



Research article

## Sensitivity tests of dimethomorph, ethaboxam and etridiazole on *Phytophthora palmivora* causing stem rot and leaf blight of durian in eastern Thailand

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### Abstract

**Importance of the work:** *Phytophthora palmivora* is a major pathogen on durians and has been found to be resistant to metalaxyl.

**Objectives:** To investigate the sensitivity of *P. palmivora* to alternative fungicides and evaluate its fungicide efficacy for controlling durian diseases.

**Materials & Methods:** The isolates of *P. palmivora* were evaluated for their fungicide sensitivity to dimethomorph, ethaboxam and etridiazole on the culture medium against mycelial growth, sporangium production and germination. The representative isolates were determined for fungicide sensitivity on durian leaf tissue. The fungicides with low values for the concentration that inhibited the mycelial growth of the isolate by 50% (EC<sub>50</sub>) were used for durian disease control.

**Results:** The mycelial growth of all *P. palmivora* isolates was sensitive to dimethomorph, ethaboxam and etridiazole, with mean ( $\pm$  SD) EC<sub>50</sub> values of  $0.233 \pm 0.116$  parts per million (ppm),  $0.007 \pm 0.002$  ppm and  $2.945 \pm 1.464$  ppm, respectively. Sporangium production and germination were also sensitive to all fungicides. The mean EC<sub>50</sub> values on durian leaf tissue were 0.696 ppm for dimethomorph, 0.089 ppm for ethaboxam and 0.766 ppm for etridiazole. Dimethomorph (100 ppm) and ethaboxam (50 ppm) completely reduced disease development on detached leaves; however, etridiazole (50 ppm) showed maximum control of the disease by 55.6%. Only ethaboxam (208 ppm) extremely suppressed the disease in a seedling experiment (92.3%), while dimethomorph (500 ppm) produced much less disease control (27%). Etridiazole (240 ppm) could not control the disease in seedling conditions.

**Main finding:** Ethaboxam had the potential to control durian disease caused by *P. palmivora* in the eastern growing areas of Thailand. Repeat fungicide application should be discussed regarding dimethomorph and etridiazole.

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## Introduction

*Phytophthora palmivora* (Butler) is a major oomycete pathogen causing root, stem, branch and fruit rot, as well as leaf blight of durian, resulting in large yield reductions over the years in all growing areas in many countries, such as Australia, Indonesia, Malaysia and Thailand (Vawdrey et al., 2005; Santoso et al., 2015; Suksiri et al., 2018). In addition, other oomycete genera, including *Phytopythium* spp. and *Pythium* spp., can cause root, stem and fruit rot diseases in durians (Thao et al., 2020; Solpot and Cumagun, 2021). *P. palmivora* also causes various diseases in many other species of economic crops, such as cacao, papaya, para rubber, jackfruit, olive, apricot and cherry (Nelson, 2008; Borines et al., 2013; Chliyeh et al., 2013; Sunpapao and Pornsuriya, 2016; Türkölmez and Derviș, 2017; Rodríguez-Polanco et al., 2020; Puig et al., 2021).

Due to fungicide applications in durian orchards, *P. palmivora* can develop resistance to metalaxyl (in phenylamide fungicides) (Kongtragoul et al., 2021; Misman et al., 2022; Somnuek et al., 2023), a current fungicide that has been frequently used to control the root and stem rot of durians for several years (Bannaphoomi et al., 1994; Phetkhajone and Songnuan, 2020). In addition, metalaxyl resistance has been documented in *P. nicotianae* (Timmer et al., 1998), *P. capsici* (Wang et al., 2021b), *P. infestans* (Matson et al., 2015) and *P. sojae* (Bhat et al., 1993), as well as in *Pythium*, *Phytopythium* and downy mildew pathogens (Porter et al., 2009; Lookabaugh et al., 2015; Feng et al., 2020; Agarwal et al., 2021; Wang et al., 2021a). Hence, alternative fungicides have been tested for sensitivity to replace metalaxyl in managing oomycete diseases.

Kongtragoul et al. (2021) demonstrated that dimethomorph (in carboxylic acid amide fungicides) and azoxystrobin (in quinone outside inhibitor fungicides), effectively inhibited the *in vitro* mycelial growth of *P. palmivora* and reduced the severity of leaf blight on detached durian. In addition, dimethomorph exhibited great suppression of the growth of *P. capsici*, *P. citrophthora* and *P. parasitica* (Matheron and Porchas, 2000).

