Advances in Weed Science
formerly Planta Daninha

RESEARCH ARTICLE

Understanding the environmental and herbicide response of *Lasiodiplodia theobromae* and *Bipolaris bicolor* isolated from infected *Eleusine indica*

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Abstract: Background: In a prior study, *Lasiodiplodia theobromae* (Pat.) Griffiths and Maubl. and *Bipolaris bicolor* (Mitra) Shoemaker., were found to suppress the growth of *Eleusine indica* (L.) Gaertn, but limited information exists on their response to environmental factors and herbicides for integrated *E. indica* control. **Objective:** This study aimed to determine the tolerance levels of *L. theobromae* and *B. bicolor* to pH, temperature, photoperiod, relative humidity, and herbicides. **Methods:** The mycelia and conidia of *L. theobromae* and *B. bicolor* were exposed to a range of environmental conditions and herbicides in a controlled setting, including different levels of temperature (25, 30, 35 °C), pH (4, 6, 8, 10), photoperiod (24 hours of darkness; alternating 12 hours of blue light followed by 12 hours of darkness; and alternating cycles of 6 hours of blue light and 6 hours of darkness), and relative humidity (75, 85, 95%). Herbicides such as topramezone, diuron, oxyfluorfen, and imazethapyr were applied at their recommended rates. Conidial germination was assessed by counting after an 18-hour incubation using a haemocytometer, while the diameter of mycelium growth was measured after 3 days of incubation, except for the herbicide effects evaluation, which were extended over 7 days. **Results:** Laboratory assays demonstrated that *L. theobromae* exhibited higher conidial germination of 85-95% and superior mycelial growth under varied pH, temperature, and photoperiod conditions compared to *B. bicolor*. *Lasiodioplodia theobromae*'s mycelia remained unaffected by herbicides at full labelled rates, but they inhibited the conidial germination of the fungus. For *B. bicolor*, the conidia were not affected by imazethapyr and topramezone, but its mycelial growth was reduced by imazethapyr, oxyfluorfen, and diuron. **Conclusions:** These results indicate that the mycelia of *L. theobromae* presents a more favorable option for tank mixing with test herbicides, offering potential for the formulation of an integrated control strategy against *E. indica*.

Keywords: *Lasiodiplodia theobromae*; *Bipolaris bicolor*; Environmental Factors; Herbicides

Journal Information:
ISSN - 2675-9462
Website: http://awsjournal.org
Journal of the Brazilian Weed Science Society

How to cite: Fakri MA, Sapak Z, Hamdani MAS, Seng CT. Understanding the environmental and herbicide response of *Lasiodiplodia theobromae* and *Bipolaris bicolor* isolated from infected *Eleusine indica*. Adv Weed Sci. 2024;42:e020240063.
https://doi.org/10.51694/AdvWeedSci/2024;42:00005

Approved by:
Editor in Chief: Carlos Eduardo Schaedler
Associate Editor: Michaela Kolářová

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Received: November 20, 2023
Approved: February 11, 2024

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1. Introduction

Eleusine indica (L.) Gaertn, an annual grass weed, thrives in diverse agricultural regions across Malaysia, encompassing oil palm plantations, orchards, and vegetable cultivations (Chuah, Sahid, 2010). However, the indiscriminate use of various herbicides has fostered resistance in this weed against multiple herbicide groups (Dilipkumar et al., 2020a). Consequently, numerous studies have sought to mitigate herbicide usage and forestall the emergence of herbicide-resistant *E. indica* by employing a blend of chemical and physical methods (Amirul et al., 2019a; 2019b; Chuah, Lim, 2021a; 2021b; Chuah et al., 2018; Dilipkumar et al., 2020b). Despite these efforts, the integration of biological and chemical strategies for controlling *E. indica* remains underexplored.

In crop fields, weeds are vulnerable to fungal colonization, with certain fungi capable of producing mycotoxins under specific conditions (Giorni et al., 2019). Several fungi, including *Lasiodiplodia theobromae* (Pat.) Griffiths and Maubl. and *Bipolaris bicolor* (Mitra) Shoemaker, have demonstrated efficacy in suppressing *E. indica* growth (Fakri et al., 2023). The colonization and mycotoxin production in fungi are influenced by eco-physiological variables such as temperature and relative humidity (Lahouar et al., 2015). Furthermore, climate change can yield diverse impacts on fungal colonization and mycotoxin production, ranging from inhibition to stimulation or no effect at all (Samapundo et al., 2007; Akbar et al., 2016; Luck et al., 2011).

