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Efficacy of Botanical Mixture and Fungicides to Combat Sigatoka Disease in Banana Cultivation

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Abstract

Black Sigatoka disease caused by *Mycosphaerella fijiensis* seriously threatens banana cultivation worldwide. This infirmity is controlled by the recurrent application of synthetic fungicides, which leads to several adverse effects. Plant extracts with antifungal potential have long been investigated as they contain secondary bioactive compounds for protecting crops from microbial infection. This study compared the antifungal activities of both botanical mixtures and recommended fungicides to control Black Sigatoka in banana. Laboratory and field studies were conducted to assess the antifungal activity of the botanical mix prepared from eight different herbal plant parts against *M. fijiensis,* with two recommended fungicides as the positive control. *In vitro* results showed that the radial growth of the fungus was significantly impaired ($P<0.05$.) by the addition of the botanical mixture, fungicides, and combination of the botanical mixture (BM) and fungicides in the PDA media. *Mycosphaerella fijiensis* differed in their reaction to the BM and fungicides, and combination of BM and fungicides, but on the whole, growth inhibition increased with the concentration. In field experiments, all the treatments reduced the disease incidence significantly compared to the unsprayed control, where the disease severity index (DSI) increased from 43.88 to 67.77 within 28 days. After the first spray, the botanical mixture and fungicides (Chlorothalonil and Carbendazim) reduced the disease severity index to 39.7, 41.19, and 44.44, respectively. Fungicides and combinations of Chlorothalonil + BM and Carbendazim + BM showed the lowest DSI, 34.07 and 35.83, respectively. The results show that combining the synthetic and botanical mixture is more effective for controlling the disease. The study reveals the possibility of utilizing a botanical mixture as an integrated approach to manage Black Sigatoka disease more efficiently and in an eco-friendly manner.

Keywords – Antifungal Activity – Carbendazim – Chlorothalonil – *Mycosphaerella fijiensis* – Plant extracts

Introduction

The banana (*Musa* spp.) is a climacteric fruit belonging to the family Musaceae. It is one of the widely cultivated and consumed fruits in many tropical and subtropical countries as a dessert and staple food.

The banana industry is worth USD 25 billion and is expected to increase at a compound annual growth rate (CAGR) of 4.5% between 2022 and 2027 (Crawford & Kueffner 2020, Mordor

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Intelligence 2022). According to the Food and Agriculture Organisation (FAOSTAT 2022), banana output increased from about 97 million tonnes (Mt) in 2008 to about 120 Mt in 2020 on 5.2 million hectares. However, pests and diseases are major barriers to banana production in many banana-cultivating countries. Fungal diseases are most prominent among the diseases affecting bananas. Members in the fungal genus *Mycosphaerella* caused Sigatoka leaf spot disease. Of which, three very closely related pathogens, *M. fijiensis* Morelet, *M. musicola* Leach, and *M. emusae*, cause Black Sigatoka, Yellow Sigatoka, and Eumusae leaf spots, respectively, in banana plants (Ramsey et al. 1990). Among these three-leaf spot diseases, Black Sigatoka disease (Black Leaf Streak) caused by *M. fijiensis* has become a limiting factor for banana production. It is known as a significant constraint for banana production wherever it occurs, and it is considered the most costly, damaging, and devastating disease among leaf spot diseases. Moreover, it has potentially threatened banana plantations globally (Carlier et al. 2000). The disease has been reported in most banana-growing countries, especially in South East Asia, Pacific, Latin America and Africa, and Asian countries (Carlier et al. 2000, Crous & Mourichon 2002).

The causal pathogen of Black Sigatoka, *M. fijiensis*, is more virulent than others, causing leaf spot diseases. After the infection, reddish-brown streaks running parallel to the leaf veins, which aggregate to form large, dark brown to black compound streaks, can be seen on the leaves. Further, those streaks eventually include fusiform or elliptical lesions that coalesce to form a water-soaked border with a yellow halo and merge to cause extensive leaf necrosis. Also, the Black Sigatoka pathogen mostly does not kill plants immediately. Still, after the infection of the pathogen, the plant is weakened by decreasing the photosynthetic capacity of leaves, causing a reduction in the quantity and quality of the fruits. Another substantial impact is that the fruits harvested from infected plants accelerate premature ripening (Ramsey et al. 1990, Marin et al. 2003, Churchill 2011)

Due to the higher effectiveness, the disease management of Black Sigatoka has been achieved by synthetic fungicides as a more straightforward aspect. However, the widespread use of agrochemicals, particularly synthetic fungicides, disrupts ecological balance, accelerates soil and water deterioration, and makes plants more vulnerable to pests and diseases (Riaz et al. 2008). Therefore, to mitigate the dependence on synthetic fungicides to overcome the adverse outcomes, directing towards an effective and environmentally sound alternative for control of the diseases will be advantageous for the future applications of controlling the black Sigatoka disease.