Ethaboxam (in thiazolecarboxamide fungicides) has been applied to control the growth of metalaxyl-resistant *P. ultimum* causing wheat damping-off (White et al., 2019) and to control the growth of *Phytophthora*, *Phytopythium* and *Pythium* causing soybean seed and seedling rot (Scott et al., 2020).

Another group of fungicides, based on an aromatic hydrocarbon compound, etridiazole, can inhibit diverse species of *Pythium*, such as *P. aphanidermatum*, *P. irregularare* and *P. ultimum* causing ornamental root rot (Krasnow and Hausbeck, 2017; Lookabaugh et al., 2021).

From this literature, dimethomorph, ethaboxam and etridiazole displayed effective inhibition of many oomycete pathogens with different modes of action, including inhibition of cellulose formation, tubulin assembly and lipid synthesis. The chemicals should also be able to control the *P. palmivora* of durians. However, study of fungicide sensitivity is a prerequisite for further disease management. Thus, the current study aimed to investigate the sensitivity of *P. palmivora* to dimethomorph, ethaboxam and etridiazole in a culture medium and on durian leaf tissue and to evaluate their fungicide efficacy for controlling the disease in detached leaves and durian seedlings.

## Materials and Methods

### Pathogen preparation

In total, 40 isolates were used of *P. palmivora* causing durian diseases isolated from eastern Thailand (Nianwichai et al., 2022). The internal transcribed spacer sequences of all isolates were deposited in the GenBank database under accession numbers ON834411–ON834450. All isolates of the pathogens were cultured on plates of half potato dextrose agar (half PDA) medium under a 12 hr photoperiod and incubated at room temperature (25±2°C) for 7 d before use.

### Fungicide sensitivity tests for mycelial growth, sporangium production and sporangium germination of *P. palmivora* on culture medium

Dimethomorph (Acrobat® 50% WP), ethaboxam (Tabox® 10.4% SC) and etridiazole (Terrazole® 24% EC) with different concentrations were used in this experiment. Dimethomorph was investigated at concentrations of 0 ppm, 0.03 ppm, 0.06 ppm, 0.09 ppm, 0.12 ppm, 0.15 ppm, 0.18 ppm and 0.21 ppm. Ethaboxam was tested at concentrations of 0 ppm, 0.02 ppm, 0.04 ppm, 0.06 ppm, 0.08 ppm and 0.1 ppm. Etridiazole was examined at concentrations of 0 ppm, 0.3 ppm, 0.6 ppm, 0.9 ppm, 1.2 ppm, 1.5 ppm, 1.8 ppm and 2.1 ppm. Six-day-old cultures of all pathogen isolates were placed at the center of a half PDA plate amended with fungicide at different concentrations. The isolates were incubated at room temperature under a 12 hr

photoperiod for 6 d. The experiment was performed twice with three replicates. The colony diameter of the isolates was measured at perpendicular lines and the percentage of mycelial growth inhibition was calculated using Equation 1:

$$\% \text{ Mycelial growth inhibition} = \left( \frac{M1 - M2}{M1} \right) \times 100 \quad (1)$$

where  $M1$  is the colony diameter of the isolate in the control plate and  $M2$  is the colony diameter of the isolate in the fungicide-amended plate.

Fungicide sensitivity analysis was conducted using the percentage values of mycelial growth inhibition plotted against the concentration levels of each fungicide. The concentration of each fungicide that inhibited the mycelial growth of the isolate by 50% ( $EC_{50}$ ) was calculated using the regression equation between the  $\log_{10}$  of each fungicide concentration and the percentage of growth inhibition was obtained based on Wei et al. (2023). Dimethomorph sensitivity was categorized following Kongtragoul et al. (2021): sensitive (S) =  $EC_{50} < 1$  ppm, moderately resistant (MR) =  $EC_{50} 1–100$  ppm and resistant (R) =  $EC_{50} > 100$  ppm. Ethaboxam sensitivity was examined using the method of Peng et al. (2019) with modifications: sensitive (S) =  $EC_{50} \leq 50$  ppm and resistant (R) =  $EC_{50} > 50$  ppm. The etridiazole sensitivity test was modified from Krasnow and Hausbeck (2017): sensitive (S) =  $EC_{50} < 10$  ppm and resistant (R) =  $EC_{50} \geq 10$  ppm.

