Effect of Cinnamon Oil on Mycelial Growth of Three Pathogens Causing Crown Rot *in vitro* Condition, on Peduncle Discs and Banana

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Abstract

Effect of cinnamon oil on mycelial growth of three pathogens causing crown rot *in vitro* condition, on peduncle discs and on banana was investigated. *In vitro* experiment was a completely randomized design (CRD) with 4 treatments; control (CK) and concentration of cinnamon oil at 1%, 2% and 10%. Peduncle discs and the crown of banana hand has 3 treatment; control (distilled water), CO 2% and CO 10%. The result shown concentrations of cinnamon oil at 10% delayed mycelium growth of *Collectotrichum musae* and *Lasiodiplodia theobromae*. In addition, this treatment inhibits mycelium growth of *Fusarium* spp. and showed no contamination when compared with other concentration. In peduncle discs, treated with cinnamon oil after inoculation of the three pathogens was more efficient to delay a fungus infection. The inhibition was not observed in peduncle discs and the crown of banana hand, the concentration of cinnamon oil at 10% only delayed a severe disease.

Introduction

Banana fruit (*Musa* spp.) is a commercially important fruit crop of the world. The banana is a tropical fruit, which is a native plant of Southeast Asia. However, it's short shelf life and suffers severe postharvest losses seriously limit the marketing of the fruits. Major losses often occur during

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shipping of bananas to their final market, mainly because of ripping during shipping (bananas should reach ripening room unripe), appearance defects, and storage decay such as anthracnose and crown rot that are caused by fungal pathogens (Lassos *et al*., 2010).

Crown rot is the most important postharvest disease of banana fruit throughout the world. A syndrome caused by several fungi, including *Lasiodiplodia theobromae, Colletotrichum musae*, *Thielaviopsis paradoxa*, and the *Fusarium* spp. complex (Alvindia *et al*., 2012). The rot causes blackening and softening of tissues, which begins at or near the cut surface of crescent shaped crown. Fungicides have provided effective control of major postharvest diseases, but this method may have the toxic to human and the environment and they are effective after prolonged use. (Abd-Alla *et al*., 2014).

In addition, many consumers prefer banana fruits which were not used any chemical as a pesticide and fungicide. (Alvindia *et al*., 2012). Medicinal plants are alternative methods that can control fungal disease, because a range of biological activities had been found in plant extracts.

Cinnamon (*Cinnamomum zeylanicum*) originated in Tropical Asia, and was particularly widely used in Sri Lanka and India. Cinnamon oil is obtained from the bark and leaves of cinnamon trees (Ranasinghe *et al*., 2013). Cinnamon oil does have various applications in aromatherapy. Due to its antifungal, antibacterial, antiviral and antiseptic properties, it is effective in treating external as well as internal infections. The objective of this experiment was to investigate the effect of cinnamon to against the pathogens causing crown rot and anthracnose disease in vitro condition.

Materials and Methods

Fungal pathogens

The crown rot causing pathogens of banana are *Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae*. Isolation method, infected crowns of banana was cut, placed on PDA surface and incubated at 25℃ for 7 days. Fungal colonies that appeared were sub-cultured and identified according to the key and description of Barnett & Hunter (1972).

Prepare the cinnamon oil (CO)

Cinnamon branches were cleaned by water and peeled. The experiment, we used peels (barks) to extract by a water distillation method for 2 hours and collected the essential oil. Kept the oil in a flask and closed by parafilm for the stock at 1℃

In vitro experiment

Potato infusion was mixed with different concentrations of cinnamon oil (CO) which used a water distillation method. Cinnamon oil was mixed with potato infusion after the potato infusion was added agar powder and sterilized by autoclaving for 35 minutes. The medium was poured into an antiseptic plate (90 × 15 mm) and waited 1-2 week to make sure the mediums are not contaminated. Each treatment has 5 plates. An isolate of each corresponding fungal pathogen was grown on full strength PDA for 7 days at 25℃. Using a sterile cork borer (diameter 4 mm), 5 mycelium discs were transferred aseptically into a plate of each treatment and kept at 25℃. Colony growth was measured for 7 days. This experiment has 4 treatments include a control (PDA), 1%, 2% and 10% of cinnamon oil.

