Growth inhibition of Black Sigatoka pathogen, *Pseudocercospora fijiensis (Morelet) Deighton*, by preharvest and postharvest treatments of Cavendish Banana

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An *in vitro* study was conducted to determine the efficacy of products applied as preharvest and postharvest treatments in banana production against *Pseudocercospora fijiensis (Morelet) Deighton*, the causal pathogen of Black Sigatoka disease. A total of seven products used in banana disease control were evaluated namely thiophanate methyl, copper hydroxide, and ionic copper for preharvest pathogens and prochloraz, calcium hypochlorite, iprodione, and potassium aluminium sulfate for postharvest pathogens. The laboratory assay was conducted following the guidelines set by the Banana Working Group of Fungicide Resistance Action Committee (FRAC) where each product was tested at two concentrations, 1 and 10 ppm. Test was laid out in Complete Randomized Design with three replicates per concentration per product. Tests revealed the inhibiting effect of the products against the fungal pathogen even at a low concentration of 1 ppm. For preharvest products, superior effect was noted from copper-based (copper hydroxide and ionic copper) treatments especially at 10 ppm. For postharvest products, treatment with calcium hypochlorite provided the best effect especially at 10 ppm. Among all products evaluated, only potassium aluminium sulfate had decreasing efficacy as concentration increases. Results of this study demonstrated the promising effect of the tested products against *Pseudocercospora fijiensis* hence providing added control to Black Sigatoka.

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INTRODUCTION

Banana (*Musa* sp.) is one of the chief staple food crops in the world. As of August 2018, the Philippines is the 6th largest banana exporter in terms of dollar value (Workman, 2018). In 2018, total banana production in the Philippines reached 9.4 million metric tons mainly with the exportation of Cavendish bananas to various markets. Cavendish banana plantations are largely concentrated in Mindanao with Davao as the largest producing region sharing 38% of the total production followed by Northern Mindanao with 21% and finally SOCCSKSARGEN with 17% (PSA, 2019).

The most devastating leaf disease of banana is Black Sigatoka, caused by the pathogenic fungus *Pseudocercospora fijiensis.* The pathogen incites necrotic lesions on leaves and serious infection may lead to a substantial reduction in the photosynthetic leaf area of infected plants and thus to yield losses. In addition to these effects on yield, Black Sigatoka was found to have an impact on fruit quality, especially because exported bananas ripen prematurely (Marin et al., 2003).

The pathogen is spread as wind-dispersed ascospores in the wind especially when conditions are favorable (Marin et al, 2003). With this means of spread, application of various fungicides through aerial spray efficiently provides good coverage thereby ensuring management of the disease on the leaves. However, these sexual spores may also land on hanging fruits where they can stay dormant until they reach market destinations. As such spores do not receive fungicides from aerial spray application, products that are being used to treat preharvest and postharvest diseases of Cavendish bananas may provide added control against *P. fijiensis*.

Preharvest products (thiophanate methyl, copper hydroxide, and ionic copper) manage the preharvest diseases diamond fruit spot caused by *Cercospora hayi* and fruit rot caused by *Erwinia* sp. while postharvest products (prochloraz, calcium hypochlorite, iprodione, potassium aluminium sulfate) prevent the manifestation of postharvest diseases crown rot and crown mold caused mainly by *Fusarium* sp. These products are applied to the fruit but their effect to *P. fijiensis* are not yet reported. This study, therefore, was conducted to determine the efficacy of preharvest and postharvest treatments against *P. fijiensis* ascospores.

METHODOLOGY

Laboratory test was done following the standard procedure of Fungicide Resistance Action Committee (FRAC) for fungicide sensitivity monitoring assay. Infected leaf samples with Stage 6 Sigatoka symptoms were collected from TADECO banana plantation. Leaves were cut and incubated for 48 hours in a plastic bag with moistened tissue paper. After incubation, leaves were attached to sterile filter paper and submerged in sterile lukewarm water for 10 minutes. Treatments were prepared by mixing test products to come up with 1 and 10 ppm concentrations. Treatment solutions at 1 ml were then amended to water agar and poured to petri plate. Leaves were placed on the cover of the petri plate to discharge ascospores and were removed after 3 hours. Petri plates were incubated for 48 hours prior to data collection. There were three replicates per concentration per treatment. After incubation, a total of 30 ascospores were measured for each Petri plate using a camera with a calibrated software (Infinity Software Version 6.5.3) connected to a compound microscope (Olympus Model CX3). Using the data on germ tube length, germ tube growth inhibition (%) for every treatment was computed relative to the measurement obtained from Untreated Control using the formula below:

$$\frac{\text{Germ tube length (Untreated)} - \text{Germ tube length (Treated)}}{\text{Germ tube length (Treated)}} \times 100$$

Normality of data and homogeneity of variances were checked using Shapiro Wilk's and Levene's tests, respectively. After these conditions were met, data were run in Analysis of Variance (ANOVA) followed by Tukey's HSD test to compare treatment means. All statistical analysis was done using R commander package for windows version 3.5.3.

