MICROBIOLOGY ARCHIVES
Original Research Article | ISSN: 3041-590X
Journal homepage: https://microjournal.researchfloor.org/

Identification and Control Treatment for Leaf Spot Diseases in Grain Corn

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ARTICLE INFO

Citation: Razean Haireen, M. R. Fatin Nurliyana, A and Halimah H. (2026). Identification and Control Treatment for Leaf Spot Diseases in Grain Corn.
Microbiology Archives, an International Journal.
DOI: https://doi.org/10.51470/MA.2026.8.2.01

Received 03 April 2026
Revised 05 May 2026
Accepted 07 June 2026
Available Online July 02, 2026

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ABSTRACT

Grain corn is the main source of feed for livestock in Malaysia, especially chickens and pigs, as most of the livestock feed products have a grain corn composition between 45% and 65%. To fulfil the needs, Malaysia targets to reduce the importation of grain corn from foreign countries such as Argentina, Brazil, the United States, Thailand, Myanmar, Pakistan and Indonesia by achieving a 30% national self-sufficiency rate (SSL) in 2040. Efforts in many aspects, including research, have been put in. In this study, the focus was given to the identification and control of fungal pathogens that caused major diseases in grain corn. Monitoring of the disease was carried out in a farmers' field at FELDA, Chuping, Perlis. The effectiveness of in vitro control methods using chemical fungicides showed the potential of Propiconazole to control the growth of Bipolaris sp. and Curvularia sp. Field trial results also showed Propiconazole significantly reduced the leaf spot diseases. The results of this study will be shared in the Grain Corn Cultivation Manual as a guide to grain corn entrepreneurs and farmers to reduce the problem of fungal disease attacks that can affect the crop yield.

Keywords: *Leaf spot diseases, Bipolaris sp., Curvularia sp. Mancozeb, Propiconazole.*

1. INTRODUCTION

Grain corn is one of the important commodities for the livestock industry in Malaysia. It is used as the main ingredient for animal feed, especially ruminants, broiler chickens and pigs [1]. Malaysia imported almost 100% of grain corn to meet 3.7 million MT of grain corn based on the market demand, which is equivalent to RM3.9 billion in 2020. To increase the production of local grain corn and to reduce the value of imports, the Malaysian Agricultural and Food Security Ministry (KPKM) has set a target to achieve a 30% national self-sufficiency rate (SSL) for grain corn by the year 2040.

Many efforts and studies are being carried out to assess its potential. One important aspect to be concerned about is pests and diseases. In Malaysia, a major threat to corn cultivation by Fall armyworm (FAW) causes a large loss for the industry; meanwhile diseases threat particularly by fungal is seem to be insignificant. Nevertheless, under the unpredictable weather nowadays, fungal disease could affect agricultural activities and food production due to its pathogenic nature that could damage plants. Fungus infects plants throughout the growing season, especially under favourable environmental conditions. According to [2], the weather conditions during the growing season have a significant impact towards the severity of the disease encountered by corn. The disease may occur in a certain growth stage of the crop; as such, some may only appear during the vegetative stage, while some appear during the tasselling stage. Fungus can infect corn either through the seed, stalk or foliar. The type of foliar diseases depends on the favourable environment surrounding the crops. Certain diseases prefer warm and humid conditions while others prevail in cool and humid conditions.

Bipolaris maydis was mentioned to cause maydis leaf blight or southern maize leaf blight problems in Pakistan, India, Nepal, Kampuchea, the Philippines, China, Indonesia, and Vietnam. This disease has caused significant yield losses in cultivars that are grown in high levels of subtropical or temperate germplasm [3]. The disease could be observed as small and diamond-shaped in the young lesions, but once they mature, the lesions start to elongate and form into a rectangular shape. The disease prefers hot and humid of which the maize-growing areas with slightly hot temperatures could enhance the pathogenic fungus spread easily to other crops [4].

