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EFFECTS OF CULTURE MEDIUM AND LIGHT DURATIONS ON THE FUNGAL GROWTH OF *Bipolaris sorokiniana*

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ABSTRACT

Bipolaris sorokiniana *has been identified as a potential indigenous fungal plant pathogen to control goosegrass (*Eleusine indica*) in oil palm plantations. However, for mass production, a suitable medium and an optimum growing environment need to be identified. This study was conducted to determine the suitable culture media and light duration requirements for optimal growth of* B. sorokiniana. *Four types of culture media: Potato dextrose agar (PDA), corn meal agar (CMA), V8 agar (V8A) and water agar (WA), were evaluated for the* B. sorokiniana *subculture. Three light durations (0, 12 and 24 hr) were tested for* B. sorokiniana *growth. The growth diameter and colony-forming units of* B. sorokiniana *were recorded. When compared to CMA, V8A, and WA media in the same light durations,* B. sorokiniana *grew at maximum mean growth diameter at 12 hr light duration in PDA (4.56 cm), 24 hr light duration in PDA (4.36 cm) and 0 hr light duration in PDA (4.21 cm).* Bipolaris sorokiniana *produced more CFU during the 0 light duration than during the 12 hr and 24 hr light durations. Hence, this study shows that* B. sorokiniana *has the potential for reproduction using PDA culture media without light.*

Keywords: *Bipolaris sorokiniana, Eleusine indica*, goosegrass, oil palm, weed biocontrol.

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INTRODUCTION

Bipolaris sorokiniana is an ascomycete fungus with a wide range of hosts in the Poaceae family. It is a causal agent of the spot blotch disease in wheat and barley, a major disease problem in warmer and humid regions of the world including India, Bangladesh, Brazil and other South Asian countries (Chauhan *et al.*, 2017; Morejon *et al.*, 2006). Spot blotch disease has caused losses of up to 50% in wheat grain yield and deteriorated seed quality (Chauhan *et al.*, 2017). The economic loss in wheat production caused by *B. sorokiniana* is due to the severe occurrence of spot blotch and has affected the livelihood of millions of small-scale wheat farmers (Acharya *et al.*, 2011). Other than spot blotch, *B. sorokiniana* is also a causal agent for diseases such as common root rot, foot rot, seedling blight and seed rot of wheat (Al-Sadi, 2021).

However, a study conducted by Ismail *et al.* (2020) found that *B. sorokiniana* is a host-specific plant pathogen that affects goosegrass (*Eleusine indica*), a weed in oil palm plantations, through the pathogenicity and host range tests but does not affect oil palm. The effort was conducted mainly to search for biological control agents to suppress *E. indica's* growth, especially in oil palm plantations (Ismail *et al.*, 2020). It is because *E. indica* has been classified as a noxious weed and the most destructive grass in oil palm plantations (Matthews, 1998). *Eleusine indica* was found in immature oil palm plantations and was also found to be the most prevalent grass (82.2%), with 30.0% having medium to high coverage (Maizatul-Suriza and Idris, 2012). Weeds can affect the growth of crops or cause yield losses (Kuan *et*

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al., 1991) by competing aggressively for nutrients, light and water. The discovery of biological weed control agents that can be used as bioherbicides has provided an alternative to chemical herbicides in biological weed management and can significantly reduce the use of chemical herbicides. This will benefit huge areas of oil palm cultivation in Malaysia, which accounted for 5.7 million hectares of land area in 2021 (MPOB, 2022).

To manage and suppress weed populations, bioherbicides are compounds and secondary metabolites derived from microbes such as fungi, bacteria and protozoa; phytotoxic plant residues; and extracts or single compounds derived from other plant species (de Souza *et al.*, 2017). The selection of pathogens with high pathogenicity and specificity (Keerthi *et al.*, 2019) for the host plants is a crucial part of the whole selection process for potential biological control agent activities. Other than that, Koch's postulates must be satisfied in order for fungal plant pathogens to serve as biological control agents (Byrd and Segre, 2016). For example, one characteristic necessitates that the fungus is cultured in the laboratory. Researchers have been using artificial media to culture fungus for mass production. Some of the fungi can be cultured on solid media for small-scale production, while others can be cultured on liquid media for large-scale production. Despite this, only a few can sporulate in liquid.