Concurrently, herbicides interact multifariously with plants and microorganisms, altering the plant-pathogen relationship while targeting weeds (Duke et al., 2007). Changes induced by herbicides to the host plant's structure and defenses may heighten susceptibility to infection (Ruuskanen et al., 2023). Understanding herbicide effects on fungal pathogens is pivotal for formulating integrated strategies for weed control (Charudattan, 2001). When herbicides are applied sequentially or as tank mixtures, it is imperative to ensure that those with synergistic potential do not impede the spores and mycelium of fungal pathogens (El-Sayed, 2005). For example, a recent study by Zhang et al. (2022) demonstrated the efficacy of combining *Bipolaris*

eleusines Alcorn and Shivas conidia with bensulfuron or penoxsulam in controlling barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], monochoria [*Monochoria vaginalis* (Burm.f.) Presl C. ex Kunth.], and small-flower umbrella sedge [*Cyperus difformis* (L.)] in rice fields. Hoagland et al. (2018) found that formulations containing technical-grade glyphosate and *Myrothecium verrucaria* (Alb. and Schwein.) Ditmar ex Fr., mycelia, effectively managed palmer amaranth [*Amaranthus palmeri (*S. Wats.)] by significantly reducing plant height by over 50% and fresh weight by 90%.

Hence, the ease of cultivating fungi in laboratory settings and their high compatibility with herbicides position them as promising candidates for integrated weed management. However, unlocking their potential necessitates a comprehensive understanding of their reproductive characteristics influenced by environmental factors, crucial for large-scale production and adaptability in field applications, and their compatibility with herbicides for effective weed suppression. Thus, this study aimed to investigate the effects of various environmental factors, including temperature, photoperiod, pH, humidity, and herbicides such as topramezone, imazethapyr, oxyfluorfen, and diuron, on the mycelial growth and conidial germination of *L. theobromae* and *B. bicolor*. These herbicides were selected because *E. indica* has not evolved resistance to them (Heap, 2023). The data obtained from this research could furnish valuable insights into the fungi's tolerance to environmental conditions and their interactions with herbicides, thereby playing a pivotal role in the integrated control of *E. indica* through both biological and chemical approaches.

2. Material and Methods

2.1 Mycelium preparation

The pure cultures of *L. theobromae* and *B. bicolor* were obtained from Fakri et al. (2023), Laboratory of Agrotechnology, Complex Star, Universiti Teknologi MARA, Arau, Perlis, Malaysia. *Lasiodiplodia theobromae* culture was sub-cultured onto fresh potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) medium in a 6-cm-diameter Petri dish using a 5-mm-diameter cork borer and incubated at 27 °C under darkness for 7 days in an incubator. The same procedure was repeated for *B. bicolor* for 14 days. Five-mm-diameter of mycelial discs taken from the margin of the colony was used for subsequent experiments into temperature, photoperiod, pH, humidity, and herbicide effects.

2.2 Conidia preparation

For harvesting conidia of *L. theobromae,* approximately 10 mL of sterilized potato dextrose broth (PDB) (Merck KGaA, Darmstadt, Germany) was poured onto a 7-day-old colony culture grown on PDA medium, and conidia were scrapped off using a sterilized glass rod. The same procedure was repeated for *B. bicolor* using a 14-day-old colony culture. The conidial suspension of the fungus was filtered through two layers of sterilized muslin cloth, and then adjusted to approximately 1.0×10^8 conidia/mL using a haemocytometer and transferred into a 50 mL universal bottle for subsequent experiments into temperature and photoperiod effects.

2.3 Temperature effect

The 5-mm diameter mycelial discs of each fungus were placed on PDA medium and incubated at temperatures of 25, 30 and 35 °C , respectively, under darkness. Diameter of mycelium growth was recorded daily for three days (Siva et al., 2017).

The conidia suspension in universal bottle was incubated at 25, 30 and 35 °C for 18 hours under darkness. Conidial germination was counted in the haemocytometer under a compound microscope where conidia was considered germinate when a germ tube was longer than the conidia diameter (Benslim et al., 2016). More than 500 conidia were counted for each treatment. The conidial germination was expressed in percentage as follows:

$$\text{Conidial germination} = \frac{\text{Number of germinated conidia}}{\text{Total number of conidia}} \times 100$$

2.4 Photoperiod effect

The 5-mm diameter mycelial discs of each fungus were placed on PDA medium and exposed to oscillate cycles of 24 hours dark; 12 hours blue light and 12 hours dark; and alternate 6 hours blue light and 6 hours dark in a growth chamber maintained at 27 °C. Diameter of mycelium growth was recorded daily for three days as described before. Meanwhile, the conidial suspension in universal bottle was exposed to different photoperiods as described above at 27 °C for 18 hours. Conidial germination was counted using the haemocytometer. Conidial germination was expressed in percentage as described in Section 2.3 (Siva et al., 2017).