Curvularia is another genus of fungi that can infect a variety of crops, including grain corn. It is primarily associated with diseases that affect corn plants, particularly in warm, humid environments [5]. Its impact on corn is significant, leading to yield reductions, quality degradation, and potential issues in grain.

Lack of control over fungal disease may result in disastrous economic consequences worldwide, as corn is one of the major crops globally. According to [6], growers lose between 10 and 23 per cent of their crops to fungal infection each year. Hence, in this study, the pathogenic fungus of grain corn needs to be identified and undergo an efficient fungicide treatment to avoid a severe loss of the corn grain yield.

2. METHODOLOGY

Analysis of disease incidence and disease severity in grain corn at farmers' field

The observation of foliar diseases in the grain corn variety P4546 was conducted at the upscaling plot, Chuping, Perlis, Malaysia. To identify the susceptibility towards fungal disease, the incidence percentage was calculated using the formula; Disease incidence percentage = (symptomatic plants/inventoried plant population) x 100. Meanwhile, disease severity was visually rated based on the symptomatic area over the total foliage of the plants as reported by [7], where 0 is no infection, 1 is 1–25% infection, 2 is 26–50% infection, 3 is 51–75% infection, and 4 is 76–100% infection. The disease severity index percentage was then calculated following the below equation and statistically analysed using SAS 9.4.

$$\text{Disease Severity Index (DSI), (\%)} = \frac{\sum(\text{P} \times \text{Q})}{(\text{M} \times \text{N})} \times 100$$

Isolation of fungus from infected grain corn

The plant which was infected severely by a fungus disease, was taken as a sample. The leaves were cut into tiny pieces in a square shape and then washed with 10% Clorox, followed by 70% ethanol. The sample were then washed with sterilised distilled water three times to clean the impurities thoroughly and air dried on filter paper. Finally, the samples were placed in the centre of a Potato Dextrose Agar (PDA) plate and incubated for seven days to promote the growth of fungi.

Microscopic observation of pathogenic fungus

The isolated fungus, which incubated for 7 days, was observed for its morphology. In order to observe the spore, staining of the fungus was carried out using methylene blue or lactophenol solution. The staining was done by dropping a drop of the staining solution on the glass slide. Then, a strip of Sellotape was used to obtain the spores of the fungus by putting it on the surface of the mycelia and slowly pulling it back up. The Sellotape was then put on top of the staining solution on the glass slide. Observation of spores was done using a light microscope with a magnification of 40X and 10X.

Inhibition test on the effect of fungicides towards *Bipolaris* sp. and *Curvularia* sp.

An in vitro antagonistic test was performed using a dual culture diffusion assay in a CRD design twice with five replications. The control of the growth of *Bipolaris* sp. and *Curvularia* sp. against commercial fungicides, Mancozeb (ai: Maneb) and Propiconazole (ai: Triazole), was evaluated using dual culture diffusion assays. 50 µL of selected fungicide (prepared at a final concentration recommended by the manufacturer) was placed in a 6 mm well, which was located 30 mm from the margin of potato dextrose agar (PDA) plates. Fresh-growing mycelium from the seven-day plate of *Bipolaris* sp. and *Curvularia* sp. was then cut (5 mm) with a scalpel and placed at a similar distance from the fungicide but directly opposite. The plates inoculated using distilled water as the antagonist served as the negative control. The plates were then incubated at room temperature for 7 days. The suppression zone was recorded by measuring the radius of fungal mycelium growth in the plates on days 5, 6 and 7. Then, the suppression percentage (%) for each treatment was calculated using the formula; Suppression (%) = (1 – Mean growth in treatment / Mean growth in control) × 100.

Data was statistically analysed using SAS 9.4 software to determine significant differences by Duncan's multiple range test, $P<0.05$.

Evaluation of the effectiveness of fungicides towards *Bipolaris* sp. and *Curvularia* sp. in the open field

The field testing was conducted at MARDI Serdang (2°59'04.2"N 101°42'16.3"E). The grain corn was planted at a spacing of 75 cm x 20 cm. Each treatment consisted of 25 plants with three replications and was laid out in a randomized complete block design (RCBD). The plants were irrigated regularly using a sprinkler system. Plot preparation, fertilizer and pesticides in this study were used according to the standard procedure for grain corn established.

