FABJ

Food and Applied Bioscience Journal

ISSN : 2286-8615
VOLUME 11 ISSUE 2
(MAY - AUGUST 2023)

FABJ

Food and Applied Bioscience Journal

TABLE OF CONTENTS

ISSN : 2286-8615
VOLUME 11 ISSUE 2
(MAY - AUGUST 2023)

Food and Applied Bioscience Journal
FABJ

The efficacy of cinnamon essential oil and vanillin in inhibiting *Colletotrichum* spp. and *Fusarium* spp. isolated from banana fruit

Naphatsanan Chailert [1] and Jurmkwan Sangsuwan [1,2,*]

[1]*Division of Packaging Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, Thailand*
[2]*Center of Excellence in Materials Science and Technology, Chiang Mai University, Chiang Mai, Thailand*
**Corresponding author. E-mail: jurmkwan.s@cmu.ac.th*
Submit: 28 March 2023, Received: 3 April 2023, Revised: 9 August 2023, Accepted: 10 August 2023, Publish online: 25 August 2023

Abstract

Colletotrichum spp. and *Fusarium* spp. are the main fungi that cause of the postharvest loss of banana fruit loss during storage. In this study, poisoned food bioassay technique and vapor phase diffusion technique were used to investigate the inhibition effect of cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) against *Colletotrichum* spp. and *Fusarium* spp. isolated from infected bananas tissue. For poisoned food bioassay technique, *Colletotrichum* spp. and *Fusarium* spp. were placed on potato dextrose agar containing 20, 100, 200 and 1000 µL/L of C and VC (vanillin 1 g/cinnamon EO 15 mL). The results showed that the growth of *Colletotrichum* spp. and *Fusarium* spp. were delay in PDA containing C and VC at 20 µL/L and above. Both strains were completely inhibited in PDA containing 100 µL/L C and VC and above. For the vapor phase diffusion technique, the 20, 100, 200 and 1000 µL of C and VC were dropped into sterile filter paper and adhered on the plate cover. At the lowest concentration studied, vanillin synergized the inhibition effect of cinnamon EO against both strains. VC had better antifungal effect against *Colletotrichum* spp. and *Fusarium* spp. compared with using C alone. Vanillin can be used to limit the stringent odour of cinnamon EO and helped to lower the amount of cinnamon EO required.

Keywords: *Fusarium* spp., *Colletotrichum* spp., Essential oils, Cinnamon, Vanillin

1. Introduction

In 2020, the export of banana from Asia declined by 12.4% (Crumpler *et al.*, 2021) due to the spread of several fungal diseases that damaged bananas. The symptoms of crown rot and anthracnose, caused mainly by *Colletotrichum musae* and *Fusarium* spp., normally represent after harvested (Triest and Hendrickx, 2016). The postharvest treatments are needed to relieve banana losses. Various synthetic chemicals like fungicides such as chloramines, dichloramines, and trichloromethanes (Fallanaj *et al.*, 2013) have been used in the last few decades to reduce the post-harvest losses (Kumar *et al.*, 2021). However, the use of synthetic fungicides is harmful to consumer health, in which chemical residues are presented in the food (López-Fernández *et al.*, 2016). Therefore, natural preservatives were used as alternatives to replace synthetic chemicals. Plant extracts, such as essential oils (EOs), and had antimicrobial properties, resulted in shelf-life extension of food products (Basavegowda *et al.*, 2021). EOs contain highly bioactive substances that are widely used in food to inhibit bacteria, viruses, and fungi. (Bhavaniramya and Baek, 2019). However, the use of essential oils is limited in intense aroma and taste affecting a negative impact on organoleptic properties (De-Montijo-Prieto *et al.*, 2021). The use of EOs in combination with other food additives might diminish these problems and led to the development of new safe and effective natural antimicrobial agents for food preservation.

Cinnamon EO were reported to suppress the growth of fungi. The main active ingredients were Cinnamaldehyde and trans-cinnamaldehyde, which shown antifungal activity (Wu *et al.*, 2017; Duan *et al.*, 2018) by inhibiting cell wall biosynthesis and destroying the cell membrane of *Fusarium sambucinum*. (Wei *et al.*, 2020). Maqbool *et al*. (2010) investigated the antifungal efficacy of cinnamon EO against infection of *Colletotrichum musae* on bananas stored at 13 ± 1°C and relative humidity 80-90% for 28 days. They reported that the maximum concentration of 0.3% cinnamon EO could be used to extend banana shelf life. In addition, cinnamon EO was applied to reduce mycelial growth and complete spore germination of *Colletotrichum acutatum* in kiwifruit (He *et al.*, 2018*). Colletotrichum acutatum* isolated from mango and strawberry is controlled by cinnamon EO. (Danh *et al.*, 2021; Duduk *et al.*, 2015). Fruit rots of avocado, mango, and papaya caused by *Colletotrichum gleosporioides*, *Fusarium solani*, and *Phytophthora palmivora* can be treated with cinnamon EO (Sarkhosh *et al.*, 2018). Cinnamon EO decreased the growth of *Fusarium oxysporum* in vitro and was found to be the most efficient in reducing conidial germination of *Fusarium oxysporum* causing *Fusarium* wilt in strawberry plants (Park *et al.*, 2017) as cinnamaldehyde in cinnamon EO causes irreversible morphological and ultrastructural changes. (Xing *et al.*, 2014).