2.5 Humidity effect

The 5-mm diameter mycelial discs of each fungus were placed on PDA medium and exposed to different relative humidity of 75, 85 and 95%, respectively, and incubated at 27 °C under darkness in a closed container sized 34 cm (L) × 24.5 cm (H) × 19.5 cm (W). The relative humidity of 75, 85 and 95% was simulated using saturated salt solution of sodium chloride (NaCl) (HmbG® Chemicals, Malaysia), potassium chloride (KCl) (QReC™, Thailand) and potassium sulphate (K_2SO_4) (SRL Chem, India), respectively (Hong et al., 2005) and confirmed using digital humidity meter. The diameter of mycelium growth was recorded daily for three days after incubation as described in Section 2.3.

2.6 pH effect

The pH levels of the PDB and PDA were adjusted accordingly using a digital pH meter by adding 1.0 N hydrochloric acid (HCl) and 1.0 N sodium hydroxide (NaOH) to attain pH 4.0, 6.0, 8.0 and 10.0. The PDA medium was sterilized and poured into Petri dish and allowed to solidify. The 5-mm-diameter mycelial disc of each fungus was placed on the centre of Petri dish and incubated at 27 °C under darkness. Diameter of the fungal mycelium was recorded daily for three days as described in Section 2.3. 10 mL of sterilized PDB with pH 4.0, 6.0, 8.0 and 10, were respectively; poured onto pre-prepared fungal culture grown on PDA medium and the conidia were scrapped off. Then, the conidial suspension was filtered, transferred into the universal bottle, and adjusted to 1.0×10^8 conidia/mL using the haemocytometer and incubated at 27°C for 18 hours under darkness. Conidial germination was counted and expressed in percentage as described in Section 2.3 (Siva et al., 2017).

2.7 Herbicide effect

Commercial grades of four herbicides examined in this experiment were topramezone, diuron, oxyfluorfen, and imazethapyr (Table 1). The herbicides were diluted with sterilized PDA or PDB to attain respective manufacturer-recommended rates. The PDA without herbicide incorporation was used as non-treated control. The herbicide containing PDA was allowed to solidify, followed by the placement of a pre-prepared 5-mm-diameter mycelial disc at the center of the agar medium in a Petri dish. Subsequently, the dish was incubated at 27°C for 7 days in darkness. (Bussaman et al., 2012). Diameter of mycelium growth was recorded and expressed as percentage of respective non-treated control as follows:

$$\text{Mycelium growth} = \frac{\text{Diameter of treated mycelium}}{\text{Diammeter of non treated mycelium}} \times 100$$

The herbicide-treated PDB at respective recommended rate was poured onto fungal culture grown on the pre-prepared PDA medium and the conidia were scrapped off (Celar, Kos, 2016). The conidial suspension was filtered with muslin cloth, transferred into the universal bottle, and incubated at 27 °C for 48 hours under darkness. The PDB without adding herbicide was used as non-treated control. Conidial germination was counted in the haemocytometer under a compound microscope. The conidial germination was expressed as percentage of respective non-treated control as follows:

$$\text{Conidial germination} = \frac{\text{Number of germinated herbicide treated conidia}}{\text{Number of germinated non treated conidia}} \times 100$$

3. Experimental design and Statistical analysis

The experiments on effects of temperature, photoperiods and humidity were laid out as split plot in a completely randomized design with five to six replications and repeated twice where main plot is temperature level, photoperiod, or humidity level whereas subplot is type of fungal pathogen. The data were checked for the normality and homogeneity of variance being subjected to ANOVA, followed by Tukey's test to compare means at 5% level of significance. The experiments on the effects of pH and herbicide were laid out as factorial in a completely randomized design with five to six replications where factor one is type of fungal pathogen, whereas factor two is pH level or herbicide type. The data were checked for the normality and homogeneity of variance before being subjected to two-way ANOVA, followed by Tukey's test to compare means at 5% level of significance. All statistical procedures were conducted using JMP software version 14.

4. Result and Discussion

4.1 Environmental effects on conidial germination

4.1.1 *Effects of temperature*

The effects of temperature on the conidial germination rates of *B. bicolor* and *L. theobromae* are illustrated in Figure 1a. Considering the conidial germination rate, a notable interaction was observed between the type of fungus and the temperature factor. These two fungi were able to germinate, with *L. theobromae* having a high conidial germination rate of 86 to 90% at temperatures between 25 and 35 °C. In comparison, *B. bicolor* conidial germination rate was just 45% to 47% (Figure 1a). The conidial germination rates of *B. bicolor* were not affected significantly ($p>0.05$) by

Table 1 - Characteristics of selected commercial grade herbicides.

