v), showed decreases in the radial growth of *Aspergillus* sp. compared to the control sample ($p<0.05$). Additionally, results of radial growth attained in lemongrass, thyme, cinnamon, and clove EOs at different levels were lower than the control and other EOs (Table 4.1). However, the CEO revealed the lowest radial growth of fungal mycelium in comparison to the control and other treated plates ($p<0.05$). A combination of EOs from clove and mustard at different concentrations of 11.57 $\mu L/L_{air}$ and 1.93 $\mu L/L_{air}$ was reported to inhibit *Botrytis cinerea* under *in vitro* conditions isolated from strawberries, respectively (Aguilar-González et al., 2015a). The values of inhibition zone of *Aspergillus* sp. PDA plates treated with different levels of EOs via disk diffusion method showed retarded growth measured as the diameter of the clear spots or zone of inhibition measured by a ruler the unit in centimeter (Table 4.2). Inhibition zones in the control sample shown no clear zone. The slight increment in the inhibition zones was marked in a dose- dependent manner, among all the EOs than the control ($p<0.05$). Additionally, the diameter of the inhibition zone was notably higher in CEO for all the tested levels compared to the control and other EO treated fungal plates ($p<0.05$). Moreover, the presence of active displayed on

the *Aspergillus* sp**.** inoculated PDA plates**.** The results were in line with the inhibition zone of fungal Aspergillus sp**.** obtained by the treatment of oregano and clove EOs (Puškárová, Bučková, Kraková, Pangallo & Kozics, 2017).

The in vitro MIC and MFC values of different levels of EOs attained in the microplate wells inoculated with serially diluted *Aspergillus* sp. culture is presented in Table 4.3. Lower values of MIC were obtained in lemongrass, thyme, and clove EOs than those in the other EOs ($p<0.05$). The MIC values of the aforementioned tested EOs could inhibit the growth of mycelium formation in a dose- dependent pattern by the different percent levels of EOs. However, the MIC values were lowest in CEO compared to the EOs ($p<0.05$). Similarly, MFC values of EOs attained in a dose-dependent manner were half of the concentrations of MIC. Moreover, the decreases in MFC values in CEO depicted that the least concentration of CEO was employed in the effective inhibition of *Aspergillus* sp. in comparison to the CEO MIC values. Puškárová et al. (2017) studied the antimicrobial effect of different EOs from oregano, clove, thyme, lavender, clary sage, and arborvitae at various concentrations of 75, 50, 25, 10, and 5% (w/v) against *Alternaria alternata* and *Aspergillus fumigatus*. The mycelial growth of tested fungal strains was retarded by oregano and clove, having MICs (0.01%, 0.025%) and MFCs (0.025%, 0.05%), respectively (Puškárová et al., 2017). A comparative study of thyme, oregano, and lemon EOs reported that the *Botrytis cinerea*, *Penicillium italicum*, and *Penicillium digitatum* were inhibited by thyme EO more effectively (Vitoratos et al., 2013). Additionally, EO from cloves during in vitro vapor phase with MIC value of 92. 56 µL/ Lair was documented to inhibit the mold growth triggered by *B. cinerea* (Aguilar- González et al., 2015a). As a matter of fact that EOs containing volatile polyphenolic compounds such as terpenes, phenolics, aldehydes, and alcohols that exhibit antimicrobial or fungicidal activity, might alter or inhibit the growth mechanism of the food born pathogenic fungal species and plant pathogenic fungi (de Aguiar et al., 2018).

Table 4.1 Effect of different levels of EOs on radial growth of *Aspergillus* sp.

EOs	Radial growth (cm)			
	0.5%	**1%**	**2%**	**5%**
Control	7.7±0.4^{a}	8.1±0.6^{a}	8.9±0.6^{a}	9.1±0.3^{a}
Lemongrass	6.7±0.7^{b}	6.1±0.9^{b}	5.5±0.8^{c}	5.4±0.7^{c}
Spearmint	7.8±0.2^{a}	6.2±0.4^{b}	6.3±0.8^{bc}	6.2±0.6^{b}
Ginger	6.9±0.8^{ab}	5.6±0.9^{bc}	6.1±0.8^{bc}	5.7±0.9^{c}
Bergamot	6.3±0.9^{b}	6.9±0.6^{b}	5.7±0.7^{c}	5.4±0.8^{c}
Lemon	7.5±0.7^{a}	7.7±0.5^{a}	7.5±0.5^{b}	6.9±0.7^{b}
Peppermint	7.6±0.4^{a}	7.7±0.9^{a}	7.4±0.8^{b}	6.7±0.7^{b}
Thyme	6.0±0.8^{b}	5.6±0.9^{bc}	4.1±0.3^{d}	3.1±0.8^{d}
Cinnamon	6.7±0.3^{b}	5.3±0.8^{bc}	4.1±0.6^{d}	3.3±0.6^{d}
Clove	5.2±0.8^{c}	4.7±0.7^{c}	2.5±0.4^{e}	1.3±0.3^{e}

Note Values are mean ± standard deviation (n = 6). Different superscripts within the same column followed by the different letters (a–e) indicate a significant difference ($p<0.05$). Concentrations of different EOs were applied in µg/ 100 µl.

Table 4.2 Effect of different levels of EOs on inhibition zone of *Aspergillus* sp.

EOs	Inhibition zone (cm)			
	0.5%	**1%**	**2%**	**5%**
Control	-	-	-	-
Lemongrass	0.4 ± 0.02^{b}	0.8 ± 0.04^{b}	1.3 ± 0.07^{b}	1.4 ± 0.2^{b}
Spearmint	0.5 ± 0.01^{b}	0.8 ± 0.03^{b}	0.8 ± 0.02^{c}	0.9 ± 0.04^{c}
Ginger	0.5 ± 0.03^{b}	0.6 ± 0.03^{b}	0.7 ± 0.09^{c}	0.8 ± 0.02^{c}
Bergamot	0.5 ± 0.01^{b}	0.7 ± 0.02^{b}	0.7 ± 0.02^{c}	0.9 ± 0.05^{c}
Lemon	0.4 ± 0.02^{b}	0.6 ± 0.01^{b}	0.6 ± 0.01^{c}	0.6 ± 0.01^{c}
Peppermint	0.3 ± 0.01^{b}	0.7 ± 0.02^{b}	0.8 ± 0.01^{c}	0.8 ± 0.02^{c}
Thyme	0.4 ± 0.04^{b}	0.7 ± 0.04^{b}	1.4 ± 0.06^{b}	1.6 ± 0.03^{b}
Cinnamon	0.5 ± 0.03^{b}	0.8 ± 0.05^{b}	1.1 ± 0.01^{b}	1.5 ± 0.08^{b}
Clove	0.9 ± 0.05^{ab}	1.5 ± 0.04^{a}	1.9 ± 0.05^{a}	2.9 ± 0.09^{a}

Note Values are mean ± standard deviation (n = 6). Different superscripts within the same column followed by the different letters (a–e) indicate a significant difference ($p<0.05$). Concentrations of different EOs were applied in µg/ 100 µl.

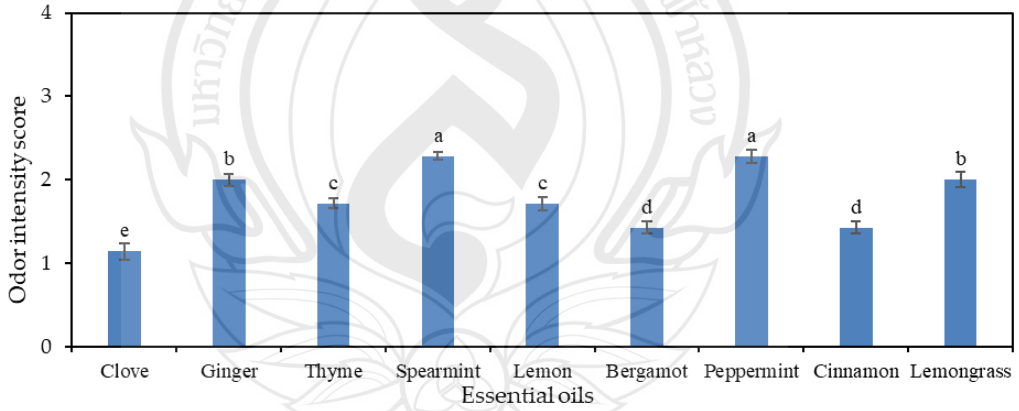
Table 4.3 Antifungal properties of EOs on *Aspergillus* sp. treated without and with different levels of EOs