Therefore, this study was conducted to investigate the best culture medium and light requirements for optimal growth of *B. sorokiniana*. It aims to obtain information that helps propagate *B. sorokiniana* through mass production. This approach will enable the development of *B. sorokiniana* as a bioherbicide capable of eradicating *E. indica*, particularly in oil palm plantations.

MATERIALS AND METHODS

Experiment Site

All experiments were conducted in the Malaysian Palm Oil Board's laboratory, located in Bandar Baru Bangi, Selangor, Malaysia.

Isolation of Microbes from Diseased *E. indica*

Eleusine indica's leaves with disease symptoms, such as brownish/yellowish spots, lesions with reddish/brownish margins, and yellow/brown lesions, were collected in the morning at the oil palm plantation in Bangi, Selangor, Malaysia and immediately transported to the laboratory to isolate the microbes. The leaf samples were surface-sterilised by rinsing them with tap water, then immersing them in 1% v/v sodium hypochlorite for 2-3 s before being washed twice with sterile distilled water. Using sterile scalpel blades, leaves were cut into small pieces (about 4 mm^2) and plated on 1% w/v water agar and potato dextrose agar (PDA) supplemented with antibiotics (50 $\mu g\ mL^{-1}$ penicillin and 100 $\mu g\ mL^{-1}$ ampicillin). After three to four days of incubation, fungal mycelia growing from plant tissue were excised and transferred to fresh PDA via hyphal tipping.

Molecular Identification of Fungal Isolates

Fungal DNA was extracted from mycelial pieces according to Izumitsu *et al.* (2012). PCR amplification was performed as described by Ogura-Tsujita and Yukawa (2008). Internal transcribed spacer (ITS) sequences were amplified using the primer pair ITS1F/ITS49 (Gardes and Bruns, 1993; White *et al.*, 1990). The purified PCR products were sequenced using an automated DNA sequencer (ABI PRISM®). DNA sequenced results were analysed using bioinformatics software, which is available in the NCBI database, by employing the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul *et al.*, 1990; 1997). The result for identification was based on the maximum scoring for identity value query coverage. The ITS gene sequences were aligned using BioEdit software version 7.2 (http://www.mbio.nscu.edu/bioEdit/bioEdit).

Fungal Subculture Media

Four types of culture media were evaluated for the subculture of *B. sorokiniana*: 1) potato dextrose agar (PDA); 2) corn meal agar (CMA); 3) V8 agar (V8A); and 4) water agar (WA) and each subculture media was tested with three light duration (0, 12 and 24 hr) for *B. sorokiniana* growth. PDA, CMA and V8A are standard media for fungal culture, and WA is a negative control without nutrients. Sub-cultured hyphal tips were transferred onto 90-mm Petri dishes containing 20 mL of each medium and were placed in the incubator at 28°C for 0 hr (total darkness), while *B. sorokiniana* exposed to continuous fluorescent lights for 12 hr and 24 hr were placed in the SANYO Versatile Environmental Test Chamber (MLR 351, Japan) at 28°C. The measurement of colony growth diameter was taken every day for six days until the plates were full of colony growth. Six replications were used in this experiment (Shtayeh *et al.*, 1998).

Preparation of Culture Media

Potato dextrose agar (PDA). A total of 39 g of PDA powder was suspended in 1 L of purified water. The solution was thoroughly mixed prior to being heated with frequent agitation, then boiled for 1 min to completely dissolve the powder. Subsequently, it was autoclaved at 121°C for 15 min. A 20 mL ready

solution with pH of 5.6 ± 0.2 was poured into a sterile Petri dish and allowed to solidify before being used to subculture the fungi at room temperature (25°C) (Garcia, 2010).

