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Bipolaris sorokiniana: A POTENTIAL INDIGENOUS PLANT PATHOGEN TO CONTROL GOOSEGRASS (*Eleusine indica)* IN OIL PALM PLANTATIONS

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ABSTRACT

Indigenous plant pathogens, namely Bipolaris sorokiniana, Phoma herbarum *and* Curvularia aeria, *were evaluated in the nursery to assess their potential of controlling goosegrass (*Eleusine indica*). Two experiments were conducted, which involved pathogenicity test and host range test. The pathogenicity test showed that* B. sorokiniana *was more pathogenic to* E. indica *compared to* P. herbarum *and* C. aeria. *Infection of* B. sorokiniana *on* E. indica *occurred on the fifth day after inoculation, with 2 x* 10^6 *CFU* ml^{-1}. *On the Day 35 after treatment,* B. sorokiniana *caused 94% disease severity, the highest damage compared to the other two fungal plant pathogens. The increase in disease severity affected* E. indica's *growth, causing dry weight losses of up to 28.5 g, which was significantly lower than the dry weight of* E. indica *treated with* C. aeria *(39.5 g) and untreated control (39.2 g). Nevertheless,* B. sorokiniana *did not infect oil palm seedlings. In other host plants, such as tomato, lady finger, sweet corn, chives, banana, eggplant, chili, sweet potato, Napier grass and spinach,* B. sorokiniana *only caused mild disease severity ranging from 10% to 20%. Therefore, this study suggests that* B. sorokiniana *may have the potential to be used as a biological control agent for* E. indica *in oil palm plantations.*

Keywords: weed biocontrol, *Bipolaris sorokiniana*, *Eleusine indica*.

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INTRODUCTION

Weed can be described as any undesirable plant growing in undesirable places, especially in the planting areas of valuable crop plants. Weeds are classified according to their groups, such as grasses, broadleaves, ferns, sedges and epiphytes. They grow aggressively and compete with the main crops for light, nutrients, water and space to survive. Most weeds survive in extreme weather conditions and disperse seeds through wind, water, and soil.

Goosegrass (*Eleusine indica),* is a weed ranked as the fifth among the 10 worst weeds in the world. The species originated in Asia and represents one of the most notorious grass weeds in crops around tropical and temperate regions of the world. It is an annual species with an average production per plant of approximately 40 000 seeds in pure stands (Kissman and Groth, 1991). The species is a serious weed in 42 countries and is frequently listed as a dominant weed species in farming systems which lack shading, for it grows vigorously and produce abundant seedlings (Holm *et al.,* 1977*).* In Malaysia,

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E. indica could be found in orchards, oil palm plantations and vegetable farms (Barnes and Chan, 1990).

The *E. indica* is a noxious weed and the most destructive grass in oil palm plantations (Matthews, 1998). It was found to be the most prevalent grass (82.2%) in immature oil palm plantations throughout Malaysia (Maizatul-Suriza and Idris, 2012). The weed competes strongly with oil palm for nutrients, water, light, and space (Holm *et al.*, 1977). It could also affect oil palm growth and yield in the long-term by disrupting the economic life cycle of oil palm (Kuan *et al.*, 1991). In addition, plantation management may be disrupted by the infestation of weed, as it could potentially host pests and diseases in oil palm plantations (Oudhia, 2004).

Herbicide application in controlling the weed population has been commonly practiced by farmers and planters for many years because it has been manually and conventionally difficult to control weed growth. In tropical countries, weed management is laborious, as the weather promotes the growth of weeds. Therefore, herbicides are widely used to suppress the growth of weeds in oil palm and rubber plantations (Chuah and Ismail, 2010).

