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Assessment of bioactive compounds and synergistic efffects from *Miliusa sessilis* plant against anthracnose diseases on mango fruits (*Mangifera indica* L. 'Nam Dok Mai Si Thong')

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Abstract Three bioactive neolignans: dehydrodieugenol A (dA), dehydrodieugenol B (dB), and miliusin A (mA) were purified from *Miliusa sessilis* (MSE). The bioactive compounds (dA, dB, mA, dB-mA, mA-dA and dB-dA) at concentrations of 50, 100, 500, 1000, 2000, 3000, 4000 and 5000 ppm were carried out to determine antifungal activity for controlling *Colletotrichum* sp. The result showed that dB-dA at 5000 ppm inhibited mycelium growth by 43.46%, while dA and dB had a high fungal inhibition effect of 43.46% and 45.50%, respectively. However, there was no significant difference in these compounds ($p<0.01$). Furthermore, the potential antifungal activity using poison food technique revealed the greatest percentage inhibition in significant difference ($p<0.01$) compared to control and all treatments at 1000 ppm of mA, dA and dB-dA as 76.07%, 75.38% and 74.79%, respectively. The MIC values of dA, dB-mA, mA-dA and dB-dA were 6.25 ppm exceptionally; dB and mA had higher MIC values (12.5 ppm). *In vivo* antifungal activity evaluation, MSE had efficacy to suppress and reduce lesions on mango fruits. These findings supported that the synergism of MSE and showed strong antifungal effects and could be developed as an alternative to synthetic fungicides.

Keywords: Mango disease, *Colletotrichum* sp., Synergism, Antifungal combination effect, Neolignans

Introduction

Mango (*Mangifera indica* L. 'Nam Dok Mai Si Thong') is one of the economically fruit crops in Thailand and the most popular cultivar. Thailand was ranked the world's largest producer and exporter of mango fruit from 2018 to 2022 (FAO, 2024). All production areas have the major problem of anthracnose disease caused by *Colletotrichum* sp., especially infects mango fruits. The anthracnose symptom in fruit is a significant economic problem in postharvest

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disease and lead to economic losses in both quantity and quality for production (Paull and Duarte, 2011, Rattanakreetakul *et al*., 2023). Currently, people concern to effect of chemical pesticides on environmental health and development of microbial chemical resistance. Thus, using the plant natural ingredients can be a good choice for consideration as alternative biological control agents to reduce synthetic chemicals. Plant extracts have been reported widely used as antimicrobial agent to inhibit the mycelial growth (Harvey, 2008). According to Amenu (2014), higer plants are source of many phytochemical compounds that demonstrated action of biolological activity against a wide variety of plant pathogenic microorganism.

Miliusa sessilis (Annonaceae) is native forest plant which located in the southern part of Thailand (Chaowasku and Kessler, 2014). The current research articles have shown that *Miliusa* genus contained various phytochemical compounds. *Miliusa* have been reported to pharmacological and phytochemical studies. The phytochemical components of this plant genus were isolated and consist of flavonoids, lignans, neolignans, alkaloids, phenolics (Son, 2019, Pootaeng-on *et al*., 2020). However, the database of these compounds has been unreported and remarkable potential for plant pathogens inhibition. Plant bioactive compounds were used to examine the biological effects that usually show efficacy of single bioactive compounds. Nowadays, activity of plant bioactive compounds combination was presented more effective than individual bioactive compounds (Efferth and Koch, 2011, Wagner and Ulrich-Merzenich, 2009). Numerous research articles have been reported the antimicrobial activity enhancement with synergistic interaction (Hosseini-Zare *et al*., 2021, Sitarek *et al*., 2020, Vaou *et al*., 2022). However, there are inadequantely scientific research to investigate the efficacy of combination effects of bioactive compounds for plant fungal pathogen controlling.

This study was aimed to investigate the antifungal efficacy of bioactive compounds from MSE *in vitro* and *in vivo* study and their synergistic effects and to determine the chemical compounds of MSE.

Materials and methods

General experimental procedure

IR spectra were measured by a PerkinElmer GX FT-IR spectrophotometer. 1D NMR experiments were recorded on a Brüker AVANCE 300 MHz spectrometer (^{1}H at 300 MHz and ^{13}C at 75 MHz) with TMS as internal standard. UV spectra were obtained on a Hewlett Packard 8453 UV–vis-spectrometer. Column chromatography (CC) was carried out on silica gel (Merck, 70–230

mesh or 230–400 mesh). Preparative TLC was performed on precoated silica gel 60_{F254} Merck Merck. Compounds were visualized under UV light or by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating. Ethanol (EtOH), *n*-hexane (*n*-Hex), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH) for extraction and chromatography were distilled at their boiling ranges prior to use. Methanol (MeOH) (analytical grade, Merck, Germany) were used for the ultraviolet analysis.

Preparation of purified bioactive compounds from Miliusa sessilis

The twigs of *M. sessilis* Chaowasku and Kessler (2014) sp. nov. (Annonaceae) were collected and deposited as described by Pootaeng-on *et al*., 2020. The air dried, ground twigs of *M. sessilis* (9.6 kg) were extracted with 95% EtOH (3 × 14 l) at room temperature. The ethanolic extract was filtered and concentrated under reduced the pressure to give the EtOH extract (481g). The EtOAc extract was prepared using the sequential liquid-liquid extraction method by dissolving in water and then successively partitioned with *n*-Hex, EtOAc and *n*-BuOH yielding *n*-Hex, EtOAc, *n*-BuOH, and water fraction as 9.92, 63.4, 51.2, and 278.7 g, respectively. The EtOAc extracte soluble fraction (60 g) was subjected to silica gel flash column chromatography (CC, 14×7 cm), using a gradient system of *n*-Hex-EtOAc (100:0 to 0:100) as the eluent to obtain 19 fractions (E1-E19). Fraction E4 (2.1111g) was purified by silica gel CC (3.5×10 cm) using (5-15%) EtOAc in *n*-Hex as the eluent to give a pale green viscous liquid as dehydrodieugenol B (**dB**) (1.0933 g). Fraction E7 (5.1290 g) was subjected to silica gel CC (4.5× 10 cm) using (5-30%) EtOAc in *n*-Hex as the eluent to obtain a pale greenish-yellow crystal as miliusin A (**mA**) (2.5624 g). Fraction E9 (3.5622 g) was purified by silica gel CC (4.5× 10 cm) using (5-35%) EtOAc in *n*-Hex as the eluent to obtain a pale-yellow needle crystal as dehydrodieugenol A (**dA**) (1.6534 g)

Dehydrodieugenol A (**dA**) Pale yellow needle crystal; UV (MeOH) λ_{max} nm (log ε): 219 (4.65), 253 (4.01), 290 (3.83); IR (neat) ν_{max}: 3252, 1639, 1599, 1490, 1467, 1454, 1424, 1327, 1257, 1229, 1145, 1047, 996, 907 and 852 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1).