Vanillin is used as flavour in many food industries. Vanillin is a phenolic aldehyde with many antimicrobial bioactive qualities that inhibit the growth and development of yeast, mold, and bacteria (Amiri *et al.*, 2021). The shelf life of fruit and vegetable can be extended when applying vanillin, such as apples (Chung *et al.*, 2009), strawberries (Yarahmadi *et al.*, 2014), lettuces (Das *et al.*, 2021), tomatoes (Safari *et al.*, 2020) and mango (Jaimun and Sangsuwan, 2019). Previous research applied a combination of cinnamon EO and vanillin in fresh-cut fruits such as Nectarines dipped in calcium ascorbate (AB) and antimicrobial agents (vanillin or cinnamic acid).

After fresh-cut nectarines were packed and stored at 5°C for 8 days. AB+vanillin and AB+cinnamic inhibited microbial counts when compared with control and AB-only samples and did not impart off-flavors. (Muche and Rupasinghe, 2011). The use of vanillin (1 or 2g/L) or cinnamic acid (0.15 and 0.3gL^{-1}) dropped on a filter paper placed inside a container with fresh-cut melon stored for 10 days at 5°C shows melon treated with vanillin (2g/L) and all cinnamon treatments had the highest polyphenol levels, lower respiration rate, and Consumers accepted the flavor of antimicrobial-treated melon. (Silveira *et al.*, 2015). The purpose of this research is to investigate the efficacy of cinnamon EO and vanillin in inhibiting *Colletotrichum* spp. and *Fusarium* spp. in bananas.

2. Materials and Methods

2.1 Analysis of the chemical composition of cinnamon essential oil and cinnamon essential oil mixed vanillin

Cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) were used in this study since there are reports that the combination of EO provide synergistic effect compared with using only one essential oil (Muche and Rupasinghe, 2011). The composition of both formulations were analyzed by GC 7890A and a MSD 5975C (EI mode) mass spectrometer (Agilent Technology, USA). A 0.1 µL volume of sample was injected onto the DB5-MS column (30 m × 0.25 mm ID × film thickness 0.25 µm). The oven temperature was set from 50°C to 280°C. The inlet temperature was 250°C and was detected to be 280°C. Helium carrier gas flowed rate 1 mL/min. For the mass spectrometer was a mass range of 50-550 amu/sec.and ms quadrupole 150°C ms source 230°C. Database of mass spectrometer references W8N08 (John Wiley and Sons, Inc., USA).

2.2 Fungal isolation

Ripe bananas were incubated at 30°C until developed dark brown anthracnose lesions and whitish moulds on banana crown rot. *Colletotrichum* spp. and *Fusarium* spp. were isolated from anthracnose lesions and the infected rots on banana fruits. The isolation of both fungi was performed according to the method proposed by Maqbool *et al*. (2010). Ripe bananas were incubated on moist sterile filter paper at room temperature for 7 days. *Colletotrichum* spp. and *Fusarium* spp. were isolated by tissue transplanting method. Lesion area on banana peel was cut into 5 × 5 mm pieces, soaked in 10% v/v sodium hypochlorite solution for 5 min, washed with sterile distilled water two times, and dried on sterile filter paper. Each tissue piece was placed on a plate containing potato dextrose agar (PDA) medium and incubated at room temperature for 7-10 days at 25 ± 2°C. After 7 days of incubation, the corresponding mycelia were then transferred to a new PDA plate.

2.3 Preparation of antifungal agents

Cinnamon EO from Thai-China Flavours and Fragrances Industry Co., Ltd, Thailand; C (100%) and combined cinnamon EO and vanillin (100%) from KH Roberts Pte., Ltd., Thailand; VC at 20, 100, 200 and 1000 µL were prepared. Vanillin and cinnamon EO mixture (VC) were prepared by adding 1 g of vanillin in 15 mL of cinnamon EO, which is the highest amount of vanillin that can be dissolved in cinnamon oil.