Botanicals are one of several non-chemical control options that recently received attention (Patrick et al. 2022). Considering the advantage of the availability of a wide range of herbal plants, introducing botanicals could be a better alternative. Botanicals are gaining popularity in organic farming due to their safety profile on crop consumption, and consumers are willing to pay a premium price for organic produce (Misra 2014). An integrated approach using plant-based extracts and synthetic fungicides can efficiently control the Black Sigatoka disease (Aman & Rai 2015). Furthermore, most herbal plants contain antimicrobial compounds that have long been recognized (Naqui et al. 2004, Riaz et al. 2008). Thus, this study focuses on the *in–vitro* screening and field evaluation of botanical mixture (BM) and combination of fungicides and BM on control of Black Sigatoka disease of banana caused by *M. fijiensis*.

Materials & Methods

Preparation of Botanical Mixture

Seven different types of herbal plants (Table 1) were collected from Mahiyangana, Sri Lanka. A mean annual temperature of 30 º C and average annual rainfall of 1937 mm characterize the area. The fresh plant parts were washed with clean running tap water and ground into fine particles, adding adequate water. All the ground plant parts were mixed and volumized up to 1l. The mixture was kept for fermentation by sealing the container properly for seven days. Then, the fermented plant materials were mixed and filtered using gauze. At the time of field application, the concentration of the botanical mixture was diluted into 1.5 ratios with water.

Table 1 Quantity of Plant used to prepare a liter of botanical mixture

Plant	Scientific name	Family	Used Plant Part	Amount
Marigold	*Tagets ereccta*	Asteraceae	Flowers	50g
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	50g
Neem	*Azadirachta indica*	Meliaceae	Leaves	50g
Neem	*Azadirachta indica*)	Meliaceae	Bark	100g
Sweet flag	*Acorus calamus*	Acoraceae	Rhizome And Leaves	100g
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome	100g
Garlic	*Allium sativum*	Amaryllidaceae	Bulbs	50g
Gliricidia	*Gliricidia sepium*	Fabaceae	Leaves	125g

Initial Screening of Antifungal Activity against *M. fijiensis*

The disk diffusion method was used to screen the antifungal activity of the treatments. Sterilized 7 mm filter paper discs (Whatman, no. 3) were impregnated with each treatment, T0 – Control, T1 – Botanical mixture –100%, T2 – Botanical mixture –50%, T3 – Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile)(Daconil®) T4 – Carbendazim (methyl benzimidazol-2-ylcarbamate)(Bavistin®), T5 – Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile)(Daconil®) (50%) + Botanical mixture (50%) and T6 – Carbendazim (methyl benzimidazol-2-carbamate)(Bavistin®) (50%) + Botanical mixture (50%), separately and allowed to air dry. *Mycosphaerella fijiensis* mycelium disc (4.7mm) placed on the surface of PDA amended with treatment and kept under 25℃ for incubation. All the treatments were conducted in triplicates.

In vitro* Evaluation of the Antifungal Activity against *M. fijiensis

Different treatments were tested for their efficiency against the pathogen using agar dilution. A Series of concentrations of the botanical mixture, 25%, 50%, and 100%, and the synthetic fungicides and their combinations with the botanical mixture (Table 2) were obtained by amending PDA. The amended medium was allowed to solidify with Cephalexin 5 mg/ml. Each plate was inoculated with 5mm mycelial discs of *M. fijiensis* taken from the advancing edges of pure cultures. Each treatment was replicated thrice. All the culture plates were incubated at 25 ℃. After five days, the radial growth of the pathogen was recorded.

Table 2 Treatments for evaluating the effect of different concentrations of synthetic fungicides and botanical mixtures on the radial growth of *M. fijiensis*.