Dehydrodieugenol B (**dB**) Pale green viscous liquid; UV (MeOH) λ_{max} nm (log ε): 205 (4.78), 230 (4.26), 277 (3.80); IR (neat) ν_{max}: 3439, 1638, 1597, 1505, 1454, 1434, 1314, 1265, 1213, 1129, 1083, 1034, 994, 914 and 832 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1).

Miliusin A (**mA**) Pale greenish-yellow crystal; UV (MeOH) λ_{max} nm (log ε): 205 (4.93), 230 (4.36), 277 (3.95); IR (neat) ν_{max}: 3421, 1740, 1610, 1513, 1495, 1329, 1262, 1238, 1131 and 1029; ^{1}H and ^{13}C NMR data (Table 1).

Isolation and identification of fungal pathogens

The ripe mango fruits (Nam Dok Mai Si Thong) showing symptoms of anthracnose disease from a local market in Huahin, Prachuap Khiri Khan, Thailand were collected for isolation. The lesions of infected mango were cut out in 5 mm^2 pieces with a sterile scalpel. The infected mango tissues were soaked in 5% sodium hyperchlorite for 3 min, then rinsed in sterile distilled water for 3 times and placed on sterile paper. Infected tissues were plated on potato dextrose agar (PDA) and incubated at room temperature for 48-72 hours. The fungal mycelia were transferred to a new PDA plate and placed in the incubator for 7 days at 25±2ºC for further study. The microscopic and macroscopic morphology were investigated and identified as described by Alexopoulos *et al*. (1996) and Barnett and Hunter (1986).

Antifungal activity test

The efficacy of bioactive compounds (MSE) on antagonistic potential against *Colletotrichum* sp. was performed on PDA medium by *in vitro* dual culture and poisoned food techniques. A ten-day-old *Colletotrichum* sp. culture on PDA was prepared to use for testing. Three bioactive compounds: dehydrodieugenol A (dA), dehydrodieugenol B (dB), miliusin A (mA) and compound combinations (dA-dB, dA-mA, dB-mA and dA-dB-mA) from *M. sessilis* were prepared at different concentrations. Dimethyl sulfoxide (DMSO) was used as a solvent to dissolve the compounds and as a negative control in treatments. All treatments with four replications for each concentration were incubated at room temperature.

Preliminary antagonistic screening was conducted by a dual culture method. A mycelial disc of *Colletotrichum* sp. was placed on the center of PDA in petri dishes. The 20 µl of bioactive compounds at various concentrations (0, 50, 100, 500, 1000, 2000, 3000, 4000 and 5000 ppm) were pipetted onto sterile Whatman filter paper discs (6 mm in diameter) and placed in PDA plate 2 cm apart from the fungal agar plug with the peripheral region. After 5 days, the rate of mycelial growth inhibition was calculated according to the following equation (Mahadtanapuk *et al*., 2007):

$$MGI = [(R_1 - R_2)/R_1] * 100$$

Where MGI represents the percentage (%) of mycelial growth inhibition. R_1 and R_2 were radial distance growth (cm) of the fungal pathogen in control and treatments, respectively.

The bioactive compounds were evaluated for the antifungal activity by the poisoned food technique (Kursa *et al*., 2022) as performed in the PDA plates amended with three bioactive compounds at different concentrations (0, 250,

500, 750 and 1000 ppm). Fungal pathogen plates without any bioactive compounds served as control. The culture disc with 6 mm diameter was placed in the middle of the PDA plate and then incubated at room temperature for 10 days. The diameter of fungal colonies was measured in cm and calculated the percentage of mycelial growth inhibition rate as follows: Inhibition percentage (I%) = $[(D_1 - D_2)/D_1] * 100$ where D_1 is the colony diameter in control and D_2 is the colony diameter of treated with MSE.

Determination of minimum inhibitory concentration (MIC)

The broth dilution method (Zanna *et al*., 2021) was used to determine the MIC values. Fungal suspension of *Colletotrichum* sp. was initially prepared by cultured in potato dextrose broth for 10 days at 26ºC using a shaking incubator at 180 rpm. Three ml of fungal suspension was transferred to sterile test tube. MSE and compound combinations at different concentrations (6.25, 12.5, 25, 50, 100, 250 and 500 ppm) were added into the test tubes. The negative control was DMSO, while the positive control was culture broth of *Colletotrichum* sp. The MIC was defined as a minimal concentration of MSE that inhibited visible growth. Microscopic morphology was evaluated under a light microscope.

In vivo detection of bioactive compounds (MSE) activities against Colletotrichum sp. on mango fruits

The antifungal potential of MSE and compound combinations to suppress *Colletotrichum* sp. growth was tested on mango fruits during the first stage of ripening. Mango fruits were washed under running water, surface disinfected with 1% sodium hypochlorite solution for 2 min, then immersed in 70% ethanol for 2 min and rinsed twice with sterile water and dried in a laminar flow hood. Fruit surface was wounded in three-point area with a sterile needle. A mycelial disc (6 mm in diameter) of *Colletotrichum* sp. that cultured on a 7-day-old PDA plate was placed on each wound. A non-colonized agar disc was used as the control. The fruits were incubated at room temperature in a clear plastic box with cotton moistened with sterile distilled water. The fruits were determined by measuring lesion diameter after inoculation by the causal fungi at 7 days. Untreated fruits with MSE were used as control and all experiments were preformed with three replications per treatment.

Statistical analysis

The measurements of the mycelial growth inhibition rate from these experiments were analyzed and compared using Duncan’s multiple range test

(DMRT) at the $p<0.01$ significance level. This study was designed in completely randomized design (CRD) and carried out with 4 replications per treatment. R statistical software was used to conduct the statistical analysis.