Peduncle experiment

The first is the preparation of conidial suspension of three pathogens causing crown rot diseases. The conidial suspensions of three fungi were obtained from 7-day-old cultures. The conidial were raked and transferred to beakers containing 200 ml of distilled water. The resulting conidial suspension was diluted serially (10^{-5} spores/ml). The second is preparation of different concentrations of cinnamon oil. Cinnamon oil experiment has 3 treatments which included control (distilled water), 2% and 10%. The last is inoculation of three fungi on the peduncle. The peduncle was cut as a disc shape that 3 mm of thinness (stem disc) and with/without immersing in conidial suspension water. This studied have two conditions; including dipped medicine before inoculated (MI), and inoculated before dipped medicine (IM). The peduncle discs were immersed in conidial suspension water for 5 min and cinnamon oil or clove extract for 5 min. They were stored at 15℃ for 3 weeks and measured.

The crown of banana hand experiment

'Pei-Chiao' Bananas (*Musa* spp., Giant Cavendish, AAA Group) were harvested with the first stage color which is an evenly green from the Viticulture Research Station, National Chung Hsing University. The bunches were cut into individual banana hands (3-4 fruits/hand). Cinnamon oil experiment has 3 treatments which included control (distilled water), 2% and 10%. The crowns of the banana hand were immersed in cinnamon oil for 5 min. Then, bananas were ripened by ethylene (C_2H_4) as above-mentioned. Finally, bananas were measured on the first day of the experiment (before inoculation) and 0 days, 2 days, 4 days after incubation by ethylene.

Statistical analysis

The data of the experiment underwent statistical analysis using SAS 9.0 (Institute Inc., 2002) and was subjected to one-way analysis of variance (ANOVA) for a completely randomized design

(CRD) statistical model. The significant differences among treatment were separated by LSD test using. Mean values among treatments, when significant, were compared by least significant difference tests at the 5% ($P \leq 0.05$) level of significance.

Results

1. *In vitro* experiment

This inhibition successively increased with the increasing of cinnamon oil concentration on all fungi. Colony diameter of *Collectotricrum musae* in different concentrations of Cinnamon oil (CO) was shown in table 1. Mycelium on PDA which treated with cinnamon oil was significantly less than other treatment during the experiment. Mycelium of *Collectotricrum musae* was not grown for 2 days. The best treatment is cinnamon oil at 10%. A significantly complete inhibition was observed in the cinnamon oil concentration at 10% in Mycelium of *Fusarium* spp. that showed in table 2. In Colony diameter of *Lasiodiplodia theobromae*, delayed action was observed in treated with 10% cinnamon oil treatments even though inhibition was not found in this fungus. Concentration of cinnamon oil at 10% could delay by 4.070 mm on the first day (Table 3.). Reduction of colony diameter (%) of three pathogens causing crown rot, in different concentrations of cinnamon oil (CO) was shown in Table 5. The treatment which was the highest reduction is cinnamon oil at 10% by 100% in *Fusarium* spp. for 7 days and in *Collectotricrum musae* for 2 days. Although the inhibition was not detected in *Lasiodiplodia theobromae*, 10% cinnamon oil had a high reduction of 99.73% on the first day.

2. Peduncle and the crown of banana hand

In botany, a peduncle is a stem supporting an inflorescence, or after fecundation, an infructescence. When the cluster was cut from the bunch, these fungi infect the crown through fresh wounds created after trimming the crown of the banana hand into a crescent shape.

In peduncle discs, effect of cinnamon oil by dipped medicine before inoculated (MI) and inoculated before dipping medicine (IM) were showed in figure 4. In *Collectotricrum musae*, peduncle discs dipped in 10% cinnamon oil showed a good appearance. Moreover, inoculated before dipped medicine part showed 10% cinnamon oil treatment was not found any fungi on peduncle discs.

In *Fusarium* spp., 10% of cinnamon oil treatment had less mycelium of fungi grown on peduncle discs all conditions (MI and IM). Treated with cinnamon oil before or after inoculation had the same effect that 10% cinnamon oil treatment is the best appearance which was less incidents of fungi

growth than other treatment. The result of *Lasiodiplodia theobromae* showed no any different between MI and IM.

In bananas, the crown of banana hands, which is a crescent shape was treated with different concentration of cinnamon oil and untreated is a control. So, it had no effect on banana's appearance. All treatments were generally ripened by incubation bananas. When considered in a crescent shape of banana hand's crown, the least mycelium of fungi were observed in 10% cinnamon oil treatment on day 2 and 4 after ripening bananas. This result was demonstrated in figure 1.