Active ingredient	Trade name	Mode of action*	Formulation
Topramezone	CLIO ™ SC	Inhibits the enzyme 4-Hydroxy-Phenyl-Pyruvat-dioxygenase (4-HPPD).	Suspension concentrate (SC)
Diuron	ANCOM DIURON F42	Inhibits photosynthesis at photosystem II.	Suspension concentrate (SC)
Oxyfluorfen	BOXY ZEENEX	Inhibition of the enzyme protoporphyrinogen oxidase (PPO or Protox).	Emulsifiable concentrate (EC)
Imazethapyr	IMAZ 5.2SL	Inhibition of branched amino acid production by inhibition of the enzyme acetolactate synthase (ALS) or acetohydroxy acid synthase (AHAS).	Soluble concentrate (SL)

the rise in temperature. By contrast, when the temperature increased, the conidial germination rates of *L. theobromae* increased marginally ($p<0.05$). Unlike *B. bicolor* isolates from Malaysia, Xiao et al. (2021) found that the optimum temperature for conidial germination of *B. bicolor* taken from a tea garden in Zhejiang, China, was 25 °C, whereas the conidial germination rate was significantly lower at 35 °C.

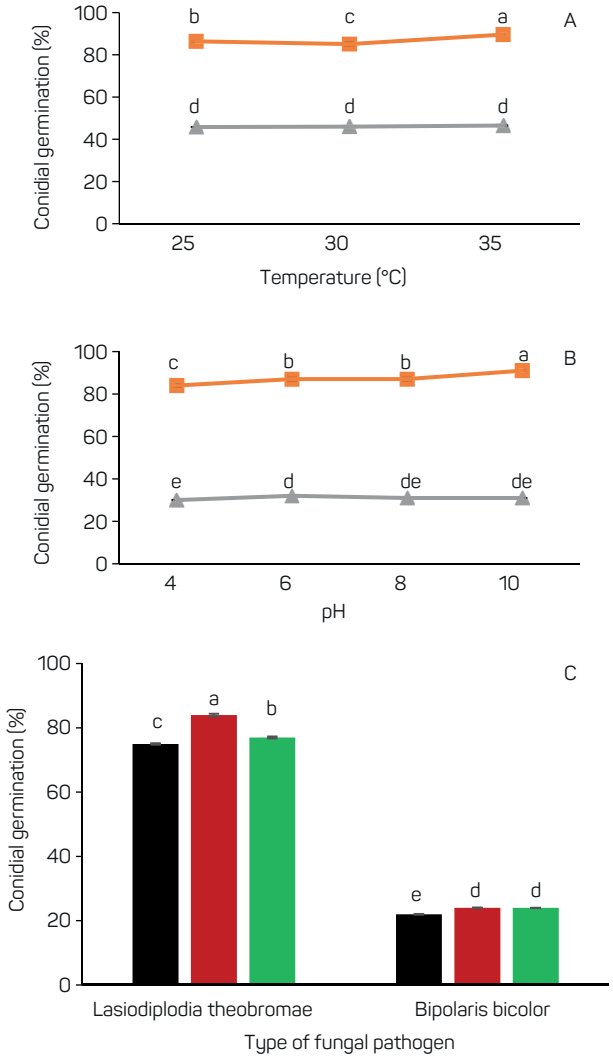

Figure 1 - Conidial germination rate of *Lasiodiplodia theobromae* (■) and *Bipolaris bicolor* (▲) under different environmental factors. Each datum is the average ± SD of six replicates. At least 500 conidia were counted for each value. A. Conidial germination rate at temperatures of 25, 30 and 35 °C for 18 hours under darkness. B. Conidial germination rate at pH 4, 6, 8 and 10 at 27 °C for 18 hours under darkness. C. Conidial germination rate at 27 °C for 24 hours under darkness (■), alternating 6-h blue light (■) and alternating12-h blue light (■). Means followed by a different lowercase letter have a significant difference at the 5% significance level

4.1.2 *Effects of pH*

The effects of pH on the conidial germination rates of *B. bicolor* and *L. theobromae* are depicted in Figure 1b. A significant interaction was observed between the conidial germination rate of the fungus type and the pH factor. The pH had a substantial effect ($p<0.05$) on the conidial germination rate of *L. theobromae*, with the conidial germination rate increasing from 84% at pH 4 to 91% at pH 10. Any increase in acidity or alkalinity had no effect on the germination rate of *B. bicolor* conidia, which constantly had a rate of 30 to 32% (Figure 1b). In contrast to present findings, Xiao et al. (2021) reported that the optimum pH for conidial germination of *B. bicolor* was 7 whereas high acidity and alkalify at pH 4 and pH 10, respectively could reduce the conidial germination rate substantially

4.1.3 *Effects of photoperiod*

The effects of photoperiod on the conidial germination rates of *B. bicolor* and *L. theobromae* are outlined in Figure 1c. There was a significant interaction between the conidial germination rate of fungus type and photoperiod factor. The effect of light exposure duration on the conidial germination rate of the test fungus was not consistent. It was discovered that *L. theobromae* treated to 6 hours of alternating blue light had a greater conidial germination rate than those exposed to 24 hours of darkness and 12 hours of alternating blue light. On the other hand, *B. bicolor*'s conidial germination rate could be reduced by 24 hours of darkness. Unlike *B. bicolor* isolates from Malaysia, conidia of *B. bicolor* from China exhibited the highest germination at fluorescent light (Xiao et al., 2021). Moreover, *L. theobromae* exhibited 75 to 85% conidial germination rate either at alternating photoperiod or complete darkness.