Corn meal agar (CMA). A total of 17 g of CMA powder was suspended in 1 L of purified water. The solution was thoroughly mixed prior to being heated with frequent agitation, then boiled for 1 min to completely dissolve the powder. Subsequently, it was autoclaved at 121°C for 15 min. A 20 mL ready solution with pH of 6.2 ± 0.3 was poured into a sterile Petri dish and allowed to solidify before being used to subculture the fungi at room temperature (25°C) (McGinnis, 1980).

V8 agar (V8A). A 200 mL amount of V8 juice was added to the 800 mL of distilled water. A 15 g amount of agar and 2 g of $CaCO_3$ were added to the solution and boiled in a microwave until all the agar was dissolved. The solution was then autoclaved at 121°C for 15 min. A 20 mL ready solution with pH of 7.2 ± 0.2 was poured into a sterile Petri dish and allowed to solidify before being used to subculture the fungi at room temperature (25°C) (Dhingra and Sinclair, 1985).

Water agar (WA). A 15 g amount of WA powder was suspended in 1 L of purified water. The solution was thoroughly mixed prior to being heated with frequent agitation, then boiled for 1 min to completely dissolve the powder. Subsequently, it was autoclaved at 121°C for 15 min. A 20 mL ready solution with pH of 7.0 ± 0.2 was poured into a sterile Petri dish and allowed to solidify before being used to subculture the fungi at room temperature (25°C) (Atlas, 1996).

Colony-forming Units (CFU) of *B. sorokiniana*

Colony-forming units (CFU) were measured on six-day-old cultures by harvesting the mycelia from each treatment in the previous experiment using 10 mL of sterile distilled water by scraping the surface of the colony. Mycelia were extracted by filtration through cheesecloth and rinsing with distilled water. The mycelial mat was gently pressed to remove the excess fluid and blended for 30 s at medium speed. A tenfold serial dilution was conducted for inoculum production. The amount of 0.1 mL liquid from the serial dilution was pipetted and spread evenly onto the PDA plate using a spatula, which allowed the liquid to absorb into the agar. The plates were incubated overnight at 28°C in the incubator. The CFU was counted by using a marking pen to check off each colony on the underside of the plate. The plates were arranged in an RCBD design with six replications. The CFU mL^{-1} was counted using the Equation 1 (Harley, 2016):

$$\text{CFU} = \frac{\text{Number of colony} \times \text{dilution factor}}{100\ \mu\text{L}} = \text{CFU mL}^{-1} \quad (1)$$

Statistical Analysis

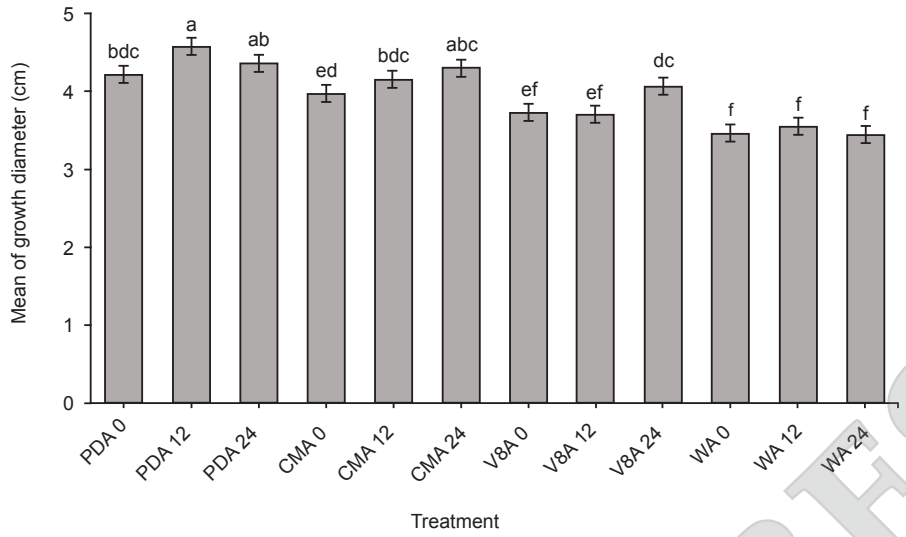
Results for the treatments were analysed using analysis of variance (ANOVA). The means at various treatments were analysed using Tukey's test at $p \leq 0.05$ using the SAS software (version 9.4) to compute the difference between treatments.