2.4 Inhibition effect of cinnamon essential oil and cinnamon essential oil mixed vanillin against *Colletotrichum* spp. or *Fusarium* spp. by poisoned food bioassay technique

Plant extract, C and VC at 20, 100, 200 and 1000 µL were added in 1 L of potato dextrose agar (PDA, Hi-Media, India) using 0.2% tween 80 as emulsifier (Maqbool *et al.,* 2010). The PDA mixture was then autoclaved for 15 min at 121°C and poured into plate to let it solidified. The pure culture of *Colletotrichum* spp. or *Fusarium* spp. was pierced with a 5 mm sterilized cork borer and put on solidified PDA. Four pieces of culture were placed on each plate. PDA with no C or VC served as control. Inoculated plates were incubated at 25°C. The mycelial growth diameter (MGD) expressed in cm (Werghemmi *et al.,* 2022), was measured every 2 days and calculate mycelial area using the following equation.

MGD (cm) = (D1 + D2)/2 (1)
D1 = Mycelial growth horizontal diameter
D2 = Mycelial growth vertical diameter
r = Mycelial growth diameter /2
mycelial area = ∏r2

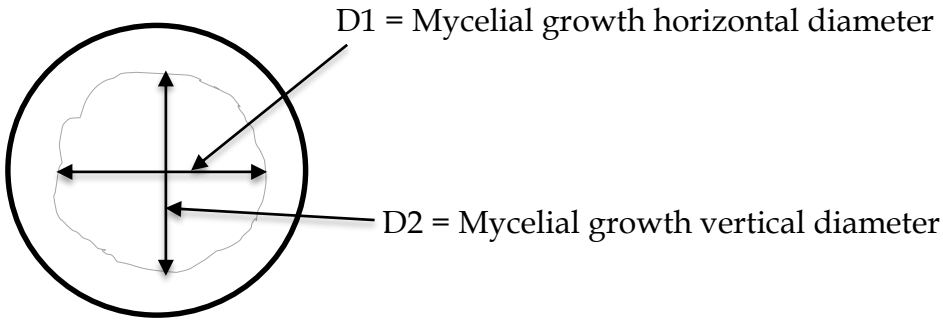

Fig 1 Mycelial growth diameter calculation

2.5 Inhibition effect of cinnamon essential oil and cinnamon essential oil mixed vanillin against *Colletotrichum* spp. or *Fusarium* spp. by vapor phase diffusion technique

The inhibition effect of EOs vaporized in the plate (vapor phase diffusion technique) was used to compare those of poisoned food bioassay (liquid essential oils were mixed in media), since vanillin and cinnamon oil are volatile. Also Sae-Eaw *et al.* (2019) reported that essential oils in vapor phase provided better antimicrobial effect than liquid phase. The fungal activity was tested according to a method proposed by Xing *et al.* (2014) with modifications. Mycelium of *Colletotrichum* spp. or *Fusarium* spp. were pierced with a sterile 5 mm cork borer and four tissue pieces were placed on solidified PDA. Antimicrobial C and VC at 20, 100, 200 and 1000 µL were dropped into a 10 mm in diameter sterilized filter paper disk and placed at the center of a plate. Inoculated plates were incubated at 25°C. Fungal growth was measured every 2 days.

2.6 Statistical analysis

The experiments were conducted with triplicate determinations, and the data were subjected to analysis of variance and Tukey's-b multiple range test ($P<0.05$) using SPSS version 11.0 by SPSS Inc., Chicago, IL, USA software.

3. Results and Discussion

3.1 Chemical Compositions of cinnamon essential oil and cinnamon essential oil mixed with vanillin

Cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) compounds were analyzed using the GC-MS technique. the main chemical compositions of cinnamon EO (C) were 3-allyl-2-methoxy phenol (71.58%) followed by benzoyl benzoate (5.64%), caryophyllene (4.19%), acetyl eugenol (3.91%), trans-cinnamyl acetate (2.23%), β-linalool (2.21%), cinnamaldehyde (1.45%), and safrole (1.41%). While the main chemical compositions of VC were 3-allyl-2-methoxy phenol (66.82%) followed by benzaldehyde,3-hydroxy-4-methoxy (7.41%), benzoyl benzoate (5.22%), caryophyllene (3.91%), δ-cadinene (3.63%), trans-cinnamyl acetate (2.09%), β-linalool (2.00%), cinnamaldehyde (1.33%) and safrole (1.30%). VC contains benzaldehyde, 3-hydroxy-4-methoxy is the main active compound of vanillin. (Sun *et al.,* 2022)

3.2 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on Colletotrichum spp. by poisoned food bioassay technique