EOs	Level of EOs							
	0.5%		1%		2%		5%	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Lemongrass	25^{b}	13^{b}	25^{c}	13^{c}	13^{c}	6^{c}	6^{d}	3^{d}
Spearmint	100^{a}	50^{a}	50^{b}	25^{b}	25^{b}	13^{b}	25^{b}	13^{b}
Ginger	100^{a}	50^{a}	100^{a}	50^{a}	50^{a}	25^{a}	50^{a}	25^{a}
Bergamot	100^{a}	50^{a}	100^{a}	50^{a}	50^{a}	25^{a}	25^{b}	13^{b}
Lemon	100^{a}	50^{a}	100^{a}	50^{a}	50^{a}	25^{a}	50^{a}	25^{a}
Peppermint	100^{a}	50^{a}	50^{b}	25^{b}	13^{c}	6^{c}	13^{c}	6^{c}
Thyme	$25b^{b}$	13^{b}	25^{c}	13^{c}	6^{d}	3^{d}	6^{d}	3^{d}
Cinnamon	100^{a}	50^{a}	25^{c}	13^{c}	6^{d}	3^{d}	6^{d}	3^{d}
Clove	25^{b}	13^{b}	13^{d}	6^{d}	6^{d}	3^{d}	3^{e}	2^{e}

Note Values are mean ± standard deviation (n = 6). Different superscripts within the same column followed by the different letters (a– e) indicate a significant difference ($p<0.05$). EOs: Essential oils; MIC: Minimum inhibitory concentration; MFC: Minimum Fungicidal concentration. MIC and MFC values presented the concentration of EOs in µg/ 100 µl.

4.2 Evaluation Odor Intensity of EOs in Table Grapes

The odor intensity scores of different EOs at 1% level in table grapes are shown in Figure 1. Desirable odor scores were given to the table grapes treated with clove, bergamot, cinnamon, lemon, and thyme EOs, sniffed by the trained panelists on the headspace of cups than the other samples ($p<0.05$). The highest scores attained in spearmint and peppermint EOs indicated a strong undesirable odor in table grapes**.** Conversely, the lowest order intensity score obtained in 1**%** CEO treated table grapes was referred to as the most desirable odor amongst all the EOs ($p<0.05$). This was a more likely feasible treatment selected for the preparation of antifungal packaging material without compromising on the sensorial properties of table grapes**.** From the previous studies, the CEO reported being rich in two major flavor compounds such as eugenol and eugenol acetate that constitutes 86% of the analyzed compounds by GC-MS in CEO (Rana, Rana & Rajak, 2011; Rodríguez, Batlle & Nerín, 2018).



Note Values are mean ± standard deviation (n = 10). Different lowercase letters on the bars indicate a significant difference ($p<0.05$)

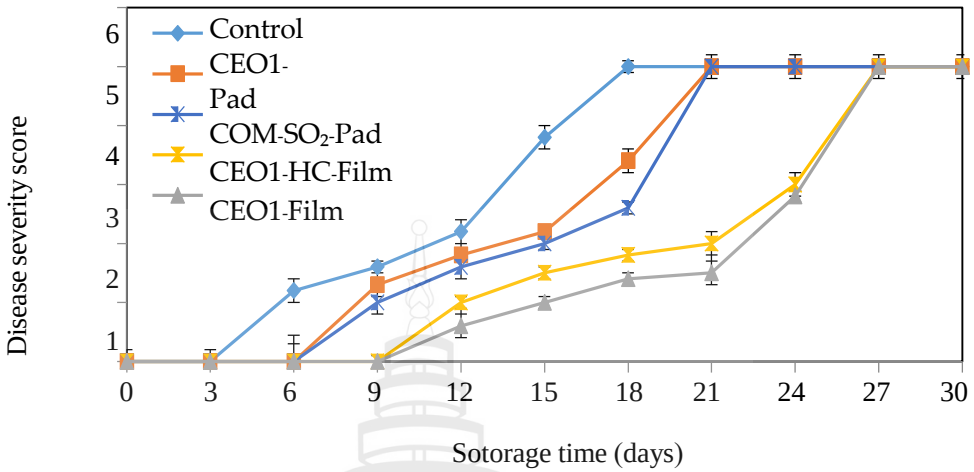
Figure 4.1 Screening of different essential oils (EOs) based on order intensity score in table grapes

4.3 Effect of Antifungal Packaging Materials Supplemented with CEO on Quality Changes of Table Grapes During Storage

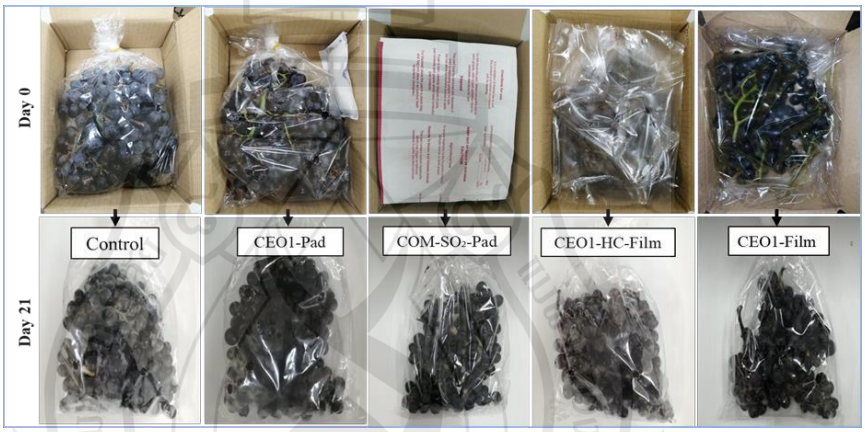
4.3.1 Disease Severity

The growth of fungal mycelium monitored by disease severity on table grapes without and with CEO packaging treatments are given in Figure 7A**.** Fungal growth was triggered in the control at day 3, while as in the CEO1-Pad sample, mycelial growth was initiated from day 6 of storage at 13 °C ($p < 0.05$). Simultaneously, the fungal growth in the COM-SO_2-Pad sample was more likely coincidental with the CEO1-Pad sample and marked a visible difference than the table grapes samples wrapped with active antifungal films fortified with 1% CEO ($p<0.05$). CEO1-HC-Film and CEO1-film samples exhibited slight fungal growth from day 9, compared to the control and pad treatments ($p<0.05$). However, the least severity of disease was visualized in the CEO1-Film sample, compared to the rest of the untreated and treated table grapes during the storage of 21 days at 13 °C. Although commercial SO_2 pads generated fumes that could aid in the decrease of mold growth to some degree in table grapes but has been reported with several constraints**.** SO_2 fumigation in seedless table grapes has been documented with the damage of berries detected by hairline cracks, bleaching, and early browning of the rachis (Blanckenberg, Opara & Fawole, 2021; Zoffoli et al., 2008). Nonetheless, the CEO1-film sample was the most effective antifungal packaging material that might release the volatile fungicidal components of CEO due to the large surface area of film**.** Furthermore, the volatile CEO components could reside in the voids between the berries of table grapes to prevent cluster growth of fungal mycelium**.** Photographs of the control and treated table grapes samples for visualization of fungal growth are presented in Figure 4.2B. Thus, the CEO contains major components of antifungal agents that are eugenol and eugenol acetate that constitutes 86% of the analyzed compounds by GC-MS (Rana et al., 2011; Rodríguez et al., 2018). That has been employed in different techniques of packaging for the prevention of table grapes decay (Sangsuwan & Sutthasupa, 2019; Servili et al., 2017).