Two Generalised Linear Mixed Models (GLMMs) were developed to examine the relationships between two response variables, a) mean growth diameter and b) CFU of *B. sorokiniana*, and the treatments. In the first model, the regression of mean growth diameter against three predictive variables, namely media, light duration and incubation day, was done. In the second model, the regression of CFU against two predictive variables, media and light duration was done. The replicate was fitted as a random factor in both models. The Poisson distribution and log-link function were used in the modelling process. Predictive variables that have significant effects on both models were determined using Wald testing. All variables in each analysis that were not significant ($p > 0.05$) were removed using the backward elimination procedure. The coefficient of determination value (R-squared, R^2) of the best model for each subset was reported. All analysis was conducted using GenStat (VSN International Ltd., Hemel Hempstead, United Kingdom).

RESULTS

Effect of Culture Media and Light Durations on *B. sorokiniana* Growth in Petri Dishes.

Figure 1 illustrates the analysis of mean growth diameter (cm) in 0, 12 and 24 hr light durations using different media, namely PDA, CMA, V8A, and WA (control). The mean growth diameter of *B. sorokiniana* in PDA 12 hr light duration (4.56 cm) was significantly different from PDA 0 hr light duration (4.21 cm), CMA 12 hr light duration (4.15 cm), V8A 24 hr light duration (4.05 cm), CMA 0 hr light duration (3.96 cm), V8A 0 hr light duration (3.72 cm), V8A 12 hr light duration (3.70 cm), WA 12 hr light duration (3.55 cm), WA 0 hr light duration (3.45 cm), and WA 24 hr light duration (3.43 cm), and showed no significant difference to PDA 24 hr light duration (4.36 cm) and CMA 24 hr light duration (4.29 cm). Meanwhile, the mean growth diameter of *B. sorokiniana* grown on WA was significantly lower than that on PDA, CMA and V8A at 24 hr light duration. *B. sorokiniana* grew well in PDA (with a maximum mean diameter), followed by CMA, V8A and WA.



Note: Light duration - 0 (dark); 12 (12 hr light); and 24 (24 hr light).

Figure 1. Bipolaris sorokiniana's *mean growth diameter in various media with different light durations. Means within columns with the same superscripts are not significantly different at 0.05% according to Tukey's test.*

Table 1 shows the results of the analysis of *B. sorokiniana*'s growth rate on PDA, CMA, V8A and WA (control) with 0, 12 and 24 hr light durations after six days of incubation. The colony growth diameter of *B. sorokiniana* varied in the four different media used in this study. In 0 hr of light, the fungus grew well in PDA and CMA, with a growth rate of 6.67 ± 0.31 and 7.17 ± 0.05 cm, respectively. In 12 hr light duration, *B. sorokiniana* grew well in both PDA and CMA, with a growth rate of 7.53 ± 0.07 and 7.49 ± 0.07 cm, respectively. In 24 hr light duration, *B. sorokiniana* grew well in both PDA and CMA, with growth rate of 6.75 ± 10.35 and 7.70 ± 0.00 cm, respectively. The growth rate in V8A was not encouraging, and it was the lowest recorded in WA.

