SCREENING OF VOLATILE COMPOUNDS FROM BRASSICACEOUS PLANTS AGAINST *Phytophthora palmivora* IN COCOA

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ABSTRACT – *Plants from the Brassicaceae family, which contain high levels of glucosinolates, have been utilized as biofumigants to control soilborne pathogens. The effectiveness of Brassica plant material is largely credited to the release of toxic isothiocyanates following the breakdown of glucosinolates. Biofumigation of Brassica tissue for controlling Phytopththora palmivora on cocoa plantation is not being discussed before. Three plants from the Brassicaceae family namely cauliflower, broccoli and cabbage were assessed for their volatile compounds potential under in vitro conditions. The macerated tissues from cauliflower, broccoli, and cabbage were individually placed on the inverted lids of PDA plates, while the plug of P. palmivora was positioned on the bottom plate. Among the three, broccoli displayed the highest suppression of P. palmivora mycelial growth at 75.32%, followed by cauliflower at 66.23% and cabbage at 57.14%. The findings of this study suggest that volatile compounds effect from broccoli exhibits promising potential in restraining the growth of P. palmivora.*

Keywords*:* Biofumigation, *Phytophthora palmivora,* cauliflower, broccoli, cabbage

INTRODUCTION

Phytophthora palmivora (Butler) is a hemibiotrophic oomycete that can infect over 200 plant species, including the economically significant *Theobroma cacao* L., commonly known as cocoa (Derevnina *et al*., 2016). It infects various parts of the cocoa plant, such as the pods, stems, and seedling leaves, causing black pod rot disease, stem canker, and Phytophthora leaf blight, respectively. Among these diseases, black pod rot is the most severe, causing significant losses of up to 30% in cocoa yields in Malaysia (Ling, 2022).

There are several methods for controlling *P. palmivora* using integrated disease management, which involves cultural practices and chemical treatments. Cultural practices include removing all infected black pods from the cocoa field and regularly pruning the canopy of the cocoa tree to increase airflow and sunlight around the trees (Luseni & Kroma, 2012). Chemical treatments involve using copper compounds (copper sulfate or copper oxide), either alone or in combination with metalaxyl. However, these methods are labor-intensive and not cost-effective. Additionally, the use of chemicals or fungicides can lead to the pathogen developing resistance.

Currently, biopesticides are gaining interest as control strategies due to their safer environmental and health impacts. One such method, "biofumigation," first introduced in the 1990s, utilizes Brassica crops like mustard and radish, which are rich in glucosinolates (GSLs) (Ashiq *et al*., 2022). Glucosinolates are non-toxic until they undergo hydrolysis upon tissue disruption. When GSLs interact with myrosinases, they are catalyzed into various biologically active substances, including toxic isothiocyanates (ITCs). These volatile compounds are known for their toxic effects on weed seeds, nematodes, bacteria, fungi, viruses, and insects. It is now documented that, in addition to Brassicas, plants within the Caricaceae, Moringaceae, Salvadoraceae, and Tropaeolaceae families also possess biofumigant properties (van Dam *et al.,* 2009).

Brassica crops are considered break crops because they can disrupt the life cycle of several soil-borne pathogens. In an *in vitro* study conducted by Charron & Sams (1999), leaf tissues of *Brassica* species were tested against Pythium and Rhizoctonia species. Forty-eight hours after being placed inverted over the neck of a 500 mL jar containing 10 g of macerated *B. juncea* leaves, the radial growth of *Pythium ultimum* and *Rhizoctonia solani* in petri dishes was reduced by 100% and 73%, respectively. In another study by Handiseni *et al*, (2016), macerated shoot tissue of *B. juncea* (3 g in a Petri dish) was found to be highly effective in suppressing the mycelial growth of *R. solani*, achieving an inhibition rate of more than 90%.