The representative isolates of the pathogens were chosen for the sensitivity test of each fungicide for sporangium production and germination. For the sporangium production assay, after measuring the colony diameter, an agar plug (0.5 cm in diameter) of a culture aged 7 d of the pathogens at a 0.5 cm distance from the original plug was transferred to a test tube containing 1 mL of sterile distilled water and then vibrated on a shaker for 2 min. Four agar plugs of each plate were investigated. The numbers of sporangia were counted using a hemacytometer. The trial was conducted with three replicates and repeated twice. The percentage of sporangium production inhibition was calculated using Equation 2:

$$\% \text{ Sporangium production inhibition} = \left( \frac{S1 - S2}{S1} \right) \times 100 \quad (2)$$

where  $S1$  is the number of sporangia in the control plate and  $S2$  is the number of sporangia in the fungicide-amended plate. A test of fungicide sensitivity against sporangium production was performed using the percentage values of sporangium production inhibition plotted against the concentration levels

of each fungicide. The  $EC_{50}$  value of each fungicide against sporangium production was calculated as mentioned above.

The fungicide sensitivity test for sporangium germination of the pathogens was conducted on half PDA medium amended with each fungicide concentration as mentioned in the mycelial growth inhibition assay. Separate samples of 20  $\mu$ L of sporangium suspension ( $5 \times 10^6$  sporangia/mL) were dropped on the culture medium surface and spread out using a triangle glass spatula. The plates were incubated at room temperature for 6 hr and the germinated sporangia were counted. The experiment proceeded with three replicates and was repeated twice. The percentage of sporangium germination inhibition was calculated according to Equation 3:

$$\% \text{ Sporangium germination inhibition} = \left( \frac{G1 - G2}{G1} \right) \times 100 \quad (3)$$

where  $G1$  is the number of germinated sporangia in the control plate and  $G2$  is the number of germinated sporangia in the fungicide-amended plate. A fungicide sensitivity test against sporangium germination was conducted using the percentage values of sporangium germination inhibition plotted against the concentration levels of each fungicide. The  $EC_{50}$  value of each fungicide against sporangium germination was calculated as mentioned above.

#### *Fungicide sensitivity tests for *P. palmivora* on durian leaf tissues*

The half-mature stage of samples of healthy durian leaves (cv. Mon Thong) was cut into small pieces ( $2.5 \text{ cm}^2$  in area). The pieces of leaves were surface-sterilized using 1% sodium hypochlorite for 3 min, rinsed twice with sterile distilled water and dried. The center of each leaf piece was punctured using a sterile needle (one wound site per piece) and the leaf pieces were separately floated in separate glass plates containing 25 mL of each concentration of each fungicide, following the protocol of Zhao et al. (2013). The fungicide concentrations for dimethomorph and etridiazole were investigated at 0 ppm, 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm and 100 ppm, while for ethaboxam, the concentrations were examined at 0 ppm, 0.001 ppm, 0.01 ppm, 0.1 ppm, 1 ppm and 10 ppm. Samples of 20  $\mu$ L of sporangium suspension ( $1 \times 10^5$  sporangia/mL) of the representative isolates were dropped onto the wound site. Inoculated leaf pieces were incubated at room temperature under a 12 hr photoperiod and 85% relative humidity for 4 d. The percentage of disease severity was assessed by estimating

the water-soaked area compared to the total area on each leaf piece. This experiment was conducted with three replicates and each replicate consisted of three pieces. This trial was repeated twice. The percentage of disease severity reduction was calculated according to Equation 4:

$$\% \text{ Disease severity reduction} = \left( \frac{D1 - D2}{D1} \right) \times 100 \quad (4)$$

where  $D1$  is the percentage disease severity in the control treatment and  $D2$  is the percentage disease severity in the fungicide treatment. The sensitivity test of fungicides against disease development was performed using the percentage values of disease reduction plotted against the concentration levels of each fungicide. The  $EC_{50}$  value of each fungicide against disease development was calculated as mentioned above.

#### Fungicide efficacy for controlling disease on detached leaves

The detached durian leaves (cv. Mon Thong) were surface-sterilized as above and dried. The leaves were separately soaked for 1 min in three fungicide suspensions (100 ppm dimethomorph, 50 ppm ethaboxam and 50 ppm etridiazole). After drying, 20  $\mu\text{L}$  of sporangium suspension ( $1 \times 10^5$  sporangia/mL) of the seven representative isolates were dropped onto the wound site of the leaf (two wound sites per leaf). Ten leaves were used for each fungicide. For the control treatment, durian leaves were immersed in distilled water instead of a fungicide suspension. Inoculated leaves were incubated in a transparent, moist plastic box at room temperature for 6 d. The experiment was repeated twice. The lesion size was measured. The percentage of disease severity reduction was calculated according to Equation 4.