TABLE 1. LINEAR GROWTH OF *B. sorokiniana's* ON DIFFERENT SOLID MEDIA AT 28°C

Media	Colony growth diameter (cm)		
	Light durations		
	0 (Mean ± SE)	12 (Mean ± SE)	24 (Mean ± SE)
PDA	6.670 ± 0.31	7.53 ± 0.07	6.75 ± 0.35
CMA	7.170 ± 0.05	7.49 ± 0.07	7.70 ± 0.00
V8A	6.016 ± 0.54	6.65 ± 0.25	6.70 ± 0.42
WA	5.850 ± 0.16	6.01 ± 0.13	5.84 ± 0.16

The media and day of incubation had a significant (p<0.001) influence on the mean growth diameter of *B. sorokiniana*, according to GLMMs. Mean growth diameter was significantly greater in PDA (slope estimate = 0.05003; mean = 4.928) compared to CMA (slope estimate = 0.00000; reference level; mean = 4.688), V8A (slope estimate = -0.08527; mean = 4.305), and WA (slope estimate = -0.14097; mean = 4.072). The predictive variable day of incubation has a positive and significant effect (slope estimate = 0.2947 ± 0.01076 SE) on growth diameter (*Figure 2*). The predictive variable light duration has no significant effect (p = 0.494) on growth diameter. The model explained 87.59% (adjusted R^2 = 87.54%) of the variation in the mean growth of *B. sorokiniana*.

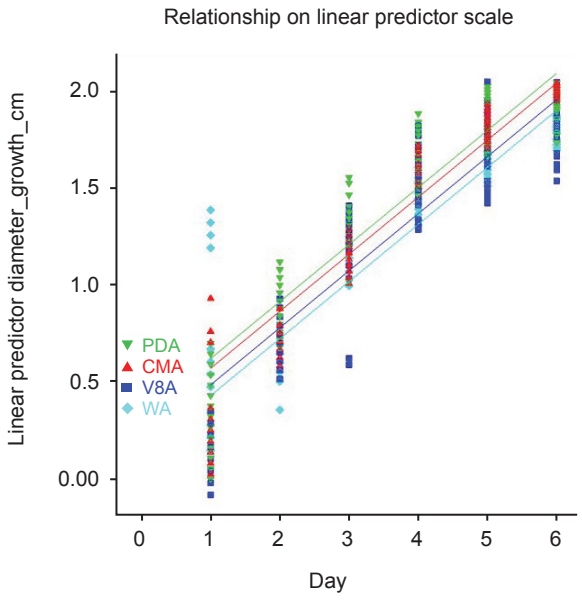
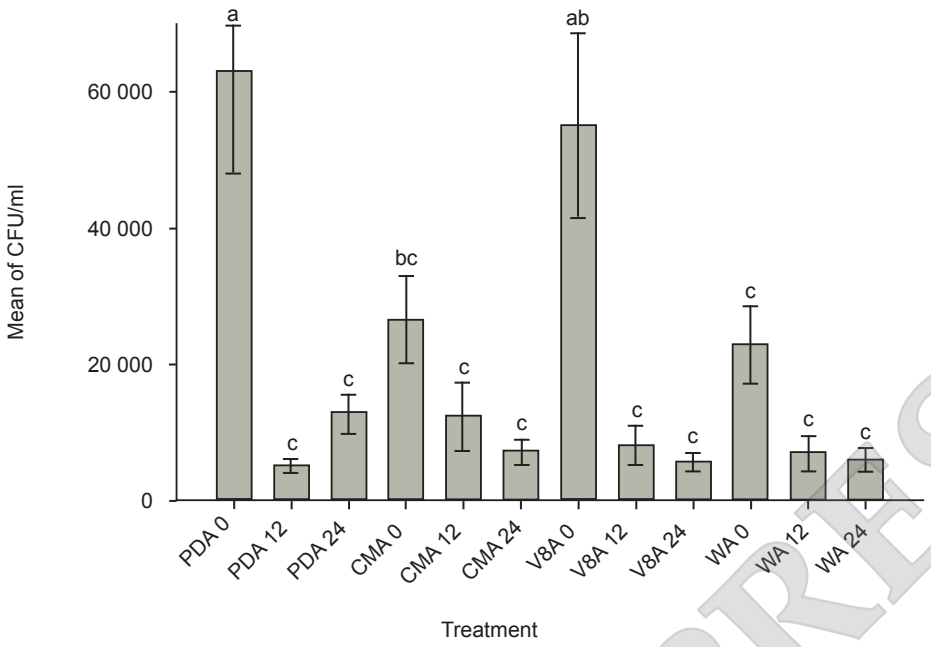


Figure 2: The effects of the predictive variable day of incubation on linear predictions of the mean growth diameter of B. sorokiniana.