Active Ingredient	Application
Thiophanate Methyl 70WP	As Preharvest Treatment
Copper Hydroxide 77WP	As Preharvest Treatment
Ionic Copper	As Preharvest Treatment
Prochloraz 45EW	As Postharvest Treatment
Calcium Hypochlorite 70	As Postharvest Treatment
Iprodione SC50	As Postharvest Treatment
Potassium Aluminium Sulfate 98%	As Postharvest Treatment

RESULTS AND DISCUSSION

Test of Preharvest Products against *Pseudocercospora fijiensis.*

At 1 ppm, thiophanate methyl and copper hydroxide obtained around 44% inhibition while ionic copper had 67% (See Figure 1). At 10 ppm, inhibition increased to 62% for thiophanate methyl and 73 to 78% for copper hydroxide and ionic copper, respectively.

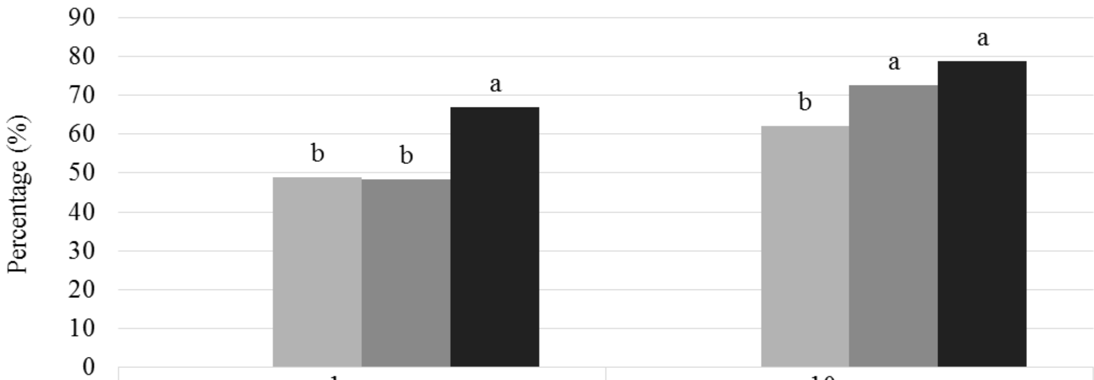
The Benzimadizoles, including thiophanate methyl, are known to inhibit *B*-tubulin assembly during mitosis (FRAC, 2018). Early Sigatoka control includes the use of these compounds in combination with dithiocarbamates and oil (Stover, 1980). However, after the use of these compounds for 2 to 3 years, the pathogen developed tolerance in Honduras and the Philippines (Stover, 1979), which discouraged the use of thiophanate methyl in the Philippines. But in this trial, thiophanate methyl showed comparable results to few products recommended for use in Black Sigatoka control not to mention that increasing the concentration also increased the efficacy of the product.

Copper, on the other hand, has a multisite activity against fungal pathogens (FRAC Code List, 2018) and is known to be reliable element for disease control (Borkow and Gabbay, 2009). Copper-based products are reported to reduce severity of other diseases such as potato late blight (Speiser et al., 2006; Giannousi et al., 2013) and grape vine diseases (Arias et al., 2004). Although most of the reported studies are against different pathogens, results of this trial showed the potential of copper in the form of copper hydroxide and ionic copper in inhibiting germ tube elongation of *P. fijiensis* that is more effective than the benzimidazoles especially at increased rate.

Test of Postharvest Products against *Pseudocercospora fijiensis*.