A



B



Note Values are mean ± standard deviation (n = 10). table grapes: Table grape; CEO: Clove essential oil. Control: table grapes without any treatment; CEO1- Pad: table grapes with 1% CEO Pad; COM-SO2-Pad: table grapes with commercial sulfur dioxide pad; CEO1-HC-Film: table grapes wrapped with a film composed of 1% CEO and halloysite clay; CEO1-Film: table grapes wrapped with 1% CEO film

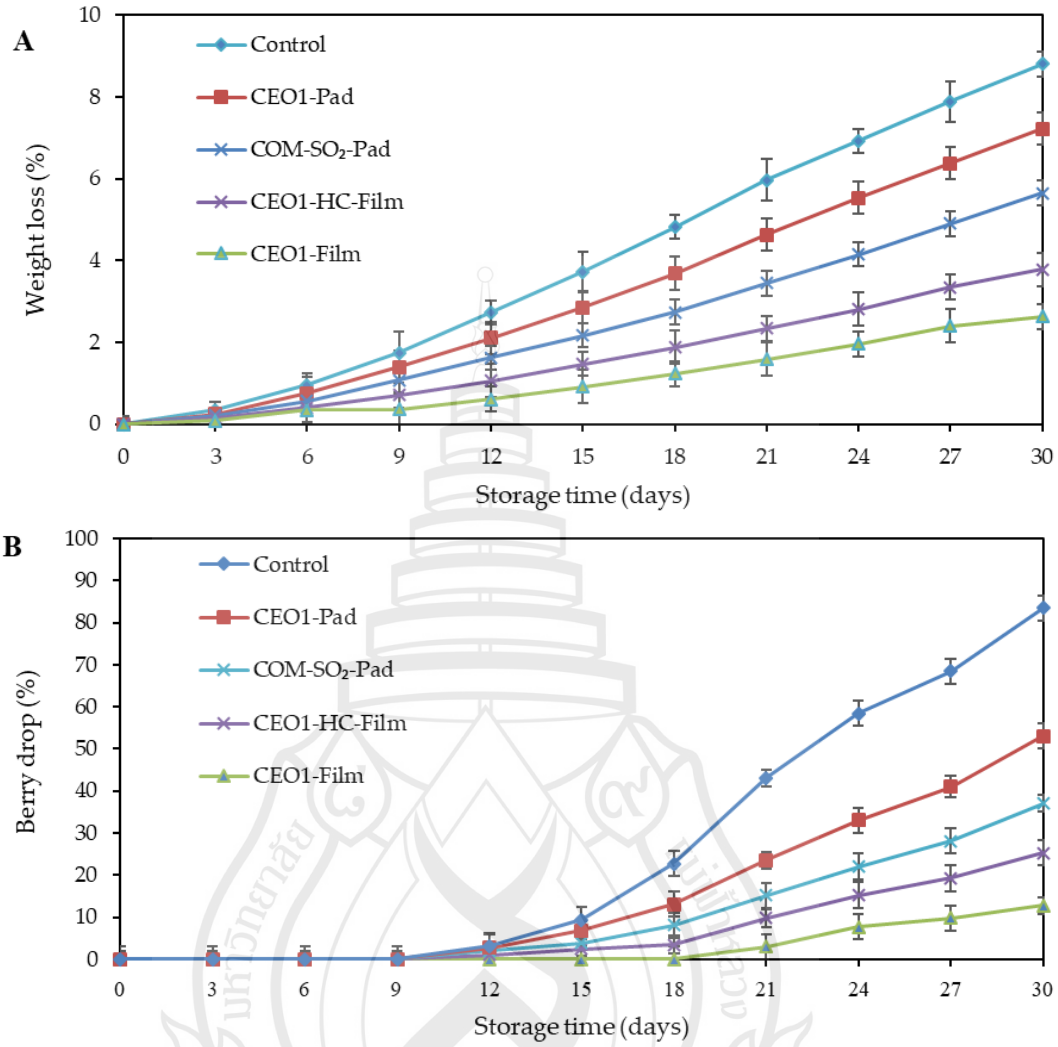
Figure 4. 2 Disease severity (A) and table grapes without and with CEO antifungal packaging (B)

4.3.2 Weight Loss

The percent weight loss of table grapes treated with different packaging materials fortified 1% CEO is presented in Figure 4.3A. Within the first 6 days of storage, all the samples displayed fresh-like characteristics without any marked loss in weight ($p<0.05$). As the storage proceeded, control (without any treatment, packaged in PP bags) showed the highest weight loss compared to the treated table grapes samples during 30 days of storage ($p<0.05$). The ascending order of weight loss was observed in CEO1-Pad > COM-SO_2-Pad > CEO1-HC-Film > CEO1-Film samples, other than the control sample ($p<0.05$). Although the COM-SO_2-Pad sample showed reduced weight loss in comparison with the control and CEO1-Pad sample ($p<0.05$). Nevertheless, the lowest weight loss values were measured in the CEO1-Film sample. The reports of increases in percent weight loss were correlated with the fungal and mold decay of table grapes (Xu et al., 2007).

4.3.3 Berry Drop

The percent berry drop during cold storage of control and treated samples of table grapes are given in Figure 4.3B. Berry drop-in control and treated samples were evidenced from day 15 of storage ($p<0.05$), accept CEO1-Film sample in which slight increment was observed at day 18**.** Control ranked highest in the berry drop followed by descending order of CEO1-Pad, COM-SO_2-Pad, and CEO1-HC-Film samples in comparison with the lower berry drop of CEO1-Film sample at 21 days of storage at 13°C ($p<0.05$). The findings of berry drop in CEO1-Film sample were in line with the disease severity and weight loss, respectively (Figure 4.2A and 4.3A). Additionally, the detachment of berries might be also related to the higher fungal disease severity causing interference in the abscission zones connecting the beery and pedicel part of the grape rachis**.** Therefore, the main mechanism of berry detachment during storage could be correlated with the berry-pedicel indentation and the gradual extension of phloem vessels and pith abscission layer creating intercellular cavities, leading to berry drop (Deng et al., 2007).



Note Values are mean ± standard deviation (n = 6)

Figure 4.3 Weight loss (A) and berry drop (B) of table grapes without and with CEO antifungal packaging

4.3.4 Sensory Acceptance

Table grapes samples without and with CEO packaging treatments were given sensory scores based on appearance, color, odor, and overall acceptability (Table 4.4). On day 0 of storage, untrained panelists (n=50) were employed to assess acceptance scores for control, CEO1-Pad, COM-SO_2-Pad, CEO1-HC-Film, and CEO1-Film samples. The control and treated samples exhibited the highest score on the first day of cold storage ($p<0.05$). It depicted the freshness and glossy appearance of table

grapes berries without any disease or desiccation. With the advancement of storage time, a gradual increase in physical and microbial quality changes, measured by disease severity, weight loss, and berry drop degraded the sensorial properties of table grapes samples. Control was scored lowest due to excessive fungal growth ($p<0.05$), compared to the CEO1-Pad and COM-SO_2-Pad samples at day 21 of cold storage.

The mist-like appearance of fungal mycelium was quite visible all over the control, CEO1-Pad, and COM-SO_2-Pad samples. Additionally, CEO1-HC-Film also exhibited higher scores than that of the aforementioned samples ($p<0.05$), but slight haziness was also visible on the grapes with less glossiness. This might be due to the slow release of CEO antifungal agents trapped by the halloysite clay particle in the PVA film. Eventually, CEO1-Film samples marked the highest score for all the tested attributes at 21 days of cold storage. The findings from the previous studies state that moisture loss may also lead to browning, desiccation, and wilting thereby affecting sensory attributes of table grapes (Harindra Champa, Gill, Mahajan & Bedi, 2015).

Furthermore, EOs from lemon, orange, and mandarin were reported to retard the physicochemical quality changes and microbial growth (molds and yeasts) to safeguard the sensory quality of strawberries during 18 days of cold storage (Shehata et al., 2020).

Table 4.4 Acceptance score of table grape without and with antifungal packaging supplemented with 1% CEO at day 0 and day 21 of storage at 13 °C and RH 75%

Samples	Day 0				Day 21			
	Appearance	Firmness	Odor	Overall	Appearance	Firmness	Odor	Overall
Control	8.5 ± 0.2^{aA}	8.6 ± 0.3^{aA}	8.5 ± 0.2^{aA}	8.5 ± 0.3^{aA}	3.5 ± 0.1^{aB}	3.8 ± 0.3^{aB}	3.2 ± 0.2^{aB}	3.5 ± 0.1^{aB}
CEO1-Pad	8.6 ± 0.1^{aA}	8.7 ± 0.1^{aA}	8.6 ± 0.3^{aA}	8.6 ± 0.2^{aA}	4.4 ± 0.3^{bB}	4.3 ± 0.2^{bB}	4.1 ± 0.4^{bB}	5.3 ± 0.3^{bB}
COM-SO_2-Pad	8.5 ± 0.2^{aA}	8.6 ± 0.2^{aA}	8.5 ± 0.3^{aA}	8.5 ± 0.2^{aA}	5.5 ± 0.2^{cB}	5.7 ± 0.1^{cB}	5.6 ± 0.3^{cB}	5.6 ± 0.2^{cB}
CEO1-HC-Film	8.6 ± 0.1^{aA}	8.7 ± 0.3^{aA}	8.7 ± 0.2^{aA}	8.6 ± 0.1^{aA}	6.3 ± 0.1^{dB}	6.4 ± 0.2^{dB}	6.1 ± 0.3^{dB}	6.3 ± 0.2^{dB}
CEO1-Film	8.8 ± 0.3^{aA}	8.8 ± 0.2^{aA}	8.8 ± 0.1^{aA}	$8.8\pm.3^{aA}$	7.2 ± 0.2^{eB}	7.3 ± 0.1^{eB}	7.2 ± 0.2^{eB}	7.2 ± 0.1^{eB}