Note: Light duration - 0 (dark); 12 (12 hr light); and 24 (24 hr light).

Figure 3. Mean CFU mL^{-1} of B. sorokiniana *in various media with different light durations. Means within columns with the same superscripts are not significantly different at 0.05% according to Tukey's test.*

The Influence of Culture Medium and Light Duration on CFU

Figure 3 illustrates the mean Colony-forming Unit per millilitre (CFU mL^{-1}) of *B. sorokiniana* in PDA with 0, 12 and 24 hr light duration; CMA with 0, 12 and 24 hr light duration; V8A with 0, 12 and 24 hr light duration; and water agar with 0, 12 and 24 hr light duration. The results show that CFU mL^{-1} of *B. sorokiniana* on PDA with 0 light duration was significantly different from CFU mL^{-1} of *B. sorokiniana* grown on PDA 12 hr, PDA 24 hr; CMA 0, 12 and 24 hrs; V8A 12 and 24 hr; and WA 0, 12, and 24 hr, but not different from CFU mL^{-1} on V8A 0hr. CFU mL^{-1} on CMA 0 hr, on the other hand, did not differ significantly from CFU mL^{-1} on PDA 12 and 24 hr; CMA 12 and 24 hr; V8A 12 and 24 hr; and WA 0, 12 and 24 hr. PDA media with 0 light duration had the highest CFU mL^{-1} (6.3 x 10^4) followed by V8A with 0 light (5.5 x 10^4) and CMA with 0 light (2.6 x 10^4) compared to the control WA with 0 light (2.3 x 10^4).

Colony-forming Units (CFU)

GLMM's result showed that media and light duration have a significant influence (p<0.001) on CFU. Colony-forming was significantly greater in PDA (slope estimate = 0.5558; mean = 35 669) compared to V8A (slope estimate = 0.3958; mean = 30 394), CMA (slope estimate = 0.00000; reference level; mean = 20 459), and WA (slope estimate = -0.2511; mean = 15 916). Predictive variable light duration has a negative and significant effect (slope estimate = -0.08472 ± 0.000063 SE) on colony formation (*Figure 4*). The model explained 35.39% (adjusted R^2 = 33.86 %) of the variation in colony formation of *B. sorokiniana.*

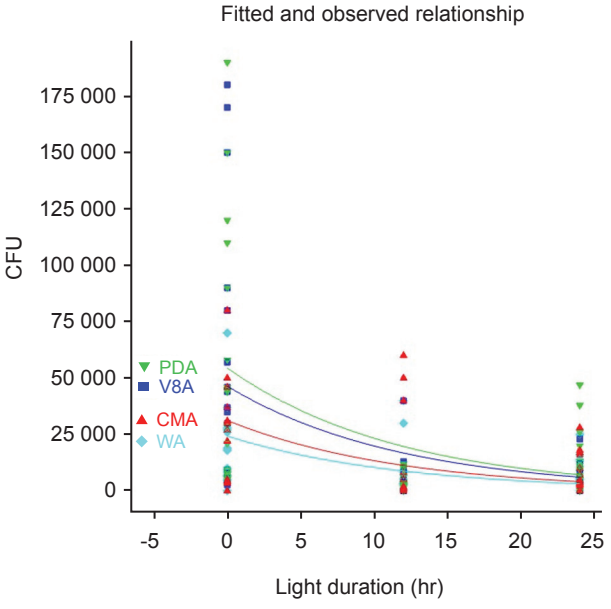


Figure 4. Fitted and observed relationships between response variable CFU and predictive variable light duration.