Table 1 shows the effectiveness of cinnamon EO (C) and cinnamon EO mixed with vanillin (VC) in inhibiting *Colletotrichum* spp. using the poisoned food bioassay technique. During the first 2 days, no growth of *Colletotrichum* spp. in all treatments was observed. On day 4, the area of *Colletotrichum* spp. on the control plate (with no antifungal agents C and VC) significantly expanded compared with other treatments. The average area of mycelium in control plate was 6.25 ± 1.59 cm^2, while those on the PDA mixed with 20 µL/L C, and VC were only 1.83 ± 2.97 cm^2 and 1.77 ± 0.31 cm^2, respectively. The mycelium areas of *Colletotrichum* spp. treated with 20 µL/L C and VC were statistically significant differences from control on Day 4 to Day 8, indicating that 20 µL/L C and VC suppressed the mycelial growth. In addition, C and VC at concentrations of 100 µL and above completely inhibited *Colletotrichum* spp. However, the effect of C and VC at the same concentration were not significantly different. Many studies reported the effectiveness of cinnamon EO in inhibiting mould. Maqbool *et al.* (2010) found that the 0.4% cinnamon EO in PDA reduced mycelia growth of *Colletotrichum musae,* which was isolated from bananas and inhibited the conidial germination by 83.2% to all other concentrations (0.1, 0.2, 0.3 and 0.4%). Danh *et al.* (2021) reported that cinnamon EO inhibited the development of *Colletotrichum acutatum* isolated from mango. Cinnamon EO 1.6 µL/mL showed the highest antifungal activity compared with basil, lemongrass, orange, mint, and coriander leaves. Sarkhosh *et al.* (2018) also evaluated the effect of cinnamon EO on mycelium growth of *Colletotrichum gloeosporioides* isolated from mango at 25°C and found that cinnamon EO at 1000 µL/L was effective in inhibiting mycelium growth, in which the cinnamon EO concentration was far higher than our study.

Table 1 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Colletotrichum* spp. by poisoned food bioassay technique

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil in PDA (µL/L)				combined cinnamon oil and vanillin in PDA (µL/L)			
		20	100	200	1000	20	100	200	1000
0	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
2	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
4	6.25±1.59^{B,c}	1.83±2.97^{A,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	1.77±0.31^{A,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
6	8.50±1.11^{C,c}	4.90±0.00^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	4.91±0.27^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
8	12.26±0.32^{D,c}	7.48±0.60^{C,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	7.07±1.10^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}

^{A} Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).

^{a} Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).

Each data point represents the mean ± standard deviations of three replications.

3.3 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Colletotrichum* spp. by vapor phase diffusion technique

Table 2 shows the effectiveness of C and VC in inhibiting *Colletotrichum* spp. using the vapor phase diffusion technique, which was intended to evaluate the antifungal activity of volatile EO. The mycelium area in all treatments remained unchanged during the first two days. On Day 4, *Colletotrichum* spp. in the control plate grew fastest, followed by 20 µL C vapor and 20 µL VC vapor. On Day 8, the control mycelium area was 12.26 ± 0.32 cm^2. While the mycelium area in the plate treated with 20 µL C vapor was 8.54 ± 1.28 cm^2, which was significantly larger than those of 20 µL VC (5.55 ± 1.13 cm^2). The addition of vanillin synergized the inhibition effect of cinnamon EO. At higher concentrations (100 µL and above), both C and VC vapor completely inhibited *Colletotrichum* spp. Many studies have been conducted to investigate the use of EO vapor to inhibit fungi. Hong *et al*. (2015) used cinnamon EO vapor to suppress *Colletotrichum gloeosporioides* in peppers by dripping 8 µL of cinnamon EO on filter paper and stored at 25°C for 10 days, which slightly showed growth and 0% conidial germination compared with control. He *et al.* (2018) demonstrated the effectiveness of cinnamon EO vapor against *C. acutatum* in kiwi at 25°C for 7 days. Vapor of cinnamon EO decreased mycelium growth at a concentration of 0.2 mL/mL and completely inhibited spore germination at 0.175 mL/mL. Furthermore, a study of the effects of cinnamon EO on the morphology of *C. acutatum* showed that cinnamon EO affected cell membranes and cytoplasm, including soluble proteins, sugars, and nucleic acids. Cinnamon EO has a high potential to be used as a natural preservative. The addition of vanillin into the cinnamon EO reduced the mycelium growth as shown in Table 2 and the results agreed with Cava-Roda *et al.* (2021). A synergistic study of vanillin and cinnamon bark oil, cinnamon leaf oil, and clove oil inhibited *L. monocytogenes* and *E. coli* O157:H7. The mixture of vanillin and cinnamon EO increases the antimicrobial effect and could be used EO at a lesser amount. Vanillin is widely used as a flavouring agent in the food and beverage industries. (Vijayalakshmi *et al.*, 2019) and developed antimicrobial properties against

bacteria, fungi, and yeast. (Triana *et al.*, 2019). Therefore, the sensory effects of EO must be minimized by incorporating vanillin.