The active pesticide ingredients used in Malaysia were about 39 407 t in 2006 and 49 199 t in 2016 (FAOSTAT, 2019) for effective weed control in oil palm and rubber plantations, as well as in direct-seeding rice farming (Jabran and Chauhan, 2015). These statistics represented an annual growth rate of 3.1% over the past 10 years for the nominal amount of agricultural pesticides used in the country. Among the various chemicals used in agriculture, herbicide had the largest usage, with 83% reported in 2014 (FAOSTAT, 2019). The common problems associated with conventional herbicides are related to their inherent ability to kill plants, harm non-target vegetation, and cause crop injury (Sherwani *et al*., 2015). The continuous use of some chemical herbicides has resulted in the manifestation of herbicide resistance in more than 140 weed species and thousands of crop fields worldwide (Heap, 1997). The presence of *E. indica* biotypes with resistance to some groups of herbicides is likely to become a concern (Chuah and Ismail, 2010). The first case of glyphosate-resistant *E. indica* has been reported in orchards in Teluk Intan, Perak, Malaysia, in 1998 (Lim and Ngim, 2000). In comparison to the more susceptible *E. indica*, the 'Teluk Intan' biotype was found to be 8 to 12-fold resistant to glyphosate. Application of glyphosate at 5760 g salt ha^{-1} had only managed to control 25% of the Teluk Intan biotype *E. indica* (Lim and Ngim, 2000).

As weed is resistant to conventional chemical herbicides, researchers tend to investigate on the more environmental-friendly alternative methods of controlling weed. Research on the use of indigenous pathogens as bioherbicides has begun since 1940s (Wilson, 1969; Julien and Griffiths, 1998).

The use of bioherbicide is deemed to be an economical, effective and environmental-friendly method of controlling weed. Bioherbicide could reduce the dependency on chemical herbicides, delay the development of herbicide-resistant weed population, and reduce the risk of herbicide contamination to the environment. Bioherbicide is a long-term solution, which is most effective as part of an integrated weed management approach. It simply aims to reunite weeds with their natural enemies and achieve sustainable weed control (Barratt *et al.*, 2018).

Examples of successful bioherbicide applications in controlling weed always involve indigenous plant pathogens. Fungal herbicides, applied by spraying them to targeted weeds, will suppress weed growth and maintain crop survival for a long period of time. Conventional chemical herbicides need to be applied repeatedly to suppress weed growth and they may harmfully affect the environment. In contrast, fungal herbicides could initiate diseases in weeds by producing phytotoxins, eventually killing the weeds within three to five weeks after inoculation (Hoagland, 2000).

The identified plant pathogens are usually easily cultured on artificial media such as potato dextrose agar (PDA), Oxoid, before mass-produced as an inoculum. They are able to produce abundant mycelia, which are stable in storage, less prone to mutation, tolerant to variation in temperature, and effective under field conditions (Charudattan, 1988; Daniel *et al*., 1973). Bioherbicide may be incorporated into on-going integrated weed management programmes for more efficient weed management (Robert, 2005). Searching for new bioherbicides for weed control in oil palm plantations is in line with Kushairi *et al.* (2018), suggesting that integrated pest management and advance biotechnology, as well as good agricultural practices, could help boost oil palm yields for both plantations and smallholdings.

In Malaysia, studies on the development of indigenous plant pathogens as biocontrol agents for *E. indica* in oil palm plantations are limited. Only few plant pathogens have been identified with the potential of controlling *E. indica*. One such example is *Phoma herbarum*. To thrust the oil palm industry forward, synergising conventional and disruptive technologies at every level of oil palm supply chain in the production of new bioherbicides for controlling weed in oil palm plantations is desirable and essential (Kushairi *et al.*, 2018). Hence, this current study evaluated the potential of three different indigenous plant pathogens isolated from *E. indica,* namely *Bipolaris sorokiniana*, *P. herbarum*, and *Curvularia aeria,* to control *E. indica* via artificial inoculation in order to develop a new bioherbicide.

MATERIALS AND METHODS

Experiment Sites

Two nursery experiments were conducted to determine the effect of indigenous plant pathogens on *E. indica.* The first experiment was conducted in a glasshouse from September until October 2016, Farm 15 at the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (2°59.056'N, 101°43.957'E). The climate was humid, with an average temperature of 26.8°C and abundant rainfall. The second experiment was conducted from January until February 2017 at the nursery, Malaysian Palm Oil Board (MPOB) located at Section 15, Bandar Baru Bangi, Selangor (2°55.645'N, 101°45.967'E). The climate was also humid, with an average temperature of 27°C and abundant rainfall, averaging 157.5 mm mth^{-1} during the experiment.