Note Values are mean ± standard deviation (n=50). Different uppercase and lowercase superscripts within the same row and same column indicate a significant difference ($p<0.05$), respectively. table grapes: Table grape; CEO: Clove essential oil. Control: table grapes without any treatment; CEO1-Pad: table grapes with 1% CEO Pad; COM-SO2-Pad: table grapes with commercial sulfur dioxide pad; CEO1-HC-Film: table grapes wrapped with a film composed of 1% CEO and halloysite clay; CEO1-Film: table grapes wrapped with 1% CEO film.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

Essential oils (EOs) from different plant sources were tested under *in vitro* conditions against *Aspergillus* sp., being one of the key indicators of fungal growth in table grapes. It was noted that clove essential oil (CEO) at different doses showed the higher fungicidal effect, measured by radial growth and disk diffusion method (zone of inhibition). Odor intensity scores by trained panelists appropriate desirable level of CEO at 1% to be employed for the preparation of antifungal packaging material. Control (table grapes without any treatment) showed the decay in 6 days of storage. Table grapes treated with antifungal pads (CEO1-Pad, COM-SO_2-Pad samples) and CEO-PVA film with and without 1% halloysite clay showed the decay in 9 and 12 days of storage, respectively, and also delayed postharvest quality deterioration during the entire storage period. However, the active release of volatile CEO components in CEO1-Film samples with excellent sensorial quality prolongs the shelf life of table grapes up to 21 days of cold storage. Therefore, CEO active packaging film could be a potential alternative for minimizing postharvest fungal decay and overall quality losses in seedless table grapes.

5.2 Suggestions

5.2.1 Film should be analyzed for mechanical and gas permeability properties.

5.2.2 Release rate of CEO volatiles should be determined by the gas chromatography technique.

5.2.3 fortified PVA film should be casting with the aid of a casting machine.

5.2.4 Antifungal bags should be developed for replacing synthetic polymers for the postharvest storage of grapes.

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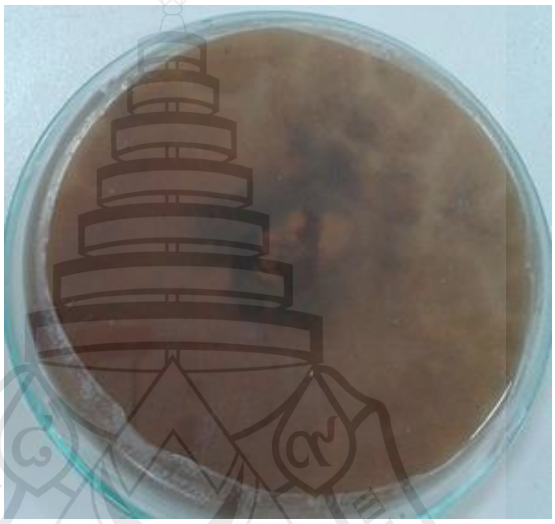
APPENDICES

APPENDIX A

ISOLATION OF *Aspergillus* sp. FROM TABLE GRAPES

A



B

Figure A1 Isolation of *Aspergillus* sp. from table grape. The characteristic of mycelium on top of plate (A) and behind (B) after storage at 25 ^{o}C for 14 day

APPENDIX B

RADIAL GROWTH INHIBITION OF ESSENTIAL OILS

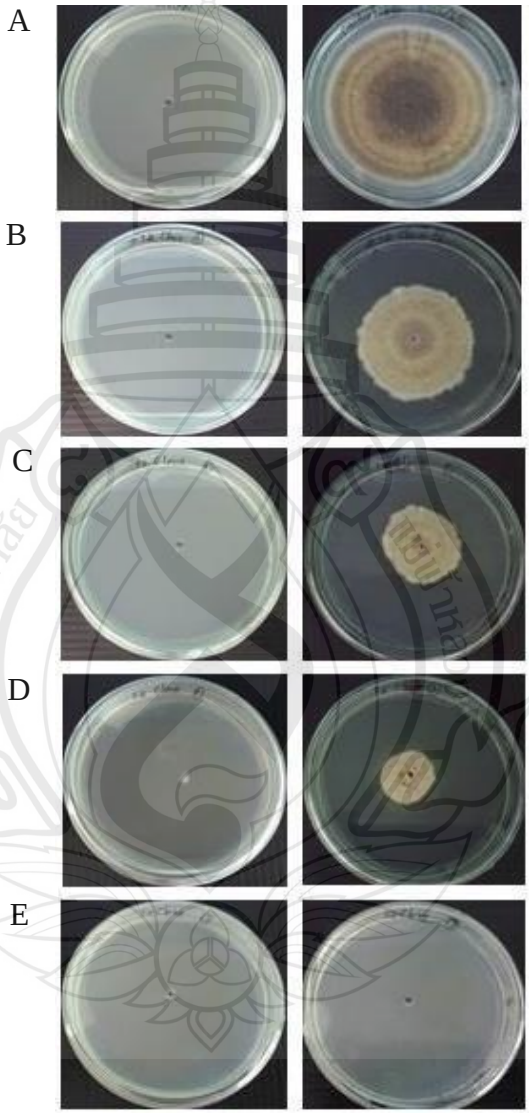


Figure B1 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of clove essential oil after storage at 25 ^{o}C for 7 days

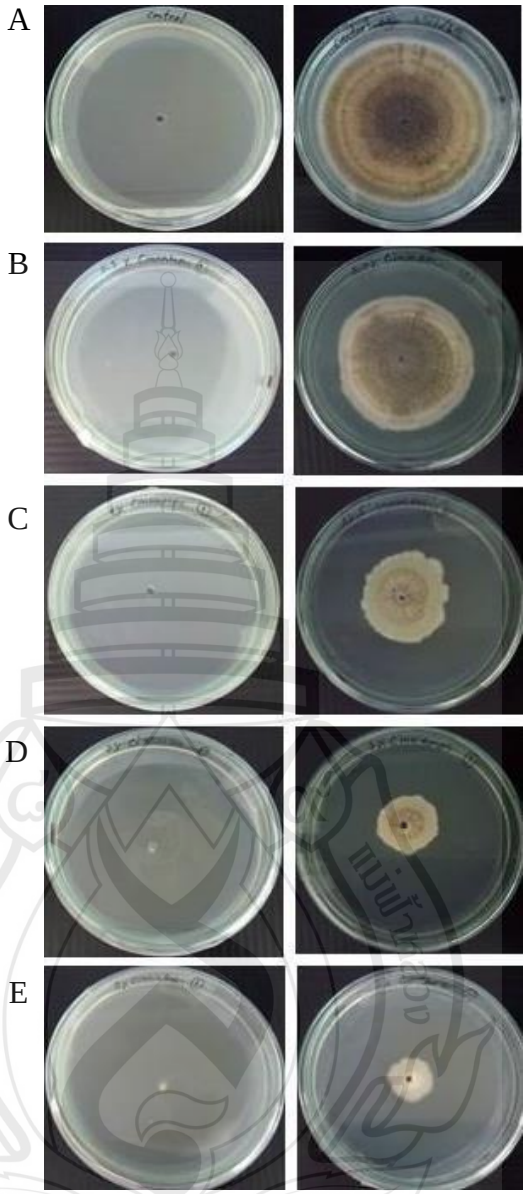


Figure B2 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of cinnamon essential oil after storage at 25 ^{o}C for 7 day