The poisoned food bioassay technique revealed that incorporation of C and VC in PDA had no significant differences antifungal effects against *Colletotrichum* spp. This technique requires Tween 80 as a surfactant to homogenize the EO to the culture medium. As a result, Tween 80 might reduce the antibacterial activity of EO in PDA emulsions (Ma *et al.*, 2016). When evaluation using the vapor phase diffusion technique, VC was more effectively in inhibiting fungal growth than applying C alone. The mycelium growth in plate treated with VC vapor was slower than those treated with C. Vanillin increased antifungal effect in the vapor technique by which vanillin has aldehyde group as the side group of the benzene ring. It functions effectively in protein and fat due to its hydrophobic nature (Li *et al.*, 2021). Vanillin vapor directly deforms the mycelium and acts on the membrane, resulting in ion gradient distribution and respiration inhibition (Fitzgerald *et al.*, 2004). The cinnamon vapor inhibited energy-generating systems. Both compounds have different sites of action in the microbial cell and thus have synergistic antimicrobial effects (Cava-Roda *et al.*, 2021).

Table 2 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Colletotrichum* spp. by vapor phase diffusion technique

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil (µL)				combined cinnamon oil and vanillin (µL)			
		20	100	200	1000	20	100	200	1000
0	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
2	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
4	$4.63\pm0.42^{B,d}$	$1.76\pm0.00^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$2.03\pm1.53^{B,c}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
6	$8.33\pm1.09^{C,c}$	$4.91\pm0.00^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$4.99\pm0.82^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
8	$12.26\pm0.32^{D,d}$	$8.54\pm1.28^{D,c}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$5.55\pm1.13^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).

[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).

Each data point represents the mean ± standard deviations of three replications.

3.4 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Fusarium* spp. by poisoned food bioassay technique

Table 3 shows the effectiveness of C and VC in inhibiting *Fusarium* spp. using poisoned food bioassay technique. During the first two days, the mycelium size in all treatments did not change. On day 4, The mycelium areas of the control PDA and 20 µL/L C and VC began to enlarge. On Day 8, The mycelium area of the control was 10.82 ± 0.39 cm^2 and did not statistically difference from those treated with 20 µL/L VC (10.10 ± 0.7 cm^2). However, the area of *Fusarium* spp. on 20 µL/L C was 5.53 ± 3.66 cm^2, which was statistically different from control and VC. The results showed that C alone inhibited the growth of *Fusarium* spp. better than VC at a concentration of 20 µL/L. Similar to *Colletotrichum* spp., C and VC at 100 µL or more completely inhibit growth of *Fusarium* spp. Many researchers demonstrated that EOs can inhibit

Fusarium spp. Horváth *et al.* (2013) studied the effect of mint and cinnamon on the growth of *Fusarium* spp. isolated from wheat using the agar dilution method. Cinnamon EO was proven to be a completely effective antifungal agent at 0.01%. Subsequently, Xing *et al*. (2014) reported that 20 mL/L cinnamaldehyde damages *F.verticillioides* cells by interfering with the enzymatic processes of cell wall production.

Table 3 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Fusarium* spp. by poisoned food bioassay technique

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil in PDA (µL /L)				combined cinnamon oil and vanillin in PDA (µL/L)			
		20	100	200	1000	20	100	200	1000
0	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
2	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
4	1.52±0.24^{B,b}	0.25±0.25^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	1.83±0.00^{A,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
6	4.47±0.44^{C,c}	4.37±4.15^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	4.91±0.00^{B,c}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}
8	10.82±0.39^{D,c}	5.53±3.66^{B,b}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	10.10±0.70^{C,c}	0.13±0.00^{A,a}	0.13±0.00^{A,a}	0.13±0.00^{A,a}

^{A} Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).

^{a} Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).

Each data point represents the mean ± standard deviations of three replications.

3.5 Antifungal Activity of cinnamon essential oil and cinnamon essential oil mixed with vanillin on *Fusarium* spp. by vapor phase diffusion technique

Table 4 shows the effectiveness of C and VC in inhibiting *Fusarium* spp. using the vapor phase diffusion technique. The mycelium area of *Fusarium* spp. in the control plate started to expand on Day 4. As storage time increased, the mycelium size increased except the plate of *Fusarium* spp. treated with C and VC vapor at 100 µL and above. On Day 8, the average size of control mycelium, mycelium treated with vapor of C and VC 20 µL were 11.05 ± 0.00 cm^2, 10.10 ± 0.70 cm^2 and 5.45 ± 1.10 cm^2, respectively. VC vapor was significantly more effective than C vapor. The result agreed with Xing *et al.* (2014), who used cinnamon EO and cinnamaldehyde to inhibit growth and analysed the morphological alterations of *Fusarium verticillioides*. A fumigation of 40 mL cinnamon EO could prevent the development of *F. verticillioides*. Romero-Cortes *et al.* (2019) also reported using vanillin in PDA to assess the prevention of *Alternaria alternata* from sorghum and barley disease plants and found that the inhibition of *A. alternata* was observed in 480 hours at a dosage of 750 mg/L. Scanning electron microscopy revealed no conidia production and morphologically abnormal body structure. Some researchers reported the synergistic impact of cinnamon EO and vanillin. The use of cinnamon EO vapor with vanillin to inhibit *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus* consistent with Sun *et al.* (2014).