Treatment	Solution	Distilled water	PDA	Final volume
T0 - Control	-	-	10 ml	10 ml
T1-Botanical Mixture 100%	1 ml	-	9 ml	10 ml
T2-Botanical Mixture 50%	500 µl	500 µl	9 ml	10 ml
T3-Botanical Mixture 25%	250 µl	750 µl	9 ml	10 ml
T4-Chlorothalonil	2.99 µl	997 µl	9 ml	10 ml
T5-Carbendazim	0.99 µl	999 µl	9 ml	10 ml
T6-Botanical Mixture 50% +Chlorothalonil 50%	500 µl+1.5 µl	498 µl	9 ml	10 ml
T7-Botanical Mixture 50% +Carbendazim 50%	500 µl+0.5 µl	499 µl	9 ml	10 ml

Data collection

Percentages of growth inhibition of *M. fijiensis* in the treatments were calculated over control by using the following formula (Vincent 1947).

$$\text{Inhibition Percentage} = \frac{\text{Diameter of control colony - Diameter of treated colony}}{\text{Diameter of control colony}} \times 100$$

Experimental Design

The research was conducted using six months aged banana plants (*Musa* spp.), Cavendish variety, showing uniform disease symptoms in all plants. Thirty-six banana plants were arranged in a Randomized Complete Block Design (RCBD) with six treatments and six replicates within three blocks with a spacing of 1.5x 2 m. The botanical mixture (BT), fungicides, and combination of the botanical mixture and fungicides were applied within 7-day intervals using a mechanical sprayer and knapsack sprayer according to the rates given in Table 3.

Table 3 The rate of application of botanical mixture (BT), fungicides, and combination of the botanical mixture and fungicides

Treatment	Description
T0	Control, without any application
T1	Daconil®/ Chlorothalonil, Contact fungicide ((900-1200 mL/ha)
T2	Bavistin® /Carbendazim, Systemic fungicide (490 -560 mL/ha)
T3	Botanical Mixture (4 L/ 50 plants)
T4	Combination of Chlorothalonil and botanical mixture (1:1)
T5	Combination of Carbendazim and botanical mixture (1:1)

Chlorothalonil 0.6 mL/plant, Carbendazim 0.25 mL/plant aerially applied within 7-day intervals by mechanical sprayer, and Botanical mixture 80mL/plant within 7-day intervals using mechanical and knapsack sprayer.

Estimation of Disease Severity Index (DSI)

The Disease Severity Index was assessed using Gauhl's modification of Stover's severity scoring system (Mobambo et al. 1993). First, both infected plants at severity stage two and ten healthy leaves from each plant were selected and numbered. Then, the leaves were given numbers according to the proportion of severity of symptoms on the leaves on a scale of 0-6: 0 - No symptoms, 1 - less than 1 % showing symptoms, 2 - 1-5 % showing symptoms, 3 - 6- 15 % showing symptoms, 4 - 16- 33 % showing symptoms, 5 - 50 % showing symptoms, and 6 - More than 50 % showing symptoms (Fig 1).

Fig 1 – Leaves showing symptoms according to the scale of numbering by using Gauhl's modification of Stover's severity scoring system (Mobambo et al.1993)

Disease Severity Index (DSI)

Initial DSI was taken before the treatment application, whereas DSI was taken after the treatment application at 5-day intervals. Then, using the graded leaves by numbering, the Disease Severity Index (DSI) was calculated according to the following formula (Gauhl 1994).

$$DSI = \Sigma(nb) \times 100 / [(N-1) T]$$

Where;
n = number of leaves in each grade, b = grade, N = number of grades used, T = total number of leaves graded on each plant

Data Analysis

All *in-vivo* and *in-vitro* experimental data were statistically analyzed using Statistical Analysis Software (SAS) 9.0 version by one-way ANOVA procedure. Duncan's Multiple Range Test (DMRT) was used to determine the significance of the treatment effects at $p \leq 0.05$ level. The Microsoft Excel (2013) computer software package was used for graphical illustrations.

Results

Initial Screening of Antifungal Activity

After five days of fungal inoculation, all the treatments have significantly shown antifungal activity by the zone of inhibition. However, the zone of inhibition showed different degrees of antifungal activity in each treatment.