Experiment 1: Pathogenicity Test

Planting material. Five emerging seedlings of *E. indica* were planted individually in each of the total 80 pots used for the experiment. Each pot (10 cm diameter x 11.5 cm height) was filled with soil in the ratio of 3:2:1 (top soil: peat: sand). The pots were arranged in eight rows, with each row containing 10 pots. The distance between pots was 65 cm inter-row, and 50 cm between rows (*Figure 1*). The age of *E. indica* used in the study was 14 days after planting.

Preparation of fungal inoculum. Young and actively growing *B. sorokiniana, P. herbarum* and *C. aeria* were individually cultured by adding 10 pieces of mycelial plugs (6 mm diameter) into a 1-litre conical flask containing 250 ml autoclaved potato dextrose broth (PDB). The flask was then shaken at 150 rpm, continuously for 15 days at 28°C. The mycelium was homogenised using a homogeniser (IKA T25 digital ULTRA TURRAX, Germany) at 10 000 rpm for 15 min. The number of colony-forming unit (CFU) per ml was determined on PDA after five days of incubation at 25°C by using serial dilution method. The concentration of mycelial suspension for each tested fungus was adjusted by diluting with water to 2 x 10^6 CFU ml^{-1} containing 0.01% Tween 20.

Treatments and inoculation methods. Four treatments were assessed in this experiment, as shown in *Table 1*. For each treatment, a group of 20 pots containing *E. indica* was inoculated by spraying method using a 2-litre hand-held pressure sprayer. Each pot was sprayed with 10 ml of fungal inoculum. The treated plants were enclosed with transparent plastic bag (44 cm length x 31 cm width) and sealed for 24 hr to allow the fungus to infect the plants. For the control, the plants were sprayed with sterile distilled water. The experiment was conducted for 35 days, using Completely Randomised Design (CRD) with 20 replications.

TABLE 1. TREATMENT IN THE STUDY

Treatment	Types of fungal pathogen
1	*B. sorokiniana* containing 0.01% Tween 20
2	*P. herbarum* containing 0.01% Tween 20
3	*C. aeria* containing 0.01% Tween 20
4	Control

Disease severity. Disease severity was rated at five-day intervals after the application of treatment. The level of severity was rated based on the rating scale as described by Kadir and Charudattan (2000) in *Table 2*, with a slight modification. The rating scale was analysed using the following formula:

$$DS = \sum \frac{\left(\begin{array}{c}\text{Severity rating x} \\ \text{Number of plants in that rating}\end{array}\right)}{\text{Total number of plants x highest rating}} \text{ x } 100\%$$

TABLE 2. DISEASE SEVERITY SCALE

Disease scale	Leaf area damaged (%)
0	0
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

***Dry weight assessment of* E. indica.** At the end of the experiment, the dry weight of *E. indica* was assessed. The shoots in the experimental pots were cut and dried at 80°C for 72 hr in a drying oven, Memmert, Germany (Mandal *et al.*, 2014). The dried plant samples were weighed using electronic balances UX6200H Shimadzu, Japan.

Statistical analysis. The dry weight of treated *E. indica* infected by the fungal pathogens was analysed using one-way analysis of variance (ANOVA). Means was separated using Tukey's Test at P=0.05.

Experiment 2: Host Range Test

Planting material. Eleven types of host plants were used in this experiment, comprising tomato, lady finger, sweet corn, chive, banana, eggplant, chili,

sweet potato, Napier grass, spinach and oil palm seedlings; additionally, *E. indica* was used as the control. They were all grown under a 70% shaded nursery and maintained at 26°C to 34°C. *E. indica* was established using seeds collected from the field and transplanted in polybags (24 cm width x 34 cm height) filled with a mixture of soil, sand, and peat in the ratio of 2:1:1, respectively. Tomato, lady finger, sweet potato, eggplant, chilli and spinach were established from seeds and transplanted in the same type of polybags containing the same soil mixture for seven days after germination and left to grow for two weeks. Chives, sweet potato and Napier grass were established from stem cuttings and transplanted in polybags and left to grow two weeks before treatment. Banana was established using three-week old tissue cultured seedlings, transplanted in polybags. For oil palm, three-month old commercial seedlings in polybags were used. Plants' age varied depending on the species, but each plant species was at a young developmental stage for treatment. Host plants were selected based on their potential to be used as integration crops, with oil palm following previous research findings (Raja Zulkifli *et al.*, 2010; Suboh and Roslan, 2004). The distance between polybags was 55 cm inter-row and 50 cm between rows.