Table 4 Effect of cinnamon oil and combined cinnamon oil and vanillin on inhibiting *Fusarium* spp. by vapor phase diffusion

Days	Mycelial Area (cm^2)								
	Control	cinnamon oil in PDA (µL)				combined cinnamon oil and vanillin in PDA (µL)			
		20	100	200	1000	20	100	200	1000
0	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
2	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
4	$2.98\pm1.84^{B,c}$	$1.63\pm0.45^{B,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$2.03\pm1.53^{B,c}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
6	$6.07\pm3.17^{C,c}$	$4.91\pm0.00^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$4.99\pm0.82^{C,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$
8	$11.05\pm0.00^{D,c}$	$10.10\pm0.70^{D,b}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$5.45\pm1.10^{D,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$	$0.13\pm0.00^{A,a}$

[A] Means± standard deviations with different upper-case superscripts in each column are shown significant differences (P<0.05).

[a] Means± standard deviations with different lower-case superscripts in each row are shown significant differences (P<0.05).

Each data point represents the mean ± standard deviations of three replications.

The poisoned food bioassay technique indicated that C at 20 µL/L was more effective than VC in retarding mycelium growth. While, vapor phase diffusion technique showed that VC provided better antifungal effect. This might be *Fusarium* spp. may use phenolic compounds as carbon sources for the development of fungal growth (Zhou *et al.*, 2012).

Both *Colletotrichum* spp. and *Fusarium* spp. fumigated with VC had significantly slower growth than those treated with C alone. Therefore, vanillin enhanced the inhibitory effects against both strains. Vanillin and cinnamon EO functioned differently. Cinnamon EO contains cinnamaldehyde, which is an addition to the direct way of action, inhibiting fungal growth (Kowalska *et al.*, 2021) and phenolic compounds in vanillin changed the community structures of *Fusarium* spp. (Derito *et al.*, 2009).

4. Conclusion

The use of cinnamon EO and cinnamon EO mixed with vanillin to inhibit the growth of *Colletotrichum* spp. and *Fusarium* spp. isolated from bananas by poisoned food bioassay technique and vapor phase diffusion technique were demonstrated. The growth of *Colletotrichum* spp. and *Fusarium* spp. was inversely related to the concentration of natural volatile EO used. Cinnamon EO and cinnamon EO combined with vanillin at concentrations of 100 µL/L PDA or 100 µL as vapor or above showed complete inhibition of both strains, evaluating by both techniques. Vanillin synergized cinnamon EO in inhibiting the growth of *Colletotrichum* spp. and *Fusarium* spp in vapor phase diffusion assay. Cinnamon EO combined with vanillin has a potential use to fumigate banana to prevent postharvest disease from *Colletotrichum* spp. and *Fusarium* spp.

Acknowledgement

This research work was supported by Agricultural Research Development Agency (Public Organization) and Chiang Mai University, Thailand.

References

Amiri, S., Moghanjougi, Z., Bari, M. and Khaneghah, A. 2021. Natural protective agents and their applications as bio-preservatives in the food industry: An overview of current and future applications. Italian Journal of Food Science. 33. SP1: 55-68

Basavegowda, N. and Baek, K. H. 2021. Synergistic antioxidant and antibacterial advantages of essential oils for food packaging applications. Biomolecules. 11(9): 1267.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R. and Baskaran, D. 2019. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain & oil Science and Technology. 2(2): 49-55.

Cava-Roda, R., Taboada-Rodríguez, A., López-Gómez, A., Martínez-Hernández, G.B.and Marín-Iniesta, F. 2021. Synergistic Antimicrobial Activities of Combinations of Vanillin and Essential Oils of Cinnamon Bark, Cinnamon Leaves, and Cloves. Foods. 10(6): 1406.

Chung, H., Toivonen, P. and Moon, K. 2009. Effect of high vanillin treatment on storage quality of fresh-cut apples. Food Science and Biotechnology. 18(3): 636-640.

Crumpler, K., Abi Khalil, R., Tanganelli, E., Rai, N., Roffredi, L., Meybeck, A., Umulisa, V., Wolf, J. and Bernoux, M. 2021. Global update report: Agriculture, Forestry and Fisheries in the Nationally Determined Contributions. Food and Agriculture Organization of the United Nations Rome Italy.

Danh, L., Giao, B., Duong, C., Nga, N., Tien, T., Tuan, N., Huong, B., Nhan, T. and Trang D. 2021. Use of Essential Oils for the Control of Anthracnose Disease Caused by *Colletotrichum acutatum* on Post-Harvest Mangoes of Cat Hoa Loc Variety. Membranes. 11(9): 719.

Das, S., Vishakha, K., Banerjee, S., Mondal, S. and Ganguli, A. 2021. Antibacterial and antibiofilm effectiveness of bioactive packaging materials from edible sodium alginate and vanillin: Assessment on lettuce. Journal of Food Processing and Preservation. 45(9): e15668.