The present findings on the optimal temperature, pH and photoperiod for conidial germination of *B. bicolor* do not align with the results of Xiao et al (2021). These discrepancies could be attributable to diverse geographic regions, potentially reflecting adaptation of the fungal pathogen (Kumar et al., 2010). Besides, in addition to displaying high pathogenicity against target weeds, a mycoherbicide must be highly adaptable to environmental conditions such as temperature, pH and photoperiod (Hassan et al., 2021). The formulation of choice for majority of effective mycoherbicide is conidia. A fungal mycoherbicide agent must also have a rapid sporulation rate (Verma et al., 2021). In this study, conidia of *L. theobromae* can be harvested from 7-day-old cultures, whereas *B. bicolor* requires 14-day-old cultures to produce similar conidial density, indicating that *L. theobromae* has advantages over *B. bicolor* for mycoherbicide development. The current investigation also revealed that *B. bicolor* exhibited a low conidial germination rate of 20 to 50% whereas *L. theobromae* had a higher conidial germination rate ranging from 75 to 95% across various levels of temperature, pH and photoperiod. These results indicated that *L. theobromae* outperformed *B. bicolor* and was more adaptable to a wide range of environmental conditions, implying its promise as a better mycoherbicide candidate.

4.2 Environmental effects on mycelial growth

4.2.1 *Effects of temperature*

Figure 2a presents the effects of temperature on mycelial growth of *B. bicolor* and *L. theobromae*. Intriguingly, the mycelial growth patterns of the test fungus differed from the conidial germination rate. An interaction between the fungus type and temperature factor influenced the mycelium growth significantly. Mycelia of *L. theobromae* and *B. bicolor* grew more slowly at 35 °C, decreasing in diameter from 5.5 to 4.5 cm and 2 to 1 cm, respectively, demonstrating that the rise in temperature from 25 to 35 °C could inhibit the growth of both fungi. Thus, mycelia of *L. theobromae* and *B. bicolor*, were clearly not tolerant to temperature fluctuations.

Channakeshave and Pankaja (2018) observed that the optimal temperature for the radial growth of *Bipolaris oryzae* (Breda de Haan) Shoemaker isolated from paddy was 30 °C, with lower and higher temperatures inhibiting the growth of the fungus. Arshad et al. (2013) also noticed that the *B. oryzae* performed optimally between 25 and 35 °C. Saha et al. (2008) demonstrated that *L. theobromae* could develop at temperatures between 8 and 36 °C, with optimum growth occurring at 28 °C. Alam et al. (2001) also observed that *L. theobromae* grew at temperatures ranging from 10 to 40 °C, with the ideal temperature range falling between 25 and 30 °C. In accordance with the findings of Alam et al. (2001), the optimum temperature for mycelia development of *L. theobromae* was found to be between 25 and 30 °C in current research.

4.2.2 *Effects of pH*

Figure 2b depicts the effects of pH on mycelial growth of *B. bicolor* and *L. theobromae*. Similar to temperature effect, the mycelial growth patterns of the test fungus varied from the conidial germination rate. The mycelium growth was significantly influenced by the interaction between the fungus type and pH factor. The mycelial growth of *B. bicolor* was marginally enhanced when the pH was increased from 4 to 10. By contrast, Channakeshave and Pankaja (2018) revealed that the optimal pH range for *B. oryzae* was between 6 and 7. On the other hand, the mycelial growth of *L. theobromae* was optimum at pH 6 but significantly hindered at pH 8 and 10. Saha et al. (2008) also found that *L. theobromae* could thrive in a pH range from 3.5 to 8.0 but the ideal pH range was