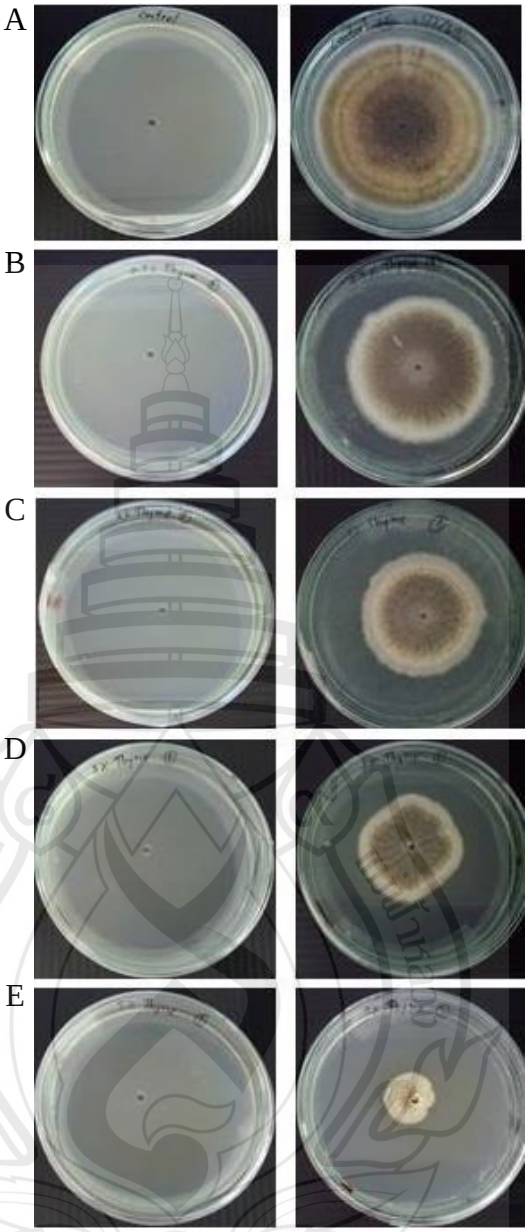


Figure B3 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of thyme essential oil after storage at 25 ^{o}C for 7 days

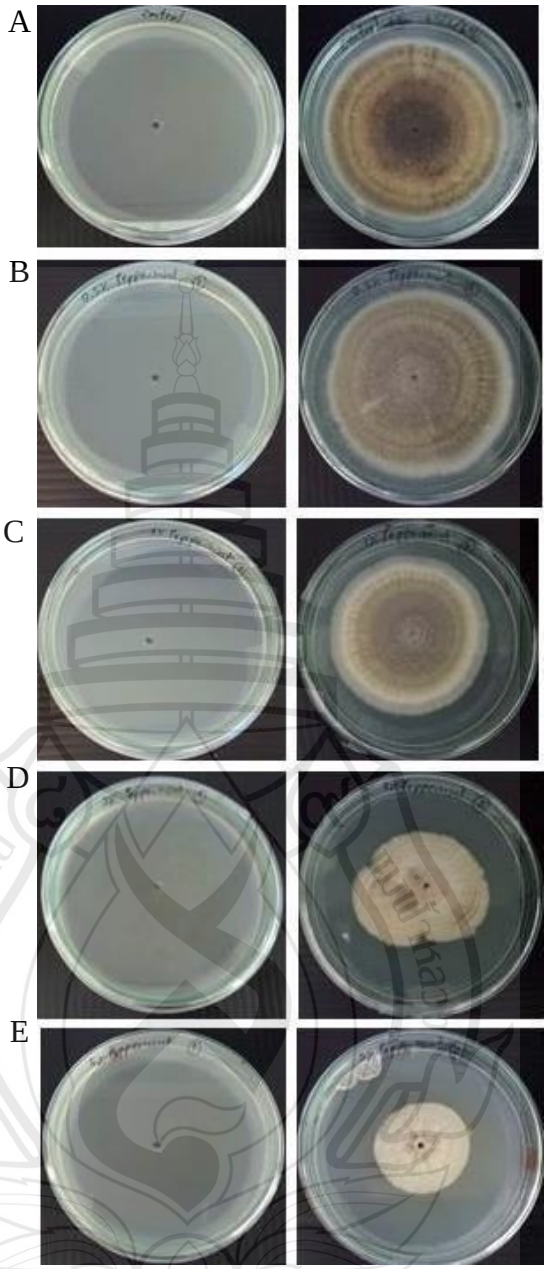


Figure B4 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of peppermint essential oil after storage at 25 ^{o}C for 7 days

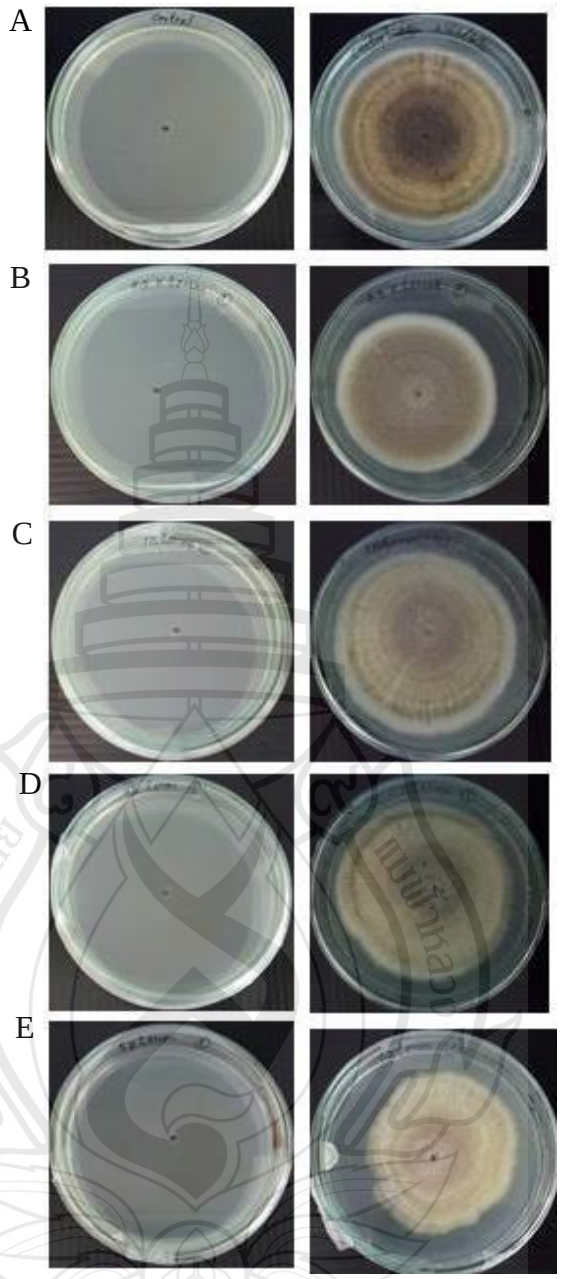


Figure B5 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of lemon essential oil after storage at 25 ^{o}C for 7 days

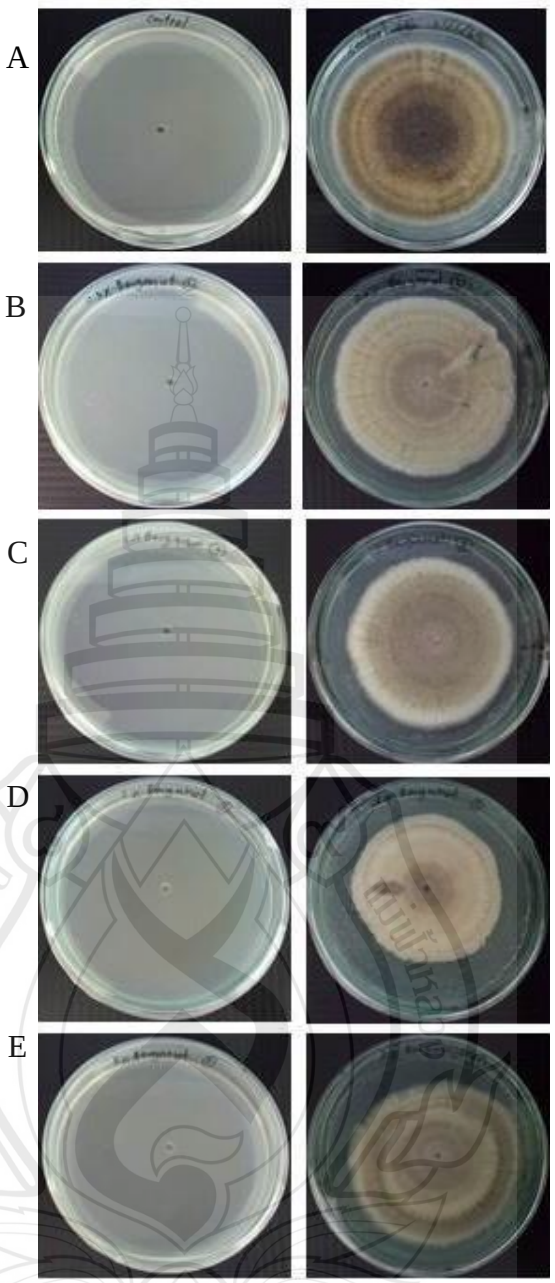


Figure B6 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of bergamot essential oil after storage at 25 ^{O}C for 7 days