De-Montijo-Prieto, S., Razola-Díaz, M. D. C., Gómez-Caravaca, A. M., Guerra-Hernandez, E. J., Jiménez-Valera, M., Garcia-Villanova, B., Ruiz-Bravo, A. and Verardo, V. 2021. Essential oils from fruit and vegetables, aromatic herbs, and spices: composition, antioxidant, and antimicrobial activities. Biology. 10(11): 1091

Derito, C. and Madsen, E. 2009. Stable isotope probing reveals *Trichosporon* yeast to be active in *situ* in soil phenol metabolism. The ISME Journal. 3(4): 477-485.

Duan, X., OuYang, Q. and Tao, N. 2018. Effect of applying cinnamaldehyde incorporated in wax on green mold decay in citrus fruits. Journal of the Science of Food and Agriculture. 98(2): 527-533.

Duduk, N., Markovic, T., Vasic, M., Duduk, B., Vico, I. and Obradovic, A. 2015. Antifungal activity of three essential oils against *Colletotrichum acutatum*, the causal agent of strawberry anthracnose. Journal of Essential Oil-Bearing Plants. 18(3): 529-537.

Fallanaj F., Sanzani S.M., Zavanella C. and Ippolito A. 2013. Salt addition improves the control of citrus post-harvest diseases using electrolysis with conductive diamond electrodes. Journal of Plant Pathology. 95: 373–383.

Fitzgerald, D., Stratford, M., Gasson, M., Ueckert, J., Bos, A. and Narbad, A. 2004. Mode of antimicrobial action of vanillin against *Escherichia coli, Lactobacillus plantarum,* and *Listeria innocua*. Journal of applied microbiology. 97(1): 104-113.

He, S., Wang, Y., Sun, Y., Chen, S., Zhang, Y. and Ying, M. 2015. Antimicrobial activity and preliminary characterization of κ-carrageenan films containing cinnamon essential oil. Advance Journal of Food Science and Technology. 9(7): 523-528.

He, J., Wu, D., Zhang, Q., Chen, H., Li, H., Han, Q., Lai, X., Wang, H., Wu, Y., Yuan, J., Dong, H. and Qin, W. 2018. Efficacy and mechanism of cinnamon essential oil on inhibition of *Colletotrichum acutatum* isolated from 'Hongyang' kiwifruit. Frontiers in Microbiology. 9: 1288.

Horváth, A., Kovács, B. and Nagy, G. 2013. Application of mint and cinnamon against fusarium disease of winter wheat. Episteme Czasopismo Naukowo-Kulturalne. 18: 297-304.

Hong, J., Yang, H., Jung, H., Yoon, D., Sang, M. and Jeun, Y. 2015. Application of volatile antifungal plant essential oils for controlling pepper fruit anthracnose by *Colletotrichum gloeosporioides*. The Plant Pathology Journal 31(3): 269.

Jaimun, R. and Sangsuwan, J. 2019. Efficacy of chitosan-coated paper incorporated with vanillin and ethylene adsorbents on the control of anthracnose and the quality of Nam Dok Mai mango fruit. Packaging Technology and Science. 32(8): 383-394.

Kowalska, J., Tyburski, J., Matysiak, K., Jakubowska, M., Łukaszyk, J. and Krzymińska, J. 2021. Cinnamon as a useful preventive substance for the care of human and plant health. Molecules. 2(17): 5299.

Kumar, D., Arya, S. K., Shamim, M., Srivastava, D., Tyagi, M. and Singh, K. N. 2021. In vitro and in vivo activity of essential oils against postharvest pathogen *Colletotrichum musae* of banana. Journal of Postharvest Technology. 9(1): 53-63.

Li, Q. and Zhu, X. 2021. Vanillin and its derivatives, potential promising antifungal agents, inhibit *Aspergillus flavus* spores by destroying the integrity of the cell membrane rather than the cell wall. Grain & Oil Science and Technology. 4(2): 54-61.

López-Fernández, O., Rial-Otero, R., González-Barreiro, C., Simal-Ma, Q., Davidson, P. M. and Zhong, Q. 2016. Antimicrobial properties of microemulsions formulated with essential oils, soybean oil, and Tween 80. International Journal of Food Microbiology. 226: 20-25.

Maqbool, M., Ali, A., Ramachandran, S., Smith, D. R. and Alderson, P. G. 2010. Control of postharvest anthracnose of banana using a new edible composite coating. Crop Protection. 29(10): 1136-1141.

Ma, Q., Zhang, Y. and Zhong, Q. 2016. Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil, and ethylenediaminetetraacetate. LWT-Food Science and Technology. 65: 173-179.

Muche, B. and Rupasinghe, H. 2011. Natural antimicrobial agents of cinnamon (*Cinnamomum zeylanicum* L. and *C. cassia*) and vanilla (*Vanilla planifola*, *V. pompona* and *V. tahitensis*) for extending the shelf-life of fresh-cut fruits. Ethiopian Journal of Applied Science and Technology. 2(1): 1-13.