Preparation of fungal inoculum. Only one plant pathogen was used in this experiment, namely *B. sorokiniana.* The selection was based on results from Experiment 1, which indicated that *B. sorokiniana* was the most potential biocontrol agent for *E. indica* compared to *P. herbarum* and *C. aeria*. The fungal inoculum was prepared using the same method as described in Experiment 1.

Inoculation method and disease severity. Each host plant was sprayed to run-off (10 ml) with 2×10^6 CFU ml^{-1}, containing 0.01% Tween 20 of *B. sorokiniana* using a 2-litre hand-held pressure sprayer. Control plants were sprayed with sterile distilled water. The treated plants were arranged in Randomised Complete Block Design (RCBD) with 10 replications (*Figure 2*). Disease severity was rated at five-day intervals after application of treatment, following the method described in Experiment 1. The experiment was conducted for 40 days.

RESULTS

Experiment 1 - Pathogenicity Test

Figure 3 shows the percentage of disease severity of *E. indica* after being inoculated with *B. sorokiniana, P. herbarum,* and *C. aeria* at 2×10^6 CFU ml^{-1} containing 0.01% Tween 20. Results showed that *B. sorokiniana* was more pathogenic to *E. indica* than the other two plant pathogens, *P. herbarum* and *C. aeria*. At five days after treatment (DAT), *B. sorokiniana* caused the highest disease severity at 10%, compared to *P. herbarum* (4%) and *C. aeria* (8%). However, at 10 DAT, disease severity by *B. sorokiniana* (18%) had not increased significantly compared to other treatments with *P. herbarum* (30%), *C. aeria* (30%) and the control (22%). The disease infection for all tested fungal pathogens increased at 20 DAT, with the highest rating shown by *B. sorokiniana* (60%), followed by *P. herbarum* (50%), *C. aeria* (54%) and the control (40%). At 35 DAT, the percentage of disease severity of *E. indica* treated with *B. sorokiniana* and *P. herbarum* was 94% and 90%, respectively. At this stage, the severity level caused by both plant pathogens was higher compared to *C. aeria* (66%), and the control (56%). Although this experiment suggested that *B. sorokiniana* and *P. herbarum* have a potential of controlling *E. indica, B. sorokiniana* is chosen for further study because *P. herbarum* is currently under investigation by another group of researchers

Figure 1. The experimental layout for Experiment 1.

Figure 2. Layout of host plants used in Experiment 2.

at the GanoDrop Unit at MPOB. Meanwhile, *C. aeria* developed the lowest disease severity to *E. indica* in comparison to *B. sorokiniana* and *P. herbarum*. Therefore, *P. herbarum* and *C. aeria* were not evaluated further in Experiment 2. *Figure 4* shows the symptom of disease on *E. indica* caused by *B. sorokiniana*, *P. herbarum* and *C. aeria*.

The effects of disease caused by plant pathogens in reducing the dry weight of *E. indica* was also determined. The study recorded that the dry weight of *E. indica* inoculated with *B. sorokiniana* at 2 x 10^6 CFU ml^{-1} was 28.5 g; this was significantly lower ($P<0.05$) compared to *E. indica* inoculated with *C. aeria* (39.5 g) and the control (39.2 g) (*Figure 5*). However, the dry weight of *E. indica* inoculated with *B. sorokiniana* and *P. herbarum* did not demonstrate any significant difference. The lowest dry weight of *E. indica* yielded by the plant pathogen *B. sorokiniana* suggested that the fungus was able to inhibit the growth of *E. indica*.

Experiment 2 - Host Range Test

The disease severity of tested host plants treated with *B. sorokiniana* at 2 x 10^6 CFU ml^{-1} containing 0.01% Tween 20 is shown in *Figure 6*. The level of disease severity of lady finger, eggplant, sweet potato, Napier grass and spinach, treated with *B. sorokiniana* was below 10% throughout the experiment. At 10 DAT, the fungus infected chives up to 36% severity, but the plant was able to recover from the disease after 10 days of inoculation, and the disease remained localised without any infestation to other new developing leaves.