The fungicide treatment (mancozeb and propiconazole) was applied using the spraying technique at 26 days and 47 days after planting. Grain corn without any treatment was used as the control plant. The incidence and severity assessment for leaf spot diseases was carried out at intervals of every two weeks. The calculation for leaf spot disease incidence and severity index is as mentioned in the earlier section.

RESULTS AND DISCUSSION

Incidence and severity of fungal disease in grain corn at farmers' field

Leaf spot disease caused by fungi was evaluated significant being the major disease in grain corn variety P4546 at the farmers' field. The fungus infects corn through seeds, foliage and stalks. Curvularia leaf spot disease is likely to occur during the vegetative growth of grain corn approximately 14 to 30 days after sowing. The leaf spot symptoms can be seen as a circular spot surrounded by a yellow circle, sized 1 mm-2 mm (Figure 1). Initial symptoms usually occur on the first leaf and then develop on the upper leaves. The isolated fungus was identified as *Curvularia* sp. based on the morphology and microscopic observation. *Curvularia* sp. showed a woolly colony and olivaceous green circular margin morphology when observed from the top of a PDA Petri plate, while a circular form of brown pigment could be seen from the bottom side. Microscopic observation showed a septate hypha with oval macroconidia (Figure 2). Based on the field observations the percentages of incidence and severity for Curvularia disease in variety P4546 were 50.25 ± 28.25 and 23.47 ± 13.67, respectively.

Another symptom of leaf spot disease was observed as a lesion with a rectangular shape (Figure 3). Fungal morphology and microscopic observation identified the pathogenic fungus as *Bipolaris* sp. The fungal morphology displayed of a cottony colony with a greyish to dark grey center and margin when observed from the top of the Petri plate while an irregular form with black undulate margin could be seen from the bottom side of the Petri plate. Microscopic observation showed a septate hypha with straight or slightly curved canoe shaped macroconidia (Figure 4). The incidence and severity of Bipolaris leaf spot were 57.91 ± 16.74 and 15.77 ± 5.10 respectively.

These diseases are likely to develop in a conducive environment around the plant especially in high temperature, high humidity, and frequent rainfall [8]. We noticed the symptoms of leaf spot as early as 14 days after infection of which then caused severe infection in grain corn. Leaf spot disease has been reported to significantly threaten and cause severe losses in corn grain yields in other countries as it could destroy the foliage quickly especially when corn grain reaches maturity [9].

Figure 1. Symptom of Curvularia leaf spot disease in grain corn.

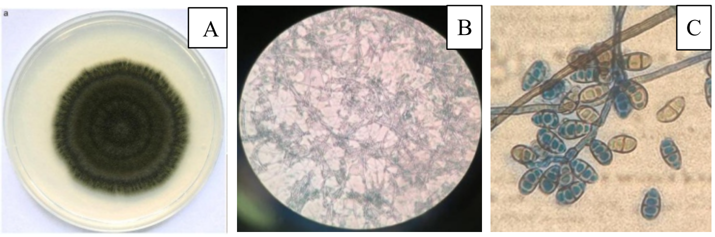


Figure 2. Fungus Curvularia sp. (A) Morphology on PDA media; (B) Microscopic observation with 40X magnification and (C) Microscopic observation with 10X magnification

Figure 3. Symptom of Bipolaris leaf spot disease in grain corn.