Results

Structure elucidation and identification

The ethyl acetate extract (MSE) obtained from the ethanolic extract of twigs of *M. sessilis* were separated and pureifide by Chromatographic fractionation to provide tree bioactive neolignans including dehydrodieugenol A, dehydrodieugenol B, and miliusin A. The IR spectral data, ^{1}H NMR, ^{13}C NMR, and DEPT spectral data of these neolignan are showed in Table 1 and Figure 2-4.

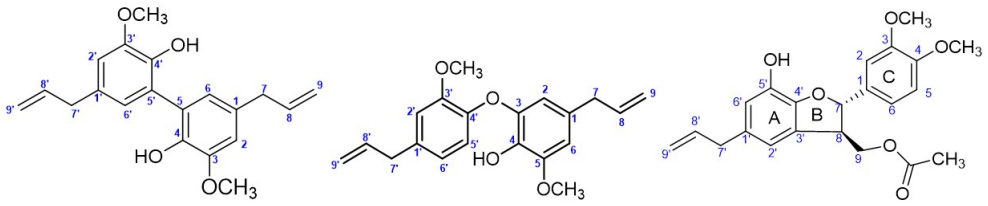

Figure 1. Structures of dehydrodieugenol A (dA), dehydrodieugenol B (dB) and miliusin A (mA) form the twigs of *M. sessilis*

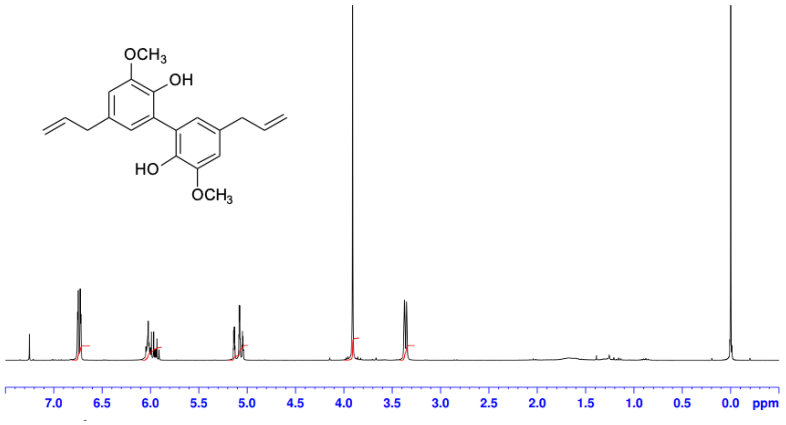

Figure 2. ^{1}H NMR spectrum of dehydrodieugenol A (300 MHz, $CDCl_3$)

Table 1. ^{1}H NMR (300 Hz) and ^{13}C NMR (75 MHz) spectral data for dehydrodieugenol A, dehydrodieugenol B, and miliusin A in $CDCl_3$ (J in Hz in parentheses)

Position	Dehydrodieugenol A		Dehydrodieugenol B		Miliusin A	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
1	-	131.1	-	131.0	-	132.9
2	6.75 (1H, d, 1.9)	110.6	6.39 (1H, d, 1.8)	111.8	6.90 (1H, d, 1.8)	109.1
3	-	147.1	-	144.4	-	149.3
4	-	140.8	-	135.2	-	149.3
5	-	124.3	-	147.9	6.85 (1H, d, 8.1)	111.1
6	6.72 (1H, d, 1.9)	123.3	6.49 (1H, d, 1.8)	107.3	6.92 (1H, dd, 8.1, 1.8)	118.8
7	3.36 (2H, d, 6.7)	40.0	3.23 (2H, d, 6.8)	39.9	5.42 (1H, d, 7.7)	88.7
8	5.98 (1H, ddt, 16.8, 9.9, 6.7)	137.6	5.91 (1H, ddt, 16.1, 9.4, 6.8)	137.4	3.80 (1H, pq, 5.5)	50.8
9a	5.09 (1H, dd, 16.8, 1.6)	115.6	5.05 (1H, dd, 16.1, 1.4)	115.7	4.45 (1H, dd, 11.1, 5.5)	65.4
9b	5.06 (1H, dd, 9.9, 1.6)		5.04 (1H, dd, 9.4, 1.4)		4.30 (1H, dd, 11.1, 7.7)	
1′	-	131.8	-	136.4	-	134.2
2′	6.72 (1H, d, 1.9)	110.6	6.79 (1H, d, 1.8)	113.0	6.68 (1H, *br* s)	116.3
3′	-	147.1	-	150.4	-	139.9
4′	-	1408	-	144.2	-	144.8
5′	-	124.3	6.89 (1H, d, 8.1)	119.5	-	127.2
6′	6.75 (1H, d, 1.9)	123.3	6.70 (1H, dd, 8.1, 1.8)	120.8	6.60 (1H, *br* s)	116.1
7′	3.36 (2H, d, 6.7)	39.9	3.36 (1H, d, 6.8)	39.9	3.30 (2H, d, 6.6)	39.8
8′	5.98 (1H, ddt, 16.8, 9.9, 6.7)	137.5	5.99 (1H, ddt, 17.0, 10.4, 6.8)	137.3	5.92 (1H, ddt,16.8, 9.9, 6.6)	137.7
9′a	5.09 (1H, dd, 16.8, 1.6)	115.6	5.11 (1H, dd, 17.0, 1.5)	115.9	5.07 (1H, dd, 16.8, 1.8)	115.7
9′b	5.06 (1H, dd, 9.9, 1.6)		5.10 (1H, dd, 10.4, 1.5)		5.08 (1H, dd, 9.9, 1.8)	-
OCH_3-3	3.91 (3H, s)	55.9	-	-	3.86 (3H, s)	56.0
OCH_3-4	-	-	-	-	3.88 (3H, s)	56.0
OCH_3-5	-	-	3.88 (3H, s)	56.2	-	-
OCH_3-3′	3.91 (3H, s)	55.9	3.86 (3H, s)	56.0	-	-
OH-4	6.04 (1H, *br* s)	-	-	-	-	-
OH-4′	6.04 (1H, *br* s)	-	-	-	-	-
O<u>C</u>(O)CH_3-9	-	-	-	-	-	170.9
OC(O)<u>C</u>H_3-9	-	-	-	-	-	20.8