DISCUSSION

When the fungi were grown on different culture media and light durations, their growth and development varied. The predictive microbiology that studies the behaviour of *B. sorokiniana* under different physico-chemical conditions, such as media and light durations, including the pH and temperature used to propagate it on the laboratory scale, helps the identification of critical points in the production and the optimisation of production for *B. sorokiniana*. However, the study's limitation was that it primarily focused on the selection of culture media and identifying the optimal light exposure period for *B. sorokiniana* growth. Other factors that may affect the growth of *B. sorokiniana* colonies, such as temperature and humidity, were not manipulated and were kept uniform in all treatments. Therefore, *B. sorokiniana* has played an important role in the selection of biological control agents for weeds. It needs to be able to grow on artificial media (Charudattan and Burge, 1988; Jenkins *et al*., 1998; Trujillo, 1992) because, for the fungus to work as a biological control agent, it should be able to produce an abundant and long-lasting inoculum in artificial culture (Charudattan, 1991).

Culture media such as PDA, CMA and V8A are standard media for fungal culture and contain nutrients, growth factors, energy sources, buffer salts, minerals, metals and occasionally solidifying or gelling agents (agar) that are beneficial to the culture of microorganisms such as bacteria, yeast, fungi, animal cells and plant cells (Atlas, 1996). Fungi, according to Samson (2016), prefer carbohydrates as a carbon source for food in their growth, infection and reproduction. It can readily absorb and metabolise a variety of soluble carbohydrates, such as glucose, xylose, sucrose and fructose. The culture media are also easy to obtain for *in vitro* laboratory tests.

Bipolaris sorokiniana was found to have excellent growth on PDA media compared to CMA and V8A media and WA as a control. This finding is in line with the findings from Nur *et al.* (2019), where, in their study, *B. sorokiniana* had the highest vegetative growth and sporulation using the PDA. It may be due to PDA media supplying appropriate nutrients and environmental conditions that are conducive to *B. sorokiniana*'s growth. PDA has nutrients consisting of dextrose, which is a basic source of carbohydrates to stimulate the growth of *B. sorokiniana* and potato infusion provides a nutrient base for the luxuriant growth of fungi (Trigiano, 2016). The findings of Kim *et al.* (2005) showed that corn meal agar does not help mycelial growth for *Sphaeropsis pyriputrescens,* the fungus that causes root rot in pears and apples. Therefore, there is a possibility that the same response has also been shown by *B. sorokiniana* in this study, causing its growth to be lower in corn meal than in PDA media. Meanwhile, V8 agar medium yielded the lowest numbers of outdoor airborne fungi, which in the study shows that V8 agar lacks simple sugars and amino acids (Black, 2020). It is likely to be a factor contributing to the lower growth of *B. sorokiniana* in V8 compared to PDA media.

It was found that *B. sorokiniana* grows optimally at a temperature of 28°C, in accordance with the findings of Naresh *et al.* (2009), which stated the optimum temperature for growth and sporulation for *B. sorokiniana* was at 28°C, and in line with the finding from Kumar *et al.* (2020) that *B. sorokiniana* survives in warm temperatures of 18°C to 32°C.

In this study, *B. sorokiniana* was able to be cultured on PDA, CMA, V8A and WA media in the dark (0 hr light), 12 hr and 24 hr light durations. However, in terms of growth, *B. sorokiniana* was observed to have an excellent colony growth diameter on PDA 12 hr light duration, CMA 24 hr light duration, and V8A 24 hr light duration compared to those on WA media. *Bipolaris sorokiniana* was observed to have the least growth diameter on WA media in 0, 12 and 24 hr light durations compared to other media in the experiment. It was found that *B. sorokiniana* has good colony growth under light. The finding was supported by the study of Patsa *et al.* (2018), who claimed that *B. sorokiniana* grew faster in light conditions and had higher mycelial growth than in dark conditions. Although the growth of *B. sorokiniana* is affected by light, this does not mean that this fungus carries out photosynthesis because it has no chlorophyll (Trigiano, 2016). Nutrients are obtained by fungi through extracellular digestion due to the activity of secreted enzymes. Another study discovered that fungi require light for mycelial growth and that some fungi respond positively to light while others do not (Weitz *et al*., 2001). Therefore, the light requirements for fungal growth are different between fungi species, whereby this study found that *B. sorokiniana* positively responds to light for its growth in its growing phase but that the growth rate will slow down when exposed to prolonged light.