Moderate disease severity below 20% was observed for tomato, sweet corn and banana. For oil palm, none of the seedlings was infected by *B. sorokiniana* by the end of the experiment. For *E. indica*, the disease severity increased from 23% at 5 DAT, to 60% at 10 DAT, then rapidly increased from 25 DAT to 40 DAT to peak at 79%. This study suggested that the plant pathogen *B. sorokiniana* was highly pathogenic and host-specific to *E. indica*.

DISCUSSION

B. sorokiniana, *P. herbarum*, and *C. aeria* are indigenous plant pathogens isolated from *E. indica (*Maizatul *et al*., 2017; Rusli *et al*., 2015). The fungi, which cause disease to a wide range of hosts in Poaceae, are significant pathogens classified as high temperature fungi with the ability to survive in extreme weather and temperature. *B. sorokiniana* is known as a causal agent of common root rot disease, leaf spot disease, seedling blight, head blight drum, and black print among wheat and barley (Zhong and Steffenson, 2001; Mathre *et al*., 2003; Meldrum *et al*., 2004). The disease, which was also reported on other grasses (Kumar *et al*., 2002), was listed in the group of necrotrophic and highly virulent pathogens (Berbee *et al*., 1999). *P. herbarum* was reported to have been infecting cherry palm with leaf spot disease for the first time in Chiang Mai Province, Thailand (Kumla *et al*., 2016), whereas incidence of *C. aeria* infection was reported to have been causing rice leaf spot disease in Malaysia. *C. aeria* was listed in the group of mild plant pathogens (Kusai *et al*., 2015).

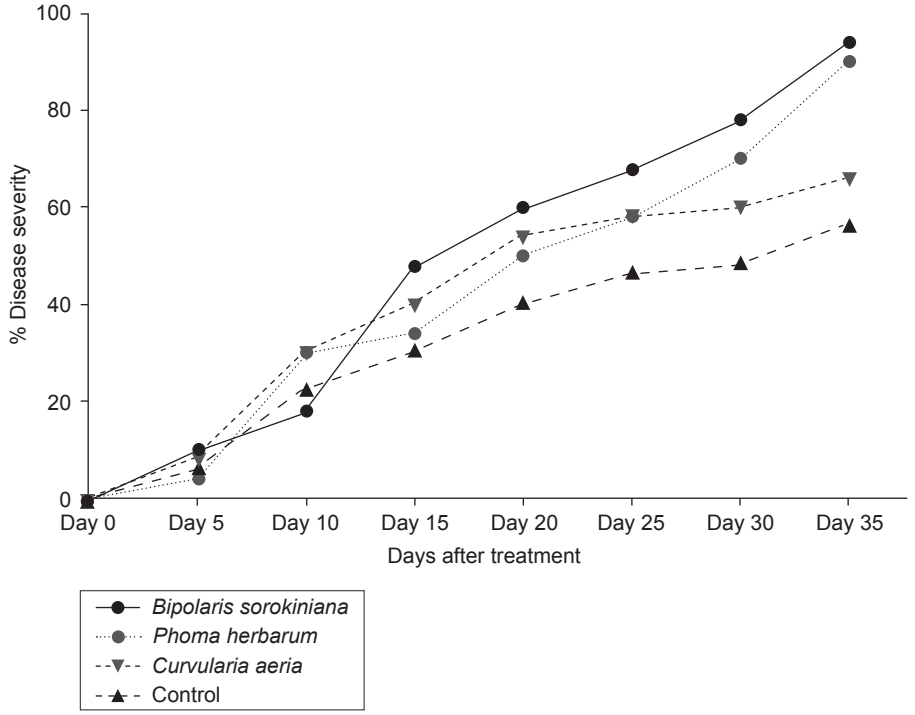

Figure 3. Percentage of disease severity of Eleusine indica *inoculated with different indigenous fungal plant pathogens.*