#### Fungicide efficacy for controlling disease on durian seedlings

Durian seedling (cv. Mon Thong) aged 7 mth were used. Leaves at the half-mature stage were inoculated with a representative isolate of the pathogen by placing the pathogen agar plugs (0.8 cm in diameter) on the wound sites of each leaf. Two wound sites per leaf were used and 10 leaves per seedling were used for each treatment. Separate inoculated seedlings were sprayed with 100 mL of each fungicide with the recommended dose (either 500 ppm dimethomorph, 208 ppm ethaboxam or 240 ppm etridiazole). The seedlings treated with distilled water served as the control. All inoculated

seedlings were incubated in moist, plastic bags for 48 hr and then kept in the greenhouse at 28–32°C for 5 d until symptoms were evident. The experiment consisted of three replicates and was repeated twice. The lesion size was measured. Then, the percentage of disease severity reduction was calculated according to Equation 4.

#### Statistical analysis

The mean  $EC_{50}$  values of each fungicide against mycelial growth, sporangium production and sporangium germination of the pathogens on the culture medium, as well as the lesion sizes on durian leaves were expressed as mean  $\pm$  SD. The data from the reduction of disease severity were determined by analysis of variance using the SPSS software version 25 (IBM Corp.; USA). Significant differences between treatment means were evaluated using Duncan's multiple range test ( $p < 0.05$ ).

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## Results

#### Fungicide sensitivity tests for mycelial growth, sporangium production and sporangium germination of *P. palmivora* on culture medium

The mycelial growth of all isolates was sensitive to dimethomorph ( $EC_{50} < 1$  ppm), ethaboxam ( $EC_{50} \leq 50$  ppm) and etridiazole ( $EC_{50} < 10$  ppm), with mean  $EC_{50}$  values of  $0.233 \pm 0.116$  ppm,  $0.007 \pm 0.002$  ppm and  $2.945 \pm 1.464$  ppm, respectively. Furthermore, the fungicide sensitivity test for sporangium production and germination of the 20 representative isolates of *P. palmivora* had mean  $EC_{50}$  values of  $0.102 \pm 0.055$  ppm and  $0.408 \pm 0.173$  ppm, respectively, for dimethomorph,  $0.007 \pm 0.003$  ppm and  $0.035 \pm 0.033$  ppm, respectively, for ethaboxam and  $0.406 \pm 0.463$  ppm and  $2.4 \pm 1.739$  ppm, respectively, for etridiazole (Table 1).

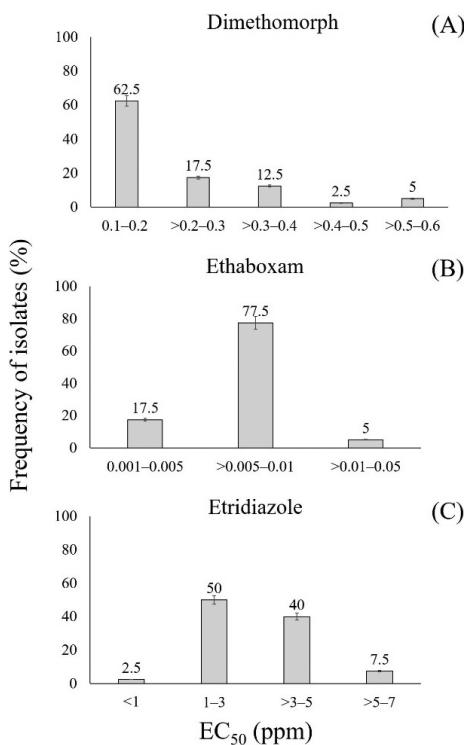
The most prevalent isolates that were sensitive to fungicides had  $EC_{50}$  values for mycelial growth inhibition in the ranges 0.1–0.2 ppm for dimethomorph (62.5%), 0.005–0.01 ppm for ethaboxam (77.5%) and 1–5 ppm for etridiazole (90%), as shown in Fig. 1.