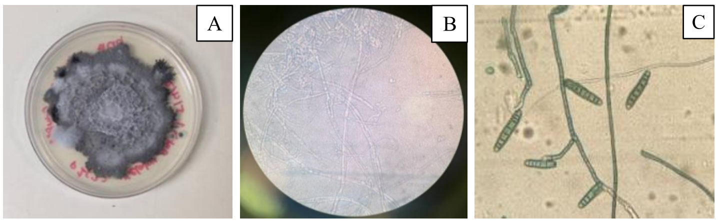


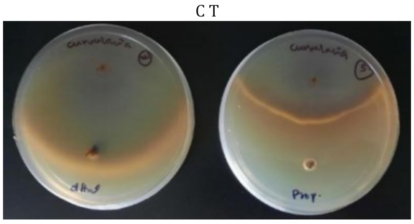
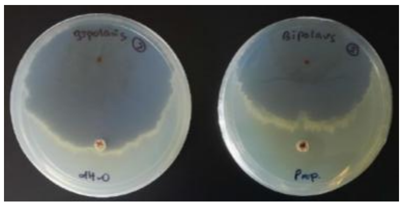
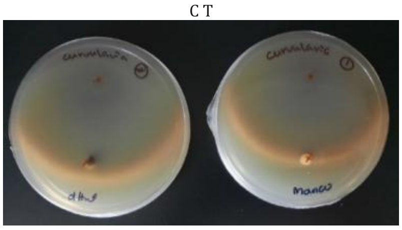
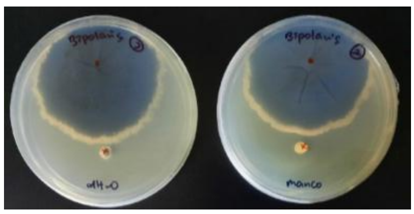
Figure 4. Fungus Bipolaris sp. (A) Morphology on PDA media; (B) Microscopic observation with 40X magnification and (C) Microscopic observation with 10X magnification.

In vitro suppression analysis on the effect of fungicides towards *Bipolaris* sp. and *Curvularia* sp.

The detection of fungal diseases in farmers' grain corn field indicated the importance of early control measures in avoiding severe effects to the plant. When leaf spot diseases are severe, fungicides serve as the primary control method [10]. In this study, the antifungal efficacy of propiconazole varied significantly between *Bipolaris* sp*.* and *Curvularia* sp*.* after a 7-day incubation period. *Curvularia* sp. exhibited high sensitivity to the treatment, resulting in a radial growth suppression of 63.06% while *Bipolaris* sp. demonstrated a much higher tolerance to the fungicide, displaying a substantially lower growth suppression rate of 32.17%. The differential susceptibility corresponds to a noticeably lesser mycelial expansion on the *Curvularia* sp. assay plates compared to the *Bipolaris* sp. (Table 1). Regardless of the suppression in different fungal species, propiconazole performed better than Mancozeb in the in vitro analysis. Triazoles penetrate fungal tissue to actively disrupt intracellular ergosterol pathways by targeting the sterol 14α-demethylase enzyme [11, 12]. The active ingredient propiconazole is widely documented in chemical and agronomic literature for its dual-action (preventive and curative) capabilities. According to [13], azoxystrobin and propiconazole were more effective when applied to maize after the flowering stage.

Conversely, mancozeb shows limited radial growth restriction against growing mycelial plugs on an agar. The assay plate with mancozeb treatment showed a low growth suppression rates observed for both *Curvularia* sp. (15.29%) and *Bipolaris* sp. (13.99%). Mancozeb is a broad-spectrum, non-systemic thiocarbamate fungicide belonging to FRAC Group M3. Mancozeb functions strictly as a surface protectant to prevent spore germination rather than penetrating active vegetative growth [14]. In that case, mancozeb is likely to be prominently applied before the grain corn reaches tasseling stage.

Table 1. Suppression percentage (%) of Curvularia sp. and Bipolaris sp. mycelial growth that subjected to propiconazole and mancozeb chemical treatments. The treatment plate (T) in comparison to control (C) placed side by side after 7 days of incubation.