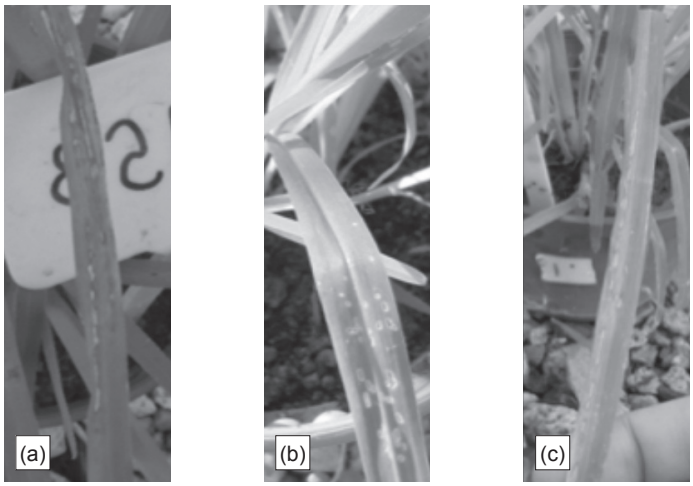

Figure 4. Symptom on Eleusine indica *inoculated with (a)* Bipolaris sorokiniana*; (b)* Phoma herbarum*; and (c)* Curvularia aeria.

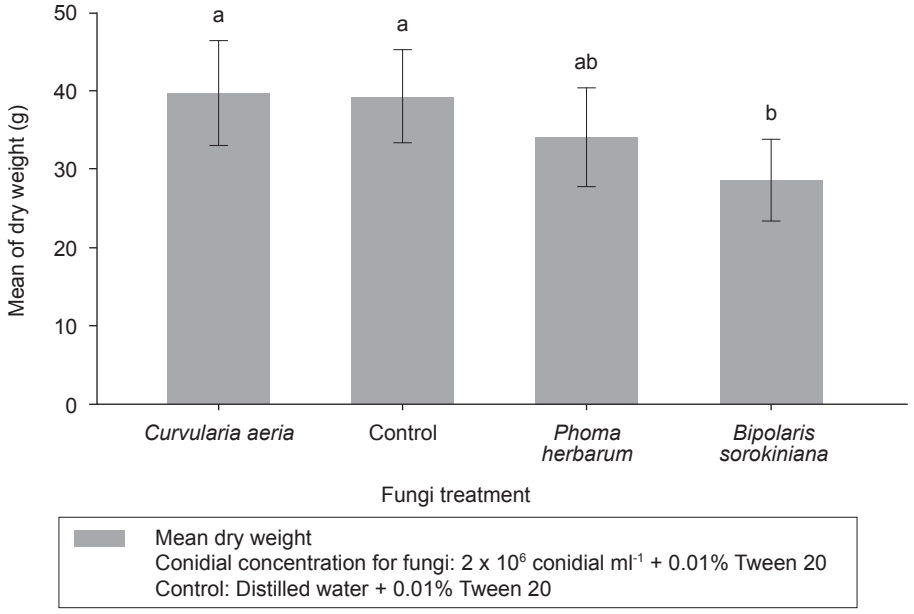

Figure 5. Mean dry weight of Eleusine indica *treated with different fungal plant pathogens. Means followed by the same letters are not significantly different at P=0.05% after Tukey's test.*