**Table 1**  $EC_{50}$  values of dimethomorph, ethaboxam and etridiazole against mycelial growth, sporangium production and sporangium germination of *Phytophthora palmivora* isolates on culture medium

Growth development	Fungicides (ppm)		
	Dimethomorph	Ethaboxam	Etridiazole
Mycelial growth ( $n = 40$ )			
EC <sub>50</sub> range	0.09–0.58	0.003–0.011	0.99–6.95
EC <sub>50</sub> mean	0.223±0.116	0.007±0.002	2.945±1.464
Sporangium production ( $n = 20$ )			
EC <sub>50</sub> range	0.004–0.17	0.003–0.01	0.006–1.23
EC <sub>50</sub> mean	0.102±0.055	0.007±0.003	0.406±0.463
Sporangium germination ( $n = 20$ )			
EC <sub>50</sub> range	0.26–0.71	0.008–0.09	0.47–4.63
EC <sub>50</sub> mean	0.408±0.173	0.035±0.033	2.4±1.739

ppm = parts per million; EC<sub>50</sub> = concentration that inhibits the mycelial growth of the isolate by 50%.

Mean ± SD within columns derived from three replicates of two trials.



**Fig. 1** Distribution of *Phytophthora palmivora* isolates sensitive to three fungicides against mycelial growth of pathogens: (A) dimethomorph; (B) ethaboxam; (C) etridiazole, where error bars represent ± SD derived from three replicates of two trials, EC<sub>50</sub> = concentration inhibiting mycelial growth of isolate by 50% and ppm = parts per million

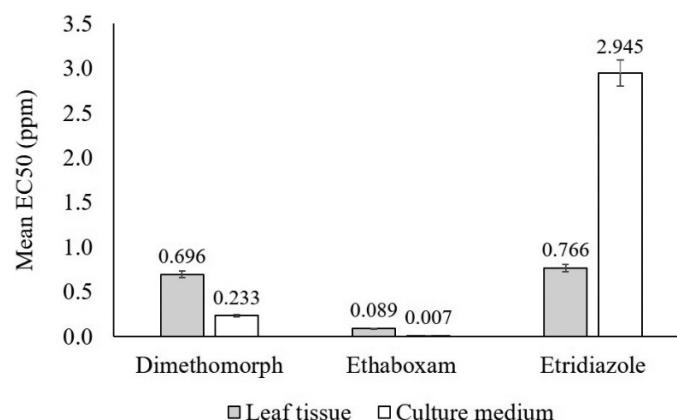
#### Fungicide sensitivity tests for *P. palmivora* on durian leaf tissues

The fungicide sensitivity of each isolate using the durian leaf tissues shifted toward higher EC<sub>50</sub> values for dimethomorph and ethaboxam compared to the EC<sub>50</sub> values on the culture medium. Notably, the mean EC<sub>50</sub> value of dimethomorph on the leaf tissue was 0.696 ppm, whereas ethaboxam was

0.089 ppm, indicating that all tested isolates were sensitive to both fungicides. In contrast, the mean EC<sub>50</sub> value of etridiazole on the leaf tissue decreased with a mean EC<sub>50</sub> value of 0.766 ppm compared to the EC<sub>50</sub> values on the culture medium (Fig. 2).

#### Fungicide efficacy for controlling disease on detached leaves

Two fungicides, dimethomorph (100 ppm) and ethaboxam (50 ppm), completely reduced the lesion sizes on detached durian leaves caused by seven representative isolates of *P. palmivora*. Etridiazole at 50 ppm could completely stop the disease caused by the CTT3 isolate but it had less efficiency in controlling the disease caused by the other isolates, with a range of 17.1–55.6% reduction (Table 2).



**Fig. 2** Comparison of values of mean concentration inhibiting mycelial growth of isolate by 50% (EC<sub>50</sub>) of dimethomorph, ethaboxam and etridiazole against disease development on durian leaf tissue and mycelial growth of *Phytophthora palmivora* on culture medium, where error bars represent ± SD derived from three replicates of two trials and ppm = parts per million

**Table 2** Lesion size and percentage of disease severity reduction on detached durian leaves after fungicide treatment followed by inoculation with representative isolates of *Phytophthora palmivora*