Park, J., Kim, S., Kim, N., Lee, S., Jeun, Y. and Hong, J. 2017. Differential inhibitory activities of four plant essential oils on in vitro growth of *Fusarium oxysporum* f. sp. *fragariae* causing *Fusarium* wilt in strawberry plants. The Plant Pathology Journal. 33(6): 582.

Romero-Cortes, T., Pérez España, V., López Pérez, P., Rodríguez-Jimenes, G., Robles-Olvera, V., José, B., and Cuervo-Parra J. 2019. Antifungal activity of vanilla juice and vanillin against Alternaria alternata. CyTA-Journal of Food. 17: 375-383.

Safari, Z., Ding, P., Juju Nakasha, J. and Yusoff, S. 2020. Combining chitosan and vanillin to retain the postharvest quality of tomato fruit during ambient temperature storage. Coatings. 10(12): 1222.

Sarkhosh, A., Schaffer, B., Vargas, A., Palmateer, A., Lopez, P. and Soleymani, A. 2018. In vitro evaluation of eight plant essential oils for controlling *Colletotrichum, Botryosphaeria, Fusarium and Phytophthora* fruit rots of avocado, mango and papaya. Plant Protection Science. 54(3): 153-162.

Sae-Eaw, A., Suppakul, P. and Chompreeda, P. 2019. Assessment of synergistic effects on antimicrobial activity in vapor-and liquid-phase of cinnamon and oregano essential oils against Staphylococcus aureus. International Food Research Journal. 26(2): 459-467.

Silveira, A., Moreira, G., Artés, F. and Aguayo, E. 2015. Vanillin and cinnamic acid in aqueous solutions or inactive modified packaging preserves the quality of fresh-cut Cantaloupe melon. Scientia Horticulturae. 192: 271-278.

Sun, J., Li, M., Wang, X., Zhang, Y., Yuan, R., Liu, H., and Zhu, H. 2014. Vanillin derivatives as the selective small molecule inhibitors of FtsZ. Medicinal Chemistry Research. 23: 2985-2994

Sun, X., Qian, Q., Xiong, Y., Xie, Q., Yue, X., Liu, J., Wei, S.and Yang, Q. 2022. Characterization of the key aroma compounds in aged Chinese Xiaoqu Baijiu by means of the sensomics approach. Food Chemistry. 384: 132452.

Triana, D., Tafdilla, M., Antika, L. and Ernawati, T. 2019. Conversion Eugenol to Vanillin: Evaluation of Antimicrobial Activity. In Proceedings of International Summit on Science Technology and Humanity, Indonesia. P-ISSN: 2477-3328 e-ISSN: 2615-1588.

Triest, D. and Hendrickx, M. 2016. The postharvest disease of banana caused by *Fusarium musae*: A public health concern. PLoS Pathogens. 12(11): e1005940.

Vijayalakshmi, S., Disalva, X., Srivastava, C. and Arun, A. 2019. Vanilla-natural vs artificial: a review. Research Journal of Pharmacy and Technology. 12(6): 3068-3072.

Wei, J., Bi, Y., Xue, H., Wang, Y., Zong, Y. and Prusky, D. 2020. Antifungal activity of cinnamaldehyde against *Fusarium sambucinum* involves inhibition of ergosterol biosynthesis. Journal of Applied Microbiology. 129(2): 256-265.

Werghemmi, W., Abou Fayssal, S., Mazouz, H., Hajjaj, H. and Hajji, L. 2022. Olive and green tea leaves extract in *Pleurotus ostreatus* var. florida culture media: Effect on mycelial linear growth rate, diameter and growth induction index. In IOP Conference Series: Earth and Environmental Science. 1090: 012020.

Wu, Y. L., Duan, X., Jing, G., OuYang, Q. and Tao N. 2017. Cinnamaldehyde inhibits the mycelial growth of *Geotrichum citri-aurantii* and induces defense responses against sour rot in citrus fruit. Postharvest Biology Technology. 129: 23-28.

Xing, F., Hua, H., Selvaraj, J., Zhao, Y., Zhou, L., Liu, X. and Liu, Y. 2014. Growth inhibition and morphological alterations of *Fusarium verticillioides* by cinnamon oil and cinnamaldehyde. Food Control. 46: 343-350.

Yarahmadi, M., Safaei, Z. and Azizi, M. 2014. Study the effect of chitosan, vanillin, and acetic acid on fungal disease control of *Rhizopus stolonifer* in strawberry fruits in vitro and in vivo. European Journal of Experimental Biology. 4(3): 219-225.

Zhou, X. and Wu, F. 2012. Dynamics of the diversity of fungal and *Fusarium* communities during continuous cropping of cucumber in the greenhouse. FEMS Microbiology Ecology. 80(2): 469-478.