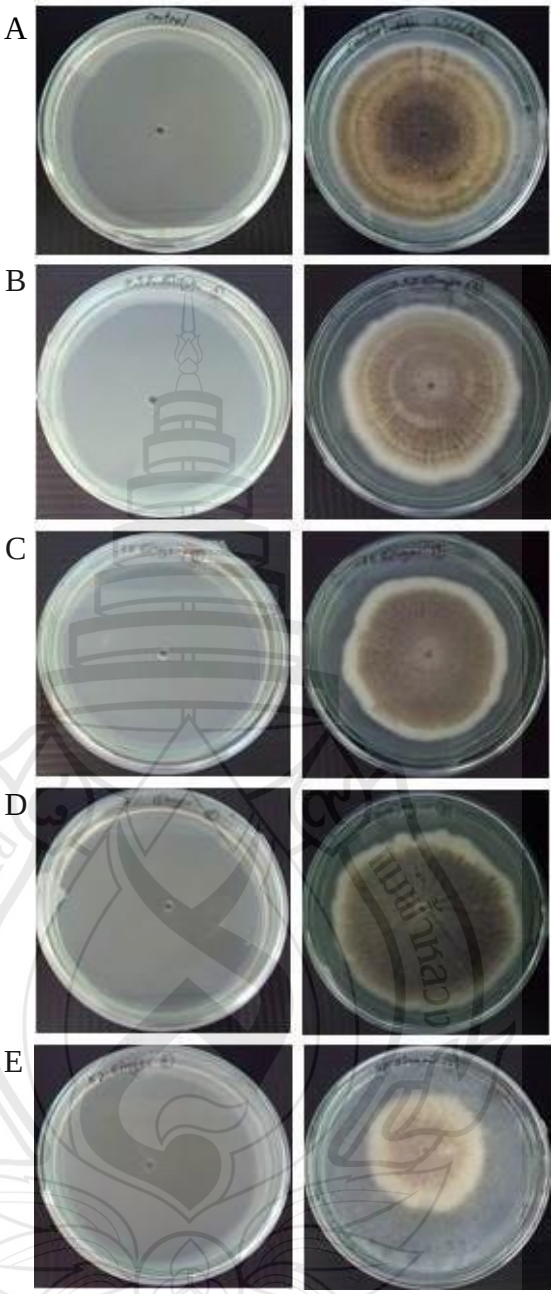


Figure B7 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of ginger essential oil after storage at 25 ^{o}C for 7 days

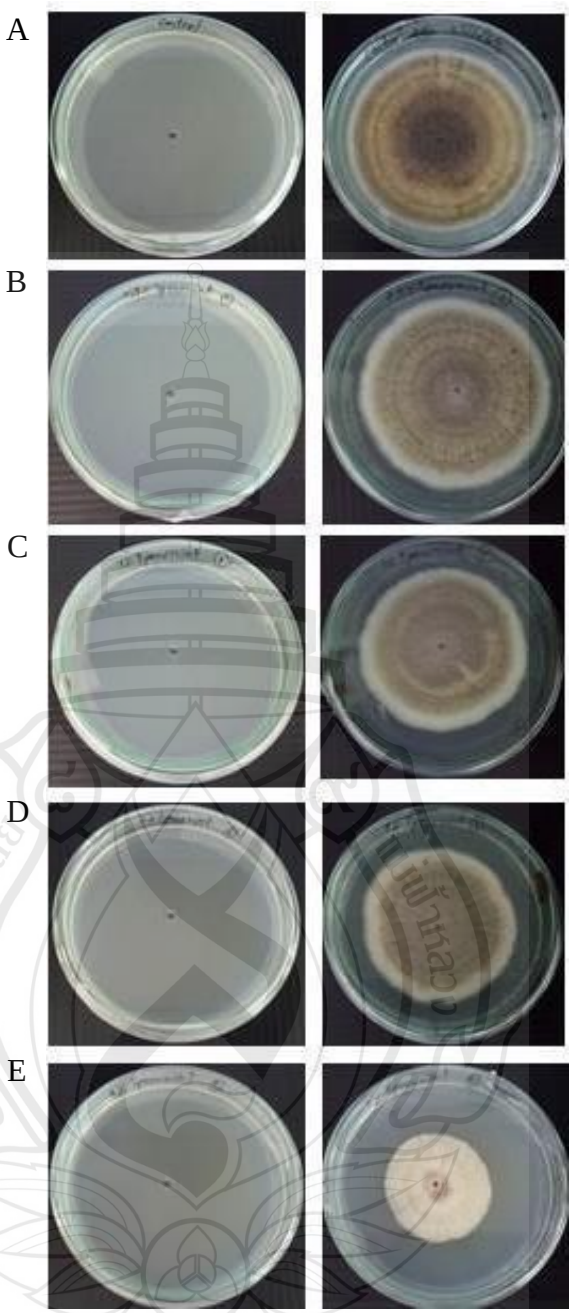


Figure B8 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of spearmint essential oil after storage at 25 ^{o}C for 7 days

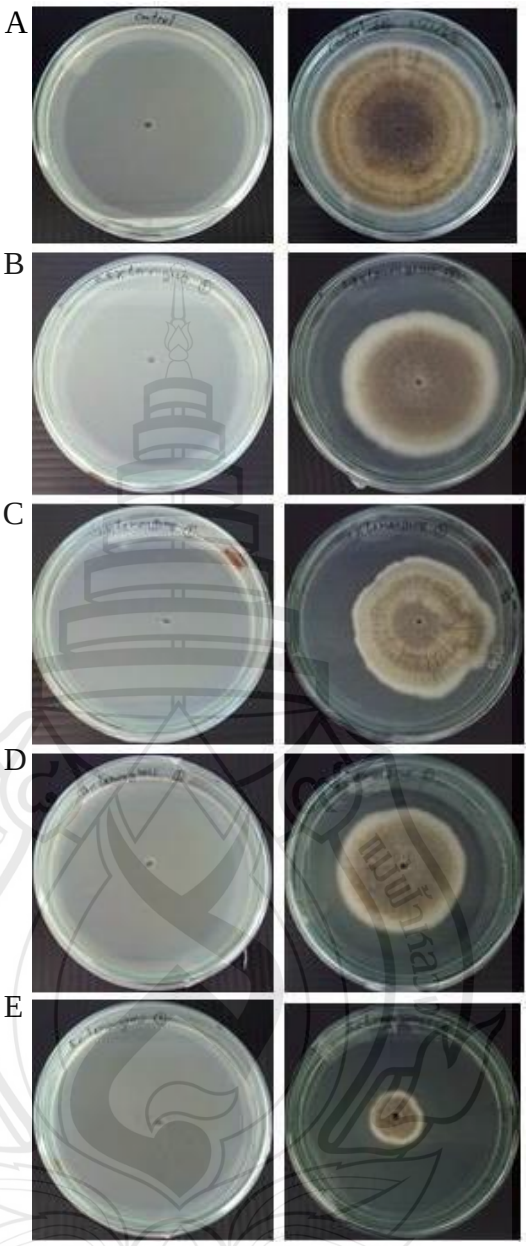
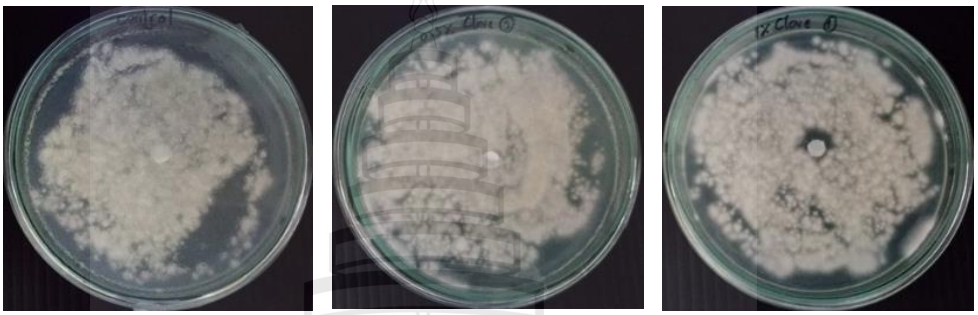