<i>P. palmivora</i> isolate	Lesion size (cm)		Reduction in disease severity (%)
	Distilled water	Dimethomorph at 100 ppm	
CTT3	3.10±0.36 <sup>c</sup>	0.00 <sup>a</sup>	100.0
CKKB2	1.97±0.42 <sup>c</sup>	0.00 <sup>a</sup>	100.0
CKLB1	2.66±0.53 <sup>d</sup>	0.00 <sup>a</sup>	100.0
RKT2	1.97±0.23 <sup>c</sup>	0.00 <sup>a</sup>	100.0
RWT2	2.17±0.29 <sup>c</sup>	0.00 <sup>a</sup>	100.0
TBL3	2.08±0.53 <sup>c</sup>	0.00 <sup>a</sup>	100.0
TKT1	1.66±0.56 <sup>b</sup>	0.00 <sup>a</sup>	100.0
Mean	2.23±0.49	0.00	100.0
Coefficient of variation (%)	23.80	0.00	
	Distilled water	Ethaboxam at 50 ppm	
CTT3	2.01±0.54 <sup>bc</sup>	0.00 <sup>a</sup>	100.0
CKKB2	1.97±0.42 <sup>bc</sup>	0.00 <sup>a</sup>	100.0
CKLB1	2.66±0.53 <sup>d</sup>	0.00 <sup>a</sup>	100.0
RKT2	1.97±0.23 <sup>bc</sup>	0.00 <sup>a</sup>	100.0
RWT2	2.17±0.29 <sup>c</sup>	0.00 <sup>a</sup>	100.0
TBL3	1.97±0.36 <sup>bc</sup>	0.00 <sup>a</sup>	100.0
TKT1	1.87±0.36 <sup>b</sup>	0.00 <sup>a</sup>	100.0
Mean	2.09±0.27	0.00	100.0
Coefficient of variation (%)	7.20	0.00	
	Distilled water	Etridiazole at 50 ppm	
CTT3	1.46±0.39 <sup>bc</sup>	0.00 <sup>a</sup>	100.0 <sup>a</sup>
CKKB2	1.97±0.42 <sup>de</sup>	1.15±0.29 <sup>b</sup>	41.6 <sup>bc</sup>
CKLB1	2.66±0.53 <sup>f</sup>	1.18±0.51 <sup>b</sup>	55.6 <sup>b</sup>
RKT2	1.97±0.23 <sup>de</sup>	1.35±0.43 <sup>cd</sup>	31.5 <sup>c</sup>
RWT2	2.17±0.29 <sup>c</sup>	1.29±0.22 <sup>b</sup>	40.6 <sup>bc</sup>
TBL3	2.08±0.53 <sup>de</sup>	1.72±0.47 <sup>cd</sup>	17.1 <sup>e</sup>
TKT1	1.72±0.54 <sup>cd</sup>	1.33±0.24 <sup>b</sup>	22.8 <sup>d</sup>
Mean	2.00±0.37	1.15±0.54	40.0
Coefficient of variation (%)	14.00	29.00	

ppm = parts per million.

Mean ± SD within columns superscripted with different lowercase letters are significantly ( $p < 0.05$ ) different.

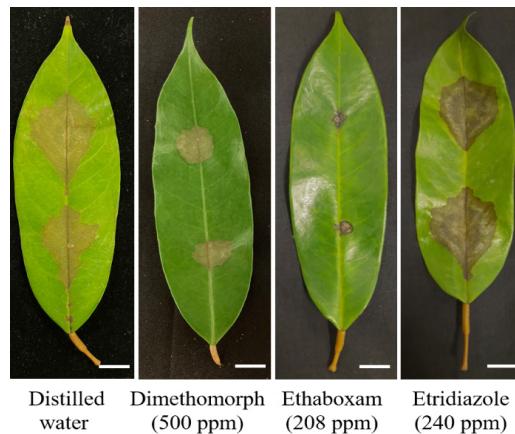
#### Fungicide efficacy for controlling disease on durian seedlings

Ethaboxam at the recommended dose of 208 ppm significantly reduced the severity of blight disease on durian seedlings by more than 90%. In contrast, the other two fungicides displayed less reduction or even failed to control the disease (Table 3 and Fig. 3).

**Table 3** Lesion size and percentage of disease severity reduction on durian leaves after inoculation with representative RKT2 isolate of *Phytophthora palmivora* and fungicide spraying on durian seedlings

Fungicide	Lesion size (cm)	Reduction in disease severity (%)
Distilled water	3.00±0.81 <sup>c</sup>	0.0
Dimethomorph at 500 ppm	2.19±0.71 <sup>b</sup>	27.0
Ethaboxam at 208 ppm	0.23±0.58 <sup>a</sup>	92.3
Etridiazole at 240 ppm	3.06±1.14 <sup>c</sup>	-2.0

Mean ± SD within columns superscripted with different lowercase letters are significantly ( $p < 0.05$ ) different.