Inhibition of Radial Growth of Mycelium at Different Concentrations of Treatments

According to the initial screening, the antifungal activity of synthetic fungicides and concentration series of the botanical mixture was assayed, and the effect of the treatments on the growth of the fungi was observed. Results revealed significant inhibition in different degrees by all treatments against *M. fijiensis*, compared with the control. Results showed radial growth inhibition by all treatments comprising different concentrations of the botanical mixture and synthetic fungicides. The highest growth inhibition was observed in Chlorothalonil, Carbendazim, and the combination of Chlorothalonil + botanical mixture. The percentage of inhibition is 100% while exhibiting more significant antifungal activity. Further, percentage of inhibition in the mycelium growth were 59.67C ± 0.59 %, 81.20 b ± 4.12 %, and 86.93 b ±7.14 % in 25%, 50%, and 100% concentrated botanical mixtures, respectively. However, the inhibition levels of 50% and 100% concentrated botanical mixtures are not significantly different. The lowest growth inhibition was shown by the 25% concentrated botanical mixture, which is significantly different from all other treatments (Fig 2).

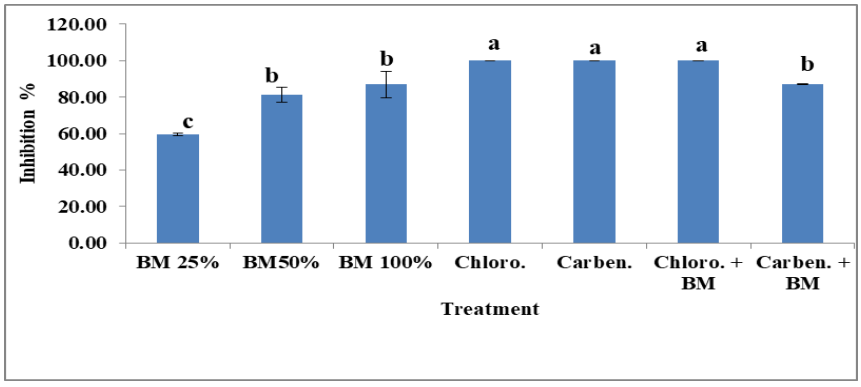


Fig. 2 – Inhibition percentages of different Treatments: 25% concentrated Botanical mixture, 50% concentrated Botanical mixture, and 100% concentrated Botanical mixture, Chlorothalonil,

Carbendazim, Combination of Chlorothalonil + Botanical mixture, and Carbendazim + Botanical mixture. Means denoted by the same letters are not significantly different.

Variation of Disease Severity in Field Experiment

The results indicated that after starting the application of the treatments, DSI changed in different levels (Table 4) with controlling the disease at several intensities, except for the control (T0). By the 28th day, DSI by T0 has increased from an initial DSI of 43.888 to 67.778, increasing the disease while showing a significant difference from the other five treatments.

Table 4 Disease Severity in Field Experiment.

Treatment	Time					
	Day 1 (Initial)	**Day 6**	**Day 11**	**Day 19**	**Day 24**	**Day 28**
T0 - Control	43.888^{a}	45.278^{a}	49.445^{a}	58.610^{a}	63.333^{a}	67.778^{a}
T1 - Chlorothalonil	38.882^{a}	36.452^{ba}	38.078^{b}	41.720^{b}	42.028^{b}	41.193^{b}
T2 - Carbendazim	42.778^{a}	38.057 ^{ba}	38.332^{b}	41.112^{b}	41.112^{b}	44.445^{b}
T3 - Botanical Mixture	35.277^{a}	35.277^{b}	37.498^{b}	38.612^{b}	40.278^{b}	39.723^{b}
T4 - Chlorothalonil+ BM	39.088^{a}	35.912 ^{ba}	32.875^{b}	33.452^{b}	34.077^{b}	34.077^{b}
T5 - Carbendazim+ BM	37.800^{a}	33.548^{b}	35.247^{b}	35.000^{b}	36.112^{b}	35.833^{b}

Means denoted by the same letters are not significantly different according to Duncan's Multiple Range test ($p<0.05$)

On the sixth day, T1, T2, and T4 did not show significant differences in disease severity, whereas T3 and T4 showed significant differences compared to T0. Further, all other treatments have reduced the disease severity from the 11th to the 28th day except for T0. They were not showing significant differences in each other. On the 28th day, the DSI of plants treated with T1 (Chlorothalonil) increased from 38.882 to 41.193, observing a smaller increment of the disease severity than the initial day. DSI of plants treated with T2 (Carbendazim) also showed a small increment of DSI from 42.778 to 44.445. Similarly, the DSI of the plants treated with T3 (Botanical mixture) also increased the disease from 35.277 to 39.723. However, the Synthetic and Botanical combinations differed from the above, reducing the infection and showing the symptoms. The DSI of plants treated with T4 (Chlorothalonil + Botanical mixture) has decreased from 39.088 to 34.077, and the DSI of plants treated with T5 (Carbendazim + Botanical mixture) has reduced from 37.800 to 35.833.