Bipolaris sorokiniana was observed to produce the highest CFU mL^{-1} in PDA media without light (dark). A high CFU mL^{-1} concentration was also found in V8A (0 hr) and WA (0 hr) when compared to *B. sorokiniana* with the same media culture in 12 and 24 hr light durations. It was discovered that a light duration of 0 hr produced the highest CFU reading on all media and the highest CFU mL^{-1} reading. CFU is very important to determine the number of living cells in a culture compared to the total number of living and dead cells (Washington, 2012). The living cells are potentially culturable. The production of CFU mL^{-1} of *B. sorokiniana* decreased as the light duration increased. *Bipolaris sorokiniana* was seen to grow and produce abundant CFU without light and

when supplied with nutrients from artificial media. The findings were similar to those of Aggarwal *et al.* (2009), Bashyal *et al. (*2010), Dinesh *et al.* (2013) and Patsa *et al*. (2018), who discovered that *B. sorokiniana* did not sporulate under continuous light conditions, but numerous sporulations were observed under dark conditions (without light). It has been shown that *B. sorokiniana* is productively producing cultivable cells under dark conditions because fungi can grow in the dark as long as there is an external food source and water for them (Webster and Weber, 2007).

Hence, in this study, there was an interesting finding where *B. sorokiniana*, which has excellent mycelial growth in PDA media at a light duration of 12hr, did not produce a high CFU compared to *B. sorokiniana* that was cultured in the dark, which produced the highest CFU of all treatments. It shows that, although the growth of *B. sorokiniana* is good under light, cultivable living cells are the most abundant in the *B. sorokiniana* treatment that is cultured in PDA without light. It shows that *B. sorokiniana* is actively producing living cells without light.

The use of bioherbicides in weed control is an important effort to reduce pollution and the incidence of weed resistance to herbicides. Previous studies have shown that one of the important sources of pollution in the agricultural sectors around the world is caused by agrochemicals, including herbicides (Krebs *et al.*, 1999; Sharma *et al.*, 2019). In this regard, Ruzmi *et al.* (2017) claimed that as many as 15 weed species have been found resistant to chemical herbicides in Malaysia. In addition, many previous studies have also found that the continuous use of chemical herbicides can cause health problems for workers who are involved in herbicide spraying activities (Alavanja *et al.*, 2003; Matich *et al.*, 2021). Hence, *B. sorokiniana* has a role as a bioherbicide for controlling *E. indica*, especially in oil palm plantations, as claimed by Ismail *et al.* (2020). The use of bioherbicides to manage weeds can minimise the application of conventional herbicides and may lower the occurrence of weed resistance.

CONCLUSION

Bipolaris sorokiniana was found to be able to grow on PDA, CMA, V8A and WA. The best growth, however, was found on PDA media incubated at 28°C with a pH of 5.6 ± 0.2. It also requires an environment without light to produce the most culturable living cells, which will provide three times as many CFU mL^{-1} as other treatments. Therefore, the findings of this study show that *B. sorokiniana* has the potential to be reproduced and has the distinctive aspect and concept of being a good candidate for a biological control agent. The use of *B. sorokiniana* in bioherbicide is expected to reduce the dependency of the agricultural sector on the use of herbicides, especially in oil palm, to control *E. indica*. In order to allow *B. sorokiniana* to be used commercially, a specific formulation is required through further research to determine a suitable and economic medium to be used on a large scale. In addition, further research on the response surface methodology (RSM) may be necessary to understand the optimisation of *B. sorokiniana* productivity.

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