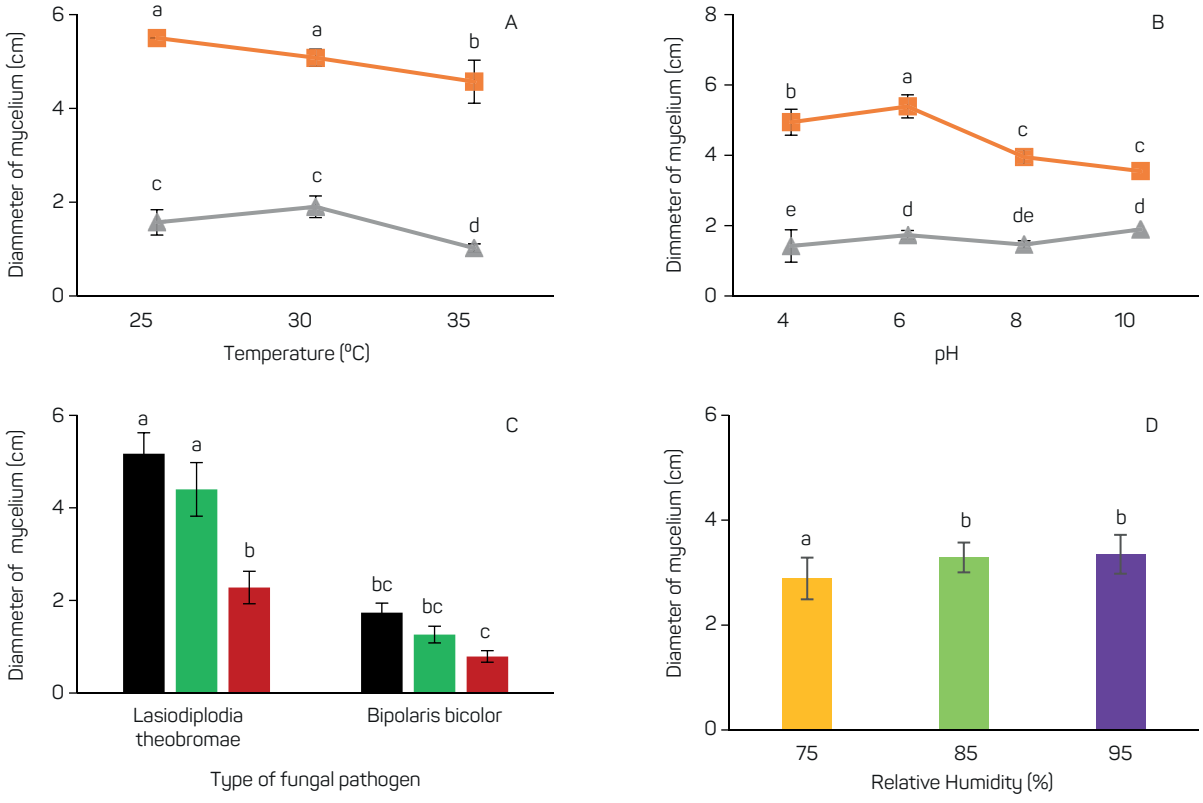

Figure 2 - Mycelial growth of *Lasiodiplodia theobromae* (■), and *Bipolaris bicolor* (▲) under different environmental factors. Each datum is the average ± SD of five replicates. A. Mycelial growth at temperatures of 25, 30 and 35 °C for 24 hours under darkness. B. Mycelial growth at pH 4, 6, 8 and 10 for 24 hours at 27 °C under darkness. C. Mycelial growth at 27 °C for 24 hours under darkness (■), alternating 6-h blue light (■) and alternating12-h blue light (■). D. Main effect of relative humidity on mycelial growth at 27 °C for 24 hours averaged across fungus type under darkness. Means followed by a different lowercase letter have a significant difference at the 5% significance level

between 5.5 and 6.5. Dheepa et al. (2018) discovered that *L. theobromae* grew most rapidly at pH 7.

4.2.3 *Effects of photoperiod*

Figure 2c illustrates the effects of photoperiod on mycelial growth of *B. bicolor* and *L. theobromae*. The mycelial growth patterns of the test fungus differed from the conidial germination rate. The interaction between the fungus type and photoperiod factor had a significant impact on the mycelium growth. Mycelial growth of *L. theobromae* was greater in the dark than under alternating blue light, indicating that darkness was more conducive to the growth of the fungus. However, Saha et al. (2008) found that light had no significant influence on the mycelial growth of *L. theobromae* under full light, complete darkness, and alternate 12-hour light treatments. Dheepa et al. (2018) also showed that there was no correlation between light regimes and the mycelial development of *L. theobromae*. The photoperiod had no marked influence ($p>0.05$) on the mycelial growth of *B. bicolor*. The findings are not in accordance with results of Channakeshave and Pankaja (2018) who found that *B. oryzae* exhibited greater mycelial growth when exposed to complete darkness as opposed to alternating light.

4.2.4 *Effects of humidity*

Figure 2d presents the effects of humidity on mycelial growth of *B. bicolor* and *L. theobromae*. The interaction between mycelium growth of fungal type and humidity effect was not significant. Since the main effect of humidity on the mycelial growth of the test fungi was not statistically significant ($p>0.05$), only the main effect of humidity level was presented. The data demonstrated that the test fungi were tolerant of humidity changes between 75% and 95%. However, mycelia of *B. bicolor* and *L. theobromae* grew better with humidity levels ranging from 85% to 95%.