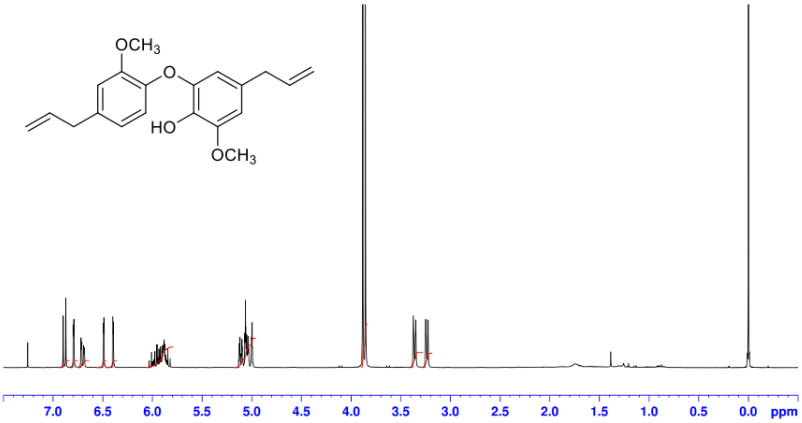

Figure 3. ^{1}H NMR spectrum of dehydrodieugenol B (300 MHz, $CDCl_3$)

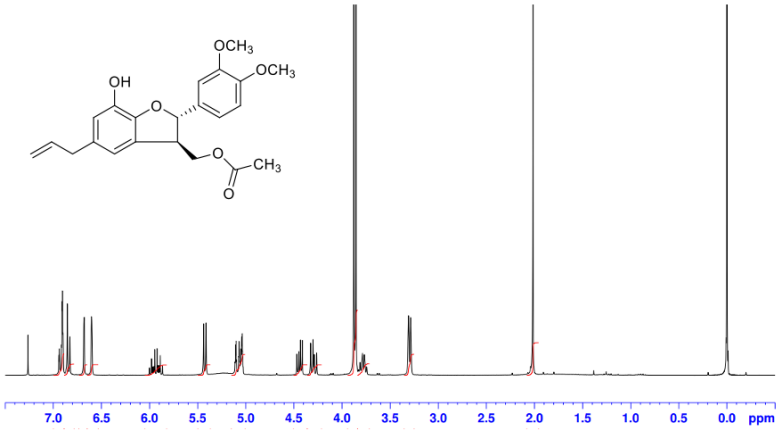

Figure 4. ^{1}H NMR spectrum of Miliusin A (300 MHz, $CDCl_3$)

Isolation and identification of fungal pathogen

Based on colony morphology, *Colletotrichum* sp. obtained from symptomatic mango tissues exhibited white to grayish color of mycelium (Figure 5, A and B). Conidia were one-celled, cylindrical shape, smooth-walled with hyaline and produced dark brown sharp setae (Figure 5, C and B).

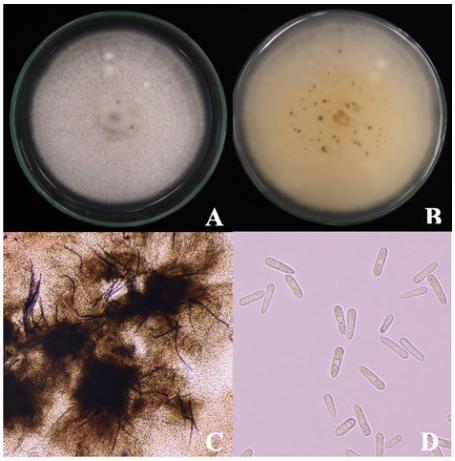

Figure 5. Macroscopic and microscopic studies of *Colletotrichum* sp. A; upper view of colony on PDA, B; reverse view of colony, C; acervulus with setae, D; conidia

Antifungal activity of isolated bioactive compounds

The efficacy of MSE and synergistic activity against *Colletotrichum* sp. were determined by dual culture and poisoned food assay. Based on the dual culture assay, bioactive compounds; dB, dA and dB-dA at 5000 ppm showed the highest antifungal activity against *Colletotrichum* sp. with 45.50%, 43.46% and 43.46% inhibition, respectively, when compared to control and other treatments in significant difference ($p<0.01$) (Table 2). Further study, the poisoned food technique was used to evaluate the antifungal activity and revealed that mA, dA and dB-dA (5000 ppm) had the lowest fungal growth rate and showed a significant difference ($p<0.01$) in rate of inhibition as 76.07%, 75.38% and 74.88%, respectively compared with control (Table 3). Obviously, the percentage of fungal growth inhibition was enhanced by increasing concentration (Figure 6).

Determination of minimum inhibitory concentration (MIC)

In order to determine the MIC of these compounds, a serial dilution of MSE and compound combinations at a concentration of 500 ppm showed an effect to inhibit visible growth of *Colletotrichum* sp. by slight turbidity in sterile culture tube. The MIC of bioactive compounds (dB, mA and dA) were 12.5, 12.5 and 6.25 ppm, respectively. As for compound combinations, the MIC value was shown as the lowest concentration. In addition, MSE and compound combinations had the growth inhibiting effect on the hyphae that represented hyphal deformation with bulbous swelling and lysis (Figure 7).

Table 2. Percentage (%) of growth inhibition of MSE and compound combinations *in vitro* was evaluated by dual culture test

Concentration (ppm)	Percentage (%) inhibition[1/]							
	Bioactive compounds							
	DMSO	dB	mA	dA	dB-mA	mA-dA	dB-dA	CV
	0.15							
50		15.53^{Y}	4.63^{Z}	8.72^{Z}	9.40^{Y}	8.72^{Z}	23.02^{Z}	70.79
100		16.21$^{BC/Y}$	12.81$^{C/Z}$	30.52$^{A/Y}$	10.08$^{C/Y}$	8.04$^{C/Z}$	24.39$^{AB/YZ}$	43.26
500		20.98^{Y}	23.02^{Y}	34.60^{Y}	29.16^{X}	30.52^{Y}	31.88^{XYZ}	21.69
1000		25.75^{Y}	26.43^{XY}	36.65^{XY}	30.52^{X}	32.56^{Y}	33.24^{XY}	28.10
2000		38.69^{X}	29.16^{XY}	37.33^{XY}	31.88^{X}	34.60^{XY}	34.60^{VX}	11.05
3000		40.06^{X}	31.88^{XY}	38.01^{XY}	33.92^{X}	35.97^{XY}	38.01^{VX}	13.38
4000		42.10^{X}	33.24^{X}	42.10^{X}	34.60^{X}	35.97^{XY}	41.42^{VX}	10.15
5000		45.50$^{A/X}$	34.60$^{C/X}$	43.46$^{AB/X}$	39.37$^{B/X}$	39.37$^{B/X}$	43.46$^{AB/V}$	6.46
CV		21.75	24.87	19.74	24.15	14.52	17.98	