Fungicide	Mode of Action	*Curvularia* sp. suppression (%)	*Bipolaris* sp. suppression (%)
Propiconazole	Systemic / Ergosterol Biosynthesis Inhibitor	C T  63.06 %	C T  32.17 %
Mancozeb	Contact / Multi-site Protective Inhibitor	C T  15. 29 %	C T  13.99%

Effectiveness of fungicides towards *Bipolaris* sp. and *Curvularia* sp. in open field

Further evaluation of the fungicide effectiveness in grain corn variety P 4546 was tested at a hotspot area where leaf spot fungus was potentially infected the plants naturally without inoculation in Serdang Selangor. The propiconazole treated plants showed a significantly lower average of leaf spot incidence (33.0%) and severity (8.41%) compared to the mancozeb and control treated plants (Figure 5). Propiconazole is absorbed across the leaf cuticles and translocated upward and outward within the xylem to safeguard newly emerging foliage from high pathogen pressure. This is supported by [15], who found PropiconazolePro® provided better control and resulted in trees with highest concentrations of propiconazole in their xylem. This systemic fungicide actively suppresses latent vegetative hyphae of leaf spot fungus that establish within the mesophyll layer before visible lesions erupt. Figure 6a shows the least infected of grain corn plants at 60 days after treatment with propiconazole in open field.

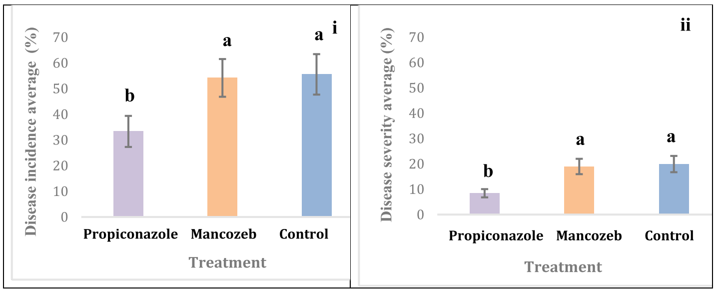


Figure 5. The effect of leaf spot incidence (i) and severity (ii) in grain corn variety P4546 applied with different treatment. Error bars represent the standard error of the mean (SEM).

In contrast, the plants that treated with contact fungicide (mancozeb) showed a similar level of incidence (>50%) and severity (>10%) to the untreated plants (Figure 5). Figures 6b and 6c show the infected grain corn plants at 60 days in open field after treated with mancozeb and untreated respectively. When applied by spraying at 26 and 47 days after planting, mancozeb forms a protective film strictly on existing leaf surfaces but unable to protect the newly emerged tissue against *Curvularia* or *Bipolaris* conidia. Furthermore, mancozeb is unstable in water and is rapidly decomposed in the presence of light, heat and moisture [16]. Based on the findings, therefore mancozeb is suggested to be applied more as a field preventative designed to stop baseline spore germination before fungal attachment occurs rather than curative for fungal disease management in grain corn.

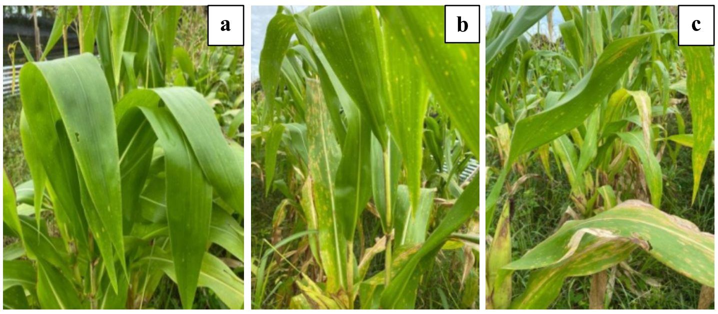


Figure 6. The grain corn plants treated with propiconazole (a), Mancozeb (b) and untreated (c) in open field.

CONCLUSION

In vitro suppression analysis and effectiveness evaluation of fungicides towards *Curvularia* sp. and *Bipolaris* sp. in an open field demonstrated that propiconazole; a systemic fungicide is essential to be used against leaf spot diseases rather than mancozeb in high-canopy, fast-growing crops like grain corn.

ACKNOWLEDGEMENT

This study was funded by The Ministry of Agriculture and Food Security under Special project (K-RM167). Author would like to express her gratitude to all who are directly and indirectly involved in this study.

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