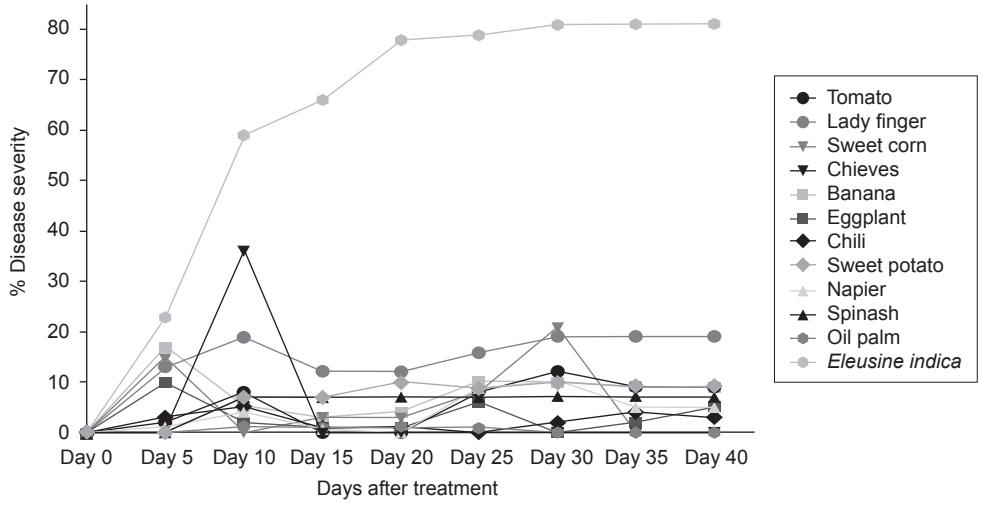

Figure 6. Percentage of disease severity of different host plants after treated with Bipolaris sorokiniana *at 2 x 10⁶ CFU ml⁻¹.*

Observation on the high percentage of disease severity caused by *B. sorokiniana* and *P. herbarum* was supported by Maizatul *et al*. (2017), suggesting that both pathogens could damage the whole plant; Rusli *et al*. (2015) also reported that *P. herbarum* was highly pathogenic to *E. indica*. Although these two types of plant pathogens were isolated from the diseased stems, the fungi had been able to cause lesions on the *E. indica* leaves (Maizatul *et al.*, 2017). Meanwhile, mainly due to the naturally occurring infestation of leaf-spotting pathogen, infection in the control group, which severely damaged the leaves, recorded up to 50% disease severity. This was supported by the research on biological control for *E. indica* in South Carolina, which identified the fungus *Bipolaris setaria* as the fungal leaf-spotting pathogen that had naturally infected *E. indica* in field plots in summer, whereas the fungal pathogen *Piricularia frisea* had infected *E. indica* in a greenhouse in spring (Figliola *et al.*, 1988).

The diseased shoots of weed infected by bioherbicide agents had reduced the weed biomass at the end of the experiment. In the experiment, treatment with *B. sorokiniana* had resulted in the lowest dry weight of *E. indica* shoots, indicating that the fungus had effectively suppressed the growth of *E. indica*. New leaves were inhibited from developing, thus reducing the biomass weight through lesion, wilting, and drying of the leaves. Initial symptoms of *B. sorokiniana* infection on the leaves of *E. indica* were observed based on the appearance of dark-brown elliptical flecks, developing 24 hr after inoculation. Lesions expanded to large areas of the leaf. Expanding lesions were deep brown with a chlorotic halo and turned the leaf to yellow before wilting and drying, causing the leaf to die. Drying of the leaves started from the bottom part of *E. indica* and moved up to the crown region. The infected plants were commonly smaller compared to the control plants. Reduction in dry weight was also reported on the weed *Cyperus rotundus* when treated with the fungus *Dactylaria higginsii* by spraying with 10^6 conidia ml^{-1}. *Cyperus rotundus* is the world's worst weed, infecting rice, sugarcane, cotton, corn and vegetables (Kadir and Charudattan, 2000).

To ensure that candidates of plant pathogens for bioherbicide do not affect other important plants, host range test is a prerequisite. This study provided the assessment of the host range test for *B. sorokiniana*. The results demonstrated that *B. sorokiniana* was a specific plant pathogen which caused severe disease only to *E. indica*. The fungus could also infect other plants, though the impacts would be considerably mild since it only caused 20% to 25% disease severity to other host plants. More importantly, *B. sorokiniana* did not infect oil palm seedlings. This study proved that *B. sorokiniana* has a potential to be used as a biocontrol agent for *E. indica* in oil palm plantations.

CONCLUSION

B. sorokiniana has a potential as a biocontrol agent for *E. indica*. The treated *E. indica* plants were severely damaged with suppressed growth and reduced the biomass. The host plants test showed that *B. sorokiniana* was a specific plant pathogen, only affecting *E. indica* and not causing disease to non-target crops. More importantly, the fungus did not infect oil palm seedlings, indicating that *B. sorokiniana* may be used as biocontrol agent for *E. indica* in oil palm plantations. Further study is required to investigate the efficacy of *B. sorokiniana* under conditions in the field. Successful development of weed biocontrol will reduce environmental pollution, such as ground water and soil contamination.

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