**Fig. 3** Symptom characteristics on Mon Thong durian leaves at 5 d after inoculation with representative RKT2 isolate of *Phytophthora palmivora* and fungicide spraying on durian seedlings, where scale bar = 10 mm and ppm = parts per million

## Discussion

Based on the sensitivity test of the fungicides on the mycelial growth of *P. palmivora* on the culture medium, all isolates of the pathogens were sensitive to dimethomorph, ethaboxam and etridiazole, which was in agreement with the findings of Kongtragoul et al. (2021), Peng et al. (2019) and Krasnow and Hausbeck (2017), respectively. Furthermore, the current data showed that these fungicides could suppress sporangium production and germination of the pathogens with lower EC<sub>50</sub> values, possibly indicating sensitive isolates. Wu et al. (2020) reported that the mycelial growth and sporangium formation, but not zoospore release, was sensitive to dimethomorph. Sensitivity to dimethomorph has also been reported with other species of *Phytophthora*, such as *P. litchii* (Gao et al., 2022), *P. capsici* (Siegenthaler and Hansen, 2021), *P. nicotianae* (Cui et al., 2018) and *P. parvispora* (Van Tran et al., 2023).

In the current study, all the *P. palmivora* isolates were susceptible to ethaboxam, with mean EC<sub>50</sub> values of 0.007 ± 0.002 ppm. *In vitro* sensitivity to ethaboxam of *P. palmivora* on durian was first reported in the current study, which was in accord with previous reports that ethaboxam strongly inhibited the mycelial growth of several species of *Phytophthora*, including *P. agathidicida* (Thurston et al., 2022), *P. sojae* (McCoy et al., 2022a), *P. cinnamomi* (Belisle et al., 2019) and *P. agathidicida* (Thurston et al., 2022).

Etridiazole was another fungicide used in the current study that also considerably restrained the *in vitro* growth of *P. palmivora*, particularly for sporangium production, with a mean EC<sub>50</sub> value of 0.406±0.463 ppm, which was the first record of the *in vitro* growth inhibition of *P. palmivora* on durians in Thailand. Other support for this has been noted in the studies by Chan and Kwee (1986), Lim and Nio (1983) and Rosa–Márquez et al. (2000), showing that etridiazole greatly inhibited the mycelial growth or zoospore germination of *P. palmivora* causing diseases in durians, cacaos, orchids and arracachas. Additionally, etridiazole was reported to have notably decreased the mycelial growth of *P. nicotianae*, with an EC<sub>50</sub> value of less than 1 µg/mL (Kuhajek et al., 2003). In the current study, the ranges of EC<sub>50</sub> values of the three fungicides could be used as fungicide sensitivity baselines of *P. palmivora* on durian in Thailand.

However, the mean EC<sub>50</sub> value obtained from the culture medium was lower than on the durian leaf tissue, particularly for dimethomorph and ethaboxam. This finding indicated that both fungicides were more effective in inhibiting the pathogen growth on the culture medium than on the durian leaf tissue,

perhaps because the plant cells could detoxify the fungicidal chemical compounds. (Yu et al., 2022; Zhang et al., 2022).

In contrast, the sensitivity test of etridiazole showed that the EC<sub>50</sub> value was four-fold higher on the culture medium than on the durian leaf tissue. This result may be dependent on the fungicide group interacting with the type of pathogens and the species of plants. Fortunately, the mean EC<sub>50</sub> values of etridiazole in both tests were less than 10 ppm, which can be classified as sensitive isolates to this fungicide according to Krasnow and Hausbeck (2017).

The results from leaf tissue tests would be the fungicide sensitivity baselines. In general, fungicide sensitivity of plant pathogenic fungi have also been tested on plant tissues, as shown in the leaf disk assay of *Plasmopara viticola*, the obligate pathogen of grapevine (Genet and Jaworska, 2013; Massi et al., 2021), as well as in non-obligate parasites, such as *P. infestans* against metalaxyl (Fontem et al., 2005) and *Alternaria solani* against boscalid (Shi et al., 2015).

In the disease control assays, 50 ppm ethaboxam completely suppressed the disease development of all tested isolates based on the detached leaf technique. Like the seedling experiment, ethaboxam at the recommended dose (208 ppm) strongly decreased the severity of the disease by greater than 90%. These results were consistent with reports that ethaboxam effectively controlled seed-borne diseases caused by diverse genera of oomycete pathogens, such as *Phytophthora*, *Phytopythium* and *Pythium* (Scott et al., 2020; McCoy et al., 2022b; Vargas et al., 2022). This fungicide has been introduced to treatment programs for minimizing downy mildew caused by *Pseudoperonospora cubensis* on cucurbits (D'Arcangelo et al., 2021). However, *P. palmivora* may develop resistance to ethaboxam in the future due to a point mutation linked to the β-tubulin gene. (Peng et al., 2019). Sooner or later, *Phytophthora* isolates distributed in durian orchards need to be monitored for ethaboxam resistance.