Whilst biofumigation has attracted significant interest, there is a need to look on their potential against *P.palmivora*. This study investigated the effects of volatile compounds

released from three Brassica crops which are cauliflower, broccoli and cabbage on the mycelial growth of *P. palmivora.*

MATERIALS AND METHODS

Pathogen isolation and morphological identification

Cocoa pods showing typical symptoms of brownish lesions were randomly collected from a cocoa field at CRDC Jengka, Pahang. The pods were kept in paper envelopes and brought to the laboratory for further studies. Pod fragments (5×5 mm) were excised from the margins of the lesions, followed by surface sterilized using a 0.5% sodium hypochlorite solution for 30 seconds, and rinsed three times using sterile distilled water. The pod fragments then were dried on sterilized tissue paper and placed in potato dextrose agar (PDA) and were incubated at 27-30°C for 24-48 hours. The first hyphae from the plated diseased tissue were subcultured onto new PDA plates and incubated for 7 days to obtain a pure culture (Latifah *et al*., 2018).

For identification, the morphology of the obtained isolates was examined at 100x and 400x magnifications using a light microscope (Rax Vision, model Y-100). Structures such as sporangia, oogonia, hyphae, and chlamydospores were captured using a microscope digital camera (SCMOS, ToupView) imaging software.

Sample preparation

Cauliflower (*B. oleracea* var. botrytis), broccoli (*B. oleracea* var. italica) and cabbage (*B. oleracea* var. capitate) were obtained from a local supermarket. The plant tissues were disinfected in a 10% ethanol solution for 10 seconds, rinsed in sterile distilled water for 5 minutes, and dried on autoclaved filter paper. They were subsequently macerated using a sterile mortar and pestle.

In vitro test

In vitro experiments were carried out to test the direct effect of volatiles inhibitors released from macerated tissues of three Brassica plants on mycelial growth of *P. palmivora* using a method adapted from Ojaghian *et al*. (2012). Two gram of each fresh macerated tissues was placed in petri plates (90 mm) lid, and the bottom is PDA plate that inoculated centrally with 5mm mycelial discs of 7 days-old pure culture of *P. palmivora.* Each petri dish was sealed under sterile conditions with paraffin tape to prevent contamination and the escape of volatile materials. Since volatile substances always move upwards, the macerated tissues were placed at the bottom of the petri dishes, with the PDA containing the pathogen positioned inverted above. As a control, the plates were inoculated with the pathogen only, without any macerated tissues. All plates were incubated at 24 ± 1° C for ten days.

The radial growth of *P. palmivora* in all plates was measured, and the percent inhibition of diameter growth (PIDG) for each treatment was calculated seven days after inoculation using the formula $I = (C-T)/C \times 100$, where I represents the percent growth inhibition, C is the colony diameter of the pathogen in the control, and T is the colony diameter of the pathogen in the treatment.

RESULTS AND DISCUSSIONS

Morphological structure of *P. palmivora* was observed after ten days of incubation. The colony of *P. palmivora* were grew slowly on PDA, shown a round with uneven edges, had a cotton-like texture, and white in color and they felt somewhat resilient when sliced using a scaple (Figure 1A). The micro morpholocal characteristics of *P.palmivora* on their presence and the shape of sporangia, oogonia, hyphae and chalamydospores were observed by doing a slide cultures (Wongwan *et al*., 2021)

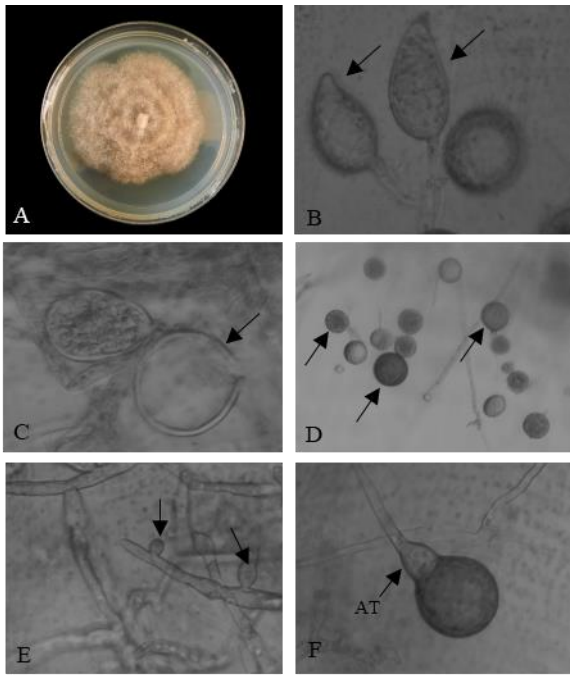

Figure 1: A: Colony morphology of P. palmivora on PDA at 10 days old. B: Sporangia with spores; C: Empty sporangium; D: Chlamydospores; D: Hyphal swelling; E: Oogonium.