[1/]The letters indicate significant differences between different treatments. (ABC); within the same row (P<0.01); (VXYZ); within the same column (P<0.01)

Table 3. The potential antifungal activity of MSE and compound combinations using poison food technique

Concentration (ppm)	Percentage (%) inhibition							
	Bioactive compounds							
	dB	mA	dA	dB-mA	mA-dA	dB-dA	dB-mA-dA	CV
250	45.66 A/Z	32.03 C/Z	44.47$^{B/Z}$	44.55 B/Z	43.10 B/Z	52.73$^{A/Z}$	51.37$^{A/Z}$	6.00
500	60.22 A/Y	42.76 F/Y	55.28^{D} C/Y	53.44 D/Y	51.20 E/Y	58.18^{A} B/Y	57.07$^{BC/Y}$	2.94
750	67.04 A/X	55.71 E/X	64.14$^{B/X}$	60.05 D/X	59.37 D/X	64.31$^{B/X}$	62.10$^{C/X}$	1.74
1000	71.64 B/V	76.07 A/V	75.38$^{A/V}$	72.23 B/V	71.84 B/V	74.79$^{A/V}$	71.81$^{B/V}$	1.55
CV	4.70	2.80	2.18	3.76	2.19	1.89	2.21	

[1/]The letters indicate significant differences between different treatments. (ABCDEF); within the same row (P<0.01); (VXYZ); within the same column (P<0.01)

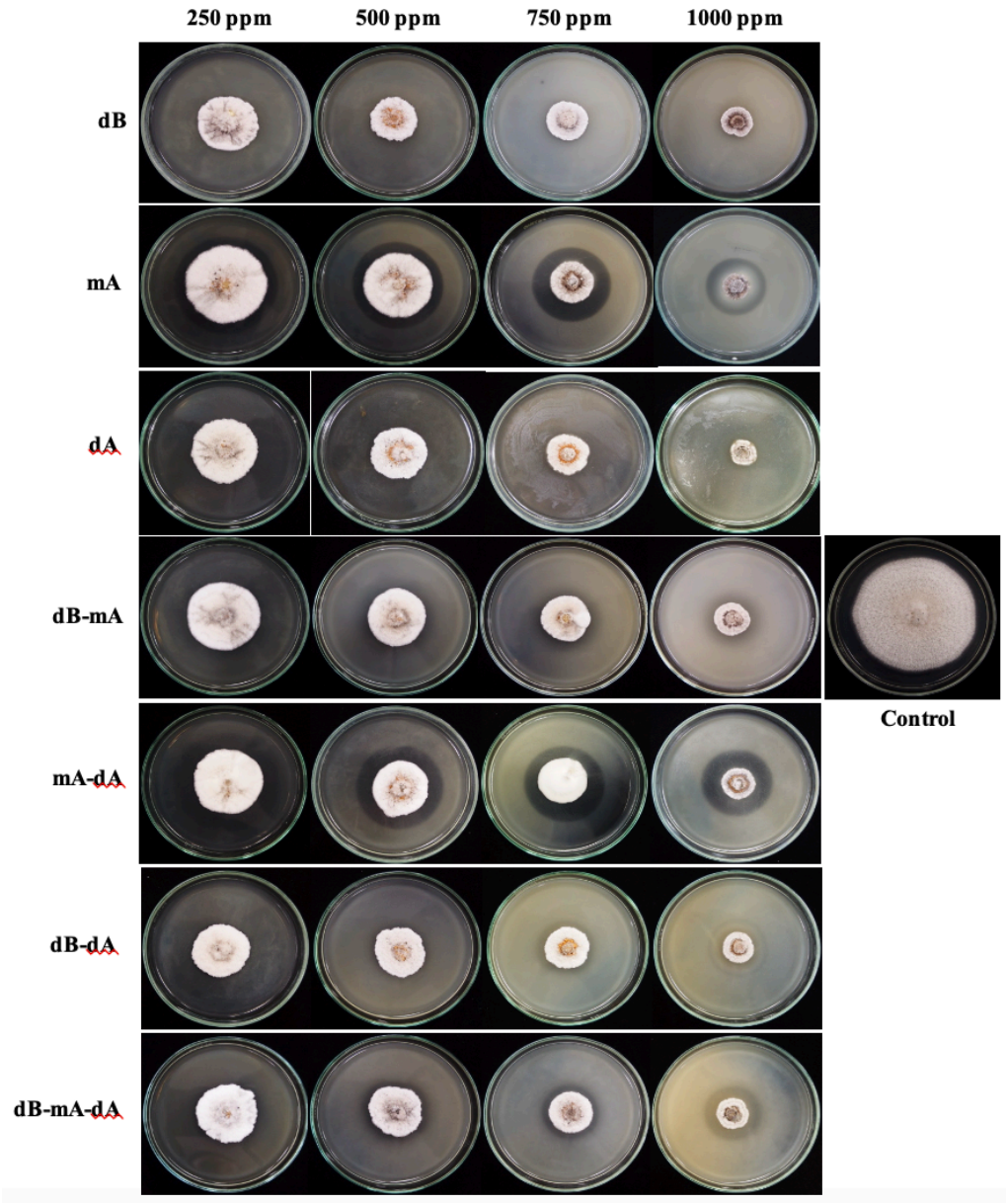

Figure 6. Inhibition of mycelial growth in *Colletotrichum* sp. by bioactive compounds from the ethyl acetate extract of *Miliusa sessilis* (MSE) and compound combinations after 10 days of incubation

