Antifungal activities of cocktail fungicide between cassia oil and sodium bicarbonate against *Penicillium digitatum* and its mode of action

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ABSTRACT: *Penicillium digitatum*, the most devastateone types of green mold is the most damaging postharvest disease on citrus fruits such as orange. The present study demonstrated the synergistic antifungal potential of GRAS (Generally Regarded As Safe) fungicide by using cassia oil and sodium bicarbonate against *P. digitatum* and further established its mode of action in orange. A cocktail fungicide of cassia oil and sodium bicarbonate was prepared and tested for its antifungal activity besides several other analysis. The treatment has successfully 100 % inhibited the growth of *P. digitatum*. The treatment also break down *P. digitatum's* cell membrane. For *in vivo* studies, the treatment significantly decreased the disease incidence of green mold from 100 % to 58.33 % for up to 10 days in room temperature as compared to control. Moreover, the treatment did not affect the quality parameter (colour index, soluble solid content and total acidity). The mixture might be a promising alternative to synthetic fungicide for controlling postharvest diseases of *P.digitatum* on citrus fruits.

Keywords: *Penicillium digitatum, GRAS, orange fruit*

1. INTRODUCTION

Citrus is one of the major economically important fruit crops produce worldwide and it was estimated that more than 170 million tons of citrus was produced annually (FAOSTAT, 2015). The huge demand for citrus fruits came from the health benefits as they contains beneficial primary and secondary metabolites in addition to great sensorial properties of the fresh fruit and juice (Lado, *et al*., 2018). Despite of its premier health benefits, the citrus's quality is affected by one of the main postharvest diseases in citrus industries involving *Penicillium digitatum (P.digitatum)*, a green mould diseases. The infection accounted for most of the citrus decay during storage and transport (Rodov *et al*., 2011). Synthetic chemical fungicides such as prochloraz, imazalil, thiabendazole, and pyrimethanil are commonly used to control the infection (Shi *et al*., 2018). However continuous application of synthetic fungicide caused development of fungicidal resistant strains to the postharvest pathogens. The residue also associated with environmental pollution and human health problems (Ruiz *et al*., 2016). Due to the high public concern regarding the issues, it has led to the discoveries of safe fungicides as an alternative over chemical synthetic fungicides.

Natural fungicides formulated from GRAS (Generally Regarded as Safe) materials such as salts and essential oils are preferable due to its no or low toxicological effects towards humans or animals and minimal impact to the environment (Palou *et al*. 2016).

2. METHODOLOGY

The pure essential oil of *Cinnamomum cassia* was purchased from local company in Kuala Lumpur, Malaysia. The green mould (*Penicillium digitatum*) was isolated from infected oranges and cultured on potato dextrose agar (PDA) at 25 ± 2 °C. The pure culture was maintained on PDA at 4 °C for further analysis.
Cassia oil with a concentration of 0.25 mL/L was mixed with different concentration of sodium bicarbonate, SB (HmbG® Chemicals, Malaysia) in the range of 1-3 %. The treatment will be used in antifungal activity, cell membrane analysis, in vivo studies on unwounded oranges as well as orange's quality.

3. RESULT AND DISCUSSION

3.1 Antifungal activity of cocktail fungicide

In this study, the antifungal activity of a cocktail fungicide containing CO and SB was evaluated against the growth of *P. digitatum*. The use of CO alone or in addition with 1 % to 3% of SB showed significant inhibition ($p < 0.05$) on the growth of mycelium (Figure 1). CO alone (0.25 mL/L) showed 44.80 % inhibition of *P.digitatum*. However, the mixture of 0.25 mL/L of CO in 1, 2 and 3 % of SB showed a 100 % inhibition towards the growth of *P.digitatum.* The control did not show any inhibition as no fungicide was added. Treatment with 0.05% Tween 80 on *P.digitatum* showed 12.32% of

inhibition.

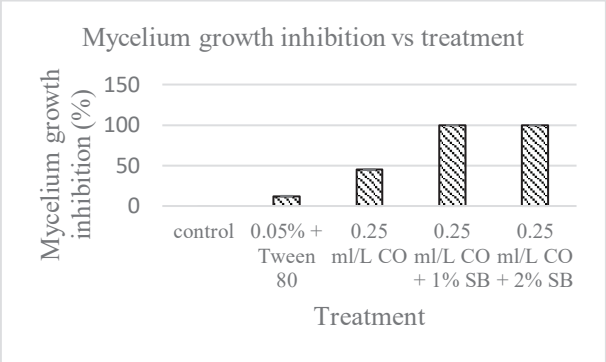

Figure 1: Effect of cocktail fungicide on mycelium growth inhibition of *P.digitatum.*

3.2 Effect of green fungicide on spore germination

The mode of action of the formulated fungicide against *P. digitatum* is evaluated based on spore germination, mycelium dry weight and cell membrane's integrity. Exposure to essential oil resulted in visible damage of the spore coat (Lawrence, H.A., Polombo, E., 2009). When assessing the fungicidal effect on the rate of spore germination as depicted in Figure 2, the results showed a complete inhibition on *P. digitatum* spore germination when cassia oil was use alone or in combination with 1,2 and 3% (w/v) sodium bicarbonate.

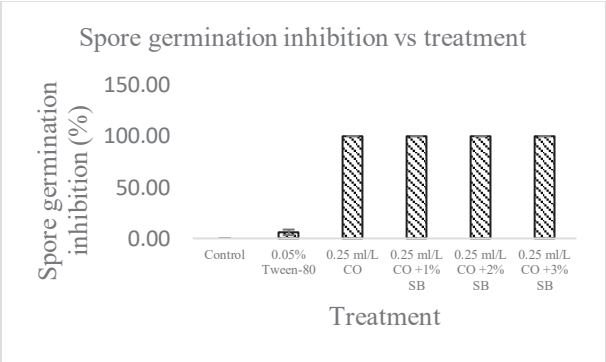

Figure 2: Effect of green fungicide on spore germination inhibition.

4. CONCLUSION

The result from this study shows that the cocktail fungicide, which consist of cassia oil and sodium bicarbonate salt has shown a significant inhibition towards fruit pathogen, *P.digitatum.* The mode of action is through the inhibition of spore germination, thus resulted in reduction of dry mass of mycelium and increment in cell constituents released in growth media, consequently affect the *P. digitatum*'s growth. Based on the finding, the cocktail fungicide offers a good alternative over chemical fungicide against post-harvest disease particularly mold of *P. digitatum* in orange post-harvest treatment.

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