The presence and activity of mycelium play a significant role in influencing pathogenic fungus infections in plants. Following the initial infection by the conidia of pathogenic fungi in weeds, the mycelium serves to perpetuate and spread the infection. As conidia mature into mycelium, they execute the invasion process. Therefore, the survival of mycelium in various environmental conditions such as temperature, photoperiod, pH, and humidity is crucial, similar to the survival of conidia. Understanding the impact of environmental factors on the growth of fungal pathogens' mycelium is also essential for optimizing manufacturing processes. The greater mycelium growth observed in *L. theobromae* across a wide range of environmental conditions suggests its superiority over *B. bicolor*, thus positioning *L. theobromae* as a highly promising candidate for mycoherbicide applications.

4.3 Herbicide effects on conidial germination and mycelial growth

Figure 3 shows the effects of imazethapyr, topramezone, oxyfluorfen and diuron on conidial germination rate and mycelial growth of *B. bicolor* and *L. theobromae*. There was a significant interaction between fungus type and herbicide treatment on conidial germination rate (Figure 3a). It was noted that *L. theobromae* and *B. bicolor*, all reacted differently to herbicide treatments. *L. theobromae* was extremely susceptible to all test herbicides because the fungus only gave a 15–21% conidial germination rate following herbicide treatments. On the other hand, *B. bicolor* was tolerant to both imazethapyr and topramezone treatment; nevertheless, oxyfluorfen and diuron treatment significantly reduced the conidial germination of this fungus by 30–35%. In contrast to the trend in conidial germination rate, herbicide treatments had only a little (0–6%) inhibitory effect on *L. theobromae* mycelial growth (Figure 3b). Surprisingly, topramezone promoted mycelial growth of *B. bicolor*, although other test herbicides reduced the fungus' growth by 50–60%.

Based on the findings of this research, only topramezone had no detrimental impact on *B. bicolor*, but imazethapyr, oxyfluorfen, and diuron hindered conidial germination and mycelial growth. Likewise, Nemoto et al. (2002) demonstrated that glyphosate suppressed the mycelial growth of *Bipolaris euphorbiae* (Y. Nisik.) by 97%, regardless of dosage applied. Imazethapyr was also proven to diminish the biomass of the fungus. By contrast, bentazon had no effect on the fungus, but lactofen, a protoporyhyrinogen oxidase (PROTOX) inhibitor like oxyfluorfen, reduced mycelium growth by 85%. As revealed by Sanyal and Shrestha (2008), fungi possessed the same PROTOX enzyme targeted by the herbicide in plants, therefore it was not surprising that oxyfluorfen had a fungistatic impact on *B. bicolor*. Since *B. bicolor* was not affected by topramezone, this result suggested that the fungus was likely capable of degrading the herbicide. Although a diuron-degrading endophyte fungus isolated from sugarcane root has been reported to degrade diuron by up to 99% in three days under optimal conditions (Wang et al., 2017), *B. bicolor* isolated from the leaves of the *E. indica* plant was thought to lack the ability to degrade diuron, resulting in a decrease in mycelial growth and conidial germination.

Herbicide studies on *L. theobromae* are scarce in the literature. Vazquez et al. (2020) reported that glyphosate had a substantial impact on soil fungi, especially *Diplodia* species. The scientists were able to isolate one species of *Diplodia* from untreated soil, but none from soil treated with glyphosate at twice the recommended dose or at the recommended dose. Similar to glyphosate, imazethapy, topramezone, oxyfluorfen, and diuron significantly reduced *L. theobromae* conidial germination. Surprisingly, the mycelial development of *L. theobromae* was either unaffected or minimally influenced by the test herbicides, suggesting that *L. theobromae* is inferior to *B. bicolor* due to the high inhibition of conidial germination by test herbicides.