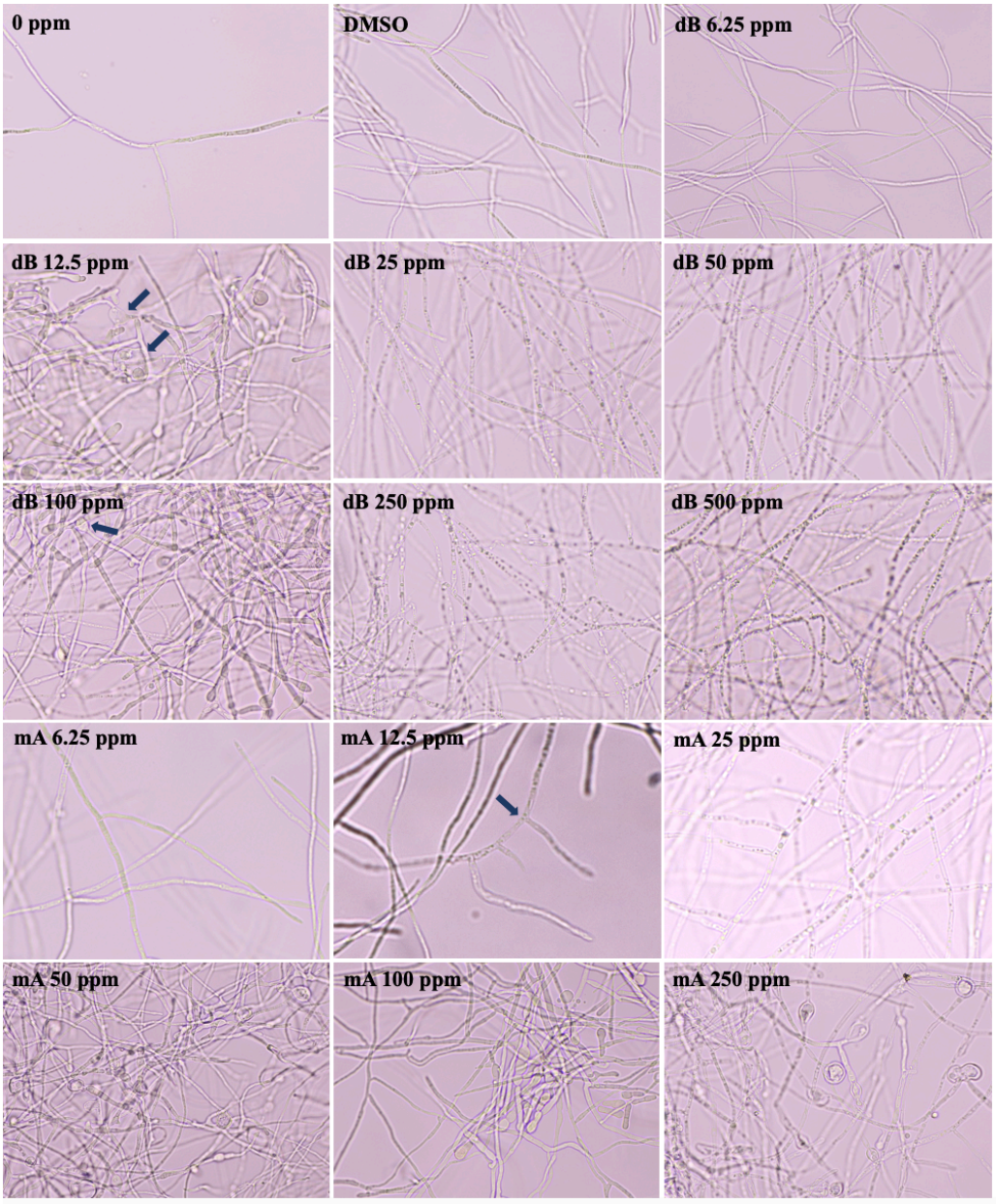

Figure 7. The antifungal minimum inhibitory concentration (MIC) of MSE from *M. sessilis* and compound combinations against *Colletotrichum* sp. at concentrations of 6.25, 12.5, 25, 50, 100, 250 and 500 ppm

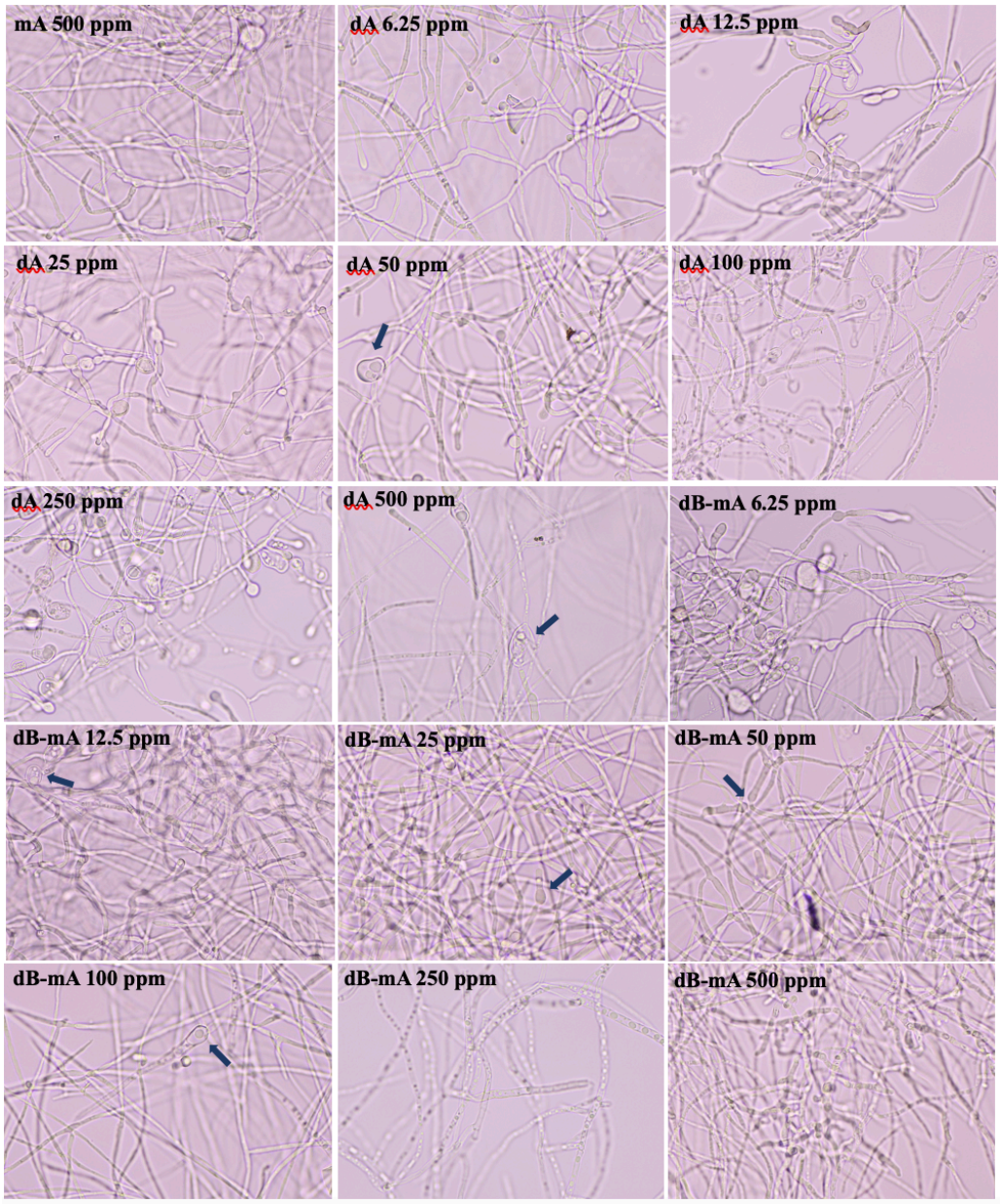
mA 500 ppm
dA 6.25 ppm
dA 12.5 ppm
dA 25 ppm
dA 50 ppm
dA 100 ppm
dA 250 ppm
dA 500 ppm
dB-mA 6.25 ppm
dB-mA 12.5 ppm
dB-mA 25 ppm
dB-mA 50 ppm
dB-mA 100 ppm
dB-mA 250 ppm
dB-mA 500 ppm