Table 1. Colony diameter of *Collectotricrum musae* grown on media containing different concentrations of cinnamon oil (CO)

Treatments	Colony diameter (mm)						
	1[z]	2	3	4	5	6	7
CK	8.6a[y]	17.3a	28.2a	39.7a	50.8a	62.1a	75.9a
1%	7.8b	15.6b	25.9b	37.1b	47.8b	59.3b	72.7ab
2%	6.7c	14.5c	25.3b	34.8c	45.3c	57.2b	69.6b
10%	4.0d	4.0d	9.0c	16.8d	25.0d	33.8c	45.8c

Table 2. Colony diameter of *Fusarium* spp. grown on media containing different concentrations of cinnamon oil (CO)

Treatments	Colony diameter (mm)						
	1[z]	2	3	4	5	6	7
CK	11.0a[y]	27.3a	46.6a	70.0a	86.0a	90.0a	90.0a
1%	9.4b	24.3b	42.6b	60.4b	75.0b	84.1a	85.5b
2%	7.5c	21.7c	39.4c	57.0c	73.4b	84.3a	90.0a
10%	4.0d	4.0d	4.0d	4.0d	4.0c	4.0b	4.0c

Table 3. Colony diameter of *Lasiodiplodia theobromae* grown on media containing different concentrations of cinnamon oil (CO)

Treatments	Colony diameter (mm)						
	1[z]	2	3	4	5	6	7
CK	29.3a[y]	68.3a	90.0a	90.0a	90.0a	90.0a	90.0a
1%	25.7b	60.0b	90.0a	90.0a	90.0a	90.0a	90.0a
2%	24.0b	59.5b	89.3a	90.0a	90.0a	90.0a	90.0a
10%	4.1c	7.6c	18.8b	33.9b	51.7b	67.1a	72.8a

[z] Indicated about days of experiment.

[y] Means followed by the same letter in a column are not significantly different according to LSD test ($P < 0.05$).

Table 4. Effect of Cinnamon oil on peduncle discs with /without three pathogens causing crown rot stored at 15°C during after 3 weeks

Pathogens	Concentrations	Experiments	
		Dipped medicine before inoculated (MI)	Inoculated before dipping medicine (IM)
Collectotricrum musae	CK (0%)	5	3
	2%	4	2
	10%	4	1
Fusarium spp.	CK (0%)	4	3
	2%	2	3
	10%	1	1
Lasiodiplodia theobromae	CK (0%)	2	2
	2%	2	2
	10%	1	1

[z] the fungi growth was categorized on a scale from 1 to 5. 1 = no mycelium growth or mycelial growth on surface of the peduncle discs 0-20%; 2 = mycelial growth on surface of the peduncle discs 21-40%; 3 = mycelial growth on surface of the peduncle discs 41-60%, 4 = mycelial growth on surface of the peduncle discs 61-80%; 5 = mycelial growth on surface of the peduncle discs 81-100%.

Table 5. Reduction of colony diameter (%) in three pathogens of crown rot in different concentrations of cinnamon oil (CO) during 7 days of experiment stored at 25°C

Concentration	Pathogen	Reduction of colony diameter (%) during experiment[z]						
		1[y]	2	3	4	5	6	7
1%	*Collectotricrum musae*	17.59	12.66	9.52	7.29	6.40	4.90	4.39
	Fusarium spp.	22.40	12.83	9.41	14.56	13.36	6.85	5.22
	Lasiodiplodia theobromae	14.37	12.87	0.00	0.00	0.00	0.00	0.00
2%	*Collectotricrum musae*	41.68	20.93	11.96	13.70	11.78	8.53	8.73
	Fusarium spp.	49.45	24.19	16.95	19.63	15.33	6.60	0.00
	Lasiodiplodia theobromae	21.02	13.67	0.86	0.00	0.00	0.00	0.00
10%	*Collectotricrum musae*	100.00	100.00	79.27	64.22	55.18	48.79	41.94
	Fusarium spp.	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	Lasiodiplodia theobromae	99.73	94.40	82.76	65.23	44.54	26.62	20.00

[z] Reduction of colony diameter (%) = colony diameter of the control - colony diameter of treatment/ colony diameter of the control x 100