According to the variation of the DSI (Fig 3), Black Sigatoka disease has increased gradually in control, whereas DSI is reduced with all treatments for up to two weeks and remains constant afterwards. After applying treatments, all treatments except the control have shown a significant reduction of the disease severity by the sixth day.

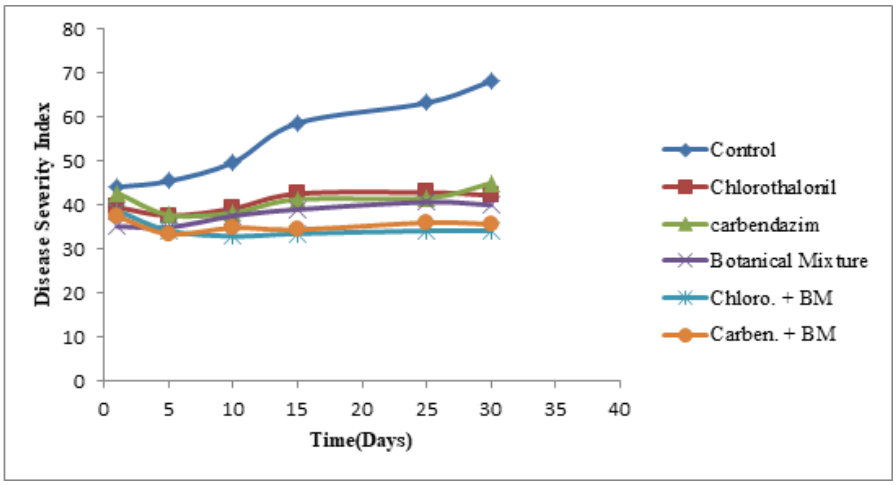


Fig. 3 – Variation of Disease Severity Index with time in different treatments: Control,

Chlorothalonil, Carbendazim, Botanical mixture, Chlorothalonil + Botanical mixture, and Carbendazim + Botanical mixture.

Evaluation of the Most Efficient Control Method for Black Sigatoka Disease

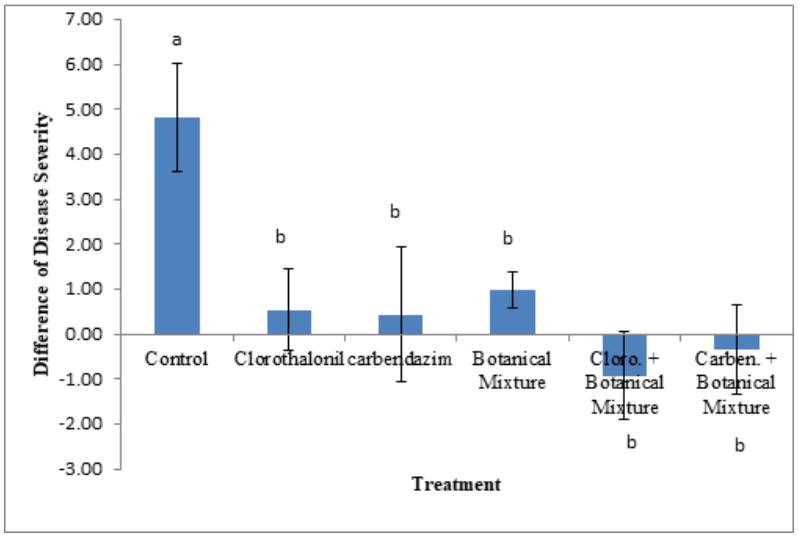
Analysis of the mean differences of the DSI has shown the variation between the initial and final DSI (Table 5). The T0, T1, T2, and T3 led a positive difference indicating the increment of the disease, whereas T4 and T5 showed a negative difference indicating the control of the disease. Among the negative values, T4 recorded the highest negative difference (-0.92 ±0.97), and T5 indicates the lowest contrast (-0.35 ±1.00).

Table 5 Differences in disease severity of the treatments.