Dimethomorph at 100 ppm could completely reduce the disease on detached durian leaves, while in the greenhouse, the recommended dose (500 ppm) of dimethomorph slightly suppressed the disease in durian seedlings (27% reduction). This result indicated that the plants may have a poor ability to absorb dimethomorph into cells, resulting in reduced effectiveness of the fungicide in the plants. Likewise, the report by Patil et al. (2023), emphasized that dimethomorph (mixed with other fungicides) greatly inhibited *Phytophthora* zoospore release *in vitro*, but it only reduced the fruit rot of palm trees by less than 50%. In addition, Higgins et al. (2023) stated that dimethomorph was ineffective in controlling downy mildew on cucurbit plants and Uribe–Gutiérrez et al. (2022)

reported that using a single chemical, such as dimethomorph, exhibited less reduction of gray mold disease caused by *Botrytis cinerea*. These results may indicate that the initial concentration of fungicide application decreased with time due to environmental effects in the greenhouse experiment. Yang et al. (2022) demonstrated that the concentration level of dimethomorph was reduced by 4–6 times within 5–7 d after fungicide spraying on cucurbit leaves in field conditions. In addition, Chen et al. (2018) noted that dimethomorph rapidly decreased 3 d after spraying on potato plants, with the residues remaining at <10% of the initial concentration. Therefore, enhancement of dimethomorph to control plant disease in the greenhouse and field conditions can result from it being applied before pathogen inoculation, being combined with other chemicals or being used with synthesized nanoparticles (Wang et al., 2009; Rashid et al., 2014; Neupane and Baysal-Gurel, 2022; Yang et al., 2022).

With etridiazole, there was less reduction in disease severity in almost all isolates on the detached leaves but it failed to affect a representative RKT2 isolate in the greenhouse trial. This may be explained by one application of etridiazole throughout the experiment not being able to control the disease adequately. This was consistent with other reports that etridiazole could not suppress root rot disease caused by *P. capsici* in peppers in a greenhouse (Segarra et al., 2013). Etridiazole was reported to slightly suppress diseases caused by diverse species of *Pythium* (Martinez et al., 2005; Lookabaugh et al., 2021). This fungicide hydrolyzes membrane-bound phospholipids in mechanisms of action (Radzuhn and Lyr, 1984); repeat fungicide application or fungicide rotation treatment with different modes of action throughout the season is a necessary action to minimize diseases (Krasnow and Hausbeck, 2017; Lookabaugh et al., 2021).

In general, after application in natural circumstances, fungicides are degraded by various environmental factors. Over time, there is a greater reduction in fungicide residues. For example, a foliar spray of metalaxyl on durian seedlings in a greenhouse caused more dissipation of the fungicide over time (Phetkhajone et al., 2021). In addition, washing durian leaves 1 d after metalaxyl application resulted in a fungicide loss of almost 6 times the original amount (Phetkhajone et al., 2021). Fungicide degradation by fungi was reported by Escudero-Leyva et al. (2022), who noted that *Trichoderma* species grown on living leaves of coffee plants could remove the residues of some chemicals 14 d after fungicide application.

Occasionally, prolonged application of a fungicide using the same mode of action could increase fungicide resistance in the pathogen population. Mutation at the pathogen's target genes and detoxification metabolisms have been implicated

in fungicide resistance by *Phytophthora* (Peng et al., 2019; Dai et al., 2022; Gao et al., 2022).

From the current results, all *P. palmivora* isolates were sensitive to dimethomorph, ethaboxam and etridiazole against mycelial growth, sporangium production and germination on the culture medium. There were low EC<sub>50</sub> values of the fungicides on durian leaf tissues. Dimethomorph completely suppressed the disease on durian detached leaves, whereas it had slight inhibition potential against the disease on durian seedlings. Etridiazole produced low to moderate effects against the disease caused by almost all isolates on detached leaves, while it was not effective on durian seedlings. However, ethaboxam strongly decreased the disease on both the detached durian leaves and seedlings, making it an excellent alternative fungicide for managing metalaxyl-resistant *P. palmivora* in durian orchards in the eastern region of Thailand.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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