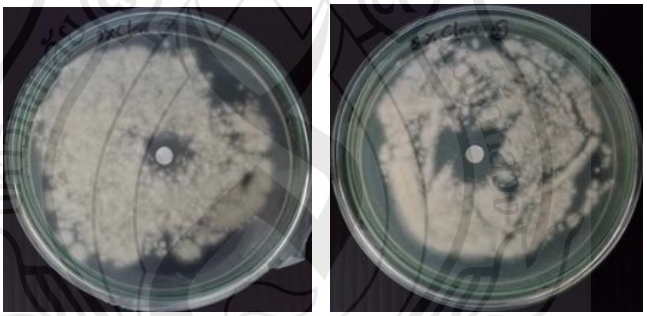
Figure B9 Radial growth measurement of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of lemongrass essential oil after storage at 25 ^{o}C for 7 days

APPENDIX C

DISC DIFFUSION TEST

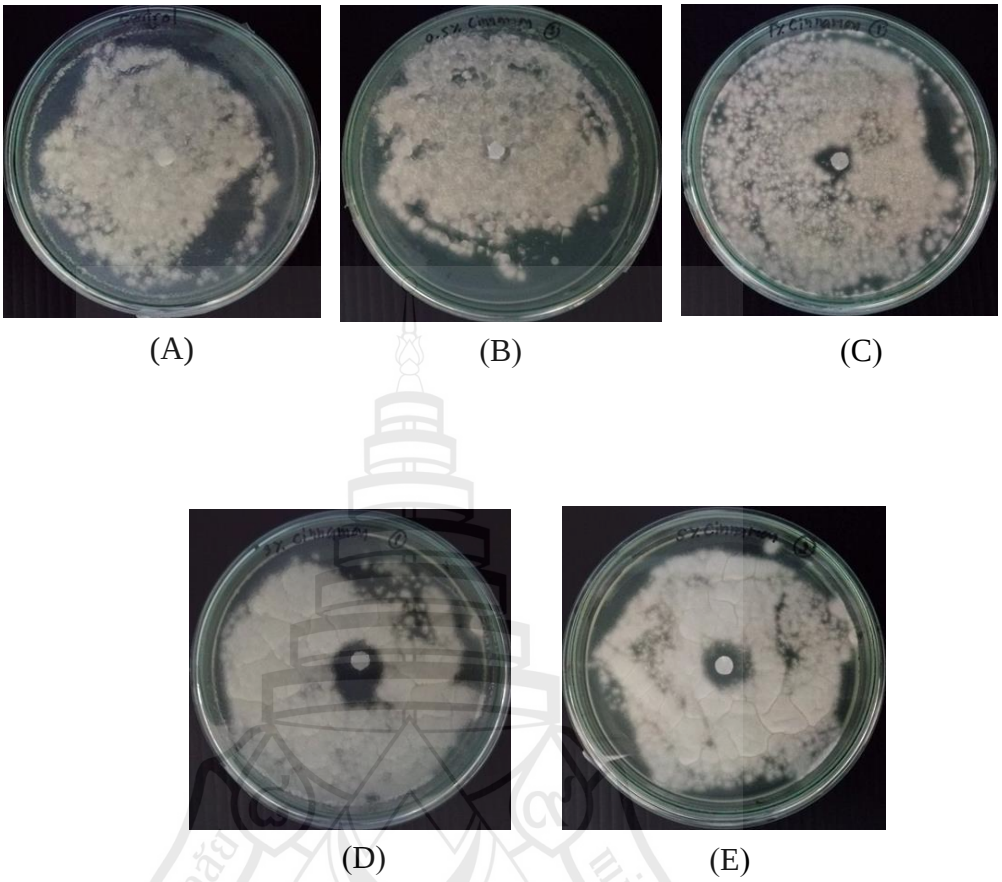


(A) (B) (C)



(D) (E)

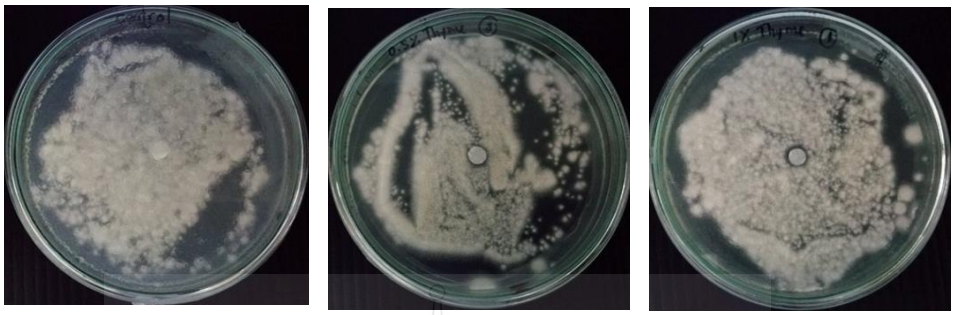
Figure C1 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of clove essential oil after storage at 25 ^{O}C for 72 h



(A) (B) (C)

(D) (E)

Figure C2 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of cinnamon essential oil after storage at 25 ^{o}C for 72 h



(A) (B) (C)

(D) (E)

Figure C3 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of thyme essential oil after storage at 25 ^{o}C for 72 h

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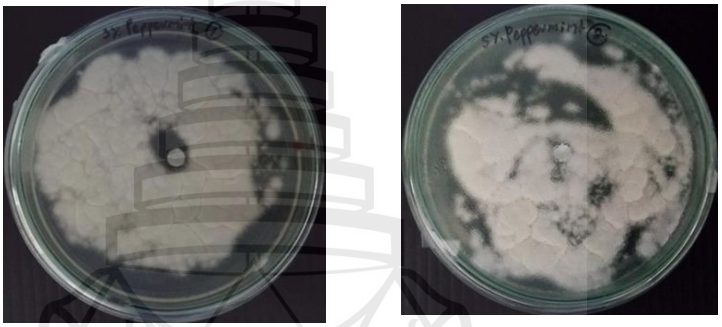
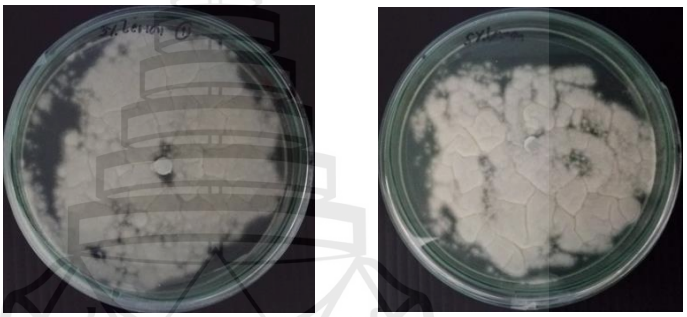


(D) (E)

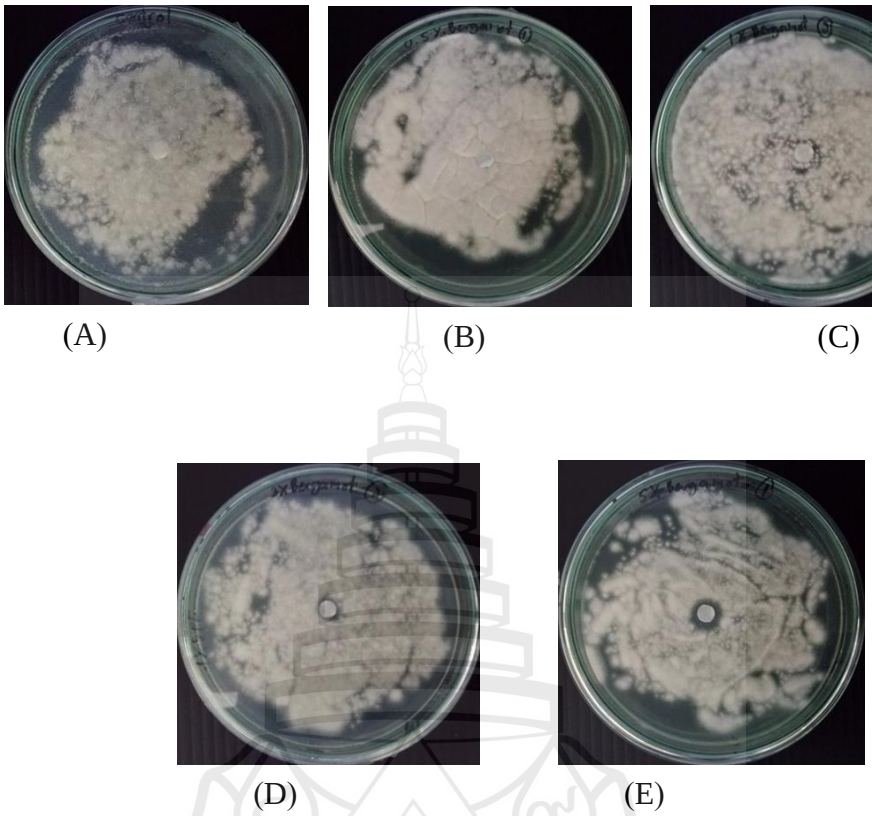
Figure C4 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of peppermint essential oil after storage at 25 ^{O}C for 72 h