Figure 7. (Con.)

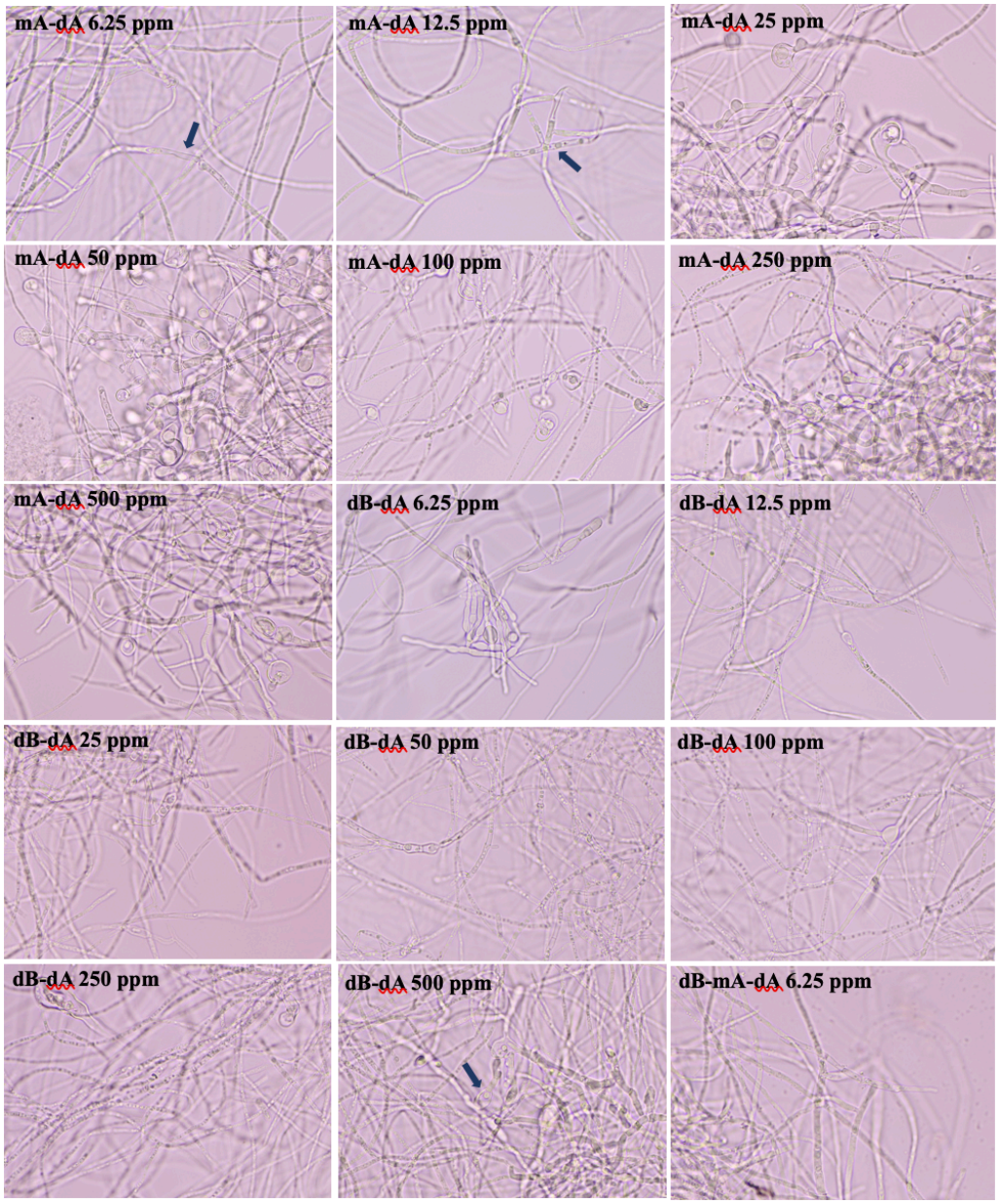
mA-dA 6.25 ppm
mA-dA 12.5 ppm
mA-dA 25 ppm
mA-dA 50 ppm
mA-dA 100 ppm
mA-dA 250 ppm
mA-dA 500 ppm
dB-dA 6.25 ppm
dB-dA 12.5 ppm
dB-dA 25 ppm
dB-dA 50 ppm
dB-dA 100 ppm
dB-dA 250 ppm
dB-dA 500 ppm
dB-mA-dA 6.25 ppm

Figure 7. (Con.)