Treatment	Mean Differences in DSI	Standard Deviation	Standard Error
T0-Control	4.81[a]	2.72	1.21
T1-Chlorothalonil	0.54[b]	2.04	0.91
T2-Carbendazim	0.44[b]	3.34	1.49
T3-Botanical mixture	0.98[b]	0.92	0.41
T4-Clorothalonil + Botanical mixture	-0.92[b]	2.17	0.97
T5-Carbendazim + Botanical mixture	-0.35[b]	2.23	1.00

Means denoted by the same letters are not significantly different according to Duncan's Multiple Range tests ($p<0.05$)

The DSI difference is significantly higher in T0 compared to all other treatments. In contrast, the differences in DSI of different treatments, T1, T2, T3, T4, and T5, have not shown a significant difference in each other (Fig 4). The difference in DSI of Chlorothalonil was obtained (0.54b ±0.91), and the difference in DSI of Carbendazim was obtained as (0.44b ±1.49). The difference in DSI is high in Chlorothalonil compared to Carbendazim. Chlorothalonil is less effective than systemic fungicides in reducing the production of pseudothecia in infected tissue. The efficient and long-lasting control has been shown in the synthetic fungicides + botanical mixture treatment combination due to the ability to act as a fungicide and a plant protectant inhibiting conidia germination on banana leaves. Among those two, the highest control has shown Chlorothalonil + botanical mixture comparatively Carbendazim + botanical mixture. The botanical mixture may act as an agent to deposit the fungicide on the leaf surface while providing the fungicidal quality on the plants with the combination of Chlorothalonil.



Figs 4 – Differences of the Disease Severity Indexes of Control, Chlorothalonil, Carbendazim, Botanical mixture, Chlorothalonil + botanical mixture, and Carbendazim + botanical mixture.

Means denoted by the same letters are not significantly different.

Discussion

In the present study, an aqueous mixture comprising extracts from a combination of seven different types of herbal plants showed an antifungal effect against *M. fijiensis* under laboratory conditions. There are large numbers of research findings on the antifungal potential of plant extracts against plant pathogenic microbes (Fiori et al. 2000, Ghosh et al. 2002, Choi et al. 2004, Sangeetha et al. 2013). Almost all research findings revealed the antimicrobial potential of plant extracts alone, not as a combination. The present study revealed the significant antimicrobial effect of combining seven plant extracts against *M. fijiensis*. The present study is the first to report on the antifungal effect of a combination of seven different aqueous plant extracts against fungal pathogens. The BM is organic preparation made by one of the organic farming instructors using a series of experiments (unpublished data). Kumakech (2017) revealed that the aqueous extract of Neem (*Azadirachta indica*) alone could protect bananas against Black Sigatoka caused by *M. fijiensis*. No work has been reported on the use of the extract of Marigold (*Calendula officinalis*), Ginger (*Zingiber officinale*), Sweet flag (*Acorus calamus*), Turmeric (*Curcuma longa*), Garlic (*Allium sativum*), and Gliricidia (*Gliricidia sepium*) for control of black sigatoka disease in bananas. However, there are several reports on the antifungal activities of Marigold (Riaz et al. 2008, Singh et al. 2020),Ginger (Kapoor 1997, Sowley & Kankam 2019), Sweet flag (Dissanayake & Jayasinghe 2013, Sugha 2013), Turmeric (Kapoor 1997), Garlic (Abdullahi et al. 2020), and Gliricidia (Abdulaziz et al. 2019) against different plant pathogenic fungi.

In the field experiment, botanical mixture, fungicides (Chlorothalonil and Carbendazim), and the combination of BM and fungicides showed efficacy against *M. fijiensis*. The difference in DSI is high in Chlorothalonil compared to Carbendazim, showing Chlorothalonil is less effective than systemic fungicides. However, Washington et al. (1998) reported that Chlorothalonil arrested hyphal growth when applied to banana leaves after ascospores had already germinated, and reduced the rate of lesion expansion when applied to the abaxial leaf surface after symptom appearance. Therefore, the correct stage and appropriate placement of chlorothalonil application are essential for better control of the disease. Washington et al. (1998) reported that the reason for the variation of the controlling disease among Chlorothalonil and Carbendazim might be the effectiveness of the contract (protectant) fungicide and systemic fungicide. Even though Chlorothalonil and Carbendazim inhibited the growth of the pathogen, a combination of fungicides and BM are more effective for reducing disease incidence than each treatment alone. The efficient and long-lasting control has been shown in the treatment combination of synthetic fungicides + Botanical mixture due to the ability to act as a fungicide and a plant protectant inhibiting the germination of ascospores on banana leaves.

Conclusion

Present work suggested that a combination of fungicides and BM can be used in the eco-friendly management of Sigatoka disease in banana plantations by reducing the number of fungicide spraying cycles. Isolation and characterization of active molecules responsible for antifungal activity from BM are required.

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