(A) (B) (C)



(D) (E)

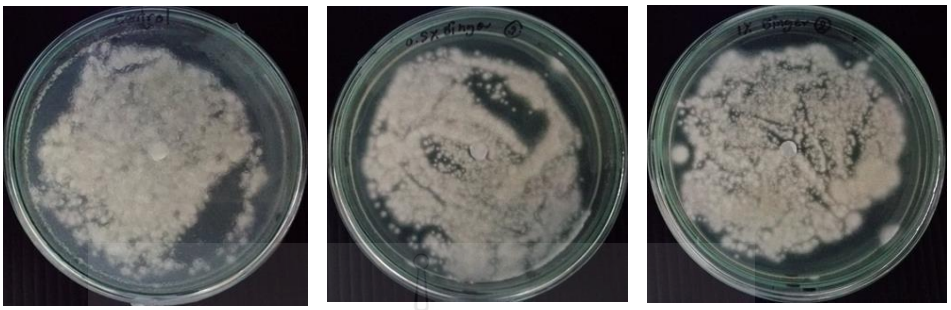
Figure C5 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of lemon essential oil after storage at 25 ^{o}C for 72 h



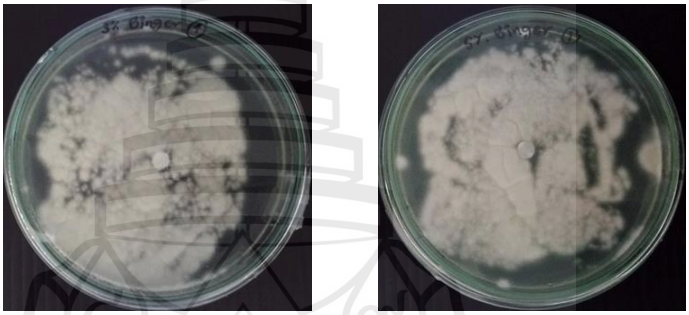
(A) (B) (C)

(D) (E)

Figure C6 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of bergamot essential oil after storage at 25 ^{O}C for 72 h

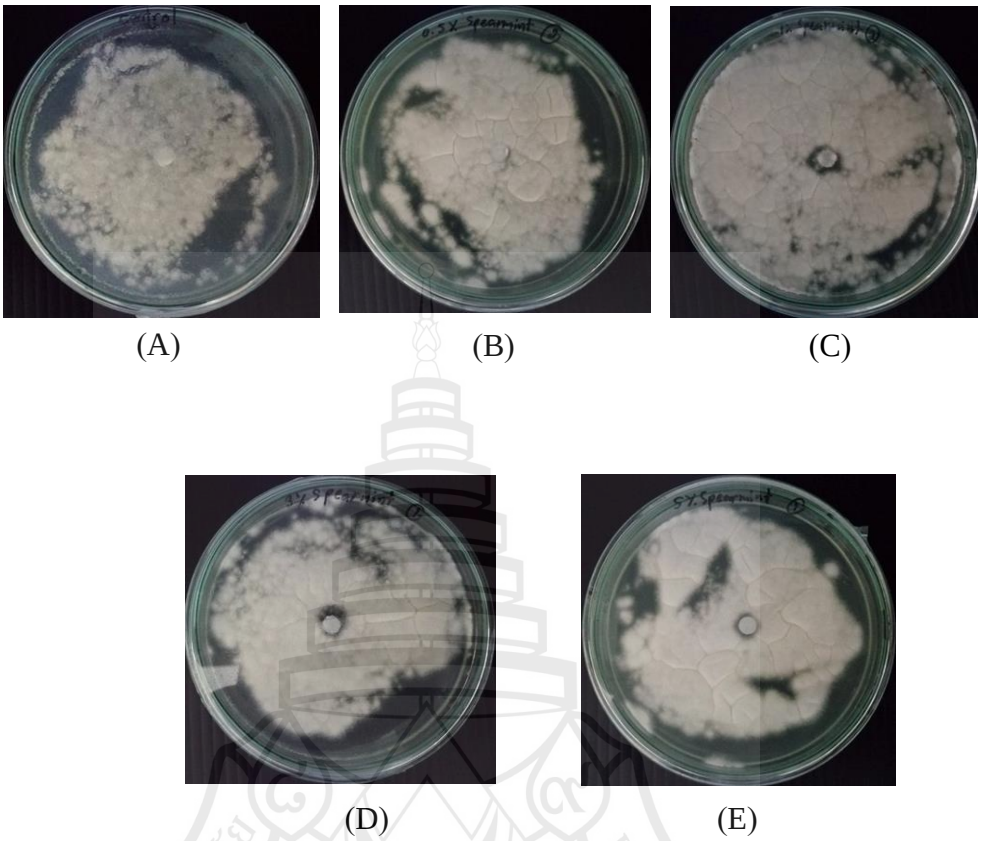


(A) (B) (C)



(D) (E)

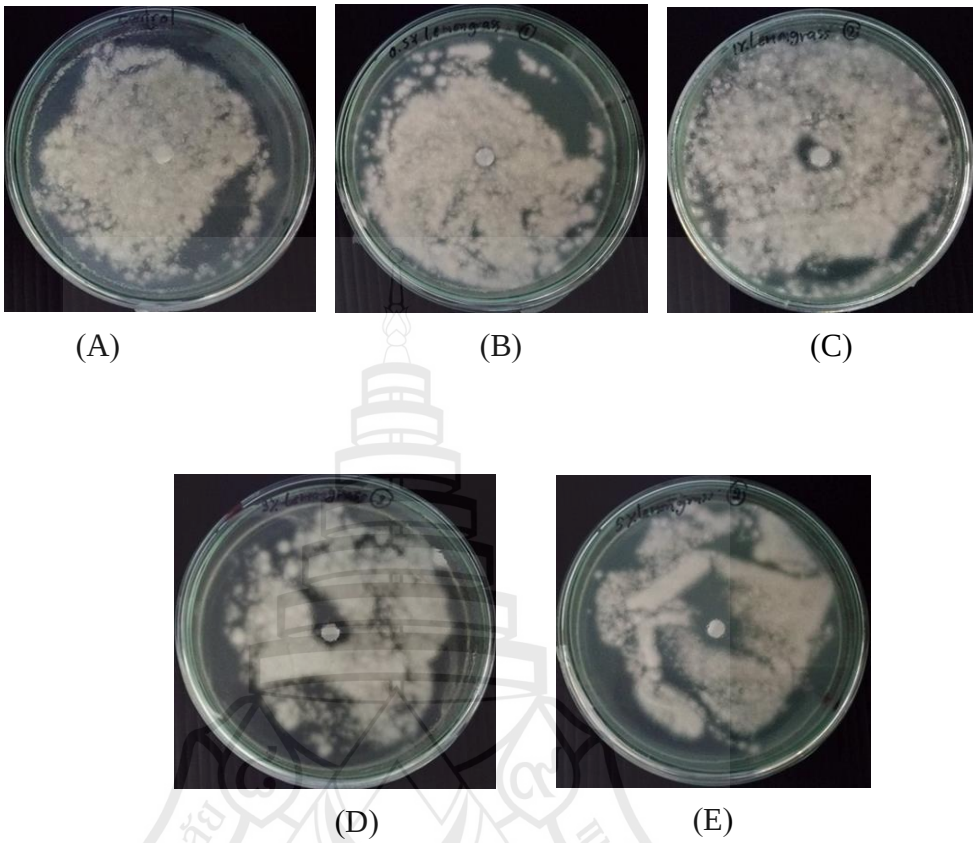
Figure C7 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of ginger essential oil after storage at 25 ^{o}C for 72 h



(A) (B) (C)

(D) (E)

Figure C8 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of clove essential oil after storage at 25 ^{o}C for 72 h



(A) (B) (C)

(D) (E)

Figure C9 Disc diffusion method of inhibition of *Aspergillus* sp. treated with 0% (A), 0.5% (B), 1% (C), 2% (D), and 5% (E) of lemongrass essential oil after storage at 25 ^{O}C for 72 h

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