[y] Indicated about days of experiment

[x] Minus sign and zero means no effect and cannot inhibit pathogens

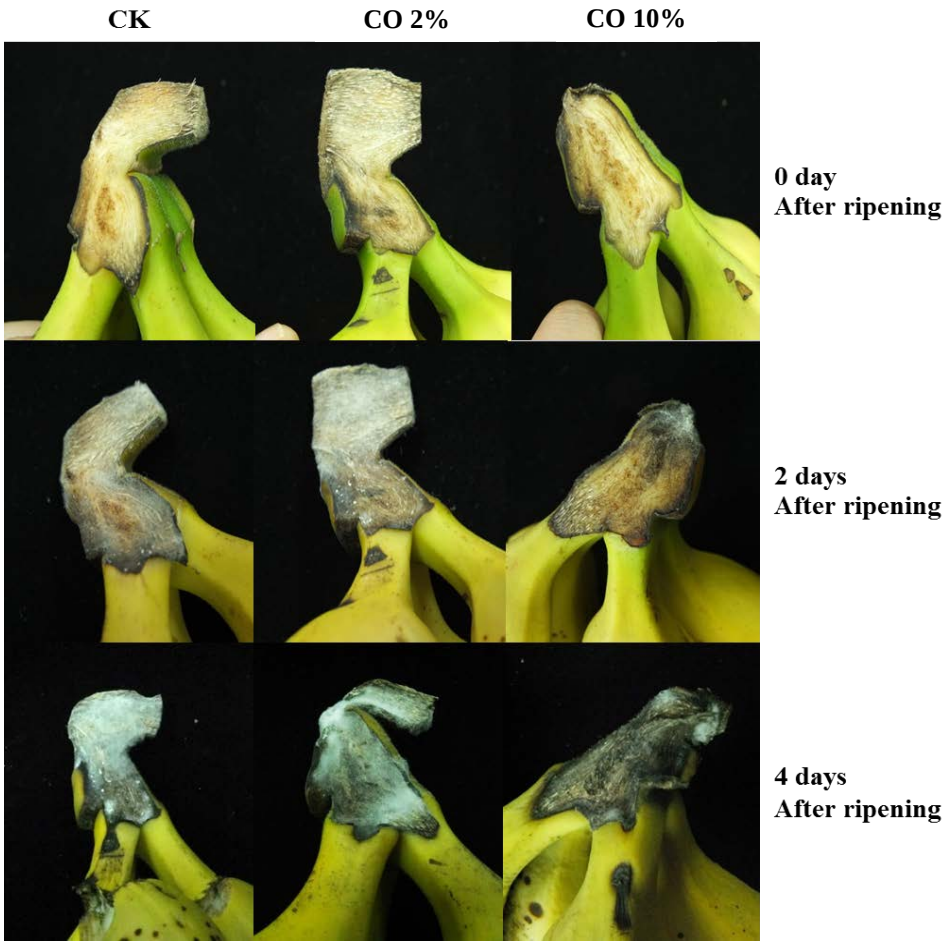

Fig. 1. Effect of different concentrations of cinnamon oil in crown rot disease of banana on the day after ripening

Discussion and Conclusion

Our study has shown cinnamon oil had an antifungal activity. The inhibitory effect of cinnamon, citral, litsea cubeba oil, clove, eucalyptus, anise, spearmint and camphor oils on *F. verticillioides* was investigated, and cinnamon oil proves to be the most effective of inhibition (Xing *et al*., 2014). Scanning electron microscopy and transmission electron microscopy of *F. verticillioides* treated with

cinnamaldehyde showed irreversible deleterious morphological and ultrastructural alterations, such as lack of cytoplasmic contents, loss of integrity and rigidity of the cell wall, plasma membrane disruption, mitochondrial destruction and folding of the cell (Xing *et al*., 2014). The main compound is cinnamaldehyde which is the compound containing an aldehyde group and conjugated double bond outside the ring. This compound possesses much stronger antifungal activity (Wang *et al*., 2005). It may be a potential main compound to obstruct β -(1,3)-glucan and chitin synthesis in yeasts and molds (Bang *et al*., 2000).

So, cinnamon oil can be used commercially as a safe method for treating banana fruits. Such control methods for postharvest diseases are more imperative due to the development of new resistant pathogens to conventional fungicides, whilst increasing concerns of the consumers on the use of permissible fungicides on fruits. However, we should use in appropriate dose because of a tolerance of fruits and an allergy in some people.

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肉桂油對三種造成香蕉軸腐病菌菌絲生長之影響

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關鍵字：香蕉、肉桂油、菌絲生長、軸腐病

摘要：本試驗目的為了調查肉桂油對三種造成香蕉軸腐病的真菌菌絲生長、對香蕉果軸及果實之影響。在真菌離體培養試驗中採完全隨機設計，共有四組處理，分別為1%、2%和10%肉桂油處理和對照組；對香蕉果軸圓片和果軸共有三種處理：對照組(蒸餾水)、2%和10%肉桂油處理。結果顯示10%肉桂油對 *Collectotrichum musae* 和 *Lasiodiplodia theobromae* 之菌絲生長有延遲的效果，此外，10%肉桂油處理也抑制 *Fusarium* spp.菌絲生長；接種三種病原菌之果軸圓片上，再處理肉桂油，有效的延緩真菌的感染；10%肉桂油處理並沒有抑制，僅延緩果軸及果手果梗上病害的發生。

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