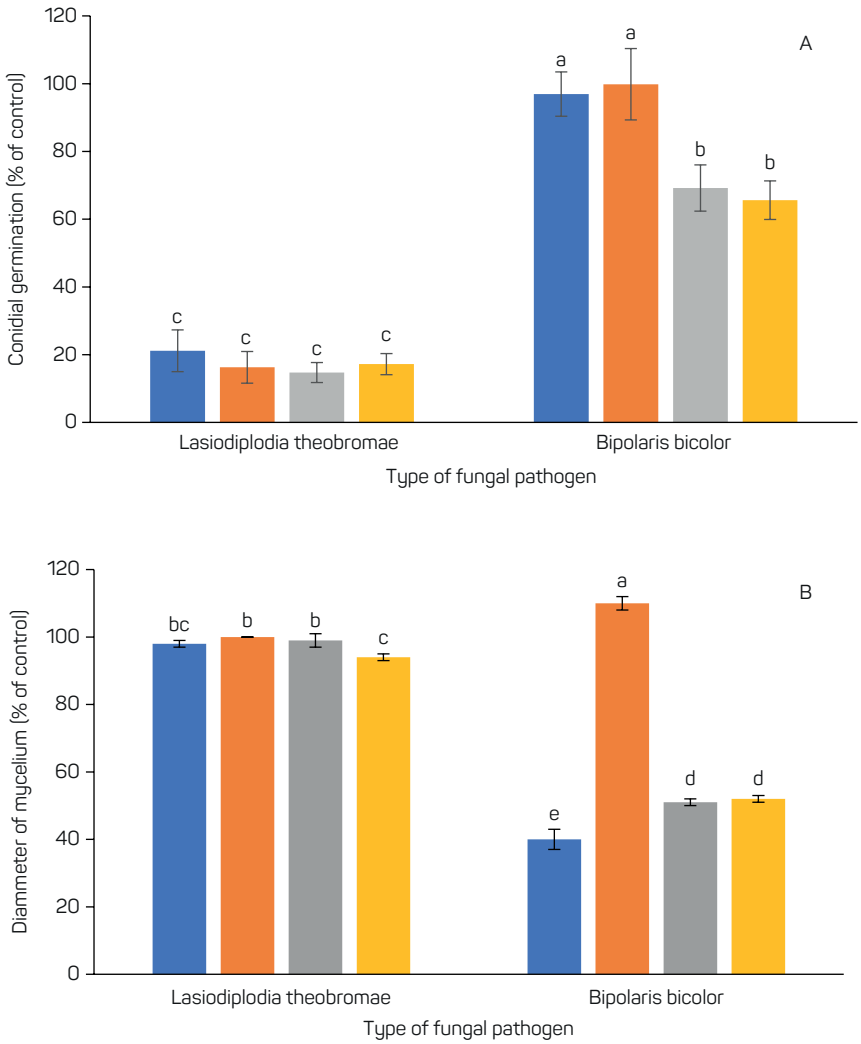

Figure 3 - Responses of *Lasiodiplodia theobromae* and *Bipolaris bicolor* after herbicide treatment of imazethapyr (■), topramezone (■), oxyfluorfen (■) and diuron (■) at respective commercial recommended rates. A. Conidial germination rate at 27 °C for 48 hours under darkness after herbicide treatment. Each datum is the average ± SD of six replicates. At least 500 conidia were counted for each value. B. Mycelium growth at 27 °C for seven days under darkness after herbicide treatment. Each datum is the average ± SD of five replicates. Means followed by a different lowercase letter have a significant difference at the 5% significance level

In the experiment conducted by Hoagland et al. (2018), it was demonstrated that formulations containing technical grade glyphosate and *Myrothecium verrucaria* mycelia significantly enhanced the control of Palmer amaranth (*Amaranthus palmeri*). This result implied that *L. theobromae* might outperform *B. bicolor* when their mycelia were used in combination with topramezone, oxyfluorfen, and diuron since no inhibitory effects were observed in these mixtures. Therefore, the combination of *L. theobromae*'s mycelia with these selected herbicides shows promise as a potential mycoherbicide and warrants further testing against *E. indica*.

5. Conclusions

Environmental factors had minimal impact on the conidia and mycelia of *B. bicolor*, and its conidia were compatible with topramezone and imazethapyr when used in combination. However, this species exhibited notably slow growth and low conidial germination, which could pose challenges for mass production in mycoherbicide development. On the other hand, *L. theobromae* is a fast-growing species, although its mycelium showed some sensitivity to temperature and pH fluctuations. Nevertheless, this sensitivity is not expected to hinder future mass production of mycoherbicides. Significantly, *L. theobromae*'s mycelia demonstrated high compatibility with various herbicides, offering flexibility in pathogen-herbicide combinations. Considering these factors, *L. theobromae* stands out as a suitable candidate for tank mixing with topramezone, imazethapyr, diuron and oxyfluorfen for development of an integrated control of *E. indica*.

Author's contributions

All authors read and agreed to the published version of the manuscript. ZS, MSAH, and CTS: conceptualization of the manuscript and development of the methodology. MAF: data collection. MAF and CTS: data analysis. MAF and CTS: data interpretation. MSAH and CTS: funding acquisition and resources. MSAH: project administration. CTS: supervision. MAF: writing the original draft of the manuscript. ZS, MSAH, and CTS: writing, review, and editing.

Acknowledgements

The authors are grateful and would like to thank Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA for providing all the facilities to conduct this research, and Universiti Putra Malaysia for partially funding this research with a research grant, vot numbered 6388500-10201.

Funding

This research received no external funding.

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