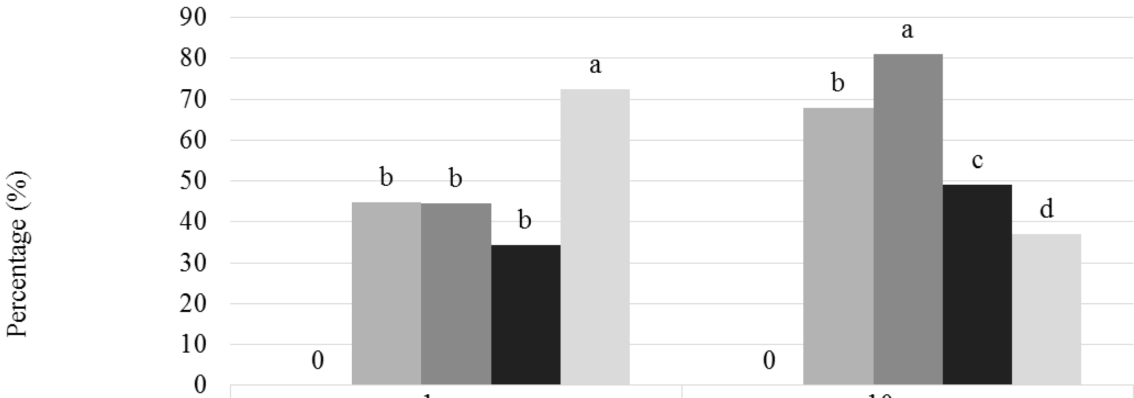
At 1 ppm, an inhibition of 72% was recorded from potassium aluminium sulfate while the rest of the PPPs obtained an inhibition ranging from 34 to 45%. At higher concentration of 10 ppm, inhibition was greatest from calcium hypochlorite with 81% followed by prochloraz with 68%, iprodione with 49% and then potassium aluminium sulfate with 37.13% (See Figure 2). Among the treatments, only potassium aluminium sulfate exhibited a decreased of efficacy at higher concentration.

Prochloraz and iprodione are known active ingredients inhibiting sterol biosynthesis in membranes and signal transduction (FRAC 2018), respectively, while calcium hypochlorite and alum are common disinfectants. Prochloraz, iprodione, calcium hypochlorite, and potash alum are well documented in terms of postharvest disease control of several fruits especially banana (Jones, 1991; Johanson and Blazquez, 1992; Abeywickrama et al., 2009; Siriwardana et al., 2017). However, there were no known reports on the efficacy of these



	1 ppm	10 ppm
Untreated control	0	0
Thiophanate methyl	48.87	62.21
Copper hydroxide	48.37	72.61
Ionic copper	66.96	78.90

Figure 1: Germ tube growth reduction (%) of *Pseudocercospora fijiensis* with the application of preharvest PPPs. Means with the same letter are not significantly different form each other at 5% level of significance.



	1 ppm	10 ppm
Untreated control	0	0
Prochloraz	44.72	67.79
Calcium hypochlorite	44.52	81.14
Iprodione	34.41	49.03
Potassium aluminum sulfate	72.39	37.13

Figure 2: Germ tube growth reduction (%) of *Pseudocercospora fijiensis* with the application of postharvest PPPs. Means with the same letter are not significantly different form each other at 5% level of significance.

products against *Pseudocercospora fijiensis* or its related species to the best of knowledge of the authors. This may be the first time that these postharvest products were explored against the Black Sigatoka pathogen. Nevertheless, the results of this trial addressed the objective on determining the effect of the pre- and postharvest products to *P. fijiensis.*

CONCLUSION

This research demonstrated the effect of the pre- and postharvest products to the growth of *P. fijiensis*. Very limited studies are available exploring the effect of products used in pre and postharvest operations against the Black Sigatoka pathogen. In this study, a growth inhibition of more than 62% was recorded by pre-harvest PPPs while postharvest products obtained as high as 81%. Even at very low concentrations, test products inhibited the germ tube elongation of *Pseudoscercospora fijiensis*. Results generated from this study add in the body of knowledge on the effect of products used in banana production to non-target pathogens. As we aim towards maximizing integrated disease management, the effect of these products to other banana pathogens will be determined including their efficacy in the field.

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CONTRIBUTIONS OF THE AUTHORS

AMRN wrote the manuscript of the article and formulated the treatments of this study. MLC provided the data analysis and supervised the conduct of the experiment. GSS and CMSA did the laboratory bioassay. BMC conceptualized the research project.

CONFLICT OF INTEREST

The authors affirm that the study was done without any financial or commercial relationships that could be interpreted as a potential conflict of interest.

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