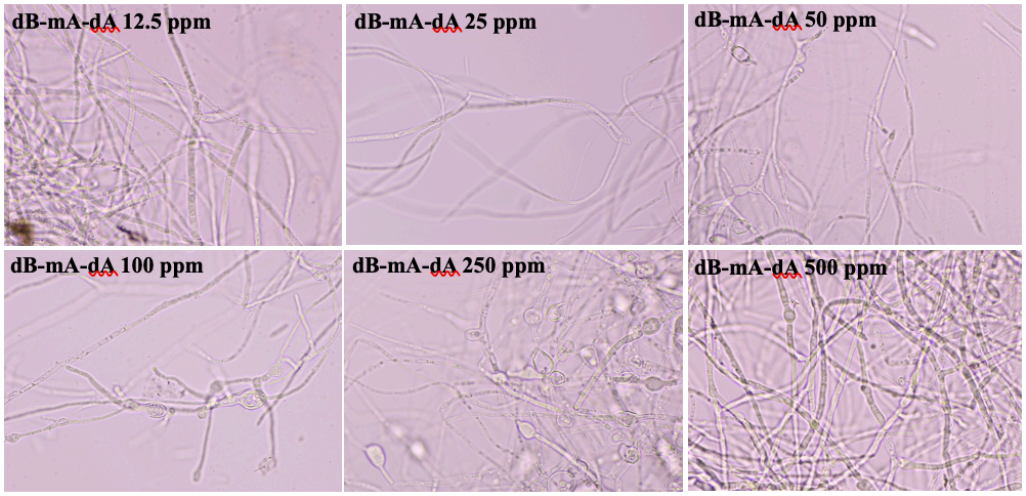

Figure 7. (Con.)

In vivo detection of bioactive compounds (MSE) activities against Colletotrichum sp. on mango fruits

The surface of mango fruits treated with MSE had efficacy to suppress the symptom of anthracnose**.** The bioactive compound combination at varied concentrations of 5000 and 1000 ppm suppressed lesion development on fruits in the treatments with dB-mA (1.05±0.14 and 1.60±0.28 in cm), mA-dA (0.87±0.10 and 0.78±0.08 in cm) and dB-dA (0.95±0.13 and 1.05 in cm) compared to control (2.68±0.24 in cm), respectively (Figure 8).

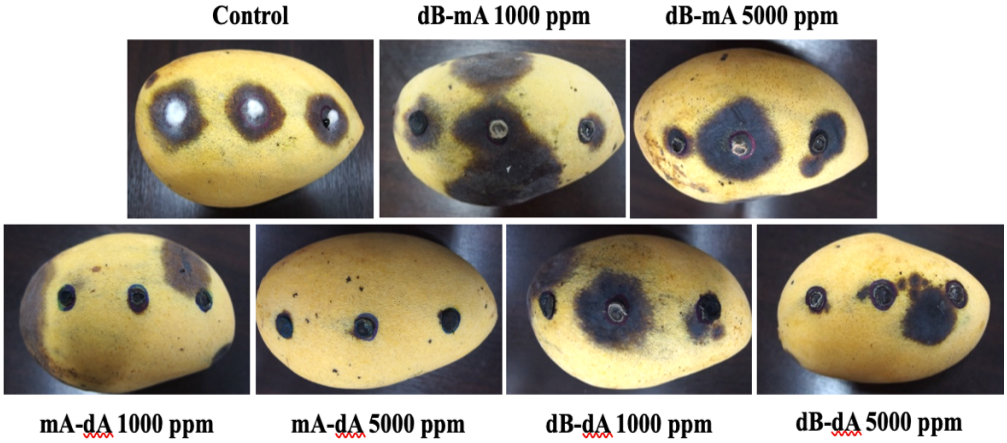

Figure 8. Synergistic effect of MSE in mango ripening, 7 days after post-inoculation with *Colletotrichum* sp.

Discussion

The anthracnose disease on mango fruits is a significant economic problem in widely mango production caused by *Colletotrichum* sp. Currently, this disease is one of severe postharvest disease management due to adaptation of pathogens to climate change and chemical resistance (Peralta-Ruiz *et al*., 2023). The present study shows the morphological characteristics of fungal pathogen were isolated from infected mango tissue that related to *Colletotrichum* sp. (Barnett and Hunter, 1986). In addition, the isolated fungal pathogen was confirmed as casual agent by pathogenicity test on mango fruits that showed the symptom of anthracnose.

The potential of bioactive compounds from the ethyl acetate extract of *M. sessilis* (MSE) against *Colletotrichum* sp. *in vitro* revealed the inhibitory effect on mycelial growth compared to the control. The bioactive compounds dA (De Diaz *et al*., 1980) and dB (De Diaz *et al*., 1980; Da Costa-Silva *et al*., 2015) were significantly more active than mA (Pootaeng-on *et al*., 2020). The maximum growth inhibition percentage was 43.46 to 45.50% in the dual culture assay while the poisoned food technique showed the greatest mycelial growth inhibiting from 74.88 to 76.07 percentage of inhibition. Similarly, de Guzman Alvindi *et al*. (2020) demonstrated that the *Allium longicuspis* extracts have antifungal activity against anthracnose of mango fruit and mycelial development. Furthermore, this plant extract has shown potential for mycelial growth inhibitory by cytoplasmic discharge and mycelial blast. This research experiment supports the present study (MIC test), which investigated that the MSE had an antifungal effect on mycelial development as evidenced by damaging the hyphal morphology as clearly observed deformation with bulbous swelling and lysis. Among different concentrations of MSE investigated, the potential of fungal growth inhibition increased with concentration.

In addition, the compound combinations of MSE were able to suppress lesion development on mango fruits cv. 'Nam Dok Mai Si Thong' after inoculation. Also, the bioactive compound combination displayed the highest inhibitory effect in vitro and *in vivo* than the pure compound combination. Nevertheless, the pure compounds clearly demonstrated the potential of antifungal activity in controlling *Colletotrichum* sp. was increased at a high concentration. Similar considerations have been investigated the synergistic effects of plant oil extracts to inhibit fungal pathogens causing postharvest decay on grapes. It has been reported that two oil combinations were more effective to control fungal pathogens than separately (Sukatta *et al*., 2008).

In conclusion, these bioactive compounds are potentially novel for future antifungi agents as an alternative choice to control anthracnose disease on

mango. This focused study was primary research to develop biological fungicides leading to sustainable agriculture for a better life.

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