The sporangia of *P. palmivora* are generally pear-shaped (ovoid). They have a distinct papilla and are caducous (easily detached from the

sporangiophore) with short stalks (Figure 1B). The sporangia produced and released zoospores, which are motile swimming spores, leaving behind empty sporangia. (Figure 1C). Chlamydospores and hyphal swellings are found either intercalary (in the middle of a hypha) or terminal (at the end of a hypha), and they vary in shape (Figure 1D-E). Additionally, *P. palmivora* isolates produce spherical oogonia with amphigynous antheridium (AT) (Figure 3E). Similar morphological characteristics of *P. palmivora* were also observed by Umayah & Purwantara (2006). They isolated *P. palmivora* from cocoa black pod rot and stem canker, confirming the identification with molecular techniques by amplifying the ITS region of rDNA followed by restriction enzyme analysis.

The results from *in vitro* experiments showed that the effect of volatile inhibitors produced from fresh macerated Brassica tissues against *P. palmivora* was significantly different from untreated controls after seven days of incubation (Table 1). Highest effect on radial growth of *P. palmivora* was observed in broccoli with inhibition rate of 75.32%, followed by cauliflower (66.24%) and cabbage (57.14%). This shows that volatiles inhibitors (potentially ITCs) produced by Broccoli (*Brassica oleracea* var. italic) is the most effective on restrain the growth of *P. palmivora.* This result is supporting another study by Mirsam & Masluki (2017), where the waste of Brassica can reduce the spore concentration of *P. palmivora* and suppressing the severity of Phytophthora leaf blight disease by 40%.

Table 1: Inhibition Percentage of *P. Palmivora* Growth on PDA by the Volatile Compounds of Three Brassica Plants after 10 Days at 24°C

Treatment	Diameter growth inhibition (%)[d]
Control	0.00[a]
Cabbage (*B.oleracea* var. capitate)	57.14 ± 1.81[b]
Cauliflower (*B.oleracea* var. botrytis)	66.24 ± 1.21[c]
Broccoli (*B. oleracea* var. italic)	75.32 ± 0.81[d]

[d]Within columns, means followed by a common letter do not differ significantly at the P < 0.05 level of confidence according to Duncan test. Values in the table indicate means ± standard error

Figure 2 illustrates the inhibition effect of volatile inhibitors secreted from broccoli (Figure 2A), cauliflower (Figure 2B), and cabbage (Figure 2C against *P. palmivora* growth. After 10 days of incubation, volatile compounds secreted by broccoli inhibited the growth of *P. palmivora* up to 2.7 cm diameter followed by cauliflower and cabbage with diameter of 3.4 cm and 4.1 cm, respectively.

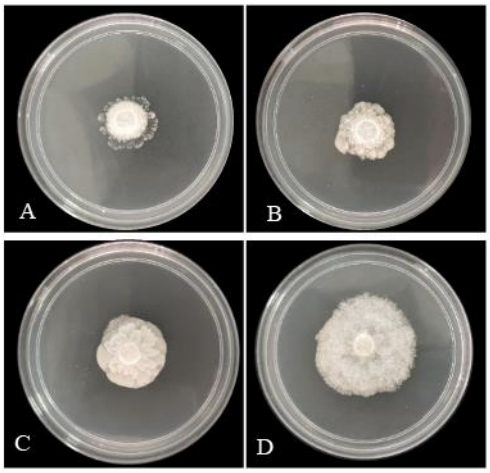

Figure 2: Inhibitory Effect of Volatile Inhibitors Released from Fresh Macerated Tissues of B.Oleracea (Placed in the Lid of Plates) Against Mycelial Growth of P. Palmivora. Compared with the Control (D). The Radial Growth of P. Palmivora was Significantly Reduced after 10 Days of Exposure to Volatile Inhibitors of Broccoli (A), Cauliflower (B), and Cabbage (C)

CONCLUSIONS

This study unveiled the potential of volatile effect from three Brassicaceae family namely cauliflower, broccoli and cabbage in suppressing the growth of *P. palmivora* under *in vitro* conditions. Among them, broccoli demonstrated the most potential in restraining the growth of *P. palmivora* in a laboratory.

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